30th ANNIVERSARY OF
MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

Special Issue June 2018

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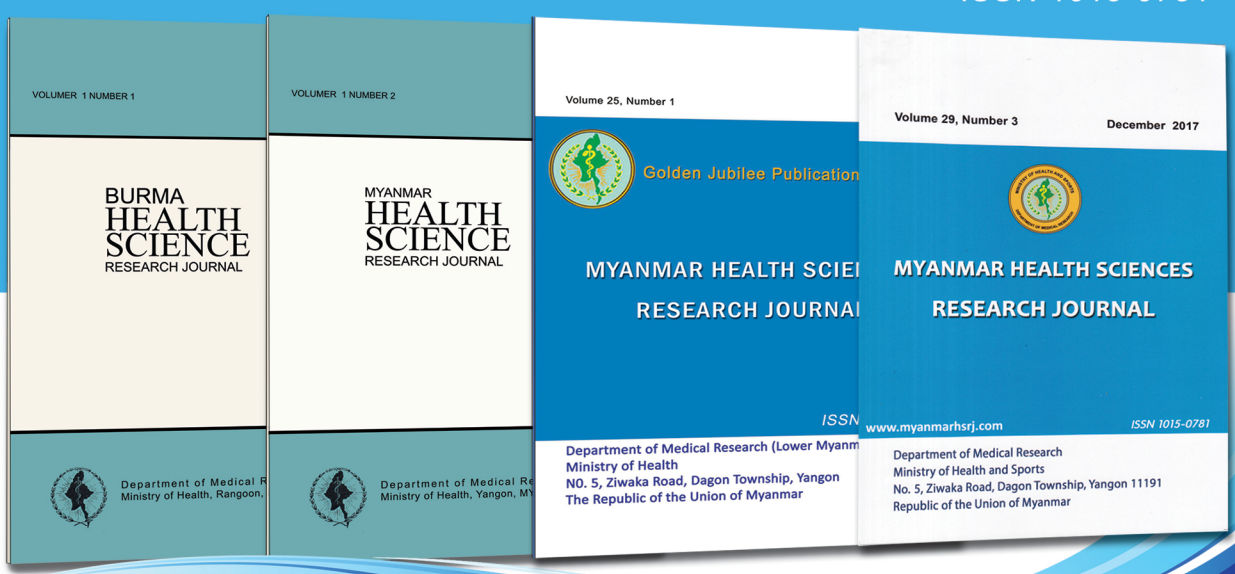

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AIMS OF THE JOURNAL

- To serve as an important medium for the publication of original research works in the field of medical science and health research, thus filling gaps in health knowledge for effective utilization of research findings
- To disseminate recent basic, applied and social research findings among health personnel of different strata for enhancing nation-wide health development in Myanmar
- To offer current medical knowledge and updated scientific information obtained from research to health professionals for better and appropriate health care management

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FOREWORD

"Myanmar Health Sciences Research Journal (MHSRJ)" is now approaching the 30th year of record as a leading academic medical research journal in Myanmar. It is published by the Department of Medical Research, Ministry of Health and Sports. In this journal, original research articles, review articles, short reports and correspondences in the field of biomedical and health sciences have been published every 4th month in a year after thorough double blind peer-review.

First issue of the journal was appeared as "Burma Health Science Research Journal" in April, 1989. Starting from second issue, the name of "Myanmar Health Science Research Journal" has been used. A total of 883 articles on various research areas have been published within 30 years of the Journal serving an important medium for the publication of original research works in the field of medical science and health research. In accordance with the aim of the Journal, it contributes to fill the gaps in health knowledge for effective utilization of research findings by disseminating recent basic, applied and social research findings among health personnel of different strata for enhancing nation-wide health development in Myanmar. It is no doubt that current medical knowledge and updated scientific information obtained from research to health professionals for better and appropriate health care management are providing to the audience of the Journal.

As a special issue celebrating the 30th year anniversary of the MHSR Journal, commentaries on various scope on the major research disciplines are invited from the experts and leaders of the medical research in Myanmar and published in which include invaluable information to the audience of the Journal covering the indispensable experiences and opinions on medical research as well as the milestones of major research areas such as hepatitis B, cancer, snake bite, malaria, dengue and tuberculosis.

Moreover, we selected 20 original research articles published in previous issues of the Journal based on the contribution and impact of the findings to the respective research fields that include the research on communicable diseases such as malaria, tuberculosis, hepatitis, HIV/AIDS, cholera, dengue and human papillomavirus, and non-communicable diseases including snake bite, diabetes, hypertension, needle disposal practice, and health system research findings. To be memorable and respectful, original referencing styles are maintained without reformatting in these selected articles.

We would like to acknowledge the researcher who submitted their research findings to our MHSR Journal, anonymous reviewers for their kind contribution for peer-review process and all staff from the Publication Division, Department of Medical Research for their dedicated effort. Without their contributions, it would be unable to continue up to 30 years of the Journal. As a Chief Editor, I hereby endorse to accelerate the effort of the MHSR Journal to achieve the high bibliometric measures in academic citation index enhancing the publication culture among the Myanmar researchers after successful implementation of the research projects.

Dr Kyaw Zin Thant
Editor-in-Chief
Myanmar Health Sciences Research Journal

COMMENTARIES FOR CELEBRATING THE 30 YEARS OF MHSR JOURNAL

Commemorative Issue for 30th Anniversary of MHSRJ, 2018

I HAVE A DREAM

Prof Aung Than Ba Tu
Emeritus Medical Researcher
Formerly: Director-General, Department of Medical Research (Myanmar)
Director (Research & Human Resources) WHO SEA Region
Professor of Medicine, Institute of Medicine 2, Yangon

I have a Dream - that the Department of Medical Research will become one of the premier medical research institutions in Asia, renowned world-wide for its scientific activities and the vigor and enterprising spirit of its scientists.

I have a dream that DMR will become an autonomous medical research institution similar to many others world-wide. That DMR will be able to grow and expand according to its scientific capability and opportunity and to acquire proffered aid for the advance of research - from any source, national or international; men, money or material; while keeping paramount the twin aims of improving the health of the people and advancing medical science in the country.

I dream that though young and small in comparison with many others, DMR will contribute significantly towards fundamental scientific knowledge in its chosen field, and towards advance of medical science in the country, by undertaking more of innovative research at the fringes and intersection of medical science with other sciences.

DMR must be innovative and avoid rigidity and stalemate. It should strongly advocate adjustment of national health research policy and venture into new and emerging medical technological fields such as genetic editing and CRISPR.

But dreaming is not enough. Dreams must be made to become reality.

How? How not to?

DMR should continue the important work it is now doing - importing scientific knowledge and technology, interpreting it and transferring it to others so that it may be used for improving the health of the people and advancing medical science in the country. But this is not enough.

DMR must not rest on its laurels. There must be a judicious balance between Exploitation and Exploration.

To repeat what I said before, the structure and function of DMR should be adjusted according to changes rapidly taking place at national level, and at an accelerating pace in the scientific world. Departments, units, programs and persons that have outlived their usefulness must be terminated. Occam's razor should be courageously and sharply applied in this context, in making choices between the old and the new, between following worn-out tracks and exploring fresh and exciting paths - but with understanding and compassion.

This commemorative volume is to mark the 30th anniversary of the publication of the Myanmar Health Sciences Research Journal, a descendent of the Union of Burma Journal of Life Sciences and the only medical journal solely devoted to publication of original medical research and research related material nowadays.

It is very commendable that the Myanmar Health Sciences Research Journal has an International Board of Editors and that some foreign scientists are beginning to publish their research findings in this Journal. The Journal should occasionally examine the Citation Index of DMR and other Myanmar medical scientists as well as other authors in related journals of neighboring countries and elsewhere to see how much attention the contents of MHSRJ is able to attract. The MHSRJ plays an important role in furthering research in the country and therefore should not be limited. It is an appropriate forum for Review of advancing medical scientific knowledge relevant to Myanmar and for comprehensive, critical Reviews of all research done in Myanmar in a particular field which has reached a certain stage of maturity; more such Reviews should be done. It is an appropriate forum for stimulating research in certain fields where more should be done. It is the proper vehicle for advocating nationally appropriate, forward-looking research strategies that should be adopted by health policy makers to take advantage of new openings in science and technology, and for discouraging those which scientists think are scientifically unsound and inappropriate.

Such are my Dreams, Vision and Aspirations for DMR.

REMINISCENCE OF A WANDERING BIOCHEMIST

Dr Aye Kyaw
Director (Retired)
Department of Medical Research

I received an e-mail from Dr. Ni Thet Oo, Associate Editor of the Myanmar Health Sciences Research Journal (MHSRJ), on 19 March 2018 requesting me to write an article for MHSRJ, which is commemorating its 30th anniversary volume in April 2018.

Actually, I have been away from research activities for nearly twelve years since my retirement in July 2006 and I am afraid that I may not be able to write an article of research alike. Instead, an article of a kind of reminiscence of my involvement with the esteemed journal of the Department of Medical Research (DMR), at which I have spent for four decades, may be feasible for me to write, I feel. When I sent this message to Dr. Ni Thet Oo, I was delighted that she instantly approved.

We have a Myanmar saying 'While treading forward, try to look back' (အရှေ့ကို လျှောက်ပါလို့ အနောက်ကိုတဲ့မျှော်လိုက်ရဲ). Sincerely, there are not many years ahead left for me to tread forward but when I try to look back, it has been a long journey I have travelled.

My memory snippets went back to some fifty years ago when I reported for duty at the then Burma Medical Research Institute (BMRI) (now, Department of Medical Research – DMR) as a Research Officer. The date, as far as I remember, was 6th August 1966.

At the BMRI, I first met U Than Pe, Executive Officer, who brought me along with him to the Director to report personally. (The title of the post of Director was later changed to Director-General as a result of the country's new administration in 1974). At that moment the Director, Dr. U Mya Tu, was busy with a microscope in his Physiology Research Laboratory (also called Director's Laboratory) situated next to the Director's Office. But he received to meet and posted me to Biochemistry Department, which had been recently opened. After circulating me for a few months through various departments of BMRI to study their research activities, I was assigned a research project. The title of the project was 'Study of urinary nitrogen partition in Burmese subjects'. Dr. U Mya Tu, himself, supervised me in performing the project. He was deemed to be my 'first teacher' (လက်ဦးဆရာ) in my research career.

When the project was complete, a paper was prepared and read in the Research Congress (Medical Sciences Division) held in March 1968 at the University of Rangoon (now, Yangon) prior to publication.

In 1969, I published my very first paper in the Union of Burma Journal of Life Sciences, which I think, was the predecessor of MHSRJ. The paper [Aye Kyaw and Tin Oo: Nitrogen partition in urine of Burmese subjects. Union of Burma J. Life Sci. **2**, 217-225 (1969)] appeared in volume 2 of the journal and probably the first volume had been published in 1968. The journal published research papers in the field of allied Life Sciences, namely

Agriculture, Medicine and Forestry. Most of the papers published in the journal were those that had been presented in the research congresses held annually in the University of Rangoon campus at that time under the umbrella of Research Policy Direction Board of the Ministry of National Planning. To the best of my knowledge, the journal stopped coming out in 1973 after publishing its sixth volume.

During the years 1974 to 1989, researchers from the DMR had to wander around other national (like Burma Medical Journal) and international journals for publications of their research papers. However to record their research activities, DMR Newsletter was published as a monthly issue from January 1974 to November 1975. Under such circumstances, I also had to wander around international journals pertinent to biochemistry to publish my research papers carried out at the DMR. I think it was a good opportunity for me to acquire experiences of trying to step diffidently towards an international level. May I recall what Dr. U Mya Tu said to us urging to publish research papers in international journals. He said, 'In this way scientists from abroad will know and recognize our Institute'.

In 1986, DMR Bulletin, a quarterly periodical, came out with the objective to disseminate information about developments and activities in medical and health research in Myanmar and with a style distinct from other routinely published research journals. It was headed by a review article on one discipline in the field of medical research written by a noted Myanmar scientist, who had extensive knowledge in that particular area. The Bulletin (vol. 1, No. 1), in booklet form, was first published in April 1986 with the review article "Recent studies of Russell's viper bite in Burma" written by our Director-General, Dr. U Aung Than Batu. But the DMR Bulletin stopped coming out after vol.3, No. 1 April 1988 issue due to unavoidable circumstances of political crisis in the whole country. During that period, the country was in a turmoil facing rapid turnover of scientists. The DMR too ran short of scientists and in this very period, I became an editorial board member of Burma (later, Myanmar) Health Sciences Research Journal and the editor of DMR Bulletin. The MHSRJ started its first issue as volume 1, number 1 in April 1989 and published original articles, short reports, and correspondences in the field of medical and health sciences. The Journal has been published four-monthly (that is, three times a year) at the DMR and has reached volume 29, number 3, in December 2017. The Journal has now stepped on its 30th volume in the year 2018 and the DMR is now publishing its MHSRJ as **Volume 30, Number 1** in April 2018 to commemorate the journal's **30th Anniversary**. The Journal has obtained the prestigious International Standard Serial Number ISSN 1015-0781 from the Centre for the International Serial Data System based in France in 1992. The number has been printed on the cover page of every issue of the MHSRJs since volume 4, number 1 (that is, April 1992 issue).

The DMR Bulletin resumed its issue in January 1990 after having a gap of over a year and a half (that is, from May 1988 to December 1989). However, to keep up without breaking continuity of the numbers, the January 1990 issue was printed as volume 4, number 1. From its first publication in April 1986 (volume 1, number 1) to 42nd publication in October 1997 (that is volume 11, number4), a total of 42 review articles covering a wide range of areas were published in the Bulletin. With the introduction of doctoral programs in the Institutes of Medicine, the number of researchers in medicine and allied fields considerably increased and

consequently such review articles were quite in demand. As the time passed on and with the frequent use by researchers, copies of these review articles might be scattered from medical libraries and hence, it was decided to compile these articles and consolidate into a book-form for ready use by researchers. As the editor of DMR Bulletin, it was my privilege and pleasure to be involved in editing "Review Articles Compiled from DMR Bulletins (1986-1997)". At this juncture, I would like to acknowledge the cooperation of my colleague U Aung Myint (then Head of Publications Division), who passed away a few years ago.

The DMR Bulletin continued its publication with the topics, such as Review Article, News Related to Medical Research Activities in Myanmar, Un-reviewed Reports on Recent Research Findings, List and Abstracts of Medical Research Papers from or Concerning Myanmar, till 2004. I retired in July 2004 and served for another two years as Advisor and took full retirement in July 2006. I learnt that due to shortage of review articles, the bulletin temporarily stopped from November 2004 to August 2006. However, the bulletin was resumed again in September 2006 as a monthly issue with a new format.

Thanks to successive Heads and Staff of Publications Division, I did not lose contact with DMR after my retirement. I have been receiving continually MHSRJs, DMR Bulletins and Newsletters, which updates my knowledge and keeps me abreast with latest information of DMR in which I spent forty years as a biochemist wandering in and out of various divisions around the DMR complex.

SNAKE BITE RESEARCH IN PAST THREE DECADES

Dr Tun Pe[1] & Dr Aye Aye Myint[2]
[1]Director (Retired)
[2]Research Scientist (Retired)
Department of Medical Research

Snake bite research was initiated in early 70 by Dr. Aung Khin, *et al* at Pathology Research Division, Department of Medical Research (DMR). Russell's viper venoid was developed and found to be immunogenic and induced satisfactory antibody response in rabbits, monkeys and human volunteers. However antibody level wanes with time and frequent boosting at 6 weeks intervals will maintain antibody at peak level which is essential in case of snake bite where a substantial amount of venom is injected in one bite. The cost effectiveness and feasibility of giving frequent booster doses of the venoid year round are main challenges to achieve the goal.

Another contribution to snakebite research is study of pathophysiology of Russell's viper bites in early 1980. Clinical Research Unit on Snakebite headed by Prof Dr. Myint Lwin in collaboration with scientists from different disciplines of the Department of Medical Research namely Immunology, Pathology, Biochemistry, Nuclear medicine and Clinical Research and Prof David A. Warrell from Mahidol-Welcome trust. The pathophysiology, haemostatic, vascular, renal disturbances and response to treatment in Russell's viper bites was studied in the Clinical Research Unit on Snakebite in Thayawaddy with laboratory support from respective disciplines of the DMR. A number of publications related to the topics and useful guidelines for management of Russell's viper bite were published. Twenty-minute whole blood clotting bedside test is recommended as a useful test for detecting development of blood incoagulability in Russell's viper bites and indication for giving antivenom.

Since traditional first aids are not effective in retarding local spread of venom, more effective first aid" local compression with immobilization" was developed and it was found to retard local spread of the venom while the pad is in place in Russell's viper bite cases admitted to Taungdwingyi hospital.

Since early administration of antivenom within ½ hr after the bites could not prevent development of renal failure and death, we thought of inventing fang's proof boots/ and gloves for the risk population in collaboration with staffs from Myanmar Pharmaceutical Factory and Boots/ Gloves Factory of Ministry of Industry (1). It took two years to develop it. The feasibility and acceptability study of the boots and gloves were studied in local users (farmers) of Taungdwingyi. The final feedback of the studies from the users are it provides a sense of security against snake bite while working in the field and could wear it on for a long hour. Fangs proof efficacy of the boot is also confirmed by the users who happened to step on the snakes while at work.

Laboratory-based research on Russell's viper venom and antivenom were carried out by Immunology, Biochemistry, Pathology and Nuclear Medicine of the Department of Medical Research. A large volume of publication was generated from this study.

Amount of venom injected by an adult Russell's viper as well as the quantity of venom used in catching prey were determined. Generally the amount of venom injected by a snake depends on the nature of the bite whether it is effective bite or not. Dry bites are often observed. Venom yield per milking correlated well with the length of the snake.

An effective bite of a snake will lead to systemic envenoming irrespective of the length of the snake. It is highlighted that an effective bite of a juvenile snake is equally as potent as that of an effective bite of an adult in inducing systemic envenoming. The degree of envenoming (amount of venom injected) does not depend on the gut status of the snake whether it has recently fed or not. Snakes can still cause envenoming after a series of bites. Therefore time of bite, either day or night does no difference in degree of envenoming,

Since there is geographical variation of venom properties, antivenom used for treating Russell's viper bite cases should be raised with widely pooled venoms from local snakes. Mono specific antivenom manufactured by Myanmar Pharmaceutical Factory is best for treating local snakebite cases. It is highlighted that imported antivenoms raised with different species of snakes are not effective in treating local snakebite cases. Giving a large volume of antivenom could induce hypersensitivity to equine immunoglobulin.

Unknown bite accounts for 29% the total bites for which a test kit for identification of snake species will be helpful. A rapid test kit dot blot immune assay (DBI) and colloidal dye immunoassay (DIA) was developed in 1998 and 1999, respectively. It could detect a venom level as low as 10ng/ml and result will be ready in 20 minutes. However dye antibody conjugate needs cold storage facility which may not be feasible in remote areas. In 2011, a rapid test kit based on lateral flow system was developed by Dr Aye Aye Myint in collaboration with Miprolab in 2011. It is species specific (100%) and does not cross react with other snake venoms.

The feasibility of raising Russell's viper antivenom (*Daboia russelli siamensis*) not available in the antivenom market was attempted in goat and in laying hens. Chicken immunoglobulin (IgY) extracted from egg yolk yields 1.85 gm per month which is equivalent to total IgG obtained from bleeding 8 rabbits or two goats per month. Pure potent IgY antibody (avian antivenom) could be used for treating Russell's viper bite cases and can supplement the current antivenom production of the country.

Bites by other species of snakes were also documented namely Cobra (*Naja kaouthia*), Green pit viper (*Cryptelytrops erythrurus*), King cobra (*Ophiophagus hannah*), banded krait (*Bungarus fasciatus*), Chinese krait (*Bungarus multicintus*), Malayan pit viper (*Calloselasma rhodostoma*), sea snake and venom ophthalmia following spitting of venom from spitting cobra (*Naja mandalayansis*).

[References are available in Tun Pe, Aye Aye Myint & Aung Myo Min. Bibliography of Research Findings on Snakebites in Myanmar (1967 to 2010), Ministry of Health, Department of Medical Research (Lower Myanmar), 2010.]

HEPATITIS B VACCINES RESEARCH, DEVELOPMENT AND PRODUCTION AT DEPARTMENT OF MEDICAL RESEARCH

Dr Khin Pyone Kyi
Director-General (Retired), Department of Medical Research
President, Myanmar Liver Foundation
Core Member of National Technical Advisory Group on Immunization

"An ounce of prevention is worth a pound of cure" (Benjamin Franklin)

My career as a researcher, developer and producer of hepatitis B (HB) vaccines started in 1989 when I entered the Department of Medical Research (DMR) as Senior Research Officer at the Experimental Medicine Research Division. I was transferred from the National Health Laboratory to DMR to participate in the "Development of Plasma-derived Hepatitis B (HB) Vaccine in Myanmar (Pilot Scale) Project" which was funded by UNDP and technically supported by World Health Organization. Actually I was recruited as Co-Principal Investigator for the HB Vaccine Project because I had experience in purification of HBsAg from plasma during WHO Fellowship training for HB diagnostics in 1985 at the Center for Disease Control (CDC) in Atlanta, USA.

The research work on HB vaccines at DMR consists of two categories – vaccine clinical trials and vaccine development/production.

1. **Clinical trials of Hepatitis B Vaccines produced in other countries**.
   The clinical trials of HB vaccines produced in other countries have been carried out at DMR since 1982.

   1.1 Efficacy trial of plasma-derived hepatitis B vaccine from Merk, Sharp and Dohme (MSD), USA.

   This was a randomized controlled double blind trial. The test HB vaccine or placebo was applied to newborn babies of chronic HBsAg carrier mothers with and without HBeAg. The results showed that Anti-HBs were present in 83.87% of vaccinees (who did not develop antigenemia). The vaccine efficacy was calculated to be over 70%.

   1.2 Efficacy trial of yeast derived recombinant HB vaccine from MSD, USA.

   This clinical trial followed the first one and the vaccine efficacy showed 90 % in preventing high risk babies from becoming chronic HBsAg carriers.

   1.3 Efficacy of Mammalian-cell derived DNA hepatitis B vaccine.
   This DNA HB vaccine produced from Chinese hamster ovarian (CHO) cells is tested for efficacy in preventing HBsAg carrier state in infants born to HBeAg positive mothers. The vaccine produced by Pasteur, France, contained Pre-S antigens and was claimed to be more protective. In this study the vaccine provided an efficacy of 85.7% (DMR unpublished report)

1.4 Pentavalent Vaccine trial

An open randomized controlled trial was undertaken with 269 children allocated to two groups to receive 3 doses of the Pentavalent vaccine which contained five different antigens to reduce the number of injections to the baby. The five antigens are – diphtheria, pertussis, tetanus and hepatitis B (DTP-HBV) in one container and a lyophilized Hemophilus influenza type b in separate container. One group received either a syringe mix of the five antigens in one arm or as separate injections in opposite arms of healthy infants at 1.5, 3, and 5 months of age. The final results showed the mixed vaccine to be safe and well tolerated with high immunogenicity against all component antigens while decreasing unnecessary injections for the infants.

1.5 Safety and immunogenicity trial of recombinant hepatitis B vaccine from China Tiantan Company.

In 2015, safety and immunogenicity trial was carried out on recombinant hepatitis B vaccine produced by Tiantan Company, China. Safety trial was carried out on adult volunteers according to the methods recommended by the World Health Organization. The vaccine was found to be safe and highly immunogenic with Anti-HBs detected in 98% of the vaccinees ranging from 10 mIU to >1000 mIU per ml.

2. Hepatitis B Vaccines Development and Production by DMR

Two types of hepatitis B vaccines; plasma-derived hepatitis B vaccine and recombinant hepatitis B vaccine have been developed and produced on a large scale by DMR

2.1 Plasma-derived hepatitis B vaccine

2.1.1 The development of plasma-derived hepatitis B vaccine in Myanmar (Pilot Scale) Project (1989-1996)

HB vaccine was developed from HBsAg positive plasma which was followed by the clinical trial to test the HB vaccine that was developed. The initial funding from UNDP was US$ 0.63 million followed by additional funding of US$ 0.2 million for Chimpanzee Safety Testing at the end of vaccine development and laboratory scale production.

In 1991 during the initial phase of the project, five key scientists from the project, U Hla Pe, Dr Aye Kyaw, Dr Khin PyoneKyi, U Maung Maung Khin and Daw Khin Khin Aye were trained for 6 months each, for the development of plasma-derived hepatitis B vaccine at the Centers for Disease Control in Atlanta, at the FDA at the National Institutes of Health in Bethesda and at New York Blood Center in New York, USA. The training included management aspects, development of HB vaccine from plasma, inactivation, adjuvanting and quality control procedures for the vaccine.

On their return, all five scientists prepared for vaccine development at DMR during 1991-1992. The vaccine laboratory was renovated and the equipments ordered for the Project were installed. Project Manager U Hla Pe, Deputy Project Manager Dr Khin Maung Win and Principal Investigator Dr Aye Kyaw took responsibility for management, international communications and advisory role for the project. The technical working group headed by Dr Khin Pyone Kyi with the two senior technicians U Maung Maung Khin, Daw Khin Khin Aye and two junior project

technicians worked in the Vaccine laboratory and Animal Laboratory for the development of the plasma-derived hepatitis B vaccine.

During 1992 and 1993, three batches of hepatitis B vaccines were produced from HBsAg high titer positive plasma. All 3 batches passed the quality control tests carried out at DMR according to the guidelines recommended by the WHO.

From 1991 to 1999 WHO Consultants for the Project – Dr James Maynard (PATH), Dr Alfred Prince (NYBC), Dr Howard Fields (CDC), Dr John Vnek (NYBC), Dr Louis Baker (Australia), Dr John Curling (UK) visited DMR to train and guide the Technical Working Group at the Vaccine Laboratory and Animal Laboratory.

Flow Diagram for plasma-derived hepatitis B vaccine development process

Whole Blood (HBsAg positive blood collected from blood banks)
↓
Plasma separation
↓
Selection for HBsAg high titer (CEP method)
↓
Plasma pool (25 liters)
↓
PEG precipitation
↓
HAP Adsorption
↓
Concentration
↓
Isopyknic ultracentrifugation
↓
Ultra-filtration
↓
Purified HBsAg
↓
Dilution & Double step heat inactivation
↓
Pasteurization
↓
Adjuvanting & Vialing

Quality control tests recommended by WHO and CDC

- Tests for purity by Electron microscopy
- Tests for identity by SDS PAGE, PCR for HBV DNA
- Tests for sterility by bacteria, virus, fungus tests
- Tests for general safety by animal tests in mice
- Tests for pyrogen by rabbit pyrogen test, LAL test
- Test for protein content by Lowry method
- Test for immunogenicity by animal test (in mice)
- Test for HBsAg content by Specific Activity (CDC)

After passing the Laboratory Quality Control tests in Myanmar, two batches of plasma-derived hepatitis B vaccine were sent to the New York Blood Center (NYBC) to carry out the final Quality Control procedure, the "Chimpanzee Safety Test" in Liberia as recommended by the WHO before carrying out the human clinical trial. The safety test took nearly one year and in 1994, the NYBC issued the Certificate of Safety for the two batches of plasma-derived hepatitis B vaccines and also for future lots produced at DMR for use in human trials.

In 1995, the Project Manager retired, the Deputy Project Manager moved to the Department of Medical Science and the Director General of DMR became the Project Manager.

2.1.2 Human reactogenicity, safety and immunogenicity study of the plasma-derived hepatitis B vaccine produced at the Department of Medical Research.

Since this is the first hepatitis B vaccine produced in Myanmar, the protocol to carry out the human clinical trial had to be submitted to the National Ethical Committee chaired by the Attorney General of the Union of Myanmar. The protocol was approved by the National Ethical Committee in 1995.

With Dr Khin Pyone Kyi as the Principal Investigator, the "Human safety, reactogenicity and immunogenicity trial of the plasma-derived hepatitis B vaccine produced at DMR" Project was carried out in Yangon on 100 adult human volunteers from 1995 to 1996. The 100 human adult volunteers were innoculated with 3 doses of hepatitis B vaccine containing 10 ug of HBsAg per dose, by intramuscular route in the deltoid region according to 0, 1, 6 month schedule. The results of the trial showed that the HB vaccine was safe with local pain as the only side effect. The immunogenicity showed that Anti-HBs was detected in over 90% of the vaccinees.

2.1.2 Scale-up production of plasma-derived hepatitis B vaccine at DMR

The successful development of the plasma-derived HB vaccine (pilot scale) led to the approval by the National Health Committee to construct a new facility in the DMR compound: a four storey Diagnostics and Vaccine Research Center (DVRC) to increase the HB vaccine production from laboratory scale to commercial scale. Thus the DVRC was constructed in 1996-1997 and the plasma-derived hepatitis B vaccine production increased to 100,000-150,000 doses annually. Another one storey building was set up in 1997 for the Hepatitis B Vaccine Clinic near the gate and wall of the DMR compound. Since then, the HB testing and HB vaccination have been commercially available at DMR HB Vaccine Clinic for the general population (public) at affordable price.

In 1999, the HB vaccine Technical Core Group of four headed by Dr Khin Pyone Kyi with Vaccine Research Officers U Maung Maung Khin, Daw Khin Khin Aye and Daw Sandar Nyunt went on a study/training visit to Cheil Je Dang Hepatitis B Vaccine Plant in Republic of Korea (ROK), to study the large scale production of plasma-derived hepatitis B vaccine (the same method used at DMR but produced at a smaller scale).

2.1.3 Outstanding achievements

The research paper "Human safety, reactogenicity and immunogenicity trial of the plasma-derived hepatitis B vaccine produced at DMR" was presented at the Myanmar Health Research Congress in 1996 and was awarded the first prize for the Best Paper in Applied Research.

In 2002, the two senior scientists Dr Aye Kyaw, Director (Vaccines) and Dr Khin Pyone Kyi, Deputy Director and Head, Vaccine Production and Distribution Division, were awarded the "Honorary Medal of the State for Excellent Performance in the Management Field" for the successful development and production of plasma-derived hepatitis B vaccine on a commercial scale, the first of its kind in Myanmar.

Several more research studies/clinical trials were carried out, using the plasma-derived HB vaccines in different doses (1/2 the normal dose), different schedule (0, 1, 2 months), different route (intradermal) and different composition (reduced adjuvant) in different groups of adult volunteers and all the results showed the local HB vaccine to be safe and immunogenic.

2.2 Recombinant Hepatitis B Vaccine Production Plant Project (2002-2006)

2.2.1 Justification to produce recombinant hepatitis B vaccine on a large scale in Myanmar instead of plasma-derived HB vaccine.

According to the National Health Plan, 5 million doses of HB vaccine were needed to incorporate the vaccine into the UCI Program to vaccinate all newborns in Myanmar. Despite a good record of safety, efficacy and many advantages with the plasma- derived HB vaccines, problems for large scale production existed such as difficulty in raw material collection for high titre HBsAg plasma and relatively expensive production cost. Also the potential presence of infectious agents like HIV and HCV viruses in the plasma source which could escape inactivation during the manufacturing process, caused unnecessary (theoretical) fear even though plasma negative for these infectious agents were used for vaccine production. The double step heat inactivation method also ensured the inactivation of all infectious agents in the final step of production. Thus it became necessary to change the type of HB vaccine production in Myanmar from plasma-derived to recombinant DNA HB vaccine for mass production.

2.2.2 EDCF Loan Agreement and Supply Contract

On 31-10-2000, the signing of the "Arrangement, Pledge and EDCF Loan Agreement" between the Republic of Korea and Government of Myanmar was carried out to establish the HB Vaccine Production Plant in Myanmar. The Supply Contract was signed between DMR and Samsung Corporation in September 2001 to construct the Hepatitis B Vaccines Plant on turnkey basis, according to WHO GMP requirement, with the capacity to produce five million doses of recombinant HB vaccine and two million doses of plasma-derived HB vaccine annually. The HB Vaccine Plant site was 3 acres of land at Ywarthargyi, Hlegu Township and the project period was 3 years. The total value of the EDCF loan was US$ 12.6 million.

The Supply Contract and the Amendment of the Loan Agreement was approved by Korea EXIM Bank in March 2002.

2.2.3 Construction of the HB Vaccine Plant.

Preparation of groundwork was started in April 2002 by the Korean Company named Golden Midas Construction. The construction works started for station house buildings for transformers and generators, animal house and cafeteria etc. By May, the HB Vaccine Plant construction was 98% completed including utility facilities such as electricity, water supply, air ducts installation, air cooling units, cafeteria, animal laboratory and enclosing brick wall. Landscaping and equipment installation and construction of staff quarters continued. The construction of the HB Vaccine Plant was completed in September 2003 and the installation of all equipment was completed in December 2003. The road repair for No. 7 Highway was completed in January 2004. Four more buildings were constructed for staff quarters.

2.2.4 Training at the Cheil JeDang (CJ) HB Vaccine Plant in ROK .

On November 2002, the first team of trainees led by Dr Aye Kyaw, Director (Vaccines) which included two administrative staff and two engineers started their two weeks training at CJ HB Vaccine Plant in Inchon. Two senior Research Scientists in the team Dr Win Aung and Dr Moh Moh Htun were trained for Basic Molecular Biology Recombinant DNA Technology in Recombinant HB vaccine production for six months.

On 1-12-2002, the ten member Technical Team headed by Dr Khin Pyone Kyi, started their training in CJ HB Vaccine Plant for 8 weeks: training for large scale production of recombinant and plasma-derived HB vaccines, Quality Control tests for the HB vaccines, finished products & vialing.

The technical team arrived back in Myanmar at the end of January 2003.

In 2004, two senior scientists; Dr Win Aung and Dr Zaw Myint attended the International GMP Audit Training in DONGSIN Tube Glass Ind. and PARA Machinery Co., Ltd, ROK.

In 2005, three senior scientists; Dr Win Aung, Dr Zaw Myint and Dr Win Maw Tun attended the 4-week Training Course for Quality Assurance (QA), Quality Control (QC) and Good Manufacturing Practice (GMP) in Korea FDA and CJ HB Vaccine Plant, ROK.

2.2.5 First test run at the HB Vaccine Plant in Myanmar

From the 2nd week of December 2003, the Myanmar Technical Team worked under the supervision of the 8 Korean Experts for 3 months and started the test run and test production of both plasma-derived and recombinant HB vaccines at the Plant together with QA and QC procedures. In March 2004, test run for vaccine formulation, vial washing, filling, capping and inspection of vaccine vials were carried out. During 2004 and 2005, 3 lots of purified HBsAg for 1.5 million doses of recombinant HB vaccine was produced at the HB Vaccine Plant.

2.2.6 Progress at the Plant

In 2003, the ferry bus from ROK and two passenger buses purchased locally were used for daily transportation of the staff. The cold van from ROK was used to collect the HBsAg positive blood from 12 hospitals in Yangon Division for the production of plasma-derived HB vaccine at the HB Vaccine Plant. Manpower at the Plant was increased. Initially in 1997, there were 36 staffs at the Vaccine Production Division which was increased to 82 in 2002 at the Vaccine Plant. In 2004 a new set up would add 36 more staff.

2.2.7 Recombinant DNA HB Vaccine Production

The recombinant HBsAg was purified from transformed ***Hansenula polymorpha*** (yeast) which has been referred to as the alternative yeast of maximum yield producing about 4-6 times more soluble protein than the currently widely used yeast ***Saccharomyces cerevisiae.*** To summarize the recombinant HB vaccine production process – the Working Cell Banks (WCB) were produced from the Master Cell Bank which was provided by CJ HB Vaccine Plant. The WCB was processed by subcultures – from Seed 1 culture to Seed IV culture in the 5 liter Seed Fermenter. Then main culture was performed in the 50 liter Main Fermenter, which was conditioned at 25° C, 200 rpm (increasing progressively) and pressure of dissolved oxygen (DO) of media was measured to determine the stage for methanol addition (feeding) to induce antigen expression. Cultivation time varied from 100 to 140 hours and then the cells were harvested, washed and homogenized under high pressure repeatedly to release HBsAg particles from the cells, centrifuged to remove unbroken cells and debris and supernatant was collected. Then diafiltration, concentration, pH precipitation and centrifugation to remove precipitate materials followed by KBr gradient ultracentrifugation carried out for HBsAg purification. The purified HBsAg was collected as bands from the centrifuge tubes, pooled and further purified by gel filtration, filtered and stored. Recombinant HB vaccine was produced by adjuvanting the purified HBsAg, vialing and carrying out quality control procedures during production process according to WHO requirements.

2.2.8 Human safety trial for the local recombinant HB Vaccine

In 2006, human safety trial was carried out on 20 adult volunteers for the locally produced recombinant HB vaccine. The adult volunteers were tested for cardiac function, liver function and renal function such as ECG, urine for routine examination, blood for complete picture, ALT, AST, and creatinine before the HB vaccination. After the vaccination these parameters were tested again to detect if there were adverse effects of the HB vaccine. No side effects were noted and the locally produced recombinant HB vaccine passed the safety trial.

2.2.9 Reactogenicity and immunogenicity trial of the locally produced recombinant HB vaccine in newborns

The Principal Investigator of this clinical trial was Research Scientist, Dr Myat Phone Kyaw. After passing the safety trial, the locally produced recombinant HB vaccine was immunized in 134 newborns at Thingangyun Sanpya Hospital and South Dagon

Township Hospital in 2006. The babies were given the HB vaccine 3 doses on the anterolateral thigh at 0, 1 and 6 month schedule. Two months after the vaccinations, babies were tested for the vaccine response and Anti-HBs was positive in 100% of the vaccinees. The babies developed protective antibody with no side effects.

2.2.10 Safety and immunogenicity trial for recombinant HB vaccine in high risked population

The Principal Investigator of this clinical trial in adults carried out in 2006-2007 was Dr Win Maw Tun. A total of 287 monks and nuns living in monasteries and nunneries (institution - like) in Yangon were screened for HBsAg and those who were negative were given three doses of locally produced recombinant HB vaccine by intramuscular route on the deltoid region at 0, 1, 2 month schedule. Post vaccination investigations showed the protective antibody response in 98.25% of the vaccinees with no adverse reactions.

2.2.11 Transfer of Hepatitis B Vaccine Plant to Ministry of Industry (1)

In August 2006, according to the decision of the higher authorities, the Hepatitis B Vaccine Plant was officially handed over from the Ministry of Health to the Ministry of Industry (1). This included transfer of all the equipment and materials in the HB Vaccine Plant, the allocated budget as well as the staff except thirteen research officers (9 doctors and 4 non-medical staff). The remaining 77 staff which consisted of personnel from Administration, Engineering and Technical Team, were absorbed by the Ministry of Industry (1). The 13 scientists who were to be transferred back to the Ministry of Health, stayed at the HB Vaccine Plant temporarily for one year for technical transfer of the Hepatitis B vaccine development to the replaced staff of Myanmar Pharmaceutical Industry and DCPT from the Ministry of Industry (1).

2.2.12 Outstanding Achievements

The research paper on “The safety and immunogenicity of the DMR recombinant hepatitis B vaccine” won the Best Paper Award in Applied Research at the Myanmar Health Research Congress, 2007.

In 2008, seven senior scientists from the Hepatitis B Vaccine Production Plant were awarded the “Honorary Medal of the State for Excellent Performance in the Medical Field” for the successful production of recombinant hepatitis B vaccine for the first time in Myanmar. The recepients of the Honorary Medals were Dr Khin Pyone Kyi, Dr Moh Moh Htun and Daw Khin Khin Aye from Recombinant HB Vaccine Production Division, Dr Khin May Oo and Dr Win Maw Tun from Quality Control Division, Dr Win Aung from Quality Assurance Division and Dr Zaw Myint from Finished Products Division.

2.2.13 Conclusion: Prevention is better than cure

Approximately 100 research papers on the locally produced hepatitis B vaccines have obtained both local and international publications and presentations by DMR scientists dedicated to vaccinology. With the local development and production of hepatitis B vaccines, hundreds of thousands of Myanmar people have been prevented from

contracting the HB infection and its fatal complications. It is the greatest achievement for our relentless and dedicated effort of nearly 30 years.

"Treatment without prevention is simply unsustainable" (Bill Gates)

"He who cures a disease may be the skillfullest but he that prevents it is the safest physician" (Thomas Fuller)

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THE ACTION STUDY: FROM THE INCEPTION TO THE CONCLUSION

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Cancer is a leading cause of disease worldwide. It has been cited as the leading cause of mortality globally, accounting for 13% (or 7.4 million) of all deaths annually with 70% of these occurring in low and middle income countries (WHO, 2010). In 2012, there were an estimated 14.1 million new cases of cancer and 8.2 million deaths from cancer worldwide.[1] It is also projected that mortality from cancer will increase significantly over the coming years with expected 13 million annual deaths worldwide in 2030. The trend is even more striking in Asia where the number of deaths per year in 2002 of 3.5 million is expected to increase to 8.1 million by 2020.[2] Although incidence rates remain highest in more developed regions, mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities.[3]

In 2012, the most common cancers worldwide (for both sexes) were lung cancer (13% of all cancers diagnosed) followed by breast cancer (12%), colorectal cancer (10%) prostate cancer (8%), stomach cancer (7%), liver cancer (6%) and cervical cancer [4%]. Among men, the 5 most common sites of cancer diagnosed in 2012 were lung, prostate, colorectum, stomach, and liver cancer. Among women the 5 most common sites diagnosed were breast, colorectum, lung, cervix, and stomach cancer.[4] In 2012, the most common causes of cancer death worldwide (for both sexes) were lung cancer (19% of all cancer deaths) followed by liver cancer (9%), stomach cancer (9%), colorectal cancer (9%), breast cancer (6%), cancer of the esophagus (5%) and pancreatic cancer (4%).[5]

ASEAN region contains more than half a billion people, almost 9% of the world population, spread over highly economically diverse countries. The burden of cancer is increasing in the ASEAN region, due to population ageing and growth and the adoption of cancer-associated lifestyle behaviors such as more sedentary lifestyle, eating more red meat and fat, consumption of more alcohol, more environmental pollution, and continued use of tobacco. It was estimated that there were over 700,000 new cases of cancer and 500,000 cases of cancer deaths in the ASEAN region in the year 2008.[6]

A large prospective study on Economic and Social Impacts of Cancer in the ASEAN was planned with the purpose of triggering and/or accelerating the translation of current cancer control knowledge into public health action in the ASEAN and to compile the existing evidence and to generate new evidence on socio-economic aspects of cancer. It was expected to assist the ASEAN decision-makers at different levels to make informed choices on cancer control policies and resource allocations. The ACTION (Asean CosTs In Oncology) Study is one of the largest observational studies of the burden of cancer ever conducted in Asia. Eight

low- and middle-income countries within the ASEAN region (Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Viet Nam) participated in the study. It is implemented through partnerships between the George Institute for Global Health, the ASEAN Foundation and Roche.[7]

Several researchers and clinicians from the participating countries including Myanmar attended the Conference on "The Burden of Cancer in ASEAN Communities" which was held on the 7th of July, 2011 in Singapore. In that meeting the Landmark Project "The Burden of Cancer and its Economic and Social Impacts in ASEAN Countries" was launched. Based on the Project, the ACTION (Asean CosTs In Oncology) Study was initiated to assess the impact of cancer on the economic circumstances of the patients and their households, patients' quality of life, costs of treatment and survival. Hospitalized cancer patients with a first time diagnosis of cancer were recruited. The patients were followed up throughout the first year after their cancer diagnosis, with interviews conducted at the base line, and at 3 and 12 months. The primary outcome was the incidence of financial catastrophe following treatment of cancer, defined as out-of-pocket health expenditure of 12 months exceeding 30% of household income. Secondary outcomes such as illness induced poverty, quality of life, psychological distress, survival and disease status were also studied. The detailed procedures were outlined. Ethical approval was obtained from the University of Sydney's Human Research Ethics Committee and the Ethics Committees of each participating countries. The study was funded through an unrestricted educational grant from the Roche Asia Pacific Regional Office.[8]

The Director General of the Department of Medical Research lead the Myanmar Project and 8 Clinicians and 4 researchers participated as investigators. The Myanmar ACTION Study project was initiated on 2nd February 2012. The total number of participants was 1178 (489 males and 689 females). Data entry was internet-based and data collection was performed over a password-protected database (GDS) using encryption software. Data entry at 3 months, and at 12 months were included in the data base. The last date of recruitment was 15th September, 2014. Every detailed step was observed and aligned with the approved Study Protocol. Data analysis was carried out by the local investigators and the Project Completion Report of 3045 pages separated into three volumes was submitted to the Ministry of Health in August 2016.[9-11]

Findings from the study were published in international peer review journals and also at local conferences. Four papers were published in the International Journals,[12-15] and five papers were presented at local conferences.[16-20] The findings were also made available to the public. Three articles in Myanmar language were published in newspapers. Two booklets were published and distributed at local conferences.

The results of the ACTION study highlighted the fact that among the ASEAN countries, 75% of cancer patients from LMIC (Lower Middle Income Country) countries such as Myanmar, Laos and Cambodia experience death or financial catastrophe within one year. Also in all participating ASEAN countries, 28% of affected families resorted to taking personal loans and 20% had to sell their assets. Moreover, 5% of the families who were living above their national poverty line were pushed into poverty. One-year risk of medical impoverishment

attributed solely to direct medical costs was found to be 25%. The study also highlighted that investment in early detection may be most effective strategy to address the negative impact of cancer. The findings were also reported at the ASEAN Health Ministers' meeting. This has also instigated the need for health care financing to support the cancer victims.

Previously, cancer has been a low health priority in Myanmar. The National Cancer Strategy is not well written and only 4 local hospital-based cancer registries exist. Public screening programs for cervical and breast cancers are in infancy and their impact has not been assessed as yet. Many areas are needed to be addressed. The biggest obstacle to developing future policies in cancer care is the lack of reliable data. Thus, encouragement for the development of a reliable cancer registry is essential to achieve the World Cancer Declaration Targets by 2025. A Cancer Registry using ICD classification should be developed for use in all hospitals throughout Myanmar to obtain the complete listing of cancer and cancer-related deaths in the hospitals. A complete death registry with the cause of death filled by a qualified health person is the key element for obtaining the total number of cancer deaths in the community. Based on the hospital and community data, a vivid description of the problem of cancer could be outlined and specific policies should be adopted for the control of cancer in Myanmar. A National Cancer Control Plan consisting of five pillars; a) prevention, b) early detection, c) better diagnosis and treatment, d) promotion of quality of life for cancer patients, and e) promotion of a healthy environment has been proposed. This could be followed by development of work plans for cancer care. Research on cancer should also be strengthened to support the identification of areas for cancer care activities. A net-work on cancer should be encouraged to transfer knowledge amongst the cancer care givers, policy makers, researchers and public health personnel.

Surveys should be carried out to determine the knowledge of the community on the risk of cancer and the obtained information should be used in the formulation of the cancer control plan. Guidelines on the diagnosis and cancer treatment should be developed and shared within the Region for maximizing the cancer care, training and education programs. Establishment of a National Cancer Institute that can act as a source of expertise on issues such as advance cancer treatment options such as gene therapy or targeted therapy, novel diagnostic methods, and innovative research on surveillance, epidemiology and economic burden of cancer should be considered in the later years.

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A JOURNEY OF MALARIA RESEARCH

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Myanmar Health Research Scientific Journal of DMR is celebrating the thirtieth anniversary of its publication this month of the year 2018. In commemoration of the occasion, this article is written to document some key historical moments of the establishment, sequential development, and achievement of the department in malaria research process shouldered by many scientists who served dedicatedly under the mission of DMR and Ministry of Health. It is a long journey that DMR has been striving on Malaria Research. Since the time DMR was born in 1963, malaria has been the priority research area in which a lot of researchers from various medical scientific disciplines put enormous effort. DMR has always performed its malaria research activities according to the National Health Plan and WHO guidelines in collaboration with National Malaria Control Program together with all the stakeholders and partners of national and international bodies concerned, with the ultimate aim of eliminating malaria. National Strategic Plan (2016-2020) and National Malaria Elimination Plan (2016-2030) have been developed and highlighted the priority research needs for the malaria control and pending research questions for malaria elimination.

Malaria is a life-threatening perennial tropical disease which develops due to transmission dynamics triad of malaria parasites, mosquito vectors and human behavior, which in turn depend on the environmental, ecological, genetic, pathological, immunological and pharmacological factors. The clinical patterns, severity, complications and response to antimalarials are greatly varied. Treatment needs to give according to species and stage of parasite infected. On top of it, the drug resistance has set in and spread widely. Therefore, **Early Diagnosis and Prompt Treatment with appropriate strategy** become essential. Challenges in preventive measures as well as control and elimination plans are seriously being dealt by the WHO and partner organizations together with the respective governments and global malaria community. With the emerging and re-emerging threats of malaria infection, the medical science also has become tremendously advanced in different areas.

In order to achieve sustainable malaria control and elimination, the following factors are being identified as impeding factors for elimination. Nowadays, no single strategy is applicable to all malaria endemic areas. Different strategies need to be appropriately used in accord with the existing malaria situation. As malaria infection induces various pathologies and manifests a wide range of signs and symptoms, the disease develops in very complex nature. Because of its heterogeneity of parasite, vector and human populations, local differences in environment and social structure, commitment of health services, cross border transmission and malaria resurgence, parasite and vector adaptability, the control measures, strategies and methods become varied. Continued intervention policies as well as case

management with appropriate changes and modifications as necessary also are big challenges in this endeavor. The changing climate and population migration patterns develop increased risk of epidemics.

Appropriate application of tools such as enabling technologies, transdisciplinary approaches, Translational research, basic biology of parasite and vector, drugs, vaccines and diagnosis becomes vital to achieve sustainable malaria control.

In the early days of the journey, DMR had started to recruit researchers, expand its facilities, extend its communication and network and build up its research capability. At first, clinicians from the hospitals started with part time single project research. As the time advanced, the more they did research, the more they got physically and intellectually involved until setting up the clinical research units at their hospitals. The same is true for other health personnel from various departments. Now a day, the research culture is being gradually developed and the research community is getting bigger.

Leading scientists: Eleven Directors General together with responsible Directors for malaria and Heads of Clinical Research Units (Malaria), who have served over the period of 55 years, led malaria research. Among whom, Dr. Aung Than Ba Tu pioneered and established malaria research in DMR. Dr. Myint Lwin, Director General of DMR (Upper Myanmar), Professor Pe Than Myint, Dr. Tin Shwe, Dr. Thein Maung Myint, Dr. Myo Paing, Dr. Willoughby Tun Lin, Mr. A.A. Sebastian, Dr. Myint Oo, Dr. Myint Myint Soe, Dr. Htay Aung, Dr. Ye Htut, Dr. Myat Phone Kyaw, Dr. San Shwe, Dr. Pe Than Tun, Dr. Khin Thet Wai and many others played essential role in building DMR to stand as one of the top research centres in South-east Asia region.

