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AIMS OF THE JOURNAL

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

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EDITORIAL

The year 2015 is the great milestone of our Department of Medical Research (DMR) because DMR (Lower Myanmar) established in 1963 at Yangon and DMR (Upper Myanmar) established in 1999 at PyinOoLwin were combined to form as one Department under the Ministry of Health on 1st April, 2015. Accordingly, it is time to undertake research more "streamlined" with concerted effort of researchers ever than before symbolizing health sector reform wave of Myanmar.

At the 43rd Myanmar Health Research Congress held in January, 2015, the Editorial Committee performed the best possible mean for regular publication of MHSR Journal. The presenters were asked for respective research papers presented at the Congress for publication in the MHSR Journal after peer reviews. It made the researchers get the great chance to be more practical in disseminating their research findings to the public as soon as they had presented at the Congress. Among the total of 138 research papers presented, we received 61 papers for MHSR Journal.

For this issue, there are 13 long articles which are of wide range of findings through operation research, implementation research, health policy and system research targeting prioritized health problems. They are about Japanese Encephalitis (JE), high school students' attitudes to adolescent pregnancy, type 2 diabetes mellitus, children with acute diarrhoea/ dengue haemorrhagic fever, bioefficacy of bed-net treated with insecticide, bioequivalence study of generic and innovator metronidazole tablets in Myanmar healthy volunteers, PCR in diagnosis of tuberculous pleural effusion, bone mineral density in adult women, predictive model of birth weight using simple anthropometric measurements, injury due to road traffic accidents and knowledge and practice on MDR-TB among MDR-TB patients.

The leading article of this issue concerns about the Japanese Encephalitis (JE), a vector-borne zoonotic disease caused by the bite of *Culex* mosquitoes with nearly 68,000 clinical cases every year. It occurs in 24 countries in the WHO South-East Asia and Western Pacific regions that have endemic JE transmission, exposing more than 3 billion people to risks of infection. Although symptomatic JE is rare, the case-fatality rate among those with encephalitis can be as high as 30%. Permanent neurologic or psychiatric sequelae in 30%-50% of those with encephalitis can occur due to the infection. In fact, treatment is only focused only on relieving severe clinical signs and symptoms, supporting the patient to overcome the infection as there is no specific treatment for the disease.

Despite the WHO recommends safe and effective JE vaccination as the disease is a recognized public health problem, JE vaccination has been started in Rakhine State where many cases were found in 2015 in Myanmar. Therefore, the present study is emphasized on getting the baseline data through prevalence and genotypic studies in Myanmar. However, it is critical to have strong data of JE prevalence, natural immune status among the normal population, and mortality among the confirmed JE cases. In the near future, depending on these data, widespread implementation of JE vaccine through Expanded Program on Immunization will be carried out successfully.

Finally, we sincerely congratulate to all the scientists; both authors and co-authors for outstanding contributions of their research findings to our MHSR Journal.

Myanmar Health Sciences Research Journal, Vol. 27, No. 1, 2015

Isolation and Identification of Japanese Encephalitis Virus from Piglets in Thakayta Township, Yangon

Aung Zaw Latt[1], Khin Mar Aye[1], Hay Mar Win[1], Khaing Moe Aung[1], Khin Khin Oo[1], Hla Myo Thu[1], Mo Mo Win[1], Hlaing Myat Thu[1] & Sutee Yoksan[2]*

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Japanese encephalitis is a vector-borne zoonotic disease caused by the bite of *Culex* mosquitoes. Japanese encephalitis virus circulates among the wild birds, and transmitted to porcine, equine and human. In 2010, there were two reported cases of Japanese encephalitis infection in Thakayta Township, Yangon and the study was initiated within a month to get the JE virus isolate. A small pig farm was selected which was close (<2 km) to the home of JE cases. Blood samples were collected from five piglets in a selected pig farm, weekly for 14 times. JE virus was isolated in C6/36 mosquito cells, identified by Immunofluorescent Assay and Reverse Transcriptase-Polymerase Chain Reaction, and then sequenced by DNA sequencer. The isolate was found to be genotype I. In 2009, the first JE virus isolate in Myanmar was genotype III. This study was the second isolation of JE virus from piglets in Myanmar.

Key words: Japanese encephalitis, Virus isolation, Gene sequencing, Phylogenetic tree

INTRODUCTION

Japanese encephalitis virus (JEV) is the most important cause of epidemic encephalitis worldwide, with an estimated 35,000 to 50,000 cases and 10,000 deaths annually.[1] The case fatality rate of JE virus infections is approximately 25%, with 50% of survivors developing permanent neurological and psychiatric sequelae.[2] JEV is transmitted between vertebrate hosts by *Culex* mosquitoes, principally of the *Culex tritaeniorhynchus.*[3, 4] In Asia, pigs as well as birds are important natural hosts for Japanese encephalitis virus because these animals are often kept close to human dwellings, they serve as amplifying or bridging hosts that transmit the virus to humans.[5, 6]

It is an RNA virus of the genus *Flavivirus*, family *Flaviviridae.* It has a genome of 11 kb which encodes for three structural and seven nonstructural proteins. The envelope (E) protein is the most important among the structural proteins because it has neutralizing properties to the virus. According to phylogenetic analysis, there are five known genotypes of JEV worldwide according to the envelope (E) gene sequence.[7, 8]

In Myanmar, in 1982, a study on vector, amplifier, and human infection with JEV was done in a Yangon community (Dawbon Township). JEV infection was detected in 52.1% of pigs. The known JEV vector mosquito species, especially *Culex tritaeniorhynchus,* were found in the study area but no concurrent human JEV infections were elicited.[9] An investigation on JEV infection in Bogalay Township, Myanmar was done in 1999. Findings showed that JEV antibodies were detected in 33% of the pigs. They also found the Culex vector mosquitoes especially *C. vishnui* followed by *C. tritaeniorhynchus.*

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JE virus antibodies were not detected among the villagers during the study.[10] Non-epidemic investigation was done in Yangon, 1968. Two hundred and thirty-eight human sera were tested at Virus Research Centre in Poona, India. Sixteen percent of the sera had detectable neutralizing antibody against JE virus.[11] Attempts to isolate virus from the blood of patients with flavivirus encephalitis are usually unsuccessful because of transient viraemia and low titers. But, it is occasionally isolated from cerebrospinal fluid of patients who do not yet have antibody, particularly those who subsequently die,[12, 13] and from post-mortem brain tissue.[13- 15]

In the 2006 JE outbreak in China, isolation and sequencing of JE virus was attempted from CSF and mosquitoes. Eleven sequences were obtained and further analysis showed genotypes I and III.[16] JE virus was isolated from human and human brain in Chiang Mai (Northern Thailand) in 1964 and 1982, respectively. They were found to be genotype III. But in 1984 and 3-year survey (2003-2005) in pigs changes in JE virus genotype pattern showed genotype I.[17]

JE is a vaccine preventable disease and currently used vaccines are derived from JEV strain representative of genotype III. Clinical trials with genotype III vaccines have demonstrated effectiveness in areas where heterologous JEV genotypes cause human disease, supporting the hypothesis that immunity induced by genotype III vaccines protects against infection with JEV belonging to other genotypes.[18] In Myanmar, isolation of JE virus from pigs in DikeOo pig farm was done in 2009 and the isolate was identified as genotype III.[19]

MATERIALS AND METHODS

Study site

A small pig farm within a 2 km radius from houses of confirmed human cases of JE virus infection was targeted for sample collection (Fig. 1).

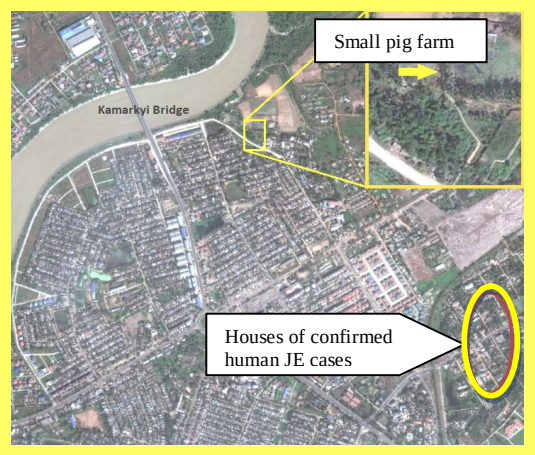

Fig. 1. A Google map showing the location of pig farm where the JEV was isolated, and houses of confirmed human JE cases

Sample collection

Blood samples were collected weekly from five piglets starting from 8 weeks of age to 22 weeks. Two to three milliliters of blood sample were collected from anterior vena cava of each piglet. A total of 70 blood samples were collected during 14 weeks.

Antibody testing by JE ELISA

Antibody was checked by Dengue-JE IgM Combo ELISA kit, Panbio Australia.

Virus isolation and identification

Each pig serum (50 µl) was diluted in 1,000 µl of serum free Modified Eagle's Medium, and 200 µl of diluted serum was inoculated onto 3 day old C6/36 mosquito cell line in a 25 cm^2 culture flask and incubated at 32ºC for 90 minutes. During incubation, culture flasks were kept on gentle rocking by a rocking machine. Then, 5 ml of MEM media was added to the flask and incubated in an incubator at 32ºC for 7 days. On day 7 of incubation, media was aspirated and one side of the flask was tapped gently to detach some cells.

Then, the loosened cells were fixed onto glass slide by using acetone for 10 minutes. Virus detection was carried out by indirect immunofluorescent staining using anti-flavivirus monoclonal antibody (4G2) and anti-JE monoclonal antibody as the first antibodies and, FITC conjugated goat anti-

mouse IgG as the second antibody. Then, the flasks were replenished with MEM 5 ml and incubation was continued, then the above steps were repeated for the next two consecutive weeks.

Gene sequencing

RNA extraction was done, by using QIA amp Viral RNA extraction kit (QIAGEN, Germany) according to the manufacturer's protocol, from a supernatant of JEV-positive cell culture, after the first passage, according to manufacturer's protocol as well as RNA reverse transcription-PCR (RT-PCR) [TPersonal (Biometra)]. RT-PCR was performed on 4 µl of cDNA template by using 2.5 units of Ampli-Taq Gold DNA Polymerase [RobusTT^{TM}II RT-PCR Kit (Finnzymes)]. Overlapping of JEV E gene fragments was amplified with 2 sets of primers: Ea forward primer, Ea reverse primer; and Eb forward primer and Eb reverse primer, respectively. The 1,500 nucleotides generated partial sequences of the JEV E gene that were compiled by using Sequence-Alignment Editor software version 5.0.9; pair-wise genetic distances were calculated with MEGA software version 4.0.

RESULTS AND DISCUSSION

After ELSA testing, two out of five serum samples collected at 14th week were JE antibody positive. The OD value of one sample was higher among the two. After virus isolation, one sample from 13th week showed cytopathic effect (CPE). This sample is also the one which was ELISA positive and with the higher OD value. After IFA testing, the sample that shown CPE was Flaviviral polyclonal antibody positive and dengue polyclonal antibody negative. After confirmation with JE monoclonal antibody and RT-PCR, the isolate was Japanese encephalitis virus.

After sequencing, the new Myanmar JEV sequence was analyzed with a group of 27 previously published JEV strain sequences which include 11 from Thailand, 5 from Japan, 6 from Indonesia, 2 from Malaysia, and one each from Korea, Australia and China. A phylogenetic tree was generated, and the new Myanmar JE virus isolate fits into the same GI cluster (Fig. 2).

