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- 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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General Information

Journal description

The Myanmar Health Sciences Research (MHSR) Journal is published by the Department of Medical Research, Ministry of Health and Sports, Republic of the Union of Myanmar. It publishes original articles, review articles, short reports and correspondences in the field of biomedical and health sciences. It has been published since April 1989 with ISSN 1015-0781. The submitted manuscript is double blind peer-reviewed by two referees with expertise in the field of research work related to the paper. The Journal is published every 4th month (i.e. April, August and December) in a year. The annual meeting of the MHSR Editorial Board is usually held in September of the year. The distribution of journal is limited and free of charge around the country and to some international institutions and libraries.

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  3. **More than 6 authors:** Khin May Oo, Yi Yi Kyaw, Ohmar Lwin, Aye Aye Yee, San San Oo, Khine Win, *et al*. Hepatitis B surface antigen sero-prevalence in two border towns near Thailand. *Myanmar Health Sciences Research Journal* 2009; 21(2): 83-87.
  4. **Book:** Thaw Zin. Role of Analytical Toxicology Laboratory in the prevention, control and management of poisoning: Principle and guidelines. In: *Guidelines on Poison Prevention, Control and Management*. Department of Medical Research (Lower Myanmar), Ministry of Health, 2003; 76-98.
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EDITORIAL

"Myanmar Health Sciences Research Journal (MHSRJ)" is now approaching it's 30th year, as the first issue of the journal was released in April, 1989. It is published by the Department of Medical Research, Ministry of Health and Sports. Nearly 900 articles on various research areas have been published within 30 years of the Journal serving as an important medium for the dissemination of original research works in the field of medical science and health research.

In celebration of the 30th anniversary of MHSRJ, a special issue has been released in June 2018. This special issue included the commentaries encompassing a wide scope of major research disciplines from leaders and pioneers of medical research in Myanmar. In this publication, invaluable information was shared to the audience of the Journal covering their indispensable experiences and opinions on medical research as well as the milestones of major research areas on common health threats in our country. Furthermore, 20 previously published original research articles that showed a high academic contribution in their respective fields, were selected and re-printed in this special issue.

In this Volume 30, Number 2 issue, we are delighted to publish an important finding on detection of heavy metal on rice samples in Myanmar. The article, "Quantification of Heavy Metals in White Rice and Brown Rice from Thapaung Township" highlighted that nearly all rice samples contained lead (Pb). Fortunately, the paper mentioned that arsenic, one of the highly toxic metal, was not detected in all rice samples collected in this study. This study had alerted the readers to be aware of the importance of the presence of heavy metal in our daily consumed rice in Myanmar.

Moreover, a variety of recent research findings are also published in this issue. A total of 15 articles comprising of 12 original research articles and 3 short reports that cover the communicable diseases such as malaria and Human Respiratory Syncytial Virus as well as non-communicable

diseases including diabetes, lung cancer, obesity, inheritance disease, cardiovascular disease and ageing are covered. Furthermore, research findings on herbal medicine and laboratory animal are also included in the current issue.

In our MHSRJ, original research articles, review articles, short reports and correspondences in the field of biomedical and health sciences have been published three times a year after thorough screening of anti-plagiarism and double blind peer-review. Moreover, submission, reviewing and editorial works are being processed online at http://www.myanmarhsrj.com and previous issues can be assessed online easily, free of charge. We would like to invite all researchers again to submit your important research findings in our Journal and we would appreciate your academic contribution not only to our Journal but also to the scientific community in Myanmar.

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Quantification of Heavy Metals in White Rice and Brown Rice from Thapaung Township

Phyo Wai Zin[*]*, Khin Phyu Phyu, San San Htwe, Mya Marlar, Tin Tin Han, Khin Moe Latt, Ohnmar Win, Aye Thida Tun & Thet Htet Aung*

Department of Medical Research

Rice is the major staple food of Myanmar. In general, there are two kinds of rice as white and brown rice. The evidence suggested that brown rice may contain more heavy metals especially arsenic than white rice. When the permissible concentration of heavy metals in the body becomes exceeded, they can cause serious health disorders. This cross-sectional study was aimed to determine the concentrations of heavy metals in white and brown rice from four villages of Thapaung Township, Ayeyawady Region. Six milled rice and six un-hulled rice samples were collected as white rice and brown rice samples, respectively. The concentration of ten kinds of heavy metals was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer) Optima 8000. In brown rice samples, lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), iron (Fe) and manganese (Mn) were detected above their respective maximum allowable concentration (MAC). However, arsenic (As), chromium (Cr) and cobalt (Co) were below their MAC. In white rice samples, Pb, Zn, Cu and Mn were above their respective MAC. However, these concentrations were lower than those of brown rice samples. Cadmium (Cd), As, Cr and Co in white rice samples were below their MAC as in brown rice samples. Among the highly toxic metals, As was not detected in both samples. However, one of the toxic metals, lead (Pb) was present above MAC in all samples of brown rice and white rice except one sample of white one and these concentrations were much lower than those of brown rice. Cd was present in all samples of brown and white rice but only one sample of brown rice had the concentration above MAC. Therefore, it can be highlighted that the studied white rice contained less heavy metals concentration than the studied brown rice.

Key words: White rice, Brown rice, Heavy metals, ICP-OES, MAC

INTRODUCTION

Rice, the seed of the monocot plants (*Oryza sativa*), is the major staple food of Myanmar. In general, there are two kinds of rice as white and brown rice; white rice is the result of milling process in which it removes the outer bran layer of the brown rice.[1] As it is claimed that brown rice contains many nutritional contents, some people prefer to eat brown rice than white rice. However, large amount of heavy metals in rice can cause serious health problems in people. Moreover, evidence suggests that brown rice may contain more heavy metals especially arsenic than white rice.[2, 3]

In Ayeyawady Region of Myanmar, Thapaung Township plays a dominant role in cultivation of rice in rich alluvial soil. The uptake of heavy metals depends on the plant species and bioavailability of the metal in the soil.[4] Rice has been identified as one of the major sources of cadmium (Cd) and lead (Pb) intakes for humans especially in Asia.[5] Up to 50% of the ingested Cd was from rice and its products in Asian countries.[6] Malidareh and colleagues reported that the value of arsenic (As), Cd and Pb concentration in rice in Iran were <0.005-

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0.051 mg/kg, <0.05-0.113 mg/kg and <0.05-0.135 mg/kg, on dry weight, respectively. They also stated that in all samples of polished white rice, Cd and Pb contents were lower than the maximum permitted level for rice compared with Standard Codex/EU/WHO and lower than the maximum permitted level Standard of Iran. However, arsenic contents in white rice were not compared because standard for arsenic in the polished white rice had not been determined yet. Moreover, the weekly intake of As, Cd and Pb from rice was lower than that of total dietary As, Cd and Pb intake, and the maximum weekly intake recommended by WHO/FAO.[7] The total arsenic concentrations in white rice were 0.501, 0.19, 0.25, 0.21, 0.07, 0.08 and 0.14 μg/g as dry basic weight in China, Japan, Vietnam, India, Bangladesh and Thailand, respectively.[8]

Heavy metals can reach into the body system through food, air and water and bioaccumulate over a period. When the permissible concentration becomes exceeded, they can cause serious health disorders; immunological defenses, intrauterine growth retardation, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer.[4] Metals such as Cd, Pb, Cr and Ni are considered as adverse effect to human and environment, thus receiving global attention for environmental contamination.[9] Although brown rice consumption is less significant than white rice consumption in Myanmar, the increased amount of heavy metals in brown rice becomes important in considering the safety for brown rice consumers.

Therefore, the quantification and comparison of ten heavy metal (As, Co, Cd, Pb, Cr, Zn, Cu, Ni, Fe and Mn) contents in white and brown rice from Thapaung Township, Ayeyawady Region, were carried out.

MATERIALS AND METHODS

A cross-sectional study with one year duration was done. White rice samples are meant to be milled rice grains. Brown rice samples are meant to be un-hulled rice grains and these are pounded to remove the husk in order to get brown rice. A total of 12 samples (6 milled rice and 6 un-hulled rice grain samples) were collected from 4 villages of Thapaung Township in Ayeyawady Region from October to December, 2016 after harvesting the paddy crops. Two milled rice grain and two un-hulled rice grain samples were collected from Konetangyi (ကုန်းတန်းကြီး) and Yaylegyi (ရေလဲကြီး) villages, each; one milled rice grain and one un-hulled rice grain samples from Daleet (ဒလယ်အဲ) and Shannkwin (ရှမ်းကွင်း) villages, each. The samples collected belong to 3 varieties of rice grain namely Yaysintheehtat (ရေဆင်းသီးထပ်), Mheekaut (မီးကောက်) and Shwe-thweyin (ရွှေသွယ်ရင်).

Twenty primary samples of rice were taken from about 50 bags of rice from warehouses of respective villages. These 20 samples were mixed thoroughly to get the one composite sample, the representative one. The composite sample was analyzed triplicately for heavy metal concentrations in the laboratory. The same procedure was done for un-hulled and milled rice of different varieties of rice from different villages.[10] Each sample was kept into each plastic bag with concise label, sent to the laboratory, Chemical Toxicology Research Division, Department of Medical Research and stored at room temperature until analysis.

For digestion of sample before analysis, 30 ml of concentrated nitric acid were mixed with 5 gm of rice sample and it was gently boiled until 3-6 ml of digest remained. Then, 25 ml of concentrated hydrochloric acid were added and heated gradually until 10-15 ml of volume remained. After cooling, the residue was filtered with 45 μm size Whatman filter paper. The sample was diluted to 50 ml with deionized water. The blank digestion was carried out as described previously.[11] ICP-OES (Perkin Elmer) Optima 8000 was used for measurement by applying the standard operating condition, including ICP-OES multi-element-standard-solution, nitric acid (69%), hydrochloric acid and deionized water.

Statistical analysis

Data were analyzed by using Microsoft Excel. Concentrations of ten heavy metals were presented as mean±SD and compared with maximum permissible level according to codex/WHO.

RESULTS AND DISCUSSION

Toxic elements

Table 1 shows that As was not present in all samples of brown and white rice. The finding was contradict to the fact that brown rice may contain especially arsenic than white rice.[2, 3]

Arsenic level in rice depends on irrigated water which comes from arsenic contaminated source.[12] The absence of As in the samples of the study may be due to the fact that the water used for rice cultivation is rainwater and water from the Gawon river (one of the branches of Ayeyawady River) but not from the deep well. Cadmium was present above the MAC (Cd=0.2 µg/g, CODEX standards) in one sample of brown rice but below in all samples of white rice.[13] The milling process reduces Cd concentration in grain.[14] In general, Cd in plants may relate to the fertilizer used and it reduces the growth both in roots and stems. This effect is partly due to the suppression of the elongation growth rate of cells, especially in the stem.[15]

Khairiah, *et al.*[16] found that the application of various types of pesticides and fertilizers served as contributor to increasing toxic metal contamination of crop. All samples of brown rice and except one sample of white rice had lead (Pb) concentration above MAC (Pb=200 ηg/g, CODEX standards).[13] However, the levels of Pb in white rice were much lower than that of brown rice. Shabbir, *et al.*[17] described that the lead content was found significantly higher in brown rice than in white rice. Lead contamination in long term may lead to anemia and brain damage.[4]

Chromium was present in all samples of brown rice and 3 samples of white rice, which were below MAC (Cr=1 µg/g, CODEX standards).[13] On milling of the grain, Cr was lost to the extent of 57%.[18]

Diet is the primary route of Cr entry into humans though humans can absorb Cr by inhalation or dermal contact.[19] Nickel ranged from 4.03±0.01 to 59.04±1.09 µg/g in brown rice samples and from 0.55±0.01 to 2.69±0.02 µg/g in white rice samples. Nickel concentration was above MAC (Ni=1.5 µg/g) in all samples of brown rice and one sample of white rice.[13] Dermatitis due to nickel toxicity is not uncommon.

Table 1. Distribution of concentrations of toxic elements (mean±SD) in brown and white rice grains

	Rice	As	Cd	Pb	Cr	Ni
Konetangyi						
Yaysintheehtat	Brown	ND	205.8±9.33	7.50 ±0.03	0.25±0.00	4.03±0.01
	White		49.27±2.18	0.66±0.01	0.01±0.01	1.58±0.00
Mheekaut	Brown	ND	78.06±1.38	3.88 ±0.03	0.11±0.00	5.00±0.07
	White		43.59±0.64	0.05±0.00	0.15±0.18	1.48±0.01
Yaylegyi						
Mheekaut	Brown	ND	104.6±0.24	5.26 ±0.05	0.24±0.01	10.57±0.07
	White		55.00±1.43	0.67±0.00	ND	0.91±0.02
Shwethweyin	Brown	ND	59.91±1.43	6.58 ±0.05	0.23±0.01	10.88±0.14
	White		61.79±1.04	1.40±0.00	0.55±0.02	1.50±0.02
Daleet						
Shwethweyin	Brown	ND	81.46±0.71	4.03 ±0.04	0.13±0.00	4.82±0.09
	White		29.76±0.40	0.26±0.01	ND	0.55±0.01
Shannkwin						
Shwethweyin	Brown	ND	188.4±4.39	98.29 ±0.86	0.51±0.01	59.04±1.09
	White		78.93±0.14	3.86±0.03	ND	2.69±0.02
Reference value		200 ηg/g	200 ηg/g	0.2 µg/g	1 µg/g	1.5 µg/g

As=Arsenic, Cd=Cadmium, Pb=Lead, Cr=Chromium, Ni=Nickel, ND=Not detected

Table 2. Distribution of concentrations of microelements in brown and white rice grain samples

	Rice	Zn	Cu	Fe	Mn	Co
Konetangyi						
Yaysintheehtat	Brown	371.4±1.44	223.10± 2.03	5.51±0.29	16.24±0.37	ND
	White	71.98±0.15	41.87±0.18	2.40±0.05	19.75±0.13	ND
Mheekaut	Brown	239.8±5.45	117.2±1.03	8.86±0.43	27.11±0.35	ND
	White	65.64±1.10	20.69±0.11	1.98±0.08	25.89±0.36	ND
Yaylegyi						
Mheeka ut	Brown	303.5±8.10	181.60±2.84	139.0±2.34	23.73±0.00	ND
	White	67.53±1.48	29.72±0.48	1.75±0.10	21.03±0.22	ND
Shwethweyin	Brown	329.80±6.15	199.10±3.72	8.11±0.11	9.21±0.08	ND
	White	80.74±1.47	49.66±1.09	6.16±0.34	14.18±0.17	0.005±0.0008
Daleet						
Shwethweyin	Brown	212.10±6.40	120.90±1.87	9.02±0.4	11.78±0.07	ND
	White	50.53±1.73	22.21±0.17	0.67±0.3	18.65±0.19	ND
Shannkwin						
Shwethweyin	Brown	4170±30.35	421.1±1.69	14.20±0.61	18.38±0.07	ND
	White	158.3±3.62	121.0±1.28	ND	9.61±022	ND
Reference value		50 µg/g	10 µg/g	5 µg/g	5 µg/g	0.01 µg/g

ND=Not detected, Zn=Zinc, Cu=Copper, Fe=Iron, Mn=Manganese, Co=Cobalt (mean±SD)

Microelements

Table 2 indicates that apart from one sample of white rice, cobalt (Co) was not detected in both brown and white rice samples. Cobalt is a natural element that is essential for the healthy functioning of many plants and animals. Rice contained only small amount of Co.[20]

Zinc (Zn), copper (Cu) and manganese (Mn) levels in brown rice samples ranged from 212.1±6.40 to 4170±0.35 µg/g, from 117.2±1.03 to 421.1±1.69 µg/g and from 9.21±0.08 to 27.11±0.35 µg/g, respectively. Zn, Cu and Mn levels in white rice samples ranged from 50.53±1.73 to 158.3±3.62 µg/g, from 20.69±0.11 to 121.0±1.28 µg/g, from 9.61±0.22 to 25.89±0.36 µg/g, respectively. These were above their respective MAC (Zn=50 µg/g, Cu=10 µg/g, Mn=5 µg/g) in both brown rice and white rice.[13]

Iron (Fe) ranged from 5.511±0.29 to 139.0±2.34 µg/g in brown rice samples and from 0.67±0.3 to 6.16±0.34 µg/g in white rice samples. Fe concentration was above MAC (Fe=5 µg/g) in all samples of brown rice and one sample of white rice.[13]

Zn was found to be the most abundant microelement, followed by Cu, Fe, Mn, Ni and Co in the brown rice samples, and followed by Cu, Mn, Fe, Ni and Co in white rice samples. The metal ions distribution in the rice grain is still not definitely known. Some studies found out that microelements (Cu, Fe, Mn, and Zn) were probably to be equally distributed in the grain.[21-23] Some authors reported that there was much of iron concentration in the outer layers (aleurone and pericarp) of rice. Milling reduced the iron concentration of the white rice.[24]

Conclusion

In this study, among toxic elements (As, Cd, Pb, Cr), Pb was found in all brown rice and white rice but except one. Zn, Cu and Mn were found in all brown and white rice. Most of the Ni and Fe were found in brown rice only. Cobalt was not found in all brown rice. It was found in one white rice sample, but it was very much lower than that of MAC limit.

Microelements, as well as toxic elements, were more contaminated to a certain extent in brown rice grains than those in white rice grains in this study. Although the increased concentration of microelements required for nutrition in brown rice may be pleasant for health, the increased concentration of toxic metals may concern the safety for brown rice consumers. In this study, there was no consideration on estimated daily intake (EDI) of metals and daily consumption of rice. Therefore, further study should be performed for assessing the risks of white and brown rice consumers by applying the reference values for estimated daily intake of metals and daily consumption of rice.

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Comparative Study of Active Curcumin Content from Four *Curcuma* Species in Myanmar

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Curcumin is a well- known anticancer agent, dietary supplement and possessed several medicinal properties. In Myanmar, rhizomes of four selected Curcuma species: *Curcuma longa* Linn. (eEG), *Curcuma comosa* Roxb. (eEGcg), *Curcuma petiolata* Roxb. (rmrm) and *Curcuma amada* Roxb. (o&ufuif) are widely used in dietary food and traditional medicine. Botanical identification of four selected Curcuma species was done by morphological characters of flowers and microscopical characters of dry rhizome powder. In this study, qualitative identification was carried out by Thin Layer Chromatography (R_f=0.68), UV spectrum (425.5 nm) and FT-IR spectrum (3356.2 cm^{-1}, 2924.18 cm^{-1}, 2854.74 cm^{-1}, 1635.69 cm^{-1}, 589.4 cm^{-1}, 1519.96 cm^{-1}, 1419.66 cm^{-1}). Quantitative determination of active curcumin was performed by TLC scanner -4 (Densitometer, CAMAG), in the absorbance mode at 425.0 nm. The analysis data of the calibration plots showed good linear relationship with R^2=0.994 and percent recovery is within 89.0% to 119.0%. Among the four Curcuma species, curcumin concentration was 2.4±0.04 mg/g in *C. longa*. It is the highest amount. Curcumin concentrations were 0.24±0.006 mg/g and 0.05±0.001 mg/g in *C. comosa* and *C. petiolata*. The lowest amount of curcumin was found in *C. petiolata* rhizome. Curcumin was not present in *C. amada* but demethoxycurcumin was present. According to OECD (423), LD_{50} cut-off values of *C. petiolata* and *C. amada* were more than 5000 mg/kg body weight (non-toxic) but those of *C. longa* and *C. comosa* were (>2000 mg/kg-5000 mg/kg) body weight (slightly toxic).

Key words: Curcuma longa Linn., *Curcuma comosa* Roxb., *Curcuma petiolata* Roxb., *Curcuma amada* Roxb., *TLC scanner-4, Curcumin*

INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global interest. Current estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care.[1] Curcuma species is widely distributed in Asia especially Thailand. The rhizomes of these species are used in traditional medicines which contain curcuminoid as major constituents. It is generally regarded as the most active constituent which has a widely range of biological and pharmacological activities including: anti-tumor, anti-inflammatory, neuro-protective, treatment of wide variety ailment and efficient inhibition of enzyme tyrosinase.[2] *Curcuma* species exhibit inter- and intra-specific variation for the biologically active principles coupled with morphological variation with respect to the above-ground vegetative and floral characters as well as the below-ground rhizome features besides for curcumin, oleoresin and essential oil.

Curcuma is gaining importance world over as a potential source of new drug(s) to combat

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a variety of ailments as the species contain molecules credited with anti-inflammatory, hypocholesterolemic, choleretic, anti-microbial, insect repellent, antirheumatic, antifibrotic, antivenomous, antiviral, anti-diabetic, antihepatotoxic as well as anti-cancerous properties.[3]

Curcuma longa Linn. (eEG) (turmeric) is used as a spice, food preservative and colouring material, in religious applications as well as a household remedy for biliary and hepatic disorders, anorexia, diabetic wounds, rheumatism and sinusitis in India, China and South-east Asia and in folk medicine. Turmeric has also been widely used in Ayurvedic medicine for its anti-oxidant, anti-septic, analgesic, anti-malarial and anti-inflammatory properties.[4] *Curcuma comosa* Roxb (eEGcg) is used for its anti-inflammatory properties in the treatment of postpartum uterine bleeding and uterine inflammation. Numerous studies have been conducted on the effects of *C. comosa* showing that it can reduce blood cholesterol, increase the thickness of epithelial cells lining the vagina, and decrease uterine smooth muscle contraction.[5, 6] *Curcuma petiolota* Roxb. (rmrm) is widely cultivated as an ornamental plant and has long been used as a folk botanical in Asia. The stem and rhizome of *C. petiolota* is selected to paste in external wound skin as traditional medicine and used for herbal stem sauna.[2] *Curcuma amada* Roxb. (o&ufuif) grows wild in parts of Bengal, Konkan and Tamil Nadu, also cultivated throughout India. Drug consists of the dried rhizome of *C. amada*.[7] It is used as medicine, and in a variety of culinary preparations, pickles and salads.[5] The mango ginger rhizome has been extensively used as appetizer, alexiteric, antipyretic, laxative and also, in the ancient Indian system of medicine known as Ayurveda, to cure biliousness, itching, skin diseases, bronchitis, asthma, hiccough and inflammation as a result of injuries.[8]

Curcumin ($C_{21}H_{20}O_6$) is orange yellow crystal powder, insoluble in water and soluble in alcohol and glacial acetic acid.[9] Curcumin has been consumed as a dietary supplement for centuries and is considered pharmacologically safe.[10] Curcumin is cancer chemopreventive and chemotherapeutic on different types of cancer such as lung cancer, breast cancer, colon cancer, prostate cancer, stomach cancer, liver cancer, pancreas cancer, kidney cancer, bladder cancer, blood/lymph cancer, skin cancer, esophagus cancer, brain/head and neck cancer, uterus cancer and ovary cancer.[11] Biliary tract obstruction patients are advised to avoid curcumin because it is known to enhance biliary flow from one's liver. To protect against gastric irritation high curcumin doses should never be taken on empty stomach.[12] Clinical trials of oral curcumin supplementation in patients with early Alzheimer's disease are under-way. The results of 6-month trial in 27 patients with Alzheimer's disease found that oral supplementation with up to 4 g/day of curcumin was safe.[13]

The study was aimed to elicit the important characteristics of four *Curcuma* species in Myanmar. *Curcuma longa* Linn. (eEG), *Curcuma comosa* Roxb (eEGcg), *Curcuma petiolota* Roxb. (rmrm) and *Curcuma amada* Roxb. (o&ufuif) were identified and confirmed botanically. Qualititative and quantitative determination of active curcumin compound were done.

MATERIALS AND METHODS

This descriptive study was conducted at Pharmacology Research Division, Department of Medical Research and Botany Department, Yangon University.

Botanical identification of four Curcuma species

C. longa was collected from Kyaukse Township, *C. comosa* from Kyaukpadaung Township, *C. petiolata* from Dawei Township and *C. amada* from South Dagon Township. Botanical identification for all was done by morphological characters of plant and microscopical characters of dry rhizome powder.[14, 15]

Investigation of phytochemical constituents and characterization of physicochemical test

Phytochemical investigation of four Curcuma species was done by Harborne method (1984) and physico-chemical characterization was done by Quality Control of Medicinal Plant Materials, WHO (1998) method.[16, 17]

Extraction and isolation of curcumin compound from four Curcuma species

Hundred grams of four *Curcuma* species were extracted with 97% ethanol by Soxhlet apparatus for 8 hours and filtered. Filtrate was evaporated by rotary evaporator at 70°C. Curcumin compound was isolated from 97% ethanolic extract of four *Curcuma* species by Preparative Thin Layer Chromatographic method (PTLC). Thin layer chromatogram was done by using Silica gel F_{254}TLC plate (Merck) and dichloromethane: methanol (98:2), mobile phase compare with standard curcuminoid.[18, 19]

Identification of curcumin compound by spectroscopy

Isolated curcumin compound was identified by thin layer chromatogram, UV spectrophotometer (UV-1600, Shimadzu) and FT-IR spectrophotometer (FT-IR, 8400, Shimadzu).[19, 20]

Quantitative determination of curcumin compound from four Curcuma species

Five serial concentrations from stock standard curcuminoid (10 mg/ml) and 97% ethanolic extracts of four *Curcuma* species were applied on TLC plate using dichloromethane: methanol (98:2) solvent system. TLC plate was determined by Densitometer (TLC scanner 4) at absorbent mode 425 nm. Mean peak area of triplicate determination was calculated in excel method.[21-23]

Acute toxicity study of four Curcuma species

Acute toxicity test was done according to the OECD guideline 423. Eighty female albino mice (ddy strain) weighing 25-30 gm were used for the acute toxicity study. They were randomly divided into one control group and two treated groups containing six animals per group and were on standard normal diet provided with water ad libitum. They were allowed to acclimatize for five days to the laboratory condition before the experiment. The treated groups received single dose of 2000 mg/kg body weight and 5000 mg/kg body weight. Control group animals were treated with distilled water (10 ml/kg body weight). They were continuously observed for 24 hours to detect any changes in autonomic or behavioral responses. They were observed daily, for a total of 14 days. Any mortality during these 14 days was also recorded and vital organs were dissected and sent for histological examination.[24]

RESULTS AND DISCUSSION

Botanical identification of four Curcuma species

Zingiberaceace, one of the largest monocotyledon families is found worldwide especially in the tropics. It comprises of 53 genera and more than 1500 species. The genus Curcuma is rhizomatous perennial herbs with at least 120 species distributed in tropical and sub-tropical Asia especially in South and Southeast Asia.