It would not be complete if the active collaboration and contribution of scientists from various departments and institutions are left out. Among them Brigadier General Kyaw Win, Professor Col. Marlar Than, Professor Col. Ye Thwe, Professor Ko Ko Hla, Dr. Soe Aung, Professor Khin May Ohn, Dr. Saw Lwin and Dr. Khin Lin should not be forgotten for their immense effort.

Academic Committee, Malaria Scientific Group and Research Activities

Under the DMR Academic Committee which organizes and gives guidance to all the academic activities, Malaria Research Scientific Group (MRSG) has been one of the most active among 14 scientific research groups. That MRSG is formed with a Senior Deputy Director as a leader and one Research Scientist as secretary and senior and junior scientists as members. Physicians, malaria control program personnel and any interested persons from medical field are invited to join its monthly meetings, weekly journal clubs and adhoc activities. Initial clinical research were started in at Yangon General Hospital and North Okkalapa General Hospital and then expanded to Defence Services Military Hospital and No. 2 Military Hospital.

Clinical Research Unit (CRU) (Malaria) was first established in 1983, with Professor Pe Than Myint as head of the unit. Among many other clinical trials, Injection artemether trial in severe and complicated malaria cases conducted at Tharyarwaddy General Hospital was a milestone. Because it was the first trial in the world testing artemether injection in

severe and complicated malaria cases outside China. At that time, back up *in vitro* susceptibility testing of antimalarials using WHO standard *in vitro* plates was investigated by me. Two to three years later, 3 more Clinical Research Units of Malaria were opened at Defence Services General Hospital (DSGH) Mingalardon, No. 2 Military Hospital and North Okkalapa General Hospital (NOGH) where researchers and post-graduate students got opportunities to undergo training and conduct proper clinical research with standard facilities.

Capacity building of malaria research in DMR

DMR as the first time in history, was awarded **Institutional Strengthening Grant from World Health Organization/Tropical Diseases Research (WHO/TDR) programme for the period of 5 years (1983 - 1987).** A total of US$ 532,659 was used for strengthening the laboratory facilities, research working groups, their disciplines and capability and the financial status. Through the Grants I.D. 800446 and I.D. 820067, both field and laboratory aspects of Entomology, Epidemiology, Leprosy and Malaria research works were able to establish and many useful and applicable results had been produced. *In vitro* falciparum parasite cultivation, isoenzyme characterization of malaria parasites, monitoring of drug resistant falciparum malaria, seroepidemiological studies in different malaria endemic areas, studies on anopheline vectors (Identification, mounting and preservation of vector mosquitoes of Burma, Cytogenetic, hybridization and biochemical studies of *An. balabecensis* species complex). Study of the behavioral and socioeconomic factors influencing malaria incidence, studies on dapsone resistant *M. leprae* in Burma were successfully carried out.

WHO/TDR personnel, experts and consultants (Dr. Wernsdorfer, Dr. Ramachandra, Dr. Mollineaux, Dr. Cheztinovec, Dr. C.C. Draper, Dr. R. M. Anderson, Professor David Warrel, Dr. V.S. Orlov, etc.) had also been very helpful in strengthening DMR's research capability in every aspects and disciplines such as epidemiological modeling, immunological techniques, basic principles and concepts of population dynamics and biomathematics as applied to the epidemiological studies of infectious disease agents, study designs, severe and complicated malaria studies, etc. With the support of that grant DMR scientists had published 34 international papers.

As second time, **WHO / TDR Programme Based Grant I.D. 920379 was awarded as Institution Grant for the period for the period of 3 years (1993-1996).** The title of the projects was " Assessment of malaria intervention strategies in Myanmar ". A total of US$ 236,000 was granted. The fund was used for upgrading the peripheral health facilities, environmental control measures, health care management system research, training of the local health staff who were involved in the malaria control. With the aim of reducing the malaria morbidity and mortality in the country: (i) The impact of improved case management on the incidence of severe and complicated malaria and malaria mortality in a township, (ii) The effect of local larvivorous fish on well-breeding *An. dirus* and on malaria transmission, (iii) Establishment of simple *in vivo* drug sensitivity monitoring method for falciparum malaria and (iv) Efficacy of artemisinin derivatives in treatment of severe and complicated malaria research projects had been conducted. WHO/TDR Director Dr. Tori Godal, Dr. Steven Wayling,

Professor Mollineaux and other scientists also visited DMR and study areas and gave full support for the TDR funded projects. With the support of Programme Based Grant, Myanmar scientists had published more than 10 international papers. Many scientists from DMR, DOH, DHP and DMS had undergone training including master and doctoral degree courses regionally as well as extra-regionally in different fields of medical science.

During the period between 1993 and 1997, 12 doctoral trainees were sent: one pathologist to Pasteur Institute, Paris, France. 4 candidates, each from Public Health, Health Economy, Pharmacology, Social Science disciplines to University of Queensland, Tropical Health Programme, Brisbane, Australia, one public health trainee to James Cook University, Australia, 4 from nursing science to University of Adelaid, Australia and 2 on Malariology to University of Colombo, Sri Lanka. Since then, DMR succeeded many types of research grants and awards for research capacity strengthening and many more scholars were trained. Currently Parasitology Research Division is carrying out the projects supported by Malaria Elimination Scientific Alliance research grant, WHO/DFC, US-Japan NIH funding , WHO/GMS (2016-2018) and WHO/TDR Small Grant.

Promotion and recognition of researchers

DMR held Research Paper Reading Session starting from 1978, then more extensive Medical Research Conferences (later in 2000 change the name to Health Research Conference) have been held annually where scientists and researchers not only from the medical faculty but also from other related faculties presented their research works. As the quality and quantity of the papers become improved, starting from the year 1993, researchers have been recognized by presenting best paper awards on Applied Research, Basic Research, Clinical Research and Health System Research as well as best poster award. Since 2010 Young Researcher Award has also been given to encourage young researchers. Malaria researchers almost every year never fail to win one or more awards.

Basic and applied research facility upgrading and training

Basic research laboratory facilities of key research divisions namely Parasitology, Medical Entomology, Epidemiology, Biochemistry, Pharmacology, Pathology and Immnunology Research Divisions were first filled up with standard equipment and instruments. At the same time, facilities of Central Biomedical Library, Computer Research Division and Laboratory Animal Services Division had to be upgraded. Then DMR gradually upgraded the modern and updated equipment and instruments through government investment, WHO contribution, taking international grants of various kinds. Electron microscope, *In vitro* and rodent malaria parasite cultivation system and monoclonal facilities, insectary for taxonomical identification of mosquito species and malaria infection studies, Immunopathological facilities, pharmacokinetics (Population pharmacokinetics Software); Kinetica and pharmacodynamics research facilities, Antimalarial Drug Assay Facilities (High Performance Liquid Chromatography (HPLC), Ultra-violet and visible spectrophotometer (UV/VIS), Fluorescence spectrophotometer, Fourier transform infra-red spectrophotometer (FT-IR), Capillary Electrophoresis (P/ACE MDQ), Electrophoresis system for detection of parasite enzymes, abnormal haemoglobins and G6PD, Spectrophotometry for measurement of organic

compounds, β Scintillation counter for efflux assays and facilities for testing of toxicity and antimalarial activity (*In vitro, In vivo* and clinical studies) of selected traditional drugs and reputed Myanmar medicinal plants are some of the instances.

As a result of concerted effort of malaria scientists and the achievements made in many important aspects, WHO recognized DMR as a centre for international research and training on malaria in 2003. **WHO Collaborating Centre for Research and Training on Malaria,** Department of Medical Research, Lower Myanmar (DMRLM) (WHO CC No. 205), Ministry of Health was first designated as WHO-CC for Research and Training on Malaria on 25 September 2003. It was re-designated on 2nd November 2009 up to October 2014. DMR has again been re-designated as WHO-CC on 8th July 2016 for 4 year term. DMR is now engaged in 33 research projects in clinical, parasitological, entomological, epidemiological, pharmacological, pathological, immunological and molecular-biology disciplines.

Advanced Molecular Research Centre, donated by Korea Overseas International Cooperation Agency (KOICA) has been established in 2014, where advanced molecular investigations including different PCR methods for detection of drug resistant gene on malaria and gene sequencing are being made. Now, it has been used not only for research but also for training both national and international students. In order to strengthen malaria diagnosis in Myanmar, regional malaria officers of high risk areas of drug resistance namely Kachin, Shan, Kayin, Rakhine and Mon States and Tanintharyi region were given training on basic malaria microscopy and malaria microscopy quality assurance. Refresher Training Course on Therapeutic Efficacy Studies (TES) methodology was also given to medical doctors under the facilitation of experienced facilitators and/or WHO staff.

DMR is organizing technical seminars regularly for private providers on case management of malaria.

Utilization of research findings

Every research finding bears its scientific merit and application. Among which some essential research which had changed the policy or strategy or treatment or management should be note worthy. The following papers are such of a kind and mentioned here as examples. (i) Pe Than Myint *et.al.* (1985)A preliminary clinical study comparing artemether (Quinghaosu derivative) with standard antimalarial drugs in the treatment of falciparum malaria in Burma [Malaria Research Findings Reference Book Myanmar (1990- 2000) page 211], (ii) Myint Lwin and Ye Htut, (1991) Study of the malaria situation in forested foothill and nearby plain areas of Myanmar, [Malaria Research Findings Reference Book Myanmar (1990- 2000) page 168], (iii) W Tun Lin, *et. al.*, (1995) Hyperendemic Malaria in a forested hilly Myanmar village [Malaria Research Findings Reference Book Myanmar (1990- 2000) page 192], (iv) W Tun Lin *et.al.* (2000) Key wells: How important are these *Anopheles dirus* breeding sites for malaria transmission in coastal Myanmar. [Malaria Research Findings Reference Book Myanmar (1990- 2000) page 78] v5)Marlar Than *et. al.* (2001) A double blind comparative trial of two dosage regimens of artesunate suppository in combination with oral mefloquine in severe falciparum malaria, [Dimensions of Malaria Research: A Collection of Abstracts (2001-2011) page 5], (vi) Marlar Than *et. al.,*(2001) A meta-analysis of efficacy of artemisinin derivatives in severe falciparum malaria, [Dimensions of Malaria Research: A

Collection of Abstracts (2001-2011) page 7], (vii) South East Asian Quinine Artesunate Malaria Trial (SEQUAMAT) group (including more than 10 Myanmar authors), (2005) Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. [Dimensions of Malaria Research: A Collection of Abstracts (2001-2011) page 31]. Recent findings of molecular and genetic studies conducted at DMR within last 5 years have provided important molecular epidemiology and genetic information of drug resistance.

In Myanmar, targeted mass drug administration (MDA) is being evaluated using ultrasensitive reverse transcription PCR (usPCR) testing that is thousands-fold more sensitive than rapid diagnostic tests (RDT) and hundreds-fold more sensitive than standard PCR. In collaboration with seven governmental and non-governmental malaria implementing partners, cross-sectional surveys of malaria prevalence in 43 villages located in 13 malaria endemic rural townships of nine State and Regions was conducted during 2017. The findings showed that this usPCR method could be used as a tool for identifying areas of pocket transmission and low prevalence falciparum malaria, which are prioritized areas for malaria elimination. *P. vivax* burden is significant and comparatively refractory to interventions that are decreasing *P. falciparum* transmission, posing a complex problem for malaria elimination.

Publications and references

DMR researchers can send their research papers either to Myanmar Health Sciences Research Journal, DMR Bulletin, DMR E- Newsletter or to Myanmar Medical Journal or Myanmar Journal of Current Medical Practices of Myanmar Medical Association (MMA) or to any regional or international journal of their choice.

DMR has compiled 2 malaria reference books for the malaria scientific community within last 2 decades. Malaria Research Findings Reference Book (1990-2000) was published in June 2002. It presented 429 malaria articles, those published in national and international journals and those presented in various international symposium/conferences. For easy reference, index by subjects has been presented in chronological order and presented under four categories namely Health Education and Health Promotion (22 abstracts), Surveillance, Prevention and Control of Malaria (74 abstracts), Drug Resistance, Early Diagnosis and Case Management of Malaria (308 abstracts) and Programme Management (25 abstracts).

Another reference book was published in 2013 as Dimensions of Malaria Research: a collection of abstracts (2001-2011) in commemoration of Golden Jubilee Event of DMR. Out of 236 abstracts in the collection, 91 represented therapeutic efficacy studies, biochemical, immunological and clinical studies, followed by 76 studies related to public health, health economics and social science, 40 studies related to diagnostics, drug resistance and molecular techniques, 19 studies in traditional medicine and pharmacokinetics and 10 malaria vector studies.

Current malaria research highlights

DMR had also contributed in conducting **Myanmar Artemisinin Resistance Containment** (MARC) Baseline survey which consisted of three components: 1) household, 2) health facility and 3) drug outlet, targeting Tier 1 and Tier 2 areas of Mon State, Kayin State, Kayah

State, Tanintharyi Region, and Bago East Region(excluding Kachin State). Many applicable findings were reported and timely taken up by the control program in both public and private health facilities in the areas of malaria diagnosis and specific antimalarial treatment, ITN distribution, IEC provision and supervision of volunteers.

With strong collaboration and support of WHO, therapeutic efficacy studies on currently used artemisinin combination and *in vitro* antimalraial effects of currently used drugs and reputed traditional medicines are being carried out. At molecular aspect, DMR is the only place in Myanmar, capable of performing falciparum malaria parasites genotyping to confirm drug resistance and also capable of doing K13 gene analysis by sequencing in collaboration with University of Maryland. Parasitology Research Division is now taking responsibility to detect asymptomatic malaria by ultrasensitive quantitative PCR method to explore the asymptomatic malaria burden in malaria pre-elimination phase. Real time reporting to NMCP focusing on therapeutic efficacy status of currently using drugs and updated molecular information on drug resistant malaria are also being made. The molecular method and results of genotyping have been validated biannually under External Quality Assurance Programme of World Wide Antimalarial Resistance Network (WWARN).

Some ongoing projects include (i) Mapping of G6PD deficiency among malaria patients in Rakhine and Chin national groups, and availability and use of primaquine in real practice in Myanmar (ii) Assessment of the bio-efficacy of long- lasting insecticidal mosquito nets (LLINs) on malaria vector Anopheles mosquitoes as well as the durability of LLINs in malaria endemic areas and (iii) Mapping of malaria vectors and monitoring of insecticide resistance (iv) Study of personal protection among rubber tappers and treatment seeking behavior for malaria among the migrant population, (v) entomological situation including vector bionomics and (vi) Village Health Volunteers (VHVs) knowledge and practices for malaria case management and prevention.

As regards to therapeutic efficacy studies, 11 sentinel sites in Myanmar have been investigated since 2009 following WHO protocol and the findings are instrumental for changing in treatment guidelines. The findings has shown the efficacy of >95% PCR confirmed adequate clinical and parasitogical response of the ACTs (A-L, DHA-PIP and AS+MQ) against Plasmodium falciparum (though day 3 parasitaemia were found >10% in some sentinel sites) and high efficacy of chloroquine against *Plasmodium vivax*. The research findings on migrants showed that they are at risk of malaria due to their behavior and nature of work and are important population to be addressed in elimination. As regards to personal protection, only 30% of migrant rubber tappers utilized personal protection throughout night. Therefore, residual malaria transmission and malaria in migrant people pose challenge for malaria elimination. The Information Education and Communication and Behavior Change and Communication (IEC, BCC) activities targeting migrant rubber tappers need to be intensified in order to increase use of personal protection as well as to increase their knowledge regarding malaria diagnosis, treatment and prevention. A larger proportion of migrants (71.6%) initially sought treatment from quacks, drug sellers and self-medication.

Assessment of the prevalence of markers for artemisinin resistance and correlation with Therapeutic Efficacy Studies (TES) in Northern Shan and Kachin States, Myanmar was

conducted. It was found that K13 mutation rate was 3.2% in Mu-Se of Shan State and 68.4% in Myitkyina of Kachin State. The F446I mutation was reported to be associated with day3 persistent parasitemic cases and it was the most common mutation detected in both study sites. The finding of the previous study done in Rakhine State confirmed the absence of K13 mutation among ACT sensitive samples and this study approved the prevalence of K13 point mutations with different degree of ACT failure and day 3 parasitemic cases. The finding was also consistent with the finding of Chinese's study. Continuous monitoring of drug resistance by therapeutic efficacy and molecular surveillance is strongly recommended as K13 mutation could emerge independently.

International collaborations

DMR is carrying out its research in collaborations with WWARN (World Wide Artemisinin Resistant Network), Global Diseases Control and Epidemiology, Department of International Health, Johns Hopkins Bloomberg, School of Health, Baltimore University, Faculty of Medicine, Mahidol University, Thailand and Korea International Cooperation Agency (KOICA). China CDC, NIPD (National Institute of Parasitic Diseases), WHOCC for Tropical Diseases, Nagasaki University, Japan, James Cook University, Cairns, Queensland, Australia.

University of Maryland/Duke University

DMR initiated collaboration with University of Maryland, Baltimore (UMB) to conduct "Pilot studies of the molecular epidemiology of drug-resistant malaria in Myanmar" on 14th October 2011 taking the sub-recipient grant for the period of 2012-2014.

Under the Memorandum of Understanding, signed between the Ministry of Health and UMB, signed on 7th February 2014, DMR continued collaboration with University of Maryland, Baltimore by carrying out the project "New surveillance tool for malaria elimination in Myanmar" in 2015-2017 as Phase 1. Then, DMR extended phase 2 of the project "New surveillance tool for malaria elimination in Myanmar" with the study entitled " Evidence to Action for Malaria Elimination in Myanmar" from 2017 – 2021 with Duke University.

ICEMR

A 7 year cooperative grant entitled "Myanmar Regional Center of Excellence for Malaria Research." (June, 2017 to May, 2024) is under way to commence which is funded by National Institute of Allergy And Infectious Diseases. Together with DMR, collaborating partners include. Defense Services Medical Research Centre, Directorate of Medical Services, Ministry of Defense, Myanmar, National Institute of Parasitic Diseases, China CDC, China, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and Duke Global Health Institute, Duke University.

KOICA

DMR received funding from KOICA starting from 2011 to conduct "Establishment of Laboratory for Research on Communicable Diseases. Establishment of monitoring system for drug resistant malaria" conducted in collaboration with Kangwon National University, Republic of Korea (ROK) was one of the three major research plans. The collaboration with Kangwon National University, was continued by performing "Monitoring of drug resistant

malaria by therapeutic efficacy trial and molecular tool (DMR-KOICA Follow up project)" 2015-2016. Under the training program, 6 methodology trainings, 2 doctoral degree course trainings were successfully completed.

Academic training and services

Academic training and services are being given by DMR. The Training programmes include Ph.D., Dr. P H, other post graduate degrees and Long/short term training programmes. Services provided are advocacy and training on health education, vector control & disease management, monitoring of drug resistant malaria, community empowerment and on malaria diagnostic methods. Further details of the research findings as regards to results, evaluation and applications can be found in the list of references.

As a national task, DMR is committed to continue **This Journey of Malaria Research** by all means in collaboration with all partners from national and international organizations and departments until malaria is eliminated. Drs Kay Thwe Han, Mar Mar Kyi, Khin Phyu Pyar, Khin Thet Wai, Tin Oo, Myat Htut Nyunt, Khin Myo Aye, Yan Naung Maung Maung are among the key players of current malaria research activities and many young researchers are in the pipeline undergoing training to make the journey of malaria research of DMR more smooth and successful.

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MYANMAR HEALTH SCIENCES RESEARCH JOURNAL AS A KNOWLEDGE TRANSFER PLATFORM FOR OPERATIONAL/ IMPLEMENTATION AND PROGRAM EVALUATION RESEARCH: A WAKE-UP CALL

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What is knowledge transfer in evidence-based research?

In evidence-based research, knowledge transfer is the only feasible way to significantly reduce the gap between knowledge creation and knowledge use. The use of knowledge generated from well-designed and well-conducted research in a public health domain is of paramount importance to serve as a bridge between researchers and decision-makers in a developing country setting like Myanmar.[1, 2] Apart from advocacy and communication of health messages and dissemination meetings at different levels of public sector health care system, strengthening knowledge transfer mechanisms to improve an access to key research results by dissemination through published articles is essential. However, there is a gap between the production of new knowledge through operational, implementation and program evaluation research and further acceptance and adoption by policymakers and program planners.

Dealing with research gaps and promoting program operations

Within the context of 5-year cycles of National Health Plan (NHP) in Myanmar, program managers are responsible for cost-effective implementation and monitoring of program interventions to reach the target beneficiaries at grass-roots. Research gaps are given priority to socio-behavioral aspects of demand creation for available health services especially among least developed and marginalized populations, remote sites and vulnerable areas inclusive of conflict zones and disaster-prone areas. Concomitantly, identifying solutions for bottlenecks in specific program operations followed by strengthening knowledge translation and transfer to the target audience is imperative for positive impact. In this connection, researchers are observing the scientific and methodological rigor inclusive of ethical considerations while passing their manuscripts through the standard peer review process of Myanmar Health Sciences Research Journal (MHSRJ).

Contributing program significance through publications in Myanmar Health Sciences Research Journal (2011-2017)

A desk review was conducted to analyze the original articles and short reports published in public health domain in MHSRJ across 2011 to 2017 that covered the NHP cycle (2011-2016) and the initial years of the recent cycle (2016-2021). The eligible articles were retrieved from the web portal of MHSRJ http://www.myanmarhsrj.com/. This brief review aimed to identify the different categories of, operational/implementation research and

program evaluation research that will also include applied field experiments which were partly beneficial for program monitoring. Altogether 54 articles which were of direct concern to health and health-related program interventions in the variety of settings *viz*: community, clinic, hospital and other confined settings such as schools, industries/work sites were included in the analysis. Most of these studies used quantitative cross-sectional designs and some included measurements and procedures to collect and analyze biological samples with some methodological limitations. Studies using mixed methods were few. Operational research (OR) studies focused mainly on improved program performance and evidence-informed improvements in program operations. Implementation research (IR) intended to optimize implementation of available interventions.[3] The major research question(s) addressed in program evaluation research (PER) focused program activities, coverage, short-term outcomes (changes in knowledge, skills, attitudes, opinions), medium-term outcomes (adoption of behavior or action that result from new knowledge) and improved health and wellness status and quality of life as a long-term outcome.[4] Key issues highlighted for each specific project included context/settings, research design and methods, and applicability towards policy, programs, regulations, and guidelines (Table 1).

Table 1. Applicability and relevance of published original articles in public health domain in MHSRJ (2011-2017) to program interventions

No.	Article	Year	Context	Study design	Method	Sample size	Program relevance
1.	The burden of care-giving in caregivers of stroke patients attending the Out-patient Follow-up Clinic of Neuromedical Unit, Yangon General Hospital	2011	Clinic	Cross-sectional (OR)	Structured interviews	100 care-givers	Stroke Rehabilitation Program
2.	Association between the use of insecticide-treated nets (INTs) and parasitaemia and presence of malaria antibody in Thanbyuzayat Township, Mon State	2011	Villages	Cross-sectional comparative (PER)	Structured Interviews & Biological sample	183 villagers	National Malaria Control Program (NMCP)
3.	Socio-economic and health consequences among HIV/AIDS affected families and orphans in Hlinetharyar Township	2011	Ward	Cross-sectional Qualitative (OR)	Indepth inter-views (IDI), Key informant interviews(KII)	16 parents/ guar- dians, 18 Basic Health staff (BHS) & volunteers	National AIDS Program (NAP)
4.	Community-based control of *Aedes aegypti* larvae by using *Toxorhynchites* larvae in selected townships of Yangon Division, Myanmar	2011	Ward	Quasi-experimental (IR)	Observation structured interviews	50 households each in test and control areas	Dengue prevention program
5.	Community acceptance on insecticide treated bed-nets in selected rural communities.	2011	Villages	Cross-sectional qualitative (OR)	Focus Group Discussions (FGD)	24 villagers	NMCP
6.	Patients' perspectives on public-private mix initiatives in tuberculosis control	2012	Clinic	Cross-sectional, Mixed methods (PER)	Structured interviews IDI	300 TB patients	NTP
7.	Knowledge on cervical cancer and opinion towards screening service among attendees at Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar)	2012	Clinic	Cross-sectional (PER)	Structured interviews	151 women aged 21-65 years	Cervical Cancer Screening Program

No.	Article	Year	Context	Study design	Method	Sample size	Program relevance
8.	Prevalence and correlation of obesity, hypertension and type 2 diabetes mellitus in selected townships of Upper Myanmar	2012	Ward & Villages	Cross-sectional (OR)	Structured interviews	3200 adults	NCD program
9.	Adherence to the recommended regimen of artemether-lumefantrine for treatment of uncomplicated falciparum malaria in Myanmar	2012	Villages	Cross-sectional comparative (PER)	Structured interviews	248 uncom-plicated falci-parum malaria patients	NMCP
10.	Utilization pattern of traditional medicine in rural community in Pyin Oo Lwin and Naungcho Townships	2012	Villages	Cross-sectional (OR)	Structured interviews	1132 villagers	Traditional Medicine
11.	Feeding practices of mothers with less than two years old children during child's illness and diarrhea	2012	Villages	Cross-sectional mixed methods (OR)	Semi-structured interviews & FGD	89 mothers (interviews) 32 mothers (FGDs)	National Nutrition Program & MCH Program
12.	Knowledge on Danger Signs and Antenatal Care Visits Made by Third Trimester Pregnant Women in Shwepyitha Township (Short Report)	2012	Ward	Cross-sectional (OR)	Structured interviews	172 pregnant women	Health Promotion Program
13.	Outlooks toward their assigned jobs of station medical officers	2012	Station Hospital	Cross-sectional (OR)	Structured interviews	32 Station Medical Officers	Human Resources for Health
14.	Knowledge on reproductive health and reproductive health problems of unmarried women (25-49 years) in three selected townships, Mandalay Region	2012	Ward	Cross-sectional (PER)	Structured interviews	600 unmarried women	Reproductive Health
15.	Knowledge and practice of safety measures on agricultural pesticide utilization among farm workers in Kyauktan Township	2012	Villages	Cross-sectional (PER)	Structured interviews	141 farm workers	Occupational Health
16.	Brucellosis: Seroprevalence and the knowledge, attitude and practice (KAP) among abattoir workers in Yangon	2012	Slaughter house	Cross-sectional (PER)	Structured interviews & Biological samples	105 abattoir workers	Occupational Health
17.	Accessibility of health services among TB patients in Kutkai Township, Northern Shan State, Myanmar	2013	Township Health Department	Cross-sectional mixed methods (OR)	Structured interviews IDI KII	120 TB patients for structured interviews 23 IDI 5 KII of Basic Health Staff	National TB Control Program (NTP)
18.	Antenatal Care and Delivery Practices among Rural Kokang Mothers, Northern Shan State, Myanmar	2013	Villages	Cross-sectional Qualitative (PER)	FGD KII	47 mothers for FGDs 6 KII of Basic Health Staff	Reproductive Maternal, Newborn, Child and Adolescent Health program (RMNCAH)
19.	Knowledge on Adolescent Reproductive Health among High School Students in Katha Township	2013	Ward	Cross-sectional comparative (PER)	Structured interviews	500 10th standard students	RMNCAH program
20.	Providers and Clients Perceptions and Problems in Providing Newborn Health Services in Project and Non-project Townships of Magway Region	2013	Villages	Cross-sectional comparative (IR)	Semi-Structured interviews	46 midwives 80 mothers in project & non-project townships	Essential Newborn Care program
21.	Currently Married Urban and Rural Women in Meiktila Township: Qualitative Study on Contraceptive Use	2013	Wards & villages	Cross-sectional qualitative (PER)	Focus Group Discussions	40 currently married women aged 15 to 49 years	RMNCAH program

No.	Article	Year	Context	Study design	Method	Sample size	Program relevance
22.	Utilization of Clean Delivery Kit in Insein Township (Short Report)	2013	Clinic	Cross-sectional (OR)	Structured interviews	100 mothers	RMNCAH program
23.	Quality of Life and Compliance among Type-2 Diabetes Patients Attending the Diabetic Clinic at North Okkalapa General Hospital	2013	Clinic	Cross-sectional (PER)	Structured interviews Biological sample	150 type 2 diabetes patients	NCD control program
24.	Use of Complementary and Alternative Medicine (CAM) in Children with Cancer at Yangon Children Hospital	2013	Hospital	Cross-sectional (PER)	Structured interviews	107 parents/guardians	NCD control program
25.	Aetiological Agents, Modifiable Risk Factors and Gamma Interferon Status of Children with Acute Respiratory Infections Attending General Practitioner's Clinics	2013	Clinic	Case-control study (OR)	Structured interviews Biological samples	Mother/care-takers of 118 cases and their 240 age-matched controls	ARI Control Program
26.	Opinion of Caregivers and Midwives towards Hepatitis B Immunization in 3-5 year-old Children from Mawlamyaing and Thaton Townships	2013	Wards & villages	Cross-sectional (PER)	Structured inter-views	1145 caregivers 23 midwives	EPI prgram
27.	Awareness on Preparedness of Community on Disastrous Storm in Kwanchankone Township, Yangon Region (Short Report)	2013	Villages	Cross-sectional (PER)	Structured inter-views	184 community members	Disaster Management
28.	Knowledge of first-year MBBS Students of University of Medicine (Magway) Rergarding Human Immunodeficiency Virus Infection	2014	University	Cross-sectional (PER)	Self-administered questionnaire	375 first year MBBS students	NAP
29.	Effect of Health Education on Changes in Dietary Habit and Cardiovascular Risk Factors among Sedentary Workers	2014	Timber Enterprise	Pre-test, post test (OR)	Structured interviews Measurements biological samples	196 employees	NCD Control Program & Occupational Health
30.	Deltamethrin Treated Clothes for Personal Protection on Malaria among Temporary Migrant Workers in Rubber Plantation, Mon State, Myanmar	2014	Villages	Quasi-experimental (OR)	Structured interviews Biological samples	Rubber plantation night time workers (50 each from intervention & control villages)	NMCP
31.	Factors Influencing Smoking, Alcohol Drinking and Betel Chewing Practices among Third-year Male	2014	University	Cross-sectional (PER)	Structured interviews	166 third year male students of University of Agriculture	Health Promotion for NCD
32.	Reproductive Tract Infections among Pregnant Women Attending the Antenatal Clinic at North Okkalapa General Hospital	2014	Clinic	Cross-sectional (PER)	Interviews & Biological samples	216 pregnant women	RMNCAH program
33.	Incidence of injury due to road traffic accidents in Lashio Township, Northern Shan State	2015	Hospital	Cross-sectional (OR)	Record review	3268 injury patients	Prevention program for Road Traffic Accidents
34.	Simple Hygienic Measures to Prevent Diarrhoea among Housewives in Mandalay	2015	Wards	Cross-sectional (PER)	Face to face interviews Observation	829 housewives	Health promotion program
35.	Antiretroviral Therapy Adherence among PLHIVs in Public and INGO Centre: Barriers and Braces	2015	Clinic	Cross-sectional mixed methods (OR)	Structured interviews IDI, KII	120 PLHIV for structured interviews, 6 IDI, 4 KII	NAP

No.	Article	Year	Context	Study design	Method	Sample size	Program relevance
36.	Healthy Eating: Teachers' Perspective and Students' Practice in Monastic Education Schools and Basic Education Primary Schools, Mingalardon Township	2015	Schools	Cross-sectional mixed methods (PER)	Structured interviews IDI, KII	458 students for structured interviews 8 KII & 13 IDI of school teachers	Health promotion program School Health Program
37.	Status of Infection with Soil-transmitted Helminths among Primary School Children in Three Selected Townships of Yangon Region	2015	Primary schools	Intervention study (IR)	Structured interviews Deworming Stool checks	1443 students (before and after intervention)	Health promotion program School Health Program
38.	Effect of Zinc Supplementation on Physical Growth and Serum Zinc Level of Primary School Children in North Dagon Township	2016	Primary schools	Quasi-experimental (OR)	Measure-ments & biological sample	Apparently healthy 76 children aged 7-9 years assigned to two groups	Health promotion program School Health Program
39.	Occupational Health Perception and Practices of Workers in the Environment of Gold Leaf (Shwe Saing) Traditional Industry in Mandalay	2016	Industry	Cross-sectional (PER)	Structured inter-views	150 workers	Health promotion program Occupational Health Program
40.	Survival of HIV Infected Children on Antiretroviral Therapy	2016	Hospital	Retrospective cohort (OR)	Record review	881 records of HIV infected children	NAP
41.	Quality Assessment of Antimalarials in Two Border Areas (Tamu and Muse)	2016	Drug shops	Cross-sectional (PER)	Quality assurance	51 samples of antimalarials	NMCP
42.	Care-seeking Behavior and Detection of Target Organ Involvement among Hyper-tensive Patients in Yangon Region (2014-2015)	2016	Clinic	Cross-sectional multimethod (PER)	Structured interviews Measurements	622 hype rtensive patients	Health promotion program NCD program
43.	Factors Associated with Anaemia in Pregnancy	2016	Clinic	Cross-sectional (PER)	Structured interviews Measure -ments	300 pregnant women	RMNCAH Health promotion program
44.	Knowledge and Lifestyle Related Perception Regarding Risk Factors of Cardiovascular Diseases among Adolescents at a Private School in Yangon	2016	School	Cross-sectional (PER)	Structured interviews	153 students (age 13-18 years)	Health promotion program NCD program
45.	Dietary Diversity and Nutritional Status of Children Aged 12-23 months from Ayeyawady Region of Myanmar	2017	Villages	Cross-sectional (PER)	Interviews & Measurements	106 children (12-23 months of age)	National Nutrition Centre
46.	Dietary Habits and Nutritional Status of the Elderly in Insein Township, Yangon Region in 2013	2017	Wards	Cross-sectional (PER)	Interviews & Measurements	Elderly (60-75 years of age)	National Nutrition Centre Elderly care
47.	Effectiveness of Health Education on Knowledge of Groundwater-dependent Rural Residents Regarding Arsenic-contaminated Water at Kyonpyaw Township	2017	Villages	Non-Equivalent Control Group (OR)	Structured interviews In-depth interviews	135 adult household members in study group and control group 6 IDIs	Health Promotion program Environment al Health
48.	Stunting and Zinc Nutritional Status among Primary School Children in North Okkalapa Township	2017	Schools	Cross-sectional (OR)	Measurements Serum samples	60 primary school children	School Health Program

No.	Article	Year	Context	Study design	Method	Sample size	Program relevance
49.	Feelings and Experiences of Pulmonary Tuberculosis Patients Who Failed to Take Regular Anti-tuberculosis Treatment Regime at No. 1 Defense Services General Hospital, Mingalardon	2017	Hospital	Cross-sectional Qualitative (OR)	Semi-structured interviews	Seven participants who failed to take anti-TB treatment	NTP
50.	Study of Stillbirths in North Okkalapa General and Teaching Hospital	2017	Hospital	Cross-sectional (OR)	Record review In-depth interview	Records of 76 women with still birth	RMNCAH program
51.	Sero-prevalence of Hepatitis B and C Viral Infections in Myanmar: National and Regional Survey in 2015	2017	Community	Cross-sectional (OR)	Short questionnaire & serum samples	5547 subjects from 18 town-ships of all States/Regions	National Hepatitis Control Program
52.	Current Practices and Problems Encountered in Emergency Obstetric Care in Rural Areas of Central Myanmar	2017	Villages	Cross-sectional, mixed methods (IR)	Structured questionnaire KII	109 midwives, two Township Medical Officers, four Health Assistants, 12 mothers, 8 community members	RMNCAH program
53.	Family Planning Practice and Reproductive Health Needs among Rural Married Women in Laukkai Township, Kokang Self-Administered Zone, Northern Shan State, Myanmar	2017	Villages	Cross-sectional, mixed methods (PER)	Structured questionnaire FGD	300 women in structured interviews 60 women in FGDs	RMNCAH program
54.	Social Network Addiction (SNA) Related Depression among Students at Kyaukse University, Mandalay Region, Myanmar	2017	University	Cross-sectional (OR)	Structured questionnaire	400 university students	Mental Health Program

Way forward to improve knowledge management through publications

Embedding of locally-relevant and demand-driven research in health systems may lead to improvements in implementation and scale-up of health policies and programs.[5] Henceforth, promoting thematic collections of relevant articles at MHSRJ in operational as well as implementation and program evaluation research context is essential to attract the attention of readers and policymakers and to promote the utilization of research results as required and enhancing relevant research to practice. In addition, there is a need to highlight specific issues for overcoming setbacks in implementing the program interventions in research articles and to strengthen the contribution of significant and relevant research evidence in developing policy briefs.

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CAPTURING THE MULTIPLE FACETS OF DENGUE IN MYANMAR: A REVIEW

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Introduction

Dengue is globally known as the most prevalent arthropod-borne viral disease and a public health problem in tropical and subtropical regions of the world. It is estimated that 3.6 billion people in over 120 countries are at-risk for infection and 500 million people infected each year.[1]In thepast five decades, the incidence of dengue fever has increased 30 times and the epidemiology of dengue in South-East Asia is undergoing a change in the virus as well as the vector bionomics. Shift in affected age groups and expansion to rural areas are evident, while the virulence and genotype of the virus determine the severity.[2] In Myanmar, dengue is a major health problem and the leading cause of hospitalization in children with all four serotypes of dengue viruses circulating in the country, making the dengue endemic situation of Myanmar, category A in the Southeast Asia region.[3]

Epidemiological facet

Dengue caught attention in Myanmar with it's first outbreak in 1970 with 1654 cases and 91 deaths. In 1973-74, a serological survey was carried out for the whole country covering all 14 states and divisions. From this nation-wide survey, it was observed that dengue antibody prevalence rate was less than 10% in Rakhine and Shan states, 10-30% in Ayeyarwaddy, Bago and Mandalay regions and also Kachin, Mon and Kayin states. In Sagaing Region (31-60%) and over 60% in Yangon, Magway and Tanintharyi regions. At that time, there was no dengue antibodies detected in Chin and Kayah states.[4] In the initial years after the first outbreak the cases were confined to Yangon, but in 1974 dengue spread to other states and regions. After 1974-2007, dengue spread to all states and regions except Chin and Kayah states. In 2008, Kayah State was first affected and Chin state in 2015 being the last to be affected in the whole country. Since then and to-date dengue viruses circulate in all states and regions of Myanmar.Outbreaks have seen to occur in increasing magnitude since the first recorded outbreak in the country in 1970 (1,654 cases reported) followed in 1975 (6,750 cases), 1987 (7,331cases), 1994 (11,647 cases), 1998 (12,918 cases), 2001(15,361cases) 2002 (16,047 cases) and in 2009 (24,285 cases).[5]

A cross-sectional study using annual records from the Myanmar vector-borne diseases control (VBDC) programme between 2011 and 2015, showed that up to the present, 2015 has been the highest year with 43,845 cases with 161 deaths.The distribution of dengue deaths each year mirrored the distribution of cases. Case fatality rate (CFR) was highest in 2014 at 7 per 1000 dengue cases, while in the other years, it ranged from 3 to 5 per 1000 cases. High CFR per 1000 were also observed in infants less than 1 year (CFR=8), adults ≥15 years

(CFR =7), those with disease severity grade IV (CFR=17), and those residing in hilly regions (CFR=9).[6]

Virological facet

One of the most unusual dengue outbreaks was in 2001 when virus isolation from patients revealed that 95% of dengue viruses were serotype 1 which was very unusual for a dengue endemic country where all 4 serotypes were circulating. This outbreak, was the first-ever example of one DENV serotype (DENV 1) displacing the other three serotypes almost completely.A phylogenetic analysis, revealed that two new lineages of dengue 1 had emerged and an earlier lineage previously circulating in Myanmar had become extinct. The DENV 1 which disappeared was genotype III and the two new viruses which appeared were of genotype I. The phylogenetic analysis further revealed that one new lineage had the closest relationship with viruses from Guangzhou, in southern China and the other one, closest with viruses from Cambodia.[7]

In another study to understand the molecular epidemiology of circulating dengue viruses (DENV) in Upper Myanmar, samples were collected from dengue (DEN) patients admitted at Mandalay Children's Hospital in 2006.Infected culture fluids were subjected to a RT-PCR to detect the DENV genome. Three DENV strains were isolated. This was the first DENV isolation performed either in Mandalay or in Upper Myanmar. Phylogenetic analyses revealed that this DENV-3 strain was clustered within genotype II, and the two DENV-4 strains were clustered within genotype I. The Myanmar strains were closely related to strains from the neighboring countries of Thailand and Bangladesh.[8]

Most of the previous studies dealing with virological aspects of dengue in Myanmar had been concentrated in Yangon. In a project funded by WHO/TDR, conducted from 2007 to 2010 entitled "Sentinel surveillance of dengue in endemic regions of Myanmar", studied the dengue situation not only Yangon, but also extended to other sites such as Lashio, Sittwe, Mandalay and Mawlamyaing. Table 1 shows the results from this study.[9]

Table 1.Sentinel surveillance of dengue in endemic regions of Myanmar (2007-2010)

Sentinel sites		2007	2008	2009	2010
Mandalay	Positive (%)	58 (n=74)	79(n=52)	70.8(n=48)	58(n=99)
	Primary(%)	16	24.4	41.2	18.3
	Secondary(%)	84	75.6	58.8	80.7
	Serotype		DEN3	-	DEN1,4&DEN1+4
Sittwe	Positive	65 (n=20)	82.1(n=39)	-	46(n=62)
	Primary	7.7	28	-	19
	Secondary	92.3	72	-	81
	Serotype		-	-	DEN1,2
Mawlamyaing	Positive	90(n=21)	78.6(n=14)	90.0(n=11)	42.5(n=41)
	Primary	21	27.3	0	17.6
	Secondary	79	72.7	100	82.4
	Serotype		-	-	DEN2,4
Lashio	Positive	48(n=81)	96(n=26)	55.6(n=18)	-
	Primary	23.1	36	40	-
	Secondary	76.9	64	60	-
	Serotype		-	DEN1,4	-

During the 2015 dengue epidemic, a hospital-based study was conducted in two widely separated regions in Myanmar (Mandalay in Upper Myanmar and Myeik in Lower Myanmar). A total of 106 virus strains were isolated (66 viral strains from patients in Mandalay and 40 from Myeik). From Mandalay, (75·8%) of the isolated strains were DENV-1, (22·7%) DENV-2 (1·5%) DENV-4. On the other hand, in Myeik, (65%) were DENV-1, (22·5%) DENV-2, (2·5%) DENV-3, (10%) DENV-4. A phylogenetic analysis of the E-protein of DENV-1 strains from both study areas showed that all strains belonged to genotype I, and closely related to strains from China, Thailand, Sri Lanka and those previously isolated in Myanmar. All DENV-2 from the two study areas were Asian I genotype and formed two lineages, closely similar to the strains from Thailand, China, and Myanmar strains.The only DENV-3 isolate from Lower Myanmar belonged to the genotype III and related to the strains from Thailand, Laos, Cambodia, and Vietnam. All DENV-4 strains belonged to genotype I. The strains were similar to the strains from Myanmar andThailand.[10]

Clinical facet

A study by Thein, *etal* in 1997 described a five-year, prospective study in two townships (Tamwe and Ahlone) in Yangon.The data from this study revealed that the risk of developing Dengue Shock Syndrome (DSS) following an anamnestic (secondary) infection in children was from 82 to 103 times greater than that of developing DSS following a primary dengue infection. It also confirmed that risk of developing DSS was 15 times greater when the host had a secondary infection with DENV-2 following a prior infection with one of the other three DENV serotypes than that of developing DSS following infection with dengue serotypes 1, 3, or 4.[11]A study in 2015 evaluated the proportion and categorized the severity of Acute Kidney Injury in dengue patients using pRIFLE (paediatric Risk, Injury, Failure, Loss of kidney function, End stage renal disease) criteria in ninety-four children, 46 boys (48.9%) and 48 girls (51.1%) ages from 2-14 years of Yangon Children's Hospital. AKI was more common in children ≤5 years age group (p=0.001). Bleeding manifestation was found significantly in AKI group (p=0.04). Children with AKI had longer hospital stay than those with non-AKI (3.50±0.67 days *vs.* 2.77±0.77 days) (p=0.01). This study highlighted that risk of AKI should be considered in paediatric dengue patients especially in younger children.[12]

A study on 13 autopsied specimens of dengue shock syndrome (DSS) cases from children at Yangon Children's Hospital, from 2005-06, showed dengue virus antigens of the structural (envelop) and non-structural (NS1) proteins were detected in 54% and 77%, respectively in both hepatocytes and Kupffer cells, using immunohistochemistry highlighting that hepatocytes and Kupffer cells may be target cells supporting virus localization and replication in DHF.In another study,dengue viruses have also been detected from the pericardial fluid and ascitic fluid from autopsied samples.[13, 14]

Studies to determine risk factors for severity of dengue in adults have also been implemented. In a study by Hlaing Mya Win, 2016 attempts were made to determine the usefulness of dengue serotypes, serum complement levels in predicting the severity of adult dengue infection. Among 48 isolates, 58.3% were serotype 1, serotype 4 contributing 25%, Serotype 2 was 12.5% .Only one of the patients was serotype 3 and one other mixed type (serotype 1 and 4). Serum complement levels (C3 and C4) were measured on the third or fourth day of

fever. There was a significant difference in C3 levels between severe and non-severe dengue patients, (p=<0.001). In predicting the severity of disease, the best cut-off for C3 was 0.79g/L with sensitivity 74.07% and specificity 71.21%. Thus, C_3 level within 4 days of fever, that is, the pre-critical phase may be used as an indicator to forecast the progression towards severe dengue.[15]

Genetic facet

Dengue viruses being RNA viruses, rapidly accumulate mutations due to the error-prone nature of RNA polymerases .Changes in the dengue viral genome can occur by mutations, recombination, genetic bottlenecks and natural selection. In 2003, the first-ever recombinant genome and both parental genomes of DENV2 viruses in a single *Aedes* mosquito host was reported in Myanmar mosquitoes. One parent (Asian 1 genotype) was related to DEN-2 viruses previously circulating in Yangon since 1995 and the other parent (Cosmopolitan genotype) was related to viruses from southern China.[16] A study by Aaskov and team in 2006 found long-term transmission of defective RNA viruses in human hosts and *Aedes* mosquitoes from Myanmar. These DENV-1populations acquired a stop-codon mutation in the surface envelope (E) protein gene. This stop-codon strain represented a defective lineage of DENV-1. It was proposed that such long-term transmission of defective RNA viruses in nature was achieved through complementation by co-infection of host cells with functional viruses.[17]

An attempt was made to reveal the genetic factor underlying the cause of emergence of two new lineages and extinction of one DENV1 lineage which occurred in the unusual 2001 dengue outbreak. To determine if genes other than the E gene were under selective pressure, an in-depth analysis was done by sequencing the whole genome (all 11 Kb) of 35 dengue 1 viruses including all the structural and non-structural proteins within the open reading frame. There was no evidence that the new lineages had arisen by recombination. There was an evidence for only weak positive selection in one protein–NS5.Amino acids NS5-127 and NS5-135 are in motif III of the RNA cap-methyl transferase portion of NS5.The second site found to be under selective pressure (NS5-669) was adjacent to a potential nucleoside triphosphate binding motif of the polymerase component at the C terminal end of NS5. Although theclade-specific nucleotide changes in the 3V UTR changed the predicted secondary structure of this region, the Delta G values for the structures were so similar that the changes were unlikely to have resulted in significant fitness advantage.Collectively, these data suggested that the extinction of the ancestral clade of viruses and the appearance of new strains was due to some stochastic event (a genetic bottleneck).[18]

Another study done by a team of scientists from over the world using a method called Antigenic cartography was applied by which antigenic maps were constructed to establish empirically how DENV types relate to one another. Viral sequences from around the world including Myanmar dengue strains were used and analyzed which showed that the four DENV types are as genetically divergent among themselves as sequences assigned to different viruses within the genus Flavivirus. The results showed that the DENV types are not antigenically homogeneous, which has implications for vaccination and research on the dynamics of immunity, disease, and the evolution of DENV.[19]

Entomological facet

Aedes aegypti is the major vector of dengue and dengue hemorrhagic fever (DHF) in Myanmar. Integration of mapping and spatial analysis model in surveillance and control of DHF was implemented using GIS based dengue vector surveillance system in Hlaingtharyar Township. Data from the GPS receiver were downloaded using Map Source software, viewed in Google Earth and image registration was processed using ERDAS imaging software. A total of 468 containers from 75 households for 2011 and a total of 1208 containers from 155 households for 2012 were examined. Moderate levels of vector infestation were evident based on House Index (HI) 29.33, 22.58, Container Index (CI) 6.41, 5.13, Breteau Index (BI) 40, 40 and Pupae per Person Index (PPI) 0.28 and 0.53 for 2011 and 2012, respectively. Based on this study, it is evident that GIS based dengue vector surveillance system should be developed to the regional level to aid in the prompt identification of local dengue hot spots for appropriate planning of prevention and control strategies in DHF high risk locations.[20] To study the insecticide susceptibility status of *Aedes* mosquitoes to Temephos (Abate),field surveys were conducted in disaster prone areas of Pathein Township, Ayeyarwaddy Region, Hlaingtharyar Township, Yangon Region and Thanbyuzayat Township, Mon State during 2014-2015. House index (HI) were 20%, 13% and 12%, container index (CI) was 7%, 2.4% and 1.4%, pupae per person index (PPI) was 0.55, 0.35 and 0.19 in Pathein, Hlaingtharyar and Thanbyuzayat, respectively. For an average temperature of 27˚C with seroprevalence of 33%, the estimate of the transmission threshold is approximately 0.71 PPI. A hundred percent larva mortality was found in the field test with Abate 0.1 gm/l (recommended field dose).[21]

A community-based intervention study was conducted from May 2009 to January 2010 in Yangon city. Six high-risk and six low-risk clusters were randomized and allocated as intervention and routine service areas, respectively. For each cluster, 100 households were covered. The strategies included eco-friendly multi-stakeholder partner groups (*Thingaha*) and ward-based volunteers, informed decision-making of householders, followed by integrated vector management approach.This study showed that the efficacy of community-controlled partnership-driven interventions was found to be superior to the vertical approach in terms of sustainability and community empowerment.[22]

Dengue vectors have developed resistance to insecticides and currently used larvicides show only short-term effectiveness. As a result, alternatives are urgently needed. A study evaluating the larvicidal effectiveness of long-lasting pyriproxyfen resin discs (SumiLarv®2MR) against dengue vectors in schools in Hlaingtharyar Township was conducted in 2017. The results showed that the proportion of mosquito-infested containers was significantly reduced in the schools using the larvicide (OR: 0.24, 95% CI: 0.12-0.48) with little reduction in the control schools (OR: 0.97, 95% CI: 0.55-1.72). The density of infested containers was also significantly reduced in the intervention schools (Beta: -1.50, 95% CI: -1.98– -1.04), with no significant reduction in control schools (Beta: -0.19, 95% CI: -0.53–0.14). The proportion of adult emergence was less than 20% in the treated water compared to over 90% in the untreated water. In addition, eight-month-old SumiLarv®2MR resin discs were still 100% effective when tested in the laboratory.[23]

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A 30 YEARS REVIEW ON TB RESEARCH PUBLISHED IN MYANMAR HEALTH SCIENCES RESEARCH JOURNAL (1989-2018)

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Introduction

TB is preventable and completely curable, however yet about 710,000 people died of TB in 2015. Incomplete treatment can lead to drug-resistant TB; over 200,000 people got drug-resistant TB in 2015. Malnutrition, smoking and diabetes aggravate TB. It thrives in poverty. TB also creates poverty; the poor have a five-time higher chance of getting TB which is the largest killer among communicable diseases in the 15 to 49 age group, when humans are most productive. TB accounts for the highest DALYs or workdays lost each year among the communicable diseases. It is the leading cause of death among people with HIV. A well ventilated room that allows sunlight reduces risk of TB transmission. Treating a drug-resistant TB case can cost as much as US$ 5000. A dollar invested in TB gives a return of US$ 43.[1]

Myanmar has a high triple burden of - TB, HIV associated TB and MDR-TB. Response was limited in the last two decades except for DOTS expansion and increasing TB notification seven times, from less than 20,000 in 1999 to 140,000 currently. National TB prevalence survey, national drug resistance survey, operational researches and strengthened surveillance system have led to a paradigm shift in TB control and care, backed by massive domestic and international support in the last five years. NTP has adopted an updated National Strategic Plan 2016-2020. High treatment success rate, 87% among new and relapse cases registered in 2014, and 83% among MDR/RR-TB cases started on second-line treatment in 2013 was reported in 2015. PMDT and TB-HIV services now available across the country; quality TB diagnostic services improving after roll-out of Xpert MTB/Rif and active case detection with portable digital X-ray equipment targeting hard-to-reach populations is expanding.[1] This article is review of research projects concerning with TB which were published in Myanmar Health Sciences Research Journal during 30 years starting from 1989 to 2018.