This strain was associated with another subcluster (GIb) strain isolated in 2005 from Southern Thailand. But, eight Thai strains also isolated in 2005 formed a subcluster to Myanmar JE virus. In Myanmar 2009, the first JE virus sequenced was genotype III which is 99.58% identical with Beijing, China strain. Isolate was obtained from a pig farm about 70 miles from Yangon.[19]

But, in this (2012) study, the isolate was genotype I. In these two isolation studies, most of the techniques were similar except sample collection pattern. In 2009 study, the serum samples were randomly collected, but in this study more systematic collection was made because the chance of getting JEV isolation was very low. A study found that the viraemia to primary JEV infection lasted for one to three days, and was detected from day 2 to day 5 post infection.[20]

In Myanmar, there have been a lot of sero-prevalence studies regarding JE virus, but there is no genotypic identification study within the last three decades. The first isolation of JE virus from the brain of a dead horse was done in 1977 and the isolate was confirmed as JEV by the WHO reference center in Poona, India.[21] In this study, the isolate was genotype I. Regarding preventive measures, Japanese encephalitis disease is a vaccine preventable disease and currently used vaccines are derived from JEV strain representative of genotype III, and this vaccine is still effective against four genotypes (I, II, III and IV).[18]

To date, the Muar strain, which was the first isolated genotype V JEV from specimens of brain tissues of patients with viral encephalitis in Malaya in 1952,[22, 23] the second finding of genotype V was from *Cx. tritaeniorhynchus* collected in Tibet, China (2009),[24] and the third report of JEV genotype V was from Republic of Korea (2010).[25]

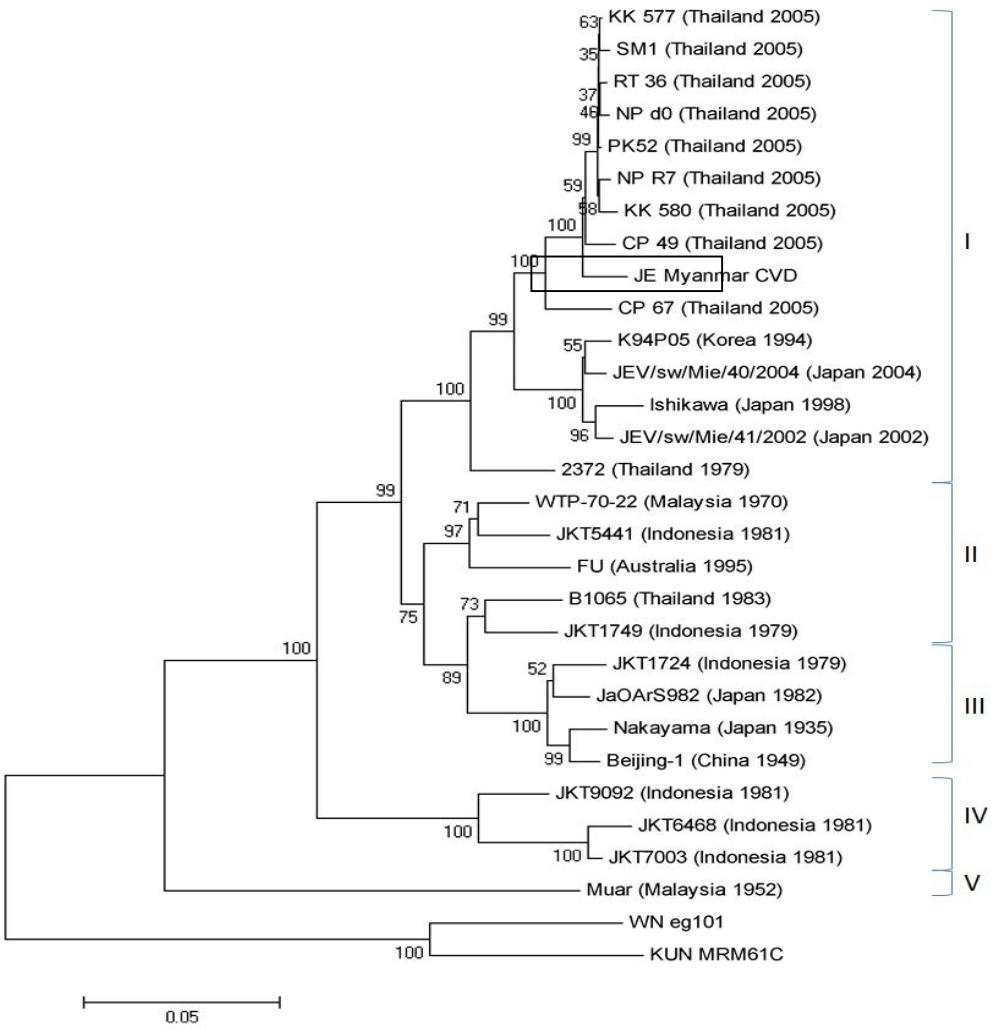

Fig. 2. Phylogenetic relationship of Japanese encephalitis viruses done by E gene (1,500 nt) analysis by MEGA version 4 software, using the neighbor-joining (p-distance) method. The length of the tree branches indicates the percentage of divergence; the percentage of successful bootstrap replicates is specified at the nodes (1,000 replicates). West Nile and Kunjin virus prototype sequences were included to root the tree.

Therefore, the genotype V is reemerged within the last three years, however, the protective efficacy of current vaccine against JEV genotype V has not been studied yet. In Myanmar, two strains of JE virus genotype I and III had been identified but it could not be concluded whether there is co-circulation or changes in genotype because of small sample size.

Conclusion

In Myanmar, there are cases of JE infection which are still reporting and, in 2008 there were 8 cases of JE ELISA confirmed patients in Yangon Children's Hospital. Among 8, 2 patients were expired, 1 suffered deafness and 1 had undergone blindness.[26] However, fever develops in only a small proportion (about 1:300) of those exposed.[27] On the other hand, the ratio of cases to undetected is 1:300.

Thus, in Myanmar, there must be a lot of undiagnosed cases and thousands of sub-clinical infections. In comparing to the dengue infection, the mortality rate is low but the neurological sequelae may be a burden to their family. Like other infectious diseases, vaccination is the most effective preventive measure against JE. In Thailand,

introduction of JE vaccine into children immunization started in 1990.[28] But, implementation of JE vaccine into Expanded Program on Immunization necessitates strong data of JE prevalence, natural immune status among the normal population, and mortality among the confirmed JE cases.

It was concluded that as long as there is no JE vaccination program in Myanmar, prevalence and genotypic studies should be continued to get the baseline data. And, to know the changes in genotype is also important because the new emerging JE virus strain may not be protected by the current JE vaccine.

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REFERENCES

1. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: Minutes of WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. *Vaccine* 2000; 18 (Suppl. 2): 1-25.
2. Hoke CH, Vaughn DW, Johnson RT, Nisalak A, Intralawan, P, Poolsuppasit S, *et al.* Effect of high-dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. *Journal of Infectious Diseases* 1992; 165: 631-637.
3. Briese T, Jia XY, Huang C, Grady LJ & Lipkin WL. Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. *Lancet* 1999; 354: 1261-1262.
4. Lanciott RS, Roehrig JT, Deubel V, Smith J, Perker M, Steele K, *et al*. Origin of the West Nile virus responsible for an outbreak of encephalitis in the Northeastern United States. *Science* 1999; 286: 2333-2337.
5. Mostashari F, Kulldroff M, Hartman JJ, Miller JR & Kulasekera V. Dead bird clusters as an early warning system for West Nile virus activity. *Emerging Infectious Diseases* 2003; 9: 641-646.
6. O'Leary DR, Marfin AA & Montgometry SP. The epidemic of West Nile virus in the United States. *Vector-Borne and Zoonotic Diseases* 2004; 4: 61-70.
7. Chambers TJ, Hahn CS, Galler R & Rice CM. Flavivirus genome organization, expression and replication. *Annual Review of Microbiology* 1990: 44; 649-688.
8. Sumiyoshi H, Mori C, Fuke I, Morita K, Kuhara S, Ondou J, *et al*. Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. *Virology* 1987; 161: 497-510.
9. Soe Thein, Htay Aung & Sebastian AA. Study of vector, amplifier, and human infection with JEV in a Rangoon community. *American Journal of Epidemiology* 1988; 128: 1376-1382.
10. Lay Myint, Kyaw Oo, Win Hlaing, Hlaing Myat Thu, Kyaw Zin Thant, Sein maung Than, *et al.* Investigation of Japanese encephalitis virus infection in Bogalay Township, Myanmar in 1999. *Tropical Medicine* 2000*;* 42(2), 47-52.
11. Review Articles Compiled from DMR Bulletins (1986-1997). 2001; 64.
12. Burke DS, Lorsomrudee W, Leake CJ, *et al*. Fatal outcome in Japanese encephalitis. *American Journal of Tropical Medicine and Hygiene* 1985; 34: 1203-1212.
13. Huang C, Skater B, Rudd R, *et al.* First isolation of West Nile virus from a patient with encephalitis in the United States. *Emerging Infectious Diseases* 2002; 8: 1367-1371.
14. Iwamoto M, Jernigan DB, Guaschi A, *et al*. Transmission of West Nile virus from an organ donor to four transplant recipients. *New England Journal of Medicine* 2003; 348: 2196-2203.
15. Geroge S, Prasad SR, Rao JA, Yergolkar PN & Setty CV. Isolation of Japanese encephalitis and West Nile viruses from fatal cases of encephalitis in Kolar district of Karnataka. *Indian Journal of Medical Research* 1987; 86: 131-134.
16. Doi Y, Adams J, O'Keefe A, Quereshi Z, Ewan L & Paterson DL. Japanese encephalitis outbreak, Yuncheng, China, 2006. *Emerging Infectious Disease* 2007; 13(7): 1123-24.
17. Nitatpattana N, Dobot-Peres A, Gouilh MA, Souris M, Barbazan P, Yoksan S, *et al.* Changes in Japanese encephalitis virus distribution, Thailand. *Emerging Infectious Disease* 2008; 14(11): 1762-65.
18. Beasley DWC, Li L, Suderman MT, Guirakhoo F, Trent DW, Monath TP, *et al.* Protection against Japanese encephalitis virus strains representing four genotypes by passive transfer of sera raised against Chimeri Vax^{TM}-JE experimental vaccine. *Vaccine* 2004; 22: 3722-3726.

19. Aung Zaw Latt, Hlaing Myat Thu, Kay Thi Aye, Hla Myo Thu, Khin Khin Oo & Yoksan S. Isolation and identification of Japanese encephalitis virus from pigs in DikeOo pig farm. *Myanmar Health Sciences Research Journal* 2011; 23(1): 21-25.
20. Williams DT, Daniels PW, Lunt RA, Wang LF, Newberry KM & Mackenzie JS. Experimental infection of pigs with Japanese encephalitis virus and closely related Australian Flaviviruses. *American Journal of Tropical Medicine and Hygiene* 2001; 65(4): 379-387.
21. Than Swe & Mi Mi Khin Soe. Japanese encephalitis outbreak in horses in Burma. *Arthropod-borne virus information exchange* 1978; 35: 43-45.
22. Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ & Barret ADT. Origin and evolution of Japanese encephalitis virus in Southeast Asia. *Journal of Virology* 2003; 77: 3091-3098.
23. Hasegawa H, Yoshida M, Fujita S & Kobayashi Y. Comparison of structural proteins among antigenically different Japanese encephalitis virus strains. *Vaccine* 1994; 12: 841-844.
24. Li MH, Fu SH, Chen WX, Wang HY & Guo YH. Genotype V Japanese encephalitis virus is emerging. *PLoS Neglected Tropical Diseases* 2011; 5(7): 1231.
25. Takhampunya R, Kim HC, Tippayachai B, Kengluecha A, Klein TA, Lee WJ, *et al.* Emergence of Japanese encephalitis virus genotype V in the Republic of Korea. *Virology Journal* 2011; 8: 449.
26. Hospital-based surveillance of Japanese encephalitis infection in Yangon, Virology Research Division. In: *Annual Report (2009),* Department of Medical Research (Lower Myanmar).
27. Vaughn DW, Hoke CH (Jr). The epidemiology of Japanese Encephalitis: Prospects for prevention. *Epidemiologic Reviews* 1992; 14: 197-221.
28. Chunsuttiwat S. Japanese encephalitis in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 1989; 20: 593-7.