The botanical description of Curcuma species is as follows: Rootstock large of palmately branched sessile annual ate tuber, aromatic with light yellow. Leaves are large, lanceolate to oblong (or) elliptic, leaf stalk as long as the blade, plain green except in the earliest, which are clouded with faint brown down the centre above, glabrous on both sides. Flowering spike are arising from the centre of the tuft of leaves. The rhizomes of *Curcuma* species known as bulb are thick and ovate or pear shaped, lateral rhizome known as finger is curved or nearly straight, ovate or oblong or pyriform or cylindrical in shape, slightly bent, outer surface is yellowish brown color and waxy appearance. Characteristic are odour and warm bitter taste. The diagnostic characters of powdered rhizome of four curcuma plants

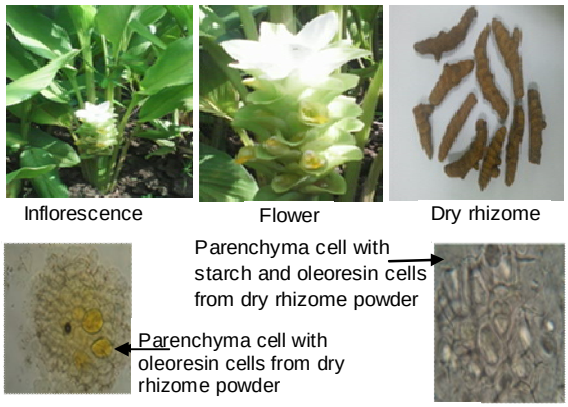


Fig. 1(a) *Curcuma longa* Linn. (eEGf)

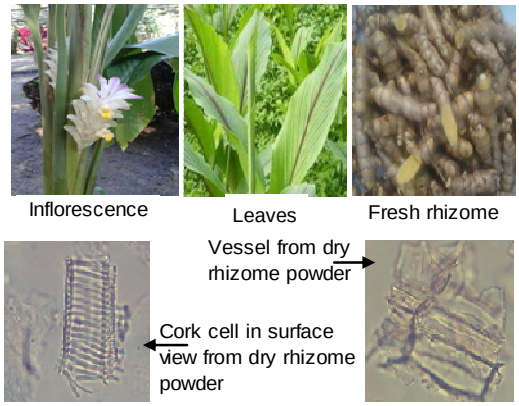


Fig. 1(b) *Curcuma comosa* Roxb (eEGfcg)

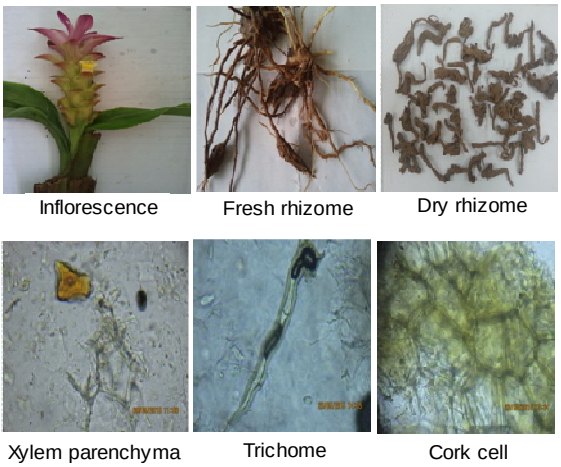


Fig. 1(c) *Curcuma petiolata* Roxb. (rmvm)

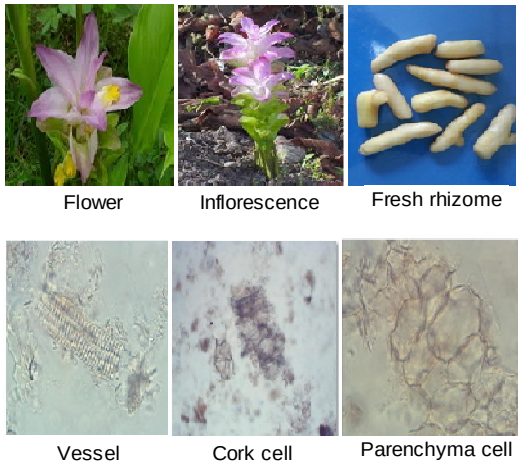


Fig. 1(d) *Curcuma amada* Roxb. (o&ufuif)

Fig. 1. Macroscopic and microscopic characters of four Curcuma species

were mainly observed vessel elements, tracheids, fiber traiheid, xylem parenchyma cells, cork cells, trichome and parenchyma cell with starch. Morphological characters of plant and microscopical characters of dry rhizome powder of four curcuma species are shown in Fig. 1.

Curcuma longa Linn.

Rhizome is thick, much-branched, golden yellow. Leaves are long, entire, long-shealthed, glabrous on both sides. Inflorescence is cylindrical, arising from the leaves, on as cape of yellow flowers and greenish or whitish bracts with pink tips.

Curcuma comosa Roxb.

Inflorescence is a dense head, spike. Flowers are bracteates, bracteolate, sessile, irregular, bisexual, zygomorphic, trimerous, epigynous, structurally unique and complicated, subtended by sheathing bracts. Calyx is three sepals, synsepalous, valvate, tubular, persistant, and superior. Corolla is three petals, apopetalous, imbricate, superior.

Curcuma petiolata Roxb.

The plant is the shape of leaf blades vary from oblong-ovate. Inflorescence is bracts green and coma bracts purple. Flowers are yellow, two rows of ovules in each locule.

Curcuma amada Roxb.

The plant is a rhizomatous aromatic herb with a leafy tuft, 60-90 cm in height, petiolate, oblong-lanceolate, tapening at both ends and glabrous. Flowers are white or pale yellow in spikes in the centre of the tuft of leaves, lip yellow lobed, the midrib emarginated.[25, 26]

Table 1. Results of phytochemical investigation of dry rhizomes of four *Curcuma* species

Phytochemical test	Reagents	Observation	Results			
			C. longa	*C. comosa*	*C. petiolata*	*C. amada*
Alkaloids	Dragendroff's reagent	Orange ppt	+	+	+	+
Steroid /Terpenes	Acetic anhydride and conc: H_2SO_4	Green blue color	++	+	+	++
Flavonoid	conc: HCl/Mg	Red color	++	+	+	+
Polyphenol	10% $FeCl_3$ solution	Deep blue color	++	+	+	+
Tannin	1% Gelatin solution	White ppt	+	+	+	+
Saponin	Distill H_2O	Frothing	+	+	+	+
Glycoside	10% Lead acetate	White ppt	+	+	-	+
Cyanogenic glycoside	Picric paper	Brown color	-	-	-	-
Amino acid	Ninhydrin reagent	Violet color	+	-	+	+
Carbohydrate	α-Naphthanol sol:	Red ring	+	+	+	+
Reducing sugar	Fehling A + B	Brick red ppt	+	+	+	+

Investigation of phytochemical constituents and characterization of physicochemical data

Results of phytochemical investigation and physicochemical characterization of four curcuma species are shown in Table 1 & 2.

Extraction, isolation and identification of curcumin compound from four Curcuma spp.

Thin layer chromatogram of ethanolic extract of four *Curcuma* species and standard curcuminoid (curcumin, demoethoxycurcumin, bisdemethoxycurcumin) under UV 365 nm are shown in Fig. 2.

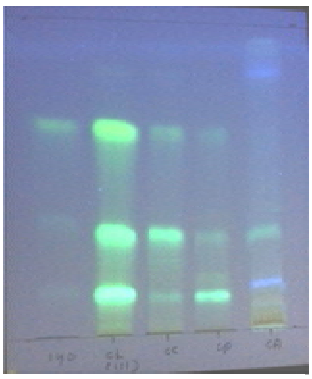
Curcumin (R_f=0.68) was present in three species and was not present in *C. amada* but demethoxycurcumin (R_f=0.34) was present in it. Curcumin was scraped from TLC plate and mixed with methanol solvent by vortex mixer followed by filteration and filtrate was evaporated by air. Dry residue of isolated pure curcumin from three *Curcuma* species was identified by UV spectrophotometer and FT-IR spectrophotometer.

UV spectrum of curcumin compound was taken in acidified ethanol and the maximum absorption was found to be 425.5 nm, which was consistent with literature value. The IR spectrums of curcumin compound from three curcuma species were shown absorption band at 3365.2 cm^{-1}, 2924.18 cm^{-1}, 2854.74 cm^{-1}, 1635.69 cm^{-1}, 1589.4 cm^{-1}, 1519.96 cm^{-1}, 1419.66 cm^{-1}.[19-21]

Quantitative determination of active curcumin was performed by TLC scanner-4, in the absorbance mode at 425.0 nm.

Table 2. Physicochemical characterization of dry rhizomes of four *Curcuma* species

Quality control parameters	Results			
	C. longa	*C. comosa*	*C. petiolata*	*C. amada*
Organoleptic character				
Taste	Slightly bitter	Bitter	Slightly bitter	spicy
Color	Yellow	Pale brown	Pale brown	Pale brown
Odour	Pungent	Pungent	Pungent	Pungent
Swelling index	6.0 ml	5.5 ml	7 ml	18.0
Foaming index	<100	143	<100	<100
pH value				
1% solution	6.6	6.2	7.3	5.2
10% solution	6.5	5.8	7.1	4.6
Ash value (%)				
Total ash	7.2	8.5	12.9	11.2
Water soluble ash	5.7	5.6	6.9	9.1
Acid insoluble ash	0.4	0.8	3.0	0.7
Water and volatile matter content	12.4	10.0	12.0	9.6
Extract values (%)				
P ether (40-60°C)	2.1	6.6	1.1	4.1
97% Ethanolic	10.2	8.7	2.9	15.2
Watery	13.2	24.2	15.3	47.0



Dichloromethane: Methanol (98.2)
Slica gel 60 F_{254} TLC plate

Fig. 2. Thin layer chromatogram of four *Curcuma* species and standard curcuminoid

The analysis data of the calibration plots showed good linear relationship with R^2=0.994 and percent recovery is within 89.0% to 119.0%. Among the four Curcuma species, the highest curcumin concentration was observed in *C. longa* (2.4±0.04 mg/g). Curcumin concentrations were 0.24±0.006 mg/g and 0.05±0.001 mg/g in *C. comosa* and *C. petiolata*.

Pothitirat reported that, *C. longa* grown in different parts of Thailand contains different amounts of various curcuminoids. Therefore, to obtain the high yield in certain curcuminoid, the area for plant collection should be considered.[21]

Curcumin dosage for healthy people is typically one 900 mg curcumin capsule once a day for cancer patients, curcumin dosage is however different since they are advised to take as much as four 900 mg capsules thrice a day for periods covering about 6 to 12 months, reducing curcumin dosage as time progress.[12]

Acute toxicity study

The 97% ethanolic extract of *C. petiolata* and *C. amada* showed no toxic signs or lethality during the observation period of 14 days with 2000 mg/kg and the maximum dose of 5000 mg/kg.

Four animals died in *C. longa* treated group and two animals died in *C. comosa* treated group within 24 hours at the maximum dose of 5000 mg/kg. Histopathological examinations showed congestion of spleen, liver and kidney in *C. longa* (5000 mg/kg b.w) treated mice. *C. comosa* (5000 mg/kg b.w) showed congestion of liver, centri-lobular necrosis, vacuolation in hepatocytes, hydropic degeneration of heptocytes, peripheral hepatitis with infiltration of lymphocytes, focal hepatocyte necrosis, few lymphocytes around portal tract and acute hepatocellular injury in liver.

Therefore, according to OECD 423 guide-line, LD_{50} cut-off values of *C. petiolata* and *C. amada* were more than 5000 mg/kg body weight but those of *C. longa* and *C. comosa* were (>2000 mg/kg-5000 mg/kg) body weight.

Sittisomwong, *et al*.[27] reported that acute toxicity study in mice indicated that oral administration of *C. longa* powder at the dose of 10 g/kg body weight produced no toxic effects. LD_{50} of 50% ethanolic extract administered orally, subcutaneously or intraperitoneally were more than 15 g/kg bodyweight.

The results of this study suggest that turmeric powder at the dose of 1.5 g/day is safe for the treatment of dyspepsia in the Primary Health Care. Khine Khine Lwin, *et. al*.[28] reported that 80% ethanolic extract of dry rhizome of *C. comosa* showed mild acute toxic effect and median lethal dose LD_{50} was determined to be 5.2 g/kg and its confident limit was (4g/kg-6.76 g/kg) on administration of mice model by Litchfield and Wilcoxon (1941).

Conclusion

Curcuma longa Lin., *Curcuma comosa* Roxb., *Curcuma petiolata* Roxb. and *Curcuma amada* Roxb. were identified by morphology and microscopical character. Physicochemical characters were used for quality control of traditional medicine.

Comparative study of the highest to lowest amount of curcumin were *Curcuma longa* Linn., *Curcuma comosa* Roxb. and *Curcuma petiolata* Roxb. Curcumin was not present in *Curcuma amada* Roxb. but demethoxycurcumin was present. LD_{50} cut-off values of *C. petiolata* and *C. amada* were more than 5000 mg/kg body weight (non-toxic) but those of *C. longa* and *C. comosa* were (>2000 mg/kg-5000 mg/kg) body weight (slightly toxic).

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Epidemiological Assessment of Climate Change and Malaria Trend

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Being a vector-borne disease, malaria transmission is determined by vectors, hosts and pathogens. Also these determinants are thought to be affected by changes in climate conditions of the environment. This study aimed to detect the trend of malaria and climatic factors change with malaria transmission. Community-based, cross-sectional descriptive study was done in Pyin Oo Lwin Township, Mandalay Region from January to December, 2014. Data of the climatic factors were collected from Meteorological Department of the studied township. Monthly data of malaria morbidity and mortality from 2004 to 2014 were collected from Vector-borne Disease Control Unit of the township. Geographical information for malaria detections was collected at endemic areas during this period. Percentage of malaria patients among the patients attending at both primary health centers and hospitals were significantly reduced (p=0.001) but, climate variables such as temperature, rainfall and humidity did not changed significantly from 2004 to 2014. In correlation analysis, percentage of malaria patients among the patients attending at both hospitals and primary health centers were significantly increased with increasing humidity (p=0.008 & 0.018). However, percentage of malaria patients among hospital admitted patients was significantly reduced with increasing monthly mean temperature. In conclusion, monthly humidity showed significant correlation with malaria prevalence in Pyin Oo Lwin. An outlook on environmental conditions favorable for the occurrence and spread of malaria could be a part of reporting and monitoring to aid future predictions on malaria occurrence.

Key words: Climate, Malaria trend, Epidemiology

INTRODUCTION

Malaria can become a re-emerging public health problem due to climatic and ecological changes, population migration, development of multi-drug resistant *Plasmodium falciparum* parasite, development of insecticide resistant vectors and changes in behavior of malaria vectors.[1]

According to the World Malaria Report, 2011,[2] malaria disease was found in about 216 million cases and 655,000 malaria deaths were estimated as malaria deaths in 2010 in 106 malaria endemic countries. It is a leading cause of death in many developing countries, where young children and pregnant women are mostly affected.

In Myanmar, according to Health in Myanmar 2014, malaria morbidities were 11.11, 9.32, 10.75, 11.68, 8.09 and 6.44 (per 1,000 populations) in 2004, 2005, 2008, 2011, 2012 and 2013, respectively. Mortalities were 3.65, 2.91, 1.84, 1.33 and 0.48 (per 100,000 populations) in 2004, 2006, 2008, 2010 and 2013, respectively. In accordance with ecological changes and malaria morbidity reduction, the micro-stratification showing high risk areas for the malaria was about 38.9% in the 1990 and it was reduced to 17.0% in 2013.

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In 2010, percentage of populations living under malaria high risk, moderate risk, low risk and no risk areas were 22%, 25%, 16% and 37%, respectively.[1, 3] Being vector-borne disease, malaria transmission is determined by vectors, hosts and pathogens. These determinants are also thought to be affected by changes in climate conditions of the environment such as temperature, humidity and rainfall.[4] During the past 100 years, the global temperature has been increasing significantly with an enhanced warming trend since the mid-1950s.[5] Global warming will enhance transmission rates of the mosquito-borne diseases including malaria, causing wider spread on its geographical distribution,[6] in particular, being identified as a potential impact of climate change.[7]

Some studies relating climate variability and malaria epidemics in East Africa in 2004, 2005 done by Zhou G, *et al*. and 2006 by Pascual M, *et al*. reported an increase in the spread of the disease in the current malaria endemic areas,[8-10] or some studies done in 1998 and 2001 in East Africa revealing reemergence of the disease in areas which have controlled transmission or eliminated the disease in the past,[11, 12] others conducted in 2012 reported no association between malaria and climate change.[13]

Climate change will increase the chances for malaria transmission in habitually malarious areas, in areas the disease has been controlled, as well as in new areas which have been previously non-malarious. A study done in 1996 showed an increase in temperature, rainfall and humidity may cause a proliferation of the malaria-carrying mosquitoes at higher altitudes, resulting in an increase in malaria transmission in areas in which it was not reported earlier.[14] At lower altitudes of some countries where malaria have been already a problem, the studies in 1996 and 1998 showed that warmer temperatures will alter the growth cycle of the parasite in the mosquito enabling it to develop faster, increasing transmission and thus having implications on the burden of disease in Southern Africa.[15, 16] Temperature plays an important role in the life cycle of malaria parasites in the mosquito. As the temperature is lower, the duration necessary to complete the cycle becomes longer for a given *Plasmodium* species. Survival of the mosquito larva and adult also depends on temperature. Increasing environmental temperature during both larval and adult stages decreases the survival of larva and adult mosquitos. Increases of 4ºC (from 23ºC to 27ºC, 27ºC to 31ºC, 31ºC to 35ºC) caused larval and adult mosquito mortality.[17] As water collections after the rains favor vector breeding, malaria transmission is highest following the rainy season. Relative humidity affects malaria transmission through its effect on the activity and survival of mosquitoes.[17]

Pyin Oo Lwin Township, located in Mandalay Region, 3538 feet above sea level, is mountainous and has malarious areas. It has become an endemic area for many years. It has many mosquito breeding sources such as forested areas, stream, swamps, wells and rivers. Malaria prevalence studies are not carried out for Pyin Oo Lwin Township in last decade.

This study aimed to detect the trend of malaria and climatic factors association with malaria transmission. Retrospective analysis of the prevalence of malaria in this township from the year 2004 to 2014 was conducted to study in relation to environmental factors like climatic conditions; rainfall, temperature and humidity.

MATERIALS AND METHODS

Descriptive study with retrospective analysis was conducted in Pyin Oo Lwin Township, Mandalay Region from January to December, 2014. Data of environmental

factors (rainfall, temperature, humidity) were collected from Meteorological Department of Pyin Oo Lwin Township. Rainfall was measured with standard rain gauge and measurements were expressed in inches. Surface air temperature was measured with air thermometer and read as Celsius.

Relative humidity was measured with hygrometer and expressed by a percentage. Data of GIS (map, tract and location) were collected using Global positioning system (GPS) and ArcGIS mapper. Garman GPS apparatus was used for collection of data of map, location and tract.

Monthly malaria morbidity and mortality of Pyin Oo Lwin from 2004 to 2014 were collected from Vector-borne Disease Control Unit of the studied township and from the rural health centers (main source). Malaria morbidity was expressed as both absolute number and relative number. Malaria out-patients and in-patients attending the hospitals and primary health care centers were measured as absolute number. Percentage of malaria cases among total out-patients and percentage of malaria cases among total in-patients were measured as relative number, i.e. percentage. The study obtained the information concerning environmental factors such as rainfall, temperature and humidity in the studied area during past 10 years.

Moreover, the information concerning monthly malaria morbidity and mortality of the studied area over 10 years were also recorded. The recorded data were compiled, coded, entered into SPSS 20.0 software and analyzed. Descriptive statistics was done for environmental factors (climatic change), malaria morbidity, mortality and demographic characteristics. Data of GIS were explained in ArcGIS mapping. Association between malaria prevalence and climatic change was analyzed using analysis of variances (ANOVA).

RESULTS

The number of malaria patients including both out-patients and in-patients attending at both the hospitals and the primary health care centers were gradually decreased from 2004 to 2014. The number of malaria cases yearly attended the Out-patients Department of hospitals and health centers were increased in 2004 and 2005 with 174 and 278 cases, respectively. Then, malaria prevalence was gradually reduced in later 9 years with 141, 79, 58, 67, 53, 80, 31, 27 and 14 cases per year.

The mean percentage of malaria cases among those attending yearly at Out-patient Departments of hospital and health centers were increased in early years, i.e. 2004 to 2006, with 6.1%, 11.0% and 6.8%, respectively. In later 8 years, i.e. from 2007 to 2014, mean percentages were gradually reduced with 4.0%, 2.3%, 2.1%, 2.0%, 3.1%, 1.2%, 0.6% and 0.2%, respectively.

According to analysis by ANOVA, percentage of malaria patients among the patients attending both Out-patient and In-patient departments were significantly reduced from the year 2004 to 2014 (p=0.001).

Table 1. Yearly rainfall in inch

Year	Month	Mean±SD	Range*	95%CI	
				Lower	Upper
2004	11	5.3±4.7	0.04-14.37	2.18	8.45
2005	10	4.9±2.3	1.54-8.79	3.21	6.48
2006	9	7.5±5.2	0.20-17.49	3.52	11.48
2007	9	6.64.4±	0.55-12.17	3.18	9.94
2008	11	4.7±4.5	0.08-14.29	1.69	7.75
2009	9	7.0±5.9	0.75-16.65	2.45	11.49
2010	9	7.4±6.6	0.16-18.84	2.30	12.48
2011	11	6.7±5.7	0.33-18.35	2.81	10.48
2012	11	4.9±3.9	0.16-12.32	2.25	7.58
2013	10	5.6±5.8	0.12-17.87	1.45	9.82
2014	10	8.0±7.2	1.10-23.47	1.40	14.65

*=Minimum-Maximum

One of the climate variables, yearly rainfall was revealed in Table 1. Yearly rain fall distribution showed that rainfalls were reduced in 2005, 2008 and 2012 with 4.9, 4.7 and 4.9 inches, respectively.

Table 2. Yearly maximum temperature in Celsius

Year	Month	Mean±SD	Range*	95%CI	
				Lower	Upper
2004	12	26.0±2.13	22.3-30.3	24.6	27.3
2005	12	26.9±2.84	21.8-31.3	25.1	28.7
2006	12	27.1±2.36	23.4-30.8	25.6	28.6
2007	12	26.3±2.47	23.3-31.1	24.7	27.8
2008	12	26.5±2.27	22.9-31.3	25.1	28.0
2009	12	27.0±2.09	23.5-30.9	25.6	28.3
2010	12	27.4±2.93	22.7-33.0	25.5	29.3
2011	12	26.0±1.86	22.2-28.7	24.7	27.1
2012	12	26.6±2.32	23.2-30.5	25.1	28.1
2013	12	27.0±2.81	21.9-32.0	25.1	28.6
2014	12	27.6±2.44	23.5-31.2	25.7	29.4

*Minimum-Maximum

Table 2 shows yearly maximum temperature in Pyin Oo Lwin Township. Yearly maximum temperature had no significant changes over study period from 2004 to 2014.

Table 3. Yearly humidity in percentage

Year	Month	Mean±SD	Range*	95%CI	
				Lower	Upper
2004	12	75.7±14.3	36-86	66.6	84.8
2005	12	80.7±9.2	59-89	74.9	86.6
2006	12	77.2±6.3	63-85	73.1	81.3
2007	12	79.2±5.1	68-85	75.9	82.5
2008	12	81.9±2.9	78-87	80.0	83.8
2009	12	76.3±10.9	56-89	69.3	83.3
2010	12	77.4±12.2	54-92	69.6	85.1
2011	12	78.6±9.4	61-92	72.6	84.6
2012	12	76.8±13.1	51-91	68.4	85.1
2013	12	78.0±12.1	56-91	70.3	85.7
2014	12	77.6±9.1	62-87	70.6	84.6

*=Minimum-Maximum

Yearly humidity over study period was expressed in Table 3. Mean humidity was reduced in the years 2004, 2009 and 2012 with 75.7%, 76.3% and 76.8%, respectively. The monthly mean rainfall was also found that rainfalls were increased from the month of May to October every year, during study period.