Diagnosis of TB

Tuberculosis is caused by *Mycobacterium tuberculosis* which is an acid fast bacillus. The highest priority for tuberculosis control is the early identification of *Mycobacterium tuberculosis* and prompt treatment to cure of infectious cases. The primary diagnosis of tuberculosis is sputum microscopy. The culture of *Mycobacterium tuberculosis* may only be feasible at a few of intermediate laboratories. According to literature review many scientists were explored the different methods of diagnosis based on bacteriology, immunology and molecular studies on diagnosis in pulmonary and extra-pulmonary tuberculosis. These studies are bacteriological

study of sputum smears positive follow-up pulmomnary TB patients, fluorescence *versus* conventional sputum smear microscopy for examination of Acid-Fast Bacilli, gluteraldehyde gelification time-a highly acceptable bedside tools in assessment of childhood TB, In-house ELISA for the diagnosis of childhood tuberculosis, the role of whole cell tube agglutination test in serodiagnosis of pulmonary TB, determination of *Mycobacterium tuberculosis* in HIV sero-positive patients with cervical lymphadenopathy using different diagnostic tools, use of IS6110 PCR assay for rapid diagnosis of genitourinary tuberculosis, role of PCR in the diagnosis of TB pleural effusion, application of PCR in diagnosis of TB meningitis and a preliminary study of CD4+T-lymphocyte count in TB patients. These findings are applicable for diagnosis of TB in different centers with different facilities.

Studies on defaulters

In Myanmar, most of the townships achieved the targets, treatment success rate 85% and defaulter rate less than 5%. Defaulter means the patient whose treatment was interrupted for two consecutive months. Defaulting TB treatment will not only reduce cure rate and treatment success rate of National TB Programme but also become high chance of transmission to community and development of multi drug resistant TB and death. Possible reasons for defaulting were categorized into two; factors related to providers (service factors) and factors related to patients (patients' factor).

The findings of service factors related to defaulting TB treatment in Myanmar highlighted that ensuring effective, complete pretreatment health education for every TB patients with reference to BHS guideline, conducting initial home visit for every TB patients, motivating BHS for effective DOT, enhancing early missed dose tracing and practicing repeated health education throughout the treatment course were essential for reducing defaulter rates and more effective TB control.[2] The following suggestions are made from study of contributing factors to treatment interruption in patients with tuberculosis in the selected townships with high default rates (i) Effective Health Education Programme including participatory approach, adequate counseling of patient and relatives and peer cohort review should be initiated and performed in a sustained action; (ii) Social and monetary supports should be contributed to ensure complete treatment by local authorities and voluntary donors; (iii) Proper transfer mechanism and opening of transit health centres should be created to enable to supply the drugs continuously especially to migrant TB patients; (iv) Drug should be supplied only after patient's address is confirmed by local authorities and health personnel concerned and alternative address is acceptable for mobile workers and (v) Cooperation between BHS and TB Office should be strengthened with a revitalization of existing default tracing programme.[3]

The study on defaulters related to patient's factors said that predictors of defaulting from anti-TB treatment were multi-factorial. Those included changeable as well as non-changeable factors. Non-changeable factors such as relatively older age group, low income patients and migratory populations should be considered as target groups in order to conduct intervention programs. For those patients, specific measures such as social support for older age group and low income patients and a flexible approach to ambulatory treatment for migratory population could be possible solutions. The changeable factors should be corrected if possible

e.g. prohibition of risk habits and behaviors such as alcohol drinking, smoking and betel chewing. Healthcare providers should consider patients' satisfaction by reducing waiting time, and practicing good social dealing. However, those factors are hard to change and may need more resources. Therefore, giving proper health education about the disease, importance of treatment regularity and danger of MDR-TB should be considered as a vital function of township TB centers to prevent defaulting from anti-TB treatment.[4] A study was performed at No. 1 Defense Services General Hospital, Mingalardon with title of feelings and experiences of pulmonary tuberculosis patients who failed to take regular anti-tuberculosis treatment regime which found that most participants suffered from side effects of anti-TB drugs. Some faced with economic problems because of long staying in hospital. Moreover, they encountered discrimination by society and worried about transmission of disease to their families. Providing adequate health information is important responsibilities of health care providers not only in hospital but also in the unit for attaining continuous care. This study suggested that health care providers of military setting must provide information on disease process, ways of transmission, side effects and importance to take full course of treatment regime to patients, family members and military persons through the unit durbar and discussion. These findings recommend to health care providers including nurse in military setting to be aware of feelings of these patients and to provide better understanding and empathetic support.[5]

Drug resistant TB

Surveillance of drug resistant TB plays an important role in determining the magnitude and trends. Estimated % of TB cases with MDR/RR-TB among new cases is 5.1% and TB cases with MDR/RR-TB among previously treated cases are 27%.[1] Study on drug resistance among tuberculosis patients who sought care at private sector before taking treatment at public health service highlighted that patients who had considerable delay while seeking treatment in private sectors before reaching public health service have greater opportunity for occurrence of drug resistance.[6] Patterns of anti-tuberculosis drug resistance among HIV patients with pulmonary tuberculosis attending the Specialist Hospital, Waibargi, Yangon showed overall anti-TB drug resistance and MDR-TB in HIV patients were high in this study. MDR-TB was associated with previous anti-TB treatment.[7] Study on determination of rifampicin and isoniazid in different formulations from market in Yangon, Myanmar concluded that the four-drug fixed-dose combination (4FDC) (rifampicin, isoniazid, pyrazinamide and ethambutol) supplied by the WHO and used by the NTP are of acceptable quality and assurance of drug quality is of prime importance in affecting a cure as well as prevention of MDR-TB. Among anti-TB drugs, rifampicin and INH are the most important drugs in the treatment of TB and resistance to at least these two has been termed as multidrug-resistant tuberculosis (MDR-TB).[8] Molecular identification of multidrug-resistant *Mycobacterium tuberculosis* strains in Myanmar Patients was done, the most common rifampicin resistance mutation was S531L (67.5%) in the *rpoB* gene and the most prevalent isoniazid resistance mutation was S315T (90.4%) in the *katG* gene. Beijing genotype was predominantly identified in 76.4% of strains (55/72). Strains belonging to Beijing geno-types are significantly associated with MDR-TB (p=0.001) as well as resistance to isoniazid,

rifampicin, streptomycin and ethambutol (all $p<0.05$).[9] Managing the MDR-TB cases that emerge is part of the STOP TB strategy and a component of all TB Control Programmes.

Health System Research on TB

TB is a major public health problem in Myanmar and it is considered as one of the priority diseases in the National Health Plan (2006-2011). To respond to this serious public health problem, the NTP started Public-Private Mix (PPM) piloting linkages with private providers in the late 1990s. A considerable number of public-private mix (PPM) initiatives have been launched since the 1990s in Myanmar. The study on patients' perspectives on public-private mix initiatives in tuberculosis control recommended that patients' knowledge levels on causation and mode of transmission of TB, free availability and effectiveness of DOTS, and awareness of PPM initiatives should be improved through Information, Education, and Communication (IEC) services using appropriate mass media channels. Patients' expectations from health care providers i.e. convenient clinic opening time, provision of symptomatic drugs, enough consultation time (about thirty minutes) and provision of Fixed Dose Combination (FDC) drugs throughout the course should be fulfilled as much as possible in order to improve the patients' compliance to anti-TB treatment in both public and private sectors.[10] Study on success and challenges of Public-Private Mix DOTS initiatives in Myanmar: a process evaluation for partnership approach of non-governmental organizations was conducted in three townships in which Public-Private Mix DOTS (PPM-DOTS) was implemented by three partners - Myanmar Medical Association (MMA), Population Services International (PSI) and Japanese International Cooperation Agency (JICA).

Majority pointed out that proper advocacy for PPM-DOTS was crucial for success of future activities and mentioned existing good personal relationship as success factor for PPM-DOTS. They also expressed trust building could be obtained by sharing information through regular contacts among each other.[11] The study on local Non-Governmental Organizations (NGOs) participation in National Tuberculosis Programme (NTP) showed strongly positive association between NGOs involvement and achievement of NTP was found. NGOs can make an important contribution by facilitating links between health services and local community. The findings of estimation of disease burden due to tuberculosis in Insein Township, Yangon indicated that overall TB disease burden can be estimated as a single metric which capture both morbidity and mortality. This study highlighted the methodological adjustments that should be considered in the estimation of burden of disease due to TB at national level.[12] The study of economic burden of TB patients attending Township TB Centre in Myanmar indicated that treatment delay increased the cost and may lead to economic burden for TB patients and their families. Transportation cost and daily wages loss due to attending TB Centre to get free drugs were found to be possible factors for economic burden of TB patients. Effective strategy to reduce delay in seeking care of TB suspects should be developed.[13] The suggestion from study of accessibility of health services among TB Patients in Kutkai Township, Northern Shan State, Myanmar was appropriate interventions should be identified to help underserved, hard-to-reach TB patients getting proper treatment without prolonged delay and enhancing treatment adherence.[14]

Risk factors

Study on smoking as a risk factor for pulmonary tuberculosis in adults was found that current active smoking was associated with development of pulmonary TB. Moreover, active smokers who started smoking at ≤20 years of age or had a duration of >10 yrs or smoked more than 10 cigarettes/day were at a higher risk of pulmonary TB compared to non-smokers. Therefore, an effective anti-smoking campaign is needed to have a positive repercussion on TB incidence.[15]

Study on importance of plasma protein binding of anti-tuberculous drugs on the response to short course chemotherapy highlights the importance of plasma protein binding of drugs and its influence on the metabolism and interaction between drugs used concomitantly in short-course chemotherapy. This study also showed that addition of pyrazinamide in short-course chemotherapy is very effective and well tolerated but increase in dosage of pyrazinamide during the twice-weekly phase may be necessary to prevent relapse.[16]

Many studies have been carried out by various researchers on different aspects of TB infection as it is a major health problem in Myanmar and regarded as a priority disease in the National Health Plan of the country. This article is a compilation of the research findings carried out in the 30 years journey of Myanmar Health Sciences Research Journal with the objective of providing information to the scientists, health care personnel, administrators and decision makers.

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PROGRESS AND IMPROVEMENT OF MHSRJ THROUGH 30 YEARS

Dr Ni Thet Oo[1] & Ms Nilar Soe[2]
[1]Associate Editor
[2]Editorial Assistant
MHSRJ Editorial Committee

The year 2018 is the time to celebrate the 30th Anniversary of the Myanmar Health Sciences Research Journal (MHSRJ) since it was established by Department of Medical Research, Ministry of Health in April, 1989 with the mission to share and disseminate health research findings to the medical personnel from various medical disciplines. It is the very first medical research journal in Myanmar and includes Long Articles, Short Reports, Review Articles and Correspondences in the field of biomedical and health sciences publishing three times a year (i.e. April, August & December).

In welcoming the auspicious occasion of this 30th Anniversary, the remarkable changes of MHSRJ over its history are consequently mentioned together as follows with a great honor.

1. "Burma Health Sciences Research Journal" was the name of the first issue (Vol. 1, No. 1, 1989 with the size of 20.7 cm x 27.7 cm. It included "Preface", "Editorial Committee", "Notice to Contributors" with a total of 12 research articles (Fig. 1).
2. Starting from the second issue (Vol. 1, No. 2, 1989), the name was changed from "Burma" to "Myanmar" so that it became "Myanmar Health Sciences Research Journal", and it is still used to date (Fig. 2).
3. In 1992, the International Standard Serial Number (ISSN-1015-0781) was assigned to the MHSR Journal by the Centre for International Serial Data System located in France. It has been printed on every issue commencing from Vol. 4, No.1, April 1992 (Fig. 3).
4. From Vol. 11, 1999 to Vol. 16, 2004, only one issue in a year was able to be published due to the shortage of articles received. Then, the regular publication (3 issues per year) has been published from Vol. 17, No.1, 2005 to date.
5. Within 30 years, the cover design of the journal has been changed for several times as follows:
    (i) DMR Logo was appeared at the bottom of the front cover from Volume. 1, No. 1 to Vol. 5, No. 2, 1993 (Fig. 1). Then, there was no logo shown on the cover from Vol. 5. No. 3, 1993 to Volume 24, No. 3, 2012 (Fig. 4).
    (ii) As the 50th Anniversary Golden Jubilee Publication (Vol. 25, No. 1, 2013), the cover with 2 logos (DMR & Golden Jubilee), and tables & figures in colorful illustration in the text were shown. Moreover, the whole book was printed in high

quality Art paper, and the journal size was changed into smaller one with the size of 18.6 cm x 25.5 cm (Fig. 5).

(iii) The DMR logo was shown in the middle of the front cover in Vol. 26, No. 1, 2014 (Fig. 6).

(iv) In Vol. 27, No. 1, April 2015, the new DMR logo with Ministry of Health was replaced at the previous one (Fig. 7)

(v) The current logo of "Ministry of Health and Sports" and DMR was again changed in Vol. 28, No. 2, 2016 and it is still used to date (Fig. 8)

6. Since the 37th the Myanmar Health Research Congress (MHRC) in 2009, the respective authors who presented at the Paper Reading Session and displayed at Poster Session were asked to their consents for publications in the MHSRJ. By taking this opportunity, more numbers of articles have been received.

7. "Aim of the Journal" and "Editorial" were firstly appeared in the Vol. 24, No. 2, 2012.

8. The MHSRJ's Official Publisher Registration No. 01263 was received issued by the Printing and Publishing Department, Ministry of Information on 8th April 2015.

9. The beautiful painting picture which is regarded as the distinct symbol of DMR was illustrated at the back cover in the Vol. 26, No. 2, 2014 (Fig. 9).

10. "Acknowledgement of Reviewers" was firstly described in Vol. 28, No. 3, 2016.

11. Website addresses of DMR have been mentioned beginning from Vol. 29, No. 2 August 2017.

12. The website of the journal (***myanmarhsrj.com)*** was launched at the Opening Ceremony of 44th Myanmar Health Research Congress on 5th January, 2016 as a part of upgrading the journal to meet the international standard and to encourage the readers: medical students, researchers and scientists to use the articles appeared in the journal as citation by accessing full text of all articles. This was a great improvement and remarkable one in the status of the MHSRJ so that Myanmar medical researchers have an easy access to submit their manuscripts to MHSRJ around the clock, using Online submission at its website.

13. In 2017, to keep abreast of the current trend of International journal publication, there were noticeably some updates for the journal:

(i) The strength of Editorial Board members was increased and upgraded consisting of 22 local scientists as well as 6 internationally well-known scientists: Prof. Shigeru Okada (Japan), Dr. Sun Dae Song (Korea), Prof. Christopher V. Plowe (USA), Prof. Anthony David Harries (UK), Prof. David Alan Warrell (UK), and Prof. Sir Roy Anderson (UK) as International Editorial Board members.

(ii) The design of journal was changed with addition of "General Information" which is transformed from "Notice to Contributors" which describes full facts about the journal in line with international standards. Some guidelines for the submitted manuscript were changed: introducing double blind peer-reviewed, requesting

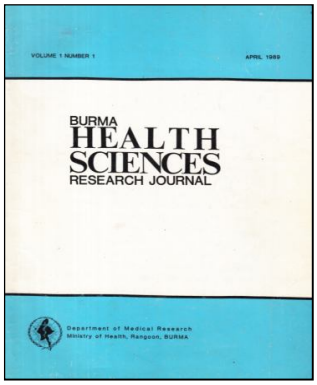


Fig. 1

Fig. 2

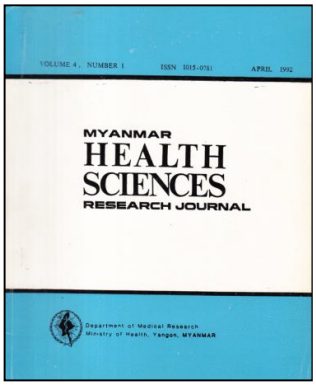
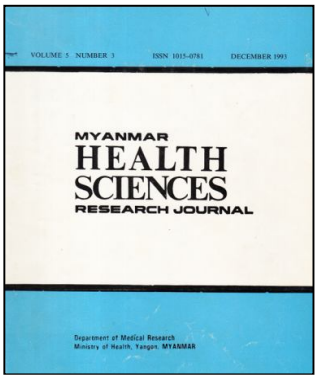


Fig. 3



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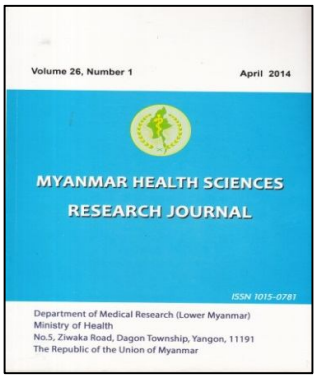

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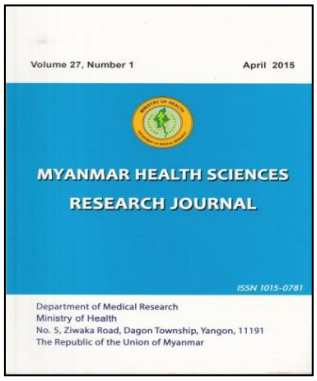

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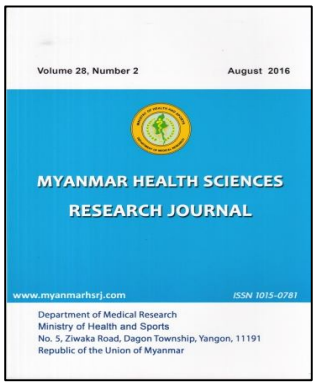

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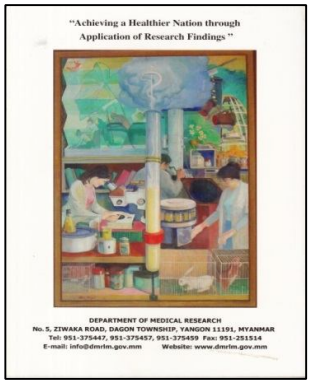

Fig. 9

Different Designs of Journal Covers through Thirty Years

ethical consideration in the manuscript, allowing up to 350 words in the abstract, providing the writing format of Conference, Thesis & Internet source in Reference section.

(iii) In June, 2017, the Online *Plagiarism Checker,* "Turnitin Software" was introduced for checking each article before peer-reviewed process, allowing maximum 30% of plagiarism.

(iv) For the very first time, two articles of international authors; from Japan and Nepal have appeared in Vol. 29, No. 2, 2017. Accordingly, in honour of international authors, the manuscript entitled "Monitoring of Water Quality in Inle Lake, Myanmar" written by Japanese scientist has been selected as a leading article in this issue.

(v) Recently, Myanmar Health Sciences Research Journal was remarkably recognized as a qualified journal for inclusion in ASEAN Citation Index (ACI) database by the ACI Steering Committee held in Malaysia on 14th November, 2017 (website: http://asean-cites.org). The ACI, a central regional database was designed and set-up to index all the bibliographic records and the citations of all quality ASEAN research outputs appearing in the ASEAN scholarly journals. To date, the MHSRJ is the one and only journal included in ACI database among other scientific journals published in Myanmar. This great achievement is an important step towards the linkage to international databases.

14. During the 46th MHRC (2018), the Annual Editorial Committee meeting was held as the distinct one among its meetings due to the attending of two International Editorial Board Members [Prof. Okada (Japan) & Dr San Dae Sung (Korea)]. During the meeting, some important decisions were made: To advertise MHSRJ more to get international awareness by taking the advantage of "Free of Charge" for journal publication; to encourage researchers from DMR as well as professors and postgraduate students of Universities for citation to MHSRJ so as to get the increased impact factor of the journal; to make plans for the peer-reviewed fees as an incentive; to encourage the prize winners of the Myanmar Health Research Congress for publishing their awarded papers at either MHSRJ/ locals or Internationals within a year, invariably; to give the credit for the publication in order to get further promotion of the researcher.

15. Starting from Vol. 30, No.1 April 2018, the printed number of the journal is increased from 500 to 700 copies per issue in order to distribute more to medical universities. Under the guidance of the Editorial Committee, the mailing list of the Journal is updated yearly as necessary. It is distributed to health departments, universities, and hospitals under the Ministry of Health and Sports and sent to some international organizations and universities including WHO, UNDP, etc.

In fact, Myanmar Health Sciences Research Journal published by Department of Medical Research truly represents the Ministry of Health & Sports. In addition, MHSRJ stands for not only the local reference resource but also the valuable treasury one related with medical research in Myanmar.

In conclusion, eight hundred and eighty-three research articles with various research disciplines have already been published in MHSRJ (up to Vol. 30, No. 1, April, 2018) by sharing an advanced knowledge of health research information to the public in accordance with the DMR's vision of achieving a healthier nation through application of research findings. The target of the MHSRJ Editorial Committee is to include our journal into the well-known international database, PubMed Central with capable and utmost effort in handing over to next generation.

[References are available in Myanmar Health Sciences Research Journal, 1989-2018]

30th ANNIVERSARY COLLECTION

Myanmar Health Sciences Research Journal, Vol. 5, No. 1, 1993, pp 1-5

Are we spraying DDT at an appropriate period of time to control malaria in Myanmar?

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DDT spraying activity has been carried out to control malaria in Myanmar for many years. Appropriateness of spray timing is being evaluated by applying moving average method on slide positivity rates in five states and four divisions of the country. It was found that, in order to cover major transmission peaks in many areas, spray timing should be adjusted.

INTRODUCTION

Malaria has been identified as the most important public health problem in Myanmar. It has been accorded first priority disease in Third PHP (People's Health Program) (1986-1990) and first National Health Plan (NHP) (1991-1992). To achieve systematic implementation of malaria control operations, the country has been divided into five distinct strata taking into consideration a variety of determining factors for the occurrence of malaria [1]. Residual spraying is one of the major activities performed in stratum II (areas under control by spraying). These areas have been sprayed for many years and it is high time that spraying activities be reviewed in-depth in the context of correct spraying technique, logistics of spraying, timing of spraying and susceptibility status of insecticide used, etc. There has been little change in the spraying time for more than two decades. This study is an attempt to elucidate whether the current spraying time is appropriate or not in the context of present epidemiological conditions. The magnitude and importance of spraying activity in the country can be envisaged by observing the fact that tons of DDT have been used yearly by the Vector Borne Diseases Control program [2].

The objectives of the study are

(a) To elucidate the transmission season of malaria in different states and division.

(b) To time the spraying activity in accord with the transmission period in respective states and divisions.

MATERIALS AND METHODS

It is a retrospective analysis of secondary data available at the Vector Borne Diseases Control Program, Headquarters, Yangon.

Study Area

States/Divisions which have complete sets of data on monthly slide positivity rates for at least 5 years (1984 to 1988) were taken [1]. A variety of different topographical areas (hilly, plain, coastal, forested) are included in the selected states and divisions. It was decided to analyze state and division- wise because factors governing the vector bionomics will be

somewhat dissimilar in different states and division. In other words, generalization for an area could not be made if overall seasonal variation of the country is examined in toto.

Study Variable

Slide positivity rates from sprayed areas have been chosen as the basic variable. The rationale for using it to determine transmission season is that the ratio of Plasmodium falciparum to Plasmodium vivax in different states and divisions from slide positive cases is in the region of 85:15 (1984-1987) [2]. There might be recrudescence of falciparum malaria but the numbers are few and far between. The distribution of blood slides examined covers all age groups and generally follows the population structure [1]. There is also no disproportionate examination of blood slides among various age groups [1]. Further elaboration of the data in terms of strata shows that there is no aggregation of falciparum malaria in one particular stratum or areas, instead it is distributed somewhat evenly [1]. These facts further justify the use of slide positivity rate.

Study Methods

Transmission season can be determined by parasitological, entomological and epidemiological methods. The parasitological methods are more sensitive than entomological methods. Infant parasite rate is one of the sensitive indicators to determine transmission season e.g., longitudinal monthly infant parasite surveys starting with the new born infants. Use of morbidity statistics alone can be misleading because relapse of vivax malaria may occur when transmission is absent. When morbidity statistics are useds, it is to be cautioned that seasonal rise starts 2 to 4 weeks after the first transmission has occurred. As in most of the infectious diseases, malaria exhibits temporal variation which is characterized by seasonal variations, cyclical variations, secular trends and random variations [3]. Seasonal variations can be defined as changes in intensity or endemicity of malaria in a particular area at different times of the year. The fluctuation of a parameter area at different times of the year. The fluctuation of a parameter value (e.g., slide positivity rate) is contributed by the above four factors. In order to depict seasonal variation, the other three factors should be suppressed.

Classical decomposition analysis usually assumes a multiplicative relationship between the four components of a time series. Time series data, which are statistical data collected or observed at successive intervals of time, were used to depict the transmission season of malaria. Thus, a trend component is denoted by T, the cyclical component by C, the seasonal component by S and the irregular or random component by I. Each observation V of the time series can be expressed as $V_t=T_t * C_t * S_t * I_t$ at time period t.

If all variations in a time series were due entirely to seasonal influences, the specific seasonal for a given month is an index number where base is a mean monthly variate. The ratio-to-twelve-month moving average method relies on a monthly time series being affected by seasonal variations which repeat regularly year after year. The malaria data pattern can be correctly assumed as a seasonal time series data and thus computation of seasonal index is justified. Two reasons support the seasonal nature of malaria data.

(a) The peaks and troughs are consistent over the years.

(b) There is an explanation for the seasonal pattern.

Computation of Seasonal Index [3, 4]

Seasonal Index is defined as the ratio of the actual value of the time series to the average for the year. There is a unique index for each period of the year. As the data are monthly data, it has 12 seasonal indices. A seasonal index of 126% in October is interpreted to mean that the number of cases (or parasite positive cases) in October is 126% of the number of cases in the average month. Similarly, an index of 65% in February is interpreted to mean that the number of cases in February is 65% of those in an average month. The following steps have been performed in order to obtain seasonal indices (Example computation is shown in Table 3).

(a) Computation of Centered 12 Month Moving Average

Computation of moving averages is based on the length of seasonality. As the seasonal index is aimed to get the monthly pattern of malaria transmission, 12 month moving totals were computed.

(b) Computation of Approximate Seasonal Index

The ratio of each month actual value to its centered 12 month moving average was computed. This is to eliminate or reduce the secular trend and cyclical variation from the data. Thus, it represents the seasonal or random component of the time series. These ratios are approximate seasonal indices.

(c) Refinement

The first refinement, in order to eliminate as much randomness as possible, was to compute the mean ratio for each month. All the ratios for a particular month were added and divided by the number of months involved.

(d) Computation of Normalizing Factor

Normalization factor was then computed using the formula, Normalizing Factor = 12/ (sum of mean ratios). This was done to adjust the mean ratios so that they summed to 12.

(e) Computation of Final Seasonal Indices (SI)

Ultimately, seasonal indices were calculated for each month by multiplying the mean ratio of each month by the normalization factor.

RESULTS AND DISCUSSIONS

Seasonal Indices for States

The following seasonal indices, based on slide positivity rates, were obtained for five states.

Table 1. Seasonal indices for five States

Month	Mon	Kayin	Kayah	Chin	Shan
Jan	*1.031	*1.199	0.826	0.954	0.671
Feb	0.937	0.951	0.464	1.081	0.637
Mar	0.908	0.973	0.464	0.831	0.744
Apr	0.812	1.122	0.671	0.885	0.642
May	0.751	0.939	1.034	*1.023	0.741
Jun	0.809	0.896	1.122	*1.216	0.974
Jul	0.950	0.864	1.105	0.982	*1.171
Aug	0.979	0.855	*1.154	0.847	*1.208
Sep	0.997	0.907	*1.195	*1.030	*1.121
Oct	*1.108	0.890	*1.241	*1.057	*1.512
Nov	*1.440	*1.283	*1.517	*1.126	*1.121
Dec	*1.341	*1.567	*1.449	0.898	*1.369

*Presumed transmission season

State-wise Analysis on Seasonal Indices

Depending on the topography and vector prevalence of an area, transmission season will vary. The seasonal indices in Mon State appear to increase starting from the latter part of the year. This is also

supported by sporozoite dissection records in which highest positive rates were found in the latter part of the year (vector: An. dirus). The transmission season seems to be in the last quarter of the year. In Kayin State, two transmission peaks are observed, minor peak in April and major peak in November and December. The seasonal indices in Kayah State start to increase in June and reach its peak in November and December where 152% and 145% of the normal occurrences are respectively observed. Malaria transmission appears to be intense during the latter half of the year. The transmission season of malaria for this state is prolonged and covers almost three quarters of the year. In Chin State there are two peaks i.e., June and November. The transmission season appears to be shorter in this state compared to other states (vectors: An. minimus and An. maculates). In Shan State, The transmission season seems to be in the latter half of the year (vector: An. minius). The first half of the year has the lowest transmission potential.

Seasonal Indices for Divisions

The following seasonal indices, based on slide positivity rates, were obtained for four divisions.

Division-wise Analysis on Seasonal Indices

In Ayeyawady Division, there are two peaks, one in April and the other one in November and December. Major transmission season appears to be in the last quarter of the year. In Mandalay Division there is only one transmission peak and spans a duration of five months. Yangon Division exhibits two peaks, minor one in April to June and the major one in November and December. Similar observations are noted in Bago Division. As per the data elucidated in the tables mentioned aboved, it appears that some states and divisions have two transmission peaks.

Table 2. Seasonal Indices for Four Divisions

Month	Ayeyarwady	Mandalay	Yangon	Bago
Jan	0.989	0.504	*1.086	0.768
Feb	0.830	0.481	1.076	0.880
Mar	0.933	0.576	1.048	1.002
Apr	*1.021	0.611	*1.090	*1.131
May	*0.991	0.633	*1.085	*1.031
Jun	0.951	0.957	*1.127	*1.235
Jul	0.797	1.016	1.086	0.905
Aug	0.839	*1.177	0.329	0.808
Sep	0.848	*1.315	0.276	1.026
Oct	0.928	*1.432	0.442	*1.215
Nov	*1.297	*1.608	*1.090	*1.258
Dec	*1.621	*1.602	*1.748	*1.047

*Presumed transmission season

Seasonal Pattern of Malaria in Myanmar

One of the factors which affects transmission season is ecological changes brought about by develop-mental activities which in turn lead to changes in vector distribution and vector bionomics especially longevity and biting rates. These changes together with various anti-malarial measures carried out over the years have significant impact on the transmission pattern of malaria. In the early eighties, transmission season of malaria usually coincides with the pre and post monsoon seasons. In some areas of Rakhine State, transmission is observed throughout the year. Practically speaking, transmission of malaria occurs in most areas throughout the year. But there is at least one major transmission peak in every state and division. At present, the density of primary vectors of malaria in Myanmar (An. minimus & An. dirus) is closely associated with seasonal pattern of malaria and hence transmission season of malaria.

Table 3. Example computation of seasonal indices for Mon State

Slide positivity rates for Mon State from 1984 to 1988 are follows:

Slide positivity rates % for Mon State, 1984-1988

Month	1984	1985	1986	1987	1988
Jan	7.92	8.79	9.76	9.70	9.08
Feb	4.62	7.27	6.28	12.20	8.30
Mar	7.52	6.96	4.95	10.20	11.20
Apr	8.27	5.98	4.18	8.75	9.37
May	7.28	5.13	5.05	7.99	9.37
Jun	8.67	7.40	5.00	7.50	9.77
Jul	9.02	9.76	5.83	9.05	9.53
Aug	10.40	10.10	5.68	8.86	8.35
Sep	9.61	11.50	6.50	8.19	8.75
Oct	8.65	12.70	8.89	9.44	13.40
Nov	16.50	14.90	10.20	10.60	14.20
Dec	15.20	11.40	10.60	11.50	13.70

12 month moving totals of SPR for Mon State

Month	1984	1985	1986	1987	1988
Jan		111.65	101.65	107.26	117.04
Feb		111.35	97.23	110.44	116.53
Mar		113.24	92.23	112.13	117.09
Apr		117.29	88.42	112.68	121.05
May		115.69	83.72	113.08	124.65
Jun	@113.66	111.89	82.92	113.98	126.85
Jul	*114.53	112.86	82.86	113.36	
Aug	117.18	111.87	88.78	109.46	
Sep	116.62	109.86	94.03	110.46	
Oct	114.33	108.06	98.60	112.91	
Nov	112.18	107.98	101.54	114.29	
Dec	110.91	105.58	104.04	116.56	

First 12 month moving total for 1984= 1984 Jan SPR +---+1984 Dec SPR =(7.92+----+15.2) = @113.66

Second 12 month moving total for 1984= 1984 Feb SPR +--+ 1985 Jan SPR =(4.62 +----+ 8.79) = *114.53

Two twelve month moving total=113.66 + 114.53= 228.19

Approximate seasonal indices (SI) for Mon State.

Month	1984	1985	1986	1987	1988
Jan		0.947	1.130	1.101	0.932
Feb		0.782	0.757	1.344	0.852
Mar		0.743	0.627	1.099	1.150
Apr		0.622	0.555	0.934	1.128
May		0.528	0.704	0.849	0.915
Jun		0.780	0.720	0.792	0.932
Jul	0.948	1.042	0.844	0.955	
Aug	1.077	1.078	0.794	0.954	
Sep	0.986	1.244	0.853	0.893	
Oct	0.898	1.398	1.107	1.014	
Nov	1.748	1.655	1.223	1.119	
Dec	1.635	1.281	1.237	1.195	

Computation

12 month centered MA

$$= \frac{\sum \text{first two 12 month moving totals}}{24} \text{(for July)}$$

= (228.19)/24

= 9.5079

Approximate SI (1984) July

$$= \frac{\text{July 1984 SPR}}{\text{(12 month centered MA for July)}}$$

= 9.02/9.5079

= 0.948

LEGEND. MA = Moving average

Mean Ratios (MR)

Mean ratios = $\sum$ (Ratios for the respective month) /# of months

MR for July = (0.948 + 1.042 + 0.844 + 0.955) / 4 = 0.947@

MR for Aug = (1.077 + 1.078 + 0.794 + 0.954) /4 = 0.976*

MR for Sep = (0.986 + 1.244 + 0.853 + 0.893) / 4 = 0.944**

Normalization Factor (NF)

NF = 12/(sum of mean ratios)

NF = 12/(1.028+0.934+0.905+-----+1.337)= .99742

Final SI = Mean ratio for the month * NF

Final SI for July = Mean ration for July * NF

= 0.947 * 0.99742

= 0.95003

Mean ratios, normalizing factor and seasonal indices for Mon State.

Month	Mean Ratio	Norm. Factor	SI
Jan	1.028	0.99742	1.03087
Feb	0. 934	0.99742	0.93694
Mar	0.905	0.99742	0.90766
Apr	0.810	0.99742	0.81228
May	0.749	0.99742	0.75124
Jun	0.806	0.99742	0.80848
Jul	0.947@	0.99742	0.95003
Aug	0.976**	0.99742	0.99716
Sep	0.994*	0.99742	0. 99716
Oct	1.104	0.99742	1.10772
Nov	1.436	0.99742	1.44031
Dec	1.337	0.99742	1.34080

Discussion in the Context of Spraying

In most of the states/divisions under study, major transmission peak seems to be in the last quarter of the year. It may be due to specific and increasing problem of mobile population engaged in forest-related economy and gem mining. These people acquired malaria, transmitted by An. dirus and return to their respective townships whereby malaria is spread by either An. minimus or local vectors such as An. annularis, An. maculates or An. sundaicus, etc. December enjoys the highest seasonal indices in most of the cases. Spraying activity was performed in all these areas from February to May. The main reason for conducting spraying activity during this period is because of favourable meterological conditions and partly it might be due to the then transmission season (Latter part of the seventies) when spraying activity was initiated. As the potency of DDT is wearing away during the latter part of its action span, it would result in more beneficial outcome if spraying could be performed during September and October i.e., it can completely cover the major transmission peak. Meterological factors must be taken into account in some areas from logistical point of view. The transmission and accessibility status of an area are also relatively good during this period. The transmission season determined by this method could be regarded as relatively crude in relation to immunological and vector studies. But in the context of the country's situation, it can be assumed as the most practicable approach to give insight into the appropriateness of spraying time. The seasonal index computed above is for the whole state/division. There might be some variation if it is determined township-wise.

Actually, an increase in slide positivity may not necessarily connote transmission season unless the slides are examined immediately after collection. Generally, the slides are examined one to two weeks after collection because these slides have to be sent to places where laboratory facilities and microscopists are available. But the positive results are registered according to the date collection rather than the date of examination. Be that as it may, interpretation of this parameter should always be guarded because there are multitude of factors [5] which can modify the results of blood slide examination.

The education of transmission season is not only for determinatation of spraying time but also for carrying out anti-malarias measures such as active surveillance, case treatment before this period. These can have dramatic influence on the size of case reservoir leading to reduction in transmission potential. One of the advantages of this method is that computation is relatively simple and can be easily taught to the peripheral staff so that s/he can monitor the situation well ahead of the crisis. In doing so, the staff will also appreciate the importance of data and how useful information can be derived from the data at hand. This can enhance their positive attitude toward timely and systematic collection of data. A growing body of experiences has shown that, once the health worker appreciates the usefulness of the data that they are collecting, it would pose no problem as to the acquisition of "quality" data. In coming to grips with the practical issues, a simple line graph depicting the

seasonal indices can greatly help in the conduct of anti-malaria activities besides preparing in advance the expected increases in malaria situation in an area. One can also know whether the situation is actual increase or just seasonal variation of malaria in an area so that necessary activities can be performed. To be more effective, an area should be sprayed every 6 months as per the seasonal indices mentioned in tables (1) & (2). But in the context of the developing country's scenario, it is far from possible.

In conclusion, ideally, in keeping with the precepts of spraying, if one could spray before the major malaria transmission peak, it would exert more beneficial effect on the containment of malaria situation. The importance of spraying time could not be over emphasized. DDT spraying should be planned on the basis of ecological and epidemiological stratification. The problem of vector resistance, reduced biological efficacy of the sprayed surfaces, reduced compliance by the community of household spraying, etc. should be considered in spray planning. In essence, the above findings clearly indicate that time of spraying should be adjusted according to the transmission season of the area concerned. Assuming that the logistics of spraying is going on smoothly and proper training has been given to the spray men, the only thing that matters most is time of spraying. If one can spray DDT at an appropriate time, one is bound to meet with success. It can therefore be stated that current time of spraying is inappropriate given the fact that transmission peaks are in the fourth quarters of every year in almost all the areas under study. In line with the current concept of malaria control, it is not justified to use major proportion of malaria budget for spraying. Impregnated (pyrethroid) bed nets and curtains are better alternative to DDT spraying. It is needless to emphasize that integrated vector control methods should be practiced wherever feasible. Be that as it may, township-wise in-depth analysis, including vector bionomics, is strongly recommended before radically switching the spraying time in respective areas.

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Syringes and needles disposal practices by House Surgeons from major hospitals in Yangon, Myanmar

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Needle stick injury (NSI) is regarded as an important cause of the transmission of blood-borne viruses to health care staff. There is lack of information on the occurrence of needle stick injury and needle disposal services among House Surgeons (HS) in Myanmar. With the aim of promoting measures for preventing NSIs, a hospital-based cross-sectional descriptive study was carried out to determine incidence of needle stick injury among HSs and to investigate their practice regarding injection instrument waste disposal. Two hundred and ten responding HSs at all medical wards of Yangon General Hospital, North Okkalapa General Hospital and Sanpya General Hospital, paediatric medical wards of Yangon Children Hospital and Sanpya General Hospital were investigated by self-administered questionnaire. Of them, 206 (98%) recapped the needle after giving injections, of which, 75 (36%) handled the cap during recapping process. Among 206 subjects who practiced recapping, 60 (29%) disclosed that they had experienced injury during recapping. A slightly higher rate of injury was observed among subjects who handled the cap during the recapping process as compared to those who recapped the needle without handling the cap (34% vs 26%). The majority 162 subjects (78%) separated the needle before discarding the syringe. Among those who separated needles 6.2% experienced injury during separation. The most commonly used container for discarding needle and syringes was plastic drinking water bottle (71%) followed by WHO Card Box (18%). However, only 28% of the respondents said the containers were within arm's reach and 72% of them stated that they had to walk to reach the container. Nearly 55% of all perceived that they are safe with the current practicing needle and syringe disposal system. This study showed that HSs are at risk of needle stick injury and blood-borne infections during their clinical activities while performing procedures on patients. Efforts need to be made to ensure greater awareness amongst House Surgeons about the risk of mucocutaneous and percutaneous injuries.

INTRODUCTION

In the midst of infections, disposing of waste properly is an often-overlooked but important aspect of infection prevention. The unsafe use and disposal of injection equipment continues to put patients, health-care workers, and the general community at risk of infections such as hepatitis B or C virus and human immunodeficiency virus. Needle stick injury (NSI) is regarded as an important cause of the transmission of such blood-borne viruses to health care staff. In Taiwan where there is a high incidence of

hepatitis infections, it has been estimated that approximately 1,000 healthcare workers out of 100,000 suffer seroconversion with hepatitis B virus and hepatitis C virus through needle sticks with hollow bore needles annually [1]. Most importantly, it was reported that in countries with a high prevalence of HIV infection, one young doctor would become infected with HIV every seven or eight years [2]. The problem of NSI is considered as an important issue even in countries with low prevalence of blood borne infections. In France, it has been estimated that one surgeon in 14 might expect to be hepatitis C contaminated during his or her career, and one in 630 might be infected with HIV [3].

Among health workers at risk, medical students and interns are those with the highest risk. Nearly a third of medical students had reported sharps injury over their clinical training of which one third of those were associated with hollow bore needles [4]. In USA, based on a survey of 3,239 participants, emergency medicine residents in their first four years of training have been reported to suffer from a high rate of exposure to blood. Residents had been exposed very often, with 56% having at least one exposure, and one in 10 having four or more exposures [5]. Medical students are at risk of acquiring infections caused by NSIs, although it is unknown when NSIs are most likely to occur during medical training. Over one third (55/157) of respondents suffered at least one needle stick injury [6]. It was also reported that life-time prevalence of NSIs was 23%, ranging from 12% in first year students to 41% in fourth year students. These accidents happened most commonly during medical internships, especially during blood-taking practices; an activity that usually starts during the third year of training [7]. Among the undergraduate students in Australia, 13.8% experienced the injury during their third year training period [8].

Although several batches of medical students have completed their years as House Surgeons at different hospitals in Myanmar, there is lack of information on the occurrence of needle stick injury and needle disposal services. The present study was conducted to explore the retrospective prevalence of needle stick injury among house surgeons and to investigate their practice regarding injection instrument waste disposal with the aim of promoting measures for preventing NSIs among the hospital medical practitioners.

MATERIALS AND METHODS

A hospital-based cross-sectional descriptive study was carried out. The frequency of injections, disposal practice of the needles and number of accidental needle stick injuries of the house surgeons at all medical wards of Yangon General Hospital, medical wards of North Okkalapa General Hospital and Sanpya General Hospital, paediatric medical wards of Yangon Children Hospital and Sanpya General Hospital were explored by self-administered questionnaire. Questionnaire included needle recapping practice, needle removal practices, and disposal practices. A total of 210 house surgeons from the four study hospitals were recruited to answer the questions. Filled questionnaires were checked by the assigned research medical officer immediately. Data recoding and entry were carried out using EpiData software. Analysis was done using SPSS version 11.5 after the data file was transferred into SPSS format. Univariate and bivariate tests were carried out to describe the occurrences and to determine differences. Differences were considered significant if $P < 0.05$.

RESULTS

Response rates

The participating house surgeons (later refer as subjects) were very cooperative and high response rates (more than 95%) were obtained for questions on needle recapping, injury obtained during recapping and

separation of needle and syringe before discarding, needle discarding practices, and disposal of containers.

Needle recapping practices and experiences of injury during recapping

Among the 210 responding subjects, 206 (98%) recapped the needle after giving injections. Of those who recapped the needle, 75 (36%) handled the cap during recapping process. The remaining 131 subjects recapped the needle without handling the cap. Among 206 subjects who practiced recapping, 60 (29%) disclosed that they experienced injury during recapping. A slightly higher rate of injury was observed among subjects who handled the cap during the recapping process as compared to those who recapped the needle without handling the cap (34% *vs.* 26%) (Table1).

Table 1. Occurrence of injury among subjects who recapped in different ways

How recap?	Injury during recapping		Total (%)
	Yes (%)	No (%)	
Handling the cap	25 (33.8)	49 (66.2)	74 (100)*
Without handling the cap	34 (26.0)	97 (74.0)	131 (100)
Total	59 (28.8)	146 (71.2)	205 100)

Chi square=1.414, p=0.234*One HS did not respond.

Table 2. Method of needle separation and experience of injury among needle separators

How separate?	Injury during separating		Total (%)
	Yes (%)	No (%)	
Two hands	9 (5.9)	143 (94.1)	152 (100)
One hand	1(10.0)	9 (90.0)	10 (100)
Total	10 (6.2)	152 (93.8)	162 (100)

Needle separation practices and experiences of injury during separation

The majority 162 subjects (78%) separated the needle before discarding the syringe. The remaining 50 subjects did not separate the needle from the syringe before discarding. Of those who separated the needle from the syringe almost all 152 subjects (94%) used both hands, whereas a few 10 (6%) used only a single hand. A small percentage (6.2%) of needle separators experienced injury during separation. Among them, 10% of those who used one hand for separation the needle from syringe had a slightly higher experience of injury during separation (10% *vs* 6%) than those who used the two hands method (Table 2).

Discarding practices

A variety of containers were listed. The most commonly used container for discarding needle and syringes was plastic drinking water bottle (71%) followed by WHO Card Box (18%). Other containers included storage bin (8%), tin can (2%) and plastic bags (1%) (Fig.1). Regarding the procedure for waste disposal, all participants stated that discarding the needle and syringe into the containers was unproblematic. However, only 59 (28%) of the respondents said the containers were within arm's reach and 150 (72%) of them stated that they had to walk to reach the container.

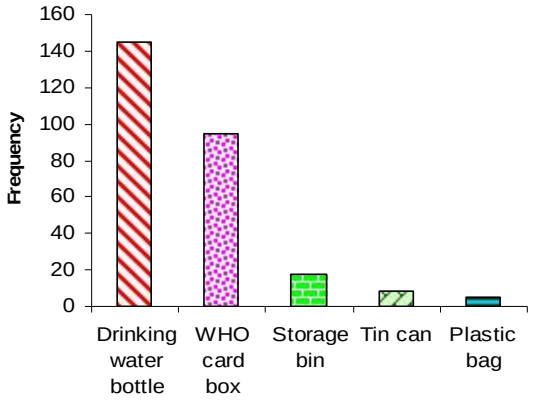

Fig 1. Types of discarding containers used by the subjects

Injury during discarding

Although it is simple to conduct, 20 % of subjects experienced that needle and/or syringes escaped from the container at the time of discarding. A very small percentage (1%) reported injury during discarding.

Practices of disposing the container

It was found that over 90% of the subjects noted that the containers were immediately emptied after being filled. It was done mainly by hospital workers (96%), although

some nurses (3%), attendants (1%) also carried out the process. Chance of injury during disposing the container was very few as only 1 % of respondents noted injury during the process.

Incidence of injury

Out of the total 211 respondents, 66 (31.3%) expressed that they had injury at least once during their HS period. The frequency of injuries was 74 amounting to 0.35 injuries per HS. Most frequent action for getting needle stick injury was during recapping (75% of all incidence injuries) (Table 3).

Table 3. Injury at various steps of disposing

Steps of disposing	N	Frequency	Percent
Injury during recapping	206	60	29.1
Injury during separating	162	10	6.2
Injury during discarding	209	2	1
Injury during emptying	209	2	1

Perceived safety on the current situation

Among the study population, nearly 55% perceived that they are safe with the current practicing needle and syringe disposal system. The remaining 45% perceived that they are less safe.

DISCUSSION

The very high response rate (more than 95%) of the subjects is very encouraging and outlines the interest of the subjects. This response rate is much higher than those obtained by investigators from United States on their medical students (77%) [4], emergency medicine residents (90%) [5]. It has been reported that only 60% of third and fourth year medical students and medical and surgical house staff replied to an anonymous questionnaire on needle stick injury [9].

