MOLECULAR TECHNOLOGY APPLICATIONS DIVISION

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The Molecular Technology Applications Division was established under the Advanced Molecular Research Centre in May 2015. The division is involved in research on communicable diseases; mainly on tuberculosis, viral hepatitis and malaria. The main research areas were establishment of molecular techniques to detect asymptomatic/ silent infections, genotyping and detection of gene mutations conferring drug resistance as well as applications of these methods for diagnosis, characterization and molecular epidemiology. The division is also responsible for maintenance of laboratory equipment and Biosafety level 2 plus laboratory.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1. TUBERCULOSIS

1.1.1. Cutaneous TB; Different clinical types and efficacies of diagnostic tests

Cutaneous tuberculosis (TB) can present with a wide range of clinical presentations. The efficacies of different diagnostic tests are varying and there is no perfect tool. In this study, skin biopsy specimens were collected from 25 clinically diagnosed cutaneous TB cases attending Dermatology Ward, Yangon General Hospital during 2014-15 and investigated for cutaneous TB by tuberculin test, smear for acid fast bacilli (AFB), histopathologic

examination, TB culture and polymerase chain reaction (PCR).The diagnostic test results were compared according to the types of cutaneous TB. Age range of the cases was 8-77 years, of which 7 were male and 18 were female. Among different clinical types recorded, lupus vulgaris was the most prevalent, (13/25, 52%), followed by tuberculosis verrucosa cutis (8/25, 32%) and the least was papulonecrotic tuberculid (4/25, 16%). When comparing the positivity of different diagnostic tests, PCR was positive in (13/25, 52%) of cases, tuberculin test was positive in (10/25, 40%). There was neither culture positive nor AFB smear positive cases. Histopathology results showed 7 cases as chronic granulomatous, 6 cases as lupus vulgaris, 1 case as tuberculid and 11 as non-specific. All PCR positive cases were chronic granulomatous and lupus vulgaris cases by histopathology examination. Among 13 PCR positive cases, 7 cases were clinically diagnosed as lupus vulgaris, 5 cases were tuberculosis verrucosa cutis and 1 was papulonecrotic tuberculid. These results showed that thorough clinical examination is still essential for the diagnosis and treatment of cutaneous TB and *Mycobacterium tuberculosis* DNA detection by PCR test can be used as a rapid diagnostic tool and confirmation test.

1.1.2 Molecular typing of *Mycobacterium tuberculosis* strains from Yangon and Mandalay Regions, Myanmar

Determining the genetic diversity of *Mycobacterium tuberculosis* (MTB) strains allows identification of the distinct MTB genotypes in different regions and also provides an invaluable tool for the study of epidemiology of TB. Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing is a fast and promising method to discriminate MTB strains in many countries. The present study was carried out to determine the genetic diversity and prevalent genotype of MTB strains isolated from pulmonary tuberculosis patients in Myanmar. A total of 210 clinical MTB strains isolates from Yangon (n=117) and Mandalay Regions (n=93) during 2012-2015 were studied. The isolates included 129 multi-drug resistant (MDR), 4 any drug resistance other than MDR and 77 susceptible strains. PCR-based typing method described by Warren *et al* 2004 was used to identify Beijing and Non-Beijing strains. Internationally standardized 15-loci and 24 loci MIRU-VNTR typing were applied for genotyping and the results were analyzed by the MIRU-VNTR plus web application. The Hunter-Gaston discriminatory index (HGDI) was used as numerical index to describe the discriminatory power. All tested 210 MTB isolates showed unique patterns and did not clustered. The combined result of two typing methods showed the genotype distributed to six lineages; Beijing (147/210, 70%), East-African-Indian (10/210, 4.8%), Uganda 1 (2/210, 0.95%), H37Rv, LAM, CAS/Delhi and NEW1 (1/210, 0.48% each) and unknown strains (47/210, 22.38%). Twelve strains showed both Beijing and non-Beijing characters and comprised as suspected co-infected cases. Out of 147 Beijing strains (75 from Yangon and 72 from Mandalay), 77.55% (n=114) were MDR. HGDI of 15 and 24 loci MIRU-VNTR were 0.9798 and 0.986, respectively. The most prevalent genotype in both Yangon and Mandalay Regions was Beijing genotype which was found to be significantly associated with MDR-TB ($P<0.0001$). As both 15 and 24 loci MIRU-VNTR genotyping showed similar high discriminatory power, 15 loci method could be more preferable in terms of cost and labour efficiency.