Data of GIS (altitude, tract and location) were collected using Garman GPS apparatus and data were viewed in ArcGIS mapper. Figure 1 shows GIS mapping displaying yearly percentage of malaria among total out-patients in each health center area in studied township from 2009 to 2014. According to the prevalence of malaria in decreasing order, malaria prevalence with >4% of total out-patients were mapped with red color which present in most of health center areas in 2009 to 2011. Before 2009, malaria prevalence was >4% in every area of study sites.

Therefore, those areas had to be colored the whole in red in those years. However, in the years of 2012 to 2014, such higher prevalence was not mapped in any health center areas as the prevalence was reduced. In 2013 and 2014, malaria prevalence with ≤0.5% of total out-patients was mapped with no color in most of health center areas. Only 3 areas in 2013 and one area in 2014 were mapped with yellow color i.e. >1 to 2% of total out-patients. Therefore, malaria morbidity was reduced in studied areas in recent years. Correlation analysis between climate factors and malaria prevalence is described in Table 4.

Table 4. Correlation between climate factors and malaria prevalence

Variables	Mean % of malaria among	
	in-patients	out-patients
Rainfall in inch		
Pearson correlation	0.41	-0.003
P value	0.678	0.972
Maximum temperature		
Pearson correlation	-0.195	-0.152
P value	0.027	0.085
Humidity		
Pearson correlation	0.232	0.208
P value	0.008	0.018

It is found that humidity is positively correlated with mean percentage of malaria among in-patients (p=0.008) and also with mean percentage of malaria among out-patients (p=0.018). The percentage of malaria patients among the patients attending both hospital and primary health centers were significantly increased with humidity increasing. However, percentage of malaria among hospital admitted patients was negatively correlated with increasing mean temperature (p=0.027).

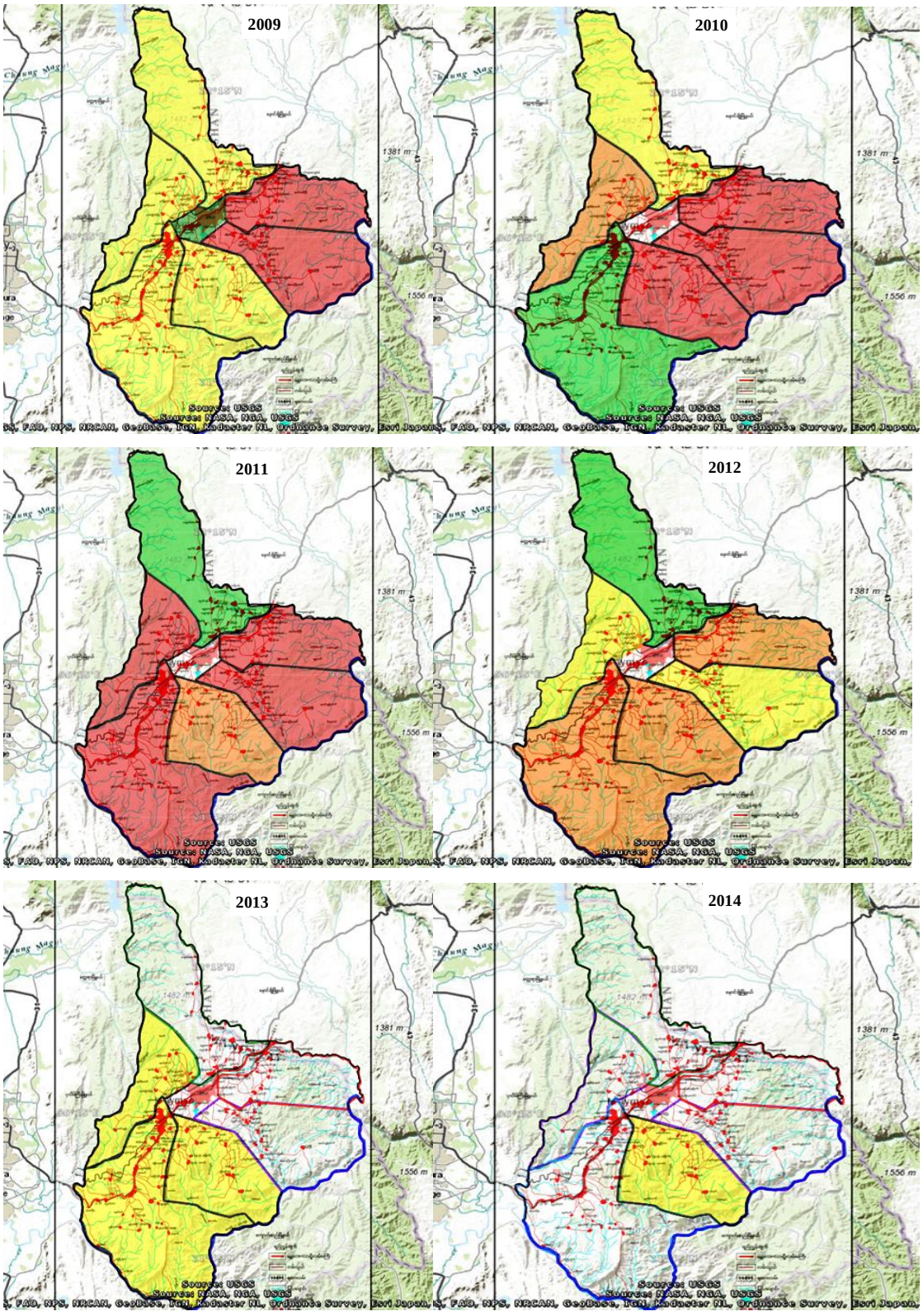

Fig. 1. GIS mapping showing malaria prevalence in health centre areas

DISCUSSION

Since late 2002, Pyin Oo Lwin Township was provided with treatment strategy through basic health staff with rapid diagnostic testing and Artemisinin Combinational Therapy (ACT). Insecticide Treated Net (ITN) for malaria endemic areas by national malaria program through the collaboration with NGOs has been started in Pyin Oo Lwin Township since 2002 also. Thus, malaria prevalence was thought to be reduced by these control activities like other regions in Myanmar. Similar explanation was also justified in Copenhagen Consensus that it may be affected by many factors such as rapid diagnostic testing and ACT provisions by local health workers, ITN supply, population and demographic dynamics, drug resistance, insecticide resistance, human activities such as deforestation, irrigation, swamp drainage, etc.[19]

This study found that humidity is positively correlated with mean percentage of malaria among in-patients and also with among out-patients significantly. Similarly, a study in India showed that high correlation was found between humidity and malaria transmission during monsoon period.[20] The different studies found that malaria incidence was positively correlated with increasing of maximum temperature.[21-23] In this study, changes in mean temperature was negatively correlated with percentage of malaria among in-patients. This is due to some limitations relating measurement of climatic variables of the study. Daily temperature may be responsible for the survival of vector and incubation period of parasites. However, temperature was collected as mean of monthly data in the study. Another limitation is that the temperature was only measured at high altitude areas where meteorological stations were based.

Rainfall was not correlated with malaria transmission in this study. The analysis could not be done between one month lag of rainfall and malaria incidence. Malaria incidence could not be assessed in this study. This study could assess the malaria prevalence. However, malaria cases were high in the rainy season from June to November. Monthly rainfall and one month lag of monthly mean temperature show significant correlation with malaria prevalence in Chennai, however the socio-economic factors and lack of awareness on sanitation and hygiene is a more contributing factor for malaria prevalence at that area.[24] Western and north-western India recorded more malaria cases with higher rainfall during La Niña in 1996 and less rain and fewer malaria cases in the same area during El Niño in 1998.[25]

Conclusions and recommendations

According to this study, malaria prevalence was gradually decreased over 10-year period from 2004 to 2014. This may be mainly due to malaria control activity with widely covered rapid dia*g*nostic testing and ACT provision through basic health staff. Humidity was significantly correlated with mean percentage of malaria from both hospital in-patients and out-patients. However, increasing rainfall and temperature were negatively correlated with malaria transmission in this study. Therefore, it may not be possible to conclude as strong relation between climate factors and malaria prevalence. Therefore, this study recommend that an outlook on environmental conditions favorable for the occurrence and spread of malaria could be a part of reporting and monitoring to aid future predictions on malaria occurrence. To identify the malaria endemicity, malaria mapping might be crucial for health centers. Future research focused on vector ecology, population and demographic dynamic, and populations' behavior will be necessary.

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Screening of Microalbuminuria and Estimated Glomerular Filtration Rate in Type 2 Diabetes Mellitus for Early Detection of Renal Dysfunction

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Monitoring patients with diabetes for microalbuminuria is now standard practice. The estimated glomerular filtration rate (eGFR) equations are based on serum creatinine level. The accuracy of these equations can be affected in certain populations such as extreme of age and body size, severe malnutrition or obesity, diseases of skeletal muscle, paraplegia or quadriplegia and vegetarian diet. The study was to find out the association between microalbuminuria and eGFR in type 2 diabetes mellitus. It was a cross-sectional, descriptive and analytic study. Urine, blood and body weight from 70 cases among type 2 diabetes mellitus patients from Diabetic Clinic, Yangon General Hospital were collected. Urine microalbumin was detected by immunometric method and eGFR was calculated by Cockcroft-Gault formula. If eGFR is less than 90 ml/min, diabetic patients will have increased risk of chronic kidney disease (CKD). With the normal urine volume, less than 20 mg/l of urine albumin was normoalbuminuria, and greater than or equal to 20 mg/l of urine albumin was microalbuminuria. Mean age of patients was 56(SD=11.2 years) and mean duration of diabetes was 7 years (SD=6.8 years). Thirty-five percent of patients had micro-albuminuria and they were in the risk of CKD, 19% had normoalbuminuria with risk of CKD, 19% had microalbuminuria with non-risk CKD. Normoalbuminuria and non-risk CKD patients were 27% of the study population. There was a statistical significant association between micro-albuminuria and eGFR (p=0.035). Patients in the CKD risk group had more risk 2.8 times (95% CI=062-7.437) to suffer microalbuminuria than non-risk CKD group.

Key words: Diabetes mellitus, Microalbuminuria, eGFR

INTRODUCTION

The World Health Organization estimated the global prevalence rate of diabetes is 9% among adults in 2014.[1] Diabetes was the 8th leading cause of death in 2012 causing 1.5 million deaths.[2] In 2014, prevalence of diabetes in Myanmar is 6.6%.[3] As the prevalence of diabetes is increasing, the complications of diabetes are important problems, especially diabetes nephropathy which accounts approximately one third of all cases of end-stage renal disease.[4] One of the earliest signs of impending glomerular nephropathy is microalbumin-uria. Monitoring patients with diabetes for microalbuminuria is now standard practice.[5] Although estimated glomerular filtration rate (eGFR) is feasible to use, the reliability is more or less questionable in other renal dysfunction. In the study of monitoring kidney function in type 2 diabetic patients from Demark, GFR is significantly under-estimated with predicted equations in micro-albuminuric patients.[6]

In a cross-sectional study done in India, results concluded that there was positive correlation between albumin excretion rate and estimated GFR in diabetic chronic kidney diseases.[7] This study was conducted

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to find out the association of microalbuminuria and estimated glomerular filtration rate using Cockcroft-Gault formula in type 2 diabetes mellitus for early detection of renal dysfunction.

MATERIALS AND METHODS

A laboratory-based, cross-sectional descriptive and analytic study was done from July 2015 to June, 2016 in Diabetic Clinic, Yangon General Hospital and Department of Medical Laboratory Technology, University of Medical Technology (Yangon). Random urine, whole blood samples and body weight from already diagnosed type 2 diabetes mellitus 70 cases, who had been consented, attending at Diabetic Clinic, Yangon General Hospital were collected. Urine samples with pus cells and red blood cells and haemolysed and lipaemic blood samples were excluded.

Urine microalbumin was measured with NycoCard U-Albumin immunometric assay. With a normal urine volume, <20 mg/l of urine albumin is normoalbuminuria and greater than or equal to 20 mg/l of urine albumin is microalbuminuria. Serum creatinine was determined by Jaffee's kinetic method and body weight was measured in kilogram. Estimated GFR was calculated by Cockcroft-Gault formula.

For male patients

$$\text{Estimated creatinine clearance}^{*} = \frac{(140\text{-age}) \times \text{BW (kg)}}{72 \times \text{Serum creatinine (mg/dl)}}$$

For female patients

$$\text{Estimated creatinine clearance}^{*} = \frac{(140\text{-age}) \times \text{BW(kg)}}{72 \times \text{Serum creatinine (mg/ dl)}} \times 0.85$$

(BW=Body weight, *= ml/min)

The risk of chronic kidney diseases (CKD) is defined by eGFR when the result is less than 90 ml/min and non-risk CKD is greater than or equal to 90 ml/min.[8]

Data entry and statistical analysis were done using Statistical Package for Social Science Software, version 20.0. The association was calculated by means of chi-square and 'p' value less than 0.05 was assumed as statistical significance. In order to assess the risk, odd ratio was calculated with 95% Confidence Interval.

Ethical consideration

This study was submitted and approved by Ethical Review Committee of University of Medical Technology (Yangon).

RESULTS

Samples from 70 cases among type 2 diabetic patients were assessed. Mean age of the study population was 56 years, minimum was 25 years and maximum was 81 years (SD=11.2 years). Mean duration of diabetes was 7 years (SD=6.8 years). Table 1 shows the demographic data of the study population.

Table 1. Demographic data

	N	Percentage
Sex		
Male	16	23
Female	54	77
Age (years)		
56 and above	36	51
Less than 56	34	49
Duration of DM (years)		
7 and over	33	47
Less than 7	37	53
Antihypertensive drug taking		
Yes	47	67
No	23	33
Race		
Myanmar	40	57
Other	30	43

Table 2. Urine albumin and eGFR

		Urine albumin, N (%)		Total N (%)
		Micro-albuminuria	Normo-albuminuria	
eGFR	Risk CKD	25(35)	13(19)	38(54)
	Non-risk CKD	13(19)	19(27)	32(46)
Total		38(54)	32(46)	70(100)

Thirty-eight patients (54%) had microalbuminuria and 32 patients (46%) had normoalbuminuria. In the case of eGFR calculated by Cockcroft-Gault formula, 38 patients (54%) were in the CKD risk group and 32 patients (46%) were in the CKD non-risk group. Thirty-five percent of the study population had both risk CKD and

microalbuminuria, while 27% had non-risk CKD and normoalbuminuria. Nineteen percent had normoalbuminuria with risk CKD, 19% had microalbuminuria with non-risk CKD. There was a statistical significant association of eGFR and microalbuminuria (p=0.035). Odd ratio of eGFR to micro-albuminuria was 2.8(95% CI=1.062-7.437).

DISCUSSION

In this study, the levels of urine micro-albumin were detected from type 2 diabetes mellitus with immunometric method and eGFR was calculated using Cockcroft-Gault formula. In terms of demographic data, 47% of the study population had disease for more than 7 years, 67% was taking antihyper-tensive drugs and 57% was Myanmar race.

In the study population, out of 70 cases, 38(54%) showed microalbuminuria. Among 38 cases, 25 participants had the risk of CKD. For normoalbuminuria group, there were 32 cases (46%), in which 13 participants had the risk of CKD.

Microalbuminuria is an indicator of incipient diabetic nephropathy.[9, 10] It is an early component in a continuum of progressive increased urinary albumin excretion and characterizes diabetic CKD.[11] However, albuminuria in type 2 diabetes mellitus may be secondary to factors unrelated to diabetes mellitus, such as hypertension, congestive heart failure, prostate disease, or infection. Therefore, detection of microalbuminuria should be repeated in type 2 diabetes mellitus to determine the overt nephropathy.[8, 12]

In this study, the CKD risk group had 38 cases (54%). Among them, 25 participants had microalbuminuria. In the CKD non-risk group, there were 32 cases (46%), in which 13 participants had micro-albuminuria.

For calculation of eGFR, several equations can be used, such as Cockcroft-Gault, MDRD and CKD-EPI formulae which are estimated based on serum creatinine levels. Estimated GFR using Cockcroft-Gault formula depends on age, body weight, serum creatinine level and sex of the patient.

Saha, *et al*.[7] proved that Cockcrock-Gault formula is more reliable than MDRD formula. It is feasible to calculate the GFR compared to measuring it. However, the precision and accuracy of eGFR equations were unacceptable in the study of Lauritsen, *et al*.[13]due to a synchronous decrease of plasma creatinine (PCr) and measured GFR in their study population. Estimates by Cockcroft-Gault equation tend to be higher than true GFR.[14] As creatinine is excreted via renal tubules, the estimated GFR with serum creatinine level may slightly increase compared to true GFR. And if the patient has large adipose tissue and edema, the calculation of weight in Cockcroft-Gault equation may overestimate the GFR. However, the estimated GFR was more reliable than serum creatinine level in patients with reduced renal function.[15]

In the study done by Saha, *et al*.[7] they found that there was a good positive correlation between microalbuminuria and eGFR in type 2 diabetes. But, the study of Rossing, *et al*.[6] showed eGFR was unacceptable and underestimated in their study population of which patients were type 2 diabetic mellitus already with incipient and overt diabetic nephropathy.

In this cross-sectional study, the patients were apparently healthy. Most of them made regular visits to the Out-patient Department of Diabetic Clinic, Yangon General Hospital. In that population, eGFR using Cockcroft-Gault formula had a significant association with microalbu-minuria (p=0.035) and risk of CKD group has 2.8 times to suffer microalbuminuria than non-risk CKD group (odd ratio=2.8, 95% CI=1.062-7.437).

Conclusion

In apparently healthy type 2 diabetes mellitus patients, eGFR using Cockcroft-Gault formula is reliable compared to microalbuminuria for early detection of renal dysfunction. Patients with risk of CKD

have more chance to have microalbuminuria 2.8 times than non-risk CKD group.

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Relationship between Coagulation Parameters and Disease Severity in Patients with Primary Lung Cancer

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Activation of coagulation and fibrinolysis is frequently encountered among cancer patients. Such tumors are supposed to be associated with higher risk of invasion, metastasis and eventually worse outcome. This study was aimed to find out the relationship between coagulation parameters and diseases severity in primary lung cancer patients. A total of 103 primary lung cancer patients attending the Out-patient Department of Medical Oncology Unit, Yangon General Hospital, were involved in the study. The median age was 59 years (range from 13 to 82 years) with male to female ratio of 1.8:1. Among them, 81% of lung cancer patients had history of smoking. Regarding the primary site of the tumour, 64% (66) of cases had right sided lung cancer and 36% (3.7) had left sided tumours. Pretreatment blood coagulation tests including fibrinogen level, prothrombin time (INR) and platelet count were determined by using automated blood coagulation analyzer CA-50 Sysmex. In one year study, according to revised WHO classification of the lung tumours, 7(6.8%) of the tumours were small cell lung cancer, 51(49.5%) squamous cell carcinoma, 35(34%) adenocarcinoma, 10(9.7%) other types (large cell carcinoma and anaplastic carcinoma). According to TNM staging, 4 cases (3.9%) were stage I, 14(13.6%) stage II, 35(34%) stage III and 50(48.5%) were stage IV. The plasma level of all coagulation tests revealed statistically significance in correlation to advanced (stage III and IV) stage of lung cancer, plasma fibrinogen level ($p<0.0001$), prothrombin time ($p=0.007$), INR ($p=0.002$) and platelet count ($p=0.009$), respectively. However, histological types of lung cancer were not significantly associated with coagulation parameters. Therefore, this study pointed out that the high levels of coagulation parameters were associated with the advanced cancer staging and these parameters might be used as the predictors for disease severity of primary lung cancer patients.

Key words: Lung cancer, Coagulation parameters

INTRODUCTION

Lung cancer is the most frequent occurring cancer and leading cause of cancer death worldwide.[1] Patients with malignant tumors often have systemic blood coagulation dysfunction, the relationship between cancer and coagulation is characterized by several mechanism pointing that tumour biology and coagulation are closely linked process.[2] It is now well-established that clotting activation is frequently encountered in cancer, typically manifestation as a low grade DIC or venous thrombloembolism either due to cancer itself or agents used for treatment. Patients with tumours of the lungs, pancrease and GI tract are supposed to be more prone to hypercoagulable state.[3] Tumours activating coagulation system supposed to behave more aggressively with higher risk of invasion and metastasis. Approximately 90% of cancer patients with metastasis disease and half of all cancer

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patients have abnormal coagulation parameters. The most common abnormalities in cancer patients are elevation of the clotting factors V, VIII, IX and XI, increased fibrin degradation products (FDP), hyperfibrinogenaemia and thrombocytosis.[4]

Fibrinogen, the most abundant plasma coagulation factor is synthesized by hepatocytes. The formation of platelet-fibrin-tumour cell aggregates may cause adhesion to endothelial cells and confers metastasis potential.[5] High levels of circulating biomarkers resembling activated coagulation and fibrinolytic system such as fibrinogen, fibrinogen split products and D-dimer have been associated with decreased survival for several tumour types in previous studies.[6-9] Research activities among lung cancer patients investigating the relationship between activated homeostasis system and prognosis have revealed similar results.[10-13]

Therefore, this study aimed to investigate the incidence of some coagulation parameters abnormalities in primary lung cancer patients and attempted to evaluate the correlation of these coagulation tests with other clinic-pathologic variables.

MATERIALS AND METHODS

This study comprised 103 primary lung cancer patients with inclusion criteria of histologically or cytologically confirmed primary lung cancer before taking chemo or radio therapy in last 6 months. Exclusion criteria included patients with history of bleeding disorder, patients who were taking anticoagulants for thromboembolic disease and patients with secondary metastasis to lung. A total of 103 primary lung cancer patients (male to female ratio with 1.8:1) attended the Out-patients Department of Medical Oncology Unit, YGH from January to September, 2016 were eligible for inclusion criteria in this study.

The pathological diagnosis of lung cancer was established in accordance with the revised WHO classification of lung tumours[14] and staged relying on TNM staging for lung cancer.[15] The pretreatment evaluation included detailed clinical history, series of biochemistry tests, complete blood cell counts and coagulation tests. Results of the investigation including Chest X-ray, USG, bronchoscopy and computed tomography were noted.

Coagulation assays

Two milliliters of venous blood sample were collected in sodium citrate tube before initiation of chemo or radiation therapy for measurement of coagulation parameters. The samples were taken to Blood Research Division, Department of Medical Research and centrifuged immediately. Within 3-4 hours after collection, plasma fibrinogen level and prothrombin time (INR) were determined by using CA-50 automated blood coagulation analyzer, Sysmex were used to measure prothrombin time (INR) (SIEMENS, Thromborel ^{R} S kit) and plasma fibrinogen level (SIEMENS, Dade^{R} Fibrinogen Determination Reagents).

Statistical analysis

Being a descriptive study, frequency and proportion for categorical data and mean and SD for continuous data were used to summarize the data. Chi square test and 't' test were used to find out the relationship between coagulation parameters and histological types and staging of the primary lung cancer. Statistical analysis was carried out using SPSS 22 software. The ‘p’ value of <0.05 was regarded as statistically significance.

Ethical consideration

This proposal was approved by Ethics Review Committee, Department of Medical Research. Informed consent was taken from all patients prior to the commencement of the study.

RESULTS

Characteristics of the patients

A total of 103 patients with a pathologically confirmed primary lung cancer were enrolled in our study. Baseline histopathological characteristics and demographic features of patients are shown in Table 1.

Table 1. Patient characteristics

Description	No. of patients	Percent
Total no of lung cancer patients	103	100
Age (years)	59	
Median (range)	(13-82)	
Gender		
Male	67	65
Female	36	35
History of smoking		
Positive	83	81
Negative	20	19
Site of primary tumour		
Right sided	66	64
Left sided	37	36
Haemoglobin level (g/dl), Mean±SD	11.69±2.12	
Total white cell count (WBC) (10^9/L)	10.43±3.89	
Creatinine (mg/dl)	72.99±20	

Table 2. Coagulation variables (n=103)

Coagulation tests	Reference range	Low (%)	Normal (%)	High (%)
Plasma fibrinogen level	150-375 mg/dl	-	73(70.9)	30(29.1)
Prothrombin time	11-14 seconds	4(3.9)	52(50.5)	47(45.6)
INR	0.9-1.2	4(3.9)	58(56.3)	41(39.8)
Platelet count	150-400x10^3/µl	-	73(70.9)	30(29.1)

Median age at diagnosis was 59 years old, ranged 13-82 years, where males constituted majority of the group (n=100, 63%). A total of 83(81%) of patients had the history of smoking. Regarding the primary site of tumors, 66 of cases (64%) were right- sided and 37(36%) were left-sided tumors.

According to revised WHO classification of the lung tumors, 7(6.8%) of the tumors were small cell lung cancer, 51(49.5%) squamous cell carcinoma, 35(34%) adenocarcinoma, 10(9.7%) other types (large cell carcinoma and anaplastic CA).