The present study demonstrates that needle stick injury among the House Surgeons is not rare. At least a third of them had an episode of NSI during their House Surgeon training period. This finding is similar to that of the risk of needle stick injury among American medical students where about a third of medical students had a sharp injury over their clinical training [4]. It has been reported that most house staff would have at least one NSI a year and NSIs most often involved disposable needles (85%), most often occurring during phlebotomy (62%), and most often when recapping a needle (54%) [10]. It has been reported that in Singapore, house officers experienced an average of 1.4 sharp and needle stick injuries per month [11].

In our study, 75% of all injuries were due to recapping the needle after injection. Both one-hand and two-hand users experienced injury in our study. The findings from our study are consistent with a study reported from Canada where 45% of needle stick injuries occurred at recapping. In that study, the results also showed between 46% and 77% of needles were being recapped and 9% to 20% of recapped needles were blood-stained [12]. Recapping devices were rarely used and two-handed recapping techniques predominated. Common reasons for recapping include inability to dispose immediately of needles properly, and sharps containers being too far away [12]. It has also been reported that recapping accounted for a higher percentage of NSI than any other activity [13]. Based on a study on medical students in India, it was found that re-sheathing or recapping the needle was responsible for causing NSI to 69% of the students [14]. Although we failed to ask the reason for recapping, more than 90% were recapping and majority of subjects (71.4%) responded that the container for discarding was placed needing to walk to reach. Only one-fourth of the subjects said the container was at within their arm's reach. This might be the reason for recapping among the subjects after practicing injection.

We failed to inquire whether the HSs reported their injury and if so any medical or psychological assistance have been

provided. They may not have reported the injury as they felt that it is not serious enough. It has also been reported that only 43% of students who were injured reported the injury, mostly because they felt it was not serious enough to constitute a serious exposure [15]. The present study focused only on NSI and did not account for other type of injuries such as those involving suture needles. In certain Institutions, injuries caused by hollow bore needles accounted for only 17% of injuries whereas surgery accounted for 70% of injuries. However, hollow bore needles could be blood filled and are usually deemed high risk [4]. Only a few incidence of injury were found during disposing and discarding container since it was not the responsibility of the house surgeons. Our study also did not take account the incidence among other staff at hospitals. .

Measures to reduce NSI have been reported by various authors. It has been reported that the introduction of a comprehensive programme to reduce NSIs led to a reduction of more than 60% over four years in a US hospital [16]. A significant, prolonged fall in needle stick injuries was demonstrated following the introduction of more, and more convenient, sharps containers at the hospital in California. More needle disposal containers were added to patient care areas and as close to the area of use as possible [15]. A similar hospital wide comprehensive multi-focused programme reduced sharps injuries by 69%. The intervention consisted of the introduction of needless systems for intravenous therapy and a new sharps disposal system. The disposal system consisted of new, wide-mouthed containers, together with a new system of changing the containers on a regular basis, and before they were full. Factors related to sharps injuries in the period before and the latest after the intervention [17]. Alternate methods for preventing needle sticks have been proposed [18]. Such measures should be outlined and adapted to local situation for use in our hospitals.

Conclusion

This study showed that House Surgeons are at risk of needle stick injury and blood - borne infections during their clinical activities while performing procedures on patients. Efforts need to be made to ensure greater awareness amongst House Surgeons about the risk of mucocutaneous and percutaneous injuries. Proper training in percutaneous procedures and how to act in case of injury should be made to reduce the number of injuries. The present study also highlights the fact that compliance with the non-recapping needle policy is poor. More education and awareness programme (lectures, videotapes, handouts); discouragement of recapping and innovative arrangements for sharps disposal should be promoted. It should be evaluated whether vaccination against hepatitis B should be offered to students before entering the clinical part of the study. It could be further stated that medical students also have a high risk for needle stick injuries, and attention should be directed to protection strategies against blood borne pathogens. Not only prevention of accidents but also post-exposure management should be frequently reiterated to the medical students at every level. Any medical practice of students should be under supervisor control.

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Study of the effect of single dose primaquine on gametocytaemia and infectivity among Amodiaquine-treated *P.* falciparum malaria patients

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One hundred and five patients who attended Thayarwady Civil Hospital during 1987 and 1988 malaria seasons were studied. They were divided into three grops. Among 35 patients who were treated with amodiaquine alone, 23-33% of the patients showed gametocytes in blood during days 14, 21 and 28 of therapy. Among those patients with gametocytes in blood, 50 to 63% of patients showed that they were viable by exflagellation method. Follow-up of one patient showed viable gametocytes in blood till 51st day of drug therapy. Among 45 patients treated with amodiaquine and primaquine (45 mg single dose given on day 3), 31% of patients showed gametocytes on day 7, later only 0-10% of patients showed gametocytes on day between 14, 21 and 28. Among the patients with gametocytes in blood 25% of the patients showed that they were viable till day 4. From day 5 they were not viable. Except one patient who showed gametocytes at day 28 was proved to be Rll resistant to amodiaquine. Among 25 patients treated with amodiaquine and primaquine (45 mg single does : given on day 1 of hospital admission) 36% showed gametocytes on day 7 and 42% showed gametocytes on day 28. Of these 26% patients with gametocytes which appeared on day 28 was proved to be viable. It is suggested that primaquine 45 mg is effective to control infectivity of gametocytes in blood. It is also suggested that primaquine should be given on 3rd day of hospital admission to get the most beneficial effect.

INTRODUCTION

To have a significant impact on the transmission of falciparum malaria, Pan American Health Oragnization in 1982 (1) made the recommendation that Primaquine should always be administered whenever a schizontocidal durg is used.

As a part of an effort to interrupt transmission of drug resistant strain, Word Health Organization Scientific Group meeting in 1984 (2) also suggested that a single dose of 45 mg primaquine base be given to sterilize the gametocytes.

They also noted that research is needed to determine the optimum schedule for such a combination.

In the months and years that have passed since these meeting, there has been little effort to follow these recommendations. A search of the files of the National Medical Library for 1984 and 1985 produced 58 references to primaquine and not one relates to these recommendation (3). Some studies on primaquine was under taken in Thailand.

Chomcharn et al (4) conducted the study of the effect of single dose primaquine on P. falciparum gametocytaemia and mosquito

infectivity in Thailand. They found that 10 patients with gametocytaemia when given 45 mg primaquine were all cleared by day 6, but among the controls gametocytes were positive up to day 17.

Bunnag et al (5) had also conducted field trial of 3 regimens of primaquine (15 mg daily X 5 days, 30 mg single dose and 45 mg single dose) on 121 patients with initial gametocytaemia. They reported that the gametocytes disappeared by day 21 in all groups. In the 194 patients without initial gametocytaemia, gametocytes developed in over 25% of them. They cleared again on day 28.

They commented that further work on the infectivity of the gametocytes is required.

But in our experience, with 45 mg primaquine given on day 1 of hospital admision to falciparum malaria patients together with 1500 mg amodiaquine or 1500 mg sulphadoxine and 75 mg pyrimethamine gametocytes persisted in blood of 7 out of 23 patients at day 7 and on 1 out of 5 patients at day 28. Thus there may be persistence of gametocytes in Myanmar patients though they were treated with 45 mg primaquine. Thus this study is needed.

PATIENTS AND METHODS

One hundred and five patients who attended Thayarwady Civil Hospital during 1987 & 1988 malaria seasons were studied. Thirty five patients in blood with parasite counts 1000-200,000/cumm were treated with amodiaquine (total dose of 1500 mg divided in 3 days). The presence and viability of gametocytes in blood were followed at day 1,3,7,14,21 and 28. One patient who had gametocytes in blood throughout this study was followed weekly till his gametocytes were disappeared from circulation.

Second group of 45 patients with P. falciparium malaria were treated with amodiaquine (dose as above) and primaquine 45 mg single dose- the later was given on 3rd day of hospital admission. The presence of gametocytes and viability were followed on days mentioned above.

Third group of 25 patients with P. falciparum in blood were treated with amodiaquine (dose as above) and primaquine 45 mg single dose given at day 1 of hospital admission. They were also followed as above.

Viability of gametocytes were tested by incubation of blood on glass slide with a drop of normal saline and storing in -20°C for 5-10 minutes. The presence of exflagellation of male microgametocyte was recorded as an indicator of viability.

RESULTS

Among the 35 patients treated with amodiaquine alone, 23-33% of the patients showed gametocytes in blood during days 14, 21 and 28 of therapy. Among those patients with gametocytes in blood, 50-63% of patients showed that they were viable by exflagellation method. Follow-up of one patient showed viable gametocytes in blood till 51st day of drug therapy.

Among 45 patients treated with amodiaquine and primaquine (45 mg single dose : given on day 3), 31% of patients showed gametocytes on 7 day, later only 0-10% of the patients showed gametocytes on days between 14 and 28. Among the patients with gametocytes in blood 25% of the patients showed that they were viable till day 4. From day 5 onwards they were not viable. At day 28, gametocytes reappeared in 1 patients. He was proved to be viable. He was also resistant at RII level to amodiaquine.

Among 25 patients treated with amodiaquine and primaquine (45 mg single dose : given on day 1 of hospital admission) 36% showed gametocytes on day 7 and 42% showed gametocytes on day 28. Of these patients with gametocytes which appeared

Table: Percentage of patients with Gametocytes and their viability among patients treated with three groups of drugs

Days	AMQ (1.5G) Only N=35		AMQ (1.5G) with Primaquine 45 mg on Day 3 N=45		AMQ (1.5G) wih Primaquine 45 mg on Day 1 N=25	
	% of patients with gametocytes	% with viable gametocytes	% of patients with gametocytes	% with viable gametocytes	% of patients with gametocytes	% with viable gametocytes
1	60	90	53	78	50	40%
3	54	90	43	25*	40	0
7	56	70	31	0	36	10%
14	33	63	7	0	-**	-**
21	23	50	0	0	-**	-**
28	30	50	10	-	42	28%

* Done on Day 4.
** Not Done.

on day 28% was proved to be viable. See Table 1 for details.

DISCUSSION

Mackerras and Ercole (7), Jeffrey et al (8) and Rieckmann et al (9), had reported the action of primaquine on gametocytes as quick action (in 6-12 hours infectivity is lost) and P. falciparum gametocytes are effectively eliminated from the circulation in 2 to 4 days.

Rieckmann et al (9), Burgess and Bray (10) and Chomcharm et al (4) had all reported disappearance of gametocytes when treated with 45 mg primaquine by day 6 (4-8) days.

Bunnag et al (5) from their studies in Thailand also reported persistence of gametocytes after various dose of primaquine till day 21. In this study there were persistence of gametocytes after primaquine even at day 28. This delay in the disappearance of gametocytes in the circulation after primaquine in patients from Myanmar and Thailand could be due to development of partial resistance of gametocytes to eliminate from circulation after primaquine.

But as all the gametocytes that are persisting in circulation are not viable (except one patient with Rll resistance to amodiaquine) we can assume that this present treatment regimen. Primaquine given on day 3 of patients admission is effective to control viable gametocytes in blood.

Among the patients treated with amodiaquine primaquine given on 1st day of hospital admission, 36-42% of patients had reappearance of gametocytes in blood between days 7 and 28. Of these two patients who had reappearance of gametocytes at day 28 was tested for viability. One of them was proved to be viable.

Since the half life of primaquine is very short probably the single dose of primaquine may have the abaility to clear the gametocytes already in the circulation but it may have no ability to remove new gametocytes which are released into circulation from the still living trophozoites of Rll level resistant patients.

Similar reasons may be given for patients who were treated with primaquine at day 1 instead of day 3.

Thus untimely administration of the drug (primaquine given on day 1 instead of day 3) may be the cause of failure of the action of primaquine. From this study, we support the idea of adding primaquine to schizontocidal drugs but we suggest to given

primaquine on day 3 of treatment, when the malaria parasites almost disappeared from circulation instead of day 1.

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Electrocardiographic effects of quinine and quinidine in the treatment of falciparum malaria

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Quinidine has been suggested as an acceptable alternative to quinine in the treatment of falciparum malaria. To compare the electrocardiographic effects of quinine and quinidine in falciparum malaria, sixty patients with more than 2% of parasitized red cells were chosen and paired as closely as possible. The study was double-blind. 15 mg/kg quinine or quinidine was given as a loading dose infused over 4 hours followed by 2 doses of 7.5 mg/kg base at 8 hourly intervals. Oral quinine or quinidine as 7.5 mg/kg base 3 times/day was then continued till day 7. Satisfactory 12 lead electrocardiograms were obtained from 58 patients on day 0, after 24 hours, on day 3 and on day 7. Overall there was no significant change in PR interval or QRS duration. The pretreatment QTc was 443±30 msec in the quinine group (n=30) and 438±25 msec in the quinidine group (n=28) with no singificant difference. Maximum QTc prolongation occured on the third day in both groups being 481±28 msec in the quinine group and this was significantly less than 532±55 msec seen in the quinidine group. QTc $\geq$ 550 msec was found in 46.6% in the quinidine group but in only one in the quinine group. T wave flatttening occured in 62% in the quinidine group but in only 25% in the quinine group. No dysrhythmias were recorded in this study but because of the excessive prolongation of the QTc caused by quinidine, we conclude that we should be cautious about the use of quinidine for falciparum malaria.

INTRODUCTION

Quinidine, an optical isomer of quinine was first described in 1848 by van Heyningen and prepared and given its present name by Pasteur in 1853, Quinidine shares all the pharmacological actions of quinine, including its anti-malarial, antipyretic and oxytocic effects (1).

Many years ago, both quinine and quinidine were used for treatment of malaria, and it was noted that patients with malaria who also had certain cardiac arrhythmias would occasionnally be cured of arrhythmia by quinidine (1). The action of quinidine on cardiac muscle was found out to be more intense than those of quinine. Quinidine came to be employed as an antiarrhythmic drug and quinine came to be used mainly for its antimalarial effect. In recent years, the use of quinidine in the treatment of falciparum malaria has been resurrected (2) and claims have been made that it is as effective and as safe as quinine (3).

It is the purpose of this investigation to compare the electrocardiographic effects of quinine and quinidine in the treatment of falciparum malaria with a view towards detecting possible toxic effects of quinidine on the cardiac muscle compared to quinine.

PATIENTS AND METHODS

Sixty patients with more than 2% parasitized red cells were chosen and paried as closely as possible (Table 1). The study was double blind. 15 mg/kg quinine or quinidine was given as a loading dose infused over four hours followed by two further infusions of 7.5 mg/kg at eight hourly intervals. This is followed by oral quinine or quinidine 7.5 mg/kg three times a day till day 7. Satisfactory 12 lead electrocardiograms were obtained from 58 patients on day 0, after 24 hours, on day 3 and on day 7.

E.C.Gs were recorded at a paper speed of 25 mm/s and were read under magnification. The QT interval was measured from the lead showing greatest T-U dissociation. The mean of 5 measure-ments was taken and corrected QT interval or QTc was calculated according to the formula

$$QTc = \frac{QT}{\sqrt{\text{R-R interval}}}$$

E.C.Gs were read without the knowledge of whether quinine or quinidine had been used on each particular case. The code was broken when all E.C.Gs had been read and interpreted. Students t test was used to analyse electrocardiographic time intervals.

RESULTS

There was no significant change in the P wave morphology, or PR interval in any of the patients in both groups treated with quinine or quinidine. Overall, there was no significant prolongation of the QRS duration in either quinine or quinidine treated cases although in 2 of the quinidine treated cases QRS duration was prolonged by 50%.

The pretreatment QTc was 443 ± 30 msec in the quinine group and 438 ± 25 msec in the quinidine group and the difference is not significant. Normal QTc is not to exceed 420 msec in men and 430 msec in women (4).

Table 1. Patient characteristics in quinine and quinidine groups

	Quinine	Quinidine
Initial parasite count	152,000 ± 253,000/cmm	153,000 ± 361,000/cmm
Number of patients	30	28
Sex ratio (male: females)	26:4	25:3
Mean age	28.8 ± 9.6 yr	34.3 ± 13.8 yr
Mean body weight	47.3 ± 5.1 kg	48.8 ± 15.7 kg
Hyperparasitaemia (>5%)	10%	11%
P.C.V. <30%	16 (2 transfused)	11 (4 transfused)
Clinical jaundice	4	10
Raised blood urea	1	2
Initial hypoglycaemia	1	2
Hyperpyrexia > 105° F	1	1
Hypoglycemia	0	0
More than one complication :	9	12

Maximum QTc in quinine treated patients occurred at the end of the third day, 481 ± 28 msec i.e. 8.58% increase over the base-line value and this was significantly less than the maximum QTc prolongation produced by quinidine, 532 ± 55 msec, i.e. 21.55% increase over base-line value.

The longest QTc in the quinine group was 550 msec seen at the end of 3rd day in one patient only whereas QTc $\geq$ 550 msec was found in 46.4% of the quinidine group. QTc of 620 msec was seen in one patient in the quinidine group. However, no significant dysrhythmias were detected in any of the patients in both the groups.

T wave flattening occurred in 62% of the quinidine group but in only 25% of the quinine group. (Table 2.3 & Graph 1).

DISCUSSION

Quinidine syncope, commonly due to TORSADE DE POINTES (a form of ventricular fibrillation) appears to occur as a result of both individual susceptibility and excessive plasma concentration. Fatalities from this complication have been reported to occur in 0.5% - 4% (5). Excessive prolongation of the QT interval may herald susceptibility to this complication. However, the actual values that constitute "excessive prolongation" are uncertain and it is regarded that a QTc of more than 550 msec is undesirable and a value of 600 msec or more is an indication to stop therapy (5).

In this study, longest QTc caused by quinine was 481 ± 28 msec and the longest QTc recorded was 550 msec seen in one patient in this group.

QTc $\geq$ 550 msec was seen in 46.4% of quinidine treated patients and QTc of 620 msec was seen in one patient.

Although no dysrhythmias were seen in the quinidine group which produced greater prolongation of the QTc, it would be prudent to say that larger numbers of patients would need to be observed to establish that quinidine is as safe as quinine in the management of malaria. The normal

Table 2. ECG changes in quinine group (n = 30)

	QRS msec	% increase over base line	p value	QTc msec	% increase over base line	p value	T wave flattening
Base line	83 ± 10			443 ± 30			
End of 24 hr	85 ± 10	2.41	N.S	468 ± 39	5.64	<0.02	
End of 3rd day	86.6 ± 10	3.16	N.S	481 ± 28	8.58	<0.001	25% of cases (8)
End of 7th day	88 ± 10	5.68	N.S	460 ± 28	3.84	<0.02	

N.B. QTc longest 550 msec in one patient (Day 3)

Table 3. ECG changes in quinidine group (n = 28)

	QRS msec	% increase over base line	p value	QTc msec	% increase over base line	p value	T wave flattening
Baseline	84 ± 9			438 ± 25			
End of 24 hr	90 ± 12	7.14	N.S	504 ± 40	15.07	<0.001	
End of 3rd day	94 ± 13	11.9	N.S	532 ± 55	21.55	<0.001	62% of cases (18)
End of 7th day	93 ± 11	10.71	N.S	501 ± 36	14.38	<0.001	

N.B. QTc ≥ 550 msec in 46.4% (13/28). Longest QTc 620 in on patient.
QRS 50% prolongation seen in 2 patients.

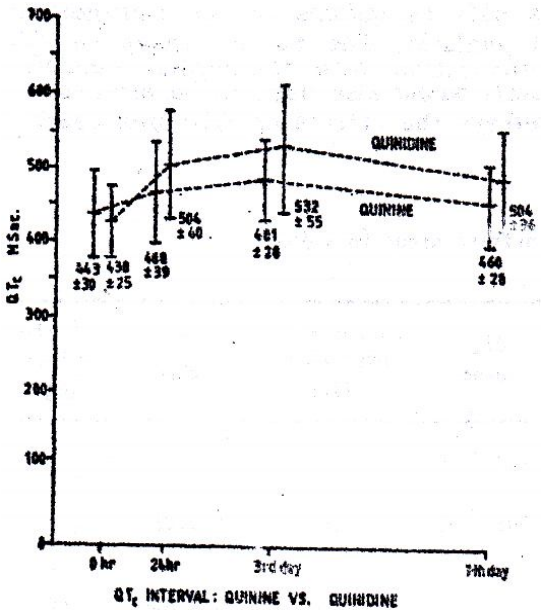

Figure 1. QTc interval in patients treated with quinine or quinidine

range of QT prolongation with therapeutic quinidine levels would also need to be established. Perhaps the effects of QTc prolongation on a previously normal heart and a diseased heart may not be the same. It is said that widening of the QRS complex is also an indication of a cardiac toxicity.

However, small changes in E.C.G. intervals could well be missed by visual inspection of traces and QRS prolongation of 50% was seen in only 2 patients in the quinidine treated group.

Thus we wish to conclude that Unless quinidine can be demonstrated to be superior that quinine in the treatment of falciparum malaria and as safe as quinine in large scale studies, we would recommend that quinidine be used cautiously in the treatment of malaria only when quinine is unavailable because of its pronounced effects on the E.C.G.

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Prevalence of hepatitis B and C infections in hepatocellular carcinoma cases in Myanmar

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Hepatitis B and hepatitis C have been implicated with chronic liver disease in many parts of the world. This study was carried out on 40 patients with hepatocellular carcinoma, diagnosed clinically and confirmed by the positivity of alpha-fetoprotein by agar gel diffusion method. Sera were tested for HBsAg by AUSZYME ELISA test kits (Abbott Laboratories, U.S.A) and for Anti-HCV by ORTHO HCV Antibody ELISA test system (Ortho Diagnostics, U.S.A.) Our finding confirmed the high incidence of hepatitis B infection (60%) and also of the association of hepatitis C infection (35%) among the hepatocellular carcinoma cases. The significance of the positivity of Anti-HCV by the ELISA tests was discussed.

INTRODUCTION

Myanmar has been listed among the top ten countries with the highest incidence of hepatocellular carcinoma (HCC) in the world [1]. Primary HCC is the second most common carcinoma in males and fourth most common in females in Myanmar [2]. Two different viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) have been implicated in the etiology of this carcinoma. Because of the similar epidemiology and disease spectrum caused by these two blood borne viruses, it is of interest and importance to study their roles in HCC in Myanmar.

Hepatitis B is highly endemic in Myanmar with a hepatitis B surface antigen (HBsAg) carrier rate of 9-12% and infection rate of 50%. Approximately, 90% of the infants and children who contracted the infection during early childhood and 10% of the acute hepatitis B cases may progress onto the carrier state, chronic hepatitis, cirrhosis and HCC [3,4,5]. HBsAg is the hallmark in the diagnosis of viral hepatitis type B.

Hepatitis C was previously designated as post-transfusion or parenterally transmitted non-A non-B hepatitis. Diagnostic test kits for hepatitis C became commercially available only in 1989. It was generally thought that hepatitis C virus (HCV) infection became chronic in 50% of the infected cases [6]. But the recent longitudinal study of post-transfusion hepatitis C patients by the National Institutes of Health in the United State showed that as many as 80% of such cases may ultimately progress to chronicity. Patients with HCV-related cirrhosis are at risk of developing HCC. A positive anti-HCV test indicates exposure to HCV and is a surrogate marker of ongoing HCV infection [7].

An increased level of alpha fetoprotein (AFP) above 400 ng/ml is an indicator of HCC and can be accomplished in suspected cases before the appearance of clinical features [8].

From 1993 to the middle of 1998, the number of HCC in-patient cases have increased fourfold according to the cancer statistics from the Cancer Ward, Radio-therapy Department, Yangon General Hospital [9]. The etiology of HCC needs to be studied for effective planning to reduce the incidence of HCC in Myanmar.

MATERIALS AND METHODS

A total of 40 serum samples were obtained from HCC patients who were diagnosed by their clinical features and the positivity for alpha fetoprotein (AFP) determined by agar gel diffusion method of Ouchterlony.

The serum samples were screened for HBsAg by using Auszyme ELISA test kits from the Abbott Laboratories, USA.

Ortho HCV Antibody ELISA test system from the Ortho Diagnostic System, USA was used to detect anti-HCV in the test simples.

RESULTS

Table 1 shows the detection of HBsAg and anti-HCV makers in HCC cases according to sex. In our study among the 40 HCC patients, the number of cases in males, predominates females by 7:1. Another finding in this study is that males predominate females in the positivity for both HBsAg and anti-HCV markers. HBsAg was positive in 60% (24 of 40) of all patients and anti-HCV was positive in 35% (14 of 40) of the study group.

Table 1. Detection of HBsAg & Anti-HCV in hepatocellular carcinoma patients according to sex.

Sex	No.	HBsAg positive HCC cases		AntiHCV positive HCC cases	
		No.	%	No.	%
Male	35	23	57.5	12	30
Female	5	1	2.5	2	5
Total	40	24	60.0	14	35

The prevalence of HBV and HCV markers in HCC patients according to age is shown in Table 2. In the 40 patients with their ages ranging from 20 years to 89 years, the peak incidence of HCC cases was seen in the 30-39 years age range (35%) where both HBsAg (27.5%) and anti-HCV (15%) were also at their highest distribution.

Table 2. Prevalence of HBsAg and anti-HCV in hepatocellular carcinoma patients according to age.

Age Groups	HCC cases		HBsAg positive		AntiHCV positive	
	No.	%	No.	%	No.	%
20-29	4	10.0	3	7.5	3	7.5
30-39	14	35.0	11	27.5	6	15.0
40-49	7	17.5	6	15.0	1	2.5
50-59	4	10.0	2	5.0	1	2.5
60-69	6	15.0	1	2.5	3	7.5
70-79	4	10.0	1	2.5	-	-
80-89	1	2.5	-	-	-	-
All ages	40	100.0	24	60.0	14	35.0

The presence and absence of hepatitis markers in the study group are shown in Table 3. One or both markers, HBsAg and/or anti-HCV, were positive in 77.5% of patients. Both markers were positive in 17.5% of cases, and both markers were negative in 22.5% of the study group.

Table 3. Hepatitis B and C markers in hepato-cellular carcinoma cases.

No. of HCC	Positive for either HBsAg and/or antiHCV		Positive for both HBsAg And anti-HCV		Negative for both HBsAg or anti-HCV		Positive for Anti-HCV only	
	No.	%	No.	%	No.	%	No.	%
40	31	77.5	7	17.5	9	22.5	7	17.5

From the data in Table 4, it could be seen that when divided into HCC patients positive and negative for HBsAg, it was positive for anti-HCV in 29.17% in the former group and 43.75% in the latter group.

DISCUSSION

The male predominance of 7:1 in HCC cases found in this study was higher than the previous study in Myanmar which was recorded to be 4:1 for male-female occurrence [3]. HCC incidence has been known to be higher in males than in females in most populations of the world. The male excess is recorded to be greatest generally between three and four to one, in places and populations in which HCC incidence is highest. It has been expressed that the sex ratio does not relate to gender differences in HBV carrier rates or to aflatoxin exposure and that sex hormones probably mediated differences in responses to the etiologic agents [10].

Globally, HBV has been recorded to be the probable etiological agent responsible for 75% to 90% of HCC cases [11]. Hepatitis C virus has also been studied to be involved in the development of HCC [12]. In this study HBsAg was positive in 60% of cases confirming the close association of HBV with HCC. A previous study in Myanmar showed a 70% positivity for HBsAg in HCC cases [2]. This study also showed that HCV played a significant role in the etiology of HCC as it was present in 35% of HCC cases. In this Study, males predominate females in the positivity for both HBsAg and anti-HCV.

This study showed that the peak incidence of HCC cases was seen in 30-39 years age range (35%) where both HBsAg (27.5%) and anti-HCV (15%) were also at their highest distribution. This was contrary to a previous study by Muir *et. al* who reported in 1987 that the HCC incidence increased with advancing age in both sexes. The incidence appeared to be linear with increasing age from early adulthood, with a steeper slope in males in most populations [13]. However, the pattern of HCC incidence in Myanmar was similar to the one in China as reported by Yeh in 1989 in which the HCC incidence appeared to rise with increasing age and then declined [14].

The high incidence of HBsAg and anti-HCV either alone (42.5% and 17.5%) or in combined forms (17.5%) in HCC cases in Myanmar showed that HCV infection has now made an additional contribution to the severe forms of liver disease either as super infection or co-infection with HBV (Table 3). A co-infection with both HCV and HBV was also reported in 26.7% of HCC cases by Liaw and Chien from Taiwan [15]. A study in Japan also established that anti-HCV correlated with clinical severity of hepatitis B chronic liver diseases and that anti-HCV was not detected in asymptomatic HBsAg carrier [16]. In this study 22.5% of cases were negative for both HBV and HCV makers which was similar to a study in China which reported 23.1% of such chronic liver diseases which was not due to either HBV or HCV.

Table 4 shows that there were 24 HBsAg positive HCC patients (60%) and 16 (40%) HBsAg negative HCC patients. HBsAg positivity was therefore 1.5 times greater than HBsAg negativity in HCC patients. Of the 24 HBsAg positive HCC cases, 7 patients were positive for anti-HCV (29.17%). These were higher than results obtained in other hepatitis B endemic countries; 13% in Singapore, 16% in Japan, 17% in Taiwan and 16.3% in Korea [18].

Table 4. Prevalence of Anti-HCV in HCC cases positive or negative for HBsAg

Total No. of HCC cases	No. tested	Anti-HCV positive	
		No.	%
HBsAg (+) cases	24	7	29.17
HBsAg (-) cases	16	7	43.75
Total	40	14	35.0

Of the 16 HBsAg negative HCC cases 7 (43.7%) were positive for anti-HCV in this study. In Singapore 71% of HBsAg negative HCC cases were anti-HCV positive, in Japan the figure was 76% and Taiwan it was 63% [12].

Some authors have also speculated upon the possibility of interaction between HBV and HCV that might alter the natural course of each separate viral disease. A possible synergistic effect of past or current HBV and HCV infection on HCC risk has also been suggested.

The prevalence of HBV and HCV infection in HCC cases varied in different countries as evident in Table 5. The incidence of HBsAg positive in HCC cases were highest in Taiwan (83.5%) where as anti-HCV prevalence in HCC patients was highest in Japan (76.2%). The prevalence of HBsAg ranged from 12% - 83.5% where as for anti-HCV it was 0-76.2% in HCC cases in different countries [19].

Table 5. Prevalence of hepatitis B and C markers in HCC cases in different countries.

No.	Country	No. of HCC cases tested	HBsAg positive (%)	AntiHCV positive (%)
1	Japan	880	21.8	76.2
2	China	52	67.3	38.5
3	Korea	75	29.3	57.3
4	Taiwan	103	83.5	32.0
5	Philippine	11	45.4	18.0
6	India	33	15.2	42.0
7	Indonesia	70	58.6	34.3
8	Thailand	20	12.0	-

CONCLUSION

The results of this study indicate that both HBV and HCV are strongly associated with HCC. These data emphasize the importance of effective planning strategies for the prevention and control of viral hepatitis in Myanmar.

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Potency assay of antivenom: Failure of Indian (serum institute) antivenom to neutralise Russell's viper (Daboia russelli siamensis) venom of Myanmar

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Neutralisation of biological properties of Russell's viper (Daboia russelli siamensis) venom of Tharyarwady by a monospecific antivenom of Myanmar (DN 86608B ex. 4/92) and a polyspecific antivenom of Serum Institute of India (Sii) (bath 109 exp. 4/96) was carried out according to WHO standard tests of neutralising activity. Neutralising potency of Myanmar antivenom was superior to the Indian antivenom and the latter required 16-126 times more antivenom than the former in neutralising haemorrhagic, necrotic, lethality, defibrinogenating and capillary permeability increasing activities of the venom. In immunodiffusion and immunoblotting experiments fewer bands were detected in both. It is concluded that the Indian antivenom (Sii) will be less effective in treating Russell's viper bite cases of Myanmar.

INTRODUCTION

Monospecific and bispecific (Russell's viper and Naja kaouthia) antivenoms manufactured by Myanmar Pharmaceutical Factory (MFP) are used for treating Russell's viper bite cases. Recently, polyspecific antivenom of Serum Institute of India (Sii) was used for treating Russell's viper bite cases in some township hospitals of Myanmar. However, its potency has not been tested. There were reports on poor clinical efficacy of some antivenoms when used in areas distant from the sources of immunising venom [1]. This report concerns the laboratory assessment of potency of the Indian (Sii) and MPF monospecific antivenom in neutralising Russell's viper venom of Myanmar.

MATERIALS AND METHODS

Neutralisation of biological properties of Russell's viper venom (RVV) (Tharyarwady) such as lethality, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing activities with a monospecific antivenom (batch no. DN 86608B exp. 4/92) manufactured by MPF and a polyspecific antivenom of Serum Institute of India (Sii) (batch no. 109 exp. 4/96) was carried out according to WHO standard tests of neutralising activity [2]. Briefly, a fixed amount of venom was mixed with a variable dilution of antivenom, incubated at 37ºC for 30 min and then injected into laboratory animals. The end point was taken as the minimum amount of antivenom required to completely neutralise the biological effects of venom. Immunodiffusion and immunoblotting using the two antivenoms were also carried out following standard methods. Details of the methods have already been described [3].

RESULTS

Results of neutralisation of biological properties of venom by the two antivenoms are shown in table 1. Neutralising potency of the MPF antivenom was superior to the Indian

Table 1. Results of neutralisation tests of the Indian (Sii) (batch no. 109) and MPF antivenom (batch no. DN 86608B)

Source of antivenom	$5LD_{50}$ iv 23.4 µg/mouse µl	10MCD 2.512 µg µl	3MHD 126.5 µg/rat µl	3MND 119.4 µg/rat µl	5MDD 12 µg/mouse µl	100MCP ID 0.724 µg µl
MPF ASV DN 86608B	2.5	0.063	2.5	2.5	0.63	2.5
Indian ASV B. no. 109	100.0	100.0	80	80	80	40

LD_{50} iv = Lethality
MCD = Minimum coagulant dose
MHD = Minimum haemorrhagic dose
MND = Minimum necrotic dose
MDD = Minimum defibrinogenating dose
MCPID = Minimum capillary permeability increasing dose

antivenom. The latter required 16-40 times more antivenom than the former to neutralise haemorrhagic, necrotic, lethality and capillary permeability increasing activity and 126 times more antivenom to neutralise defibrinogenating activity of the venom. It failed to neutralise procoagulant activity of the venom in vitro test system (Table 1). Fewer bands were recognised by the Indian antivenom in immumnodiffusion when tested against Daydaye, Kungyangon and Tharyarwady venoms (Figure 1). The Indian antivenom failed to pick up small molecular weight bands in immunoblotting experiment (Figure 2).

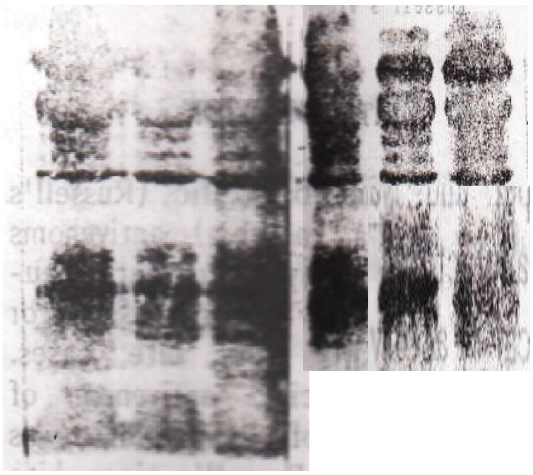

Fig. 1. A picture of immunodiffusion showing precipitin bands developed between Russell's viper venoms of (D) Daydaye, (K) Kungyangon and (T) Tharyarwady with MFP antivenom (Batch no. DN 86608B) and Indian Antivenom (batch no.109)

DISCUSSION

The current practice of potency assay of antivenom is mainly based on testing antilethality potency only. Here we have an opportunity to test the potency of the Indian and MPF antivenoms according to WHO standard tests of neutralising activity. According to the manufacturer's instructions, 1 ml of the MPF antivenom can neutralise 2 mg of RVV and that of the Indian is 0.6 mg for Vipera russelli. However, almost neat Indian antivenom was needed to neutralise all the biological properties of the venom indicating its poor efficacy. Geographical variation of venom composition of RVV [3-5] and subspecices differences may explain poor performance of the Indian antivenom.

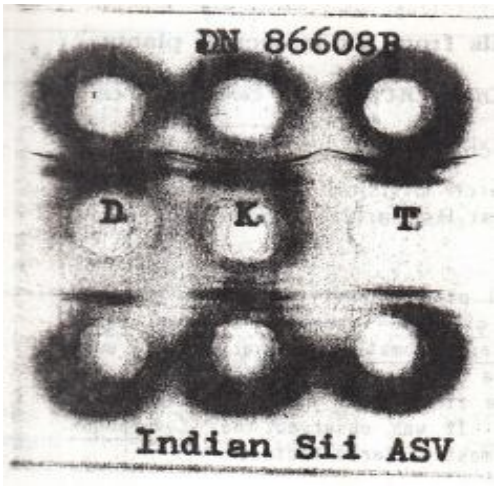

Fig. 2. A picture of immunoblot developed between Russell's viper venoms of Daydaye (D), Kungyangon (K) and Tharyarwady (T) and the Indian (Sii) (batch no. 109) and MPF (batch no. DN 86606B) antivenoms

This observation was in agreement with the finding that poor clinical efficacy of some antivenoms was observed when used in areas distant from the sources of immunising venom [1]. Variation in neutralising potency of MPF antivenom against venoms from different localities of Myanmar has been observed [6]. It is concluded that the Indian (Sii) antivenom will be less effective in treating Russell's viper bite cases of Myanmar.

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Quality of antenatal care at outpatient department of Mandalay General Hospital: time utilization and satisfaction among users

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The study was a hospital-based cross-sectional survey using recording and interviewing techniques. Average waiting time (in minutes) before seeing registration clerk, nurses, laboratory staff and medical doctors, for all first visit and follow-up visit patients, were 10, 5, 8 and 31 respectively. Average time in contact (in minutes) with registration clerk, nurses, laboratory staff and medical doctors, for all patients, were 2, 2, 11 and 3 respectively. Among first visit patients, only 21% were told of their body weights, 21% were told of their blood pressures, 66% were told of their urine examination results, and 64% were told of their baby's clinical conditions. Similar figures for follow-up patients were 29%, 26%, 83% and 80% respectively at different service points. The types of service offered and the training and performance of the service providers can be concluded as far from being adequate.

INTRODUCTION

The aim of antenatal care (ANC) should be to guide and support prospective parents, and pregnant women should be treated accordingly, Much of the benefit of regular ANC checkups would be undermined if the way services delivered at an ANC clinic is poorly organized. Long waiting times before appointments are a source of much frustration, anger and worry [1]. This can influence a woman's decision to continue attending ANC clinics. According to the European Organization for Quality Control (EOQC), quality is "the totality of features of a product or service that bears on its ability to satisfy given needs" [2]. The definition is referring to quality as the merit or excellence of a thing or activity. It is being referred to as something that can be felt than measured. Roemer and Montoya-Aguilar referred to quality of health care as the degree to which the resources for health care or the services included in health care correspond to specified standards [3]. Application of those standards could be expected to give desired results. In general notion, quality can be regarded as the merit or excellence of the system in all its aspects and this involves the resources, the activities, the management, and the outcomes of health care [4].

The model of "structure, process, and outcome" is to be applied whether to assess the quality of medical (clinical) care given to an individual patient or to assess the quality of health (public health) care provided to a population [3,5]. Structure includes tools and resources that providers of health care have at their disposal and of the physical and organizational settings in which they work. Process refers to the set of activities that interact within and between health care providers and patients/community. Outcome is a change in a patient's/ community's current and future health status

that can be attributed to the medical/health care being provided.

Studies in the past have shown that smooth patient flow is a basic requirement for providing effective health care with limited medical resources [6]. Long patient waiting times and the inefficient use of professional staff time can substantially limit the cost-effectiveness of a clinic's programmes [7]. Inefficient clinic practices that force patients to wait for long periods of time can cause patient dissatisfaction with the clinic and result in a low rate of patient return. A method of analyzing patient flow in the average clinic could provide a basis for improving clinic efficiency, not only from the viewpoint of the provider, but also from the often-neglected perspective of the patients.

The general objective of the study was to assess the quality of antenatal care (ANC) services being provided at outpatient department (OPD) of Mandalay General Hospital (MGH) through time utilization and satisfaction among the users.

MATERIALS AND METHODS

The study was a hospital-based cross-sectional survey using recording and interviewing techniques. ANC clinic of MGH opens from 8:00 am till 12:00 noon on every Monday, Wednesday and Friday. There are two senior nurses, assisted by about 15 student nurses, and 5-8 medical doctors providing ANC services at the OPD. VDRL test is performed as STD department next door to the OPD. There is no appointment system. New and old pregnant mothers coming to the OPD of MGH for antenatal care during a one-month period in March in 1998 were included in the study.

When a pregnant mother arrives at the OPD, she has to fill a registration form and ANC record at the registration point. Then, her blood pressure and body weight are taken by a nurse, usually a student nurse. There is no proper flow system as regards which measurement should be taken first. Next, blood samples of new pregnant (first visit) mothers are taken for VDRL test. Urine for albumin is also tested for these first visit mothers. Finally, patients are examined by medical doctors in five separate rooms. High-risk mothers needing special investigations such as ultrasonography are referred to the responsible units of MGH and appropriately managed.

Arrival times of each patient at the entry of the OPD, registration table, service points of nurses, laboratory staff and medical doctors, and the exit of the OPD were recorded. Each patient was interviewed at the exit. A time recording form was given to each patient at the entry point and was used for all the subsequent time recordings. Time recording at each service point was performed by trained enumerators. Interview, using a pretested structured questionnaire, was also performed by trained enumerators. Although new patients were interviewed again in their second visits during the study period, old patients were interviewed once. A total of 601 patients were included in the study. The whole process of data collection was supervised by the investigators at MGH.

RESULTS

Table 1 shows patient care at ANC clinic of MGH. It can be noted from this table that average waiting time (in minutes) before seeing registration clerk, nurses, laboratory staff and medical doctors, for all patients, were 10.25, 5.41, 7.63 and 30.54 respectively. When first visit patients and follow-up patients are separated out, the respective waiting time at different service points was found to be 14.07, 8.53, 7.16 and 30.47 for

the first visit patients, and 6.43, 2.30, 10.93 and 36.00 for the follow-up patients.

Table 1. Patient care in Antenatal Care Clinic of Mandalay General Hospital

Indicator	All patients	First visit patients	Follow-up visit patients
Average patients per day	50	25	25
Average waiting time (in minutes) before seeing			
• Registration clerk	10.25	14.07	6.43
• Nurses	5.41	8.53	2.30
• Laboratory staff	7.63	7.16	10.93
• Doctors	30.54	30.47	36.00
Average time in contact (in minutes) with:			
• Registration clerk	2.37	3.56	1.09
• Nurses	2.15	2.18	2.11
• Laboratory staff	11.79	12.17	5.50
• Doctors	3.30	4.53	2.06
Average time in clinic (in minutes)	94.23	112.44	76.09
Average proportion of patient's time spent on receiving services from OPD personnel (only for nurses and medical doctors)	5.78%	5.96%	5.48%

Average time in contact (in minutes) with registration clerk, nurses, laboratory staff and and medical doctors, for all patients, was 2.37, 2.15, 11.79, and 3.30 respectively. When first visit patients and follow-up patients are separated out, the respective contact time at different service points was found to be 3.56, 2.18, 12.17 and 4.53 for the first visit patients, and 3.56, 2.18, 12.17 and 4.53 for the follow up patients.

Average time in clinic (in minutes), for all patients, was 94.23 out of which only an average proportion of 5.78% was spent on receiving services from nurses and doctors.

Average time in clinic (in minutes) for first visit patients was 112.44, out of which 5.96% was spent on receiving services from doctors and nurses; similar figures for follow-up patients were 76.09 and 5.48% respectively.

Table 2 shows satisfaction of patients on services received. It can be observed that among first visit patients, only 21% were told of their body weights, only 21% were told of their blood pressures, 66% were told of their urine examination results, and 64% were told of their baby's conditions/ clinical conditions after being examined by a medical doctor. Similar figures for follow-up patients were 29%, 26%, 83% and 80% respectively. However, 60%-99% of the patients said that they were

Table 2. Satisfaction of patients on services received

Service point	Service received		Results/condition told		Satisfaction with the service	
	First visitors	*Follow-up visitors*	First visitors	*Follow-up visitors*	First visitors	*Follow-up visitors*
Body weight measurement	300 (100%)	*300 (99%)*	62 (21%)	*88 (29%)*	293 (98%)	*294 (98%)*
Blood pressure measurement	299 (99%)	*300 (99%)*	62 (21%)	*77 (26%)*	294 (98%)	*298 (99%)*
Blood examination for STD	286 (95%)	*8 (3%)*			240 (83%)	*6 (60%)*
Urine examination	296 (99%)	*23 (8%)*	195 (66%)	*20 (83%)*	295 (99%)	*22 (92%)*
Clinical/abdominal examination	297 (99%)	*301 (100%)*	184 (64%)	*241 (80%)*	295 (98%)	*296 (98%)*

satisfied with the services they received at different service points.

DISCUSSION

Quality can be thought of as having two aspects: technical competence and the art of care. The technical aspect, being concrete, is certainly the most easily measured. While assessing technical aspects, an opportunity can be provided to the staff being assessed to improve their approach in the art of care [8]. Retrospective chart audit after the discharge of a patient, concurrent document review while care is in progress, interviewing, administering a questionnaire to test knowledge and probe attitude, and observation are the common techniques used in collecting data for quality assessment [8]. In practice, a combination of techniques is used. The design of this study is not comprehensive enough to reveal the true quality of ANC services being rendered at ANC staff, However, it does reveal a situation that the quality of ANC services being provided at the OPD of MGH is in need of improvements.

It is found in the study that only about 6% of patients' time was spent on receiving services from nurses and doctors (Table 1). This figure for similar studies in Medico City, Panama City and Caracas were 29% 9% and 17% respectively [1]. Waiting time at each service point, except for laboratory workers, is long compared with the contact time for receiving service. Waiting time is found to be longest with the doctors.

Although the majority of patients said that they were satisfied with the services, they might have different reasons for saying this way and might not reflect their true opinions. The majority of them, as a matter of fact, did not get any feedback from service providers as regards what their examination results were. This situation is particularly true for the nurses and medical doctors. Lack of this art of communication in no way could provide true satisfaction to the patients. Health care personnel should encourage and give time for women to express their view and should respect and treat sympathetically what they say [1].

According to the findings of the study - that waiting time to see medical doctors was long but contact time with them was quite short and that the majority of patients did not receive any feedback for what they have been examined - make the investigators conclude that the types of service offered, and the training and the performance of the service providers are far from being adequate.

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Use of risk scores for screening of hepatitis C of blood donors in remote areas

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Hepatitis C virus (HCV) infection is considered as an emerging health problem in Myanmar. Seropositivity rates vary from 3% in blood donors at major blood banks in Yangon to over 10% in northeast and northwest border areas. Determination of HCV is limited in remote areas as it is expensive and some test systems need special equipment. To assist in the control of hepatitis C in Myanmar, we developed a simple system for screening of HCV infection using risk scores. The scores were based on the data obtained from HCV surveys carried out at the northeast and northwest border areas of Myanmar. The database consisted of 652 subjects (250 males, 402 females), aged 18 years to 60 years. Multivariate analyses revealed the following factors to be related to HCV infection in the subjects: more than 30 years of age, Odd Ratios (OR)=2.41 (p=0.001); a history of tattooing, OR= 1.78 (p=0.035); a history of hepatitis in the family, OR=1.58 (p=0.049). The screening scores for HCV infection were developed using risk scores. Validity was analyzed using the Receiver Operating Characteristics curve. The sensitivity of the system was 80% and the specificity 37% when a cut-off score of $\geq$2.5 was used. By increasing the cut-off score, higher specificity (up to 80%) could be achieved at the cost of decreasing sensitivity. The developed risk scores could be applied for screening of blood donors for HCV infection in areas where laboratory HCV testing could not be performed.

INTRODUCTION

Hepatitis C virus (HCV) infection is an important health problem in many countries [1]. HCV was shown to be the major causative agent of non-A, non-B hepatitis and that it is associated with blood transfusion. Although the acute infection is usually asymptomatic and may not be recognized clinically, the subsequent chronic infection is usually life-long and may lead to chronic liver disease leading to liver cirrhosis and hepatocellular carcinoma. In fact it had been proven in Japan as a single most important aetiological factor for the development of hepatocellular carcinoma, where the incidence of hepatitis B virus infection is low [2].

HCV is regarded as an emerging health problem in Myanmar. Reports in early 90's demonstrate HCV infection in one third of patients with hepatocellular carcinoma [3], and in 2.5% of apparently healthy subjects [4]. In recent years, the Myanmar-Japan cooperation study group observed that 2 cases were positive for anti-HCV (5.9% positivity rate) among 34 voluntary blood donors [5]. During May 2000 to Oct 2002, a total of 102,632 donors were screened and the overall anti-HCV positivity rate was

found to be 2.84% [6]. The prevalence of antibody to hepatitis C virus (anti-HCV) was found to be 2.8% among 569 subjects (246 males, 323 females), aged 3 months to 74 years, residing at Mayangone Township, Yangon Division [7]. A recent study showed a higher prevalence of HCV among the population in a northeast border town than blood donors and community in Yangon [8].

For the control of HCV infection in Myanmar, screening for HCV infection is essential. We carried out this study to devise a risk screening form for HCV infection for use in remote border areas where HCV testing is not easily available.

MATERIALS AND METHODS

Cross-sectional, community-based studies were carried out during 2002 and 2003 in northeast and northwest border townships of Myanmar. The northeast border (Muse Township) study population comprised of 349 subjects (137 males, 212 females) aged 12 months to 70 years. Tamu Township (northwestern border) survey included 502 persons (aged 1year to 65 years). During the surveys, consecutive samples were collected from subjects residing at the above mentioned areas. Those who refused consent were excluded from the study. A standardized proforma was used to collect biological and sociodemographic data. Clinical and family histories were carefully asked and recorded. Special emphasis on history of jaundice, history of jaundice in family members, history of dental and surgical operations, and blood transfusion history were noted. From each subject, two milliliters of blood was collected under aseptic measures and sera separated. Sera were transported back to the National Blood Research Centre of the Department of Medical Research (LM) and stored at -80ºC till further analyses. Ortho-HCV Ab PA test II (Orth-Clinical Diagnostics, Fujiredbio Inc., Tokyo, Japan) was used for determination of seropositivity to HCV. These two data bases were combined and those with ages qualified for blood donation were further selected as the data base for the present study. The data base consists of 652 subjects (250 males, 402 females), aged 18 years to 60 years residing at Muse and Tamu Townships.