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Bangkok High School Students' Attitudes towards Adolescent Pregnancy and its Prevention

Aung Aung Kyi[*] *& Khin Khin Aye*

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The causes and consequences of adolescent pregnancy impact everyone from family members to entire communities. This study investigated the attitudes and responses of high school students, who studied in a bilingual school in Bangkok, to the issue of adolescent pregnancy. Responses were assessed on three key aspects with 21 items measuring their attitudes based on a five Likert scale namely: causes, consequences and the use of school-based adolescent pregnancy awareness education programs. Data collection required students (n=63) to complete a self-administered questionnaire and the responses were analyzed by grade level and gender using both descriptive and inferential statistics. Independent sample 't' tests revealed that G-11 students perceived a drop in moral values, and teenagers' sexual behavior as leading causes of adolescent pregnancy while school drop-out and early parenthood were the most serious consequences of adolescent pregnancy. However, female students perceived school drop-out as the most serious consequence. Moreover, G-11 students were more likely than G-12 students to respond positively to a school-based adolescent pregnancy awareness education program. A one-way ANOVA test indicated that students aged 16 years were more likely to perceive a drop in moral values and teenagers' sexual behavior as the most common causes of adolescent pregnancy than those aged 17 and 18 years. This independent study can be applied to designing prevention programs in order to reduce the incidence of adolescent pregnancy among high school students in every country.

Key words: Adolescent pregnancy, Prevention programs, Bangkok High School students

INTRODUCTION

Adolescent pregnancy is defined as pregnancy that occurs in young females aged 10-19 years old.[1] Unplanned and unwanted pregnancy among adolescents is a common public health problem worldwide[2] as about 16 million girls aged 15-19 years and 2 million girls under age 15 give birth every year. Moreover, one in five girls has given birth by the age of 18 worldwide.[3]

Adolescent pregnancy occurs in all societies but the level of teenage pregnancy and childbearing varies from country to country.[4] In developing countries, about 19% of young women aged under 18 become pregnant[5], and about 95% or almost all pregnant adolescent are from low or middle income countries.[6] Several causes contribute to adolescent pregnancy. For example, in 2000, the total number of teen pregnancies was 38,600 (38 in 1000) in Canada while compared to the United States, it was 821,810 (84 in 1000). And, among examination teenage pregnancy is definitely linked with teenagers' poor relationships with their parents or parenting styles that include lack of love or concern or single parent families or absent parents or the permissiveness of such parents.[7]

A study also pointed the cultural value placed on fertility is believed to encourage teenage pregnancy.[8] Moreover, one study indicated that lack of opportunity and socio-economic disadvantage significantly contri-

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butes to teen pregnancy.[9] Teens living in poverty were more likely to get pregnant than teens who do not and teen parents often had lower lifetime earnings, as well as more social problems throughout their lives.[10, 11]

Many young people engage in risky sexual behavior which can result in unintended health outcomes including pregnancy, HIV infection and other sexually transmitted diseases.[12] Another study stated that though not always, the increase in sexual activity is often associated with an increase in the incidence of teen pregnancy.[13] Today, unwanted pregnancies, venereal disease and the feeling of emptiness are common among young people who considered living a life of immorality as normal behavior.[14]

There are many consequences of teen pregnancy that impact not only on the adolescent but also everyone else from family members to entire communities. Teen pregnancy is a major contributor to high school drop-out.[15] Previous studies also found educational failure and school drop-out are negative results of teenage pregnancy.[16] It reduces the chances of high school completion and attending college.[17] Therefore, researchers conclude that many such adolescent mothers are unable to obtain any form of higher education and so are at a disadvantage.[18]

Moreover, in many cases, teenage mothers are not in a position to go back to school as they have to look after their children after delivery and in some cases, their physical condition is not conducive for them to go to school.[19] Also, in comparing to their non-pregnant peers, pregnant secondary students are less likely to get a job or attend college and as a result, adolescent mothers often experience the lack of meaningful or equal career opportunities leading to the high rate of teen unemployment.[18]

Teen childbearing has also serious consequences for teen mothers, their children, and society as a whole. For example, mothers aged younger than 17 faced an increased risk of maternal mortality because their bodies are not yet mature enough to bear children. Most teenagers showed high levels of parenting stress and are less responsive and sensitive in interaction with their infants than adult mothers that caused lack of parental skills.[20] Therefore, early childbearing may be life-threatening to both the mother and the child.[21]

Today, there are hundreds of adolescent pregnancy programs available. However, it is difficult to know which programs can be served as the best practice for educators and practitioners.[22] Although sexual education is part of the national school curriculum, teaching is clearly insufficient in Thailand. The Education Ministry limits the instruction on sex education to eight hours a year despite changing attitudes towards sex among the young and the consequences of unplanned pregnancy are often left out of them leave the classroom teaching too despite the lack of legal option.[23] Moreover, many researchers and advocates argue that teenage pregnancy prevention should target attitudes towards pregnancy.[24, 25]

Considering the above, the purpose of this study was to investigate the attitudes and responses of high school students, who study in a bilingual school in Bangkok, and no previous studies have been identified on the issue of adolescent pregnancy at this school. The questions to be answered in this study were: What are the leading causes of adolescent pregnancy? ; What are the most serious consequences of adolescent pregnancy? ; What efforts can help to address this issue?

The findings from this study will be helpful to broaden our understanding of the issue before implementing school adolescent pregnancy prevention programs. By exploring students' attitudes on this matter, the information can be used to determine how best to incorporate such attitudes into the overall goal of developing school teen pregnancy prevention programs. Beneficially, this independent study can assist in designing targeted future prevention programs to be applied by community organizations or by schools in Thailand or elsewhere.

MATERIALS AND METHODS

A cross-sectional study was conducted to examine the attitudes and responses of high school students. Approximately 300 students of G-11 and G-12 classes were enrolled in the semester 2 of 2013. Permission to sample the high school students was obtained from the school director prior to the study. Protecting the students' privacy, confidentiality and anonymity were ensured by not indicating their names and school identity code on questionnaires.

A total number of 100 students from both classes were selected by random sampling technique according to their school identity code which was assigned by the school registration office. The students who were selected from simple random table for each class were given self-reported questionnaire by their teachers. When they received the questionnaire, they were asked to complete the questionnaire that related to adolescent pregnancy in order to describe their attitudes. Sixty-three randomly selected students sent completed questionnaires back to their teachers' office.

Students' responses were assessed on three key aspects with 21 items measuring their attitudes based on a five Likert scale namely: causes, consequences and the use of school-based adolescent pregnancy awareness education programs as the initial assessment. Responses based on the scale were coded as 1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree.

The data collected was analyzed by using both descriptive and inferential statistics. The descriptive statistics showed the characteristic of the data (e.g., frequency, percentage, average, etc.). The inferential statistics was done by using an independent sample 't' test and a one-way ANOVA test that measured significant mean differences among the above-stated three key aspects of adolescent pregnancy classified by gender, student's level of schooling and age. Criteria of class interval are shown in this study (Table 1).

Table 1. Criteria of class interval mean range for this study

1.00-1.49	1	Strongly disagree
1.50-2.49	2	Disagree
2.50-3.49	3	Neutral
3.50-4.49	4	Agree
4.50-5.00	5	Strongly agree

In order to avoid the absence of responses in this study, the following options were included: 7=do not know; 8=refuse to answer; 9=question does not apply and were assigned as missing data.

RESULT

Table 2 shows the overall mean for the three different key aspects of adolescent pregnancy stating the range of the mean score. Among them, the last key aspect showed a high mean score of (3.5-4.49) while the first two key aspects showed a mean score range of (2.50-3.49). However, it indicated that the higher the mean, the more likely students are to think about adolescent pregnancy and their openness to school-based prevention programs.

Table 2. Overall descriptive statistics on adolescent pregnancy

Key aspects of adolescent pregnancy	Mean	Standard deviation	Conclusion
Causes	3.2952	0.66513	Neutral (2.5-3.49)
Consequences	3.4802	075709	Neutral (2.5-3.49)
School-based prevention program	3.8794	0.76836	Agree (3.5-4.49)

Table 3 shows the mean differences between causes for adolescent pregnancy and level of school (grade). The highest mean score was related to teenager's sexual behavior while the lowest mean score was related to low socio-economic status for both G-11 and G-12 students. It also showed that both G-11 and G-12 students had the highest mean score for teenagers' sexual behavior. However, the independent sample 't' test indicated that G-11 students were more likely to perceive a drop in moral values, and teenagers' sexual behavior as

leading causes of adolescent pregnancy than the other ($p<0.05$).

Table 3. Independent samples 't' test for causes of adolescent pregnancy by grade

Causes	Grade	Mean	Standard deviation	't' statistic (p value)
Low socio-economic status	11	3.2400	0.92556	1.954
	12	2.7895	0.87481	(0.055)
Cultural factors	11	3.1600	1.10604	1.332
	12	2.8421	0.78933	(0.188)
Permissiveness of parents	11	3.2400	1.09087	-.608
	12	3.3947	0.91650	(0.546)
Drop in moral values	11	4.2000	0.64550	5.340
	12	2.9211	1.07506	(0.000)
Teenagers' sexual behavior	11	4.3200	0.69041	3.558
	12	3.4211	1.13021	(0.001)

Table 4. Independent samples 't' test for consequences of adolescent pregnancy by grade

Consequences	Grade	Mean	Standard deviation	't' statistic (p value)
School drop-out	11	4.0400	1.01980	2.326
	12	3.3158	1.31735	(0.023)
Low self-esteem	11	3.7600	1.01160	1.701
	12	3.2895	1.11277	(0.094)
Unemployment	11	3.0000	0.81650	-0.845
	12	3.1842	0.86541	(0.401)
Early parenthood	11	4.2000	0.76376	3.069
	12	3.4211	1.10604	(0.003)

Table 4 shows the mean differences between four different consequences and level of school (grade). It indicated that G-11 students had higher mean score than G-12 students for almost all consequences. The independent sample 't' test also indicated that two kinds of consequences, school drop-out and early parenthood are meaningful difference regarding consequences of adolescent pregnancy and grade. Mean comparisons revealed that G-11 students are more likely to perceive school drop-out and early parenthood as the most serious consequences of adolescent pregnancy compared to G-12 students ($p<0.05$).

In gender differences of consequences, it was found that the result of Independent sample 't' test was only significant for one consequence (school drop-out) which indicated female students perceived school drop-out as the most serious consequence ($p<0.05$). However, mean comparisons revealed that female students were more likely to perceive all four consequences of adolescent pregnancy as serious consequences compared to male students (Table not shown).

Table 5 shows the different age groups and their responses to the causes of adolescent pregnancy. It was found that students aged 16 years had the highest mean score for the four different causes of adolescent pregnancy. When comparing age groups, using a one-way ANOVA test, the results showed that there was a significant relationship between age and the two different causes of adolescent pregnancy. This suggests that students aged 16 years were more likely to perceive a drop in moral values and teenagers' sexual behavior as the most common causes of adolescent pregnancy compared to others ($p<0.05$).

Table 5. ANOVA test for causes of adolescent pregnancy by age

Causes	Age	Mean	Standard deviation	'F' statistic (p value)
Low socio-economic status	16	3.1364	0.88884	0.660
	17	2.9091	0.87905	(0.52)
	18	2.7500	1.16496	
Cultural factors	16	3.0000	1.06904	0.032
	17	2.9394	0.86384	(0.968)
	18	3.0000	0.92582	
Permissiveness of parents	16	3.1364	1.20694	1.691
	17	3.3333	0.81650	(0.193)
	18	3.8750	0.83452	
Drop in moral values	16	4.0909	0.81118	8.213
	17	2.9697	1.07485	(0.001)
	18	3.5000	1.19523	
Teenagers sexual behavior	16	4.2273	0.75162	4.343
	17	3.4242	1.19975	(0.017)
	18	4.0000	0.75593	

Regarding the differences of means in school-based prevention program by level of school (grade), the Independent sample 't' test was significant for only one variable which indicated G-11 students perceived that every individual in a school community needed to be knowledgeable about adolescent pregnancy ($p<0.05$). However, the majority of mean comparisons revealed that G-11 students were more likely than G-12 to respond positively to a school-based

suicide awareness education program (Table not shown).