According to TNM staging for lung cancer, 4 of the cases (3.9%) were stage I, 14(13.6%) stage II, 35(34%) stage III and 50(48.5%) were stage IV. The values of coagulation variables are shown in Table 2. The coagulation tests and their correlation with histological types of primary lung cancer are described in Table 3. The relationship between coagulation parameters and the histological types of lung cancer are summarized in Table 3.

There was no statistically significant relationship between them. In Table 4, it was observed that there was a statistically significant association between all the coagulation parameters and stages of lung cancer. Patients with advanced (extensive) stages of lung cancer exhibited evidently higher level of plasma fibrinogen, platelet counts and prothrombin time (INR). ($p<0.0001$*, $p=0.009$*, $p=0.007$*, $p=0.002$*, respectively).

Table 3. Relationship between coagulation parameters and histological types of lung cancer

Histological types of lung cancer	Coagulation tests n(%)							
	Platelet count (p=0.37)		Plasma fibrinogen level (p=0.85)		Prothrombin time (p=0.79)		INR (p=0.78)	
	Normal	High	Normal	High	Normal	High	Normal	High
Small cell carcinoma	3 (42.9)	4 (57.1)	3(42.9)	4(57.1)	5(71.4)	2(28.6)	5(71.4)	2(28.6)
Squamous cell carcinoma	37(72.6)	14(27.4)	26(51.0)	25(49.0)	28(54.9)	23(45.1)	32(62.8)	19(37.2)
Adenocarcinoma	25(71.4)	10(28.6)	15(42.9)	20(57.1)	18(51.4)	17(48.6)	20(57.1)	15(42.9)
Others	8(80.0)	2(20.0)	4(40.0)	6(60.0)	5(50.0)	5(50.0)	5(50.0)	5(50.0)

Table 4. Relationship between coagulation parameters and stages of lung cancer

Stages of lung cancer	Coagulation tests n (%)							
	Platelet count (p=0.009*)		Plasma fibrinogen level (p<0.0001*)		Prothrombin time (p=0.007*)		INR (p=0.002*)	
	Normal	High	Normal	High	Normal	High	Normal	High
Stage I	4(100.0)	0	4(100.0)	0	4(100.0)	0	4(100.0)	0
Stage II	13(92.9)	1(7.1)	13(92.9)	1(7.1)	12(85.7)	2(14.3)	14(100.0)	0
Stage III	28(80.0)	7(20.0)	19(54.3)	16(45.7)	19(54.3)	16(45.7)	20(57.1)	15(42.9)
Stage IV	28(56.0)	22(44.0)	1(24.0)	38(76.0)	21(42.0)	29(58.0)	24(48.0)	26(52.0)

'p' value of <0.05 was regarded as statistically significant.

DISCUSSION

A systemic activation of clotting system has been observed in cancer patients which is usually reflected by subclinical abnormalities of conventional coagulation tests.[16, 17] There were some evidences that activation of coagulation system by neoplastic cells facilitates invasiveness and metastasis.[13] Thus, the extend of such activation has been associated with tumor stage and prognosis in some malignancies such as breast, colorectal and lung cancers.[8, 18, 19] As the occurrence of the lung cancer is strongly associated with the smoking, a total of 83(81%) of the patients had history of smoking in this study. Most of the patients were found to be in advanced stages, 35(34%) of the cases were stage III and 50 cases (48.5%) were in Stage IV. Thrombocytosis, hyper-fibrinogenemia and elevated D-dimer levels have been demonstrated in different types of cancer involving head and neck, colon, prostate and lung cancer.[20, 21]

Pedersen, *et al.*[22] reported increased platelet count in 32% of 1,115 patients with primary lung cancer, and showed that thrombocytosis was prognostically significant. In 2013, Faruk Tas, *et al.*[23] found that patients with extensive stage SCLC exhibited evidently higher levels of D-dimer, INR and platelet count. In this study, increased in platelet count above $400x10^3$, was found in 30 cases (29.1%) among 103 patients and increased in platelet was statically significantly associated with advanced stages of the lung cancer (p=0.009). Fibrinogen, one of the major protein, is synthesized in the liver and secreted into the circulation. Although fibrinogen synthesis is significantly up regulated by inflammatory stimulation, the precise mechanism in malignancy has not been elucidated yet.

Inflammatory cytokines such as IL6 secreted from cancer cells are supposed to induce production of fibrinogen from the liver. Thus, hypersecretion of fibrinogen may overcome depletion of coagulation tests by ongoing DIC process.[24] Meehan, *et al.*[25] studied 119 untreated SCLC patients and showed that higher pretreatment fibrinogen levels correlated significantly with advanced stages of the disease and reduced survival. Pavey, *et al.*[26] and Maeda, *et al.*[27] found that plasma fibrinogen was associated with decreased survival and also strongly with stages of NSCLC.

So, these findings are in concordance with the study[25] that high pretreatment plasma fibrinogen level was strongly correlated with the advanced (extensive) stage of lung cancer ($p<0.0001$) and also found that 55 patients (53.4%) of lung cancer patients have high pretreatment plasma fibrinogen level. However, the survival analysis was not studied because of limitation of time and budget. The coagulation cascade initiated by tissue factors (procoagulants) triggers a number of events which in turns converts prothrombin to thrombin and generates the insoluble fibrin clots. Assuming that fibrinogen level is normal, a prolong prothrombin time signifies deficiency, depletion of coagulation factors or presence of a specific inhibitor in this cascade.[28]

Prolongation of PT was strongly associated with poor prognosis in NSCLS (Non-small cell lung cancer) patients in previous study but in that report multivariate did not confirm the prognostic relevance of any coagulation factors.[29] The study had also revealed the significance of prolong prothrombin time (INR) in correlation with advanced stages of the lung cancer p-0.007 (p-0.002). In clinical practice, prolongation of PT with a PTT due to depletion of coagulation tests is a well-known markers for DIC.[30] A total of 47 cases (45.6%) of primary lung cancer patients had prolong prothrombin time (INR) in this study. Therefore, this finding supports the presence of low-grade DIC in lung cancer patients. A number of studies have also shown relationship between coagulation changes and natural history of malignancies. Studies conferring evidence of anticoagulants

for cancer patients have revealed conflicting results. However, anticoagulants, particularly heparin with low molecular weight have an anti-tumour effect without fatal bleeding and venous thromboembolism.[31]

This study was a descriptive study and information on post treatment coagulations parameters and survival analysis had not emphasized. A prospective study is required to determine the prognostic significance of coagulation parameters. Anyway, in the present study, extensive (advanced) stages of disease were strongly associated with each of the coagulation parameters (platelet count, fibrinogen level, PT(INR), respectively, although the histological types of lung cancer were not correlated with them. Further large studies on specific subgroups of lung cancer are needed to better define the effective prognostic values of the clotting abnormalities and we may recommend the use of coagulation assays particularly, fibrinogen, PT (INR) in all new lung cancer patients to provide the foresight about outcome, so constitute a surrogate marker for treatment with novel anticoagulants in the near future.

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Relationship between Serum Leptin and Insulin Resistance in Persistent Obese and Current Obese People

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Adiposity and duration of obesity are important for development of type 2 diabetes. The present study, a cross-sectional one, aimed to investigate the relationship between serum leptin and insulin resistance in persistent overweight/obese subjects (n=46) and current overweight/obese people (n=48) (BMI ≥25, duration of obesity >15 and ≤15 years) from Sanchaung and Dagon townships, Yangon. Serum leptin and serum insulin levels were determined by ELISA method. Fasting plasma glucose level was determined by glucose oxidase, phenol, 4-aminophenazone method. Although serum leptin level was not statistically significant between two groups, serum insulin was significantly different between 2 groups (18.94±10.67 μIU/ml *vs.* 14.79±7.10 μIU/ml), respectively ($p<0.05$). Homeostasis model assessment of insulin resistance of the persistent overweight/obese group was significantly higher than that of the current group [median value (interquartile range) [4.46(3.00-5.44) *vs.* 3.40(2.66-4.57)]. Mean homeostasis model assessment (HOMA) of β-cell function in the persistent overweight/obese group was 179.28±84.54% and that of current overweight/obese group was 148.31±72.57%. A difference in mean HOMA β-cell function between two groups subjects was not significant ($p=0.06$). But, there was a significant association between duration of obesity and HOMA β-cell function impairment; current obesity having about 3 times (OR: 3.73, 95% CI=1.23-11.32) increased risk of β-cell function impairment. Correlation between serum leptin and HOMA-IR of all subjects was weak but statistically significant (Spearman's $\rho=0.240$, $n=94$, $p<0.05$). Therefore, the findings indicated that those with longer duration (i.e. persistence overweight/obese) might be more related to insulin resistance. However, those with lesser duration (i.e. current overweight/obese) were found to be more related with impairment of β-cell function. It seems that rapid rise in BMI within short duration is more likely to be associated with impairment of β-cell function.

Key words: Leptin, Insulin resistance, Persistent, Current

INTRODUCTION

Adiposity and duration of obesity are important for development of type 2 diabetes. Duration of obesity was defined as the time since body mass index (BMI) was first known to be at least 30 kg/m^2.[1] The number of years lived with obesity is directly associated with the risk of mortality. Longer exposure to obesity might be expected to lead to a longer exposure to endogenous production of reactive oxygen species and oxidative DNA damage, alterations in carcinogen-metabolizing enzymes, alteration in endogenous hormone metabolism and partial exhaustion of β-cells, with the resultant insulinopenia causing depressed glucose oxidation and impaired glucose tolerance.[1-3] The current obese people who had a medium number of years lived with obesity (5-14.9 years) have

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the risk of mortality more than doubled than normal weight people and the risk was almost tripled for those with the longest duration of obesity (≥15 years).[4]

Obesity enhances insulin resistance.[5] Insulin resistance (IR) is defined as resistance to the effects of insulin on glucose uptake, metabolism or storage. Insulin resistance in obesity is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle, and by impaired suppression of hepatic glucose output.[6] Leptin, the satiety hormone, is a hormone produced by the fat cells in the body, which regulates the amount of fat stored in the body. Obesity promotes hyperleptinemia, which in turn self-promotes leptin resistance and further obesity, making leptin resistance both a consequence and cause of obesity.[7]

Leptin acts as an insulin sensitizer when leptin levels are at low and normal levels but it may contribute to insulin resistance when leptin is chronically activated.[8] Although insulin resistance and leptin resistance increase in parallel with the rise in adiposity, they differ in the timing of their development and their relationship to white adipose tissue mass.[9-11] The association between hyperinsulinemia, hyperleptinemia and insulin resistance is still controversial. Therefore, it was necessary to find out that serum leptin level can increase along with duration of obesity and hyperleptinemia can effect on insulin secretion and insulin resistance.

The aims of the present study were to determine and compare serum leptin, fasting blood glucose level, fasting insulin level and HOMA-IR in persistent and current overweight/obese people and to find out the relationship between serum leptin and HOMA-IR in persistent overweight/ obese and current overweight/obese people.

MATERIALS AND METHODS

A cross-sectional study carried out in persistent overweight/obese subjects (n=46) (BMI ≥25, duration of obesity >15 years) and current overweight/obese subjects (n=48) (BMI ≥25, duration of obesity ≤15 years) from Sanchaung and Dagon Township, Yangon. Plasma glucose level was determined by glucose oxidase, phenol, 4-aminophenazone (GOD-PAP) method. Serum insulin level was determined by ELISA method using DRG Insulin ELISA. Insulin resistance was calculated by formula.[12] Serum leptin concentration was determined by ELISA method using "Human Leptin ELISA Duoset".

$$\text{HOMA-IR} = \frac{\text{Insulin (}\mu\text{IU/ml)x Glucose (mmol/l)}}{22.5}$$

$$\text{HOMA } \beta\text{-cell function} = \frac{20 \text{x FPI (}\mu\text{IU/ml)}}{\text{FPG (mmol/l)-3.5}}$$

Data were presented as mean±SD. Skewed data were presented as median and interquartile range. Data analysis was done by using Statistical Package for Social Sciences (SPSS) software version 16. The difference between the mean of persistent overweight/ obese group and current overweight/ obese group was assessed by Student's t test and Mann-Whitney U test. Pearson's correlation and Spearman's correlation coefficient were computed to explore strength and significance of the relationships among variables. The statistical significance was set at $p<0.05$.

RESULTS

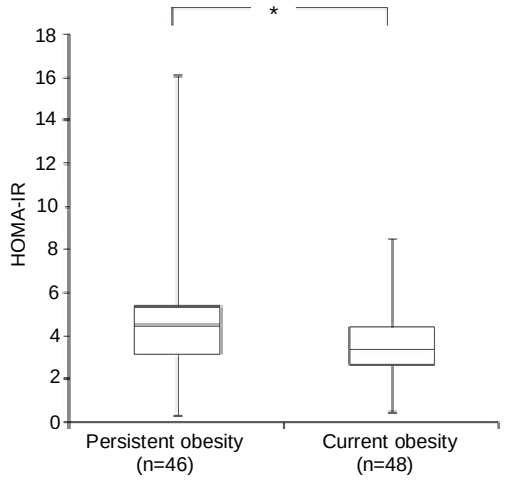
General characteristics of both study groups are shown in Table 1. Median value (interquartile range) of HOMA-IR was 4.46 (3.00-5.44) in the persistent overweight/ obese

Table 1. General characteristics of the study groups

	Overweight/obese groups		p
	Persistent	Current	value
Age (years)	44.57±7.57	48.90±6.90	<0.05
Height (m)	1.53±0.07	1.54±0.05	>0.05
Weight (kg)	69.50±8.39	68.06±8.72	>0.05
BMI (kg/m^2)	29.53±2.72	28.38±3.11	>0.05
Duration of obesity (years)	28.30±8.01	9.42±4.12	<0.01
Body fat percent (%)	44.91±4.89	43.01±5.98	>0.05
Serum leptin (ng/ml)	33.85±19.10	34.30±22.79	>0.05
Serum insulin (µIU/ml)	18.94±10.67	14.79±7.10	<0.05
Fasting plasma glucose (mmol/l)	5.59±0.55	5.58±0.56	>0.05

Data are shown in mean±SD

group and 3.40(2.66-4.57) in the current overweight/obese group. HOMA-IR of the persistent overweight/obese group was significantly higher (p=0.04) than that of the current overweight/obese group (Fig. 1). Mean HOMA β-cell function in the persistent overweight/obese group was 179.28± 84.54% and that of current overweight/obese group was 148.31±72.57%.



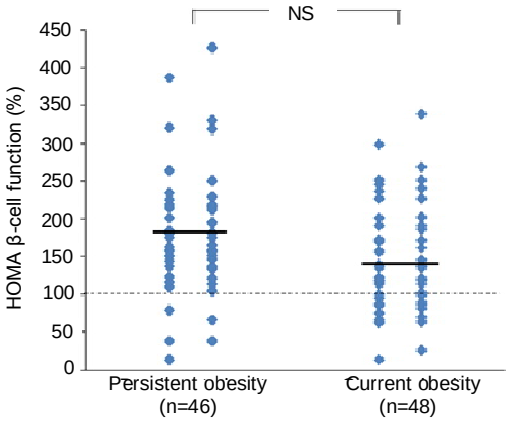
*****indicates significant difference between two groups (p<0.05)

Fig. 1. Comparison of HOMA-IR level of the study groups

A difference in HOMA β-cell function between persistent overweight/obese and current overweight/obese subjects was not significantly different (p=0.06) (Fig. 2). No correlation was found between serum leptin and serum insulin in all subjects and in two study groups. Correlation between serum leptin and HOMA-IR of all subjects in the present study was weak but statistically significant (Spearman's ρ=0.240, n=94, p<0.05) (Fig. 3). However, no correlation between serum leptin and HOMA-IR was found in the persistent overweight/obese group (Spearman's ρ=0.198, n=46, p>0.05) and the current overweight/obese group (Spearman's ρ=0.260, n=48, p>0.05).

Association of β-cell function between persistent and current overweight/obese groups

Five out of 46 persistent overweight/obese subjects (i.e. 10.87%) and 15 out of 48 current overweight/obese subjects (i.e.



NS indicates no significant difference between two groups (p>0.05).

Solid line (—) indicates mean value of different groups.
Dash line (- -) indicates normal HOMA β-cell function.

Fig. 2. Comparison of HOMA β-cell function of the study groups

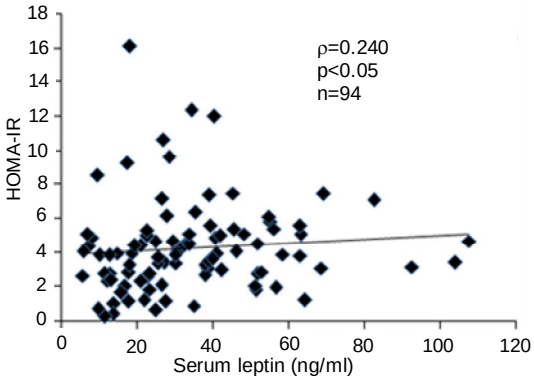


Fig. 3. Correlation between serum leptin and HOMA-IR in overweight/obese subjects

31.25%) had HOMA β-cell function values lower than 100% (i.e. normal HOMA β-cell function).[13] There was a significant association between duration of obesity and HOMA β-cell function, current obesity having about 3 times (OR: 3.73, 95% CI=1.23-11.32) increased risk of β-cell function impairment.

DISCUSSION

Some researchers have focused on the relationship between serum leptin and serum insulin, but the exact relationship between leptin and insulin is not clear and

is sometimes controversial. Chu, *et al.*[14] reported that the relationship between leptin and insulin level is complicated and may be bi-directional.

In the present study, serum leptin and fasting plasma glucose were not significantly different between persistent and current overweight/obese groups. The persistent overweight/obese group showed a significantly higher value in serum insulin and HOMA-IR than the current overweight/ obese group. No correlation was found between serum leptin and serum insulin in all subjects of two study groups. A weak positive correlation between serum leptin and HOMA-IR was found only in all subjects (Spearman's ρ=0.240, p=0.02, n=94) whereas no correlation between serum leptin and HOMA-IR was found in both groups.

Al-Sultan and Al-Elq (2006) also investigated in 89 non-diabetic healthy subjects with normal BMI between 20-25 kg/m^2 (n=43) and obese BMI >30 kg/m^2 (n=46) and their result was a significantly positive correlation (r=0.344, p=0.001, n=89) between leptin and HOMA-IR.[15] Although correlation was found between leptin and HOMA-IR in the present study, it was a weak correlation. In addition, the cross-sectional study design limits to evaluate causal relationships between leptin and insulin resistance. Therefore, the present study could not show any evidence regarding relationship between serum leptin, fasting serum insulin and insulin resistance in overweight/obese subjects with varying duration of obesity. However, the present findings pointed out that the relationship between serum leptin and insulin and the relationship between serum leptin and insulin resistance seems to be independent of duration of obesity.

The finding of the present study was inconsistent with the findings of Zimmet, *et al.*[16] and Mohamed, *et al.*[17] Zimmet, *et al.*[16] found that there was a significant positive correlation (r=0.63 in men and 0.64 in women, p<0.001 in both) between leptin and insulin concentration. They assumed that insulin may directly affect leptin concentration or that leptin reduces insulin values. In addition, Mohamed, *et al.*[17] showed that there was a significant positive correlation (r=0.5174, n=50, p<0.05) between leptin and insulin in obese and metabolic syndrome group.

In the present study, all participants were apparently healthy overweight/obese subjects with different duration of obesity, but these aforementioned two studies included those with normal BMI and those with metabolic syndrome. This difference in subjects' baseline characteristics could explain inconsistent findings between the present and their studies.

Evidence suggested that there is a close link between serum leptin and serum insulin level. However, there are many other independent factors relating to serum leptin and serum insulin level. Thus, the relationship between leptin and insulin resistance was not completely clear. In fact, both the degree of obesity and its central or peripheral fat mass distribution were important determinants of leptin levels; central (visceral) fat was associated with hyperinsulinemia and insulin resistance while peripheral (subcutaneous) fat was associated with hyperleptinemia, indicating that leptin and insulin resistance probably reflect two different metabolic compartments.[18]

In the present study, there was no significant difference in mean HOMA β-cell function between persistent and current overweight/ obese groups. However, a significant association was found between HOMA β-cell function impairment and duration of obesity in all subjects; current obesity having about 3 times increased risk of β-cell function impairment (OR: 3.73, 95% CI=1.23-1.32).

It was found that both persistent and current overweight/obese groups lead to a rise in obesity-related metabolic parameters such as fasting insulin level, HOMA-IR and HOMA-β cell function. The persistent overweight/obese group had higher degree of obesity, as well as obesity-related

metabolic parameters. There were some limitations in the present study: cross-sectional design of the study and participation of apparently healthy subjects with BMI between 25 and 29. In fact, longitudinal study is more appropriate to explore the effect of duration. Previous longitudinal studies found that the longer the duration of obesity, the lower the insulin secretory rate. Thus, the discrepancy in the findings might be explained by difference in study design.

Conclusion

The present findings indicated that those with longer duration (i.e. persistent overweight/obese) might be more related to insulin resistance, but they were found to probably be still in compensatory state. However, those with lesser duration (i.e. current overweight/obese) were found to be more related with impairment of β-cell function. It seems that rapid rise in BMI within short duration is more likely to be associated with impairment of β-cell function.

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Plasma Malondialdehyde Level, Serum High Sensitivity C-reactive Protein Level and Cognitive Ability in Elderly People

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Oxidative stress and low grade inflammation are associated with cognitive impairment in aging. This study aimed to determine plasma malondialdehyde (MDA) level and serum high-sensitivity C-reactive protein (hs-CRP) level in three groups of elderly people with normal cognitive ability, with minor cognitive impairment and with dementia, to compare both parameters among three studied groups, and to find out the relationship between each of these two parameters and cognitive ability in all elderly people. This study was conducted in elderly people with normal cognitive ability (n=24), elderly people with minor cognitive impairment (n=22) and elderly people with dementia (n=24). Cognitive ability was assessed by Mini Mental State Examination (MMSE) and the cut-off score for dementia was MMSE ≤23, that of minor cognitive impairment was MMSE 24-27, and that of normal cognitive ability was MMSE ≥28. Plasma MDA and serum hs-CRP were determined by spectrophotometric method using thiobarbituric acid and enzyme linked immunosorbent assay, respectively. Plasma MDA level of the elderly people with dementia (2.64±0.66 μmol/l) was significantly higher than that of elderly people with normal cognitive ability (1.26±1.03 μmol/l), ($p<0.001$) and those with minor cognitive impairment (1.86±1.10 μmol/l), ($p<0.05$). There was a significant negative correlation between plasma MDA level and MMSE score (Pearson's $r=-0.469$, $p<0.001$, $n=70$) in all elderly people. The median and interquartile range of serum hs-CRP level of the elderly people with normal cognitive ability, those with minor cognitive impairment and those with dementia were 2.03 (0.89-4.06), 2.95 (0.95-4.34), and 4 (1.45-8.65) mg/l, respectively. There was no significant difference in serum hs-CRP levels among three studied groups. A significant negative correlation was seen between serum hs-CRP level and MMSE score (Spearman's $\rho=-0.247$, $p<0.05$, $n=70$) in all elderly people. The present findings indicated that oxidative stress might be involved in pathogenesis of cognitive impairment but the role of low-grade inflammation in cognitive impairment was still equivocal.

Key words: Oxidative stress, Low grade inflammation, Cognitive ability

INTRODUCTION

At present, the average life expectancy of human is increasing. The number of older persons was 841 million worldwide in 2013 and it will be almost triple by 2050.[1] Although the higher life expectancy reflects a positive development, it also brings new challenges. In particular, age-related diseases such as cognitive impairment and dementia, have become more and more prevalent for both the individual and for society.[2] Cognitive impairment in the elderly exists many forms, ranging from subtle impairments through mild cognitive impairment (MCI) to dementia and Alzheimer's disease (AD).[2] According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules (nucleic acids,

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lipids, proteins or carbohydrates).[3] In the aging brain and in the case of several neurodegenerative diseases, there is a decline in the normal anti-oxidant defense mechanisms, which increases the risk of oxidative damage to the brain.[4]

Some studies reported that oxidative damage to biomolecules occurs early in the pathogenesis of AD and precedes pronounced neuropathological alterations.[5-7] Most of the studies described an increased level of the peripheral MDA in AD patients [8-10] as well as in red blood cells of both MCI and early stage AD patients (mild AD).[7] However, in some studies, it was shown that there was no difference in peripheral level of MDA between AD patients and healthy control subjects.[11,12] Therefore, the association between oxidative stress and cognitive function is still controversial. It was suggested that an important mechanism for age-related cognitive impairment-chronic low-grade inflammation may play a role in age-related cognitive impairment.[13] High sensitivity C-reactive protein (hs-CRP) can reflect the presence of inflammation and can be induced by cytokines.[14] Thus, Arai, *et a*l. in 2006 suggested that hs-CRP can be used as a candidate biomarker for screening patients with cognitive impairment.[15] Some studies described that increased serum CRP levels are associated with concurrent cognitive impairment[16] and with poorer cognitive performance at baseline in elderly.[17]

However, some studies showed that there was no association between serum CRP and baseline cognitive performance.[18, 19] Thus, this issue of possible role of serum CRP in age-related cognitive impairment may be still needed to clarify.