Statistical methods

Data analysis was performed with SPSS (Statistical Package for Social Scientists Ver 10.1; SPSS Corporation, Chicago, IL, U.S.A.) on a IBM computer. Univariate and bivariate tests were carried out to determine differences between groups. Differences were considered significant if $p < 0.05$. The associated factors were further analysed using odds ratio and multiple logistic regression analysis [9].

RESULTS

General characteristics

Of the 652 subjects, 250 were males and 402 were females. Their ages ranged from 18 years to 60 years with a mean (SD) age of 34.53 (10.9) years. Twenty- five percent of the study population (45%) has achieved high school and university status. Bamars constituted 60% of the study population and the remainder was ethnic groups including Shans, Chins, and Kachins.

HCV antibody prevalence

One hundred and four subjects were found to be seropositive to hepatitis C infection (15.9%). Males had a higher prevalence of anti-HCV seropositivity than females (18.8% vs 14.2%; Student's 't' test, $p=0.12$). No significant difference in age was found between males and females (35.36 ± 11.5 vs 34.02 ± 10.4, $p=0.13$). The anti-HCV seropositivity rate significantly increased with increasing age group. It was found to be the lowest (9.4%) in 18-20 years age group and highest (24.1%) in the 51-60 years age group. Anti HCV seropositivity in 21-30 years, 31-40 years and 41-50 year age groups were found to be 10%, 18.3% and 21.1% respectively.

Associated risk factors for anti-HCV seropositivity

After univariate analysis, it was found that the significant associated factors among the population were: (a) age 30 years and above, (b) presence of tattoos, (c) history of jaundice in the family.

Table 1. Associated factors for anti -HCV sero-positivity by univariate analysis

Associated factors	Anti-HCV seropositivity			Remarks
	No. of tested	No. of positive	Percentage	
Gender				
Male	250	47	18.6	p=0.117
Female	402	57	14.2	
Education group				
Primary & below	271	49	18.1	p=0.418
Secondary & above	327	51	15.6	
Marital status				
Never	265	38	14.3	p=0.339
Ever	367	63	17.2	
Family size				
6 members & below	432	67	15.5	p=0.614
More than 6 members	217	37	17.1	
History of liver diseases				
Yes	139	26	18.7	p=0.317
No	513	78	15.2	
History of transfusion				
Yes	52	8	15.4	p=0.907
No	600	96	16	
History of tooth extraction				
Yes	271	41	15.3	p=0.619
No	380	63	16.6	
History of surgery				
Yes	210	33	15.7	p=0.891
No	440	71	16.1	
History of ear piercing				
Yes	302	45	14.9	p=0.486
No	349	59	16.9	
History of tattooing				
Yes	101	23	22.7	p=0.043
No	550	81	14.7	
History of hepatitis in the family				
Yes	176	35	19.9	p=0.047
No	475	69	14.5	
Age 30 years & above				
Yes	388	78	20.1	p=0.001
No	264	26	9.8	

There was no significant association with gender, education, marital status, and size of the family, history of liver diseases, and history of transfusion, history of tooth extraction, history of surgery, and history of ear piercing (Table 1).

Multiple logistic regression was applied for controlling confounders and for evaluating the effects of associated factors on HCV infection in the studied group. After analysis, 3 variables: age of more than 30 years, Odd Ratios (OR) =2.41 (p=0.001); a history of tattooing, OR= 1.78 (p=0.035); a history of hepatitis in the family, OR=1.58 (p=0.049) were found to have effect on HCV infection (Table 2).

Table 2. Associated factors for anti-HCV sero-positivity among the studied population by multivariate analysis

Associated factors	Adjusted OR	95% CI of OR	p value
Age 30 years & above			
Yes	2.41	1.49 - 3.90	p=0.001
No	1		
History of tattooing			
Yes	1.78	1.05 - 3.03	p=0.033
No	1		
History of hepatitis in the family			
Yes	1.58	1.00 – 2.51	p=0.049
No	1		

Development of a simple risk screening form for screening of HCV infection using risk scores

The risk screening form for HCV infection was developed by using scores from Table 2 as follows: risk score = scores of age 30 years and above plus a history of tattooing plus history of hepatitis in the family. Score of age 30 years and above = 2.5, score of a history of tattooing = 2, score of family history of hepatitis = 1.5. The calculation of risk scores was analyzed and the validity of this model for predicting the risk of HCV infection was calculated by Receiver Operating Characteristics curve (ROC curve). The

sensitivity of this model was 82% and the specificity was 39% when the cut-off score of ≥ 2. If the cut-off score was increased to ≥ 4, the specificity increased to 80% but the sensitivity was greatly reduced to 40%. The optimal cut-off score was determined by the ROC curve (Fig. 1) and was found to be ≥ 2.5, with a sensitivity of 80% and a specificity of 37%. A risk screening form for HCV infection in population aged 18 to 60 years is proposed in Fig. 2.

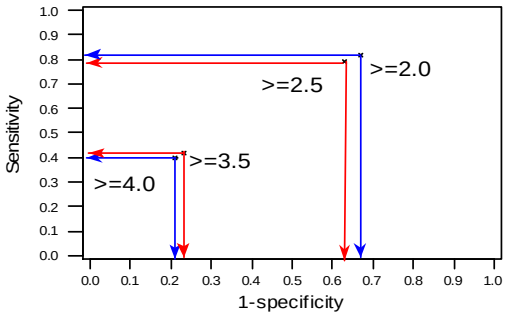

Fig. 1. ROC curve for prediction of HCV infection

Risk screening form for HCV infection among blood donors in remote areas (*where HCV screening is not possible*)				
Name		Age		Years
Gender	Male/Female	Marital status		
Residence				
Risk factors		Status	Full scores	Check list scores
Age 30 years & above		Yes	2.5	
		No	0	
History of tattooing		Yes	2	
		No	0	
History of hepatitis in the family		Yes	1.5	
		No	0	
		Total	6	
Interpretation	Total check list scores ≥ 2.5 indicates the risk for HCV infection at the sensitivity of 80%. Please do not allow blood transfusion unless confirmed to be negative by HCV serological test.			

Fig. 2. Risk screening form for HCV screening among blood donors in remote areas

DISCUSSION

The problem of hepatitis C infection in Myanmar is well recognized and efforts to control it in the blood donor population have been initiated since the year 2000. With the support of Japan International Cooperation Agency under the Control of hepatitis C in Myanmar project, hepatitis C screening of 154161 blood donors during May 2000 to April 2004 had demonstrated the prevalence of HCV infection to be 2.6% in Myanmar blood donors [10]. Data from Europe demonstrated lower than 1% HCV seropositivity in blood donors [2], and 1-2% of blood donors were found to be HCV seropositive in the Far East [11]. However, high rates of HCV infection had been reported with 4% of the blood donors being positive for anti-HCV antibodies in Egypt with higher prevalence rate of 15% in the rural areas of the country [12].

We have studied the HCV prevalence among 349 subjects (137 males, 212 females) aged 12 months to 70 years residing at the Muse Township, Northern Shan State. The overall prevalence of anti-HCV postivity was found to be 13.5% [13]. In northwestern border towns, Tamu and Kalay, a field survey carried out during 2003 revealed that 12.7% of the study population of 502 persons (aged 1 year to 65 years) to be anti-HCV seropositive [14]. Although HCV prevalence surveys could not be considered to be representative of the population at large, it could be concluded that significant higher rates of HCV infection exists among apparently healthy populations residing in border areas.

A study carried out in Yangon blood donors had also outlined similar associated factors. Higher prevalence of anti-HCV positivity was found in those with history of surgical operation, tooth extraction, ear piercing and tattooing. The seropositivity increases with age. In addition, education levels and previous history of blood transfusion had been regarded as possible associated factors [7, 15].

HCV screening tests are expensive and are not easily available in all parts of Myanmar, especially remote areas. The developed risk

screening form will be very helpful in such situations. The screening form is cost free and could be easily used by a medical person, in charge of blood collection. The facts are also in consistent with those mentioned in the donor self deferral form but the proposed form is very simple and could be handled by a person with a little medical knowledge.

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Association of *pvmdr1* Y976F mutation and *in vitro* chloroquine sensitivity of *Plasmodium vivax* in Kawthaung

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Plasmodium vivax is prevalent throughout tropics accounting for 25-40% of global malaria burden. Chloroquine (CQ) is the first-line treatment though chloroquine-resistant *Plasmodium vivax* had been reported since 1989. Vivax malaria has long been considered as benign but some recent reports on complicated and fatal forms of vivax malaria draw a great attention to monitor emergence of drug resistance in *Plasmodium vivax*. To find out *in vitro* CQ status and its association with polymorphism of *pvmdr1* gene of *P. vivax*, a total of 26 *P. vivax* isolates from Kawthaung District were investigated during transmission season of 2009. Polymerase chain reaction could detect *pvmdr1* Y976F mutation in 30.8% of isolates. Mean effective concentrations 90 (EC_{90}) of CQ was noted as 307.18 nM (95%CI, 162.46-580.84). Mean EC_{90} of CQ among isolates carrying *pvmdr1* Y976F was higher than that of wild types (444.14 nM vs. 281.87 nM). Minimum inhibitory concentration (MIC) of CQ and number of isolates harboring *pvmdr1* Y976F mutation were found to be directly associated (R^2=0.457). The findings of the study could report applicability of molecular tool in relation to *in vitro* test for monitoring of CQ-resistant vivax malaria.

INTRODUCTION

Plasmodium vivax is widely distributed among tropics and affecting 40% of the world's population which accounts for 147-436 million clinical infections each year [1]. Chloroquine (CQ) is the first-line treatment since 1946 though chloroquine-resistant *Plasmodium vivax* had been reported starting from 1989. CQ-resistant *P. vivax* was first reported from Papua New Guinea [2]. Reports from Indonesia [3, 4], Myanmar [5, 6], and India [7] confirmed the emergence of CQ-resistant vivax malaria. Development of dormant hypnozoite stages of *P. vivax* in the liver resulting in relapse few weeks after the initial episode is the major obstacle for control measures.

In vitro susceptibility assays provide an alternative mean of assessing drug susceptibility of Plasmodium spp. without confounding effect of host immunity, relapse, and reinfection [8]. Lack of continuous culture system for *P. vivax* is another obstacle to monitor CQ-resistant *P. vivax*. To better define the drug-susceptibility profile of *P. vivax*, a standardized *in vitro* assay was recently developed and validated [9, 10]. Using an *in vitro* susceptibility assay, a spectrum of chloroquine susceptibility in *P. vivax* can be defined. The findings of *in vitro* studies suggested *in vitro* test as useful tool in monitoring for the emergence of CQ-resistance [8].

The *in vivo* test fails to detect a reduction of drug sensitivity before the manifestation of overt resistance [11]. In 2005, the *mdr*-like gene in *P. vivax* was first identified and presence of two *pvmdr1* mutations at codon 976 and 1076 of the isolates from CQ-resistant *P. vivax* were found [12]. These two mutations were proposed as early molecular markers for CQ-resistant *P. vivax*. A study reported the association of *pvmdr1* Y976F mutation and 4-fold higher chloroquine IC50, and *pvmdr1* Y976F mutation

was reported as a useful tool to monitor the emergence of chloroquine resistance [10]. Usefulness of molecular markers may vary in different geographical areas and their validity needs to be evaluated before applying them into drug resistance surveillance [13].

Diagnosis of CQ-resistant *P. vivax* requires a proof of adequate compliance to and absorption of therapy, given under supervision or, ideally, by determination of the levels of drug in blood [14]. A recurrent parasitaemia should not occur within 35 days of standard CQ therapy, if so, it is resistant to ordinary lethal exposure of CQ. Therefore, recurrent parasitemia, regardless of their origin, within 35 days of CQ therapy supports a provisional diagnosis of resistance [1]. Vivax malaria has long been considered as benign but some recent reports on complicated and fatal forms of vivax malaria draw a great attention to monitor emergence of drug resistance in *Plasmodium vivax*. In the studies conducted in Thailand and Papua New Guinea (PNG), 21-27% of patients with severe form of malaria were due to *Plasmodium vivax* monoinfection [15]. CQ-resistant vivax has faster growth rate and found phenotypically correlated with severe falciparum malaria [16].

Therefore, the study was conducted with the objective to determine the *in vitro* CQ sensitivity of *Plasmodium vivax* and to find out the prevalence of the molecular marker and its association to CQ sensitivity status, in Myanmar.

MATERIALS AND METHODS

Study site and study period

The study was conducted in Kawthaung Township during malaria transmission season of 2009.

Sample collection

Two milliliters of venous blood were collected aseptically from a total of 26 *Plasmodium vivax* infected patients who met inclusion criteria after obtaining written informed consent.

One milliliter of collected sample was transferred onto 3M Whattman filter paper and then the filter paper was air dried and kept in self-sealed plastic bag individually. Collected filter paper samples were transported to Parasitology Research Division, Department of Medical Research (Lower Myanmar) and stored at -20°C till they were processed by molecular assay.

One milliliter of collected venous blood sample was transferred into sterile test tube containing 0.5 ml of Waymouth medium and 40 IU heparin, for *in vitro* chloroquine susceptibility test.

Inclusion criteria for sample collection

- Age over 6 months
- Monoinfection with *P. vivax* detected by microscopy
- Asexual parasite count >250/µl
- Axillary temperature ≥37°C or history of fever during 48 hr before recruitment
- Patients with no history of hypersensitivity to antimalarial drug
- Patients with negative pregnancy test or not lactating

Exclusion criteria

- Patient with concomitant medical illness
- Patient with mixed malaria infection
- Patient who does not give consent to participate in the study

Ethical approval

The study was approved by The Institutional Ethical Committee for Research Involving Human Subjects in 2002.

In vitro susceptibility test

In vitro susceptibility test for CQ was conducted according to method of Congpuong [17], using WHO pre-dosed microtitre plates. Different concentrations of chloroquine predosed in the plate were 20, 40, 80, 160, 640, and 1280 nM, respectively. The procedure in brief was to add 0.1 ml of parasitized blood to 1.9 ml of medium containing RPMI medium plus Waymouth

medium at ratio of 1:1. Fifty microlitre aliquots were dispensed into each scheduled well of microtitre plate. Then, the microtitre plate was incubated at 37.5°C±1°C for 36 hr applying candle jar method. After 36-hr incubation, thick smears were made from each well of plate. Microscopic differenttial counting of 200 asexual parasites of the smears made from different drug concentrations was performed. Effective concentrations were calculated using Probit calculus computer programme, provided by WHO. MIC (minimum inhibitory concentration) was defined as the minimum concentration of a drug at which more than 99% of parasites, relative to control, were inhibited from developing schizonts (parasites with six or more chromatin dots).

Molecular method

DNA was extracted from collected filter paper samples by applying Chelex method [18].

Polymorphism in *pvmdr1* gene was detected by polymerase chain reaction primer mismatch method described by Suwanarusk [10]. Primers used were *Pvmdr* 976F (5'-GGA TAG TCA TGC CCC AGG ATT G), *Pvmdr* 976R (5'-CAT CAA CTT CCC GGC GTA GC), and *pvmdr* Internal (5'-CGG CTG TAC TGA CCG GAA CGT A). A fifty microliters of PCR premix was prepared containing 10x PCR buffer, 2.5mM $MgCl_2$, 0.20 mM of each dNTP, 1µM each primer and 1.25 U of Taq DNA polymerase and 1 µl of genomic DNA. Mutant and wild controls were included in each PCR reaction. The reaction was performed at following cycling condition; 95°C for 10 min. and 40 cycle of at 94°C for 40 second, at 55°C for 1 min. and at 72°C for 2 min. PCR products then underwent agarose gel electrophoresis and examined under UV light by BioDot documentation system. Discrimination of wild *pvmdr1* Y976 and mutant *pvmdr1* F976 was made based on the band size. Mutant alleles show single band of 560 base pair and wild-type alleles show double bands having 560 base pair and 400 base pair.

Data analysis

Data analysis was made by applying SPSS version 12.0.

RESULTS

In vitro sensitivity status of Plasmodium vivax isolates

A total of 26 criteria-matched *P. vivax* isolates were investigated for their sensitivity status against chloroquine using WHO microtiter plates predosed with CQ. Mean effective CQ concentration 90 (EC 90) of 26 tested isolates was found to be 307.1884 nM (95% CI, 162.4631-580.8377).

Prevalence of pvmdr1 976 alleles

Polymerase chain reaction detected *pvmdr1* Y976 (wild type) in 69.2% (18 out of 26 isolates) and *pvmdr1* F976 (mutant type) in 30.8% (8 out of 26 isolates) (Plate 1).

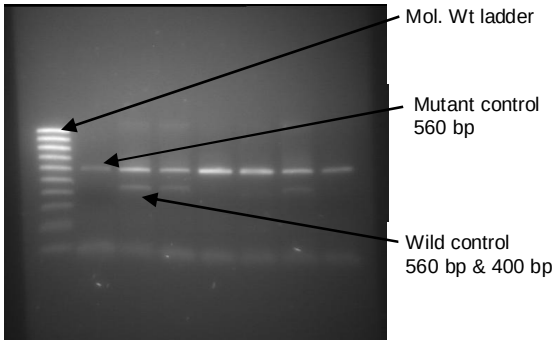

Plate 1. Photographic documentation of *pvmdr1* 976 alleles on agarose gel electrophoresis

Distribution of pvmdr1 976 alleles in MIC (minimum inhibitory concentration) status

More than 50% of all isolates showed growth inhibition at the MIC level of 320 nM. Most of the mutant isolates (63.33%; 5/8 mutant isolates) were distributed in the higher MIC level (>320 nM) (Table 1).

Mean EC90s (effective concentration 90) of chloroquine against pvmdr1 976 alleles

Mean EC90 of chloroquine against *P. vivax* isolates harboring *pvmdr1* F976 (mutant type) was noted to be 1.6 times higher than that of wild type isolates (Table 2).

Table 1. Distribution of *pvmdr1* 976 alleles in different chloroquine MIC status

Pvmdr1 976 allele	MIC in nM of chloroquine					
	80	160	320	640	≥1280	Total
Wild type (no of isolates)	4	4	3	2	5	18
Mutant type (no. of isolates)	1	0	2	2	3	8
Total (no of isolates)	5	4	5	4	8	26

Table 2. Effective concentration of chloroquine against *Plasmodium vivax* isolates harboring different *pvmdr1* 976 alleles

Pvmdr1 976 allele	Mean EC90 of chloroquine in nM	95% confidence Intervals	
		Lower	Higher
Wild type (n=8)	281.8717	138.8607	572.1681
Mutant type (n=18)	444.1423	90.8351	2171.6533

Association between numbers of Plasmodium vivax isolates harboring different pvmdr1 976 alleles and chloroquine MIC status

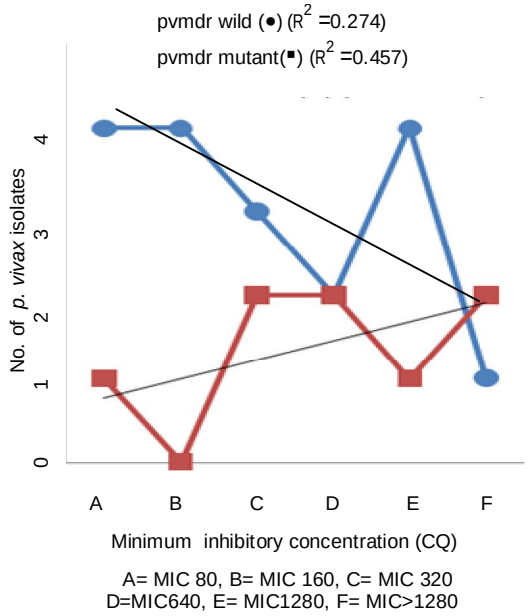

Fig. 1. Association between number of *P. vivax* isolates and chloroquine MIC

The number of *Plasmodium vivax* isolates harboring *pvmdr1* F976 muations was found to have direct linear association with chloroquine MIC status (R^2 = 0.457) (Fig. 1).

DISCUSSION

Antimalarial sensitivity of *P. vivax* is significantly influenced by initial parasite stage and duration of incubation. Regarding parasite stage, the ratio of ring to trophozoite (RT ratio) is negatively correlated with inhibitory concentration of chloroquine because of relative insensitiveness of trophozoite to CQ. Duration of incubation is also negatively correlated with inhibitory concentration [9].

In this study, the average proportion of ring stage parasites was 40% and incubation time was 36 hours. Mean effective chloroquine concentration 90 (EC90) of overall tested isolates was noted as 307.1884 nM (95% CI, 162.4631 - 580.8377). A study tested on *P. vivax* isolates from northwestern Thailand in 1998-99, reported mean EC90 as 358 nM. [11]. Another study from Thailand conducted in the same region in 2001 presented mean EC90 of 511 nM [20].

In Myanmar, *in vitro* drug testing system for *P. vivax* using WHO pre-dosed microtitre plates was first established in laboratory setting in 2002 and field applicability was tested on 19 *P. vivax* fresh isolates in Butheedaung Township in 2004 and mean EC90 of chloroquine was noted to be 107 nM (Ye Htut, unpublished data). This is the first report of *in vitro* sensitivity status of chloroquine against *P. vivax.*

Monitoring of *P. vivax in vitro* sensitivity in Thailand showed an approximate 4-fold decrease in CQ sensitivity in Thailand within a couple of decades [21]. Although the sites of two studies in Myanmar were not the same, CQ sensitivity status on *P. vivax* was found to be decreasing in parallel with therapeutic efficacy (107 nM vs. 307 nM). As CQ-resistance has been evident since 1993 in Myanmar, regular monitoring of drug-resistant *P. vivax* is strongly recommended to track the spread of drug resistance. Among tested isolates, 30.8% (8 out of 26 isolates) harbored *pvmdr1* F976 (mutant type) while 69.2% (18 out of 26 isolates) harbored wild type.

Regarding prevalence rate, a wide range of rates has been reported by different studies.

The prevalence rate of mutant *pvmdr1* 976F was reported as 39% of isolates from PNG [19]. A Thailand study investigated a limited number of isolates and found mutant *pvmdr1* in 3 out of 5 Myanmar isolates and 1 out of 11 Thai isolates [22]. *Pvmdr 1* mutation was found in 93% of Papuan isolates and 25% of Thai isolates [10]. The discrepancy in mutation rates may be due to the geographical origin of isolates tested, having different CQ sensitivity status and different degrees of drug pressure.

Results of *in vitro* drug sensitivity test are usually presented with IC (inhibitory concentration) or EC (effective concentration). In this study, EC values calculated by Probit calculus computer programme were presented. Decreased sensitivity of CQ was observed among mutant *P. vivax* isolates (n=8) in this study. Mean EC90 (90% effective concentration) of the *P. vivax* isolates harboring mutant *pvmdr1* 976F was found 1.6 times higher than that of wild type (*pvmdr1* 976Y) carrying isolates. Similar finding on reduced CQ sensitivity of mutant isolates (*pvmdr1* 976Y) than wild type was reported by a Thailand study [23].

Chloroquine resistance can occur in *P. vivax* isolates harboring wild type (*pvmdr1* 976Y) and clinical cure can be achieved in mutant isolates [15]. Among mutant isolates, 3 out of 8 showed lowest MIC while 5 out of 18 wild isolates showed highest MIC in the study. This finding supported the above statement.

Molecular assay using genetic marker is an important tool for antimalarial resistance monitoring [8]. Usefulness of molecular markers may differ for different geographical areas and their validity needs to be evaluated before applying them into drug resistance surveillance [16]. Association of *pvmdr1* 976 allele and *in vitro* CQ susceptibility was first reported by Suwanarusk [10]. A total of 81 *P. vivax* isolates from Thailand and 45 isolates from Papua, Indonesia were included in that study and *pvmdr1* 976F was proposed as useful tool for chloroquine-resistant *P. vivax.*

In this study, a moderate linear association was found between the number of *P. vivax* isolates harboring *pvmdr1* 976F muations and chloroquine MIC status (R^2=0.457). The more the mutant isolates, the higher the MIC of chloroquine is. It states that as the number of mutant isolates increases, the higher concentration of chloroquine is required to kill the parasites.

Linking molecular tool with *in vitro* susceptibility tests would be of value to map out drug-resistant *Plasmodium vivax* particularly chloroquine resistance.

The report of the study is the first step towards monitoring and surveillance of drug-resistant *P. vivax* malaria emergence and spread.

Conclusion

This study is the first report on *in vitro* susceptibility status of *Plasmodium vivax* in Myanmar. It could provide the molecular evidence of chloroquine resistance in *P. vivax,* in terms of *pvmdr1* 976F mutation which has been proposed as marker of CQ-resistant *P. vivax.* The results also proved the direct association between the number of isolates bearing *pvmdr1* 976F mutation and minimum inhibitory concentration of chloroquine

Recommendation

1. Chloroquine sensitivity of *Plasmodium vivax* should be monitored regularly by means of both *in vivo* and *in vitro* tests.
2. Field as well as laboratory-based research works focusing on emergence and spread of CQ-resistant vivax malaria should be encouraged.

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Body composition of Myanmar elderly people from home for the aged (Hninsigone), Yangon

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To find out body composition of Myanmar elderly people, the study was conducted on 154 (67 males and 87 females) apparently healthy elderly people, age ranged from 70 to 103 years at the home for the aged (Hninsigone), Yangon. Body composition was measured using skins-fold thickness. Body fat percent was calculated by equation described by Durnin and Rahamen (1967). Mean and SD of Body Mass Index (BMI) of elderly males and females were 19.18 ± 2.75 and 20.48 ± 4.03 respectively. 22.38% of males and 19.54% of females were found to have BMI less than17, and 6.2% of females had BMI more than 25. The older the age, the higher the reduction was observed in body fat percent, fat mass and fat free mass. Significant difference was evident in fat mass of females and fat percent of males. However, elderly people with low BMI (BMI<17) had significantly lower body weight, fat percent, fat mass and fat free mass than those of their counterparts with normal BMI.

INTRODUCTION

In many Southeast Asian countries, continuous economic growth in recent decades has led to improved living condition for large proportion of the population. Partly this improvement has resulted in increased life expectancy and the proportion of elderly in the population has increased. Therefore, the health care of elderly people takes an important part in the national health program.

Changes in body composition such as Increase in fatness especially in central region increase the risk of atherosclerosis, hypertension, hypercholesterolemia and insulin resistant diabetes mellitus and insulin resistant diabetes mellitus and conversely, changes in body composition are secondary to many diseases such as acromegaly, postmenopausal osteoporosis, Paget's diseases etc.[1] Furthermore, change in body composition is an indicator for the assessment of nutritional status. A Body Mass Index (BMI) of less than 18.5 has been proposed to indicate chronic energy malnutrition (CEM) in adults whereas those with BMI lower than 17 have added health risk and reduction in physical work capacity [2]. On the other hand, a BMI of more than 25 is regarded as obesity.

In elderly people, there are many controversies regarding changes in body composition. It is generally accepted that age-related changes in body composition such as loss of fat free mass with increase in total and central fatness occur in elderly people [3]. Reduction in fat free mass and increase in fat mass have considerable health have implication by contributing to frailty and functional impairment [4].

However, the two longitudinal studies of changes in FFM suggested lower rate of changes than observed in the cross-sectional studies [5, 6]. Furthermore, changes in body composition that are associated with increasing age may not be related and the levels of physical activity are important in determining body fat accumulation [5]. In addition, the composition (the mixture of FM and FFM on the frame) can differ by ethno-geographic situation e.g. for height-matched Asians and Caucasians, the latter have higher body mass indices but the former have more total body fat [1].

Since little information is available on the body composition of Myanmar elderly people, the present study is a preliminary one aimed at finding out the body composition of Myanmar elderly people.

MATERIALS AND METHODS

Subjects

The study was conducted on 154 (67 males and 87 females) apparently healthy elderly people, age ranged from70 to 103 years at the home for aged (Hninsigone), Yangon. None of the subjects was suffering from over diseases and all were able to walk and dress unaided.

Anthropometric and body composition assessment

Body weight was measured with bathroom scale weighing machine, calibrated with standardized weight. Subjects were taken two hours after breakfast and the weight was recorded to the nearest 0.1 kg.

Standing height was measured to the nearest 0.1 cm using a stadiometer. Subjects stood barefoot on a flat horizontal surface, their heads held in the Frankfurt plane and with their heels, buttocks and shoulders touching the wall.

Body Mass Index (BMI) was calculated as weight/height^2 (kg/m^2)

Body composition was assessed through measurements of skin-fold thickness. Skin-fold thickness at sites of biceps, triceps, sub-scapular and supra iliac was measured on the left side of the body using a Harpenden caliper by a well-trained technician. Body fat percent was calculated by using the formula described by Durnin and Rahaman, 1967 [7].

Body fat %=(4.95/Y-4.5) ×100
Y=1.1610- 0.0632 X in men
Y=1.1581- 0.0720 X in women.
(Y is body density and X is the log of the sum of skin-fold thickness at all four sites in mm)

Statistical analysis

Data were expressed as mean ± SD. Comparison were made using Student 't' test for unpaired samples (two tailed). Differences were considered significant if $p<0.05$.

RESULTS

Table 1 shows comparison of body composition of elderly people with that of middle-aged people [8]. Old-age people had lesser height, weight, and body fat percent, fat mass and fat free mass than the middle-aged people.

Table 2 shows body composition of elderly males at different ages. The older the age, the higher the reduction was observed in body fat percent, fat mass and fat free mass. Significant difference was evident in fat percent.

Table 3 shows body composition of elderly females at different ages. The changes were similar to those of the males but significant difference was evident in fat percent and fat mass.

Table 1. Comparison of body composition of elderly people with that of middle-aged people

Sex	No.	Age (yr.)	Height (cm.)	Weight (kg.)	Fat (%)	Fat mass (kg.)	Fat free mass (kg.)	Body Mass Index
Male	294	49.92 ± 6.06	162.35 ± 5.73	57.32 ± 12.24	14.18 ± 6.10	9.10 ± 5.90	48.25 ± 8.35	21.70 ± 4.18
	67	81.03 ± 5.05	157.64 ± 6.30	47.82 ± 8.05	18.93 ± 4.37	9.42 ± 3.50	38.70 ± 5.10	19.18 ± 2.75
Female	329	48.99 ± 5.65	151.48 ± 5.67	52.44 ± 12.39	27.77 ± 6.56	15.41 ± 7.11	37.02 ± 6.66	22.75 ± 4.74
	87	80.85 ± 5.55	145.52 ± 5.63	43.29 ± 8.63	29.80 ± 4.53	13.19 ± 4.31	30.10 ± 4.67	20.48 ± 4.03

Table 2. Body composition of elderly males at different ages

Age groups	All	70-79 yrs	80-89 yrs	90-above
No. of subjects	67	27	37	3
Height (cm)	157.64 ± 6.30	159.01 ± 6.36	156.62 ± 6.25	157.3 ± 3.15
Weight(kg)	47.82 ± 8.05	50.14 ± 6.89	46.45 ± 4.83	43.83 ± 3.70
Body Mass Index	19.18 ± 2.75	19.85 ± 2.66	18.83 ± 2.77	17.58 ± 1.49
Body Fat Percent	18.93 ± 4.37	20.51 ± 3.88	18.21 ± 4.33	15.27 ± 2.67
Fat Mass (kg)	9.42 ± 3.50	10.43 ± 3.11	8.89 ± 3.65	6.77 ± 1.54
Fat Free Mass (kg)	38.7 ± 5.10	39.71 ± 4.71	37.82 ± 5.40	37.07 ± 2.33

Table 3. Body Composition of elderly females at different ages

Age groups	All	70-79 yrs	80-89 yrs	90-above
No. of subjects	87	37	46	2
Height (cm)	145.52 ± 5.63	147.51 ± 5.16	143.92 ± 5.54	143.65 ± 3.55
Weight(kg)	43.29 ± 8.63	45.52 ± 8.79	41.77 ± 8.10	34.75 ± 0.75
Body Mass Index	20.48 ± 4.03	20.97 ± 4.18	20.22 ± 3.88	16.89 ± 1.20
Body Fat Percent	29.80 ± 4.53	31.33 ± 3.90	28.81 ± 4.56	22.69 ± 0.54
Fat Mass (kg)	13.19 ± 4.31	14.35 ± 4.34	12.33 ± 4.17	7.88 ± 0.02
Fat Free Mass (kg)	30.10± 4.67	31.05± 5.03	29.44± 4.25	26.87± 0.77

Table 4. Incidence of elderly people with BMI <16, 16-16.9 and 17-18.4 in different age groups

Age(yr)	70-79	80-89	90 and above
BMI (Male)			
17-18.4	18.52%	21.62%	
16-16.9	3.70%	10.81%	66.67%
<16	3.70%	18.92%	
BMI (Female)			
17-18.4	15.39%	13.04%	50%
16-16.9	7.69%	8.70%	
<16	5.13%	15.20%	50%
>25 - <30	7.69%	6.52%	
>30	5.13%	2.17%	

Table 5. Comparison of body composition of elderly from different ethnic groups

Ethnic groups	No. of subjects	Age (Yr)	Weight (kg)	Body fat (%)	Fat mass (kg)	Fat free mass (kg)
Male						
Caucasian(11)	15	65 - 72	75.40 ± 2.50	28.00 ± 1.50	21.50 ± 1.70	53.90 ± 1.30
Chinese(10)	64	70 - 74	55.61	29.31	16.30 ± 5.30	39.31 ± 5.83
Myanmar	67	71 - 92	47.82 ± 8.05	18.93 ± 4.37	9.42 ± 3.50	38.70 ± 5.10
Female						
Caucasian(12)	10	68 - 79	60.00 ± 7.22	21.70 ± 3.90	21.70 ± 3.90	38.30 ± 4.40
Chinese	95	70 - 74	49.86	34.61	17.26 ± 6.24	32.60 ± 4.67
Myanmar	87	70 - 103	43.29 ± 8.63	29.80 ± 4.53	13.19 ± 4.31	30.10 ± 4.67

Table 4 shows incidence of elderly people with BMI 17 to 18.4, 16 to 16.9, <16, .25 and >30 in different age groups. Some elderly women, not elderly men, had BMI. 25 and >30.

Table 5 shows comparison of body composition of elderly from different ethnic groups. Although height, weight, fat mass and far free mass were lower in Chinese and Myanmar elderly than those of Caucasians, body fat % was higher in Chinese and Myanmar elderly women than that of Caucasians.

DISCUSSION

It was found that Myanmar elderly people had lower body weight, height and fat free mass but higher body fat percent when compared with those of middle-aged people. This finding was in agreement with the concept that age related changes in body composition were loss of fat free mass with increase in total and central fat. However, when the subjects were divided into three groups (i.e. 70-79 years, 80-89 years and their 90 years & above) and compared their body weight, fat percent, fat free mass, all reduced with increasing age.

BMI is an early measurable nutritional status indicator for adult and BMI of <8.5 has been proposed to indicate chronic energy malnutrition (CEM). In the present study, 41.79% had a BMI <18.5 and 22.38% had a BMI <17. These findings were a little higher than those found in Indonesian urban elderly where 33% had a BMI <18.5 and 15% had a BMI <17 [9]. Considering high prevalence of low BMI individuals, CEM may be a public health problem among elderly or alternatively, the BMI cut-off point to define CEM may not be valid for elderly in Myanmar. Woo *et al*. reported that the BMI of elderly Chinese was lower than that of other elderly with different ethnic backgrounds, namely Caucasian, Maori and Scandinavian. The fiftieth percentile of BMI values for the Chinese elderly fell on the twenty-fifth percentile BMI values for the Caucasian elderly in United Kingdom [10]. Therefore, many researchers recommended conducting further investigation of general applicability of the BMI classification for different populations

Wang *et al.* reported that, despite lower BMI values, Asians had higher body fat percent than did Caucasian [6]. In the present study, Myanmar elderly woman also had higher body fat percentage than Caucasians but Myanmar elderly men had lower body fat percentage.

When comparison was done between elderly with normal BMI and elderly with BMI less than 18.5, body weight, body fat percent, fat mass and fat free mass of normalelderly were significantly greater than those of elderly with low BMI value.

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Association of chronic complications of diabetes mellitus and presence of risk factors

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Chronic complications of diabetes are known to be associated with certain risk factors. Once complications develop, they will never regress, denoting that reduction of these factors as much as possible, is the best way to deter the complications. To determine such risk factors, a study was done on diabetic patients with a sample size of 315 from Diabetic Clinic and Wards 1+2, Yangon General Hospital. There were 281 NIDDM and 341 IDDM patients. The study on NIDDM patients shows that physical inactivity and age of the patients were associated with the development of retinopathy. The following factors were identified as being associated: hypertension, high serum cholesterol and age of the patient. Smoking is also associated with neuropathy. The possible risk factors for nephropathy were age of patient, hypertension, alcohol and hyperuricaemia. Hypertension, age of the patient, male sex and physical inactivity were found to be important risk factors for CVA. For IHD, contraceptive pills, physical inactivity and age of patients were possible risk factors. On the other hand, physical inactivity, hypertension, age of patient and duration of disease were important for peripheral vascular disease. Out study spotlighted that age factor is the mainstay in the development of chronic complications in both NIDDM and IDDM. In NIDDM Physical inactivity predisposes to retinopathy and atherosclerotic complications. Hypertension enhances neuropathy, nephropathy, CVA and peripheral vascular disease. However, in IDDM, the duration of diabetes and fasting blood sugar levels are significant risks in the development of retinopathy and nephropathy.

INTRODUCTION

Diabetes mellitus is a disease of metabolic dysregulation of glucose, accompanied by characteristic long term complications. The complications that are specific to diabetes include microvascular complications like retinopathy, nephropathy and neuropathy. Diabetes is also accompanied by substantial increase in macrovascular atherosclerotic disease of large vessels, including cardiac, cerebral and peripheral vascular disease. Progression of these complications represents a major threat to the mortality and morbidity of diabetes, with loss of life, limb or vision. New possibilities have appeared for limiting the progression of these complications. Evidence suggest that improved control of the diabetic state and alteration of risk factors will reduce the complications.

Effectiveness of methods of prevention and treatment of complications depends upon their timely application. Therefore it is crucial for early identification of risk factors and removal or modification of these if possible.

The aim of this study was to analyse several clinical and biochemical parameters,

1. to identify those subsets of patients likely to develop these complications
2. to elucidate whether the incidence of diabetes complications were related to any of twelve variables (risks).

MATERIALS AND METHODS

A total of 315 diabetic patients of the diabetes clinic and in-patients of wards 1 & 2, Yangon General Hospital were included in this study over a three year period starting from first January 1991. A complete history and physical examination including neurological examination and dilated ophthalmoscopy was performed. Laboratory workup included blood sugar estimation, serum cholesterol, urea, uric acid, urinalysis, ECG, and CXR. Patients with chronic complications were correlated with twelve clinical laboratory variables. Data were analysed statistically using the Chi square test, Student's 't' test and Odds ratio.

RESULT

1. Age and Sex Distribution: in NIDDM, the female male ratio was 1.9:1. In IDDM, the male female ratio was 1.6:1. In NIDD, mean age of males was 59.34 years and females 54.72 yr. In IDDM, mean age of males was 27.85 years and females 28.3 years.
2. Mean weight in pounds in NIDDM: was 125.7 lbs (95% CI 123.31-128.09) accounting for 30.8% being obese.
3. Duration of diabetes in years: In NIDDM ranged from 1-20 years with mean 4.44 ± SE0.30. In IDDM ranged from 1-4 years with mean 2.7 ± SE0.41.
4. Age of onset: In NIDDM ranged from 30 to 78 years with mean 48.66 ± SE0.66. In IDDM ranged from 13 to 37 years with mean 25.25 ± SE1.80.
5. A family history of diabetes: In one or both parents was found in 20.64% of NIDDM and 23.58% of IDDM, overall in 20.9% of patients.
6. Race 69.2% in Myanmar, 22.85% were Indians and 4.18% were Chinese.
7. Complications (Table 1): 72.95% of NIDDM and 35.29% of IDDM had complications. Among patients with NIDDM, commonest complications were hypertension 46.62% neuropathy 33.45%, ischaemic heart disease 28.83% and retinopathy 20.28% and neuropathy 19.93%. Among patients with IDDM, the commonest complications were neuropathy 14.71%, retinopathy 11.76%, nephropathy and hypertension 8.82%.
8. Risks: In NIDDM, significant risks for RETINOPATHY were physical inactivity (Odd ratio 4.25) and age of patient ($p < 0.025$) (Fig. 1). In NIDDM, significant risks for NEUROPATHY were hypertension (OR 2.20), hypercholesterolaemia (OR 4.41), smoking (OR 1.96) and age ($p < 0.001$) (Fig. 2). In NIDDM, risks factor for NEPHROPATHY were hypertension (OR 3.32), alcohol (OR 2.18), hyperuricaemia (OR 2.78) and age ($p < 0.001$) (Fig. 3). Significant risk factors for atherosclerotic complications. Risks for cerebrovascular accidents were hypertension (OR 3.58), physical inactivity (OR 4.08), age ($p<0.01$) and male sex (OR 2.47) (Fig. 4). Risks for peripheral vascular disease were hypertension (OR 2.41), physical inactivity (OR 4.56) and age ($p < 0.01$).

Table 1. Percentage distribution of chronic complications in diabetes

	NIDDM (281)				IDDM (34)			
	Male	Female	Total	%	Male	Female	Total	%
Retinopathy	18	39	57	20.28	3	1	4	11.76
Background	16	33	49	17.44	3	1	4	11.76
Proliferative	2	4	5	1.78	0	0	0	0.00
Advanced	1	2	3	1.07	0	0	0	0.00
Atherosclerosis	58	107	165	58.72	4	2	6	17.65
Hypertension	45	86	131	46.62	2	1	3	8.82
I.H.D.	25	56	81	28.83	0	1	1	2.94
P.V.D.	5	13	18	6.41	0	1	1	2.94
C.V.A.	13	10	23	8.19	0	0	0	0.00
Neuropathy	36	58	94	33.45	3	2	5	14.71
Nephropathy	24	32	56	19.39	0	3	3	8.82
Anyone or more	70	135	205	72.95	8	4	12	35.29

I.H.D. = Ischaemic heart disease
P.V.D.= Peripheral vascular disease
C.V.A.= Cerebrovascular accident

Risk for ischaemic heart disease were OC pills (OR 1.12), physicial inactivity (OR 7.08) and age ($p < 0.01$).

Significant risk factors for complications in IDDM (Fig. 5). Risk for NEPHROPATHY were duration of diabetes ($p < 0.05$), age ($p < 0.05$) and fasting blood sugar levels ($p < 0.001$). Risks for RETINOPATHY were family history (OR 4.0), smoking (OR 2.75) alcohol (OR 5.0), age ($p < 0.001$), duration of diabetes ($p < 0.05$) and fasting blood sugar ($p < 0.005$).

DISCUSSION

There was predominance of NIDDM 89.2% in our study. This is comparable to a study in primary health care setting by Lee in Singapore where 91.5% were NIDDM [1]. In an Audit of diabetes in General Practice in Bristol by Kemple 75.8% were NIDDM [2].

In NIDDM patients the female male ratio was 1.9:1 comparable to Sudan study by Elmahdi with identical ratio [3]. Mean age of NIDDM was 57.03 years while mean age of IDDM was 28.3 years. 30.8% of NIDDM were obese.

Duration of diabetes in NIDDM ranged from 1 to 20 years while in IDDM ranged from 1 to 4 years.

A family history of diabetes was present in 20.9%, 69.2% of patients were Myanmar.

72.95% of NIDDM and 35.29% of IDDM had complications. In NIDDM the commonest complications were hypertension 46.62%, neuropathy 33.45%, ischaemic heart disease 28.83%, retinopathy 20.28%, and nephropathy 19.93%. In Primary health care setting in Singapore by Lee [1], hypertension was found in 38% of diabetics. Comparable results were found in Sudan study by Elmahdi [3] where commonest complication was neuropathy 31.5% followed by retinopathy 17.4%.

Significant risks for retinopathy in NIDDM in our study was increasing age and physical inactivity. Chen reported that risk of retinopathy correleated with duration of diabetes and age onset and higher serum creatinine levels [4].

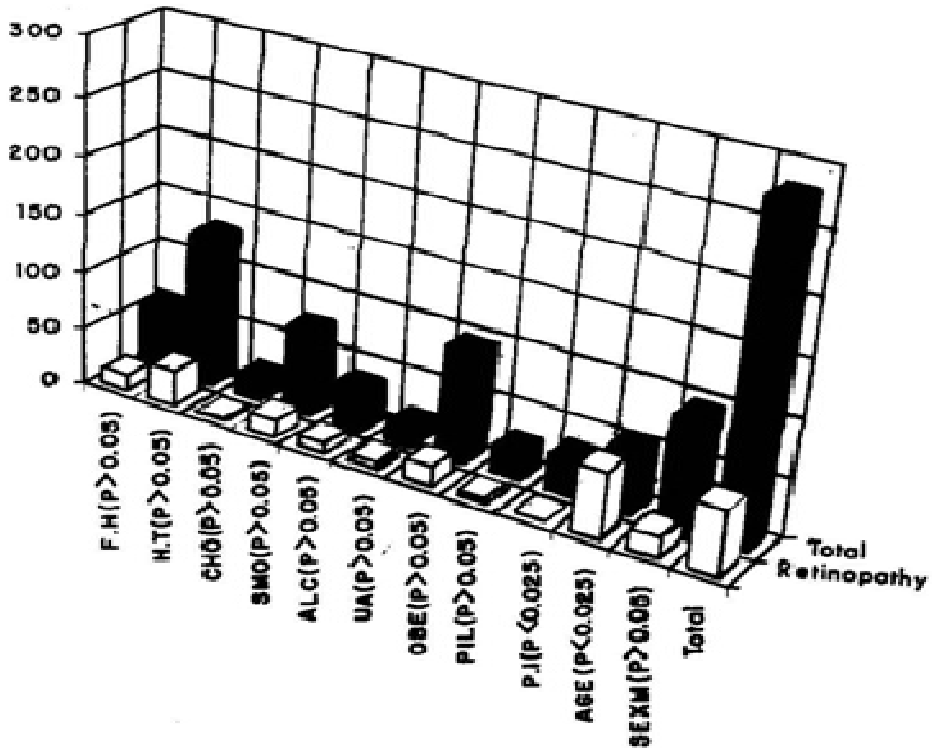

Fig. 1. Presence of risk factors and retinopathy

FH = family history; HI = hypertension; CHO = hypercholesterolaemia; SMO = Smoking; ALC = alcohol; UA = hyperuricaemia; OBE = obesity; PIL = OC pill; PI = Physical inactivity

Duration of diabetes was the most important risk, supported by the Wiscosin Epidemiologic study of Diabetic Retinopathy [5]. Risks for neuropathy in NIDDM in our study were hypertension, hypercholesterolaemia, smoking and age. Said reported that the most important risk for neuropathy was longer duration of diabetes [6].

Risks for nephropathy in NIDDM in our study were hypertension, alcohol, hyperuricaemia and age. Our understanding of nephropathy in NIDDM is complicated by uncertain duration of NIDDM, and high prevalence of coexisting hypertension. Babolola reported that diabetes with hypertension had a greater degree of proteinuria [7].

Risks for CVA cerebrovascular accident (disease) were hypertension, physical inactivity, age and male sex. Kuller reported that there is a five fold risk of CVA in diabetics and hypertension is a major risk factor [8].

Risks for peripheral vascular disease in NIDDM were hypertension, physical inactivity and age. Alcolodo reported that hypertension and dyslipidaemia are important risks for peripheral vascular disease in, NIDDM [9]. Migdolis reported that risks of peripheral vascular disease is related to duration of disease [10].

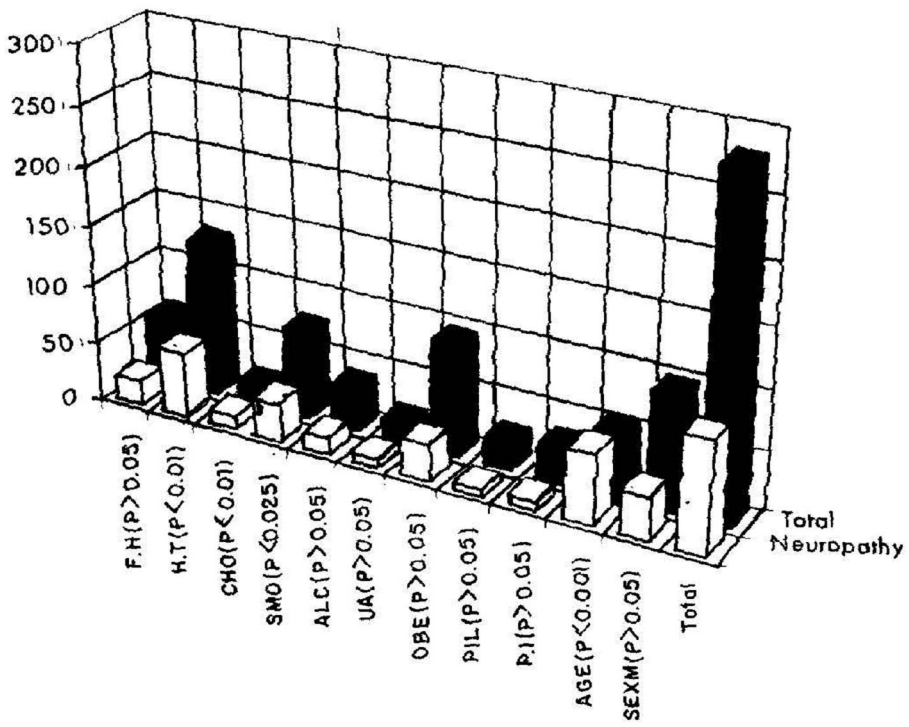

Fig. 2. Presence of risk factors and neuropathy

Risks of ischaemic heart disease in NIDDM were OC pill, physical inactivity and age. Ford reported that coronary artery disease is leading cause of mortality among diabetes [11].

Ford reported that age, male sex, current smoking, hypertension and physical inactivity were associated with all cause mortality. Singer reported that the level of chronic glycaemia, determined by the measurements of glycosylated haemon-globin is an independent risk factor for coronary artery disease [12].

In IDDM significant risks for retinopathy in our study are age, duration and fasting blood sugar. Similarly Krowleski reported that developement of retinopathy in IDDM depends on duration of disease [13]. Klein reported that there is a relation between level of glucose control and prevalence and incidence of retinopathy [14].

Risks for nephropathy in IDDM were age, duration of disease and fasting blood sugar. In support of our findings Monske reported that there is association of both higher Haemoglobin AIC levels and number of years of diabetes in patients with nephropathy [15]. Beyer reported that in high risk group early identification of nephropathy included assessing glycaemic control, mean arterial pressure, urinary albumin excretion rate and glomerular hyperfiltration [16].