DISCUSSION

This study investigated how high school students, who study in a bilingual school in Bangkok, perceive and respond to the issue of adolescent pregnancy. The three key aspects of adolescent pregnancy, namely: causes, consequences and the use of school-based adolescent pregnancy awareness education programs were measured as an initial assessment. Comparing the causes of adolescent pregnancy by school level (grade), the highest mean score was related to teenagers' sexual behavior while the lowest mean score was related to low socio-economic status for both G-11 and G-12 students. However, the independent sample 't' test showed a significant mean difference between G-11 and G-12 students and suggested that G-11 students are more likely to perceive a drop in moral values and teenagers' sexual behavior as the leading cause of adolescent pregnancy compared to the others ($p<0.05$).

For the different responses for the consequences of adolescent pregnancy with respect to the school grade level and gender, an independent sample 't' test was used. The results again indicated G-11 students were more likely to perceive school drop-out and early parenthood as the most serious consequences of adolescent pregnancy ($p<0.05$). It may be because most adolescents with unexpected pregnancy postpone their education and are likely to lack sufficient skills and training.

As a result, this may lead to inadequate parental care to support their children with miscarriage. The independent 't' test results also confirmed a significant difference in the mean score by gender with female students perceiving school drop-out as the most serious consequence of adolescent pregnancy. Pregnant adolescents might feel excluded from school activities and feel discouraged and experience disapproval from their school community as well as society at large.

Moreover, G-11 students are more likely than G-12 students to respond positively to a school-based adolescent pregnancy awareness education program. They may believe that a pregnancy prevention program can influence adolescents' perception of sexually risky behavior and also it can encourage precautions to reduce pregnancy and sexually transmitted infections in the future.

To examine the different responses to the above mentioned four key aspects with respect to age group, a one-way ANOVA test was used. It indicated that students aged 16 years are more likely to perceive a drop in moral values and teenagers' sexual behavior as the most common causes of adolescent pregnancy compared to those aged 17 and 18 years. The pregnant teenager can be socially isolated with little notion of morality from family or their environment which increases the risk of becoming pregnant.

In addition, the results of this research certainly suggests the benefits of implementing pregnancy prevention programs in the surveyed school as adolescent pregnancy has become an issue of national concern in Thailand.[26]

Conclusion & Recommendation

In Thai society, generally the issue of adolescent pregnancy is taboo and not discussed openly in public. Currently, sex education in schools is limited. Therefore, an important public health issue is mostly shielded from discussion. This study revealed that G-11 students are more likely than G-12 students to respond positively to a school-based adolescent pregnancy awareness education program. It also suggests that G-12 students need to understand how adolescent pregnancy prevention programs are important and necessary in all schools. In addition, schools need to implement effective school-based adolescent pregnancy awareness education

programs to reduce the risk of pregnancy among students and as part of promoting healthy sexual behavior and life skills generally not only in Bangkok but in schools everywhere.

REFERENCES

1. World Health Organization. Adolescent pregnancy: Issues in adolescent health and development. Geneva, Switzerland: WHO, 2004. Available from: URL: http://whqlibdoc. who. int/ publications/ 2004/ 9241591455_ eng. pdf.
2. Ramos S. Interventions for preventing unintended pregnancies among adolescents: RHL commentary. The WHO Reproductive Health Library; Geneva: WHO, 2011. Available from: URL: http://www.apps.who.int/rhl/ adolescent.
3. Boonstra HD. Young people need help in preventing pregnancy and HIV; How will the world respond? *Guttmacher Policy Review* 2007; 10(3): 2-8.
4. Sarantaki RM & Koutelekos RN. Teenage pregnancy. *Journal of Health Science* 2003; 1(2): 29-30.
5. United Nations Population Fund. *The State of World Population, 2013.* Available from: URL: http.//www.who.int/pmnch/about/governance/partnersforum/youth_unfpa.pdf.
6. World Health Organization. A report on early marriages, adolescent and young pregnancies for the Sixty-fifth World Health Assembly. The WHO reproductive library: Geneva, 2012. Available from: URL: http.//www. apps.who. int/ gb/ebwha/pdf_files/WHA65/A65_13-en.pdf.
7. Parent teenage pregnancy. Available from: URL: http://www. ukessays. com/ essays/ childcare/ parents- teenage-pregnancy.php.
8. Macleod, C. Teenage pregnancy and its negative consequences: Review of South African research, Part 1. *South African Journal of psychology* 1999; 29(1): 1-7.
9. Ross DP, Roberts PA & Scott K. Variations in child development outcomes among children living in lone-parent families. Ottawa: Applied Research Branch, Human Resources Development Canada, 1998.
10. Wilkins R & Sherman GJ. Low incomes and child health in Canada. In: *Health and Canadian Society: Sociological Perspectives*. D. Coburn, GM Torrance and CD' Arcy (Eds.), Toronto, University of Toronto Press 1998; 102-109.
11. Grogger J. Incarceration-related costs of early childbearing. In: *Kids having kids: Economic costs and social consequences of teen pregnancy.* Maynard R, ed., Washington, DC: Urban Institute Press, 1997; 231-255.
12. Center for Disease Control and Prevention. Sexual risk behavior: HIV, STD and teen pregnancy prevention, 2013. Available from: URL: http://www.cdc.gov/healthyyouth/ yrbs/ cdcreports.htm.
13. Kohli V & Nyberg KL. Teen pregnancy prevention through education. Sacramento, CA: Center for California Studies, California State University, 1995.
14. Campbell JC. Manners and morals of today's children and teens. Available from: URL: http://www.lifepaths360.com/index.php.
15. Uche RD. Drop-out syndrome among girls in secondary schools and human resources development in Nigeria. *Journal of Education and Practice* 2013; 4(2): 25-30.
16. Robin EW. Teen pregnancy. Available from: URL: http://www.about.com.
17. Hofferth SL, Reid L & Mott FL. The effects of early childbearing on schooling over time. *Family Planning Perspectives* 2001; 33(6): 259-267.
18. Domenico DM & Jones KH. Adolescent pregnancy in America: Causes and responses. *Journal for Vocational Special Needs Education* 2007; 30(1): 4-12.
19. Sodi EE. *Psychological Impact of Teenage Pregnancy on Pregnant Teenagers*. Polokwane, South Africa. University of Limpopo, 2009.
20. Nkwanyana TR. A study of the high rate of teenage pregnancy in high schools in the Lembe District. Available from: URL: http://www. uir. unisa.ac.za.
21. Tunick B. Risks and realities of early childbearing worldwide. (Issues in Brief). NewYork: Alan Guttmacher Institute, 1996.
22. Franklin C & Corcoran J. Preventing adolescent pregnancy: A review of programs and practices. *Social Work* 2000; 45(1): 40-53.
23. Lefevre AS. Thailand struggles with teen pregnancy. Bangkokpost. Available from: URL: http://www.bangkokpost.com, accessed 8 March 2013.
24. Witte K. Preventing teen pregnancy through persuasive communications: Realities, myths, and the hard-fact truths. *Journal of Community Health* 1997; 22(2):137-154.
25. Stevens-Simon C, Kelly L, Singer D, *et al*. Why pregnant adolescents say they did not use contraceptives prior to conception? *Journal of Adolescent Health* 1996; 19(1): 48-53.
26. Neamsakul W. Unintended Thai adolescent pregnancy: A grounded theory study. Michigan: ProQuest. Available from: URL. http:// books. google.com.

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Coagulation Profile in Type 2 Diabetes Mellitus Patients Attending the Diabetic Clinic, Yangon General Hospital

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Diabetes mellitus is a chronic disease that causes serious health complications including renal failure, heart disease, stroke and blindness. In Myanmar, the overall prevalence of diabetes mellitus was 12.6% of females and 11.5% of males. Type 2 diabetes mellitus patients had been known to have a hypercoagulable state and hypofibrinolysis. The coagulation profile, glycosylated hemoglobin (HbA1c) were determined in 187 patients with type 2 diabetes mellitus (44 males, 143 females, mean age 55±11 years) attending the diabetic clinic of Outpatient Department of Yangon General Hospital. In these patients, prothrombin time (PT) (12.2±1.4 sec), activated partial thromboplastin time (APTT) (32.2±4.7 sec), thrombin time (TT) (18.1±2.6 sec) were within normal ranges. One hundred and two (55%) patients have increased in fibrinogen concentration (4.2±1.3 g/l) just exceeded the upper limit (2-4 g/l). Fibrinogen concentration was significantly higher in female (4.4±1.2 g/l) than in male (4.0±1.3 g/l) ($p<0.001$). In diabetic patients with HbA1c levels more than 6.4%, mean fibrinogen concentration and TT were significantly increased ie., 4.4±1.3 g/l and 18.9±2.7 sec, respectively. Plasminogen activator inhibitor-1 (PAI-1) was measured in 77 diabetic patients with increased fibrinogen levels by using Human PAI-1 ELISA Kit. Forty-nine (64%) had PAI-1 level more than 50 ng/ml (references). PAI-1 level had positive correlation ($r=0.29$) with fibrinogen concentration in type 2 diabetes mellitus patients. The findings on coagulation status and fibrinolytic activity of type 2 diabetes mellitus patients would be beneficial for management of diabetic patients with thrombotic complications.

Key words: Coagulation profile, Plasminogen activator inhibitor-1, HbA1c, Type 2 diabetes mellitus

INTRODUCTION

Diabetes is a metabolic disorder of multiple causes characterized by chronic hyperglycemia and disorders of carbohydrate, fat and protein metabolism. It is caused by defects in insulin secretion (type 1), insulin actions (type 2) or combination of these causes.[1] Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence rate is expected to occur in Asia and Africa.[2] In Myanmar, the prevalences of diabetes were 12.6% of females and 11.5% of males among the sample population in rural and urban areas of Yangon Region, 2003-2004 according to the diabetes project of Ministry of Health.[3]

Eighty percent of diabetic patients die with thrombotic disease and 75% of deaths are due to cardiovascular complications and

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remainder due to cerebrovascular events and peripheral vascular complications. Diabetes is associated with increased risk of atherosclerosis and thromboembolic disease. Hypercoagulability as evidenced by increased fibrinogen levels and hypofrinolysis as evidenced by increased Plasminogen activator inhibitor-1 (PAI-1) levels contribute to procoagulant state observed in diabetes.[4] Patients with diabetes mellitus have a high risk of atherothrombotic events.

Many studies have shown a variety of diabetes mellitus-related abnormalities in hemostasis and thrombosis. Venous thrombosis has also been found to occur more frequently in diabetic patients.[5] Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor.[6]

Prothrombin time (PT) was originally thought to measure prothrombin, but is known to depend on reactions with factor V, VII and X and the fibrinogen concentration. Activated partial thromboplastin time (APTT) clotting assay, like PT, failed to detect the hypercoagulable state of diabetes because this test depends on the reactions with factor V, VIII, IX, X, prothrombin and fibrinogen. TT clotting assay depends upon the concentration reaction of fibrinogen.[7] Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin.[6] TT reveals the deficiency or abnormality of fibrinogen. Fibrinogen concentration is the determinant of plasma viscosity.[8]

The fibrinolytic system is natural defense against thrombosis. Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor and evidence suggests that it is the major regulator of the fibrinolytic system.[9] An important protein that inhibits fibrinolysis is PAI-1, which blocks the conversion of plasminogen into active plasmin.[10] There is evidence in the literature indicating that hyperglycemia can affect PAI-1. Furthermore, it has been shown that hyperglycemia stimulates activation of the PAI-1 gene promoter in vascular smooth muscle cells. A positive linear correlation has been demonstrated between hemoglobin A1c and PAI-1 activity in type 2 diabetic patients.[11]

Glycosylated hemoglobin (HbA1c) provides a practical assessment of long-term glycemic control in patients with diabetes (normal range is 3.3-6.4%). HbA1c is hemoglobin which has combined permanently with the glucose present in the blood. This result is stable for 3 months as for the life of red blood cells and it is a measure of the risk for the development of diabetes complication (micro and macrovascular complications).[12]

The aim of this study was to determine the coagulation profile, PAI-1 and HbA1c in type 2 diabetes mellitus patients and to compare the results of HbA1c, coagulation tests and PAI-1 of type 2 diabetic patients.