MATERIALS AND METHODS

A cross-sectional, comparative study was carried out from April 2015 to February 2016. Elderly people of age 65 years and above were recruited from Day Care Centre for the Aged, Department of Social Welfare in Mayangone Township and Home for the Aged Poor (Kandawkalay), Mingalar Taungnyunt Township, Yangon. Seventy elderly people were selected according to inclusion and exclusion criteria. History taking and physical examination including anthropometric measurements were done according to proforma.

After the 10-hour fasting (from 8:00 pm to 6:00 am), about 5 ml of fasting blood sample were taken: 2 ml of blood sample were collected into anticoagulant (EDTA) containing tube for plasma MDA assay and another 3 ml of blood were collected in plain tube for serum hs-CRP assay. Blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma MDA level was determined at the day of sample collection. The serum was kept in a separated tube which was stored at -20°C until analysis for hs-CRP. Plasma MDA level was determined by spectrophotometric method using thiobarbituric acid.[20] Serum hs-CRP level was determined by enzyme linked immunosorbent assay according to manufacturer's instruction from DRG instruments, Germany.

Cognitive ability was assessed by Mini Mental State Examination (MMSE) and the cut-off score for dementia was MMSE$\leq$23, that for minor cognitive impairment was MMSE 24-27, and that for normal cognitive ability was MMSE $\geq$ 28. After asking the questions individually, scoring was done according to the instructions given consistency and cognitive ability score was recorded with code number.

Statistical analysis

Data were presented as mean±SD. Data analysis was done by using the Statistical Package for Social Sciences software (SPSS) version 16. The difference between data of the elderly people with normal cognitive ability, those with minor cognitive impairment and dementia was assessed by one way ANOVA test. Pearson's and Spearman's correlation coefficients were computed to explore strength and significance of the relationships among

variables. The statistical significance level was set at $p<0.05$.

Ethical consideration

Ethical consideration was done according to the guideline of Board of Studies (Physiology), University of Medicine 1, Yangon. The elderly people were explained first, and written informed consent was obtained.

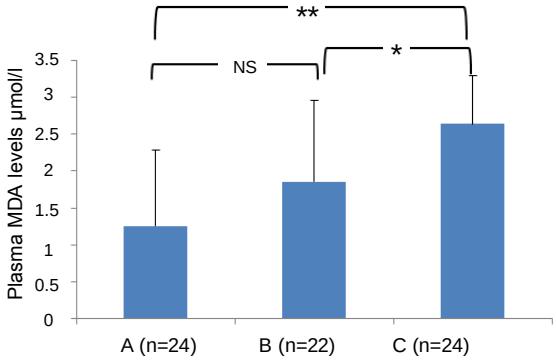
RESULTS

Baseline characteristics of the three different groups of elderly people are shown in Table 1.

Table 1. Baseline characteristics of three different groups of the elderly people

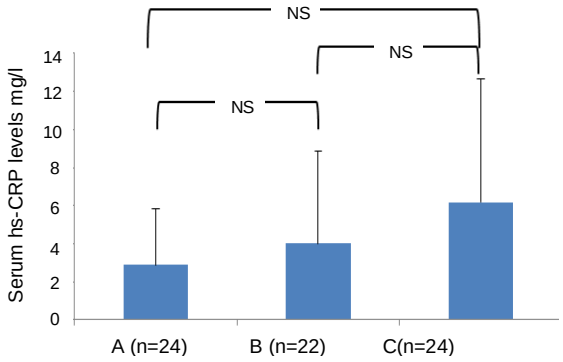
	Elderly people with			P value
	Normal Cognitive Ability (n=24)	Minor cognitive impairment (n=22)	Dementia (n=24)	
Age (years)	76.7±5.10	77.7±4.17	80.1± .29	0.049
Weight (kg)	51.59±8.59	52.68 ±1.34	46.88± 3.04	NS
Height (cm)	158.6±7.54	158.1±7.33	150.4±9.40	0.001
BMI (kg/m^2)	20.51±3.06	21.12±4.66	20.69±5.10	NS
Heart rate (beats/min)	79.8±4.44	81.5±5.32	80.9±4.64	NS
Resting SBP (mmHg)	123.5±8.07	131.7±11.97	126.3 13.99	NS
Resting DBP (mmHg)	77.3±5.43	78.2±5.55	76.9 .75	NS

Data are presented as mean±SD.
NS=No significant difference



A= Elderly people with normal cognitive ability
B= Elderly people with minor cognitive impairment
C= Elderly people with dementia
**=Highly significant difference ($p<0.001$)
*=Significant difference ($p<0.05$)
NS=No significant difference

Fig. 1. Comparison of plasma malondialdehyde levels among three different groups of the elderly people



A=Elderly people with normal cognitive ability
B=Elderly people with Minor cognitive impairment
C=Elderly people with Dementia
NS=No significant difference

Fig. 2. Comparison of serum high sensitivity C-reactive protein levels among three different groups of the elderly people

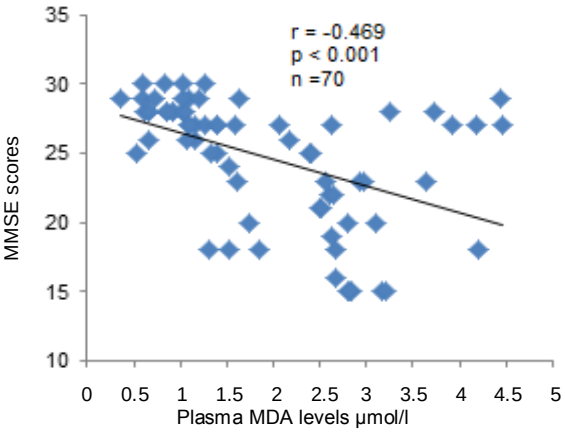


Fig. 3. Correlation between plasma MDA levels and Cognitive ability (MMSE scores) of all elderly people

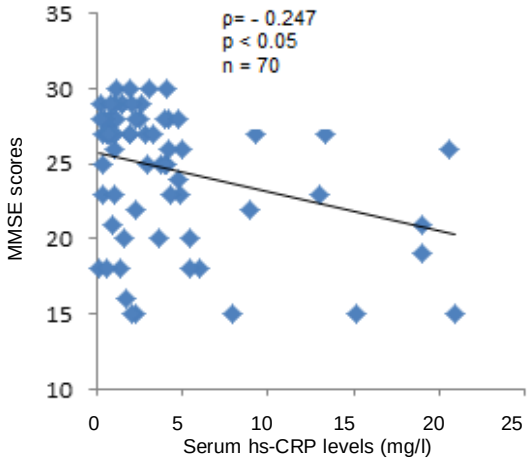


Fig. 4. Correlation between serum hs-CRP levels and Cognitive ability (MMSE scores) of all elderly people

Plasma MDA level of the elderly people with dementia (2.64±0.66 µmol/l) was significantly higher than that of the elderly people with normal cognitive ability (1.26± 1.03 µmol/l), ($p<0.001$) and those with minor cognitive impairment (1.86±1.10 µmol/l), ($p<0.05$) (Fig. 1). There was no significant difference in serum hs-CRP level between three studied groups (Fig. 2).

There was a significant negative correlation between plasma MDA level and MMSE score ($r=-0.469$, $p<0.001$, $n=70$) in all elderly people (Fig. 3). A significant negative correlation between serum hs-CRP level and MMSE score ($p=-0.247$, $p<0.05$, $n=70$) in all elderly people (Fig. 4).

DISCUSSION

In the present study, mean plasma MDA level of the elderly people with normal cognitive ability was 1.26±1.03 µmol/l, those with minor cognitive impairment was 1.86±1.10 µmol/l and those with dementia was 2.64±0.66µmol/l. The normal reference interval of plasma MDA level (20-79 years) is 0.34-1.37 µmol/l.[21] Plasma MDA levels of the elderly people with normal cognitive ability were within normal reference range, whereas that of the other two groups were found to be higher than this reference range. In a study[22] in 2005, plasma MDA level of thirty eight apparently healthy elderly people (63-75 years) was 1.96±0.66 µmol/l before antioxidants supplementation.[22] The plasma MDA level of elderly people with normal cognitive ability in the present study was slightly lower than the other study.[22]

In the present study, plasma MDA levels of the elderly people with dementia were significantly higher than that of the elderly people with normal cognitive ability and minor cognitive impairment. The findings of the present study agreed with Torres, *et al.* in 2011 which demonstrated that average MDA level AD patients was higher than that of MCI patients and healthy aged controls.[23] The present finding was also consistent with that of Greilberger, *et al.* in 2008 and Padurariu, *et al.* in 2010.[11, 24] Greilberger, *et al.* in 2008 reported a significance difference ($p<0.05$) in plasma MDA level of control group ($n=15$; 1.15±0.32 µmol/l) and the neurodegenerative group ($n=16$; 2.62±1.27 µmol/l) in elderly subjects.[11] Padurariu, *et al.* in 2010 also found that a significant increase of the MDA level ($p<0.0005$) was found in the serum of MCI ($n=15$) and AD ($n=15$) patients compared to the age-matched control group ($n=15$).[24]

Many studies showed an association between pathogenesis of AD or neurodegenerative disorders with oxidative stress, which might be responsible for the resulting dysfunction and death of neuronal cells. Thus, the present study also gave supportive evidence that plasma MDA level increases in cognitive impairment such as AD or dementia subjects. There was also a significant negative correlation between plasma MDA level and MMSE score in all elderly. It indicated that low cognitive performance was related to elevated plasma MDA level in aging. The oxidative stress theory of aging postulated that a progressive and irreversible accumulation of reactive oxygen species (ROS) impacts on the senescence process leading to impaired physiology function and increasing incidence of age related pathologies.

Thus, the observed findings of the present study indicated that an oxidative stress might be involved in cognitive impairment in the elderly people. Many studies had been demonstrated that a positive correlation between decreased antioxidant defense and increased lipid peroxidation in MCI and AD patients.[7, 24] It indicated that the progression of AD or dementia may be related to an inadequate capability of the antioxidant defense system to counterbalance the oxidative attack. One of the limitations of the present study was that antioxidant defense parameters (i.e. blood antioxidant enzymes or vitamin levels) related to aging were not determined and assessed in the present study. Thus, it is

recommended that antioxidant enzymes or vitamins should be determined to get insight into oxidative stress and antioxidant imbalance in cognitive impairment in future studies.

In the present study, the median and inter-quartile range of serum hs-CRP levels of the elderly people with normal cognitive ability, those with minor cognitive impairment and those with dementia were 2.03(0.89-4.06 mg/l), 2.95(0.95-4.34 mg/l) and 4(1.45-8.65 mg/l), respectively. There was no statistically significant difference in serum hs-CRP level between the three studied groups.

The present study was in consistent with the findings of Kim, *et al*. in 2015 in which there was no significant difference in serum CRP levels between AD, MCI and control groups.[14] Contrary to the present findings, Dimopoulos, *et al.* in 2006 reported significantly higher serum concentration of adhesion molecules and hs-CRP in patients with dementia (n=37) compared to controls (n=33).[25] Although the present study did not find significant difference in serum hs-CRP among three studied groups, the involvement of low-grade inflammation in the pathogenesis of cognitive impairment could not be excluded. Cross-sectional study design and smaller sample size could be the possible confounding factors for this issue. Since the distribution of hs-CRP was highly skewed, and it is possible that sample size of the present study was not sufficient to detect any significant difference across MMSE score groups.

Thus, longitudinal study with larger sample size would be suggested to determine causal inference and increased hs-CRP with risk of cognitive decline. Another possibility is that the present data showed wide variation in serum hs-CRP level. Selection error may in part explain these results. In the present study, a major effort was made to avoid inclusion of participants with major inflammatory conditions. However, the participants in the present study included hypertensive elderly people except those with malignant hypertension. Previous studies suggested that hypertension may lead to multiple inflammatory stimuli at the vessel wall which in turn promote the production of a number of proinflammatory cytokines such as tumor necrosis factor-α(TNF-α), interleukin-6 (IL-6) and CRP as a defense against injurious factors.[26]

In the present study, there was no correlation between serum hs-CRP level and MMSE score in all three studied groups. However, a significant negative correlation was seen between serum hs-CRP level and MMSE score in all elderly people (Spearman's p=-0.247). There was limited evidence of cross-sectional study to find correlation between hs-CRP and cognitive function in dementia or AD. However, some studies showed increased concentrations of serum hs-CRP has been associated with increased risk of vascular dementia[27] and Alzheimer's disease at follow up.[28] Weuve, *et al.* [29] in 2006 showed there was no evidence of a link between hs-CRP and decrements in cognitive function in older women.

Conclusion

Based on the results of comparison correlation studies, the present study stress might be involved in pathogenesis of cognitive impairment but it could not provide definite evidence that relatively high levels of serum hs-CRP has a negative impact on cognitive impairment.

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Effect of Different Room Temperatures on Breeding Performance of icr Strain Mice

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Temperature is one of the environmental factors and the laboratory mice may have physiological changes because of the environmental temperature. Food and temperature availability have a strong interaction that influences the reproductive performance of female mice throughout the reproductive cycle. This study aimed to analyze the effect of different room temperatures in the housing room on breeding performance of icr mice. In this study, healthy 15 males and 45 females with 1:3 mating ratio of icr strain mice, weighing 25-30 gm, were used for breeding performance under different room temperatures; 18-22˚C, 23-25˚C and 26-32˚C conditions. The monitoring and evaluation were done during 21 days, from birth to weaning for each group. Fertility rate, delivery rate, litter size, birth weight, weaning rate, weaning weight and mortality rate were monitored at the first, the second and the third consecutive gestations. It was found that fertility rate 100%, delivery rate 100%, litter size 9, weaning rate 99.75% and mortality rate 0.24% in group one condition; fertility rate 100%, delivery rate 100%, litter size 7.67%, weaning rate 98.67% and mortality rate 1.32% in group two condition; fertility rate 71.11%, delivery rate 71.11%, litter size 5.33, weaning rate 80.63% and mortality rate 19.37% in group three condition; were found in the first, the second and the third gestations in this study. Birth weight 1.5±0.1 gm and weaning weight 9.5±0.1 gm were found in every room condition. The findings showed that high temperature has effect to lower growth rate and impaired fertility. It was found that the high temperature (26-32˚C) is not suitable for breeding performance and well-being of the animals.

Key words: Temperature, Reproductive performance, Mice

INTRODUCTION

Temperature is one of the environmental factors for reproductive performance in mouse. The laboratory mice may have physiological changes by the temperature variation. The environment of breeding room is an important factor for the productivity. Animals are very sensitive to environmental changes such as sharp fluctuations in temperature, humidity, light, sound and ventilation. A constant room temperature is essential because variation in room temperature causes change in food and water intake. The temperature also affects fertility and lactation.[1] In animals, alteration in the environmental temperature can produce subtle changes in respiration, cardiac rate and behavior in order to maintain a constant body temperature. Body temperature is regulated by a balance between heat production and heat loss. Behavioral adaption include alterations of body position to change the ratio of surface area to mass, hair raising, huddling together of animal in cold and grooming in heat.[2]

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Extreme temperatures usually lead to lower growth rate and impaired fertility. At 32˚C, productivity declines as a result of intra-uterine fetal death.[3] Food and temperature availability have a strong interaction that influences the reproductive performance of female mice throughout the reproductive cycles.[4] The aim of this study was to find out effect of different room temperature conditions on breeding performance of icr strain mouse (*Mus musculus*) by determining the delivery rate, fertility rate (pregnancy rate), number of suckling (litter size), birth weight, weaning weight, weaning rate and mortality rate and to compare those parameters of icr mice under different room temperatures.[5, 6]

MATERIALS AND METHODS

This study was a laboratory-based, experimental study. Mice male (n=15) and female (n=45) (*Mus musculus*) of icr strain were obtained from Laboratory Animal Services Division, Department of Medical Research (Pyin Oo Lwin Branch). Cleaned polypropylene cages (24 cm x 18 cm x 13 cm), covers, water bottles were provided throughout the study. Body weight of animals was measured by balance Scout Pro SPS402F (USA). Breeding room temperatures were measured by standard thermometer (UK).

Three different room temperatures were adjusted and stabilized at 18˚C to 22˚C, 23˚C to 25˚C and 26˚C to 32˚C before starting the experiment and experiment were kept in well-ventilated rooms with exhaust fan. Standardization of thermometer was carried out. Thermometers were placed in each experimental room. Conventional pellet diet and water were provided *ad libitum* for experimental animals. The bedding materials were changed twice a week. These conditions as above were maintained throughout the whole experiment. For acclimatization of mice, 15 male mice were kept in one cage and 45 female mice were kept in another cages for one week before experiment.

In next week, the animals were divided into three groups for three different room temperatures (18˚C to 22˚C, 23˚C to 25˚C and 26˚C to 32˚C) with the mating ratio of 1:3 (5 males and 15 females) for each room temperature. A total of five cages were prepared for three different rooms, in each cage, one male and three females were kept together for mating purpose. When pregnancy occurred, the female was transferred into a separated cage until weaning. After that, the female mice were mated again with their male mice partners. The experiment was continued with the same procedure until 3rd litter.

The 1st, 2nd and 3rd litters mean that total number of offspring per a female in the first, second and third deliveries. Weaning weight means body weight at weaning period. Fertility and delivery rate were measured and recorded as the data of reproductive performance of icr strain mouse. The reproductive performance of icr mouse was calculated under different room temperatures as follow:

$$\text{Litter size} = \text{Total no. of offspring per female mouse (at birth)}$$

$$\text{Fertility rate} = \frac{\text{Total no. of pregnant female}}{\text{Total no. of mated females}} \times 100$$

$$\text{Delivery rate} = \frac{\text{Total no. of delivery}}{\text{Total no. of pregnant}} \times 100$$

$$\text{Weaning rate} = \frac{\text{Total no. of weaned}}{\text{Total no. of offspring}} \times 100$$

$$\text{Mortality rate} = \frac{\text{Total no. of deaths}}{\text{Total no. of offsprings}} \times 100$$

Birth weight and weaning weight were measured individually.

Data analysis

The findings were described by mean and standard deviation. Analysis of variance (ANOVA) was used to determine the significant difference among three tested groups. The statistical comparison of the data was performed by analysis of variance followed by Bonferroni test. 'p' value less than 0.05 ($p<0.05$) was considered significant.

RESULTS AND DISCUSSIONS

According to the study, the significant differences in fertility, delivery rate, litter size, birth weight, weaning weight, weaning rate and mortality rate were found among three tested groups.

Table 1. Comparison of fertility and delivery rate among three tested groups

Group	Room temperature (°C)	1st litter (%)	2nd litter (%)	3rd litter (%)	Mean±SD (%)
I	18-22	100	100	100	100±0.00
II	23-25°	100	100	100	100±0.00
III	26-32°	93.33	66.67	53.33	71.11±20.37

Table 1 shows comparison of fertility and delivery rate. Fertility and delivery rate were 100±0.00% in group one and group two. Fertility and delivery rate were observed 71.11±20.37% in group three. The significant difference in fertility and delivery rate was not found between group one and group two. The significant difference in fertility and delivery rate was found between group one and group three and between group two and group three ($p<0.05$).

Table 2. Comparison of litter size and birth weight among three tested groups

Group	Room temperature (°C)	Litter size Mean±SD (gm)	Birth weight Mean±SD (gm)
I	18-22	9.0 ±1.00	1.6±0.00
II	23-25	7.6±1.53	1.5 ±0.087
III	26-32	5.33±1.53	1.43±0.058

Table 2 shows comparison of litter size and birth weight. Litter size was recorded at birth of icr strain mouse. Litter size was 9.0±1 gm in group one, 7.6±1.53 gm in group two and 5.33±1.53 gm in group three. Litter size of group one was greater than those of the other two groups. The significant difference in litter size was found between group one and group three ($p<0.05$). Birth weight was measured for individual group in same manner. Birth weight was 1.6±0 gm in group one, 1.5±0.087 gm in group two and 1.43± 0.058 gm in group three. Birth weight of group one was greater than those of the other two groups. The significant difference in birth weight was found between group one and group three ($p<0.05$).

Table 3. Comparison of weaning weight and weaning rate among three tested groups

Group	Room temperature (°C)	Weaning weight Mean±SD (gm)	Weaning rate Mean±SD (%)
I	18-22	10±0.44	99.7±0.43
II	23-25	9.6±0.26	98.67±1.25
III	26-32	8.9±0.10	80.63±.61

Table 3 shows comparison of weaning weight and weaning rate. Weaning weight was measured for individual group in same manner. Weaning weights were 10±0.44 gm in group one, 9.6±0.26 gm in group two and 8.9±0.10 gm in group three. Weaning weight of group one was greater than those of the other two groups. The significant difference in weaning weight was found between group one and group three ($p<0.05$). Weaning rates were measured for having reproductive performance of icr strain mouse. Weaning rate of group one and two were 99.75% and 98.67%, respectively. In group three, weaning rate was observed as 80.63%. According to this study, group three had less weaning rate than group one and group two among three tested groups. The significant difference in weaning rate was found between group one and group three and between group two and group three ($p<0.05$).

Table 4. Comparison of mortality rate in three tested groups

Group	Room temperature (°C)	1st litter (%)	2nd litter (%)	3rd litter (%)	Mean±SD (%)
I	18-22	0.00	0.00	0.74	0.24±0.43
II	23-25°	0.00	1.48	2.50	1.32±1.26
III	26-32°	14.29	15.71	28.12	19.37±7.61

Table 4 shows comparison of mortality rate. Mortality rate was also measured for different room temperatures in breeding of

icr strain mouse in this study. The mean mortality rates of all three litters was 0.24±0.43% in group one, 1.32±1.26% in group two and 19.37±7.61% in group three, respectively. Group three had the highest mortality rate among three tested groups.

The significant difference in mortality rate was found between group one and group three, between group two and group three ($p<0.5$). The mortality rate in 3rd litter was greater than those in 1st and 2nd litters.

Conclusion

The results showed that changes of the environmental temperature influence the evaluated parameters above significantly. Group three condition is not suitable for breeding of icr strains mice and the high temperature (26-32˚C) should be avoided. As room temperature is one of the important factors for breeding performance of mice room temperature should be maintained at the condition of (18-22˚C).

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Antimicrobial Activity of *Justicia adhatoda* L. Leaf Extracts (မုယားကြီး)

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Justicia adhatoda L. is a well-known medicinal plant and widely distributed in Myanmar. In this study, antimicrobial activity of different extracts (95% ethanol and methanol) of *Justicia adhatoda* L. leaf was studied on *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* Ceftriaxone was used as positive control. Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. Ethanolic and methanolic extracts showed zone of inhibition, i.e. 10 mm for *Escheriachia coli*. Broth dilution method was used for determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf. MIC of ethanolic and methanolic extracts were observed in 200 mg/ml and MBC of both was observed in 200 mg/ml. Phytochemical analysis of *Justicia adhatoda* L. leaf was also carried out and alkaloids, glycosides, steroids, phenolic compounds, amino acids, starch, flavonoids, proteins, resins, phenols, tannin and carbohydrates were detected. The presence of phenolic compound seemed to be exert antimicrobial activity. So, this study provided referential information about the antimicrobial activity of different extracts of *Justicia adhatoda* L. leaf.

Key words: Leaves extracts

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.[1] It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin. In recent years, pharmaceutical companies have spent a lot of time and money to develop natural products extracted from plants and to produce more cost effective remedies that are affordable to the population.[2]

Medicinal plants used traditionally produce a variety of compounds which have known therapeutic properties. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants.[3]

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity of *J. adhatoda*. *Justicia adhatoda* L. (Family Acanthaceae) is a shrub, widespread throughout the tropical regions of Southeast Asia.[4] Moreover, it is widely used as a medicinal plant and extensively grown in American, India, Nepal

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and Pakistan. It is commonly known as Vasaka or Malabar nut. It is a perennial, evergreen and highly branched shrub (1.0 m to 2.5 m height) with unpleasant smell and bitter taste. It has opposite ascending branches with white, pink or purple flowers. It is a highly valuable Ayurvedic medicinal plant used to treat cold, cough, asthma and tuberculosis.[5] Its main action is expectorant and anti-spasmodic (bronchodilator).[6] Moreover, the importance of Vasaka plant in the treatment of respiratory disorders can be understood from the ancient Indian saying, "No man suffering from phthisis needs despair as long as the Vasaka plant exists."

Thus, the frequent use of *J. adhatoda* has resulted in its inclusion in the WHO manual "The Use of Traditional Medicine in Primary Health Care" which is intended for health workers in Southeast Asia to keep them informed of the restorative utility of their surrounding flora.[5] The major alkaloids of the plant, vasicine and vasicinone, have been found to be biologically active and are the area under discussion of many chemical compounds and pharmacological studies. The source of the drug 'Vasaka' is well-known in the indigenous system of medicine for its beneficial effects, particularly in bronchitis.[7]

The present study was aimed to determine the antimicrobial activity of different extracts of *Justicia adhatoda* L. on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

MATERIALS AND MOTHODS

Place of study

Justicia adhatoda L. leaves were collected from Mandalay Region. Identification of plant was performed at Department of Botany, University of Mandalay by using The Flora of Ceylon (Dassanayake, 1998).[8] The study was conducted at Pharmacology Research Division and Bacteriology Research Division, Department of Medical Research (Pyin Oo Lwin Branch).