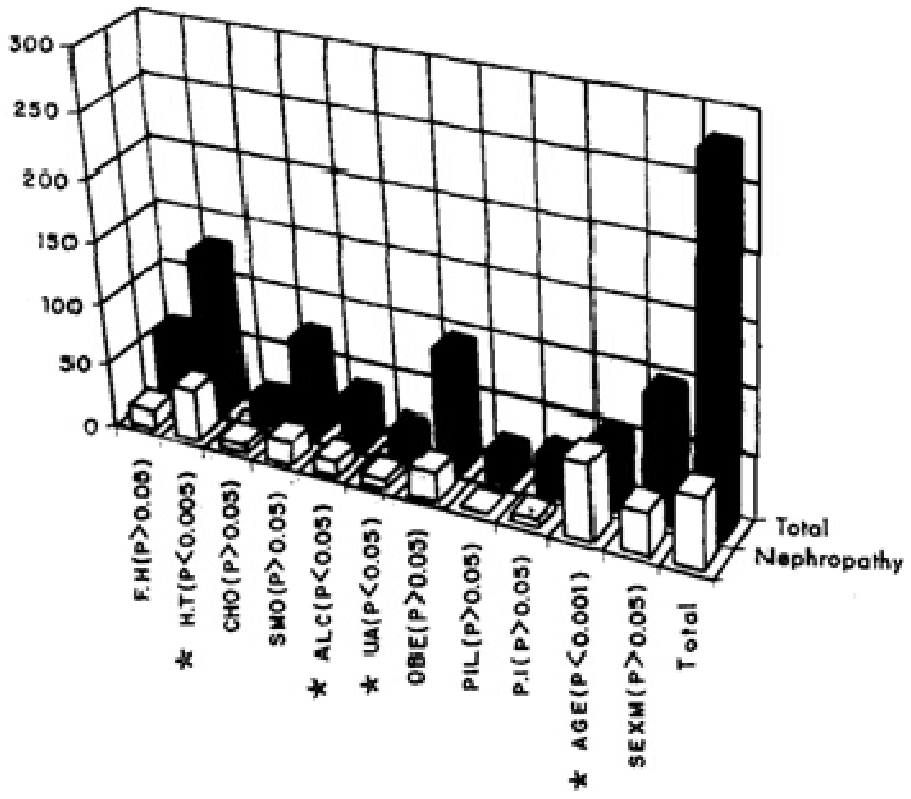

Fig. 3. Presence of risk factors and nephropathy

*Risk factors

CONCLUSION

Our study highlighted that age ie. (increasing age), indirectly reflecting duration of diseases, is the main risk factor in the development of chronic complications in both NIDDM and IDDM. In NIDDM, hypertension enhances both micro and macrovascular complications and should be strictly controlled. In IDDM, the age, duration of disease and fasting blood sugar levels are significant risks in development of nephropathy and retinopathy. Thus effective glycaemic control is essential in their management.

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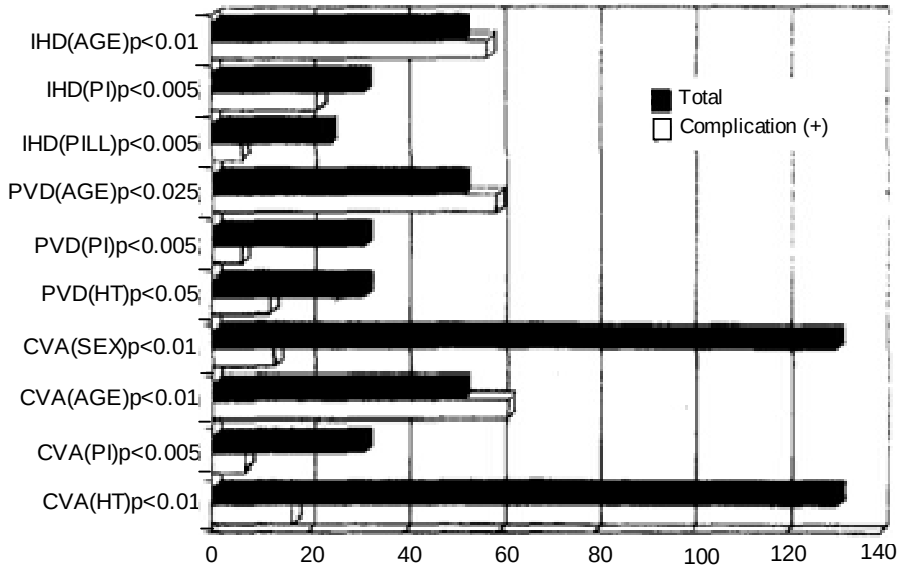

Fig. 4. Significant risk factors for atherosclerotic complications

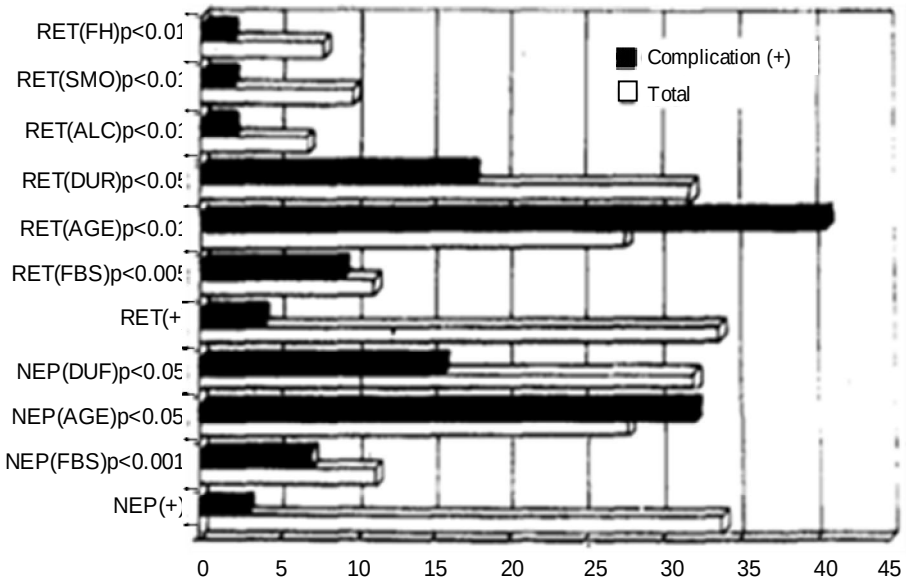

Fig. 5. Significant risk factors for complications in IDDM

RET = retinopathy; NEP = nephropathy; DUR = duration of diabetes; FBS = fasting blood sugar

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Role of intramuscular anti-snake venom administration as a first-aid measure in the field

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Effectiveness of intramuscular (i.m) anti-snake venom (ASV) administration immediately after bite as a first-aid measure in the field followed by standard hospital treatment in the management to Russell's viper bite patients was studied in 12 victims and was compared with that of standard hospital management alone in 82 victims. There was a marked reduction in the number of patients with systemic envenomation ie. disseminated intravascular coagulation (DIC), clinical proteinuria, oliguric acute renal failure (ARF), systemic bleeding, hypotension and fatality rate of Russell's viper bite victims who received initial i.m ASV prior to the hospitalization compared with those who did not. It is recommended that i.m ASV could be administered to the Rusell's viper bite patients at the site of incident as a first-aid method in places where no facility for giving intravenous ASV therapy prior to hospitalization.

INTRODUCTION

Vast amount of research and clinical trial on Russell's viper (Daboia russelli siamensis) bite envenomation have been conducted by many researchers and authorities concerned from Ministry of Health in order to reduce morbidity and mortality of snake bite in Myanmar. In spite of the availability and wide-spread use of potent anti-snake venom (ASV) produced by Myanmar Pharmaceutical Factory (MPF), snake bite fatality rate still remains considerably high in our country. One of the reasons is delayed in getting antivenom probably due to late arrival to hospital since almost all the snake bite cases occur in paddy fields which are far away from hospital and where an immediate administration of intravenous (i.v) antisera is impossible. Therefore, an alternative measure to i.v ASV therapy is the intramuscular (i.m) injection of ASV, to be given immediately after bite at the site of incident prior to hospitalization as a first-aid measure in situation where i.v adminstration is impossible or transportation to hospital is likely to be delayed. Preliminary studies on i.m administration of ASV on experimentally envenomed mice and rats revealed that immediate administration of antisera by i.m route could reduce the lethality [1, 2]. A study on clinical trial of i.m antisera administration as a first-aid measurein the field in Thayawady Township, Bago Division, Myanmar during 1991-1994 showed that there was a definite reduction in the number of patients with systemic envenomation, complications and fatality in 34 Russell's viperbite victims who had received i.m ASV prior to ospitalization [3]. Therefore this promising study was extended as a research-cum-action program in other areas of Myanmar where snake bite morbidity and mortality rates are high.

EXPERIMENTAL DESIGN AND METHODOLOGY

The study was carried out in Meiktila Township, Mandalay Division for 2 successive years, 1994 and 1995 with the active participation of the Basic Health Staff (BHS) in the field and Medical Officers in Meiktila District Hospital. The BHS from all villages served as a mobile task force prior to the snake bite season. They were trained for giving i.m ASV and provided with 3 pairs of lyophilised antisera, disposable syringes with needles and methylated spirit swabs. They were stationed at the respective village tract and were instructed to give a total of 10 ml ASV (5 ml to each buttock) intramuscularly to a victim within 2 hours after Russell's viper bite at the site of incident in the field. Those who received i.m ASV at the field and were transferred immediately to the district hospital for further standard hospital management were regarded as "test cases". Those victims who did not receive i.m ASV were regarded as "control cases". Patients of both groups were given the same hospital management and were kept in the hospital for a minimum of 5 days. Blood and urine samples were taken at the time of admission, and then daily for 5 days to detect development of disseminated intravascular coagulation (DIC) and clinical proteinuria by 20 minute whole blood clotting test [4] and boiling test [5] respectively. The complications following Russell's viper envenomation such as oliguric acute renal failure (ARF), systemic bleeding, hypotension and final out-come were noted. Appropriate statistical tests such as Student's unpaired 't' test, Chi-square test and Fisher's exact test were used for analysis of data.

RESULTS

A total of 94 Russell's viper bite patients confirmed by snake seen and/or dead snake brought were avilable for the study. Of which 12 received 10 ml of i.m ASV at the field (test) and 82 did not (control). Comparison of bio-physical data of the two groups is shown in Table 1. The initial serum venom antigen levels of the subjects could not be determined in our study. As shown in Table 2, the number of patients who developed systemic envenomation (ie. DIC) was significantly less in the tests and the other parameters such as clinical proteinuria, oliguric ARF, systemic bleeding, hypotension, change from coagulable to in-coagulable state in hospital and death were also found to be markedly reduced in the tests compared to the controls.

Table 1. Bio-physical data of control and test cases

	Control n=82	Test n=12	Significance
Age (years)	32±1.63 (12-66)	29±4.42 (13-59)	NS*
Sex (M:F)	54:28	8:4	NS**
Snake length (mm)	378±34 (200-680)	345±58 (150-1200)	NS*
Distance from site of incident to hospital (miles)	8.3±1.04 (3-25)	10.7±2.15 (10-22)	NS*
Interval between bite and admission (hours)	2.3±0.24 (0.5-7.5)	2.9±0.35 (2.0-4.75)	NS*

- Each value represents the mean ± SEm.
- Values in the parenthesis indicate the ranges.
- Data between the two groups were compared using Student's unpaired 't' test* and Chi-square test**
- NS means not significant at the 5% probability level.

DISCUSSION

Although the majority of the juvenile or young snake bites result in blank bite and the snakes responsible for the bite in this study were juvenile snakes, their venom have a greater lethal, defibrinogenating and edema-inducing activities than adult viper consequently leading to more severe envenomation [6].

Table 2. Clinical data between control and test cases

	Control n=82	Test n=12	Significance
DIC (incoagulable blood) (no.).	46 (56.1%)	1 (8.3%)	p<0.01
Clinical protein-uria(no.)	35 (42.7%)	3 (25%)	NS*
Oliguric ARF (no.)	23 (28.1%)	1 (8.3%)	NS*
Systemic bleeding (no.)	8 (9.1%)	Nil	NS**
Hypotension (no.)	7 (8.5%)	Nil	NS**
Change from coagulable to incoagulable state in hospital (no.)	1 (1.2%)	Nil	NS**
Death (no.)	7 (8.5%)	Nil	NS**

-Data between the two groups were compared using Chi-square test* and Fisher's exact test**.

-NS means not significant at the 5% probability level

Since initial venom antigen levels of the victims could not be determined, the true proportion of systemic envenomed cases could not be ascertained. Moreover, the lengths of the snakes of the control and the test were not significantly different in the study. There were also no significant differences in the other clinical criteria between two groups indicating the validity of experimental design (Table 1).

It is well known that administration of specific antisera in adequate dose intra-venously and immediately after the bite is an ideal and effective therapy for Russell's viper bite victims. However, i.v antisera could be given by trained medical personnel only at the hospital and at the later hours (ie. about 2-4 hours after bite). Therefore systemic envenomation and mortality resulted from Russell's viper bite remain high in our country. In our study, 10 ml i.m administration of antisera within 2 hours after the bite as a first-aid measure in the field to the Russell's viper bite victims could significantly prevent the onset of systemic envenomation. Besides, less number of patients developed clinical proteinuria and oliguric ARF. There were no cases of systemic bleeding, hypotension and death in those who received i.m ASV prior to hospitalization. Although most of the findings were not statistically significant, marked clinical significance was observed in the tests compared to the controls.

From our present and the past studies [3] it can be concluded that in the management of Russell's viper bite victims, administration of i.m ASV immediately after the bite may be of value as a first-aid measure in the field where i.v route of ASV is impossible or transport to hospital is likely to be delayed for more than 2 hours. Hence, these findings could be used in formulating national guide lines in the management of Russell's viper bite patients in Myanmar.

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Detection of *Mycobacterium leprae* by the polymerase chain reaction (PCR) in nasal swabs of leprosy patients and their contacts

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In the light of current leprosy control strategies, non-invasive samples such as nasal swabs may be more important than skin slit specimens as a source of material for epidemiological study. The objective of this research project was to investigate the use of a polymerase chain reaction (PCR) test to detect *M. leprae* in samples of nasal mucous from leprosy patients and asymptomatic household contacts of those patients. Nasal swabs from 15 paucibacillary (PB) and 55 multibacillary (MB) patients attending the Central Special Skin Center, Yangon General Hospital and 137 of their household contacts were tested for the presence of *M .leprae* by PCR and 33% of the samples of both patients and contacts were found to contain *M. leprae.* One of 32 (3.1%) swabs and 21 of 105 (20%) swabs were positive for *M. leprae* among contacts of PB and MB patients respectively ($p<0.05$). Among the patients, PCR positivity for nasal swab was 3 out of 15 (20%) in PB patients and 45 out of 55 (81.82%) in MB patients ($p<0.001$). Therefore, total 48 out of 70 (68.57%) in clinically diagnosed patients was PCR positive. PCR positivity of MB is significantly higher than PB in both patients and contacts. Although nasal carriage does not necessarily imply infection or excretion of bacilli, the finding of nasal carriage supports the theory of a disseminated occurrence of *M. leprae* in populations for which leprosy is endemic. This study is part of the molecular epidemiological study of leprosy in Myanmar.

INTRODUCTION

Following the introduction of the multidrug therapy (MDT) program in 1980, there has been a significant reduction in the estimated prevalence of leprosy worldwide, from around 12 million in early 1980, to approximately 1 million now. However, this decline in prevalence has not been mirrored by a concomitant fall in the observed incidence of the disease: over half a million new cases are still detected annually, a figure similar to that of 1985 [1]. This apparent discrepancy suggests that the widespread use of MDT is having little impact on the transmission of leprosy. Although the causative agent of leprosy, *Mycobacterium leprae*, has long been known, the exact mode of transmission of the disease remains to be fully elucidated [2]. There appears to be few natural animal hosts of this bacterium in endemic areas: human-to-human contact is, therefore, thought to play a major role in providing a reservoir of infection. In addition, the inability to culture *Mycobacterium leprae in vitro* makes assessment of subclinical infection rates difficult.

It is thought that the nose is the usual site of primary infection with *M. leprae*, as a result of airborne infection [3]. The advent of polymerase chain reaction (PCR) technology has afforded the opportunity to specifically detect small amounts of DNA, and a procedure, which indicate the presence of DNA equivalent to as few as 20 *M. leprae* cells, has been developed by Hartskeerl, *et al*. [4]. Studies using this technique have detected *M. leprae* DNA on

swabs taken from nasal mucosa of clinically normal individuals in family contacts of leprosy patients. The significance of the presence of such DNA is as yet unresolved, but may represent a form of sub-clinical infection or transient carriage of *M. leprae*, which may in turn be important in the transmission of the disease.

The technique described here is simple, sensitive, and specific for use in large-scale epidemiological studies. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination programs in countries where the disease prevalence has been significantly reduced. This study is part of the molecular epidemiological study of leprosy in Myanmar.

MATERIALS AND METHODS

Sample collection

Patients and contacts: After taken informed consent, nasal swab samples were collected from leprosy patients attending the Central Special Skin Center (CSSC), Yangon General Hospital and their family contacts. Fifteen PB and fifty-five MB patients with clinically and bacteriologically documented disease, as well as 137 household contacts (HHC) agreed to participate in the study. HHC were defined as persons sleeping during the night under the same roof. Leprosy patients were classified clinically and microscopically according to WHO classification [5] which consists of two categories, paucibacillary (PB) and multi-bacillary (MB). PB leprosy is defined as five or fewer skin lesions with no bacilli in skin smears, and MB leprosy cases have six or more lesions and may be skin smear positive.

Nasal swabs: Nasal swabs were taken by introducing cotton tip swabs (sterilized *JCB* MENTIP, Japan) 2-3cm into each nostril successively, and rubbing gently on the lateral and median sides of each cavity. Swabs were immediately chilled and transported to the Immunology Research Division, DMR (Lower Myanmar) and analyzed.

Specimen preparation

The collected nasal swab samples were dipped in 1.5 ml eppendorf tubes containing 1ml PBS with 0.05% Tween 20 to release the bacilli from the cotton swab by turning and squeezing, then subjected to high speed centrifugation 14,000 rpm for 10 minutes. The supernatant was discarded and the sediment was resuspended in 1 ml 70% ethanol and prepared for DNA extraction by Klaster's Method [6].

DNA template preparation (DNA extraction)

DNA was prepared from nasal swabs according to the method of Klatser *et al* [6]. Briefly, nasal swabs immersed in 70% ethanol were centrifuged at 14,000 rpm for 10 minutes.After discarding the supernatant, the precipitate was washed with PBS and centrifuged again at 14,000 rpm for 10 minutes to remove remaining alcohol. The washed precipitate was suspended in 50µl of lysis buffer containing proteinase K 10mg/ml in 1M Tris-HCL, pH 8.5 and 0.5% Tween 20 and incubated at 60°C for 18 hours. Five microlitre of mineral oil was over-layed microlitre to prevent evaporation of water from the mixture. After heating at 97°C for 10 minutes, the suspended solution was treated with freezing and thawing twice to extract DNA and also to inactivate proteinase K, which inhibits Taq polymerase during PCR.

The DNA polymerase chain reaction (PCR)

A set of primers (5'-AAA AAA TCT TTT TTA GAG ATA CTC GAG-3' and 5'-CAA GAC ATG CGC CTT GAA-3') was used for amplification of the specific region of *M.leprae* 16S rRNA gene. *M. leprae* chromosomal DNA was kindly supplied by Dr. M. Matsuoka, Leprosy Research Center, NIID, Japan. This DNA served as a positive control in all PCR experiments.

The 50 µl reaction mixture contained 10 µl of template solution, 0.2 µl of *Ex Taq* DNA polymerase (Takara Shuzo Co., Shiga, Japan), 1 µM of each primer, 5 µl of 10x DNA PCR buffer, 8 µl of dNTP solution and 25.8 µl of water. The reaction mixture was overlayed with mineral oil 5 µl. The reaction was performed with a Mastercycler personal Eppendof AG, Hamburg, Germany. The reaction mixture was heated to 94°C for 1 min, rounds of amplification consisted of a 30 second denaturation step at 94°C, a 2 min annealing step at 44°C and a 3 min elongation step at 72°C for 45 cycles. The amplified DNA fragments were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels in Tris-Borate EDTA (TBE) buffer.

RESULTS

A total of 70 leprosy patients (55 MB and 15 PB) attending the Central Special Skin Center (CSSC), YGH and 137 of their house hold contacts (HHC) were studied to detect *M. leprae* from nasal mucosa by PCR using the 16S ribosomal gene amplification. In 55 MB patients including both Bacillary Index (BI) positive and negative cases, 45 patients (81.82%) were PCR positive. Out of the 15 PB patients (BI negative) 3 cases were found to be PCR positive, that is 20% (Table 1). Table 2 shows 21 cases (20%) out of 105 contacts of MB cases were PCR positive and 1 (3.12%) out of 32 contacts of PB patients were PCR positive.

Table 1. Detection of *M. leprae* by PCR from nasal swabs in different types of leprosy

Types of Patients	PCR Results		Total
	Positive	Negative	
PB	3 (20%)	12 (80%)	15 (100%)
MB	45 (81.82%)	10 (18.18%)	55 (100%)
Total	48 (68.57%)	22 (31.43%)	70 (100%)

PB = Paucibacillary MB = Multibacillary
Pearson chi^2 (1) = 20.8988 Pr = 0.00001
Odds ratio = 18.0 95% CI =3.19-101.56

Table 2. Detection of *M. leprae* by PCR from nasal swabs in House Hold Contacts (HHC) of different types of leprosy

HHC in different types of patients	PCR Results		Total
	Positive	Negative	
HHC of PB	1 (3.12%)	31 (96.88%)	32 (100%)
HHC of MB	21 (20.00%)	84 (80.00%)	105 (100%)
Total	22 (16.06%)	115 (83.94%)	137 (100%)

HHC = House Hold Contacts, PB = Paucibacillary, MB = Multibacillary, Pearson chi^2 (1) =5.1812 Pr = 0.023, Odds ratio = 7.75, 95% CI =0.95-63

DISCUSSION

For more than a century, the excretion of *M. leprae* through the nasal mucosa of MB patients has been documented, and it represents the most important portal of exit for the organism. Whether *M. leprae* invades the human body after deposition of aerosolized organisms on the nasal mucosa is unknown. In the past, many attempts were made to detect *M. leprae* in Ziehl-Neelsen-stained smears from nasal swabs, but this technique is always open to criticism, particularly because of its lack of specificity.

PCR targeting species-specific sites of *M. leprae* DNA offers promise, in terms of both specificity and sensitivity. We investigated the household contacts of a number of PB and MB leprosy patients. Sampling of the nasal mucosa through swabbing is not the optimal technique because it is impossible to standardize. However, under field conditions it is the only practical possibility; a nasal washing procedure as performed by Shepard [7] would be impossible.

M. leprae was detected by PCR in 1 (3.12%) out of 32 samples from contacts of PB patients and in 21 cases (20%) out of 105 samples from contacts of MB patients.

The difference is significant ($p<0.05$). Among the patients, PCR positivity for nasal swab was 3 out of 15 (20%) in PB patients and 45 out of 55 (81.82%) in MB patients. The difference is highly significant ($p<0.0001$, odds ratio=18.0, 95% CI=3.19-101.56). Therefore, PCR positivity of MB is significantly higher than PB in both patients and contacts. This might be an indication that carriage of *M. leprae* in the nose is indeed related to exposure to type of leprosy.

The results of this study provide evidence that a majority of MB patients are carrying *M. leprae* in their noses and that carriage of *M. leprae* occurs among healthy people living an area where leprosy is endemic. Nasal carriage by apparently healthy people might have an impact on leprosy control and thus be an important phenomenon from the public health point of view. New studies have to be undertaken to investigate whether and to what degree nasal carriage occurs in the general population of areas of endemicity and what might be the role of carriers in the maintenance of infection reservoirs and transmission of leprosy.

Our results differed from those published by Stefaan R, *et al* [8], who did not find a difference among contacts of PB and MB patients. The finding was 1 of 52 (1.9%) swabs and 13 of 164 (7.9%) swabs were positive for *M. leprae* among contacts of PB and MB patients. However, De Wit, *et al* [9] suggested that the percent positivity of the patients group was significantly higher than in both the group of occupational contacts and endemic controls. The amplification products were found in 55% of untreated patients, in 19% of occupational contacts, in 12% of endemic control, and in none of the non-endemic controls.

Although nasal carriage does not necessarily imply infection or excretion of bacilli, the finding of nasal carriage supports the theory of a disseminated occurrence of *M. leprae* in populations for which leprosy is endemic.

The technique described here is simple, sensitive, and specific for use in large-scale epidemiological studies. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination programs in countries where the disease's prevalence has been significantly reduced. This study is part of the molecular epidemiological study of leprosy in Myanmar.

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Genetic population structure of *Aedes aegypti* mosquitoes at various spatial scales in Myanmar

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Dengue fever/dengue haemorrhagic fever (DF/DHF), the most common mosquito-borne viral disease, is a re-emerging public health problem in the world particularly in the tropics. At present, dengue vector control still depends on the selected use of certain insecticides in targeted dengue patient areas particularly during outbreaks in disease endemic countries including Myanmar. An understanding of genetic population structure and dispersal ability in dengue vector *Aedes aegypti* populations may provide useful information for effective vector control strategies. It is useful to understand the genetic variability of local mosquito population for the development of current vector control purposes. The study therefore has attempted to infer the genetic population structure and the extent of dispersal ability of *Aedes aegypti* at various spatial scales using different statistical models. Based on 13 microsatellite loci data, a significantly low level of genetic structuring was found at all spatial scales among three clusters of mosquito populations. Average pairwise F_{ST} values ranged from 0.026 within 5 km to 0.043 across >500 km distance. Landscape genetics results showed different genetic heterogeneity among and within regional clusters. The findings of restricted gene flow and small scale population structure support the nature of limited dispersal ability of *Aedes aegypti* and possible effect of different insecticide usages on local mosquito populations. On the other hand, passive mosquito dispersal which is a problem for dengue vector control also plays an important role in shaping large scale genetic structure.

INTRODUCTION

Dengue/dengue haemorrhagic fever (DF/ DHF) is one of the major re-emerging public health problems in the world, particularly in the tropics and subtropics with an estimated 50-100 million people infected annually which can lead to hospitalisation in half a million of severe cases [1]. Dengue virus is mainly transmitted to humans through the bite of *Aedes aegypti* mosquitoes. Southeast Asia (SEA) including Myanmar has experienced severe dengue epidemics since the 1950s, particularly in urban areas [2-4]. The first DHF case in Myanmar was reported in 1970 in Yangon [5]. In the following decades, dengue outbreaks occurred in 1987, 1991, 1994, 1998 and the largest in 2001 with 15,361 reported cases of DHF/DSS, including 192 deaths [6]. The spread of dengue viruses and *Aedes aegypti* in SEA was mainly via trade and transport between populations by shipping [7, 8]. Because it is a highly domestic species that feeds on humans and breeds in and around human habitation, *Aedes aegypti* and the disease it transmits have proliferated along with human population growth, economic development, increased mobility and uncontrolled urbanisation.

At present, the reduction of dengue transmission relies on appropriate vector control as vaccine development is still underway [9]. It is useful to understand the genetic variability of local mosquito population for the development of current vector control purposes. Genetic population structure usually results from a combination of several contemporary and historical processes such as dispersal ability, mating patterns, environmental barriers and demographic history [10]. Since we know relatively little about the population history of *Aedes aegypti*, the study has attempted to infer the genetic population structure and the extent of dispersal ability of *Aedes aegypti* at various spatial scales using different statistical models.

Microsatellite markers have been recently used for determining genetic population structure in *Aedes aegypti*. Most of these small scale studies generally conclude low levels of genetic differentiation [11-14]. We do not have any statistics on genetic population structure of *Aedes aegypti* in Myanmar to date. The aims of this study are therefore to infer the genetic population structure of *Aedes aegypti* at a hierarchy of spatial scales across mainland Myanmar and to determine the factors shaping genetic structure at each scale. In addition to using conventional population-based methods we also apply landscape genetics approaches [15]. The findings are interpreted for their significance for vector control in Myanmar and for the future utility of landscape genetics to identify the factors shaping the genetic structure of this species on a wider scale.

MATERIALS AND METHODS

Mosquito sampling

Aedes aegypti mosquito larvae from natural populations were sampled at a hierarchy of spatial scales (5 km, 50 km and 500 km) during 2004-2005. The sampling strategy, which is fundamental in population genetics, was carefully designed and arranged in clusters to optimise a range of spatial scales over an extensive geographical area representing different ecological regions. There were three main collection regions (clusters): Yangon, Meiktila and Myitkyina, each of which comprised four collection sites, three that were ~5 km apart and a fourth that was ~50 km apart. A site covered an area of ~500m in diameter as a reasonable estimate of neighbourhood size based on the flight range of *Aedes aegypti* [16, 17].

Within this area third and fourth stage larvae and pupae were collected from 50 different water storage containers (such as tanks, indoor cisterns, tyres etc.) in and around the houses. Larvae and the adults (which hatched from the collected pupae) were morphologically identified using standard taxonomic keys. Identified larvae were preserved in 95% ethanol and adults were placed in silica gel. The locations of the sampled containers were recorded using a global positioning system (GPS). For microsatellite genotyping a single individual was selected at random from each container to avoid incidental sampling of close relatives.

DNA extraction and microsatellite genotyping

DNA was extracted from individual mosquitoes using a standard phenol/ chloroform method [18]. Thirteen microsatellite loci which have been characterised and found to have suitable levels of variation in *Aedes aegypti* [19] were amplified in two sets of multiplex PCR. Each reaction comprise of dried DNA template (1 µl of a 1:400 dilution); 1 µl of Primer Mix (containing 0.2 mM of each primer with the forward primer of each pair fluorescently labelled with HEX, FAM or NED); and 1 µl of Qiagen Master Mix (QIAGEN). The reaction conditions were an initial denaturation step at 95˚C for 15 mins; 35 cycles at 94˚C for 30 sec, 55˚C for 90 sec and 72˚C for 90 sec; and a final extension of 10 mins at 72˚C. The amplified products were run on an ABI 3730 capillary sequencer (Applied Biosystems Inc.) and were genotyped using the GeneMapper software 3.7 (Applied Biosystems Inc.).

Genetic analyses

Allelic richness (R_S), observed and expected heterozygosity (H_O and H_E) were estimated for each locus in each population using ARLEQUIN 3.01 [20]. Genetic differentiation between pairs of populations was also estimated using F_{ST} as a distance matrix. Significance was estimated at the 5% level by 1,000 permutations of the genotypes among populations. Mantel test was implemented to test for isolation by distance using $F_{ST}/(1- F_{ST})$ as an estimate of pairwise genetic distances between populations [21].

A Bayesian clustering method, TESS 2.0 [22], was used to estimate genetic clusters. It is based on the assumption that spatially close individuals are likely to be genetically related. For each value of clusters (k) from 2 to 12, five replicates of 10 independent MCMC chains each with 12,000 sweeps and a burn-in period of 2,000 sweeps was run. The optimal number of k can be determined from the runs with the highest posterior likelihoods. The populations were tested for population bottleneck using the intra-locus k-test and inter-locus g-test [23]. Both tests were conducted using the Excel Macro KGTESTS [24].

RESULTS

Variation in 13 microsatellite loci for 304 individuals from three clusters was analysed. All loci were polymorphic in all populations. Average allelic richness (R_S) were 5.6, 5.42 and 5.38 for Yangon, Meiktila and Myitkyina populations, respectively. Average observed heterozygosity (H_O) (0.065) and average gene diversity over loci (0.73) were more or less the same in all populations. The F_{ST}-based estimates for all pairs of populations revealed that even sites that were only ~5 km apart were significantly genetically differentiated although the level of differentiation was low (average F_{ST} value of 0.026) (Table 1). It was possible that there were some false positives but these would have minimal effect on overall findings since the majority of tests were positive (62 out of 66, P≤0.0001).

Table 1. Pairwise population differentiation of *Aedes aegypti* mosquitoes from Myanmar

	Within clusters		Between clusters	
	~ 5 km	~ 50 km	~ 500 km	>500 km
Average pairwise F_{ST}	0.026	0.032	0.039	0.043
Range	0.001 - 0.039	0.013 - 0.045	0.029 - 0.088	0.021 - 0.083
Significant pairwise	8/9	9/9	30/32	15/16

As spatial scale increased, the average F_{ST} value and level of significance also increased. This was consistent with the signal of isolation by distance found in the populations (Fig. 1). The Mantel test showed that genetic and geographic distances were significantly correlated although the level of correlation was low ($R^2 = 0.07$; P = 0.041).

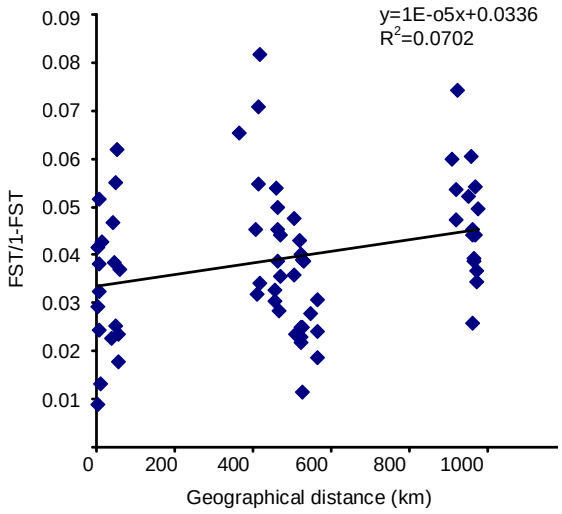

Fig 1. Correlations of genetic and geographic distance for 12 populations of *Aedes aegypti* in Myanmar

When TESS was run with the spatial interaction parameter (ψ) set to 0, for k=2-12, all the populations were always mixed. With ψ set to 0.6, the likelihood of TESS increased with increase in k, reflecting the low but significant differentiation

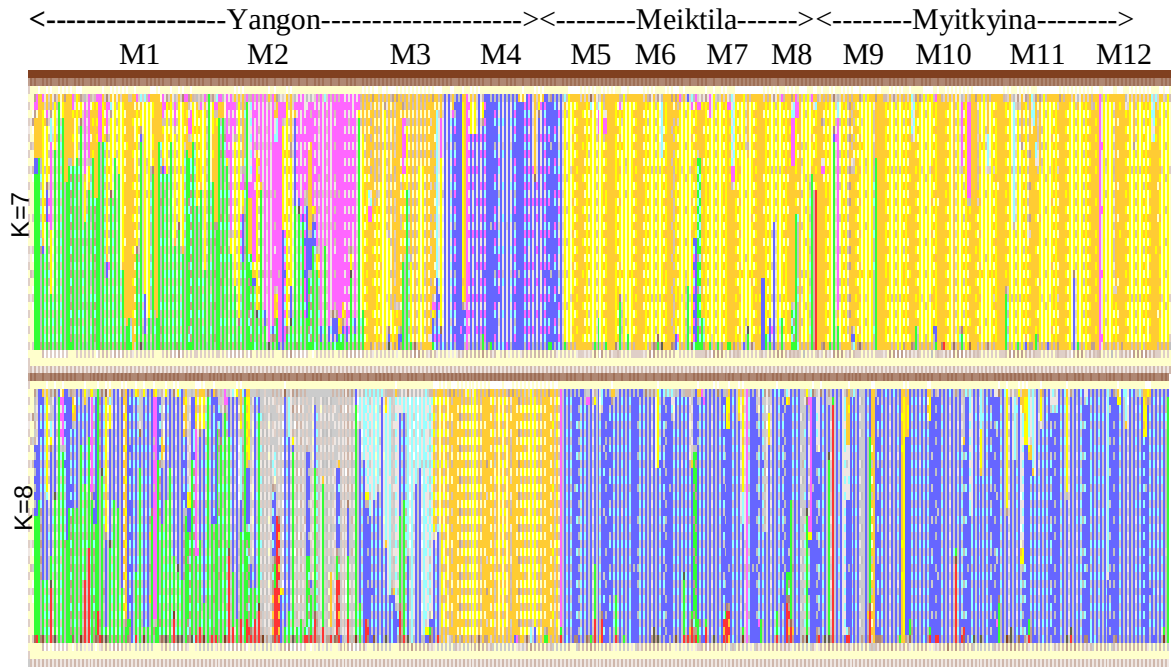

Fig. 2. Memberships of individuals to seven (upper panel) or eight (lower panel) genetic clusters estimated by TESS

between all populations. In TESS at k=8, the M4 population on the outskirts of Yangon became distinct. It was also found that the Yangon populations (M1-M3) were genetically heterogeneous compared to other populations (Fig. 2).

The results of the *k*- and *g*- tests for population bottlenecks are shown in Table 2. The number of loci that had positive values of *k* decreases when three clusters were pooled (five out of 13 loci) but none of the results were significant. All the *g* values were also all positive indicating that a model of constant population size could not be rejected for any of the population groupings.

Table 2. Population expansion analysis of *Aedes aegypti* microsatellite data in 13 loci

Sites	*k*-test (Number of positives)	*g*-test
Cluster 1 (Yangon)	7/13 NS	0.64
Cluster 2 (Meiktila)	7/13 NS	0.84
Cluster 3 (Myitkyina)	6/13 NS	0.65
Total (Myanmar)	5/13 NS	0.7

NS = not significant

DISCUSSION

The results show significantly low genetic structuring in *Aedes aegypti* at all spatial scales, which is consistent with the expectation that all these mosquitoes have a recent common ancestry in Africa [8]. The tests of population expansion indicate that there has not been a substantial bottleneck so that there have been multiple introductions from several different sources. However, the presence of genetic structuring within the groups may be the reason why the tests cannot detect a population bottleneck even if one has occurred.

Although the overall level of genetic differentiation is low, populations that are 5 km apart are significantly different from each other. The data indicate genetic structuring on a very small spatial scale (<500 m) in *Aedes aegypti*. The lack of signal of isolation by distance within a collection site indicates that individuals are not continuously distributed throughout the area (data not shown). Data from mark-release-recapture studies show that *Aedes*

aegypti mosquitoes have a limited flight range but can move up to a few hundred metres around their larval habitats [17].

Although such dispersal could be sufficient to genetically homogenise clusters within a 500m area, there are several factors which may prevent this. Dispersal rates may be reduced where there is increase in oviposition sites [25]. The genetic clustering of *Aedes aegypti* may therefore be due to clustering of oviposition sites and hosts around human habitation coupled with low dispersal.

Average F_{ST} values in this study were 0.026 and 0.032 at ~5 km and ~50 km scales (Table 1), respectively. Other microsatellite-based studies on a similar spatial scale had overall F_{ST} of 0.056 [26] and 0.053 [14] with many individual values being larger. One reason for low levels of genetic differentiation in this study compared to others could be due to the 500m sampling areas with multiple demes of highly clustered *Aedes aegypti* structure [27].

However, it is also possible that some other studies have overestimated as the larvae were sampled from areas as small as two to three [13] or four to five houses [26]. This could result in the over-representation of siblings in the sample. Overall, it is clear that *Aedes aegypti* has a highly clustered distribution and restricted gene flow on a very small spatial scale.

Although a signal of isolation by distance is detected, it is very slight (Fig. 1), indicating that the restricted mosquito dispersal at a small spatial scale does not explain larger scale population structure. This is in accord with results from the TESS analysis. It shows that although there is some regional clustering with similarities among populations, genetic clusters do not correspond obviously to spatial distance; some close populations are highly divergent (e.g. M4 population 50 km away from the main Yangon cluster). Conversely, populations that are distant may be very similar (e.g. Meiktila and Myitkyina clusters) (Fig. 2).

The genetic similarity of geographically distant populations, coupled with the greater genetic heterogeneity detected in the port city, Yangon, is indicative of some large scale dispersal events. The data provide good evidence that passive transportation of *Aedes aegypti* along human transportation routes is important in shaping large scale structure of this species. This has also been suggested based on previous population genetic studies in the southern United States [28] and SEA [13]. Passive dispersal likely involves the movement of immature stages of mosquito as well as adults; eggs, larvae and pupae could easily occur in water containers transported by people and the eggs can withstand desiccation for several months [16].

Our results have several implications for vector control. Conventional control measures such as insecticides or the removal of larval habitats are often implemented following a dengue outbreak [29]. The limited dispersal of *Aedes aegypti* indicates that this approach should be effective in removing infective populations and preventing their spread. It may also help to delay the spread of new insecticide resistance genes which is a major problem for vector control. However, the high selection pressure on such genes means that even a very small amount of dispersal enables the spread of insecticide resistance. To prevent this, effective monitoring of the emergence of insecticide resistance is essential. On the other hand, passive mosquito dispersal also plays an important role in shaping large scale genetic structure.

Conclusion

This study has shown low levels of genetic differentiation on all spatial scales in *Aedes aegypti*. It is still necessary to determine the exact spatial scale of genetic clusters and which environmental features may form the barriers between them. An effective approach will be landscape genetic studies in which individuals are sampled on a fine scale throughout

extensive urban and rural areas. It is also important to determine the environmental (including spatial) factors shaping large scale population structure and the extent to which these are historical or contemporary effects.

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Synthesis of Health Systems Research under the framework of Health Research Programme: a decade work of Department of Medical Research, Lower Myanmar (2000-2009)

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Prime purpose of Health Systems Research (HSR) is "to help improve health of people through improvement not only of conventional health services but also of other services that have a bearing on health". With this aim, divisions within Department of Medical Research (Lower Myanmar) (DMR-LM) have been conducting HSR-related projects since 1986. However, there is a gap of information on linkage between these research projects and Health Research Programme (HRP) of National Health Plan (NHP) and their utilization. Thus to fulfil this gap, research summaries from Annual Reports of DMR-LM from 2000 to 2009 were reviewed and categorized into 7 projects under HRP using content analysis. During this decade, 16 research divisions and one research unit of DMR-LM conducted 160 HSR-related projects. About 61% of the projects were conducted by social science research divisions. Number of projects conducted over the years had increased due to involvement of clinical and laboratory-based research divisions in HSR projects particularly in 2004. Of them, about half were communicable diseases followed by reproductive health, non-communicable diseases and environmental health. Majority was health systems-related projects under HRP (83.8%), of which 67.9% were conducted by social science-related divisions. It could be due to nature of work of the respective divisions. Collectively, most findings were academically utilised (85.6%), and about 13% were utilized by project managers (n=21) for various purposes. The findings indicate that there is still a need to promote result utilization by service departments by involving both researchers and service managers.

INTRODUCTION

World Health Organization (WHO) had defined a health system as 'consists of all organizations, people and actions whose primary intent is to promote, restore or maintain health' [1]. To improve health systems, three research domains including operational, implementation and health system play an important role in conducting the research or projects [2]. Of these three domains, Health Systems Research (HSR) is defined broadly as the production of new knowledge to improve how societies organize themselves to achieve health goals [3]. Prime purpose of HSR is to help improve health of people through improvement not only of conventional health services but also of other services that have a bearing on health. Accordingly, divisions within the Department of Medical Research (Lower Myanmar) (DMR-LM) have been conducting HSR-related projects since 1986. More or less, these researches were related to 7 projects under Health Research Programme (HRP) of National Health Plan (NHP) 2006-2011, namely, communicable diseases (CD), non-communicable diseases (NCD), health systems, environmental health (EH), traditional medicine, academic and technology development and capacity strengthening. However, there are limited information on the linkage between these research projects and HRP and their

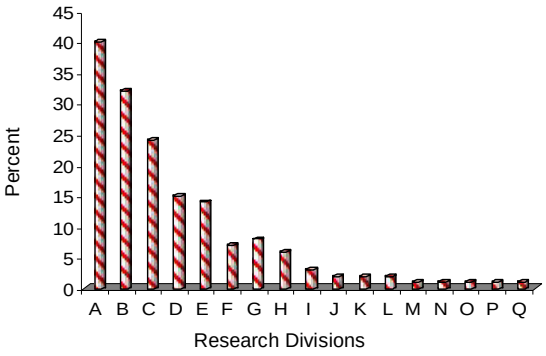
utilization. With this aim, this review study was conducted to determine types of HSR-related projects under the framework of HRP conducted by divisions within DMR-LM from 2000 to 2009 and the degree of research utility.

MATERIALS AND METHODS

For consistency of source of information, Annual Reports of DMR-LM from 2000 to 2009 were used [5]. All HSR-related projects in these reports were analysed. Based on the year of reporting the research findings, the summaries of the research findings were reviewed and categorized into seven projects under HRP of NHP using content analysis. Regarding the situation of utilization of research findings, scientists from the respective divisions of DMR-LM were requested to fill in a form on research utilization.

RESULTS AND DISCUSSION

During a 10-year period, 16 of 22 research divisions and one of seven research units of DMR-LM conducted 160 HSR-related projects.



A = Epidemiology
B = HSR
C = Statistics
D = Nutrition
E = Clinical
F = Bacteriology
G = Parasitology
H = Entomology
I = Pharmaceutical Toxicology
J = Pharmacology
K = Virology
L = Immunology
M = Blood
N = Chemical Toxicology
O = Clinical Unit
P = Experimental Medicine
Q = Physiology

Fig. 1. HSR-related projects conducted at DMR-LM during 2000-2009 by research divisions

Social science-related research divisions (i.e., Epidemiology, Health Systems Research and Medical Statistics research divisions) conducted 60.6% of the projects and clinical and laboratory-based research divisions conducted 39.4% of the projects, respectively (Fig. 1).

Yearly HSR-related projects conducted by divisions

During the study period, while number of projects conducted over the years had increased, number of projects conducted by social science-related divisions had decreased between 2001 and 2006, particularly in 2004. It could be due to increased involvement of clinical and laboratory-based research divisions in HSR projects, more collaboration among divisions with different disciplines of DMR-LM and most researchers from social science-related research divisions were out of office for further studies during 2001 and 2006 (Fig. 2).

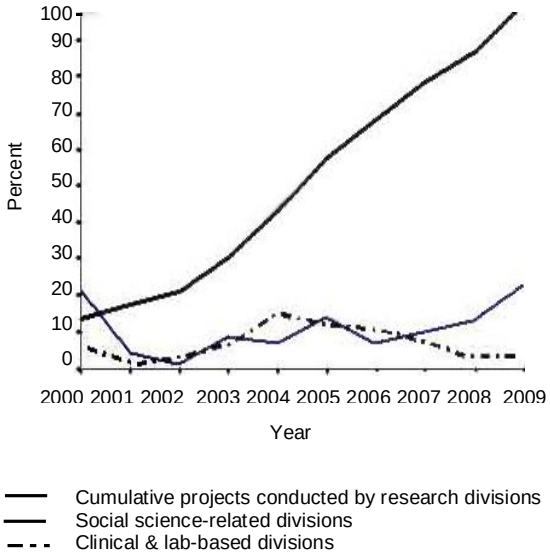


Fig. 2. Yearly HSR-related projects conducted by divisions from 2000 to 2009

Types of HSR-related researches conducted by divisions

During this decade, social science-related research divisions conducted 97 projects (60.6%) and clinical and laboratory-based research divisions conducted 63 projects (39.4%), respectively (Table 1).

Table 1. Type of disease-related projects by Health Research Programme conducted by research divisions

Project under Health Research Programme	Type of disease	Research Division		
		Social science-related divisions	Clinical & lab-based divisions	Total no. (%)*
Health systems	Communicable diseases	51	21	72
	Reproductive health	23	1	24
	NCD	0	3	3
	Environmental health	0	2	3
	Other	16	16	32
Sub total		91	43	134(83.8)
Non-communicable disease	Non-communicable diseases	1	6	7
	Reproductive health	0	1	1
	Other	2	3	5
Sub total		2	10	13(8.1)
Communicable disease	Communicable diseases	2	7	9
Sub total		2	7	9(5.6)
Environmental health	Environmental health	0	3	3
Sub total		0	3	3(1.9)
Other	Other	1	0	1
Sub total		1	0	1(0.6)
Total (%)**		97 (60.6)	63 (39.4)	160

*Column percent **Row percent

By type of disease-related research, about half (50.6%) were communicable diseases including AIDS, TB, malaria, leprosy, ARI, DHF and HCV. Next came reproductive health (15.6%) followed by non-communicable diseases (6.3%) and environmental health (3.8%). About one-fourth were other research areas such as smoking practice, elderly, research implementation, cost study, snake bite, minor ailments, vital statistics, dietary pattern, food behaviour, iron status, health information, etc. Both social science-related research divisions and clinical and laboratory-based divisions focused more on communicable diseases (53/97=54.6% and 28/63=44.4%, respectively).

With respect to the projects under HRP, the majority of researches were concerned with health systems (83.8%), and these were relating to treatment seeking behaviours, establishment of community-based surveillance system for women cancer, intervention studies for sustainable dengue control and malaria prevention. Of which, 67.9% were done by social science-related research divisions. Clinical and laboratory-based research divisions also emphasised on NCD, communicable diseases and environmental health relating to HRP. Environmental health-related studies were done by Pharmaceutical Toxicology, Chemical Toxicology, Entomology and Pharmacology research divisions.

Utilization of research findings

Collectively, most findings were academically utilised by either one of the following channels (n=137, 85.6%): presentation at Myanmar Health Research Congress, reporting to Department of Health and international agencies, dissemination at seminars, thesis and publications in local and international journals. Of which, reporting and presentation at the congress were the commonest. The situation of result finding of 2 researches was not known.

About 13% of the research findings were utilized by project managers (n=21) as a citation or reference, guidance for patient treatment and guidelines for health providers of the respective programme and general practitioners. Among them, 11 and 10 researches were conducted by social science-related research divisions and clinical and laboratory-based research divisions, respectively. Since the findings were based on the personal contact of the respective divisions of DMR-LM, we could not know the real utilization status from the service department's aspect. It is assumed to be larger than this extent.