MATERIALS AND METHODS

A cross-sectional descriptive study was carried out in 187 type 2 diabetic patients including 44 males and 143 females with age ranged 23-79 years in Diabetic Outpatient Clinic of Yangon General Hospital (YGH). After getting approval from the Ethical Review Committee of Department of Medical Research (Lower Myanmar), medical and drug history of these cases were recorded according to the proforma.

And then, total 6 ml of blood were collected from antecubital vein of all subjects. Among them, 3.6 ml of blood were put into plastic test tube containing 0.4 ml of 3.13% trisodium citrate dehydrate salt (1:10 dilution) for coagulation profile. One milliliter of clotted blood was added to vacutainer tube for PAI-1 and other 1.4 ml of blood was added to a glass test tube containing ethylenediaminetetraacetic acid, tripotassium salt (K_3EDTA) tube for hemolysate

preparation of HbA1c test. These samples were kept on crushed ice and transported to the laboratory within 2 hours.

Platelet-poor plasma (PPP) was prepared by centrifugation at 3000 rpm for 10 minutes at 4°C. The plasma and serum of all samples were put into microcentrifuge tube to perform PAI-1 and coagulation profile by PT, APTT, TT, fibrinogen concentration. PT, APTT, TT were estimated by standard methods as described by Dacie and Lewis. PT test measured the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system.

After mixing 0.1 ml citrated plasma and 0.1 ml in-house brain thromboplastin reagent[13] in a glass tube, it was placed in 37°C waterbath for 1 minute. Then, 0.1 ml of pre-warmed 25 mmol $CaCl_2$ was added to the mixture and the end-point of clotting was recorded. APTT test measured the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin, and so indicates the overall efficiency of the intrinsic pathway. Equal volumes 0.1 ml of citrated plasma and the kaolin 5 g/l were mixed in a glass tube and kept in the waterbath at 37°C for 9 minutes. Then, 0.1 ml of the phospholipid reagent was added to this glass tube and waited for 1 minute. Pre-warmed 25 mmol $CaCl_2$ 0.1 ml was put into the mixture and the clotting time was recorded.[7]

Thrombin is added to plasma and the clotting time was measured. TT is affected by the concentration and reaction of fibrinogen. At 37°C, 0.1 ml of barbitone buffered saline, pH 7.4 and 0.1 ml of citrated plasma were placed in a glass tube. Then, 0.1 ml thrombin was added and the clotting time was recorded with the stopwatch. Fibrinogen concentration was measured by dry clot weight method in all subjects. Fibrinogen in plasma is converted into fibrin by clotting with thrombin and calcium. The resulting clot is weighted.[7]

PAI-1 was measured by using the OmniKine™ Human PAI-1 Enzyme-Linked Immunosorbent Assay (ELISA) Kit. This PAI-1 ELISA Kit allows for the detection and quantification of endogenous levels of natural and recombinant human PAI-1 proteins within the range of 23-3000 pg/ml. This particular immunoassay was utilized the quantitative technique of a "Sandwich″ ELISA where the target protein (antigen) was bound in a "sandwich″ format by primary capture antibodies coated to each well-bottom. The serial dilution of protein standard and 100 ul serum of each sample was put into the well and incubated for 2 hours. After incubating, this plate was washed. The diluted Biotin-Conjugated Detection Antibody was added to this plate and incubated for 2 hours and then, washed. The Streptavidin-HRP was added to each well and incubated at room temperature for 30 minutes and washed. The substrate was added for color development and measured by a spectrophotometer at 450 nm.[14]

HbA1c was measured by high performance liquid chromatography (HPLC) analyzer. Whole blood 20 ul was mixed with 1000 ul of distilled water for at least 3 hours at room temperature before determining with HbA1c analyzer.[15] In analysis, the results of coagulation tests were summarized in mean and SD. Mean values were compared by gender and HbA1c level. Probability values were computed using Student ‘t’ test or Mann-Whitney U test.

RESULTS

In this study, the mean age of 187 type 2 diabetes mellitus patients was 55±11 years ranging between 23 and 79 years. Of 187 diabetic patients studied, 44 males (24%) and 143 females (76%) were included. The commonest age group was 40-59 years (106 patients, 57%) among the three age groups, ie., 20-39, 40-59, 60-79 years for analytical purpose. The PT, APTT, TT, fibrinogen concentration and PAI-1 were not significantly different between three age groups of these patients. According

to proforma of these patients, 46% had hypertension, 19% heart diseases, 42% renal complication and 8% eyes complication. The commonest complications were hypertension and renal complication in this study.

Table 1. Coagulation profile in type 2 diabetes mellitus patients

Coagulation tests	Normal range	Patient (n)	Mean±SD	Positive No. (%)
PT (sec)	11-16	187	12.2±1.4	0(0)
APTT (sec)	30-40	187	32.2±4.7	8(4)
TT (sec)	15-19	187	18.1±2.6	55(29)
Fibrinogen (g/l)	2-4	187	4.2±1.3	102(55)

PT=Prothrombin time
APTT=Activated partial thromboplastin time
TT=Thrombin time
Fibrinogen=Fibrinogen concentration

Table 1 shows the coagulation profile of the diabetic patients compared to the reference normal ranges. The mean results of PT, APTT, TT were within normal ranges. The mean fibrinogen concentration was increased in these patients just exceeded the upper limit of normal (2-4 g/l). High level of fibrinogen concentration was found in 102 type 2 diabetes mellitus patients (55%) in this study.

Table 2. Sex distribution of coagulation profile in type 2 diabetes mellitus patients

Coagulation tests	Female (n=143)	Male (n=44)	P value
HbA1c (%)	7.2±2.0	7.2±2.5	0.558
PT(sec)	12.1±1.4	12.3±1.4	0.37
APTT(sec)	31.9±4.8	33.2±4.2	0.046
TT(sec)	18.1±2.6	18.0±2.7	0.91
Fibrinogen (g/l)	4.4±1.2	4.0±1.3	0.001
PAI-1(ng/ml)	84.7±81.9	70.9±45.1	0.78

PT=Prothrombin time
APTT=Activated partial thromboplastin time
TT=Thrombin time
PAI-1=Plasminogen activator inhibitor-1

Table 2 shows that fibrinogen concentration was significantly higher in females than in males (p=0.001). Seventy-seven diabetic patients with increased fibrinogen levels were measured PAI-1 level by using Human PAI-1 ELISA Kit. PAI-1 level was higher than normal reference unit (50 ng/ml) in 49(64%) of these diabetic patients (Table 2).

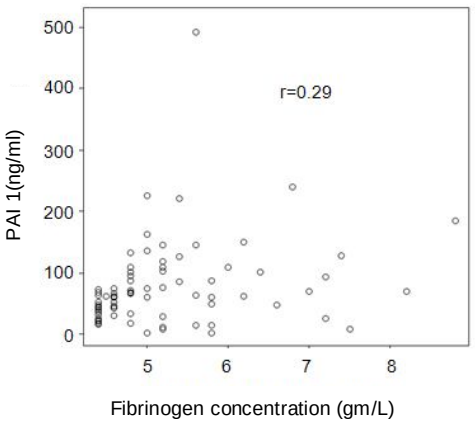

Fig. 1. Correlation between PAI-1 level and fibrinogen concentration in type 2 diabetes mellitus patients

Figure 1 shows that type 2 diabetes mellitus patients had positive correlation (r=0.29) between fibrinogen concentration and PAI-1 level in this study.

Table 3. Coagulation profile in type 2 diabetes mellitus patients groups according to HbA1c levels

Coagulation tests	HbA1c		P value
	Group I (≤6.4%)	Group II (>6.4%)	
PT(sec)	12.1±1.2	12.2±1.5	0.88
APTT(sec)	32.9±5.0	31.7±4.5	0.124
TT(sec)	17.5±2.5	18.9±2.7	0.001
Fibrinogen(g/l)	3.9±1.2	4.4±1.3	0.047
PAI-1(ng/ml)	58.9±40.8	93.6±87.3	0.843

Table 3 shows the value of coagulation tests and PAI-1 for two groups of type 2 diabetes mellitus patients according to HbA1c level. The results of PT, APTT and PAI-1 showed no significant differences between two groups. Fibrinogen concentration and TT values were significantly different in group II diabetic patients with higher HbA1c (>6.4%) in this study.

DISCUSSION

In our study, the commonest age group of type 2 diabetic patients was 40-59 years (57%) in all patients. In developing countries, the majority of people with diabetes are in the 45-64 year age range.[16] Type 2 diabetic patients were 76% female

and 24% male in this study. The prevalence of type 2 diabetes mellitus was higher in female than in male (78% *vs.* 22%) in all age groups in previous study.[15]

The commonest complications were the hypertension (46%) and renal complication (42%) according to clinical history in this study. One study described that commonest complications of type 2 diabetes mellitus patients were hypertension (46.6%) and nephropathy (20%).[17] Diabetes mellitus is the most common cause of adult kidney failure worldwide.[2]

In this study, the mean PT, APTT and TT were not significantly different but fibrinogen concentration and PAI-1 level were increased in these diabetic patients. Other study described that fibrinogen values above the reference range (fibrinogen >4.0 g/l) were more frequent in the diabetic group[5] and PT, APTT, TT did not show significant difference with control. There is general agreement that fibrinogen concentrations in diabetic patients are significantly increased as compared to the normal controls.[18] Increased plasma fibrinogen may contribute to a hypercoagulable state in non-insulin dependent diabetes mellitus.[8] Patients are considered to have a hypercoagulable state if they have laboratory abnormalities or clinical conditions that are associated with increased risk of thrombosis; diabetic patients meet these criteria.[5] Moreover, plasminogen activator inhibitor may increase the risk of thrombotic complications.[9] In the study, the fibrinogen concentration was significantly higher ($p=0.001$) in female than in male (female 4.4±1.2 g/l *vs.* male 4.0±1.3 g/l). Fibrinogen levels were correlated with sex and HbA1c. Other study showed that female and male had mean fibrinogen (6.6±1.4 g/l *vs.* 6.5± 1.3 g/l) which were higher but was not statistically significant.[19]

Our study showed high levels of fibrinogen and PAI-1 in type 2 diabetic patients compared to normal ranges. There was a positive correlation ($r=0.29$) between PAI-1 and fibrinogen level in these diabetic patients. Evolving evidence of the central role of PAI-1 in fibrosis and thrombosis increasingly supports the theory that it is a significant risk factor for macrovascular complications and cardiovascular disease, particularly in patients with diabetes.[20]

Patients with type 2 diabetes mellitus had a high prevalence of hyperfibrinogenemia. There is an increase in a number of coagulation factors such as PAI-1, fibrinogen, factor VII and thrombin antithrombin complexes particularly in association with macrovascular and microvascular disease and glycemic control.[19] PAI-1 showed a strong and consistent relation to incident diabetes.[21] PAI-1 levels (but not fibrinogen) further increase with the rising glucose levels and the development of diabetes. These findings extend the current knowledge on the relation of fibrinolysis and coagulation abnormalities to the development of type 2 diabetes mellitus.[22]

In this study, fibrinogen levels and TT value were significantly increased in type 2 diabetic patients with HbA1c more than 6.4% group. The coagulation abnormalities observed in diabetic patients seem to be caused by the hyperglycemia, which also contributes the distinguishing feature of this disease. In a hyperglycemic environment, fibrinogen can become hyperglycosylated.[5]

Fibrinogen level was independently associated with HbA1c values, which suggests that fibrinogen may be involved in the increased cardiovascular risk of patients with type 2 diabetes mellitus.[19] Evidence shows that in diabetic patient fibrinogen may be a cardiovascular risk factor because it is correlated to increased thrombin formation. Both fibrinogen levels and thrombin formation suggest a possible link between coagulation derangement and atherosclerosis in diabetes.