Phytochemical analysis

Phytochemical analysis was also done by phytochemical techniques.[9]

Extraction of leaves

Justicia adhatoda L. leaves were collected and thoroughly washed with water and then air-dried for about two weeks. The dried leaves were powdered and extracted by percolation method.[10] The powdered leaves of 70 g were percolated with 600 ml of 95% ethanol for a week in a percolator. The liquid extract containing plant constituents was filtered and evaporated on the water bath at 50°C until to get constant weight and stored in the desiccators. Methanolic extract of *Justicia adhatoda* L. leaves was obtained by the same procedure.

Determination of antimicrobial activity of different extracts of Justicia adhatoda L. leaves

Antimicrobial activity of different extracts of *Justicia adhatoda* L. leaves was determined by agar disc diffusion technique according to modified Kirby and Bauer method.[11]

- Preparation of medium
  Muller-Hinton agar plate was prepared and sterilized by moist heat at 121°C for 15 minutes. After autoclaving, 25 ml of the media was poured into 9 cm diameter petridishes and allowed to set at room temperature. It was prepared freshly before use. When the agar had solidified, the plates were dried at 50°C by placing them with the upright position in the incubator with the lids tilted. The plates were then labeled.
- Preparation of bacterial suspension
  A few colonies of organisms from the sub-culture to be tested were picked with a wire loop and introduced into test tube containing peptone solution. These tubes were incubated at 37°C for 3-4 hours to produce the growth turbidity.
- Preparation of impregnated disc of different plant extracts
  The sterile discs, 6 mm in diameter, were spread out separately in petridishes so that

each disc was not less than 2 mm from its neighbours. They were sterilized by dry heat at 160°C for 1 hour. Ethanolic extracts of *Justicia adhatoda* L. leaf (6.25 mg, 12.5 mg, 25 mg, 50 mg, 100 mg, 200 mg etc.,) were dissolved in each of 1 ml of 95% ethanol. From the different stock solutions, 20 µl of each solution was impregnated to discs, respectively and dried in the incubator at 37°C to evaporate the solvent.

Discs for methanol extract of *Justicia adhatoda* L. leaves were done by the same procedure. Cefriaxone (30 µg) was used as positive control reference standard. Disc as negative control was prepared using the same solvent employed to dissolve the plant extract.

- Antimicrobial susceptibility test

Antimicrobial susceptibility test was determined by a standard disc diffusion technique using Muller-Hinton agar according to the recommendations of Clinical and Laboratory Standards Institute (CLSI).

A sterile cotton swab was dipped into bacterial suspension (1% turbidity of Mac Ferland tubes). Freshly grown liquid cultures of the test pathogens were seeded over the Muller-Hinton agar (MHA) plates with a sterile cotton swab. The swab was streaked in at least three directions through the angle of 60° over the surface of the Muller-Hinton agar to obtain uniform growth. A final sweep was made around the edge of the agar surface.

After the inoculum has dried for a few minutes, the sterile filter paper discs impregnated with plant extracts were placed on the seeded MHA plates at equidistance with a sterile forceps and gently pressed down to ensure contact with the medium. The plates were incubated at 37°C for 24 hours. Following overnight incubation, a zone of inhibition occurred around the discs in the plates. The inhibition zones were recorded as millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

The determination of the MIC involves a semi-quantitative test procedure which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. MIC/MBC values can be determined by a number of standard test procedures. The most commonly used methods are the tube dilution method and agar dilution method. Serial dilutions are made of the products in bacterial growth media. The test organisms were then added to the dilution of the products, incubated and scored for growth.

Determination of MIC and MBC of Ethanolic and Methanolic extracts of Justicia adhatoda L. leaf

The ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf were proceeded for Minimum Inhibitory Concentration by broth dilution method.[12]

Different concentrations of ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf ranging from 3.125 mg/ml to 200 mg/ml were tested for different test organisms. A series of ten tubes for each test organisms were prepared. Each tube contained 20 µl of test organisms in 1 ml of Muller-Hinton broth. The different dilution of 1 ml of ethanolic extract of *Justicia adhatoda* L. leaf was added to the tubes. The eighth tube was used as control tube which contained Muller-Hinton broth, 95% ethanol with test organisms. The ninth tube was used as test extract control and the tenth tube, test organisms only. Then, the different dilution of methanolic extract was

also done and incubated at 37°C for 24 hours. After incubation, MIC was recorded as tube with lowest concentration at which no visible turbidity was observed. For determination of MBC, one loopful from each tube of above dilutions was streaked on Muller-Hinton agar plate and incubated at 37°C for 24 hours.

RESULTS

The plant was identified as *Justicia adhatoda* L. belonging to the family Acanthaceae. The yield percentage of plant extracts of *Justicia adhatoda* L. leaf are shown in Table 1.

Table 1. Determination of yield of different extracts of *Justicia adhatoda* L. leaves

Solvent	Yield (%)
95% Ethanol	53.9
Methanol	63.44

Phytochemical analysis

The results of qualitative tests of leaves are shown in Table 2.

Table 2. Results of phytochemical test on *Justicia adhatoda* L. leaves

Type of compound	Results
Alkaloid	(+)
Carbohydrates	(+)
Glycosides	(+)
Phenols	(+)
Amino Acids	(+)
Saponin	(-)
Starch	(+)
Tannins	(+)
Flavonoids	(+)
Steroids	(+)
Cyanogenic substance	(-)
Proteins	(+)
Resin	(-)
Cardiac glycosides	(+)

(+) Detected, (-) Not detected

Antibacterial activity of different extracts of Justicia adhatoda L. leaves

Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. Both 95% ethanolic extract and methanolic extract showed zone of inhibition, i.e. 10 mm for *Escherichia coli.* These results are shown in Table 3.

Table 3. Antibacterial activities of different extracts of *Justicia adhatoda* L. leaves

Test organism	Diameter of inhibition zone of different extracts of studied leaves	
	95% Ethanolic	Methanolic
Escherichia coli.	10 mm	10 mm
Staphylococcus aureus	<8 mm	<8 mm
Pseudomonas aeruginosa	<8 mm	<8 mm

Table 4. MIC and MBC of ethanolic and methanolic extracts of studied leaves

Test organism	Ethanolic extract (mg/ml)		Methanolic extract (mg/ml)	
	MIC	MBC	MIC	MBC
Escherichia coli	200	200	200	200
Staphylococcus aureus	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-

MIC=Minimum Inhibitory Concentration
MBC= Minimum Bactericidal Concentration

Determination of MIC and MBC of Justicia adhatoda L. leaf extracts

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Justicia adhatoda* L. leaf extracts were determined by broth dilution method. MIC and MBC of ethanolic extract and methanolic extracts of *Justicia adhatoda* L. leaves were 200 mg/ml for *Escheriachia coli.* These results of ethanolic and methanolic extracts are shown in Table 4.

DISCUSSION

From phytochemical investigations, it was observed that alkaloids, glycosides, steroids, cardiac glycosides, amino acids, starch, flavonoids, proteins, phenols, tannin and carbohydrates were significantly present and cyanogenetic substant was absent in the leaf. The phenolic compounds were among the most active components against gram positive and gram negative bacteria.[13] Regarding the medicinal value of *Justicia adhatoda* L. leaves, antimicrobial properties may be due to the presence of phenolic compounds.

Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. The inhibition zones ranged between 7 mm to 10 mm on

Escherichia coli, Pseudomonas aeruginosa and *Staphylococcus aureus.* MIC and MBC values of methanolic and ethanolic extracts were each of 200 mg/ml on *Escherichia coli*. The previous study showed that MIC and MBC values of methanolic extract *Justicia adhatoda* L. was 3.125 µg/ml and 6.25 µg/ml on *Escherichia coli* and did not detected on *Pseudomonas aeruginosa.*[14] This difference may be due to the disparity of extraction methods, species of strains and resistance of organisms.

Conclusion

In this study, the plant *Justicia adhatoda* L. was identified. The phytochemical analysis revealed the presence of various phytochemical constituents such as alkaloids, glycosides, steroids, phenols, amino acids, starch, flavonoids, proteins, tannin and carbohydrates. Both methanolic and ethanolic extracts of *Justicia adhatoda* L. had antibacterial activity at the dosage of 200 mg/ml on *Escherichia coli*.

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Potential Risk Factors of Cardiovascular Diseases among Adolescent Students at Two Selected Schools in Yangon

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A cross-sectional descriptive study was conducted at the No. 2 State High School, North-Okkalapa Township and No. 4 State High School, Ahlone Township, Yangon in 2016. Of 230 students, 108 and 122 students were from North-Okkalapa and Ahlone, with the mean age of 14.24± 1.05 years were recruited to determine the proportion of selected cardio-vascular disease (CVD) risks and the relationship between students' background characteristics and cardiovascular risks. Data were collected on students' background characteristics and lifestyle related CVD risks. Anthropometric measurements, blood pressure and venous blood sample were taken using standard procedures. Regarding the CVD risks, the most prevalent risks were having of inappropriate diet like fried snack and fast food (about 95%, each), followed by salty food preference (77.4%) and physical inactivity (51.7%). Overall, 28% and 15% of students had high blood pressure and overweight/obesity, respectively. About 3.5% of the students had high serum total cholesterol and no one had high blood sugar as a CVD risk. Relationships were found between salty food preference and gender ($p<0.05$), students whose mothers are less educated and dependants ($p<0.05$). Smoking/betel chewing practice was significantly associated with parents education ($p<0.05$) and gender ($p<0.01$). Having fried snacks were significantly related to students whose mother are less educated ($p<0.05$) and father are working outside ($p<0.01$). The most prevalent risks are modifiable, therefore, changing lifestyles play an important role in the prevention of CVDs in their later life. School-based educational intervention are required to increase knowledge and awareness of students regarding about CVDs risks for reducing the burden of CVDs.

Key words: Adolescent, CVD, Risks

INTRODUCTION

Globally, prevalence of non-communicable diseases (NCDs) is increasing and cardio-vascular diseases (CVDs) play a major role in these NCDs.[1] In low-income and middle-income countries, CVDs are the leading causes of mortality and morbidity, and are one of the main contributors to economic burden.[2, 3] Family history, age, sex, smoking, raised blood cholesterol and glucose, high blood pressure, physical inactivity, excessive alcohol consumption, overweight and obesity are the related risk factors to develop CVDs.[4] CVDs may not be only due to one factor but a combination of many which may originate through behavioral and lifestyle factors. Prevention of these factors has positive effects on reducing NCD rates and mortality.[5, 6] It was reported that up to 80% of deaths due to heart disease, stroke, and type 2 diabetes could be prevented by eliminating known lifestyle risk factors.[7] Although CVDs typically occur in middle age or later, the development of these risks starts early in life.[8-11]

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During adolescence, teenagers start to make individual choices and develop personal lifestyles, and many of lifestyle choices are related to risk factors for CVDs. Therefore, it is better to inculcate healthy behaviors at a younger age rather than to modify behaviors at later ages or after the onset of disease, as for the primary prevention of CVDs.

Several studies have reported the prevalence of CVDs risk factors but the majority of them have been conducted among adults.[12, 13] Likewise, studies on the CVDs risk factors in Myanmar have been done among adults rather than adolescents. Despite differences in the individual risk factors, the prevalence of the metabolic abnormalities was high among the study population in Myanmar.[14, 15] Adolescents in developing countries consume more high fat, energy dense foods and are less engaging in physical activity, which mimic the lifestyle of developed countries.[16] In Myanmar, owing to the advertisements on media and changing lifestyle, adolescents are expected to be exposed to the unhealthy foods and lifestyle factors. It was reported that adolescents lacked knowledge regarding the risk of CVDs and did not perceive themselves at risks for CVDs.[17] Therefore, detection of CVD risk factors is needed among adolescents. Early identification of these risks may help to prevent the burden of CVDs in future.

The objectives of the study were to determine the proportion of students with some CVD risk factors at the selected schools in Yangon, and to find out the relationship between the cardiovascular risks and students' background characteristics.

MATERIALS AND METHODS

Subject recruitment

A cross-sectional, descriptive study was conducted among students (13-18 years) at No. 2 State High School, North-Okkalapa Township and No. 4 State High School, Ahlone Township, Yangon in 2016. Of all eligible students, both sexes from each school who agreed to participate were randomly selected till the required sample size was obtained.

Data collection and procedure

Face-to-face interview method was used. Data was collected on background characteristics (age, sex, parental education and occupation) and students' lifestyle related risk factors (smoking, unhealthy food, physical activity, etc.) by using the pretested structured questionnaire.

Heights and weights were taken to calculate BMI as weight (kg)/height (m^2). Waist and hip circumference measurements were taken using standard procedures. The blood pressure was taken on the right arm of subjects seated and at rest for at least 5 minutes using mercury sphygmomanometer. BP measurement was done twice and the mean value was taken.

Venous blood was taken from the arm under aseptic conditions for random blood sugar and total cholesterol measurements. Serum cholesterol was measured by enzymatic colorimetric method using spectrophotometer. Glucometer and test strips (Glucocard II, Japan) were used to measure blood glucose.

Student with BMI $\geq 85^{th}$ and $<95^{th}$ percentile, and BMI $\geq 95^{th}$ percentile for age and sex were considered overweight and obese.[18] Hypertension is considered when average systolic and/or diastolic blood pressure are at or above the 95^{th} percentile (based on age, sex and height).[19] High cholesterol was defined as random serum cholesterol $\geq$200 mg/dl.[20] High blood glucose was interpreted if random blood sugar was $\geq$200 mg/dl.[21]

Student who smoked any number of cigarette in last 30 days was considered current smoker. Students who ate unhealthy food for $\geq$3 days per week were recognized as at risk for CVDs. Physical inactivity was defined as <30 minutes of moderate activity per day on at least five days per week.[22]

Statistical analysis

Data were analyzed with the Statistical Package for Social Studies (SPSS) for window version 16.0. Mean and standard deviation were used for continuous data. Categorical variables were expressed as percentage. Chi square and Fisher exact tests were used to determine the association between the selected cardiovascular risks and students' background characteristics. A 'p' value <0.05 was considered statistically significant.

Ethical consideration

This proposal was submitted to the Ethics Review Committee, Department of Medical Research for approval. Before the study, informed consents and assents were taken from student and their parents. Students were allowed to quit during the study if they did not wish to participate.

RESULTS

Of 230 students, 108(47%) and 122(53%) students were from No. 2 State High School, North-Okkalapa Township and from No. 4 State High School, Ahlone Township, respectively. Among them, 108(47%) were boys and 122(53%) were girls with the mean age of 14.24±1.05 years. Regarding prevalence of the CVD risk factors, the most prevalent risk factors were having inappropriate diet like fried snack and fast food (about 95%, each), followed by preference of salty food (77.4%) and physical inactivity (51.7%). Overall, about 28% and 15% of students had high blood pressure and overweight or obesity, respectively. About 3.5% of the students had high serum total cholesterol and no one had high blood sugar level as a risk for CVD (Table 1).

Table 1. Prevalence of cardiovascular risk factors among students (n=230)

Risk factors	No (%)
Behavioral risks	
Betel chewing	9(3.9)
Smoking	17(7.4)
Preference of salty food	178(77.4)
Having fast food	218(94.8)
Having fried snack	219(95.2)
Physical risks	
Overweight and obesity	35(15.2)
Hypertension	64(27.8)
Physical inactivity	119(51.7)
Biological risks	
Raised blood cholesterol	8(3.5)
Raised blood sugar	0

Relationships were found between girls and preference of salty food like fish-paste, dried fish, dried prawn ($p<0.05$). Students whose mothers were less educated and dependants also had more preference of salty food ($p<0.05$). Smoking/betel chewing practice was found in boys compared to girls ($p<0.01$) and students whose parents were less educated was associated with their smoking practice ($p<0.05$). The significant

Table 2. Relationship between selected CVD risk factors and students' background characteristics

Risk factors	Gender		Father's education		Mother's education		Father's occupation		Mother's occupation	
	Boy (n=108)	Girl (n=122)	≤High school (n=162)	>High school (n=68)	≤High school (n=156)	>High school (n=74)	No (n=27)	Yes (n=203)	No (n=126)	Yes (n=104)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Smoking	17(100)**	-	16(94.1)*	1(5.9)	16(94.1)*	1(5.9)	3(17.6)	14(82.4)	11(64.7)	6(35.3)
Betel chewing	9(100)**	-	8(88.9)	1(11.1)	8(88.9)	1(11.1)	-	9(100)	4(44.4)	5(55.6)
Fried snack	104(47.5)	115(52.5)	154(70.3)	65(29.7)	145(66.2)*	74(33.8)	20(9.1)	199(90.9)**	121(55.3)	98(44.7)
Fast food	100(45.9)	118(54.1)	151(69.3)	67(30.7)	145(66.5)	73(33.5)	26(11.9)	192(88.1)	119(54.6)	99(45.4)
Preference of salty food	77(43.3)	101(56.7)*	128(71.9)	50(28.1)	127(71.3)*	51(28.7)	20(11.2)	158(88.8)	106(59.6)*	72(40.4)
Physical inactivity	58(48.7)	61(51.3)	80(67.2)	39(32.8)	75(63)	44(37)	12(10.1)	107(89.9)	71(59.7)	48(40.3)
Overweight & obesity	17(48.6)	18(51.4)	22(62.9)	13(37.1)	24(68.6)	11(31.4)	4(11.4)	31(88.6)	17(48.6)	18(51.4)
Hypertension	33(51.6)	31(48.4)	44(68.8)	20(31.2)	43(67.2)	21(32.8)	5(7.8)	59(92.2)	34(53.1)	30(46.9)
Raised blood cholesterol	2(25)	6(75)	3(37.5)	5(62.5)	4(50)	4(50)	-	8(100)	6(75.0)	2(25.0)

*=p<0.05, **=p<0.01

relationship was found between fried snack eating and students whose mother were less educated ($p<0.05$), and father worked outside ($p<0.01$). High serum cholesterol, BP and BMI of students were not found in relationship with their background characteristics (Table 2).

DISCUSSION

In the present study, the proportion of selected CVD risk factors was assessed among students at two schools from urban and sub-urban areas. Most students (about 95%) were eating unhealthy food and for those whose mothers were less educated and fathers worked outside. This result was consistent with the study conducted in Western India (94%)[23] and in Parkistan (over 80%).[24] In United States, the prevalence of soft drink consumption among children and adolescents increased from 37% to 56% in a 20-year period.[25] It may be due to easily available fast food, eating out practices, affordable prices and market appeal. Restriction of available unhealthy food outlets, especially in the school compound. Advertisement of fast food should be prohibited. Not only students but also parents should be educated to replace fast food with more nutritious food and drinks in order to have correct dietary knowledge so that they can adopt healthy eating habits.

Hypertension is an important risk factor for heart disease and stroke. Approximately, 28% of students from this study had high blood pressure although they were relaxed to reduce stress as much as possible. The proportion of high blood pressure from this study was found to be a half of the Myanmar National (Adult) prevalence of hypertension (57%)[15] and coincided with the previous study conducted among adolescent students.[26] It was similar to Malaysia study (27.8%)[27] but lower than Portugy study (34%).[28] The possible causes for high blood pressure were that BP was measured on single visit whereas hypertension should be based on reading taken on several visits. However, most other epidemiological studies of BP in children also relied on single-visit readings.[29-32]

This finding should be viewed with much concern because of the tendency of high blood pressure may track into later adult life,[33] and also because of the possibility of secondary hypertension in this age group.[34] Regular BP measurement in younger age groups should be considered to detect hypertension in early age and for BP control in their later life. By doing so, it can reduce the further development of CVD risk.

The prevalence of salty food preference was found in 77% of students in this study. Salt has dose response relationship with hypertension. Data from household budget survey for Brazil population also reported increasing consumption of food with high salt content.[35] Significant relationships were also found between preference of salty food with girls and students whose mothers were less educated and dependants in this study. It might be the fact that they have insufficient knowledge regarding harmful effects of salty food. Moreover, mothers' behaviors were likely to influence their children' eating habit due to the close link between them, in general.

In this study, about 52% of students were physically inactive and it is consistent with the study from Pakistan (54%).[24] It could be the fact that teenagers spent more time in front of television, social media and computer games. Another possible reason is being stress by lessons in the school and, even at home, that lead to physical inactivity. Physical training and activity classes also tend to be on the wane in schools due to competing subjects. Hence, physical activity and sports should be restored in school curriculum. Parental role modeling of physical activity behaviors is important to promote physical activity behaviors of offspring. Thus, parents' encouragement to the children for doing physical activities is advised and limiting sedentary behaviors should be provided.

The prevalence of overweight or obese (15.2%) observed in this study is lower than the previous studies conducted in Yangon (23%).[26, 36] It is also higher and slightly lower than the studies conducted in Philippines (4.8%)[37] and in Thailand (16.6%),[38] respectively. It might be explained by the fact that today's generation of young people prefer low body weight.

It was noticed that 7.4% of students had admitted having smoked and 3.9% of them reported having betel chewing. The prevalence of smoking and that of betel chewing were lower than the findings observed in Global Youth Tobacco Survey, 2016 in Myanmar (8.3% of current cigarette smoking and 5.7% of current smokeless tobacco use).[39] In this study, including only students in Yangon may be the possible reason for difference in the prevalence rate of tobacco use.

Moreover, smoking status may be under-reported because data were collected at schools where smoking is banned in school environment. The students who tried to smoke would have the high risk to become regular smokers leading to an increase risk of CVDs in their later life. Smoking practice of students was significantly associated with gender ($p<0.001$) and parental education ($p<0.05$) in this study. Higher prevalence of smoking was found in boys whose parents were less educated. It is clear that parents' rearing practice and education are important to children for building up healthy habits.

The findings from this study revealed that the most prevalent risk factors are modifiable, therefore, changing lifestyles play an important role in the prevention of CVDs in their later life. Healthy behaviors and attitudes formed during childhood lay a strong foundation for lifetime health related behavior patterns. Thus, educational interventions are required for not only children but also for parents to increase knowledge and awareness about CVDs risk factors in order to reduce the burden of CVDs.

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Phylogenetic Analysis of Human Respiratory Syncytial Virus from Children with Acute Respiratory Infection Admitted to Yangon Children's Hospital

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Human respiratory syncytial virus (RSV) is one of the most important pathogens responsible for acute respiratory tract infection (ARI) outbreaks in children worldwide. RSV is a member of the family *Paramyxoviridae* in which differentiated into two groups (A and B) based on antigenic and genetic variability. To date, 11 genotypes for RSV group A and 23 for RSV group B have been described based on changes in the G gene coding for the attachment glycoprotein. In this study, nasopharyngeal swab samples were collected from hospitalized pediatric ARI cases at Yangon Children Hospital from January to September, 2014. Of 160 cases, non-structural protein 1 (NS1) gene of RSV was detected in 16.3% (26/160), comprising RSV-A strains 52% (11/21) and RSV- B strains 48% (10/21). Furthermore, 21 NS1 gene-positive nasopharyngeal swab samples were processed for genotyping by sequencing of C terminal of the G gene, second variable region. G gene of the RSV was successfully sequenced in 61.9% (13/21) of samples. RSV-A strain was the larger group, accounting for 53.8% (7/13), followed by RSV-B, 38.5% (5/13) and one case 7.7% (1/13) was a mixed infection. The phylogenetic analysis revealed that all group-A strains clustered as the ON1 genotype. Additionally, 72 nucleotide duplication in the second highly variable region of attachment G gene was observed in all RSV ON1 genotype in subgroup-A isolates. Moreover, one isolate of ON1 genotype showed G284S substitution as a novel mutation. Molecular surveillance of RSV infection should be conducted in Myanmar.

Key words: Phylogenetic analysis, Human respiratory syncytial virus, Children with acute respiratory infection

INTRODUCTION

Acute respiratory infection (ARI) is responsible for high morbidity and mortality among under five children, especially in developing countries.[1] Viruses are considered the most important agents in ARI of the lower respiratory tract (LRT) that require hospitalization. Respiratory Syncytial Virus (RSV) is one of those viruses that tops the list among respiratory viruses responsible for annual epidemic ARI outbreaks in infants and pre-school children worldwide, frequently causing bronchiolitis and pneumonia mostly in infants less than six months old.[2] RSV is classified in the genus *Pneumovirus* belonging to the *Paramyxoviridae* family and has an envelope, non-segmented, single-stranded, negative sense RNA genome of approximately 15.2 kb and contains 10 genes encoding at least 11 proteins.[3]

RSV is differentiated into two groups (A and B) based on antigenic and genetic variability. Further studies of genetic variability among RSV strains belonging to

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A and B groups revealed the existence of different genotypes, each with a distinct genetic pattern. The G protein of RSV is a type II surface glycoprotein of about 300 amino acids in length, consisting of a cytoplasmic domain, a transmembrane domain and an ectodomain. It is associated with attachment of the virus and shows the largest antigenic and genetic differences between the two HRSV groups and is one of the targets for neutralization and protective antibody responses.[4, 5]

The G protein gene contains two hypervariable regions (HVR2); the second variable region, which corresponds to the C- terminal region of the G protein (HVR2), reflects overall G protein gene variability and has been analyzed in molecular epidemiological studies.[6-9] The G protein is heavily glycosylated with N-linked and O-linked sugars. Sequencing of the second hypervariable region at the C-terminal end of the G gene, which encodes the G protein, has been widely used to further subdivide RSV-A and RSV-B into genotypes and facilitated differentiation between RSV isolates. To date, 11 RSV-A genotypes, GA1-GA7, SAA1, NA1-NA2, and ON1,[9-11] and 23 RSV-B genotypes, GB1-GB4, SAB1-SAB3, SAB4, URU1, URU2, BAI-BAXII, and THB[9, 12-20] have been described based on nucleotide sequence analysis.