It was observed that most of the research results utilized by the service managers were communicable diseases (62%), which were among the priority diseases ranked in NHP 2006-2011 such as TB, malaria and leprosy (Fig. 3). In addition, with regards to HRP, while research findings relating to health systems were mostly used by the service departments, other types such as communicable diseases, non-communicable diseases and environmental health were

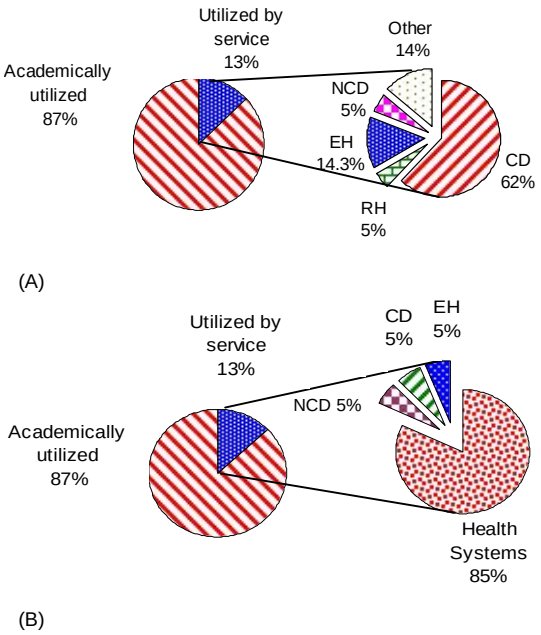


Fig. 3. Situation of research findings utilization by (A) type of disease-related projects and (B) Health Research Program

the least (5% each). Through personal communication, it was also noted that service managers participated in those researches from the beginning of protocol development.

Conclusion

During the 10-year study period, 160 HSR-related projects were conducted by more than half of the research divisions of DMR-LM. However, some of the research findings of ongoing projects were not documented in Annual Reports; in reality, number of the projects could be larger than this. However, it was observed that by effective collaboration among divisions of DMR-LM, HSR-related projects of DMR-LM contributed to health development of the country. However, it still needs to promote research areas relating to Environmental Health. It was noted that both types of research divisions of DMR-LM conducted the research, of which the results were used by the service departments. The research findings were used by the service departments for various purposes. However, the extent of result utilization was not fully satisfactory. The situation inclines to the view of Remme and his group [2] that recognition of role of research in improving health system and health care delivery had increased and concurrently, result utility which is useful and serviceable, should also be taken into consideration.

Although the real situation of research utilization was not known, the findings indicate that there is still a need to promote result utilization by service departments. Thus, not only the researchers need to be encouraged to involve service managers in implementation of research but also the service managers need to be encouraged to seek information for utilization through various channels. To achieve this, the following ways and means could be helpful to enhance the utilization of research findings:

(a) Preparing research findings in brief or executive summary for the service mangers
(b) Making this summary reach to service mangers, and
(c) Creating a situation for sharing the findings among researchers and the service managers

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Care-seeking Behavior and Detection of Target Organ Involvement among Hypertensive Patients in Yangon Region (2014-2015)

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Hypertension is a major risk factor for cardiovascular diseases (CVDs) and hemorrhagic stroke due to hypertension is one of the leading causes of death in Myanmar. The aim of the study was for assessing knowledge, attitude, and practice related to hypertension among people with known history of hypertension who seek care in CVD clinics with public health care facilities in Yangon Region. A cross-sectional study was conducted from December 2014 to March 2015 among 622 hypertensive patients seeking care at 12 CVD clinics in Yangon Region. Data collection was done by the trained interviewers by using a pretested standard questionnaire and weight, height, ECG, blood pressure (BP) were measured. Capillary blood sampling was done with calibrated standardized machines. After cleaning of data, it was entered using Epidata software and analyzed using STATA. Over 90% of respondents were ≥40 years, female-male ratio was about 2:1, majority were married, had education of middle school or lower while annual household income varied from 200,000 to 300,000 kyats per month. At least 60% of the study population has the knowledge on ways of controlling BP and some common complications of hypertension such as stroke and heart attack. The respondents' household usually added salt in cooking or preparing food despite knowing the health risk of salty food. Most of the respondents had limited consumption of processed food. The majority of the respondents had lower than 5 servings of fruits and vegetables daily and were physically inactive. Over half of the respondents were overweight and their total blood cholesterol levels were high. About 7 in 10 respondents had uncontrolled BP although they were taking anti-hypertensive drugs regularly. The study showed that about 6 in 10 respondents had at least one target organ involvement due to hypertension (renal impairment or abnormal ECG changes). This study indicates the need for strong behavior change communication programs for hypertensive patients focusing on regular monitoring of blood pressure, reduction of dietary salt consumption, more consumption of fruits and vegetables, adoption of physically active life and control of body weight.

Key words: Hypertension, CVD risk, Target organ involvement

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading non-communicable diseases (NCDs) category worldwide. CVDs include diseases of the heart, vascular diseases of the brain and diseases of blood vessels. The early half of the 20th century witnessed a rapidly growing epidemic of cardiovascular diseases as a result of industrialization, urbanization, increased prosperity, and social upheaval in the higher income countries. Therefore, CVDs are often thought to be problems of wealthy, industrialized nations. In fact, the epidemic of CVDs is a global health phenomenon nowadays. Over the past two decades, deaths from CVDs have been declining in high-income countries, but have increased at an astonishingly fast rate

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in low-and middle-income countries (LMIC). CVDs now have a major impact not only on developed nations but also on low- and middle-income countries, where it accounts for nearly 30 percent of all deaths.[1]

CVDs remain the leading cause of death in the world, far outstripping deaths due to malaria, HIV/AIDS, and tuberculosis.[2] Nearly half of the 36 million deaths due to NCDs are caused by CVDs.[3] The percentage of premature deaths from CVDs ranges from 4% in high-income countries to 42% in low-income countries, leading to growing inequalities in the occurrence and outcome of CVDs between countries and populations.

The increased prevalence of risk factors for CVDs and related chronic diseases in developing countries, including tobacco use, unhealthy dietary habit, reduced physical activity, increasing blood lipids, and hypertension, reflects significant global changes in behavior and lifestyle. These changes now threaten once low-risk regions, a shift that is accelerated by industrialization, urbanization, and globalization. The potentially devastating effects of these trends are magnified by a deleterious economic impact on nations and households, where poverty can be both a contributing cause and a consequence of chronic diseases.[1]

CVDs is now in a rising trend in South East Asia including Myanmar due to the increase in major cardiovascular risk factors in both urban and rural areas. Hypertension is a major risk factor of CVDs in Myanmar and hemorrhagic stroke due to hypertension is one of the leading causes of deaths in Myanmar. The sub-national survey on NCD risk factors done in Yangon Region in 2003 - 2004 showed that prevalence of hypertension in persons aged 20 years and above was 33.8%.[4] One survey also indicated that among hypertensive participants, a little more than half were aware of their hypertension and about one third were currently taking antihypertensive treatment, but only about one tenth had their blood pressure controlled.[4] The national survey on NCD risk factors in 2009 showed that the overall prevalence of hypertension in persons aged 15 years and above was 30%.[5] This national survey pointed out that, on an average, about 88% of males with raised blood pressure were not on medication and 8% still had high blood pressure even though they were on medication and about 75% of females with raised blood pressure were not on medication and 17% still had high blood pressure despite being on medication.[5]

In 2013, CVDs project of Myanmar conducted the survey on CVDs risk factors among 600 persons aged 40 years and above in 4 townships in lower Myanmar. This survey indicated that 52% of the respondents aged 40 year and above were hypertensive.[6]

Untreated or uncontrolled blood pressure leads to serious complications such as target organ involvement with resultant avoidable deaths or reduced quality of life. But the extent of target organ involvement and nature of care seeking pattern among hypertensive patients needs to be explored to formulate counter measures to prevent target organ involvement. So this study tried to depict the picture of care seeking pattern and target organ involvement among the hypertensive patients who sought care at CVDs clinics in Yangon Region.

MATERIALS AND METHODS

Study design and population

The facility-based cross-sectional design was used. The study was conducted with a representative sample of the adults with known history of hypertension who sought care from CVD clinics at public healthcare facilities in 12 townships from Yangon Region (Pazuntaung, Dagon, Kyauktada, Thongwa, North Okkalapa, Kyimyindine, Kamayut, Dawbon, Thanlyin, Latha, Lanmadaw and Dagon Myothit (North).

Sample size and sampling procedure

Renal insufficiency as target organ involvement of hypertension was regarded

as one of the main variables of this study and its level was used in determining the required sample size.

The sample size was calculated using the following formula.

N=Number of geographical regions x (1/1- non-respondent rate) x (Z_α^2 x P(1-P)/e^2)

N=Required sample size

Z_α=Z statistics for predetermined alpha error

P=The estimated prevalence of diabetes mellitus

e=Margin of error

P was set at 0.5 for the proportion of people with known hypertension who had renal insufficiency due to hypertension in Myanmar. Alpha error was set at 5%; so Z_α was 1.96. Margin of error (*e*) was set at 15%. Non-response rate was estimated to be 15%. Number of geographical regions was 4.

So, the required sample size for the whole survey was:

(1/1-0.15) x (1.96^2 x 0.5 (1-0.5)/0.05^2)=452

The respondents were recruited consecutively from those who sought care in CVD clinics which were regularly opened with 2 public health care facilities (3 township hospitals and 9 Urban health centers) in Yangon Region. The final total sample size added up to 622 persons.

Data collection

Data collection was done from December 2014 to March 2015. Data on socio-demographic characteristics, knowledge of hypertension, care seeking behavior for hypertension and risk behavior for cardio-vascular diseases and dietary salt intake were collected by the trained interviewers using a pretested standard questionnaire. Weight, height and blood pressure were measured with calibrated weighing machine, stadiometer and Omron blood pressure measuring device. Random capillary blood samples were tested for random blood glucose and total blood cholesterol and urine was tested for albumin on site, and ECG was also done on every patient.

Data management and analysis

For completeness and consistency, questionnaire overseen by supervisors were checked by the interviewers for data quality and validity. The data entry was done using Epi data software and analyzed by using STATA to describe the percent of the main outcome variables and 95% confidence interval and disaggregated by gender.

RESULTS

The study population included 622 adults aged 20 years and above from 12 townships. Over 90 percent of the respondents were aged 40 years and above and female-male ratio was about 2:1 in the study population. The majorities of the respondents were married and had education of middle school or lower. Most respondents' annual household income ranged from 200,000 to 3,000,000 kyats.

At least 60% of the study population knew the ways of controlling blood pressure. Table 1 shows that there was no appreciable difference in the level of knowledge on the ways of controlling blood pressure. Stroke and heart attacks were well-known complications of hypertension among the study population.

Table 1. Knowledge of hypertension

Knowledge of hypertension	Men (n=211)	Women (n=411)	Both sexes (n=622)	
	%	%	%	(95%CI)
Way of controlling blood pressure				
Reducing salt and salty food	80.1	87.1	84.7	(81.9 87.6)
Eating a balanced diet	66.8	64.0	65.0	(61.2 68.7)
Limiting alcohol	57.8	59.9	59.2	(55.3 63.0)
Doing regular exercise	73.0	68.1	69.8	(66.2 73.4)
Maintaining a healthy body weight	59.7	58.6	59.0	(55.1 62.9)
Avoiding tobacco use	60.7	60.8	60.8	(56.9 64.6)
Regular checkup of blood pressure	63.0	60.8	61.6	(57.7 65.4)
Taking medicine for treating high blood pressure as prescribed	64.5	64.5	64.5	(60.7 68.2)
Complications of uncontrolled high blood pressure				
Stroke	75.4	75.9	75.7	(72.3 79.1)
Heart attack	62.6	66.2	65.0	(61.2 68.7)
Kidney failure	46.0	47.7	47.1	(43.2 51.0)
Blindness	40.3	38.9	39.4	(35.5 43.2)
Irregularity of heart beat	34.6	35.0	34.9	(31.1 38.6)
Heart failure	36.5	36.0	36.2	(32.4 40.0)

The level of knowledge on complication of hypertension was similar between men and women. In most households, salt, salty seasoning or a salty sauce were always or often added in cooking or preparing food.

But practice of adding salt or a salty sauce to food or eating processed food high in salt were relatively rare among these households. Most of them had positive perception to salt intake. Most of them reported that they limited consumption of processed food and avoid eating food prepared outside of home. However, less than half of them reported to adopt other practices of controlling salt intake (buying low salt/sodium alternatives, looking at the salt or sodium content on food labels or using spices other than salt when cooking).

In nearly 30% of the respondents, blood pressure was controlled below 140 mmHg of systolic blood pressure and 90 mmHg of diastolic blood pressure. The women were relatively more likely to have controlled blood pressure than men. The overall level of current smoking and past-year drinking was low among the respondents.

But the men were more likely to be current smokers or past-year drinkers than the women. The majority of the respondents had taken less than five servings of fruits and vegetables per day and they were physically inactive. Over half of the respondents were overweight and had high total blood cholesterol level. Women were more likely to be overweight, had high total blood cholesterol level and increased in waist circumference. About one third of the respondents had diabetes mellitus (Table 2).

Table 3 displays the care seeking behaviour for hypertension among the respondents. The median duration of high blood pressure from first diagnosis was 4 years which was found as similar between men and women. In most cases, the first diagnosis of hypertension was made by a healthcare provider. About 7 in 10 respondents got their blood pressure checked weekly, mostly by basic health staff.

Table 2. Salt consumption pattern, way of blood pressure control and associated cardio-vascular risk factors other than hypertension

Characteristics	Men (n=211)	Women (n=411)	Both sexes (n=622)	
	%	%	%	95%CI
Salt intake pattern				
Always/often add salt, salty seasoning or a salty sauce in cooking or preparing food in the household	68.7	75.9	73.5	(70.0 77.0)
Always/often add salt or a salty sauce such as soya sauce to food right before eating it or while eating it	27.5	33.3	31.4	(27.7 35.0)
Always/often eat proce-ssed food high in salt	21.3	22.6	22.2	(18.9 25.5)
Perception to salt intake				
Think that I consume too much salt	17.1	19	18.3	(15.3 21.4)
Think that too much salt or salty sauce in my diet is important to me and could cause a health problem	96.2	98.5	97.7	(96.6 98.9)
Practice for controlling salt intake				
Limit consumption of processed food	78.7	84.4	82.5	(79.5 85.5)
Avoid eating food pre-pared outside of a home	66.8	73.7	71.4	(67.8 74.9)
Buy low salt/sodium alternatives	36.5	43.3	41.0	(37.1 44.9)
Look at the salt or sodium content on food labels	31.3	42.3	38.6	(34.7 42.4)
Use spices other than salt when cooking	30.3	29.7	29.9	(26.3 33.5)
Control of blood pressure				
Systolic blood pressure (SBP)>140 mmHg alone	35.5	31.4	32.8	(29.1 36.5)
Diastolic blood pressure (DBP)>90 mmHg alone	6.2	4.9	5.3	(3.5 7.1)
Both SBP>140 mmHg and DBP>90 mmHg	37.0	35.0	35.7	(31.9 39.5)
Both SBP<140 mmHg and DBP<90 mmHg	21.3	28.7	26.2	(22.7 29.7)
Associated cardiovascular risk factors				
Current smoking	22.3	3.6	10.0	(7.6 12.3)
Drinking during past year	14.0	2.2	6.2	(4.6 7.8)
Fruit and vegetable intake <5 servings per day	98.1	97.8	97.9	(96.8 99.0)
Low level of active physical activity (<150 minutes per week)	84.8	92.5	89.9	(87.5 92.2)
Overweight and obesity (BMI>=25)	55.0	67.4	63.2	(59.4 67.0)
Increased waist circum-ference (>102 cm in men and >88 cm in women)	13.3	56.9	42.1	(38.2 46.0)
Raised random blood sugar(>200 mg/dl)	29.9	36.5	34.2	(30.5 38)
Raised total cholesterol (>190 mg/dl)	52.6	65.0	60.8	(56.9 64.6)

Table 3. Care-seeking behaviors for hypertension

Care-seeking behavior	Men (n=211)	Women (n=411)	Both sexes (n=622)	
	%	%	%	(95%CI)
Median duration of high blood pressure from 1st diagnosis in years	3*	4*	4*	(3 5)**
Ways of detecting high blood pressure first time				
Accidental diagnosis by a health care provider	67.3	79.3	75.2	(69.2 81.6)
Diagnosis during general medical checkup	30.8	18.5	22.7	(19.5 26.0)
Self-detection by blood pressure monitoring device	1.9	2.2	2.1	(1.2 3.6)
Frequency of checking blood pressure				
Daily	4.7	4.9	4.8	(3.4 6.8)
Weekly	73.9	69.6	71.1	(67.4 74.5)
Monthly or less frequently	21.3	25.5	24.1	(20.9 27.6)
Usual way of checking blood pressure				
GP/Physician	11.8	18.5	16.2	(13.5 19.4)
Basic health staff	75.8	70.6	72.3	(68.7 75.7)
Self/family members	10.4	9.5	9.8	(7.7 12.4)
Pharmacy	1.9	1.5	1.6	(0.9 3.0)
Medication				
Taking medication regularly	90.5	89.8	90.0	(87.4 92.2)
Taking medication irregularly	9.5	10.2	10.0	(7.8 12.6)
Advice from health workers				
Received	92.9	96.6	95.3	(93.4 96.7)
Not received	7.1	3.4	4.7	(3.3 6.6)
Current taking herbal or traditional remedy for raised blood pressure				
Yes	18.5	14.4	15.8	(13.1 18.8)
No	81.5	85.6	84.2	(81.2 86.9)

*Median value, **Non-parametric 95% confidence interval

Almost all respondents reported that they had advice from health workers to change behaviour (to reduce salt intake, to lose weight, to stop smoking, to start or do more exercise) and to reduce blood pressure. They took medication regularly. Only a few (15.8%) had herbal medicine to control hypertension. The level of comorbidities was lower 10% except for stroke which occurred once in every ten respondents. Urine albumin was detected and random blood sugar was raised above 200 mg/dl in one third of the respondents. ECG abnormal voltage (the suspicious of ischaemic heart disease) was found in 1 in 8 respondents.

Table 4. Reported comorbidities, target organ involvement and 10-year risk for CVD

Characteristics	Men (n=211)	Women (n=411)	Both sexes (n=622)	
	%	%	%	95%CI
Comorbidity				
History of stroke	16.6	8.5	11.3	(8.8 13.7)
History of myocardial infarction	3.8	6.6	5.6	(3.8 7.4)
History of arrhythmia	4.7	5.6	5.3	(3.5 7.1)
History of eye problems	1.9	2.2	2.1	(1.0 3.2)
History of renal impairment	2.4	1.0	1.4	(0.5 2.4)
Target organ involvement				
Urine albumin detected (trace, + to +++)	43.1	31.1	35.2	(31.4 39.0)
ECG abnormal voltage (Left ventricular hypertrophy and other abnormal voltage)	12.8	12.4	12.5	(9.9 15.1)
ECG abnormal rhythm (Atrial fibrillation, atrial flutter and other arrhythmias)	4.7	1.2	2.4	(1.2 3.6)
10-year CVD risk score				
Low (<10%)	21.8	40.4	34.1	(34.1 34.1)
Moderate (10-<20%)	34.6	23.6	27.3	(27.3 27.3)
High (20-<30%)	13.7	13.9	13.8	(13.8 13.8)
Very high (30-<40%)	13.7	7.1	9.3	(9.3 9.3)
Extremely high (>=40%)	16.1	15.1	15.4	(15.4 15.4)

About 1 in 4 respondents had 10-year risk for cardiovascular diseases equal or more than 30%. In this respect, men had higher risk for cardiovascular diseases than women (Table 4).

DISCUSSION

This study mainly focused on the hypertensive patients in Yangon Region and demonstrated the data on 'care seeking behavior and target organ involvement of hypertension'. There was a female preponderance in the study population. Most respondents were over 40 years, married with an education level of middle school and lower.

The majority of the study population knew ways of controlling blood pressure and some common complications of hypertension such as stroke and heart attacks. However, other complications of hypertension such as renal impairment and heart failure were less known. The respondents' households usually added salt, salty seasoning or salty sauce in cooking or preparing food even

though most of them knew that too much salt or salty sauce in the diet could cause a health problem. Most of the respondents had limited consumption of processed food but rarely checked the salt or sodium content on food labels. The great majority of the respondents daily had lower than 5 servings of fruits and vegetables and they were physically inactive. Over half of the respondents were overweight and high in total blood cholesterol.

About 7 in 10 respondents had uncontrolled blood pressure although they took medication regularly. The study showed that about 1 in 4 respondents had 10-year risk for cardiovascular diseases equal or more than 30% and 6 in 10 respondents had at least one target organ involvement of hypertension (renal impairment or diabetes or abnormal ECG changes).

The study pointed out that the blood pressure was uncontrolled, although the respondents reported regular medication and most of them had target organ involvement. The reasons may be multi-factorial: lack of optimization of hypertension treatment and regular monitoring of blood pressure, addition of other risk factors such as high intake of dietary salt, low intake of fruits and vegetables, low physical activity and overweight.

Therefore, these indicated the need for strong communication programs of behavior change for persons with known hypertension and these programs should stress regular monitoring of blood pressure and reduction of dietary salt consumption, more consumption of fruits and vegetables, adoption of physically active life and control of body weight. It is also necessary to consider how to improve the optimization of anti-hypertensive medications in the community and early detection of target organ involvement.

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Genotypic Characteristics of *Vibrio cholerae* Strains from Myanmar: Comparison between Past and Recent Isolates

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Atypical E1 Tor *Vibrio cholerae*, which possesses traits of both classical and E1 Tor biotypes, has replaced the seventh pandemic E1 Tor *V. cholerae* O1 in Asian and African countries. The origin and spread of these E1 Tor *V. cholerae* in Myanmar should be tracked by genomic analysis. The genotypic characteristic of recent (2012) and past (1982-1996) clinical *V. cholerae* O1 isolates from Yangon, Myanmar were investigated. *V. cholerae* isolates were confirmed by culture, biochemical identification, serotyping and polymerase chain reaction. Eight *V. cholerae* strains isolated during 1982-1996 and 34 strains isolated in 2012 were undergone genotypic analysis by Pulse Field Gel Electrophoresis Typing (PFGE) and Multilocus Variable Number Tamdem Repeat Analysis DNA sequencing. Recent 2012 isolates were atypical E1 Tor, which carried the classical cholera toxin B subunit gene (*ctxB*^{Cla}) and E1 Tor repressor gene (*rstR*^{E1}) and exhibited a total of 10 PFGE patterns. Among *V. cholerae* O1 strains isolated during 1982-1996, 4 pulsotypes were identified and they were different from 2012 PFGE patterns. Pulsotype Y1 and Y4 isolates carried *ctxB*^{El} and *rstR*^{E1} and they are related to seventh cholera pandemic E1 Tor strains. Pulsotype Y2 isolate was atypical E1 Tor which carried *ctxB*^{Cla} and *rstR*^{E1}. Remarkably, seventh cholera pandemic prototype E1 Tor was observed only in 1982 isolates and atypical E1 Tor with *ctxB*^{Cla} and *rstR*^{E1} was found to be existed in Yangon 30 years ago. This study provided basic genetic information on past and recent cholera strains in Myanmar.

Key words: Vibrio cholerae, Genotypic characteristics, Myanmar

INTRODUCTION

Cholera is a rapidly dehydrating acute enteric infection caused by the ingestion of toxigenic serogroup (01 and less commonly 0139) of *Vibrio cholerae* present in faecally contaminated water or food. It is characterized in its most severe form by a sudden onset of acute watery diarrhea that can lead to death by severe dehydration. Cholera has spread widely and affects at least 56 countries in the world at least. Cholera remains a global threat to public health.

The global disease burden is estimated to be 3-5 million cases and 100,000-130,000 deaths per year. In 2009, a total of 221,226 cases, including 4,946 deaths, were reported to the World Health Organization from 45 countries.[1] The recent outbreaks of cholera were due to a new emerging form of *V. cholerae* O1, which possesses traits of both classical and El Tor biotypes. El Tor variants spread to several countries in

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Asia and Africa.[2-4] *Vibrio cholerae* harbours a virulence regulon consisting of genes involved in colonization, toxin production and bacterial survival within the host. In Bangladesh, all of the El Tor isolates of *V. cholerae* O1 obtained since 2001 produced classical cholera toxin. In Kolkata, India, El Tor variant strains carrying the hybrid CTX prophage, which carries the El Tor *rstR* (*rstR*^{El}) (*rstR*, CTX prophage repressor gene) and the classical *ctxB* (*ctxB*^{Cl}), have entirely replaced the El Tor type *ctxB* since 1995.[5] In northern Vietnam, the El Tor variant carrying this hybrid CTX prophage has been reported since late 2007.[6]

In Myanmar, cholera is a fifth priority disease in National Health Plan (2006-11). *V. cholerae* O1 El Tor outbreak was occurred in 1961 and spread throughout the country. In 1994, *V. cholerae* 139 outbreak occurred in some townships of Yangon Reagion and since then there was no more O139 detected and only *V. cholerae* O1 is circulating in Myanmar. According to the laboratory data of the National Health Laboratory, Yangon, there were 103 culture-confirmed cholera cases in 2011.[7]

With consideration of the increase in the global prevalence of cholera, the origin and spread of these El Tor variant strains of *V. cholerae* in Myanmar should be tracked by genomic analysis.

In the present study, the genotypic characteristics of recent (2012) and past (1982-1996) *V. cholerae* O1 isolates from Yangon were investigated. Observation of possible emergence of a new virulent variant strain will provide an effective control of cholera infections and outbreaks. Moreover, the molecular epidemiological data will provide insight knowledge of facilitating further studies to develop a safe vaccine against cholera.

MATERIALS AND METHODS

Study design and period

Cross-sectional, descriptive study was carried out during 2012-2014.

Study population

Suspected cholera cases attending hospitals in Yangon (Specialist Hospital, Waibargi, Insein General Hospital, North Okkalapa General Hospital, Thingangyun Sanpya Hospital, Yangon General Hospital and New Yangon General Hospital) in 2012 and *V. cholerae* isolates from Yangon during 1982-1996.

Laboratory procedure

Rectal swabs in Cary Blair Transport Media were collected from suspected cholera cases attending hospitals in Yangon and transported to DMR-LM. Isolation, identification, serotyping and PCR-based characterization were carried out at Bacteriology Research Division. Pulse Field Gel Electrophoresis Typing (PFGE) and genome sequencing were carried out at Thailand-Japan Research Collaboration Center on Emerging and Reemerging Infections, Nonthaburi, Thailand.

Isolation, identification, serotyping

Rectal swabs were placed in alkaline peptone water for 6 hours and then streaked on thiosulphate-citrate-bile salt-sucrose (TCBS) agar (Eiken Chemical, Japan) and incubated for 12-18 hrs at 37°C. Suspicious colonies in yellow, flat, shiny, and 2-3 mm in diameter on TCBS were tested for biochemical identification and slide aggluination with *Vibrio cholerae* specific antisera (Denka Seiken, Japan). Past *V. cholerae* isolates from stock cultures were inoculated onto TCBS after enrichment in alkaline nutrient agar for 6 hrs.

Drug susceptibility test

Drug susceptibility to 11 antibiotics (Becton Dickinson, Sparks, MD) is tested using a standard disc diffusion technique, according to the guidelines[8] of Clinical and Laboratory Standards Institute.

PCR-based characterization

The amplification of virulence-associated genes and/or biotype makers encoding the genome of the *V. cholerae* isolates is performed using LAMP method and hexaplex polymerase chain reaction (PCR).[9]

Pulsed-field gel electrophoresis (PFGE) typing

Intact agarose-embedded genomic DNA of the *V. cholerae* strains is digested with *No* I enzyme (New England Biolabs, MA, USA) and the fragments are separated in a contour-clamped homogeneous electric field apparatus (CHEF-DRIII; Bio-Rad, Hercules, CA, USA) according to the Pulsenet *V. cholerae* subtyping protocol.[10]

Multilocus variable-number tandem repeat analysis (MLVA) DNA sequencing

Five loci for MLVA are amplified using primers and PCR conditions as described in previous studies.[11] The purified PCR products were sequenced in both directions using a Big Dye Cycle Sequencing kit (Applied Biosystems) and sequencing was performed on an ABI 3770 automatic sequencer according to manufacturer's instructions.

PFGE and MLVA profiles

Not I PFGE profiles were compared digitally using Bionumerics 6.1 software (Applied Maths). Cluster analysis of Dice similarity indices based on the unweighted pair group method with arithmetic mean (UPGMA) was used to generate a den-drogram describing the relationships among PFGE profiles.

Ethical consideration

This study was approved by the Ethical Committee on Medical Research Involving Human Subjects, Department of Medical Research (Lower Myanmar).

RESULTS

Phenotypic characteristics of V. cholerae isolates

Of 20 *V. cholerae* stock culture isolates collected during 1982-1986, 8 *V. cholerae* O1 strains (serotypes: 4 Inaba and 4 Ogawa) were revived and preceded for molecular characterization. Of 312 rectal swab speci-mens collected during 2012, *V. cholerae* O1 were isolated from 72 cases (23.1%). The 34 out of 72 isolates of *V. cholerae* O1 were proceeded for molecular analysis. All isolates were *V. cholerae* O1, serotype Ogawa and tetracycline resistant.

Genotypic pattern of past (1982-1996) V. cholerae isolates

Of 8 *V. cholerae* O1 strains isolated during 1982 to 1996 in Yangon, a total of 4 pulsotypes were identified. The isolates exhibited pulsotypes Y1 and Y4 carrying *ctxB*^{El} and *rstR*^{El} and they are related to seventh cholera pandemic El Tor strains examined by the Multi-locus sequence typing.

The 5 isolates isolated in 1982, 1986, and 1996, exhibited pulsotype Y3 which were corresponding to atypical El Tor strains carrying *ctxB*^{Cla} and *rstR*^{Cla}. The remaining pulsotype Y2 was atypical El Tor which carried *ctxB*^{Cla} and *rstR*^{El} (Table 1).

Table 1. Genotypic patttern of past (1982-1996) *V. cholerae* isolates (n=8)

No.	Year	Serotype	*ctxB* gene pattern	*rstR* gene pattern	PFGE pattern
1	1982	Inaba	El	El	Y1
2	1982	Inaba	Cl	El	Y2
3	1982	Ogawa	Cl	Cl	Y3
4	1982	Inaba	El	El	Y4
5	1982	Inaba	Cl	Cl	Y3
6	1966	Ogawa	Cl	Cl	Y3
7	1986	Ogawa	Cl	Cl	Y3
8	1996	Ogawa	Cl	Cl	Y3

EL=ELTOR, CL=Classical

Genotypic pattern of recent (2012)V. cholerae isolates

All 34 *V. cholerae* O1 Ogawa carried *tcpA* (encoding the structural subunit of the toxin-coregulated pilus) and *rstR* (repressor gene in CTX phage) of El Tor biotype. All sequences toxin B subunit gene were classical type (*ctxB*^{Cla}). All recent Myanmar isolates were atypical El Tor, which carried *ctxB*^{Cla} and *rstR*^{El} and exhibited a total of PFGE 10 patterns (Y5 to Y14). Y10 pulsotype was predominantly found in 50% isolates (17/34). Multilocus variable-number tandem-repeat analysis of Y10 isolates revealed that five MLVA types were shown and they were closely related (Table 2 & Fig. 1).

Table 2. Genotypic pattern of recent (2012) *V. cholerae* O1 isolates (n=34)

No.	Date	Age	Sex	PFGE pattern	MLVA type
1	10-Feb	18	M	Y14	-[†]
2	13-Feb	24	M	Y5	-
3	13-Mar	1	M	Y6	-
4	15-Mar	12	M	Y8	-
5	16-Mar	18	F	Y9	-
6	19-Mar	68	M	Y11	-
7	20-Mar	24	F	Y10	M1[‡]
8	20-Mar	7	M	Y11	-
9	23-Mar	56	M	Y10	M1
10	26-Mar	54	M	Y8	-
11	29-Mar	62	M	Y8	-
12	29-Mar	63	F	Y11	-
13	2-Apr	18	M	Y7	-
14	2-Apr	50	F	Y10	M5
15	2-Apr	20	M	Y12	-
16	2-Apr	28	F	Y10	M1
17	2-Apr	31	F	Y10	M1
18	2-Apr	22	F	Y11	-
19	4-Apr	48	F	Y10	M1
29	4-Apr	17	F	Y11	-
21	23-Apr	35	M	Y10	M3
22	2-May	10	F	Y10	M1
23	7-May	4	M	Y10	M2
24	14-May	2	M	Y10	M2
25	15-May	2	M	Y10	M1
26	5-Jun	5	F	Y10	M1
27	5-Jun	2	F	Y10	M1
28	6-Jun	2	F	Y10	M2
29	6-Jun	4	M	Y10	M2
30	14-Jun	24	F	Y10	M1
31	28-Jun	1	F	Y12	-
32	4-Jul	1	M	Y10	M2
33	30-Jul	5	F	Y11	-
34	6-Aug	56	F	Y11	-

MLVA types, M1 (11, 6, 5, 17, 17), M2 (11, 6, 5, 17, 18), M3 (10, 6, 5, 17, 17), M4 (11, 6, 5, 12, 17), and M5 (11, 6, 5, 16, 17)

The numbers of repeats were counted and listed sequentially for the five VNTR loci (VC0147, VC0436-7, VC1650, VC0171, and VCA0283) to generate an isolate pattern.

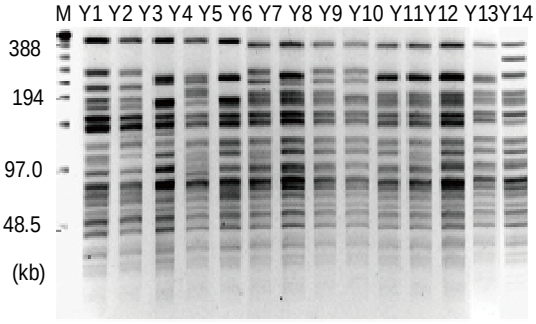

Fig. 1. Y1-Y14 PFGE pattern of *V. cholerae* isolates showing multiple bands

DISCUSSIONS

In Myanmar, morbidity rate of severe diarrhea (assumed to include cholera cases but not laboratory confirmed) is an estimated 2.6-3.5/100,000 population and mortality rate is 0.04-0.1/100,000.[12]

Yangon is the largest city and the total population of the city is 4.5 million (2014 Census). Cholera outbreaks caused by El Tor biotype were recorded in 1961 in Yangon, although it is unknown to be related with the seventh cholera pandemic wave which began in Indonesia, 1961. *V. cholerae* O139 Bengal was initially appeared in Bangladesh and India 1992,[13] In 1994, several outbreaks caused by O139 cholera pathogen were firstly recognized in some townships of Yangon.[14] In recent two decades, atypical El Tor *V. cholerae*, which possesses traits of both classical and El Tor biotypes, has replaced the seventh pandemic prototypic El Tor *V. cholerae* O1 in Asian and African counties.[15]

All 2012 isolates were serotype Ogawa, tetracycline resistant, and carried *tcpA* (encoding the structural subunit of the toxin-coregulated pilus) and *rstR* (repressor gene in CTX phage) of El Tor biotype,[16] while all sequences of cholera toxin B subunit gene were classical type ($ctxB^{Cla}$). There is no O139 Bengal isolate, O1 isolates carrying Haitian variant cholera toxin gene[17] and MS6 strain which we previously found as a new genetic line in Thai Myanmar border area.[18] Recent 2012 *V. cholerae* isolates from Yangon were of atypical El Tor, which carried $ctxB^{Cla}$ and $rstR^{El}$ and exhibited a total of 10 patterns of Pulsed Field Gel Electrophoresis (PFGE).

Between February and April, 2012, the majority of patients was adults and 9 variation of PFGE patterns were observed. Whereas, since May to August, most patients infected with *V. cholerae* O1 were children less than 5 years old and almost exhibited pulsotype Y10. This pulsotype was predominantly found in 50% of isolates. For further distinguish, we apply all isolates of Y10 for multilocus variable-number tandem-repeat analysis and

revealed that five MLVA types were shown and they were closely related. Sporadic cholera among children since May probably attributed to limited sources of infection during rainy season in Yangon.

V. cholerae O1 strains isolated during 1982 to 1996 in Yangon were analyzed. A total of 4 pulsotypes were identified and they were different from 2012 PFGE patterns. The isolates exhibited pulsotypes Y1 and Y4 carried $ctxB^{El}$ and $rstR^{El}$ and they are related to seventh cholera pandemic El Tor strains examined by the Multi-locus sequence typing. The 5 isolates isolated in 1982, 1986, and 1996, exhibited pulsotype Y3 which were corresponding to atypical El Tor strains carrying $ctxB^{Cla}$ and $rstR^{Cla}$. The remaining pulsotype Y2 was atypical El Tor which carried $ctxB^{Cla}$ and $rstR^{El}$. Nevertheless, their pulsotypes in past and present were differentiable.

The largest bands of PFGE pattern (ca. 400 kb) in Y1-Y4 of past isolates and in Y5 could be due to the insertion of bacteriophage K139 (35 kb) (referenced MJ1236 complete genome, accession no. NC_012667/ NC_012668) in the large chromosome confirming by PCR and Southern hybridization (using probes against the genes encoding phage replication (VCD_002162) and capsid (VCD_002167) proteins).

Taken together, in 1982, Yangon would had been attacked by several waves of cholera transmission, at least three cholera organisms which carried different CTX phages. Remarkably, seventh cholera pandemic prototype El Tor were observed only in 1982 isolates. In 1986-1996, *V. cholerae* O1 were only atypical El Tor with $ctxB^{Cla}$ and $rstR^{Cla}$. Notably, atypical El Tor with $ctxB^{Cla}$ and $rstR^{El}$ existed in Yangon 30 years ago. Nevertheless, this type of atypical El Tor has replaced in 2012. Variation of PFGE in adult patients and on the contrary, limited PFGE pattern of less 5 aged, could have led to understand the transmission routes in Yangon. Continuous and systematic surveillance on cholera in Yangon would contribute especially to prevention and control.

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Sero-prevalence of Hepatitis B and C Viral Infections in Myanmar: National and Regional Survey in 2015

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In the South East Asia region, there is an estimated 100 million people living with chronic hepatitis B viral infection and 30 million people with chronic hepatitis C viral infection. Estimates of how many people are likely to be affected may be made by assessing populations at high risk as well as previously documented prevalence and incidence rates. This study was aimed to determine the sero-prevalence of hepatitis B and C viral infections in Myanmar as a nationwide survey and to describe the associated factors of transmission route of these infections. A cross-sectional study was conducted among general population of 18 townships, selected from 7 States, 7 Regions and Nay Pyi Taw Union Territory in 2015. A total of 5547 subjects after taking informed consent were tested for HBs antigen and anti-HCV antibody by one-step qualitative immunochromatographic assay (Standard Diagnostic, Inc, Korea). The average prevalences for hepatitis B and C viral infections among general population was 6.5% and 2.7%, respectively. The highest prevalence of hepatitis B infection was 12.3% in Yangon Region and the lowest was 3.3% in Magway Region. Regarding hepatitis C prevalence, the highest was 10.3% in Mon State and the lowest was 0.3% in Bago Region and Chin State. The potential associated factors of transmission of infection were male gender, age >50 years, blood transfusion, dental treatment, surgery, and history of liver disease or hepatitis, significantly related to hepatitis C virus positivity. This study will provide the evidence-based prevalence data to National Hepatitis Control Program which can lead to follow-up the surveillance and to plan the screening and treatment strategies of these infections in Myanmar.

Key words: General population, Hepatitis B virus, Hepatitis C virus, Myanmar, Sero-prevalence

INTRODUCTION

Viral hepatitis is a group of infectious diseases that affects hundreds of millions of people, worldwide. Five distinct hepatitis viruses have been identified: A, B, C, D and E. Hepatitis B and C, which can lead to chronic hepatitis, are particularly prevalent; 240 million people are thought to be chronically infected with hepatitis B and 184 million people have antibodies to hepatitis C.[1, 2] According to the Global Burden of Disease estimates, hepatitis B and hepatitis C together caused 1.4 million deaths in 2010, including deaths from acute infection, liver cancer and cirrhosis.[3]

It is estimated that in the next 10 years, more than 5 million people in the countries of the WHO South-East Asia Region will die from the consequences of viral hepatitis.[1] There is an estimated 100 million people living with chronic hepatitis B viral-

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(HBV) infection and 30 million people with chronic hepatitis C viral (HCV) infection in the South-East Asia Region. Chronic viral hepatitis infections are 30 times more frequent than HIV in this region. Because of the asymptomatic nature of chronic HBV and HCV, most people infected with these are not aware of their status until they have symptoms of cirrhosis or liver cancer many years later. About 65% of those with HBV and 75% of those with HCV do not know they are infected.[4] In Myanmar, HBV prevalence in general population was 10-12%[5] and HCV prevalence in healthy population was 2.5%.[6] The prevalences of HBV and HCV infections in different regions or townships or different populations were found out by different research groups.[7]

Antiviral agents against HBV and HCV exist and new therapies are also being developed. Treatment of HBV infection has been shown to reduce the risk of developing liver cancer and death. HCV is generally considered to be a curable disease but for many people this is not the reality. Access to treatment remains a constraint in many parts of the world. Viral hepatitis places a heavy burden on the health care system because of the costs of treatment of liver failure and chronic liver disease. In many countries, viral hepatitis is the leading cause of liver transplants. Such end-stage treatments are expensive, easily reaching up to hundreds of thousands of dollars per person.[8]

In Myanmar, the Ministry of Health introduced the hepatitis B vaccine combined with routine Expanded Program of Immunization (EPI) for all infants in 2003 with assistance from Global Alliance for Vaccines Introduction (GAVI). The coverage of HB vaccination in the EPI was gradually increased 62% to 90% from 2005 to 2010 in all states and divisions, Myanmar. Recombinant hepatitis B vaccine plant at Department of Medical Research has the capacity to produce 5 million doses annually for infants and approval from Myanmar FDA was obtained in 2007.[9] Extended program of HB vaccination at birth and other successful HB vaccination strategies will provide to dramatic reduction of HB transmission and maintain the control of chronic liver diseases related to hepatitis B viral infection in Myanmar.[10]

The preventive measures, effective screening of those infections and early treatment are important to consider and perform to reduce transmission. Preventive measures include the screening and testing of blood, plasma, organ, and tissue donors, risk-reduction counseling and services, implementation and maintenance of infection control practices, identification, counseling and testing of persons at risk, medical management of infected persons, health education, surveillance and research.

National programs are required to plan screening and treatment strategies. Building on country-specific information on policies and structures will be necessary to increase the availability of treatment for those infected. This study was aimed to determine the sero-prevalence of HBV and HCV infections in general population of Myanmar as a nationwide survey, and to describe the associated factors of transmission route of these infections.

MATERIALS AND METHODS

A cross-sectional study was conducted in persons aged between 15 to 80 years, both sexes from 18 townships. The survey was carried out from May to October, 2015 and a total of 5547 subjects participated in the survey. Eighteen townships were selected from 7 States (Kachin State, Kayah State, Kayin State, Chin State, Mon State, Shan State-East, Shan State-South, Shan State-North, and Rakhine State), 7 Regions (Bago Region-East, Bago Region-West, Sagaing Region, Magway Region, Ayeyawady Region, Thaninthayi Region, Yangon Region, and Mandalay Region) and Nay Pyi Taw Union Territory. To achieve a national representative sample,

two-stage cluster sampling method was used and selection of primary sampling units (PSUs) was performed that one township was randomly selected, which is considered to have average level of viral hepatitis B or C infection in all States and Regions of Myanmar. Selection of secondary sampling units (SSUs) was performed that from each selected PSU (township), 10 wards and villages were selected according to probability to population size. From each selected SSU (ward/village), 30 households were selected using systematic random sampling. The sampling frame for this sampling is the list of households available from the Basic Health staff. One eligible participant aged between 15 to 80 years in the selected households was recruited by random sampling.

The participants' informed consent was obtained by in-field investigators who explained the purpose and procedure of the study and it was signed on-site at the time before the blood samples were taken. Past medical and surgical history, family history, and risk factors were recorded according to the proforma with code number. Blood was taken from finger tip of each subject by sterile lancet needle under aseptic condition.

For hepatitis B virus screening, SD Bioline HBsAg WB (Cat. No 01FK10W, Standard Diagnostic, Inc., Korea), and for hepatitis C virus screening, SD Bioline HCV (Cat. No. 02FK10, Standard Diagnostic, Inc. Korea) were used according to the manufacturer's instructions. The sensitivity of HB device and HC device are 100%. The specificities of HB device and HC device are 100% and 99.4%, respectively. HBV infection was defined by positivity of HBs antigen in blood by immunochromatographic test and HCV infection was defined by positivity of anti-HCV antibody in blood by immuno-chromatographic test.

The result was given to the participants individually with closed envelope. The counseling about the con-sequences of these infections, treatment options, the pamphlet of health education and the referral to the hepatology or medical department of general hospitals for early diagnosis and treatment were given to the HB or HC positive participants.

Statistical analysis

Data entry was performed and checked for double entry, incorrectness, incompleteness to validate the data. Simple descriptive analysis for each variable was done by mean, SD, minimum, maximum, range, percentage etc. Statistical analysis was done by using SPSS version 16 and Stata 13. Differences were considered significant if $p<0.05$.

Ethics approval and consent to participate

The ethics approval and consent of this study was granted by Ethics Review Committee of Department of Medical Research with letter No.16/Ethics 2015, dated 11.3.2015.

RESULTS

Demographic characteristics

The study population's age ranged from 15 to 80 years with a mean age of 36 years (SD=12) and more than half (58.6%) of the study population was 20-39 years age group. Female gender constituted about 70.2% (3894/5547) and the remaining 29.8% (1653/5547) were male. 68.3% of the study population were married and 28.9% were never married. The family members ranged from one to seventeen and average number was 4.8 (SD=2). Demographic characteristics are summarized in Table 1.

Sero-prevalence of HBV and HCV infections

An overall average sero-prevalence for HB and HC among general population from eighteen townships was 6.5% (361/ 5547) and 2.7% (147/5547), respectively. Two out of 5547 in this study had both HB and HC viruses. The prevalence of HB and HC infections by demographic characteristics is shown in Table 1.

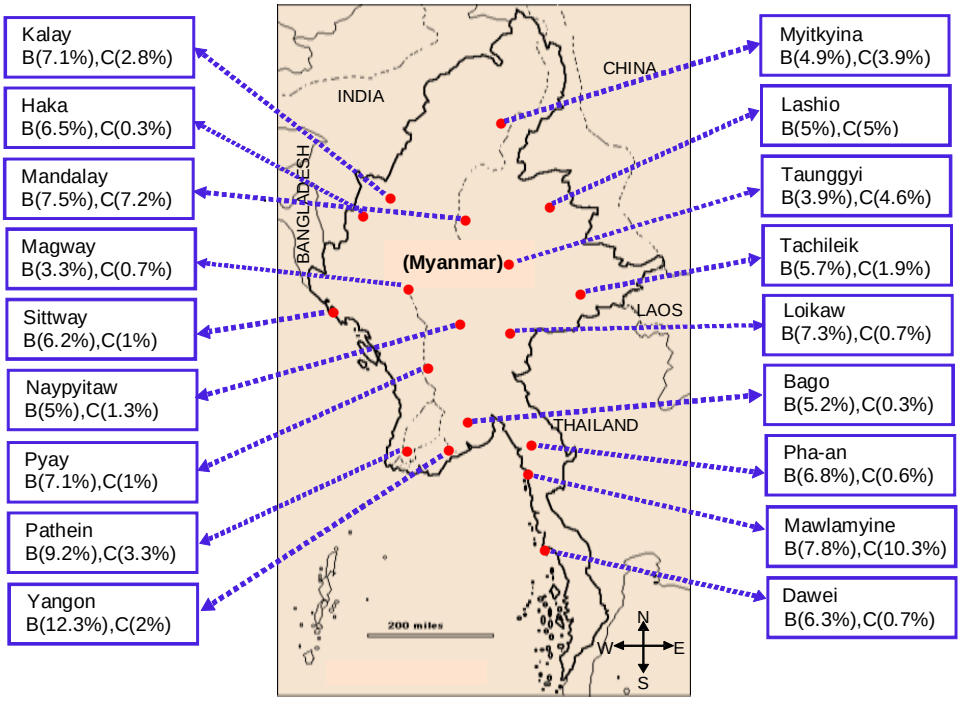


Fig. 1. HBV and HCV prevalence by States and Regions of Myanmar (2015)

Table 1. Prevalence of HB and HC infections in 18 selected townships with demographic characteristics

Characteristics	Subject	HBsAg positivity			Anti-HCV antibody positivity		
		n (%)	95%, CI	*P* value	n (%)	95%, CI	*P* value
Total population	5547	361(6.5)	5.87, 7.19		147(2.7)	2.24, 3.11	
Age group (year)							
15-19	117	6 5.1)	2.32, 10.95	0.095	0	-	<0.001
20-29	1685	113(6.7)	5.61, 8		7(0.4)	0.2, 0.87	
30-39	1563	115(7.4)	6.16, 8.76		30(1.9)	1.35, 2.73	
40-49	1409	86(6.1)	4.97, 7.48		60(4.3)	3.32, 5.45	
50-59	615	34(5.5)	3.98, 7.64		45(7.3)	5.51, 9.66	
>60	158	7(4.4)	2.13, 9		5(3.2)	1.32, 7.38	
Gender							
Male	1653	148(9)	7.67, 10.4	<0.001	58(3.5)	2.72, 4.51	0.009
Female	3894	213(5.5)	4.8, 6.23		89(2.3)	1.86, 2.81	
Marital status							
Never married	1602	106(6.6)	5.5, 7.94	0.026	25(1.6)	1.06, 2.3	0.011
Married	3790	246(6.5)	5.75, 7.32		116(3.1)	2.56, 3.66	
Separated/ Widowed	155	9(2.5)	1, 4		6(4.1)	1.06, 6.9	
Family size							
1-4	2851	176(6.2)	5.35, 7.12	0.29	77(2.7)	2.17, 3.36	0.8
>5	2696	185(6.9)	5.97, 7.88		70(2.6)	2.06, 3.27	

The highest HBV prevalence (7.4%) was found in 30-39 years age group but the highest HCV prevalence (7.3%) was found in 50-59 years age group. Male had significantly higher prevalence of both infections than female gender (9% *vs.* 5.5%, p<0.001 for HBV, 3.5% *vs.* 2.3%, p=0.009 for HCV). The prevalence of both viruses varied between townships and Table 2 and Figure 1 show that the prevalence of HBV and HCV infections by each township. There were significant differences in the prevalence between townships from all States and Regions. The highest prevalence of HB infection was 12.3% in Yangon Region (Hlinethaya Township) and the lowest was 3.3% in Magway Region (Magway Township).