1.1.3 Detection of gene mutations conferring resistance to pyrazinamide and second line anti TB drugs in *Mycobacterium tuberculosis* strains in Myanmar

Myanmar is a high tuberculosis (TB) burden country with high rates of multi-drug resistant TB (MDR-TB). MDR-TB does not respond to first line anti-TB drugs and has to be treated with second line anti-TB drugs (SLDs) which are less effective and more toxic.

Extensively drug resistant TB (XDR-TB), which is caused by MDR-TB strains that are also resistant to at least one of fluoroquinolones (FQ) and any of the injectable SLDs, amikacin (AMK), kanamycin (KM) and capreomycin (CAP), is associated with poor treatment outcomes. Pyrazinamide (PZA) is an important drug for the treatment of both MDR and XDR-TB. Mutations in several genetic loci have been implicated in the development of resistance to PZA and SLDs. Compared to conventional liquid medium-based drug susceptibility testing, which still takes about 4 to 21 days, the detection of genetic variants which mediate resistance to certain antimicrobial agents represents a more rapid alternative. The objectives of this study were to establish DNA sequencing method to detect gene mutations conferring PZA and SLDs resistance in Myanmar and to apply direct sequencing method for identification of gene mutations for PZA (pncA), FQs (gyrA and gyrB) and KM/AMK and CAP (rrs,eis) resistance in MDR Mycobacterium tuberculosis (MTB) isolates. Mycobacterial DNA extraction, amplification of resistant determining regions of specific genes, PCR purification, cycle sequencing and conventional DNA sequencing (capillary electrophoresis using ABI 3500 analyser, Applied Biosystems) method was established at Advanced Molecular Research Centre, Department of Medical Research during 2015. Liquid drug susceptibility testing using MGIT 960 automatic system was performed as standard phenotypic PZA and SLDs assay. Of initially tested 30 MDR MTB isolates, mutations in pncA gene were found in 5 PZA resistant strains. Most of FQ resistant mutations were found in codon 94 of gyrA gene and AMK resi*stant* strains carriedmutations in A1401G in *rrs*. There were 3 pre-XDR and 1 XDR-TB+PZA resistant cases among tested strains. The rapid detection of PZA and SLDs resistance prior to and during treatment is important for treatment success, early detection of XDR and implementation of increased infection control measures to prevent further transmission.

1.2. VIRAL HEPATITIS

1.2.1. Hepatitis B infection

1.2.1.1. Detection of hepatitis B virus X and core promoter mutations and gene expression study on Myanmar patients with HBV induced hepatocellular carcinoma

(Please refer to Annual Report of Experimental Medicine Research Division)

1.2.1.2 Molecular characterization of viral hepatitis in Myanmar

(Please refer to Annual Report of Experimental Medicine Research Division)

1.3. MALARIA

1.3.1. Drug Resistant Malaria

1.3.1.1 Molecular assessment of K13 (Pf3D7_1343700) in uncomplicated falciparum malaria among children in Minbu district

Efficacy and safety of artemether-lumefantrine combination in under five years was assessed between 2014-2015. A total of 115 uncomplicated falciparum children were recruited and follow-up until 28 days according to the WHO standard protocol. There was no treatment failure clinically. Among these samples, 64 out of 115 samples have been selected after excluding low level parasitaemia. Artemisinin resistance molecular marker, Kelch 13 (Pf3D7_1343700) was amplified and sequences were analysed. All isolates showed the wild type indicating that local transmitted strain in Minbu region might be artemisinin sensitive strain based on the clinical and molecular findings.

1.3.2. Field Research on Malaria

1.3.2.1 Sero-epidemiology and Malaria Antibody Kinetic Profile in Tier I area of Myanmar Artemisinin Resistance Containment (MARC) Zone

(Please refer to Annual Report of Parasitology Research Division)

1.3.2.2 Molecular detection of asymptomatic malaria infection in Shwe Kyin Township

Among the samples collected in longitudinal cohort study, molecular detection of asymptomatic infection was carried out using cost effective and high throughout pooling strategy in which combined 10 samples in each pool before DNA extraction, was used. Nested polymerase chain reaction (PCR) based molecular detection on genus specific small subunit ribosomal RNA gene was used to exclude the *Plasmodium* infection. Individual DNA extractions followed by genus and species specific PCR were done for all positive pools. Most of the asymptomatic cases were vivax and no ovale infection was detected. Detail was shown in Table. These findings highlighted the essential role of the molecular based detection method to assess the cryptic asymptomatic infection and need of action for these hidden parasitaemic cases in the residence where artemisinin resistance containment measures have been conducting.