Another important issue is the relationship between fibrinogen levels and hyperglycaemia. A significant correlation between fibrinogen and HbA1c has been frequently found. This suggests that a high level of

fibrinogen in plasma might be a risk marker for cardiovascular disease because it reflects increased thrombin formation and therefore a greater probability that a thrombotic event will occur.[23] Patients with type 2 diabetes mellitus had hypercoagulable state and hypofibrinolysis thereby indicating that the activation of coagulation and reduced fibrinolytic activity may contribute to the increased risk of vascular disease in type 2 diabetic patients.[24]

Conclusion

This study provides to assess coagulation status and fibrinolytic activity of type 2 diabetes mellitus patients. It reveals the association of the coagulation profile, PAI-1, HbA1c clinical data of type 2 diabetes mellitus patients in this study. Fibrinogen level may be useful hemostatic markers in diabetic patients especially in those at high-risk for thrombotic complication. It is very important indicator for prevention of micro- and macrovascular thrombotic diseases. It helps to more effective treatment for these patients with thrombotic complication. The findings on coagulation status and fibrinolytic activity of type 2 diabetes mellitus patients would be beneficial for management of diabetic patients with thrombotic complication.

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REFERENCES

1. Soman SS, Soman SA & Rao TKS. Diabetic nephropathy, e medicine nephrology. Available from: URL: http://emedicine. medscape. com/ article/ 238946- overvieww accessed 28 April 2009.
2. Diabetes mellitus. Available from: URL: http:// www.chemistry daily. com/ chemistry/ Diabetes
3. Prevention, control and management of non-communicable diseases. *Health in Myanmar*, Ministry of Health 2011; 108-109.
4. Madan R, Gupta B, Saluja S, Kansra UC, Tripathi BK & Guliani BP. Coagulation profile in diabetes and its association with diabetic microvascular complications. *Journal of the Association of Physicians of India* 2010; 58: 481-484.
5. Zhao Y, Zhang J & Wu J. Diabetes mellitus is associated with shortened activated partial thromboplastin time and increased fibrinogen values. Available from: URL: http://www. ncbi. nlm. nih. gov/ pmc/ articles/ PMC3030577.
6. Coagulation. Available from: URL: http://en. wikipedia.org/wiki/Coagulation.
7. Lewis SM & Dacie SJV. Investigation of haemostasis. In: *Practical Haematology* 8th ed. Churchill Livingstone 1995; 307-345.
8. Chaw Su Hlaing, Myo Win, Ne Win, Mi Mi Cho & Myat Mon Oo. Hemostatic changes in acute ischaemic stroke patients. *Myanmar Health Research Congress, Programme and Abstracts* 2004; 40.
9. Fattah MA, Shaheen MH & Mahfouz MH. Disturbances of haemostasis in diabetes mellitus. *Disease Markers, 2003*. 2004; 19: 251-258.
10. Alzahrani SH & Ajjan RA. Coagulation and fibrinolysis in diabetes. *Diabetes and Vascular Disease Research* 2010. Available from: URL: http://www.dvr.sagepub.com/content/early/2010 /09/16/1479164110383723.
11. Pandolfi A, Cetrullo D, Polishuck R, Alberta MM, Calafiore A, Pellegrini G, *et al*. Plasminogen activator inhibitor type 1 is increased in the arterial wall of type 2 diabetic subjects. Available from: URL: http://atvb. Ahajournals. org/content/21/8/1378.full.
12. Jean-Claude Mbanya. The standardization of glycated hemoglobin. *Diabetes Voice* 2005; 50(2): 27-30.
13. Myat Mon Oo, Moh Moh Htun, Kyaw soe, Minn Minn Myint Thu, Kyaw Oo, Tin Tin San, *et al*. Establishment and evaluation of in-house one stage prothrombin time test. *Myanmar Health Sciences Research Journal* 2011; 23(1): 51-56.
14. OmniKine™ Human PAI-1 ELISA Kit, United States of America. Available from: URL: http:// www.assaybiotech.com.
15. Moh Moh Htun, Yin Min Htun, Myat Mon Oo, Han Win, Khin Myo Set, Mya Thandar Win, *et al*. Analysis of haemoglobin variants and HbA1c level in type 2 diabetes mellitus patients in Yangon. *Myanmar Health Sciences Research Journal* 2012; 24(3): 163-167.

16. Wild S, Roglic G, Green A, Sicree R & King H. Global prevalence of diabetes (Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27(5): 1047-53.
17. Khin Ye Myint, Chit Soe & Thet Khine Win. Association of chronic complication of diabetes mellitus and presence of risk factors. *Myanmar Health Sciences Research Journal* 1995; 7(1): 1-8.
18. Ahmed R, Rasheed S, Farooq M, Lodhi Y & Tayyab M. Glycosylated haemoglobin and coagulation profile in diabetes mellitus. Available from: URL: http://pjmhsonline. com/ glycosylated haemoglobin and coa.html.
19. Bembde AS. A study of plasma fibrinogen level in type 2 diabetes mellitus and its relation to glycemic control. *Indian Journal of Hematology Blood Transfusion* 2012; 28(2): 105-108.
20. Lyon CJ & Hsueh WA. Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease. *American Journal of Medicine* 2003; 115(8): 62-68.
21. Festa A, Agostino RD, Tracy RP & Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes. *Diabetes* 2002; 51(4): 1131-1137.
22. Festa A, Williams K, Tracy RP, Wagenknecht LE & Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. *Circulation* 2006; 113: 1753-1759.
23. Ceriello A. Fibrinogen and diabetes mellitus: Is it time for intervention trials? *Diabetologia* 1997; 40: 731-734.
24. Erem C, Hacihasanoglu A, Celik S, Ovali E, Ersoz HO, Ukinc K, *et al*. Coagulation and fibrinolysis parameters in type 2 diabetic patients with and without diabetic vascular complications. *Medical Principles and Practice* 2005; 14(1): 22-30.

Bacteriological and Drug Sensitivity Profile of *Vibrio cholerae* Isolated from Children with Acute Diarrhoea

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Diarrhoeal diseases constitute a leading cause of morbidity and mortality in children particularly in developing countries. Cholera, caused by *Vibrio cholerae,* is one of the major epidemic diarrhoeal diseases. A cross-sectional descriptive study was carried out on 250 children under 12 years of age with acute diarrhoea attending Yangon Children's Hospital, Yankin Children's Hospital, Thingangyun Sanpya Hospital, Insein General Hospital and North Okkalapa General Hospital during the period of January to September 2013. The rectal swab samples were collected and isolation of *V. cholerae* was carried out by standard culture methods at Bacteriology Research Division, Department of Medical Research (Lower Myanmar). Suspected colonies were confirmed by biochemical tests and slide agglutination tests using specific antisera. Antibiotic sensitivity was determined by disc diffusion method and E test. Of 250 samples, *V. cholerae* was isolated from 14% (35/250). All isolates were *V. cholerae* O1 ogawa serotype and El Tor biotype. *V. cholerae* isolates were resistant to cotrimoxazole and naildixic acid (100% each), doxycycline (20%) and amikacin (14.3%). They were sensitive to chloramphenicol and cefotaxime (100% each), norfloxacin (94.3%), ciprofloxacin (85.7%), azithromycin (82.8%), and doxycycline and Amakacin (62.8%, each). Thirteen multiple drug-resistant *V. cholerae* isolates were detected. Minimum inhibitory concentration (MIC) of susceptible strains to ciprofloxacin revealed 0.125 to 0.5 μg/ml. High level azithromycin resistance (MIC≥32 μg/ml) was seen in one case. The present study highlighted the occurrence of cholera among the pediatric population and determined the drug sensitivity profile of common antibiotics which are currently used for treatment of childhood cholera.

Key words: Vibrio cholerae, Drug sensitivity, Childhood diarrhoea

INTRODUCTION

In developing countries, cholera is one of the most common causes of acute watery diarrhoea in young children especially in endemic areas. The global burden of cholera is large, particularly in developing countries. Every year, estimated 2.8 million cases of cholera and about 91,000 deaths occur in endemic countries and another 87,000 cases and 2,500 deaths occur in non-endemic countries. The burden of cholera is greatest in Africa and southern Asia.[1] Cholera caused by either *Vibrio cholerae* O1 or O139 is a major global health problem of children in cholera endemic areas in the developing world. Only *V. cholerae* serogroups O1 and O139 produce enterotoxin, cholera toxin (CT) and that have the potential to cause epidemics and global pandemics of cholera. The first 6 pandemics were caused by toxigenic strain

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of Classical serogroup O1 biotype, whereas 7th pandemic was caused by the El Tor biotype.[2]

Diarrhoea and cholera are prioritized diseases in the National Health Plan in Myanmar. Diarrhoea morbidity rate is 400 to 500/100,000 populations and mortality rate is 4 to 13/100,000 population in Myanmar.[3] The reported cholera cases among diarrhoea cases in Yangon Division were: 49 cases and 191 cases in 2008 and 2009, respectively.[4] In 2004, 138 *V. cholerae* O1 El Tor Ogawa were identified from among 339 rectal swab specimens in Mandalay Division.[5] According to the laboratory data of the National Health Laboratory (Yangon), there were 103 culture-confirmed cholera cases in 2011.[6]

Cholera kills more than 100,000 people worldwide every year, mainly infecting children between 1 and 5 years old.[7] One study observed that cholera is not uncommon in infants and young children and like children in the older age group, acute onset diarrhoea arouse suspicion of cholera in young children also.[8] In a study of India in 2010, the prevalence of cholera was 24.6% in children with acute diarrhoea.[9] A research carried out at Yankin Children's Hospital reported that culture-confirmed childhood cholera cases were found to be 21% (21/100) in children presenting with acute diarrhoea in 2012.[10]

For cholera, antibiotics play a major role in reducing the shedding of bacillus, thereby preventing the spread of diseases. But, due to rampant and haphazard use of the antibiotics in treatment of *V. cholerae* (O1 and O139) infection, there were rapid emergence of multidrug resistance (MDR) strains.[11] The antibiotics susceptibility pattern of local endemic strain is important not only in treatment of *V. cholerae* in children but also emergence of multidrug resistance strains. Information about the pattern of antibiotic sensitivity and minimum inhibitory concentrations (MICs) of frequently used antibiotics can improve patient care and save money by selecting the most suitable antibiotics. Knowledge of sensitivity pattern to *V. cholerae* is critical to antibiotic selection.

The present study was carried out to detect the proportion of culture-confirmed childhood cholera cases among children with acute diarrhoea and to determine the phenotypic characteristics of currently circulating *V. cholerae* strains. In addition, this study also revealed the antibiotic susceptibility profile of *V. cholerae* isolated from children.

MATERIALS AND METHODS

This study was a cross-sectional descriptive one. From February to October 2013, a total of 250 children (both sexes) who were less than 12 years with duration of illness not more than 14 days and with or without use of antibiotics, presenting with acute diarrhoea attending at Yangon Children's Hospital, Yankin Children's Hospital, North Okkalapa General Hospital, Insein General Hospital and Thingangyun Sanpya Hospital were studied.

Inclusion criteria were both sexes of children less than 12 years with duration of illness not more than 14 days and with or without use of antibiotics were included in this study.

Collection of specimens and transportation

The purpose of the study and procedure were explained to the parents and a written informed consent was obtained. Information on demographic data and current clinical symptoms were collected. Then, rectal swabs were collected by sterile swab sticks. The swabs were put into Cary Blair transport media and were transported to Bacteriology Research Division, Department of Medical Research (Lower Myanmar) and culture inoculations were performed within six hours of sample collection.