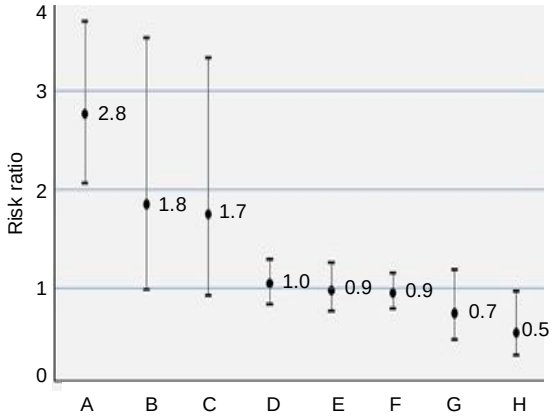
Table 2. Prevalence of HBV and HCV infections in 18 townships

State/Region	Township	Subject	HBsAg positivity		Anti-HCV positivity	
			n(%)	95%, CI	n(%)	95%, CI
Total		5547	361(6.5)	5.87, 7.19	147(2.7)	2.06, 3.27
Yangon	Hlinethaya	301	37(12.3)	9.04, 16.51	6(2)	0.9, 4.36
Ayeyawady	Pathein	306	28(9.2)	6.39, 12.93	10(3.3)	1.77, 5.97
Mon	Mawlamyine	319	25(7.8)	5.35, 11.34	33(10.3)	7.45, 14.2
Mandalay	Pyikyiitagon & Chanayetharzan	307	23(7.5)	5.03, 11.02	22(7.2)	4.76, 10.64
Kaya	Loikaw	300	22(7.3)	4.88, 10.89	2(0.7)	0.17, 2.63
Sagaing	Kalay	322	23(7.1)	4.79, 10.52	9(2.8)	1.46, 5.28
Bago-West	Pyay	310	22(7.1)	4.72, 10.54	3(1)	0.31, 2.96
Kayin	Hpa-an	324	22(6.8)	4.51, 10.10	2(0.7)	0.15, 2.43
Chin	Haka	309	20(6.5)	4.21, 9.82	1(0.3)	0.05, 2.26
Taninthayi	Dawei	304	19(6.3)	4.02, 9.59	2(0.7)	0.16, 2.59
Rakhine	Sittway	306	19(6.2)	4, 9.53	3(1)	0.32, 2.99
Shan-East	Tachileik	315	18(5.7)	3.63, 8.89	6(1.9)	0.86, 4.17
Bago-East	Bago	310	16(5.2)	3.19, 8.26	1(0.3)	0.05, 2.25
Shan-North	Lashio	298	15(5)	3.06, 8.18	15(5)	3.06, 8.18
Kachin	Myitkyina	303	15(5)	3.01, 8.05	12(4)	2.26, 6.84
Naypyitaw Union Territory	Pyinmana	301	15(5)	3.03, 8.10	4(1.3)	0.5, 3.49
Shan-South	Taunggyi	308	12(3.9)	2.23, 6.73	14(4.6)	2.71, 7.53
Magway	Magway	304	103.3	1.8, 6	2(0.7)	0.2, 2.6

Table 3. Prevalence of HBV and HCV infections with associated factors

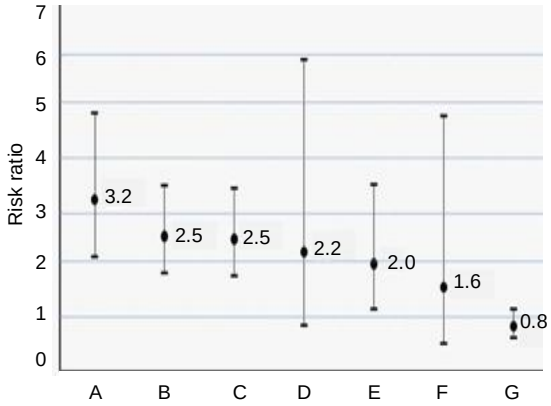
Associated/Risk factors	Subjects	HBsAg positivity			Anti-HCV antibody positivity		
		n (%)	95%,CI	*P* value	n (%)	95%,CI	*P* value
History of blood transfusion							
Yes	344	16(4.7)	2.87, 7.46	0.15	26(7.6)	5.20, 10.8	<0.001
No	5186	343(6.6)	5.97, 7.32		121(2.3)	1.96, 2.78	
History of surgery							
Yes	1069	66(6.2)	4.88, 7.78	0.63	55(5.1)	3.97, 6.64	<0.001
No	4460	293(6.6)	5.88, 7.34		92(2.1)	1.68, 2.52	
History of dental treatment							
Yes	1469	96(6.5)	5.38, 7.92	0.93	70(4.8)	1.50, 2.34	<0.001
No	4060	263(6.5)	5.76, 7.28		76(1.9)	3.79, 5.98	
History of any piercing							
Yes	3050	190(6.2)	5.42, 7.14	0.37	75(2.5)	2.31, 3.64	0.30
No	2479	169(6.8)	5.89, 7.88		72(2.9)	1.97, 3.07	
History of sharing razor							
Yes	72	8(11.1)	5.65, 20.6	0.11	3(4.2)	1.35, 12.1	0.42
No	5455	351(6.4)	5.81, 7.12		144(2.6)	2.25, 3.10	
History of sharing tooth brush							
Yes	68	8(11.8)	5.99, 21.8	0.076	4(5.9)	2.23, 3.08	0.097
No	5459	351(6.4)	5.81, 7.11		143(2.6)	2.23, 14.6	
History of HB vaccination							
Yes	298	10(3.4)	1.81, 6.12	0.024	17(5.7)	3.58, 8.98	0.001
No	5230	349(6.7)	6.03, 7.38		130(2.5)	2.10, 2.94	
History of liver disease or hepatitis							
Yes	257	39(15.2)	11.29, 20	<0.001	18(7.0)	4.46, 10.8	<0.001
No	5304	320(6.1)	5.46, 6.75		128(2.4)	2.05, 2.88	
Household contact with HBV/HCV							
Yes	253	42(16.6)	12.51, 21	<0.001	13(5.1)	3.01, 8.65	0.012
No	5274	317(6.0)	5.40, 6.69		134(2.5)	2.15, 3.00	
History of DM or renal disease							
Yes	254	17(6.7)	4.20, 10.5	0.88	13(5.1)	2.99, 8.61	0.013
No	5272	341(6.5)	5.84, 7.16		134(2.5)	2.15(3.00)	

Regarding HCV prevalence, the highest was 10.3% in Mon State (Mawlamyine Township) and the lowest was 0.32% in Bago-East Region (Bago Township) and Chin State (Haka Township). The HB and HC prevalences by urban rural populations were 6.1% (urban), 3.2% (rural) and 6.9% (urban), 2% (rural), respectively. The HCV prevalence was higher in urban population (3.2% *vs.* 2%). The associated factors of the transmission of those infections analyzed in this study with the positivity of those infections are shown in Table 3.



A=Household contact with HBV
B=Sharing tooth brush
C=Sharing razor
D=History of dental procedure
E=History of surgical operation
F=History of skin piercing
G=History of blood transfusion
H=History of HB vaccination

Fig. 2. Risk ratio and 95% CI for specific risk factors of viral hepatitis B infection



A=History of blood transfusion
B=History of dental procedure
C=History of surgery
D=History of sharing tooth brush
E=Household contact with HCV
F=History of sharing razor
G=History of skin piercing

Fig. 3. Risk ratio and 95% CI for specific risk factors of viral hepatitis C infection

It was found that the significant associated factors for HBV were: (i) male gender, (ii) history of liver disease or hepatitis (iii) history of household contact of infection and (iv) HB vaccination. For HCV, the significant associated factors were: (i) age >50 years, (ii) male gender, (iii) blood transfusion, (iv) dental treatment, (v) surgical operation, (vi) history of liver disease or hepatitis (vii) history of household contact of infection and (viii) HB vaccination. Risk ratio and 95%CI for associated factors of viral hepatistis B and C infections are shwon in Figure 2 and 3. History of household contact, sharing razor and tooth brush were two times associated with HBV infection and the history of blood transfusion, dental procedure, surgery, household contact, sharing razor and tooth brush were two to three times associated with HCV infection.

DISCUSSION

The overall prevalence of HBV infection was 6.5% in this study which was lower than previous estimate (10-12%) for overall HBV prevalence in Myanmar.[5, 7] It might be due to difference in methodology such as small sample size or narrow age group and the effective health education and awareness raising activities leading to preventive measures for HB infection such as HB vaccination and reducing risk factors. There was a variation in HB prevalence across the States and Regions and it may be due to the differences in risk factors. The highest prevalence (12.3%) was in Yangon Region and the lowest (3.3%) in Magway Region. It may be due to urban population (population density) and floating population who are probably exposed to more risk factors of transmission, geography, economic level, lack of knowledge to risk factors of transmission.

The highest HBV prevalence (7.4%) was found in 30-39 years age group and it was different from the findings reported by Yang S, *et al.*[11] that groups aged 41-60 years had the highest HB prevalence rate but there was no significant association between hepatitis B prevalence and age. HBV infection being higher in 30-39 years age group may be due to their greater exposures in society as compared to

children and aged persons. Although HBV prevalence was highest in 30-39 years age group, it was not much different (5.1%, 6.7%, 7.4%, 6.1%, 5.5% and 4.4%) in every age group and statistically not significant (p=0.095) (Table 1). But under 15 years age group was not included in this study, who received the birth-dose HB immunization and there will be decreased HB prevalence rate in that population. It will be impossible to interpret the high or low HBV prevalence with age groups because it depends mainly on the transmission routes and availability of HB vaccine prevention. HBV is mostly transmitted by vertical mother to child transmission.

Male had significantly higher prevalence than female gender (9.0% *vs.* 5.5%, p<0.001) in our study and it was also different with the report of Yang S, *et al.*[11] in which there was no association between gender and HBV infection (male 4.11% *vs.* female 3.97%). It may be due to some associated factors like their being employed outside their homes, visiting barber shops, migrants for job (floating population), and less accessible to get complete HB vaccination etc. but women are mostly involved in household activities due to religion and culture. The overall prevalence of HCV infection was 2.7% (147/5547) in this study and it was similar to previous finding in 2002 by Khin Pyone Kyi, *et al.*[6] which was 2.5% (9/379) among general population in Myanmar and the result of Wanting Cheng, *et al.*[12] that was 2.8% in >14 years of residents in Southwest China.

But our finding was different from the result of Myo Khin, *et al.*[13] which was 0.93% (0.34-2.03%) among Myanmar blood donors from 10 hospitals during 2005-2007. In this study, the highest prevalence was 10.3% (33/319) in Mawlamyine Township of Mon State and the lowest 0.3% was in Bago (1/310) and Haka (4/309) townships. It was comparable to that of blood donor population.[13] There was also a variation in HCV prevalence across the States and Regions and it may be due to geography, floating population who are probably exposed to more risk factors of transmission and, lack of knowledge to transmission risk factors. The highest HCV prevalence (7.3%) was found in 50-59 years age group and it was significantly different with other age groups. There was no anti-HCV positivity among <20 years age group (Table 1). Our finding was different from the report of Myo Khin, *et al.*[13] in which 1.9% of HCV prevalence was found among 41-50 years age group of Myanmar blood donors.

Regarding HCV prevalence of 50-59 years age group, the most associated factors were having dental procedure (71%, 32/45), history of any piercing (51%, 23/45), having surgical operation (40%, 18/45), and receiving blood transfusion (20%, 9/45) (data not shown). In 40-49 years age group, the most associated factors were having dental procedure (48%, 29/60), history of any piercing (43%, 26/60), having surgical operation (40%, 24/60), and receiving blood transfusion (15%, 9/60) (data not shown). Comparison of these factors in two age groups, having dental procedure, history of any piercing, and receiving blood transfusion factors were the more possible transmission routes for the highest HCV prevalence in 50-59 years age group. Dental procedure and piercing factor are less likely to practice in younger age group.

HCV was transmitted by blood or blood products as a well-known factor and proper blood screening is essential to prevent the transmission of HCV infection. In Myanmar, HCV screening in blood donor was started in 2000 and it may be one of the causes for increased HCV pre-valence in older age groups. HCV pre-valence is low in younger populations but increases dramatically and is sustained in older populations as a reflection of a past high risk of infection.

Moreover, it is not possible to differentiate recent infection from chronic or past infection by detection of anti-HCV antibody alone and antibody is persistently detected

in infected persons. The present study highlights that a significant higher HCV prevalence was found in male gender (3.5% *vs.* 2.3%, p=0.009) which was different from the previous finding of Myo Khin, *et al.*[13] that female blood donors had slightly higher anti-HCV seropositivity than male donors (1.06% *vs.* 0.93%). The present study of HCV prevalence in male gender was lower than the finding by Wanting Cheng, *et al.*[12] They reported that men had a higher prevalence of HCV infection than women (6% *vs.* 1%) in participants aged ≥14 years during 2014 to 2015. This may be due to the lack of awareness about the possible risk factors among the general population.

Regarding the potential associated factors of transmission of HCV infections, male gender, age over 50 years, blood transfusion, dental treatment, surgery, and history of liver disease or hepatitis were significantly related to HCV positivity. Since no vaccine is as yet available for HCV infection, the most effective control measure would be to prevent this potentially life-threatening viral disease. Male gender, history of liver disease or hepatitis and household contact were significantly related to HBV positivity. Therefore, these associated factors should be considered as effective control measures to reduce the transmission of those infections. In this study, only 5.4% (298/5528) of the population gave history of complete HB vaccination and it pointed out that the complete HB vaccination essentially needs to be recommended for all age groups for prevention of HBV infection. Hepatitis B vaccination should be focused on adults, under the consideration of policies for universal vaccination, especially in areas or regions with high hepatitis B endemicity.

Myanmar is located in South-East Asia region which has high prevalence of HBV (>8%) and HCV (>2%) infections. The overall prevalence of HBV infection in this study was lower than those of some South-East Asia region countries[14, 15] but it was higher than those of India and Thailand.[16, 17] The overall prevalence of HCV infection in this study was higher than those of China, Vietnam and Thailand[14, 15, 18] but it was lower than the HCV prevalence in Central India.[19] The large reservoir of HBV and HCV infections will cause an enormous burden of patients with cirrhosis and hepatocellular carcinoma in the future. Therefore, our findings could fulfill the needs to control those infections and reduce the burden of disease.

Recommendation

The complete HB vaccination to all age groups including adults born prior to HB vaccine integration into the EPI and the confirmation of those infections by EIA or nucleic acid detection should be carried out to consider for future treatment, especially for HCV infection.

Conclusions

This study would provide the evidence-based prevalence data to National Hepatitis Control Program which can lead to follow up of the surveillance and to plan the screening and treatment strategies of these infections in Myanmar.

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Genotyping of High-risk Type Human Papillomavirus (HR-HPV) in Women with Cervical Cytological Abnormalities

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Human papillomavirus genotype identification is important for the estimation of the impact of HPV-based cervical cancer screening and HPV vaccination. This study aimed to determine the proportion of high-risk human papillomavirus (HR-HPV) infection and genotypes in women with abnormal cervical cytology. A cross-sectional descriptive study was carried out in women attending the cervical cancer screening clinic, DMR (LM) in 2010-2011. Cervical swabs were collected from 96 women with abnormal cervical cytology and 20 with normal cytology, age ranging from 18-69 years. HR-HPV DNA testing was performed by polymerase chain reaction (PCR) with *pU1M/pU2R* primers. HR-HPV were identified in (22/62) 35.5% of inflammatory smear, (6/10) 60% atypical squamous cells of undetermined significance, (13/15) 86.7% low-grade squamous intraepithelial lesion, (3/6) 50% high-grade squamous intraepithelial lesion, (3/3) 100% squamous cell carcinoma and (1/20) 5% normal cytology. In PCR positive cases, HPV genotyping was analyzed by cleaved amplification polymorphism method. The most prevalent HPV genotypes were HPV16 (60.4%) followed by HPV 31 (14.6%), HPV18 (12.5%) and HPV 58 (12.5%). The women with abnormal cervical cytology were 10 times more likely to be HR-HPV positive than those with normal cytology (p=0.0001). Among cervical cancer cases, 66.7% was genotyped as HPV 16 and 33.3% was HPV 18. Most patients infected with HR-HPV were aged between 40-49 years followed by 30-39 years. This study suggests that HPV 16 & 18 were mainly responsible for cervical cancer in Myanmar as in other countries and the implementation of routine vaccination against HPV in preadolescent and adolescent groups will greatly reduce the burden of HPV-associated cervical cancer.

INTRODUCTION

Worldwide, cervical cancer is the third most common cancer in women and the seventh overall with an estimated 530,000 new cases in 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. Cervical cancer remains the most common cancer in women only in Eastern Africa, South-Central Asia and Melanesia. Overall, the mortality: incidence ratio is 52% and cervical cancer is responsible for 275,000 deaths in 2008, about 88% of which occur in developing countries. WHO South-East Asia region (SEARO) reported that cervical cancer incidence and mortality is an estimated 188,000 new cases and 102,000 deaths in 2008.[1] This is due to the fact that the majority of women in the world do not have access to cervical screening which can prevent up to 75% of cervical cancer.[2] In Myanmar, over the past 30 years (1976-2006) showed that among 56097 total commonest female cancers, cervical cancer accounted for 13181 cases which always being either the commonest or the second commonest female cancers all along.[3] Estimated cervical cancer incidence in 2008 was 26.4% (6434 cases per 100, 000).[1]

Worldwide, HPV 16 and 18, the two vaccine-preventable types contribute to over 70% of all cervical cancer cases, 41-67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions. In South-Eastern Asia, prevalence of HPV 16 and HPV 18 by cytology was 72.6% in cervical cancer, 33.3% HSIL, 14.2% LSIL and 3.2% normal cytology.[4]

Human papillomavirus (HPV) can be identified in virtually all 99.7% of cervical cancer cases and has been established as etiological agents of invasive cervical cancer[5] and they are the most common sexually transmitted viral infection world-wide. Persistent infection with oncogenic or high-risk HPV (HR-HPV) is necessary for the development of premalignant lesions and/or progression of the disease.[6]

The HPV prevalence and genotype distribution are important for the estimation of the impact of HPV-based cervical cancer screening and HPV vaccination on the incidence of diseases etiologically linked to HPV. Distribution of HPV genotypes varies across different populations and geographical regions.[7] In Myanmar, limited data are available on the distribution of HPV genotypes in the general population and in low-grade and high-grade lesions of cervix and cervical cancer. With the advent of preventive HPV vaccines that target HPV 16 and 18 which are responsible for 70% of invasive cervical cancer in the world, such information is crucial to predict how vaccination and HPV-based screening will influence the prevention of cervical cancer. This study aimed to determine the proportion of high-risk human papillomavirus infection and genotypes in women with abnormal cervical cytology.

MATERIALS AND METHODS

A laboratory-based cross-sectional descriptive study was carried out in women who have cervical cytological abnormalities reported by cytopathology in cervical cancer screening clinic, DMR (LM) in 2010-2011. After getting informed consent, cervical swabs were taken from 96 women with abnormal cervical cytology and 20 with normal cytology for HPV-DNA testing and genotyping. Those cervical cells were collected in the phosphate buffer saline and stored in -20ºC.

For DNA extraction, they were suspended in 300 µl of proteinase K and incubated at 50°C for 2 hours and then treated with 100 µl of 5M NaCl. After centrifuged, the supernatant was treated with 900 µl of ethanol. DNA precipitates were collected by centrifugation at 12000 rpm for 10 minutes and washed with 400 µl of 70% ethanol. DNA was dissolved in 100 µl of TE.

HPV-DNA testing was performed using polymerase chain reaction (PCR) method. Consensus sequence primer pairs within the E6 and E7 open reading frames (*pU-1M* & *pU-2R)* were used to amplify HR-HPV (HPV 16, 18, 31, 33, 35, 52b, 58). Reaction mixture was done using taq polymerase, 10X buffer, dNTPs, forward and reverse primers, distilled water and DNA. They were subjected to 35 cycles of amplification using ASTEC thermal cycler. Each cycle included a denaturation, annealing and extension steps. Detection of the PCR products was performed by electrophoresis on 6% polyacrylamide gel (PAGE), 200V, 30 minutes and silver staining.

In PCR-positive cases, HPV genotyping was analyzed by restriction fragment length polymorphism (RFLP) i.e, cleaved amplified polymorphic sequence (CAPS) method. HR-HPV genotypes were decided by polycrylamide gel electrophoresis and silver staining of the digest of PCR product with restriction enzyme(s), *Ava* II (HPV 16, HPV 18 and HPV 33), *Rsa* I (HPV 31), *Bgl* II (HPV 52b), *Acc* I (HPV 58) and *Ava* I (HPV 35). The enzymatic digestion was done under the condition recommended by the manufacturer.[8]

Statistical analysis

Data analysis was done by using Statistical Package for Social Sciences (SPSS-15).

RESULTS

In this study, high-risk human papillomavirus (HPV-DNA) testing was performed by polymerase chain reaction (PCR) with *pU1M/pU2R* primers in 96 women with abnormal cervical cytology and 20 with normal cytology, age ranging from 18-69 years. Out of 116 women, 48 (41.4%) women were positive for high-risk HPV-PCR revealing around 240 bp-260 bp bands (Fig. 1a). HR-HPV were identified in (22/62) 35.5% of inflammatory smear, (6/10) 60% atypical squamous cells of undetermined significance (ASCUS), (13/15) 86.7% low-grade squamous intraepithelial lesion (LSIL), (3/6) 50% high-grade squamous intraepithelial lesion (HSIL), (3/3) 100% squamous cell carcinoma (SCC) and (1/20) 5% normal cytology (Table 1).

Table 1. Proportion of high-risk human papillomavirus (HPV) infection in women with cervical cytological abnormalities

Cytology	HPV- PCR		Total No. (%)
	Positive No. (%)	Negative No. (%)	
Normal	1(5)	19(95)	20(100)
Inflammatory smear	22(35.5)	40(64.5)	62(100)
ASCUS*	6(60)	4(40)	10(100)
LSIL**	13(86.7)	2(13.3)	15(100)
HSIL***	3(50)	3(50)	6(100)
Cancer	3(100)	0(.0)	3(100)
Total (% within cytology)	48(41.4)	68(58.6)	116(100)

* =Atypical squamous cells of undetermined significance
** =Low-grade squamous intraepithelial lesion
*** =High-grade squamous intraepithelial lesion

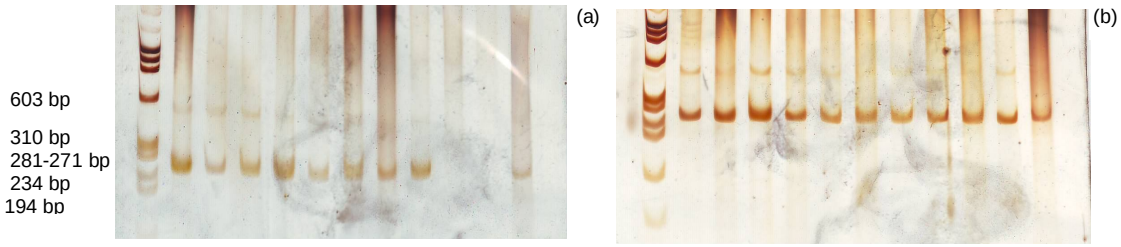

Fig. 1. Positive PCR for (a) HPV using *pU1M/pU2R* primers (b) Human DNA using *β-globin* primers, 6% PAGE with silver staining, molecular marker: ΦX174/ HaeIII digest

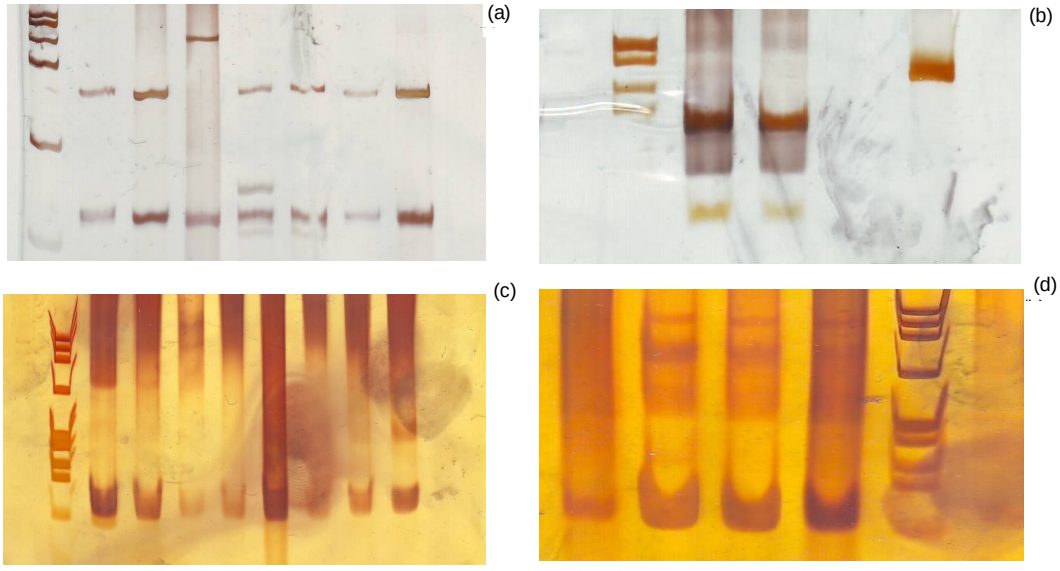

Fig. 2. Electrophoresis of PCR products with *pU1M/pU2R* primers digested with restriction enzymes (a, b) *Ava* II (c) *Rsa* I (d) *Acc* I (6% PAGE, 200V for 35-45 min)

All samples (100%) were amplified with β-globin primers which were used as human DNA integrity (Fig. 1b). The women with abnormal cervical cytology were 10 times more likely to be HR-HPV positive than those with normal cytology (p=0.0001).

HPV genotyping was analyzed by cleaved amplified polymorphic sequence (CAPS) method using restriction enzymes digestion i.e., *Ava* II, *Rsa* I, *Bgl* II, *Acc* I and *Ava* I. Among 48 HPV-PCR positive cases using *Ava* II digestion, 29 samples appeared two fragments, about 155 bp and 80 bp bands being HPV 16 genotype (Fig. 2a) and 6 appeared about 170 bp and 90 bp bands being HPV 18 genotype (Fig. 2b) but 13 samples were undigested with *Ava* II, *Ava* I and *Bgl* II in the same original size. These were further digested with *Rsa* I and *Acc* I. Seven samples using *Rsa* I appeared the broad band overlapping the two fragments, about 119 bp and 114 bp regions, being HPV 31 genotype (Fig. 2c). Six samples using *Acc* I appeared the broad overlapping band about 126 bp and 118 bp regions being HPV 58 genotype (Fig. 2d).

Table 2. Proportion of high-risk human papillomavirus (HPV) genotypes in women with cervical cytological abnormalities

Cytology	HPV genotypes				Total No. (%)
	HPV 16 No. (%)	HPV 18 No. (%)	HPV 31 No. (%)	HPV 58 No. (%)	
Normal	0(0)	0(0)	1(100)	0(0)	1(100)
Inflammatory	11(50)	2(9.1)	5(22.7)	4(18.2)	22(100)
ASCUS	4(66.7)	0(0)	0(0)	2(33.3)	6(100)
LSIL	9(69.2)	3(23.1)	1(7.7)	0(0)	13(100)
HSIL	3(100)	0(0)	0(0)	0(0)	3(100)
Cancer	2(66.7)	1(33.3)	0(0)	0(0)	3(100)
Total (% within cytology)	29(60.4)	6(12.5)	7(14.6)	6(12.5)	48(100)

The most prevalent HPV genotypes were HPV 16(60.4%) followed by HPV 31(14.6%), HPV 18(12.5%) and HPV 58 (12.5%). HPV genotypes 16, 18, 31 and 58 were in 11(50%), 2(9.1%), 5(22.7%), and 4(18.2%), respectively, in 22 cases of inflammatory smear, and 9(69.2%), 3(23.1%), 1(7.7%) and 0%, respectively, in 13 LSIL cases. HPV 16 and 58 were 4(66.7%) and 2(33.3%), respectively, in 6 women with ASCUS. All cases of HSIL were HPV 16. Among women with cervical cancer, 66.7% was genotyped as HPV 16 and 33.3% was HPV 18 (Table 2).

Table 3. Proportion of high-risk human papillomavirus (HPV) genotypes in women with cervical cytological abnormalities by age

HPV genotypes	Age range (years)					Total No.(%)
	20-29 No.(%)	30-39 No.(%)	40-49 No.(%)	50-59 No.(%)	60-69 No.(%)	
HPV 16	1 (3.4)	10 (34.5)	13 (44.8)	3 (10.3)	2 (6.9)	29 (100)
HPV 18	0 (0)	0 (0)	3 (50)	3 (50)	0 (0)	6 (100)
HPV 31	1 (14.3)	2 (28.6)	2 (28.6)	1 (14.3)	1 (14.3)	7 (100)
HPV 58	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	6 (100)
Total (% within genotype)	3 (6.3)	14 (29.2)	19 (39.6)	8 (16.7)	4 (8.3)	48 (100)

Most patients infected with HR-HPV were aged between 40-49 years (39.6%) followed by 30-39 years (29.2%), 50-59 years (16.7%), 60-69 years (8.3%) and 20-29 years (6.3%). HPV 16 was highest among women aged 40-49 years followed by 30-39 years. As for HPV 18, the detection rate was same in 40-49 years and 50-59 years age groups. HPV 31 was high in age 30-39 years and 40-49 years. HPV 58 was high in 30-39 years age group and also found to be the same in other age groups (Table 3).

DISCUSSION

Two main stages could be considered in HPV epidemiology: type distribution of cervical HPV infection in women with normal cytology and in women with abnormal cytology. Recently, a meta-analysis of relevant studies about the worldwide prevalence and type distribution of cervical HPV-DNA in women with normal cytology has been published. In this manuscript, the overall HPV prevalence was estimated to be 10.4%. However, there were some differences depending of the region of origin. HPV 16 is the most common HPV type and the five most common HPV types in HPV-positive women worldwide were

PV 16, 18, 31, 58 and 52, being 50% of all HPV infections.[7]

A study which focused the distribution of HPV genotypes in women with cervical lesions revealed HPV 16 and 18 as the most frequent HPV types identified in invasive cancers (80%) but the distribution patterns of HPV types in the intraepithelial lesions were highly varied.[9]

Another study on the HPV type distribution in females with abnormal cervical cytology reported that 75% were positive for HPV-DNA and 23.7% were negative. HPV 16 was the most common type followed by HPV 58, 51, 33, 31 and 18.[10]

In Czech women and men with diseases etiologically linked to HPV, HPV 16 was the most prevalent type both in precancerous lesions (45%) and squamous cell carcinomas (59%). HPV 16 and/or 18 were present in 76% of cervical cancer samples, 33% of CIN 1, 43% CIN 2 and 71% of CIN 3.[11]

In Japanese women, HPV genotypes were detected in 9.5% of negative for intraepithelial lesion or malignancy (NILM), 72.2% ASCUS or more cervical lesions. HPV genotypes were HPV 52 at 26.6%, HPV 16 at 25.2%, HPV 58 at 21.8%, and HPV 18 at 7.1%.[12]

In Myanmar, the prevalence of HPV in women with premalignant and malignant lesions of cervix was 77/145 (53.1%). HPV was identified in 33.3% LSIL, 60% HSIL, 58.7% SCC and 50% adenocacinoma cervix. HPV 16 was the predominant genotype followed by HPV 31 and HPV 18.[13] One study reported that HPV-DNA was detected in 27/131 (20.6%) women among 17.6% normal smear, 77.1% inflammatory smear, 0.8% LSIL and 4.6% unsatisfactory smear.[14] Another study revealed that the high-risk human papillomavirus (HR-HPV) was detected in 81.25% of cervical tissue with squamous cell carcinoma (SCC), 5.56% with CIN I and 8.33% with normal cytology.[15]

In the present study, 47/96 (49%) women with abnormal cervical cytology and 1/20 (5%) with normal cytology were positive for high-risk human papillomavirus (HR-HPV). They were identified in 35.5% of inflammatory smear, 60% ASCUS, 86.7% LSIL, 50% HSIL and 100% SCC. HPV prevalence was much higher in the present study compared to the previous one i.e, 86.7% vs. 33.3% in LSIL, 100% vs. 58.7% in SCC samples. The highest percentages of women with positive result for HR-HPV were found in patients with LSIL and cervical cancer. Therefore, HPV-DNA testing could be especially for triage of low-grade smears to improve the sensitivity of cytology alone and select women at most risk who require colposcopy.

The most prevalent HPV genotype in this study was HPV 16 (60.4%), one of the vaccine- preventable HPV genotypes, which was consistent with other studies mentioned above, followed by HPV 31 (14.6%), HPV 18 (12.5%) and HPV 58 (12.5%). Among cervical cancer cases, 66.7% was genotyped as HPV 16 and 33.3% was HPV 18. Clinical studies of HPV vaccines have demonstrated close to 70% protection against HPV 16 and HPV 18 related infections and diseases, implying potential cross-protection against HPV 31, 33, 45, 52, and 58.[16, 17]

In this study, most patients infected with HR-HPVs and HPV 16 genotype were aged between 40-49 years followed by 30-39 years. As for HPV 18, the detection rate was same in 40-49 years and 50-59 years. HPV 31 was high in age 30-39 years and 40-49 years. HPV 58 was high in 30-39 years.

In Japan, most patients infected with HPV 16 were between 20-29 years, decreasing with age thereafter. HPV prevalence by age revealed mostly in young women aged 15-25 years and a second peak was observed in 55 years or older.[12] HPV infections occur with a high-attack rate soon after sexual initiation. Follow-up studies of virgins from different countries after sexual

debut have shown up to 70% of women becoming HPV-DNA positive at least once within 48 months. The cumulative life time exposure to HPV has been estimated to be close to 80% and for HPV 16 or 18 is 20%. Thus, primary prevention with HPV vaccines should focus on the years before sexual initiation, in the adolescent and preadolescent age groups.[18]

In the future, HPV-DNA testing in conjunction with cervical cytology testing could be used for cervical cancer screening or in a follow-up program after conservative treatment of cervical lesions since 35.5% of inflammatory smear, 60% ASCUS and 86.7% LSIL were HR-HPV positive. If no intervention is implemented in the near future, a dramatic increase in the number of cervical cancer cases is predicted. HPV vaccines offer an efficient way to prevent HPV-related cervical cancers. Results of this study together with many literatures allow estimating potential benefit that can be achieved by the implementation of routine vaccination for the prevention of HPV-associated cervical cancer in Myanmar.

Conclusion

The most prevalent HPV genotypes were HPV 16 followed by HPV 31, HPV 18 and HPV 58 in this study. Among cervical cancer cases, both vaccine-preventable HPV genotypes i.e. HPV 16 and HPV 18 were 66.7% and 33.3%, respectively. The women with abnormal cervical cytology were 10 times more likely to be HR-HPV positive than those with normal cytology. This study suggests that the implementation of routine vaccination programme against HPV in preadolescent and adolescent age groups will greatly reduce the burden of HPV-associated cervical cancer in Myanmar.

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Socio-economic and health consequences among HIV/AIDS affected families and orphans in Hlinethaya Township

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With the objectives of identifying the socio-economic and health consequences among HIV/AIDS orphans, a community-based study was conducted employing qualitative research methods in 2009. In-depth interviews with 16 parents/guardians and 18 key informant interviews with the basic health staff, community volunteers and responsible persons from an international non-governmental organization were carried out in Hlinethaya Township, Yangon. There were 41 orphans comprised of 18 double orphans, 21 paternal orphans and 2 maternal orphans. Four orphans and eight guardians were HIV positive. Social consequences included family dispersion, effects on education and stigma/discrimination. Family dispersion was seen in three out of 18 families. There was no orphan currently under care of or stayed with non-relative guardians. About half of school going age orphans dropped out from the school because of economic hardship. Some older children had to work to help the single parents and younger siblings. Discrimination was uncommon among the extended family members. However, disclosure of HIV status affected the working opportunity of single parents. Although change in family possession was not significant, many families had difficulty in struggling for family's daily expenses along with the loss of the bread winner. Vertical transmission of HIV was seen in 4 children. Malnutrition and TB were common health problems for HIV infected orphans. All affected families have some extent of socio-economic consequences from HIV/AIDS. Sustainability and strengthening of support programs should be strongly considered since availability of these support networks could alleviate the negative consequences.

INTRODUCTION

Globally, HIV pandemic remains a serious challenge to public health and AIDS is the leading cause of death worldwide for people aged 15 to 49 years. It was estimated that there were 33.3 million people living with HIV at the end of 2009. In Asia, this figure increased from 4.2 million in 2001 to 4.9 million in 2009 [1].

Epidemic had left behind 16.6 million AIDS orphans by the end of 2009, defined as those aged under 18 who have lost one or both parents to AIDS [1]. Even with anti-retroviral treatment, it is estimated that the number of orphans will still be overwhelmingly high by 2015 [2]. Orphans in some sub-Saharan African countries exceed one million, and, in some countries, children who have been orphaned by AIDS comprise half or more of all orphans nationally [3]. Most AIDS orphans who live outside of Africa live in Asia, where the total number of orphans for all reasons exceeds 73 million [4]. Estimated AIDS orphans in Asia were more than 1 million [1]; however, there was no estimation of AIDS orphans in Myanmar.

HIV/AIDS has much direct and indirect impact on children's rights, including psychological impact, limited access to quality education and health services.

Previous studies focusing on HIV/AIDS orphans in China and Africa have highlighted the psychological problems [5, 7] and unmet basic needs like food inadequacy, discontinuation of schooling and inaccessible to health care services [7].

Myanmar is one of the countries afflicted by HIV/AIDS like other developing countries in Southeast Asia. According to NAP, it was estimated that adult HIV prevalence is 0.61% [8]. This study was done as there were limited studies on HIV/AIDS orphans in Myanmar.

Objectives

- To determine the socio-economic consequences faced by HIV/AIDS orphans
- To determine the health consequences faced by HIV/AIDS orphans

MATERIALS AND METHODS

A community-based, cross-sectional study was conducted using qualitative approach: in-depth interview (IDI) and key informant interview (KII). Hlinethaya Township from Yangon Region was randomly selected with a consideration of being a peri-urban township, having some community-based activities focusing on HIV/AIDS.

Parents/guardians of HIV/AIDS orphans, Basic Health Staff, community volunteers and responsible persons from World Vision (WV), an International Non-governmental Organization, were included in the study. The HIV/ AIDS orphans were defined as the orphans under the age of 15 years who lost one or both parents due to AIDS.

Data collection

A total of 16 IDI with the guardians of HIV/AIDS orphans and KII with four Basic Health Staff (BHS), six community volunteers and four responsible persons from WV were carried out. Guardians consisted of fathers, mothers, grandparents, elder siblings and aunts/uncles.

All interviews were conducted by the investigators and well-trained research assistants. Both tape-recording and note taking were made after getting informed consent.

Data analysis

Transcripts were prepared after the field data collection. The research team reviewed all the transcripts and discussed to clarify the findings. Themes and sub-themes were identified carefully and thematic analysis was performed. ATLAS.ti 5.2 demo version was used for coding and organizing the themes. Findings from IDIs and KIIs were triangulated for validation.

Ethical consideration

The proposal was approved by the Institutional Ethical Review Committee, DMR-LM. Written informed consent was obtained from the participants after thorough explanation about the study. To ensure confidentiality, only code numbers were mentioned on the interview records.

RESULTS

Background characteristics

A total of 18 affected families were included in the study. There were 41 orphans comprising of 18 double orphans, 21 paternal orphans and 2 maternal orphans. Double orphans mean orphans who lost both parents due to HIV/AIDS. Orphans' age ranged from 4 months to 15 years with the mean age of 7.2 years (SD-2.1) while guardians' age ranged from 25 to 66 years with the mean age of 47 years (SD-6.3). Among the orphans, 6 children were under 5 years old.

Four out of 41 orphans and 8 out of 9 single parents were HIV positive. Two children already died from the disease before the study. Most double orphans stayed with their grandparents and aunts/uncles. Majority of the guardians mentioned that they have the responsibility to take care as they are the close relatives.

Social consequences

Social consequences included family dispersion, effects on education and stigma/ discrimination.

Family dispersion

Dispersion of family members was seen in three out of 18 families. Most orphans could stay with their siblings and single parent together with the extended family members. Some older siblings got married and younger siblings were looked after by other relatives like grandparents, aunts. There was no orphan currently taken care or stayed with non-relative guardians.

"....we have 5 siblings…I'm the eldest...I got married before my mother died...2nd brother got married and 3rd brother was lost from home after my mum passed away... until now we couldn't find him...4th one is a sister who stays with our aunt...youngest brother stays with me..."

(An eldest sister of 5 siblings, 25 yrs)

"...3 children were left when my daughter passed away…at first all of them stay with me…now I let middle child to stay with his aunt to help her with household chores…they stay in Mandalay..."

(A grandmother of 3 orphans, 55 yrs)

Education

Continuation of schooling

Majority (34 out of 41) were the orphans of school going age. About half of them could continue their schooling despite the economic hardship. Their continuation of schooling mostly depended on the economic condition of the family, child's health and support from some organizations and relatives. Some children join the non-formal education organized by WV.

"...I couldn't afford schooling of my grand-sons but they want to attend the school…so I asked some acquaintance whether they can support for them. Now, both children can continue school. Some of our close relatives and friends give pens, pencils, books and WV arrange for school registration...."

(A grandfather of 2 orphans, 58 yrs)

"... My granddaughter can't study well. Her school performance is poor as she had to take medicines frequently. Now we let her to study at non-formal education run by WV..."

(A grandmother of one HIV-positive orphan, 53 yrs)

Reasons for school drop out were different. Few HIV-positive children could not continue because of their health condition. Some dropped out because of their parents' health condition or economic hardship. Even though WV supported for school enrolment in formal schools, some of them dropped out after few months as their guardians could not afford other expenses for schooling.

"...My brother had to quit school before the final exam as we could not provide extra expenses for schooling like clothing, books, pens and some fees..."

(An elder sister of 13 years old orphan, 26 yrs)

Some elder children quit school because they had to work to earn money for younger siblings together with their single parents or extended family members. In one family, the elder child had to quit school as he had to look after his younger sister who was HIV positive.

"...The elder boy is 13 years old…he could not continue schooling as he had to take care of 6 years old younger sister who is HIV positive...he always accompany her to the clinic to take medicine..."

(A grandmother of 2 orphans, 66 yrs)

Stigma/Discrimination

Stigma/discrimination is not very common as most families rarely disclosed their family member's HIV status to their neighbors and relatives. Extended family members rarely discriminated the affected family since most of them stayed together for long time and they had same economic background. However, discrimination from neighbors was seen in some families. In addition, disclosure of the HIV status affected their working environment and opportunity. Discrimination was seen in spouse who was still alive and infected.

Few volunteers also stated the problem of discrimination. However, many community volunteers stated that the affected families rarely disclosed the HIV status to their neighbors.

"...I have two sons…the elder boy has some problems with neighbors…he frequently fights with other children as they make a joke with his mother's death...they said…she died of "A" (at ullfw, f... my son can not tolerate and fights back... some children threw with stones to our house..."

(A father of 2 sons, 39 yrs, HIV positive)

"...I work as a street vendor…sometimes I sell snack…sometimes fruits…I can't sell in my ward…as they know my disease…but I can sell in other wards as they don't know..."

(A mother and an aunt of 3 orphans, 25 yrs, HIV positive)

Economic consequences

Economic consequences were mainly focused on change in family possession and effect on daily living. Majority was from low socio-economic background before they got HIV infection and thus change in family possession was not so significant after getting the disease. However, along with the loss of the bread winner in some families, they found difficult to struggle for family's daily expenses. Consequently, spouse and older children needed to work to earn money by doing odd jobs (*kya-ban*). Many families needed to sell household assets including cooking utensils, clothings, etc. In extreme case, spouse worked as a commercial sex worker for her family's living.

"...Before my husband died, I don't need to work. Now, I work as manual labour in day time and I also work as sex worker at night...about 2-3 times per week..."

(A mother and an aunt of 3 orphans, 25 yrs, HIV positive)

"...We've faced difficulty for living after my husband's death as woman's income can't compare with that of man. Sometimes if I can't work...we've to pawn the cloths in exchange with money..."

(A mother of 4 orphans, 37 yrs, HIV positive)

Health consequences

Of all health consequences, transmission of HIV from mother to child was the most severe one. Six children got HIV from their mother and two children already died before their 5th birthday. Common health problems among HIV infected children were mal-nutrition and TB. Consequently, they took the treatment frequently and sometimes required hospitalization. Some HIV-positive orphans and single parents are taking anti-retroviral therapy (ART) with the support of AZG. Some guardians came to know the children's HIV status only when they had to seek health care for TB. Other HIV-negative orphans were healthy apart from some minor illnesses like skin infection and worm infestation. They normally took treatment from "Thazin" clinic run by AZG.

"...She suffers from malnutrition frequently. She is very thin and...has to take treatment at Thazin...she stays there at day time... they provide her with nutritious food..."

(A grandmother, 53 yrs)

Support networks

<u>Support from extended families, relatives and neighbors</u>

The most important support from the extended families especially grandparents was to share a place to live. Immediate relatives supported for their daily living since many of them stayed together in the same house or compound. Supports from other relatives and neighbors were not regular and inadequate for their expenses. In some families, grandparents had to work outside in order to support the orphans.

"...We stay with my sister's family…we can eat together but they can't support us .... sometimes other relatives give me some money like 2-3000 kyats ..."

(A mother of 2 orphans, 25 yrs)

"...We stay in grandmother's house together with my mother and aunts...at first in a same house...now we stay in a small hut in-front but in the same compound. My mother works outside and supports us...."

(A mother of 2 orphans, 32 yrs, HIV positive)

Support from organizations

Different organizations have their own agenda to support the affected families. Organizational support can be categorized as education, health care and financial support. Some BHS participated in these activities although it was not done in collaboration with public sector. Many public health care providers stated that these programs were beneficial for the affected families although sustainability was un-certain. In contrast, some of them concerned for the problem of increasing migrant population in their township because of these programs.

"...There's a nutritional program for the children organized by WV, I've to recruit and weigh the children for their program. They invite me to participate as responsible health personnel. They provide lunch to the children...I also try to contribute by giving medicines for minor ailments..."

(A midwife, 48 yrs)

Education support

In the study township, WV supports school enrollment of the children. They arranged the program not only for the HIV/AIDS orphans but also for the children from the poor families. Additionally, these orphans can join the non-formal education program. Vocational training for HIV-positive children is also provided from WV and AFXB, an INGO working for PLWHA.

"...It's for the children who can't attend the formal school…We accept 7-15 years old children... the class usually starts from 9:00 am to 3:00 pm. We teach them till they can read and write...mainly for Myanmar, English and Mathematics...."

(A volunteer, 25 yrs, 5 yrs service)

"...We have a link with other INGO like AFXB. We send some HIV orphans and children from poor families to attend the vocational training in which girls can learn for sewing, knitting and boys can learn for carpentry..."

(A volunteer, 57 yrs, 10 yrs service)

Health care

Majority of the affected families in the study area usually seek health care at Thazin clinic run by AZG. Both HIV infected orphans and their infected parents took treatment in these clinics. The clinics provide antiTB drugs for all TB patients from the affected families and ART free of charge for some infected individuals.

Regarding general health, children can take treatment from the clinics that have been linked with WV. If they need to take treatment at the hospital, WV arranges and provides the cost.

"...My grandson contracted TB and took treatment at Thazin...now it's finished... now he needs to take only medicines for "A"..."

(A grandfather of 2 orphans, 58 yrs)

"...If a child needs to seek health care...I've to inform my superior and ...there's a token from WV...by using the token...he/she can take treatment from those clinics..."

(A volunteer, 29 yrs)

Financial support

WV supports financially by giving loans for income-generation. There are some rules to control misuse of the opportunity. Receivers have to recruit a group of five people to guarantee among themselves. If someone can not give the money back on time, other group members have to pay for that person. Some are reluctant to apply for that kind of loan since they do not want to guarantee for others although they want to borrow money for themselves. Moreover, the program is not so beneficial for those who are very poor as others do not want them to join the group.

"...To apply loan from WV…we must have a group to guarantee each other. Others do

not want to accept like us in their group since we are poor and they think that we can't give back on time..."

(A mother of 5 orphans, 43 yrs, HIV positive)

DISCUSSION

The study was done in Hlinethaya Township having community-based activities focusing on HIV/AIDS for about 5 years. Using qualitative research methods, socioeconomic and health consequences faced by HIV/ AIDS orphans were identified. Moreover, support networks for the affected families were described.

Family dispersion, effects on education, stigma/discrimination and psychological consequences were observed as the common social consequences. Of all social consequences, drop out from school was the most significant finding among the affected families. Only half of the school going age children could continue the formal education. This finding of effect on education supported the previous study but the magnitude of drop out from school was much higher than that of previous one [9]. Findings from the African studies identified that orphans are at risk of poorer educational outcomes with maternal deaths having stronger negative effects than paternal deaths [10].

Staying with extended family members was common finding not only for double orphans but also for paternal/maternal orphans. It may contribute from the Myanmar tradition of staying as extended families. Discrimination from neighbors was seen in some families but rarely observed in school environment. This may be due to the concealment of the HIV status of the family members in the schools attending by orphans.

Regarding the economic consequences, change in family possession was not so significant in most of the families. Underlying poverty of the affected families in the study township may partly contribute to that finding. In contrast, significant change in family possession was seen in the previous study [9]. Only prominent change was difficulty in struggling for family's daily expenses along with the loss of the bread winner in some families.

Transmission of HIV from mother to child was identified as the most serious health consequence faced by the children. Lack of awareness of HIV positive status and delayed seeking care of infected mother may contribute to that occurrence of transmission.

Majority of affected families received benefits from the support programs of INGOs especially for education and health care. Non-formal education programs and vocational trainings seemed to be beneficial for those who can not afford the school expenses. However, loans for income generation could not help much for poor families.

Although many providers from public sector appreciated the benefits of support programs for the affected families, some of them pointed out about the sustainability. Moreover, few concerned of increasing migrant population because of support programs in their township.

Our study highlights the situation of HIV/ AIDS orphans and their families in the community. All affected families have some extent of socio-economic consequences from HIV/AIDS. Since availability of support networks from INGOs could alleviate the negative consequences faced by the affected families, sustainability and strengthening of these programs should be strongly considered.

Recommendations

Further study with larger sample size using both quantitative and qualitative research methods should be conducted in order to identify and compare the situation of HIV/ AIDS orphans in different localities.

Non-formal education and vocational training programs should be strengthened and expanded. It was beneficial for the

orphans who can not afford the formal education. Moreover, majority of the guardians want their children to get vocational skills to earn money for their living in the future. Community and public sector involvement should also be promoted for sustainability of these education programs.

Furthermore, coordination between community, public sector and INGOs should be encouraged for implementation of better rehabilitation programs for HIV/AIDS orphans in the future.

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