The genetic variability of RSV circulating in Ontario, Canada during 2010-2011 winter seasons was investigated by sequencing and phylogenetic analysis of the G glycoprotein gene. RSV-A (55.7%) was more commonly observed than RSV-B (42.3%). Furthermore, a 72 nucleotide duplication in HVR2 nucleotide sequence was first detected in 10% (11/110) of the subgroup A strains and designated the ON1 gene.[13]

In China, 557 HRSV antigen-positive nasopharyngeal aspirates were selected during 2012/2013 to 2013/2014 seasons in Beijing for group typing. Among them, 37.2% (207/557) were group A and 62.8% (350/557) were group B. Phylogenetic analysis revealed that they belonged to either genotype ON1 66.2% (49/74) or genotype NA1 33.8% (25/74).[21] In Myanmar, a study on RSV was conducted at YCH during 2014 (January to September). A total of 160 nasopharyngeal swabs were collected from under five children attending YCH with acute respiratory infection (ARI). RT-PCR and sequencing was done for identification of Non-Structural protein 1 (NS1) gene. It was found that NS1 gene was detected in 16.25% (26/160) of ARI cases; RSV-A comprised of 52% (11/21) and RSV-B 48% (10/21).[22] Study on molecular characterization of the RSV has not yet been done in Myanmar. Therefore, there is lack of information on the prevalent genotype and subtype.

The aim of this study was to continue further genotyping of previously NS1 gene positive samples by sequencing of G gene. In this study, prevalent genotypes of RSV in Myanmar were explored and this baseline data was useful for development of vaccine against RSV, and might predict future clinical and epidemiological threat among children in Myanmar.

MATERIALS AND METHODS

Twenty-one NS1 gene-positive nasopharyngeal swab samples were used. RNA was extracted by using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

RT-PCR

RT- PCR was done for amplification of the C terminal of the G gene. The second hyper variable region at the carboxy terminal of the G gene was amplified using primers (Table 1) for A and B groups. The cycling protocol included 30 minutes of reverse transcription at 50°C, a 15-minute activation step of the enzyme at 95°C followed by 40 cycles of 30 seconds of denaturation at 94°C and 30 seconds of primer annealing at 50°C and 45 seconds of extension at 72°C, followed by a final extension step of 5 minutes at 72°C in Veriti 96-well thermal cycler (Applied Biosystems).

Nested PCR for RSV-A and RSV-B

These positive samples were subjected to nested PCR. Primers pairs used for A and B subgroups (Table 1). Thermo cycling conditions included a 5 minutes activation step of the enzyme at 95°C, followed by 25 cycles of 30 seconds of denaturation at 94°C, 30 seconds of primer annealing at 50°C, and 45 seconds of primer extension at 72°C followed by a final extension step of 5 minutes at 72°C.

Table 1. Genotyping primers

RSV A group	
GPA (511-530)	5'-GAAGTGTTCAACTTTGTACC-3'[10]
RSAG (539-558)	5'-ATATGCAGCAACAATCCAAC-3'[23]
F1A (3-22)	5'-CAACTCCATTGTTATTTGCC-3'[10]
RSV B group	
GPB (494-515)	5'AAGATGATTACCATTTTGAAGT-3'[10]
RSBG (512-531)	5'-GTGGCAACAATCAACTCTGC-3'[23]
F1B (3-22)	5'-CAACTCCATGGTTATTTGCC-3'[10]

10=Ref: 10, 23=Ref: 23

Gel documentation

The PCR products were verified by agarose gel electrophoresis and visualized under UV light Transilluminator, (BioRad). The size of the cDNA for each group of RSV: RT-PCR were 492 bp for RSV-A, 509 bp for RSV-B and 572 bp for RSV-BA and for Nested-PCR were 465 bp for RSV-A, 461 bp for RSV-B and 524 bp for RSV-BA.

DNA sequencing methodology

(i) The PCR products were purified by using Montage's Purification Method.

(ii) Cycle sequencing was done by using Big-Dye V.3.1, Applied Biosystems. Amplification was done under the following conditions: 1 minute of activation at 96°C followed by 25 cycles of 10 seconds denaturation at 96°C, 5 seconds of annealing at 50°C and 4 minutes of extension at 60°C.

(iii) Purification of Cycle sequencing product was done by ethanol purification method to remove excess Big-dye.

(iv) DNA sequencing was done by using Applied Biosystems 3500 XL Genetic Analyzer, Hitachi at Department of Medical Research.

Phylogenetic analysis

Sequence quality was checked by Bio Edit software v.7.0.5 and manual correction was done. A 462 nucleotide segment of HVR2 were aligned with that of other sequences available from GenBank by using Clustal W 1.6 method of MEGA software version 6.06. Phylogenetic tree was generated by the neighbor-joining method with 1,000 replicates of bootstrap values. RSV-A stains G gene sequences were submitted to GenBank and the accession numbers are KY320500 to KY320507.

Table 2. RSV group A G protein gene GenBank sequences used in the study

Strain	GenBank accession no.	Country of isolation	Years of isolation
A2	M11487	Australia	1961
ON67-1210A	JN257693	Canada	2010
CU2011/216	KC342446	Thailand	2011
CU2011/211	KC342444	Thailand	2011
RSVA/GN435/11	JX627336	Korea	2011
MY-2444006-11	JX256871	Malaysia	2011
CU2011/192	KC342434	Thailand	2011
AS12-047	AB808774	Japan	2011
CN-A011-12	KU681162	Korea	2012
BJ/39979	KC461212	China	2012
RXH/ON1/001	JX885730	South Africa	2012
Chiba-C/24226	AB808757	Japan	2012
NIV1212316/12/A	KF246640	India	2012
NIV1212334/12/A	KF246641	India	2012
HR3445-12	KF057865	Croatia	2012
WUE/16397/12	JX912364	Germany	2012
WUE/14576/12	JX912357	Germany	2012
KEN/Kilifi/116160/25	KF587959	Kenya	2012
BJ/43849	KM434001	China	2013
HD12114	KJ710386	Germany	2013
JPN/21413	AB918735	Japan	2013
LA2-85/2013	KJ672471	USA	2013
1308-509AN	KC858245	Italy	2013
BJ/51238	KM434024	China	2014
HN-5787	KT781390	China	2014
BJ/55543	KM434062	China	2014
BJ/52897	KM434039	China	2014
BJ/52137	KM434034	China	2014

Amino acid analysis

G protein of Myanmar RSV-A strains were compared to references strains from GenBank to identify amino acid substitutions by MEGA v6.06.

RESULTS

Detection of RSV

Among twenty-one NS1 gene-positive RSV isolates, RSV-A was identified in 53.8% (7/13), RSV-B in 38.5% (5/13), and one case (7.7%) was mixed infection of these two groups (A+B).

Sequence alignments and phylogenetic analysis

Sequences of the second hyper variable region of the G gene from 8 RSV-A (RSVA-7 and Mixed-1) and 6 RSV-B (RSVB-5 and Mixed-1) samples were aligned with sequences of reference strains and prototype strain (M11486) from GenBank. After the phylogenetic analysis (Fig. 1. A) all Myanmar RSV-A groups were clustered as the ON1 genotype and they were found to be closely related to ON67-1210A/2010/Canada, JPN/ 214.13/ 2013/ Japan, HN_5787/2014/China, CN-A002-14/2014/Korea and CU2011/216/ 2011/ Thailand, respectively.

Regarding RSV-B, only one sequence RSV-B139 Myanmar strain belonged to BA9 genotype and closely related to one strain from Japan 2006 (NG-022-06). The remaining 5 sequences belonged to RSV-B group (Fig. 1. B).

Deduced amino acid sequence analysis

Amino acid alignment of all the genotypes of RSV-A is shown (Fig. 2). The Myanmar RSV ON1 genotype has a characteristic of a 72- nucleotide duplication in the second highly variable region of attachment G gene and it was first detected in Canada in 2010.[13] The ON1 genotype could be defined by E232G or T253K substitutions. The eight Myanmar ON1 strains were compared with those strains from other countries with reference to the original strains from Canada (JN257693) and showed that there are four characteristic substitutions as given below. Thorough amino acid analysis was done except RSV-A083 because of the shortness of sequence length.

- One ON1 strain RSV-A100 Myanmar showed S250F substitution unlike the strains from Canada and all other countries. The remaining 7 Myanmar ON1 genotypes did not have such substitution like the strains compared in this study.
- There were amino acid substitutions at position (L274P, L298P, and Y304H) seen in ON1 strains from China, Japan, India, Germany, Kenya and Italy (KM434034; AB808757; KF246640; KF246641; JX912364; KF587959; KC858245). However, this type of substitution was not seen in Canada strain and all Myanmar ON1 strains.
- (G284S, Y280H) substitutions were seen only in RSV-A102 ON1 strain which was different from other Myanmar ON1 strains and other countries. This is unique for Myanmar strain. In addition, there was also E295K substitution in RSV-A102, which is similar to Germany ON1 strain (KJ210386). However, all other ON1 strains showed no such amino acid substitution.
- Seven Myanmar ON1 strains (except RSV-A083) did not show L310P substitution which was different from Japan, India and Kenya (AB808757, KF246640, KF246641 and KF587959) ON1 strains, but similar to Canada ON1 strains.

Clinical and epidemiological features of RSV infection at YCH

Among RSV-positive patients, the ratio of boys to girls was 1.2:1; the age range was 1 month to 20 months, the majority of patients (92.3%, 12/13) were under one year old. Diagnosis in the majority of patients was bronchiolitis (55%) followed by pneumonia (15.0%), severe pneumonia (15.0%) and AVI with no pneumonia (7.5%).

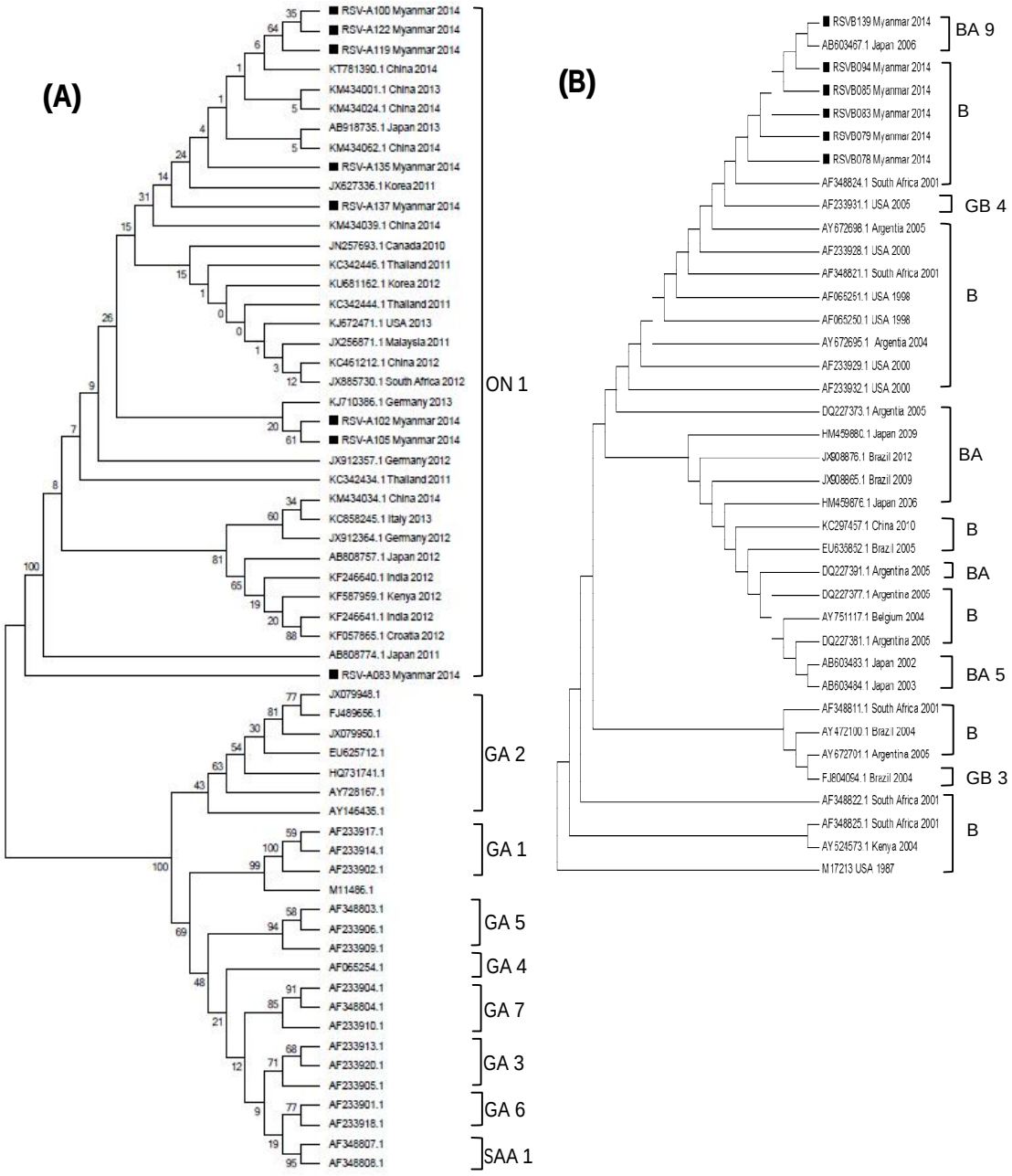

Fig. 1. Phylogenetic tree of RSV-A/RSV-B strains and reference sequences of identified genotypes. Phylogenetic trees for RSV-A (A) and RSV-B (B) strains were constructed with Neighbor-joining tree method with 1,000 bootstrap replicates using MEGA 6.06 software. RSV strains from Myanmar are indicated by "RSV-A and RSV-B" followed by their strain identification number. Number of identical strains is indicated in brackets after the strain identifier. Reference strains representing known genotypes were retrieved from GenBank and included in the tree (labels include accession number) (Table 2). The genotype assignment is shown on the right by brackets. Prototype strains (M11486 for subgroup A and M17213 for subgroup B) were used.

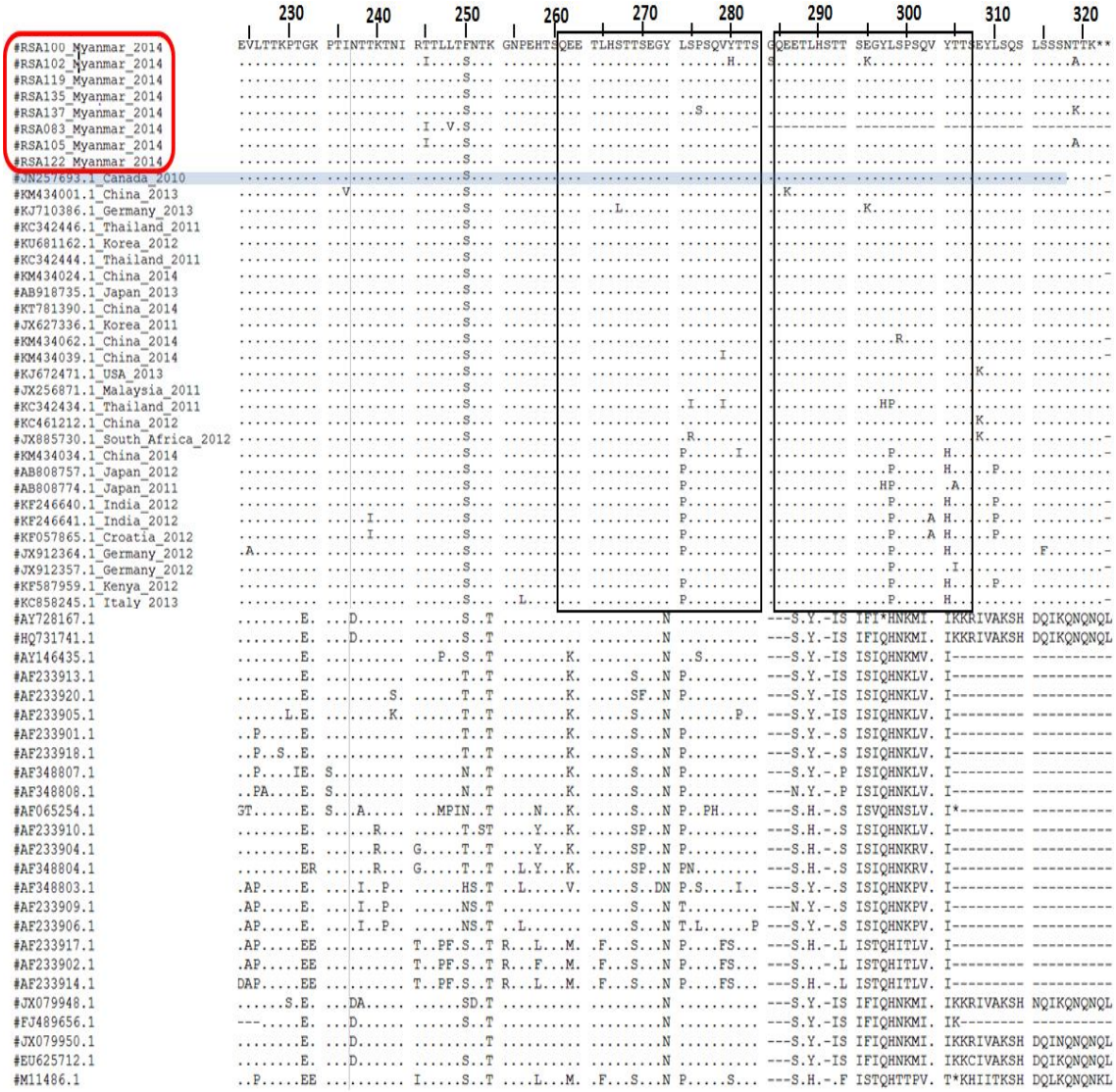

Fig. 2. Alignments are shown relative to the sequence of ON1 strain first described in Canada, JN257693. Alignment of sequences was performed using the ClustalW 1.6 method in MEGA 6.06 software. The amino acid positions correspond to positions 224 to 323 of the G protein of the prototype strain A2. Identical residues are indicated by dots, asterisks indicate the position of stop codons. Boxes frame the 23 amino acid duplicated region of the 24 amino acid insertion. Myanmar RSVA-ON1 strains were highlighted in red box.

DISCUSSION

RSV is the major pathogen of lower respiratory tract infections in infants and young children worldwide.[24] The prevalence of RSV among under five children of ARI cases in YCH, confirmed by IFA, in 2012 is 39%.[25] In the study done during January to September 2014, RSV infection was detected in 16.25% (26/160) of under five children with ARI at YCH. It was found that 52% (11/21) of RSV infection belonged to Group RSV-A and 48% (10/21) to Group RSV-B as done with NS1 gene.[22] In the present study, after sequencing of G gene of 21 RSV-positive samples, RSV-A was detected in 53.8% (7/13), RSV-B in 38.5% (5/13) and one case (7.7%) was mixed infection.

After nucleotide alignment and phylogenetic analysis, all Myanmar strains (61.5%, 8/13) of the group A clustered under the ON1 genotype which is characterized by a 72 nucleotide duplicated genetic region. Similar finding has also been reported in some countries in Asia such as China (KM434024), Korea (KU724061), Japan (AB918735), Thailand, (KC342444) and India (KF246641), in Europe such as Germany (JX912364), Italy (KC858245) and Spain (KF915266).

Most of the RSV-infected patients were younger than 6 months. This finding is similar to China report.[26] The diagnosis in the majority patients was bronchiolitis followed by pneumonia.[24] One study reported that ON 1 genotype was associated with less severe cases such as bronchiolitis. However, some countries, but not all, reported that the ON1 may be associated with greater clinical severity of ARI infection. It is unable to report any such clinical association in Myanmar because of the limited number and time period of the study done so far. More studies are needed.

Detection of ON1 strains in the present study is a first time report in Myanmar. Worldwide, the new ON1 strains has replaced all other strains in RSV-A group and is significant because this may indicate a greater resistance to host immune mechanism as reported from Germany, etc.[24] Although it cannot be definitely said that there is replacement of ON1 strains in RSV-A groups in Myanmar because DNA sequencing of RSV has never been done, the finding that ON1 strains are the prevalent strains in Myanmar is in accordance with the current prevalent strains globally.

The finding of G284S substitution in ON1 genotype of RSV-A102 in Myanmar is unique, it has not been reported from any other country so far. However, Heidelberg strain from Germany is also shown to have a unique substitution at E287K, which has not been reported elsewhere. These unique substitutions are of scientific interest and may be scientifically significant. Regarding RSV-B strains, one strain of RSV-B in Myanmar has already changed to RSV-B/BA9 strain. In other countries, this has led to replacement of all RSV-B strains by RSV-B/BA9 and it need to be confirmed whether this will also happen in Myanmar.

The important finding in the present study is that a new novel strain of RSV-A/ON1 has been detected in RSV isolated from ARI cases in under 5 children at YCH and probable replacement of all RSV-A by ON1, and the existence of the second hypervariable regions in the G protein genes of ON1 suggests the likelihood that the RSV strains in Myanmar are likely to evolve and change rapidly from time to time. It may be difficult to develop a vaccine against these rapidly varying strains. The detection of unique amino acid replacement in one strain of RSV-A/ON1 is of scientific interest and it may need further in-depth study.

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Factors Related to Glycemic Control among Type 2 Diabetic Outpatients in North Okkalapa General Hospital

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Although a variety of factors influencing the glycemic control have been identified, little is known about these factors in Myanmar context. A cross-sectional study was carried out on type 2 diabetes patients attending the Diabetic Clinic at North Okkalapa General Hospital from January to May 2015. It was aimed to identify the demographic, anthropometric and clinical characteristics related to glycemic control. Personal interviews were conducted to collect data. Some data were obtained from patient records. The blood samples were also collected for measurement of HbA1C. Poor glycemic control was defined as HbA1C ≥7%. A total of 120 type 2 diabetes patients were included in the study. Of them, 76.7% were females. The mean age of participants was 56.69 (SD=9.83) years. The mean value of HbA1C was 8.26 (SD=1.92) and 66.7% had HbA1C ≥7%. In the multivariate analysis, disease duration more than 5 years, medication type consisting of insulin, and systolic blood pressure ≥140 mmHg were significant factors ($p<0.05$) related to poor glycemic control. Health care providers should pay special attention to type 2 diabetes patients with longer duration, elevated systolic blood pressure and those on insulin treatment.

Key words: Influencing factors, Glycemic control, Type 2 diabetes, HbA1C

INTRODUCTION

Diabetes mellitus (DM) is an important public health problem worldwide. In a recent national survey in Myanmar, overall prevalence of raised blood glucose (fasting glucose or 2-hour glucose) or currently on medication for diabetes was 10.5% with 9.1% in the men and 11.8% in the women.[1]

Maintaining good glycemic control is a goal for all patients with diabetes. Several large clinical trials have demonstrated that tight blood glucose control correlates with a reduction in the microvascular complications of diabetes.[2, 3] Glycemic control remains the major therapeutic objective for prevention of target organ damage and other complications arising from diabetes.[4] The primary target of glycemic control is glycosylated hemoglobin (HbA1C) and its desirable value is below 7%.[5] HbA1C is a gold standard in analysis of patient's status, and is essential to ensure the optimal care of diabetic patients.[6] A previous research has shown that the risk of microvascular complications could be reduced by 40 percent with each one percent reduction in HbA1C.[7]

Despite the evidence from large randomized controlled trials establishing the benefit of intensive diabetes management in reducing microvascular and macrovascular complications, high proportion of patients remain poorly controlled.[8, 9] In clinical

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practice, it is difficult to obtain an optimal glycemic control on the long-term basis as the reasons for poor glycemic control are complex.[10] A variety of factors which influence the glycemic control have been identified. They include age, sex, education, marital status, body mass index (BMI), smoking, diabetes duration and type of medications among others.[10-12] However, the results are not consistent and little is known about these factors in Myanmar context. Thus, the present study was conducted to identify the demographic, anthropometric and clinical characteristics related to poor glycemic control in patients with type 2 DM in Myanmar.

MATERIALS AND METHODS

A cross-sectional study was carried out on type 2 diabetes patients attending the Diabetic Clinic at North Okkalapa General Hospital in Yangon from January to May 2015. The inclusion criteria were: aged ≥35 years, duration of diabetes ≥1 year, minimum follow-up period of 6 months at study clinic, and the absence of serious complications (e.g. renal failure, heart failure). Face-to-face interviews were carried out by trained interviewers using pre-tested questionnaire. Some of the data were obtained from patient records. The following information was collected from each patient.