Isolation and identification of V. cholerae

The rectal swab specimens were cultured directly on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar and incubated at 37°C

for 16-20 hours. Pre-enrichment of rectal swab samples in alkaline peptone broth was also carried out with subsequent subcultured on thiosulphate citrate bile salt sucrose (TCBS) agar and alkaline nutrient agar (ANA). Sucrose fermenting yellow colored colonies from TCBS were suspected as *V. cholerae* and the typical translucent colonies from ANA were confirmed by biochemical tests such as triple sugar iron test, lysin iron agar test, urease test, sulphide indole motility, citrate utilization test, salt tolerance test, string test, methyl red test and oxidase test.[12]

Serologic identification of V. cholerae O1 and O139

Serogroups was determined using a slide agglutination test with specific antisera against *V. cholerae* O1 and O139 (Denka Seiken, Tokyo, Japan). Serotype identification was also performed by Inaba and Ogawa antisera by slide agglutination test if the isolated *V. cholerae* was O1 serogroup and biotype identification was also done by Polymyxin B sensitivity test and Voges-Proskauer test to differentiate Classical or El Tor biotype.[12]

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of *Vibrio cholerae* was performed by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar (Beckton-Dickinson, USA) and E test for minimum inhibitory concentration.[13, 14] For disc diffusion test, commercially available discs (Oxoid Limited, England) such as doxycycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), amikacin (30 µg), cefotaxime (10 µg), norfloxacin (5 µg), chloramphenicol (30 µg) and azithromycin (15 µg) were used and for E test, E test strips (AB Biodisk, Sweden) of ciprofloxacin and azithromycin with the range of 0.16-256 µg/ml and 0.002-32 µg/ml were used, respectively. *Eschericia coli* ATCC 25922 strain was used as quality control strain.

Based on the zone size diameter according to the guidelines of Clinical Laboratory and Standard Institute (CLSI) and MIC values described by the manufacturer (AB Biodisk, Sweden), the isolate was interpreted as susceptible, intermediate and resistant.[15] In this study, the defining criterion for multiple drug resistance (MDR) was resistance to ≥3 of the antimicrobial agents belonging to different structural classes.[16]

Statistical analysis

Data entry was done in Excel spread sheet and data analysis was carried out by SPSS version 19.0.

Ethical consideration

Ethical approval was obtained from Research and Ethical Committee, University of Medicine 1 (Yangon).

RESULTS

Proportion of cholera cases among study population

Out of 250 children with acute diarrhoea cases, *V. cholerae* were isolated from 35 (14%) cases.

Age and sex distribution of cholera cases among study population

A total of 250 rectal swabs were tested for *V. cholerae* isolation. The age distribution of acute diarrhoea cases in this study showed that 28.4% were less than one year of age, 60% were between 1 to 5 years of age and 12% of total were between 6 to 12 years. Among them, *V. cholerae* was isolated from 8.6% (3/35) in the age group of less than one year, 57.1% (20/35) in the age group of 1 to 5 years and 34.3% (12/35) in the age group of six to twelve years. The sex distribution of cholera cases in this study revealed that 57.1% were males and 42.9% were females (Table 1).

Clinical presentations according to Vibrio cholerae culture-positive cases

Stool frequency during 24 hours prior to admission in relation to *V. cholerae* culture positivity was noted and most of the cases (62.9%, 22 cases) passed 6-10 times.

Most of the cases (71%, 20 cases) vomited 3-5 times and (8%, 2 cases) were associated with no vomiting. Other clinical presentations are shown in Table 2.

Table 1. Age and sex distribution of *V. cholerae* in study population

Variables	Cases	
	Acute diarrhoea n=250 (%)	*V. cholerae* culture-positive (n=35) (%)
Age (year)		
<1	71(28.4)	3(8.6)
1-5	149(59.6)	20(57.1)
6-12	30(12)	12(34.3)
Sex		
Male	158(63.2)	20(57.1)
Female	92(36.8)	15(42.9)

Table 2. Clinical presentation according to *Vibrio cholerae* culture-positive cases

Clinical presentations	*V. cholerae* culture-positive (n=35), No.(%)
Dehydration status	
No dehydration	2(5.7)
Some dehydration	20(57.2)
Severe dehydration	13(37.1)
Frequency of stool (times/ 24 hours)	
3-5	11(31.5)
6-10	22(62.9)
11-15	1(2.8)
16-20	1(2.8)
Frequency of vomiting (times/ 24 hours)	
No vomiting	2(5.7)
3-5	23(65.8)
6-10	10(28.5)
11-15	0(0)
Other clinical presentations	
Fever	12(34.3)
Shock	13(37.1)
Convulsion	2(5.7)
Abdominal pain	6(17.2)
Sunken eyes	33(94.3)
Loss of skin turgor	33(94.3)

Serogroups and serotype of isolated V. cholerae

All isolated *V. cholerae* in this study were identified as serogroup O1 of Ogawa serotype and biotype El Tor.

Antibiotics susceptibility pattern of isolated V. cholerae by disc diffusion method

The antibiotic susceptibility pattern determined by using disc diffusion method is shown in Fig. 1. *Vibrio cholerae* isolates were resistant to nalidixic acid and cotrimoxazole (100% each), doxycycline (20%) and amikacin (14.3%). They showed moderate resistance to antibiotics such as amikacin (22.9%) and doxycline (17.2%).

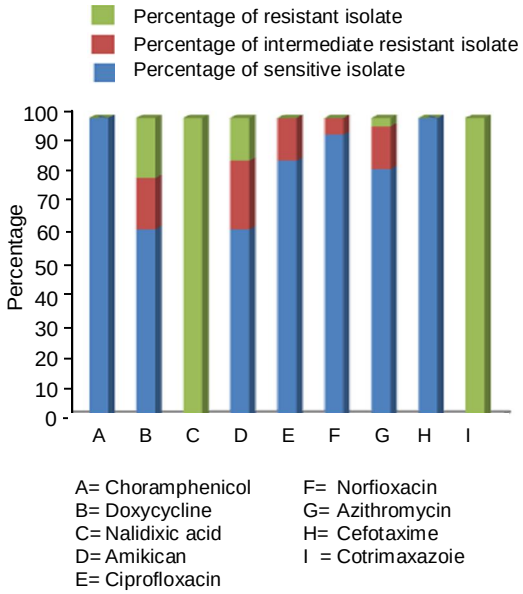

Fig. 1. Antibiotics susceptibility pattern of isolated *V. cholerae* by disc diffusion method

The isolates were sensitive to choramphenicol and cefotaxime (100% each), norfloxacin (94.3%), ciprofloxacin (85.7%) and, doxycycline and amikacin (62.8%, each). It was found that most of the isolates showed high resistance to drugs commonly used for children with acute diarrhoea such as naildixic acid and cotrimoxazole. Among the antibiotics that are prescribed for children, norfloxacin and ciprofloxacin were found to be highly effective against cholera and azithromycin was found to be (82.8%) sensitive.

Table 3. Range of MIC values (µg/ml) of ciprofloxacin and azithromycin by E test in *V. cholerae* culture-positive cases

Antibiotic (MIC range on E test strip)	Sensitivity pattern	No. of strains	MIC values of tested *V. cholerae* (µg/ml)
Azithromycin (0.016-256 µg/ml)	Susceptible	29	0.125-0.50
	Intermediate	5	0.75-1.0
	Resistant	1	32
Ciprofloxacin (0.002-32 µg/ml)	Susceptible	35	0.25-0.50
	Intermediate	0	0
	Resistant	0	0

Range of MIC values (μg/ml) of ciprofloxacin and azithromycin by E test in V. cholerae culture-positive cases

Antibacterial activities of azithromycin and ciprofloxacin to cholera were tested by E test and expressed in range of minimum inhibitory concentrations as shown in Table 3. High level azithromycin resistance (MIC=32 μg/ml) was seen in one *V. cholerae* isolate.

V. cholerae strains with multiple resistance to antibiotics

V. cholerae strains that were resistant to at least 3 or more antibiotics prescribed in current use for children were also analyzed. A total of 13 multiple drug resistant *V. cholerae* strains were detected among the drug resistant isolates. Seven isolates were resistant to nalidixic acid, cotrimoxazole and doxycycline. Five isolates were resistant to nalidixic acid, cotrimoxazole and amikacin and another one isolate was resistant to nalidixic acid, cotrimoxazole and azithromycin.

DISCUSSION

In the present study, among 250 rectal swab samples from children presenting with acute diarrhoea, 35(14%) were found to be *V. cholerae* culture positive. In a hospital-based study of India in 2006, the detection rate of *V. cholerae* in children with acute diarrhoea was 24.6%.[9] Their detection rate was higher than that of the present study. The detection rate of *V. cholerae* from children with acute diarrhoea was reported varying from 5-20% in Yangon between 1999 and 2012, which was found to be similar to the detection rate of the present study.[10, 17-19]

According to this study, proportion of cholera from children with acute diarrhoea may still constitute as one of the causes of acute diarrhoea among childhood population. In the present study, despite a target age of 0-12 years, 88% of patient with acute diarrhoea were between 0-5 years. The majority of the *V. cholerae* encountered were from children aged 1-5 years among culture-positive cases. This is in agreement with another report from children with acute diarrhoea in Myanmar in 2012, which found the most common age group to be 1-5 years, followed by age 6-12 years.[10] The youngest and the oldest age of patients affected in the present study were 3 months and 11 years, respectively. The sex distribution of cholera cases in this study revealed that male was 57.2% with female comprising of 42.9% as were in report of other countries.[20, 21] This finding is also similar with that of previous study of children with acute diarrhoea in Yankin Children's Hospital during 2012 in which 11(55%) cases were males and 9 (45%) cases were females among *Vibrio cholerae* culture-positive cases.[10]

According to this study, the majority of *V. cholerae* (94.3%, 13/35) was found in children presenting with moderate and severe dehydration and it is similar to other studies in Myanmar.[10, 21] Almost all isolated cholera cases in this study presented with vomiting but two cases were associated with no vomiting. Most patients (65.8%) presented with 3-5 times of vomiting. Regarding the frequency of diarrhoea, 65% had 6-12 times on 24 hours prior to admission. Fever was noted in 12 cases (43%), sunken eyes and loss of skin turgor were present in 82.5% of *V. cholerae* culture-positive cases. Other presentations include convulsion (7%) and abdominal cramps (21%), respectively. Another study of children with acute diarrhoea in India also reported that *V. cholerae* infection in children also presented with these clinical presentations.[21]

Isolated *V. cholerae* in this study were identified as serogroup O1 of Ogawa serotype and El Tor Biotype and in agreement with the earlier observations by other researchers.[22, 23] Although the finding of this study reflects the predominant of *V. cholerae* O1, particular attention should be done to detect that strain in children. ElTor biotype showed mild cholera

symptoms than Classical biotype. There is no mortality reported in cholera cases isolate. This finding consider into the fact that El Tor biotype had mild clinical symptoms.

All *V. cholerae* isolates were 100% susceptible to chloramphenicol and cefotaxime. Although findings of the present study showed 85.7% susceptibility to ciprofloxacin, there were reports of emergence of ciprofloxacin resistance in other parts of the world.[24, 25] Other drugs with high susceptibility were norfloxacin (94.3%), azithromycin (82.8%), doxycycline and amikacin (62.8%, each). The isolated strains were fully resistant to cotrimoxazole and naildixic acid.

These findings pointed out the changing pattern of sensitivity to cotrimoxazole and nalidixic which are commonly used in children with acute diarrhoea.[10] Class of antibiotics commonly used for *V. cholerae* included aminoglycoside, macrolide, tetracycline and quinolone. Reports from Myanmar and other countries such as India, Bangladesh, Nepal and Haiti showed that there were still highly resistance to cotrimoxazole and nalidixic acid and they were still sensitive to ciprofloxacin and azithromycin.[20-24]

Data from the National Health Laboratory, Yangon, in 2012 also revealed that *V. cholerae* isolated in laboratory were fully sensitive to chloramphenicol and norfloxacin, and resistant to cotrimoxazole. A study carried out in Yankin Children's Hospital in 2012 reported that isolated *V. cholerae* in children were sensitive to norfloxacin, chloramphenicol, ciprofloxacin and cefotaxime.[10] These findings are in agreement with the present study. Thus, the finding of this study can also validate their use in cholera treatment and prophylaxis in children with cholera.

In the present study, the MICs of ciprofloxacin and azithromycin on 35 *V. cholerae* isolates were determined by E test. All *V. cholerae* O1 isolates in this study were susceptible to ciprofloxacin not only an according to the MIC but also the disk diffusion method. Although, there was no ciprofloxacin resistance detected, by E test in the present study, the MIC level of ciprofloxacin was found to be high compared to other studies.[26, 27]

In most developing countries, there were uncontrolled uses of broad-spectrum antibiotics and inappropriate prescribing of these by unskilled health workers or by traditionally healers. In addition to these, as a public health measure, antibiotics were often prescribed on a large scale for prophylaxis during epidemics and thus the benefit to individual was usually offset by the rapid emergence of resistance.