Table. Summary of asymptomatic infection in collected samples

Collection (2015)	Total no. of samples	Total no. of positive pool	Falciparum	Vivax	Malariae
January	1004	22	3	22	2
April	920	6	1	5	0
July	960	11	0	11	0
October	905	7	0	7	0
Total	3789	46	4	45	2

2. NON COMMUNCABLE DISEASES

2.1. CANCER

2.1.1 Studies of epigenetic changes in circulating tumor cell in hepatocellular and nasopharyngeal carcinoma

(Please refer to Annual Report of Experimental Medicine Research Division)

1. ACADEMIC AND TECHNOLOGY DEVELOPMENT

3.1 Detection of hepatitis B virus DNA by real-time PCR using SYBR green dye technology

Hepatitis B virus (HBV) infection is a major cause of chronic liver diseases worldwide. In addition to genotyping of the virus, quantitative analysis of HBV infection is extensively used for monitoring of disease progression and treatment. Affordable viral load monitoring is desirable in resource-limited setting. The aim of this study is to develop an in-house real-time PCR based method, which is sensitive and efficient, offering an alternative method to conventional PCR on HBV detection at the Advanced Molecular Research Centre, Department of Medical Research, Myanmar. Core gene encoded in HBV 1.2merplasmids was used as HBV-DNA standard. To detect HBV DNA, real-time PCR based on SYBR green

chemistry was carried out following proper primer designing and PCR optimization. The core gene contained in the plasmids and clinical specimen was quantitatively measured using with real-time PCR ABI 7500 (Applied Bio-systems). The detection limit of the assay for the HBV DNA was 14 copies per microliter. Linear standard curve was obtained between 10^{-2} and 10^{-7} DNA ng/µL. None of negative samples showed false positive reactions in duplicate. The coefficient of variation for both intra and inter experimental variability was carried out. The SYBR green based detection method for HBV viral load is reliable, accurate, and reproducible. In addition, the quantification of HBV DNA is useful to monitor the efficacy of anti-HBV therapy as well as to understand the natural history of HBV in the endemic country like Myanmar.

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1	Dr. Yi Yi Kyaw	Workshop on Research Methodology (2015)	Facilitator
		Training workshop on molecular and liquid drug susceptibility testing methods for detection of pyrazinamide and second line anti-TB drug resistance	
		1st year Diploma in Molecular Biology, DSMA	Teaching
		1st Year M.MedSc (Microbiology) (UM1)	External Examiner
2.	Dr. Myat Htut Nyunt	Training on Molecular surveillance on drug resistance malaria by Kangwon National University School of Medicine	Teaching Demonstration
3.	Daw Ohnmar Lwin	1st year Diploma in Molecular Biology, DSMA	Demonstration
		Training workshop on molecular and liquid drug susceptibility testing methods for detection of pyrazinamide and second line anti-TB drug resistance	
4.	Dr. Phyu Win Ei	1st year Diploma in Molecular Biology, DSMA	Demonstration
5.	Dr. Hnin Ohmar Soe	Training workshop on molecular and liquid drug susceptibility testing methods for detection of pyrazinamide and second line anti-TB drug resistance	Demonstration

Sr.	Name	Course	Responsibility
6.	Dr. Zayar Han	1st year Diploma in Molecular Biology, DSMA Training workshop on molecular and liquid drug susceptibility testing methods for detection of pyrazinamide and second line Anti-TB drug resistance	Teaching Demonstration

RESEARCH CAPACITY STRENGTHENING

1.1. Routine services of Biosafety and laboratory equipment maintenance

Routine services on Biosafety lab and regular equipment maintenance are carried out. During the reporting period, special works at AMRC are

- Setting up to use type I water(molecular grade) from water purifier
- Maintenance of UPS from current over load
- Repair the ice making machine and regular gas filling
- Checking of air-condition safeguards which were placed on the ceiling
- Change all filters for BSL2+ lab
- Servicing of air conditions

1.2 Maintenance Schedule

- Daily check of all laboratories equipment, lighting and air conditions
- Checklist of AHU (Air handling Unit) and EFU (Exhaust Fan Unit) twice a week
- Checklist of all laboratory equipment twice a month to prolong the life of instruments and to acquire precise data and results
- Change Pre-filter twice a month for AHU
- Change Medium filter 4 times a year for AHU

Change HEPA filter once a year for AHU