- Demographic information: age, sex, education, marital status, smoking, family history and duration of diabetes
- Anthropometrics data: body mass index (BMI), and waist circumference. Based on the BMI, subjects were grouped into different categories as recommended by the WHO: normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2). Waist circumference was measured in centimeters. It was considered as increased if more than 90 cm in males and 80 cm in females.
- Clinical measures: glycosylated hemoglobin (HbA1C), systolic and diastolic blood pressure, and medication type. HbA1C was measured by using NycoCard Reader II. Blood pressure (BP) was measured using a mercury sphygmomanometer at the sitting position after the 10-minute rest.
- Other data: self-monitoring blood glucose (SMBG) and exercise. SMBG was defined as monitoring blood glucose at least once per week. Exercise was defined as a minimum of 30 minutes of aerobic exercise (e.g. walking, jogging, bicycling) performed long enough to sweat at least twice a week.

Statistical analysis

Both univariate and multiple logistic regression analyses were used to find out the association between outcome variable (good *vs.* poor glycemic control) and exposure variables (age, sex, education, marital status, smoking, BMI, duration of disease, family history, SMBG, medication type, systolic BP, diastolic BP and exercise). HbA1C values less than 7% were regarded as good glycemic control and values ≥7% as poor glycemic control.[5]

Ethical consideration

This study was approved by the Ethics Review Committee, Department of Medical Research under letter No. 77/ Ethics 2014 on 25 November 2014. Written informed consent was obtained from participants after explaining the procedures involved.

RESULTS

Participant's characteristics

Of a total of 120 type 2 diabetes patients, 76.7% were females. Their mean age was 56.69±9.83 years (range, 35-77 years), 60.8% were married, and 48.3% had BMI within normal range (18.5 to 24.9). Median duration of diabetes was 5 years (range, 1-24 years). Family history of diabetes was reported by 31.7%.

Glycemic control

The mean value of HbA1C was 8.26±1.92 and 66.7% had HbA1C ≥7%. The results

Table 1. Univariate analysis of factors associated with poor glycemic control among type 2 diabetes patients

	Good control (n=40, HbA1C <7%)	Poor control (n=80, HbA1C ≥7%)	OR (95% Confidence interval)	p value
	No (%)	No (%)		
Age (year)				
35-54	17(42.5)	36(45.0)	1(ref.)	
≥55	23(57.5)	44(55.0)	0.9(0.39-2.10)	0.79
Sex				
Male	8(20.0)	20(25.0)	1(ref.)	
Female	32(80.0)	60(75.0)	0.75(0.30-1.89)	0.54
Education				
Primary	13(32.5)	22(27.4)	1(ref.)	
Secondary	23(57.5)	47(58.8)	1.21(0.52-2.82)	0.66
Higher	4(10.0)	11(13.8)	1.63(0.43-6.17)	0.48
Marital status				
Married	24(60.0)	49(61.2)	1(ref.)	
Single	4(10.0)	8(10.0)	0.98(0.27-3.58)	0.98
Widowed/Divorced	12(30.0)	23(28.8)	0.94(0.40-2.20)	0.88
Smoking				
No	34(85.0)	74(92.5)	1(ref.)	
Yes	6(15.0)	6(7.5)	0.46(0.14-1.53)	0.21
Family history				
No	29(72.5)	53(66.2)	1(ref.)	
Yes	11(27.5)	27(33.8)	1.34(0.55-3.44)	0.49
Duration of diabetes (year)				
≤5 years	26(66.6)	36(46.7)	1(ref.)	
>5 years	13(33.3)	41(53.2)	2.28(1.15-5.56)	0.04
Medication type				
Oral monotherapy	17(42.5)	17(21.25)	1(ref.)	
Oral combined	19(47.5)	38(47.5)	2(0.84- 4.77)	0.12
Insulin alone	1(2.5)	12(15.0)	12(1.4-22.8)	0.02
Insulin and oral drugs	3(7.5)	13(16.25)	4.33(1.04-18)	0.04
SMBG				
Yes	32(80.0)	68(85.0)	1(ref.)	
No	8(20.0)	12(15.0)	0.71(0.26-1.89)	0.49
Systolic Blood Pressure (mmHg)				
<140	35(89.7)	54(69.2)	1(ref.)	
≥140	4(10.3)	24(30.8)	3.89(1.18-16.57)	0.01
Diastolic Blood Pressure (mmHg)				
<90	37(94.9)	64(82.0)	1(ref.)	
≥90	2(5.1)	14(17.9)	4.05(0.85-18.28)	0.057
BMI				
<25 (normal)	20(55.6)	38(50.7)	1(ref.)	
25-29.9 (overweight)	9(25.0)	24(32.0)	1.4(0.55-3.59)	0.48
≥30 (obese)	7(19.4)	13(17.3)	0.98(0.34-2.84)	0.97
Waist circumference				
Normal	7(17.5)	18(22.5)	1(ref.)	
Increased	33(82.5)	62(77.5)	0.73(0.28-1.93)	0.53
Exercise				
Yes	17(42.5)	38(47.5)	1(ref.)	
No	23(57.5)	42(52.5)	0.82(0.38-1.76)	0.61

SMBG=Self-monitoring blood glucose
BMI=Body mass index

obtained from univariate analysis are shown in Table 1. It shows that duration of diabetes more than 5 years (p=0.04), medication type consisting of either insulin alone (p=0.02) or combination with oral drugs (p=0.04), and systolic blood pressure ≥140 mmHg (p=0.01) were factors significantly related to poor glycemic control in patients with type 2 DM in this study. Increased diastolic blood pressure (≥90 mmHg) was also found to have marginally significant association with poorly controlled diabetes (p=0.057).

Table 2. Multivariate analysis of factors associated with poorly controlled diabetes among type 2 diabetes patients (Reduced model)

Variable	OR (95%Confidence Interval)	p value
Age (year)		
35-54	1	
≥55	0.45(0.16-1.29)	0.14
Duration of diabetes (year)		
≤5	1	
>5	3.33(1.18-9.38)	0.02
Medication type		
Oral monotherapy	1	
Oral combined	1.98(0.69-5.73)	0.2
Insulin	11.42(1.78-17.1)	0.01
Insulin and oral drugs	4.79(0.81-18.48)	0.08
Systolic BP (mmHg)		
<140	1	
≥140	4.43(1.48-19.9)	0.01
BMI		
<25	1	
25-29.9	0.67(0.21-2.07)	0.49
≥30	0.63(0.18-2.25)	0.48
Exercise		
Yes	1	
No	0.71(0.27-1.87)	0.48

Multivariate analysis of factors associated with poorly controlled diabetes

Reduced model was used with variables significant at 0.05 by univariate analysis and some clinically important confounding variables (age, BMI, physical activity). In the multivariate analysis, longer duration of diabetes (>5 years *vs.* ≤5 years) (OR=3.33, p=0.02), medication type consisting of insulin (OR=11.42, p=0.01), and elevated systolic blood pressure (OR=4.43, p=0.01) were significantly associated with poorly controlled diabetes (Table 2).

DISCUSSION

The proportion of patients with poor glycemic control was high (66.7%) which was more or less comparable to the reports from other countries; 67% in Thailand, 65% in Jordan and 66.7% in Kuwait.[13-15] In the present study, good glycemic control was defined as HbA1C level <7% following the guidelines by the America Diabetes Association (2011).[5] However, the American Association of Clinical Endocrinologists/ American College of Endocrinology (AACE/ACE) Guidelines (2015) recommended a goal HbA1C level ≤6.5%.[16] The more strict goal of 6.5% would have resulted in a higher prevalence of poor glycemic control.

Because of the progressive decline of β-cell function with time, longer duration of diabetes was known to be associated with poor control. Most patients in the long run require a combination therapy for controlling their blood glucose. The UK Prospective Diabetes Study (UKPDS) Group revealed that HbA1C level in both conventional and intensive groups decreased in the first study year but subsequently increased with each following year.[3] The result was consistent with the UKPDS which reported that the longer a patient has DM, the poorer the glycemic control will be. It was also in agreement with that reported by other studies.[14, 17, 18]

Medication type consisting of insulin, alone or in combination with oral hypoglycemic agents (OHAs), was found to be related to poor glycemic control in the study. It was in agreement with that reported by Harris and co-workers.[19] Poor glycemic control is most common among insulin-treated patients because the majority of them are patients with secondary failure of OHAs or patients presenting with chronic complications of DM. As they have more aggressive disease, they require more aggressive treatment to get their blood glucose controlled.[17] Patients with high systolic BP were found to have uncontrolled diabetes in this study. Basit and co-workers also reported the significant association of hypertension with poor glycemic control in type 2 diabetic patients.[20] Hypertension may be due to renal damage as a result of poorly controlled diabetes. However, it was also possible that the increased blood pressure might be a direct consequence of poor glycemic control very early in diabetes contributing to the onset of the renal damage.[21]

An association between age and poor glycemic control was not observed in this study which is contrary to the reports that

younger age was related to poor glycemic control.[22-24] Regarding BMI, Nichols and co-workers found that lower BMI was the strongest and most consistent factor related to poor glycemic control.[23] It was consistent with UKPDS in which the intensive group gained weight (2-5 kg) compared to the conventional group.[3] In the present study, BMI as well as waist circumference did not turn out to be related to glycemic control. Harris and co-workers also reported that BMI was not related to glycemic control, but waist circumference emerged as a determinant of poor glycemic control in a study by Ghazanfari, *et al.*[9, 19]

This study explored the factors associated with poor glycemic control in Myanmar context. However, causal relationship between the independent variables and outcome cannot be established as it was a cross-sectional study. At the same time, information on physical activity and blood glucose self-monitoring were obtained by self-report and thus subjected to recall bias.

Conclusion

Significant factors related to poor glycemic control in patients with type 2 DM were longer duration of diabetes, medication type consisting of insulin and elevated systolic blood pressure. It is recommended that health care providers should pay special attention to type 2 diabetes patients with longer duration, elevated systolic blood pressure and those on insulin treatment.

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Myanmar Health Sciences Research Journal, Vol. 30, No. 2, 2018

SHORT REPORT

Prevalence of Sibling Species Complex of *Anopheles minimus* in Pyin Oo Lwin Township, Mandalay Region and Kamamaung Township, Kayin State

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Key words: *Anopheles minimus*, Identification, Polytene chromosome, sibling species, sporozoite, Vector

The *Anopheles* mosquitoes breeding sites are very greatly depending on the climatic conditions and availability of water. In Myanmar, *An. minimus* and *An. dirus* are considered to be one of the most efficient malaria vectors. Previously, *An. minimus* was found in foothill and forest fringe but now found in rice field in plain areas. *An. dirus* is bred in deep forest areas but now their larvae were found in water wells in Mon State and Thaninthayi Region,[1] because of the certain environmental changes like deforestation and vegetation clearance for crop plantations. All these main vectors are species complex and morphologically indistinguishable, known as sibling species. Salivary gland polytene chromosome can be dissected from *An. dirus* only, although ovarian nurse cell chromosome can be dissected from other Anophelines species in different areas. About 23 *Anopheles* taxa have been identified so far as species complex.[2]

The study assessed the prevalence of sibling species complex of *An. minimus* using ovarian nurse cell polytene chromosome and determined the potential vector using ELISA methods in Pyin Oo Lwin Township, Mandalay Region and Kamamaung Township, Kayin State from October 2014 to September, 2015.

Anopheles mosquitoes were collected by animal bait K-net, human bait indoor and outdoor and light trap collection. Blood fed *An. minimus* were separated out by the help of sucking tube to paper cup with glucose for ovary development. Humidity and temperature were maintained by covering with water soak towel (27ºC, RH 90%). Christophe stage ovary specimens were dissected and preserved in Cornoy's fixative in screw type bottle. Samples were stored in 4ºC in refrigerator. Preserved ovaries of *An. minimus* were processed in 50% propionic acid and were stained with 2% lacto-acetoorcein stain according to Green and Hunt[3] for making polytene chromosome preparations. The complements of chromosome of individual mosquitoes were examined under 40x and 100x magnification lens of compound light Olympus microscope. Species diagnostic inversions were used for identification of the members of *An. minimus* complex. Head and thorax of mosquitoes were dissected to find out *Plasmodium* sporozoites in salivary gland followed by ELISA test for conforming potential vectors.

A total of 1153 Anopheles mosquitoes comprising 6 mosquito species were collected from Phonedun Village, Pyin Oo Lwin Township. Among them, the highest number (553) of *An. minimus* were collected. *Anopheles* mosquitoes 953 (524 from Katinehtit and 429 from Kinetaw) comprising 9 species were collected from Kamamaung Township. Among them,

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93 and 92 *An. minimus* were collected from Katinehtit and Kinetaw villages, respectively. All collected *An. minimus* were observed sibling species A in both areas. The ovarian nurse cell's polytene chromosomes of *An. minimus* exist as 5 arms. They were the telocentric X chromosome, the sub-metacentric chromosome 2 and the metacentric chromosome 3. The X chromosome is easily recognized by its length and shuttle-shaped zone 6. The most important characteristics are that each autosome arm has one to three big puffs.

Table 1. Identification of species complex of *An. minimus* in two different townships using ovarian nurse cell polytene chromosome

Collected mosquitoes	P	K		Total mosquitoes (sporozoite positive rate, Spp A) (%)	
	Phonedun village	Katinehtit village	Kinetaw village		
	Spp complex & sporozoite positive rate (Spp A) (%)				
An. minimus sporozoite	0/553 (0)	1/93 (1.075)		0/92 (0)	738 (0.136)

P=Pyin Oo Lwin Township, Mandalay Region
K=Kamamaung Township, Kayin State
Spp=Species

Among the 46 zones, 7A, B and 19C in 2R which is the longest arm in the complement, 28A and 20A, B in 2L, 30A, B and 37D in 3R and 46D and 38A, B in 3L are considered as characteristic zones. The ovarian nurse cell polytene chromosome of *An. minimus* collected from Pyin Oo Lwin and Kamamaung townships were found species complex A. Higher numbers of *An. minimus* were observed in animal bait collection than human bait in both townships. A two-year study in west Thailand (Kanchanaburi Province) found that higher number of 3,808(81.8%) *An. minimus* species C and A were captured on cattle collection.[4] *Plasmodium falciparum* sporozoite protein was found in one *An. minimus* A in Kamamaung Township (0.54%, 1/185), and it was not found in Pyin Oo Lwin Township (0/553) by circumsporozoite ELISA test.

In the present study, sibling species A of *An. minimus* complex was found in all studied areas in Myanmar although sporozoite positivity rate was high in Kayin State. Therefore, *An. minimus* A is a main vector of malaria in Kayin State areas.

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SHORT REPORT

Growth of Weaning Laboratory Rats (Wistar strain) using Different Formulated Diets

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Key words: Rats, Growth, Diet, Formulation

Laboratory rats originate from the wild brown rats (*Rattus norvegicus*). There are more than 70 species of the genus *Rattus* (family *Muridiae*). Males gain weight more quickly and became larger than females of the same strain.[1, 2] The main components of food for animals are water and dry matter.[3] Laboratory animals should be fed nutritionally adequate food without any contamination daily. Nutrition is an important environmental factor which can have profound effect on the quality of experimental results. Protein deficiency may cause results in poor growth, poor reproductive and lactation performance, hypoproteinemia and anemia, edema, and muscle wasting. Young rats fed diets deficient in a single amino acid had poor hair coats with areas of alopecia, weakness, lethargy and weight lost. The animal feed should contain moisture, crude fiber, crude protein, essential vitamins minerals, crude fat and carbohydrate for providing appropriate nutrition. The percentage of water in pelleted diet at normal room temperature should be between 7 and 12 percent.[4] There are many brands of ready-made feed and formulations reported in the literature which have been found to support and provide for growth. However, these formulations are either too expensive or are not practical to use in Myanmar.[5]

It is necessary to formulate an adequate ratio of locally available feed ingredients to ensure rapid growth, prevent nutritional diseases, develop and maintain resistance to infection and provide for an attractive and palatable product to adult stages. Therefore, this study was conducted in weaning stage of laboratory rats with different formulated feeds of an adequate ratio of locally available feed ingredients.

The main objective of this study was to provide the lab animals with optimum nutritional value in order to get faster growth in the shortest time with the least cost in nursery periods, and to find the effect of five formulated animal feeds on growth changes on albino rats from weaning stage to adult stage.

This laboratory-based experimental study was done in Laboratory Animal Services Division, DMR (POLB) and nutrients analysis of formulated diets was done in Food Laboratory, UMFCCI, Yangon. Myanmar. The contents of moisture, ash, crude protein and crude fiber were analyzed by AOAC-2000. Healthy weaning albino rats of 25-28 days old (thirty males and twenty females) were selected and divided into 5 groups; each group contained 10 number of rats (6 males and 4 females). Males and females were kept in separated polypropylene cages with wood shavings bedding materials. Their initial body weights were measured by the balance (Ohaus Corporation, Pine Brook,

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NJ USA). Animals were kept at 23-25°C room temperature. Cleaned cages, covers and water bottles were provided throughout this study. Each formulated diet was done by pellet machine and then these pellets were dried with big dry oven at 121°C for 30 minutes. Then, each formulated diet and clean water were provided *ad libitum* to each animal group daily. Formulated diet (32 grams) was fed to each experiment rat as daily con-sumption. The feeding trial was begun at weaning stage and ended at the onset of sexual maturity. Weight gain status by using balance weekly was recorded up to another 45 days.

Table 1. Content analysis of ingredients in different diets

Ingredients	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)	Diet 4 (%)	Diet 5 (%)
Dry fish meal	15.0	15.0	15.0	15.0	15.0
Rice powder	12.5	12.5	12.5	12.5	12.5
Rice bran	15.0	15.0	15.0	15.0	15.0
Peanut cake meal	12.5	12.5	12.5	12.5	15.0
Corn meal	15.0	15.0	15.0	15.0	15.0
Wheat bran	15.0	15.0	15.0	15.0	15.0
Variety of bean & pea meal	12.5	-	-	12.5	12.5
Niger cake meal	2.5	2.5	2.5	-	-
Soy bean meal	-	12.5	-	-	-
Shan soy bean meal	-	-	12.5	-	-
Sesame cake meal	-	-	-	2.5	-
Crude protein	12.68	16.15	14.77	14.97	12.40
Ether extract (Crude fat)	2.30	3.51	2.53	2.53	2.66
Crude fiber	3.94	4.00	5.71	4.73	5.47
Moisture	32.32	26.98	26.09	23.07	18.13
Ash	6.80	7.08	7.47	7.84	7.89
carbohydrate	41.90	42.28	43.43	46.86	41.45
Energy value (kcal/100g)	238	268	259	275	287
Moisture (Bc) Pol	4	2	2	2	2
Ash (Bc) Pol	7	8	8	7	7

In this study, it was found that the nutrient values were different from batch to batch due to the different geographical areas of the country from where they were cultivated, fertilizer used and ways of cultivation. Depending on the five kinds of formulated diet, body weights gained were different: female 81.5 gm and male 112.9 gm for diet-one, female 98.5 gm and male 147.4 gm for diet-two, female 97.9 gm and male 115.8 gm for diet-three, female 102.1 and male 104.3 gm for diet-four, female 97.3 gm and male 102.2 gm for diet-five. Among them, the best growing weight gain of Wister strain rats was received in diet-two. Formulated diet-three and diet-four are better than diet-one and -five.

In this study, group-two got the highest growth and weight due to the higher crude protein content of formulated feed although five different types of feed were acceptable for weaning stage of laboratory rat and good for growth. So, weaning rats of the group-two received the most suitable supplemental feed in their nursery period. During the study, analyscs of formulated diet-one, diet-two, diet-three, diet-four and diet-five were carried out to confirm the actual nutrient composition of formulated diet. It was found that they were not much different from those of calculated values.

In addition, sesame cake meal and Niger cake meal used in this study contained rich source of fat, vitamin and other nutrients that are essential for reproduction and growth of the offspring. There was no significance in body lengths of weaning stage of rats in all groups. The costs of five formulated diets were not much different significantly. It could be concluded that the better growth was achieved in weaning laboratory rats, feeding with diet-two which contains higher protein and quality when compared to other diets.

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SHORT REPORT

Establishment of In-house Production of Phytohaemagglutinin (PHA) Reagents for Detection of Chromosomal Disorders

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Key words: Phytohaemagglutinin, Karyotyping, Chromosome, Mitogenic activity

Phytohaemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolus vulgaris*) (ဖြဴထောကိုပဲ), contains potent cell agglutinating and mitogenic activities. It possesses the ability to stimulate lymphocytes to undergo mitosis.[1] Lectins are carbohydrate-binding glycoproteins that can react specifically with human blood cells, preferentially agglutinate malignant cells, and undergo mitogenic stimulation of lymphocytes.[2] Lectin extraction is usually achieved using different methods of diffusion in aqueous solution and ammonium sulfate precipitation.[3] PHA-induced blastogenic response is a potentially useful assay for the detection of immunocompromized persons especially in patients suffering from squamous cell carcinoma.[4] Lymphocytes, cultured with PHA, can be used for karyotype analysis. PHA is one of the main reagents for karyotyping analysis of chromosomal diseases. It has enhancing activity of cellular mitosis. This study assessed the quality of in-house PHA extract from red kidney beans for karyotyping to analyze chromosomal diseases.

After getting approval from the Ethics Review Committee of Department of Medical Research, this laboratory-based study was carried out from January to October, 2016. Red kidney beans were collected from the market and extracted in-house PHA reagent from this bean by homogenate method. Red kidney beans (20 g) were washed with sterile water and softened in 400 ml 0.15 M NaCl for 24 hours at 4˚C, then homogenized in a blender. The homogenate was diffused for another 24 hours at 4˚C, then filtered with Whatman paper 1. The filtrate was centrifuged at 9168×g for 30 minutes, and the supernatant was fractionally precipitated with ammonium sulfate at 40%, 50%, 60%, and 70% saturation, respectively. The four pellets were combined, dissolved in 1 ml of water, and dialyzed against distilled water at 4˚C.[3]

The protein concentration of the extracted PHA reagent from red kidney beans was 180 mg/ml by Bradford's method in this study. This mitotic activity was found in these reagents by using one in ten dilutions of protein concentration of extracted in-house reagents. The protein concentration of in-house PHA reagents was used in 18 mg/ml. The protein concentration of commercial PHA reagent was 10 mg/ml. In-house PHA reagents was stimulated the effect of mitotic activity that was same activity of commercial PHA reagents. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed for the standardization of in-house PHA extracted from red kidney beans by the method of Laemmli. This method used 15% separating and 5% stacking gel to confirm the effectiveness of the purification.[5] A single band of in-house PHA reagent

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appeared by SDS-PAGE in this study. Specific activity was expressed by hemagglutinating activity assay. Serial two-fold dilutions of the lectin solution in microtiter v-plates (25 µl) were mixed with 25 µl 2% chicken red blood cell suspension in saline (pH 7.2). Readings were recorded after 30 minutes at room temperature, when the blank had fully sedimented. The hemagglutination titer was defined as the reciprocal of the highest dilution exhibiting hemagglutination.[3]

Mitogenic activity of the PHA (extracted from *Phaseolus vulgaris*) was compared with commercial PHA (SIGMA) using karyotyping method. After obtaining informed consent from ten normal healthy persons, 2 ml of venous blood from antecubital vein were collected into sterile heparinized tubes to determine comparative effect of in-house and commercial reagents. Ten normal healthy volunteers were cultured with both the in-house PHA reagent and commercial reagent to determine human chromosomes. The commercial PHA reagent was compared with in-house PHA reagents in these subjects.

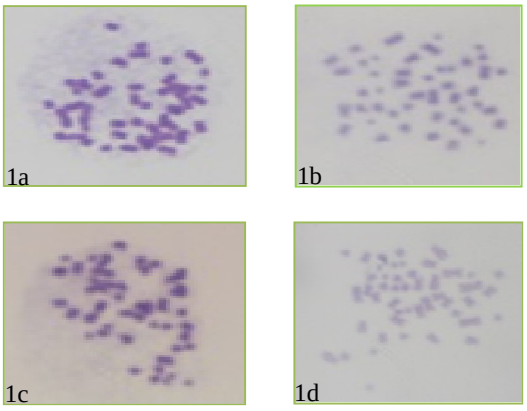

Fig. 1. Comparison between commercial PHA reagent (1a & 1b) and in-house PHA reagent (1c & 1d)

Mitogenic activity of the lectin was reasonably good compared with commercial PHA (extracted from *Phaseolus vulgaris*).[3] In this study, the number of human chromosomes was determined by using in-house PHA compared with commercial reagent (Fig. 1). This study found that the comparison between in-house PHA and commercial PHA reagent was good in mitogenic activity. The in-house PHA reagents have stimulation effect of individual cells (lymphocyte) for analysis of chromosomes (karyotyping) and it is useful for detection of autosomal defects in chromosomal disorders. PHA, a lectin extracted from red kidney bean, has been widely used as mitogen for chromosome study. In this study, preparation of indigenous in-house PHA reagent could be used for identification of chromosomal diseases. It is useful as commercial product, fresh, low cost and easily accessible. However, further investigation of in-house PHA reagent's properties should be performed.

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