Although cotrimoxazole was recommended as the first-line drug for children by WHO (2001), finding of resistance to this drug in this study highlighted the ineffectiveness of this drug in treatment of cholera for children in our locality. Since the specimens taken in this study were from children with acute diarrhoea attended to limited hospitals, further studies that encompass more children in wide areas of the countries will be necessary for more applicable information.

Multiple drug-resistant cholera is being reporting from India and elsewhere in the neighbourhood.[28-30] *V. choleare* can become multiple antibiotic resistant via the acquisition of plasmid and have repeatedly caused epidemics. In the present study, multiple antibiotics resistance was isolated from 37.1% (13/35) among drug resistant strains and it indicates the possibility of mobilization of resistance markers among isolates and calls for further studies.

The finding of antibiotic susceptibility pattern in this study is only on phenotype, so that genotyping profile of *V. cholerae* strains in children is necessary so as to detect the strains which passed a class 1 integron, a necessary vehicle for the acquisition of antibiotic resistance genes, thus suggesting that these strains may acquire resistance in the near future. Since

antimicrobial susceptibility of *V. cholerae* strains varies with geographical regions and testing method used, MIC determination by a standardized method is needed to describe reliable susceptibility patterns for both epidemiological surveillance and patient's management. Longitudinal surveillance and antimicrobial susceptibility of *V. cholerae* using MIC method can provide early detection of emergence of resistant strains. Up to our knowledge, there are no published reports on MIC of antibiotics for *V. cholerae* infection of children in Myanmar, these results could provide valuable baseline information for the infection control programs regarding management and transmission of *V. cholerae* infection in children.

Conclusion

The present study revealed the occurrence of childhood cholera in Yangon and determined the antibiotic susceptibility profile including minimum inhibitory concentration of ciprofloxacin and azithromycin which are common antibiotics currently used for treatment of childhood cholera in endemic areas. These findings may contribute to effective management and control strategies for cholera among pediatric population. The phenotypic characteristics such as serotypes and biotypes will provide information on currently circulating *V. cholerae* strains in Myanmar and choice of effective cholera vaccine program for children in Myanmar.

REFERENCES

1. World Health Organization. The global burden of cholera. *Bulletin of the World Health Organization*, Geneva, 2012.
2. Safa A, Sultana J, Dac CP, Mwansa JC & Kong RY. *Vibrio cholerae* O1 hybrid El Tor strains, Asia and Africa. *Emerging Infectious Diseases* 2008; 14: 987-988.
3. Than Htein Win. Effectiveness of oral cholera vaccination on prevention and control of severe diarrhoea disease in high risk areas. In: *Proceeding of Symposium on Effects of Environmental Changes on Health*, Myanmar Health Research Congress 2010; 40-50.
4. Ministry of Health, Epidemiological Surveillance and Response. In: *Annual Public Health Statistics Report* 2009: 25.
5. Aye Aye Han. Typing of antibiotic susceptibility patterns of isolated *Vibrio cholerae* from cholera cases in Mandalay Division. *Thesis*, M.Med.Sc (Microbiology), University of Medicine (Mandalay), 2004.
6. National Health Laboratory (Yangon). Isolated *Vibrio cholerae* strains in 2011 (Unpublished data).
7. Nishibori T, Vries GC, Rahardjo D, Wasito EB, Kinoshita S, Hayashi Y, *et al*. Phenotypic and genotypic characterization of *Vibrio cholerae* clinically isolated in Surabaya, Indonesia. *Japanese Journal of Infectious Diseases* 2011; 64: 7-12
8. Amin V, Patwari A, Kumar G, Anand V, Diwan N & Peshin S. Clinical profile of cholera in young children. *Indian Journal of Pediatrics* 1995; 32: 755-759.
9. Kuttiat VS, Lodha R & Kohli BD. Prevalence of cholera in pediadric patients with acute dehydrated diarrhoea. *Indian Journal of Pediatrics* 2010; 77(1): 67-71.
10. Nyein Nyein Zaw. Clinical profile of acute diarrhoeal diseases in children aged 2 months to 5 years with positive bacterial culture results and negative bacterial culture results. *Thesis*, M.Med.Sc (Pediatrics), University of Medicine 2, 2012.
11. Jain M, Goel AK, Bhattacharya P, Ghatole M & Kamboj DV. Multidrug resistant *Vibrio cholerae* O1 El Tor carrying classical ctxB allele involved in a cholera outbreak in south Western India. *Actalantic Tropical Medicine* 2011; 117: 152-156.
12. World Health Organization. In: *Manual of Diagnostics Laboratory Procedure for Acute Enteric Infection*, Geneva, 2003; 3.
13. Bauer Aw, Kirby WMM, Sherris JC & Turk M. Antibiotics susceptibility testing method by a standardized single disk method. *American Journal of Clinical Pathology* 1966; 4: 493-496.
14. Brown DF & Brown L. Evaluation of the E test, a novel method of quantifying antimicrobial activity. *Journal of Antimicrobial Chemotheraphy* 1991; 27:185-90.
15. CLSI, Clinical Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing; CLSI document, Clinical and Laboratory Standard Institute, Wayne, USA, 2011.
16. Wright SW, Wrenn KD, Haynes M & Haas DW. Prevalence and risk factors for multidrug resistant uropathogens in ED patients. *American Journal of Emerging Medicine* 2000; 182: 143-146.

17. Ni Ni Aye. Study of toxigenicity and antibiotics sensitivity of *Vibrio cholerae* O1 and O139 strains isolated from acute gastric cases. *Thesis*, M.Med.Sc (Microbiology), University of Medicine 1, 1997.

18. Thin Thin Mar. A study of some parasitic enteropathogen in children under five with acute diarrhoea. *Thesis*, M.Med.Sc (Microbiology), University of Medicine 1, 2003.

19. Soe Soe Myint. A study on clinical profile and aetiological agents in acute diarrhoea cases admitted to Yangon Children's Hospital. *Thesis*, M.Med.Sc (Pediatrics), University of Medicine 1, 1998.

20. Harris JB, LaRocque R, Ryan ET & Calderwood SB. Cholera. *Lancet* 2012; 379: 2466-2476.

21. Sharma NC, Mandal PK, Dhillon R & Jain M. Changing profile of *Vibrio cholerae* O1, O139 in Delhi & its periphery (2003-2005). *Indian Journal of Medical Research* 2007; 125: 633-40.

22. Kaushik JS, Gupta P, Faridi MMA & Das S. Single dose azithromycin *versus* ciprofloxacin for cholera in children: A randomized controlled trial. *Indian Pediatrics Journal* 2010: 47: 309-315.

23. Mar Mar Nyein, Wah Wah Aung, Mi Mi Htwe, Tin Ohn & Than Nu Shwe. Antibiotics susceptibility pattern of *Vibrio cholerae* O1, O139, *Salmonella typhi* and *Escherichia coli*. *Myanmar Health Sciences Research Journal* 2000; 12(3): 30-35.

24. Mukhopadhyay AK, Basu I, Bhattacharya SK, Bhattacharya MK & Nair GB. Emergence of fluoroquinolone resistnace in strains of *Vibrio-cholerae* isolated from hospitalised patients with acute diarrhoea in Calcutta, India. *Antimicrobial Agents Chemotheraphy* 1998; 42: 206-207.

25. Pancharren C, Mekmullica J, Niwattanakanjana N & Chongrisawat V. Hospital-based epidemiology of childhood cholera: A 6-year review in a University Hospital in Bangkok, Thailand. *Journal of Medical Association* Thailand 2004; 87: 59-61.

26. Khan WA, Bennish ML, Seas C, Khan EH, Ronan A, Dhar U, *et al.* Randomised controlled comparison of single-dose ciprofloxacin and doxycycline for cholera caused by *Vibrio cholerae* O1 or O139. *Lancet* 1996; 348: 296-300.

27. Saha D, KhanWA, Karim MM, Chowdhury HR, Salam MA & Bennish ML. Single dose ciprofloxacin *versus* 12 dose erythromycin for childhood cholera: A radomised controlled trial. *Lancet* 2005; 366:1085-93.

28. Bhattacharya D, Sayi DS, Thamizhmani R, Bhattacharjee H, Bharadwaj, AP, Roy A, *et al.* Emergence of multidrug-resistant *V. cholerae* O1 Biotype El Tor in Port Blair, India. *American Journal of Tropical Medicine and Hygiene* 2012; 86: 1015-1017.

29. Das S, Saha R & Kaur IR. Trend of antibiotic resistance of *Vibrio cholerae* strains from east Delhi. *Indian Journal of Medical Research* 2008; 127: 478-482.

30. Urassa WK, Mhando YB, Mhalu FS & Mjonga SJ. Antimicrobial susceptibility pattern of *Vibrio cholerae* O1 strains during two cholera outbreaks in Dares Salam, Tanzania. *East Africa Medical Journal* 2000; 77: 350-3.

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Bio-efficacy of Brand-new and Long-term Used Unwashed PermaNet 2.0 against *Anopheles dirus, Aedes aegypti* and *Culex quinquefasciatus*

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Bio-efficacy of unwashed PermaNet 2.0 in relation to the period of long-lasting insecticide nets (LLINs) against laboratory reared F1 generation of 3-5 days old *Anopheles dirus*, *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes were investigated in the laboratory of Medical Entomology Research Division, Department of Medical Research (Lower Myanmar) from February 2013 to January 2014. The bioefficacy of unwashed brand-new, 1-, 3- and 5-year duration of used PermaNet 2.0 was evaluated with the standard bioassay cone test method following WHO guidelines. It was found that the efficacy of unwashed brand-new, 1-, 3-, and 5- year old PermaNet 2.0 was fully recovered by 24 hours against *An. dirus*, i.e., 100% knockdown and 86-100% mortality. *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes were found to have 100% knockdown and mortality against brand new PermaNet 2.0 and 90-96% knockdown and 80-84% mortality against one-year duration of unwashed PermaNet 2.0. Effective bio-efficacy was reduced after 3- and 5-year duration of unwashed PermaNet 2.0 when tested with *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. Dose effect analysis of these three mosquitoes revealed that *An. dirus* was the most susceptible mosquito to deltamethrin insecticide followed by *Ae. aegypti* and *Cx. quinquefasciatus*. This study concluded that unwashed PermaNet 2.0 is suitable for long-term use against *An. dirus* in remote malaria endemic areas although it can protect for only one year from *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

Key words: PermaNet 2.0, *An. dirus, Ae. aegypti, Cx. quinquefasciatus*, Knockdown, Mortality

INTRODUCTION

Malaria is one of the priority diseases in Myanmar. It is a re-emerging public health problem due to climatic and ecological changes, population migration, development of multidrug-resistant *Plasmodium falciparum* parasite, development of insecticide-resistant vectors and changes in behavior of malaria vectors. Long-term trend shows decreasing malaria morbidity and mortality in Myanmar. The two major vectors for malaria transmission are *Anopheles dirus* and *An. minimus*. *Anopheles dirus* is mostly found in deep forest areas and also found in well breeding in coastal areas of Mon State and Thaninthayi Region. *Anopheles minimus* is mostly found in forest fringe and plain areas. *Anopheles annularis* is responsible for local malaria transmission in Rakhine State and it is highly resistant to DDT. *Anopheles sandaicus* is a main vector for malaria transmission in coastal regions. Scaling up the Long Lasting Insecticidal Nets (LLIN) and Insecticide Treated Net (ITN) programme throughout the country has been a part of the approaches to control malaria in Myanmar.

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Selective and sustainable preventive measures are carried out emphasizing on personal protection and environmental management. With limited resources, areas were prioritized for ITN programme either distribution of LLINs or impregnation of existing nets. In 2010, 78,960 LLINs were distributed and 515,200 existing nets were impregnated in 2,574 villages of 36 endemic townships particularly in hard-to-reach areas.[1]

Total population covered by ITN programme was 1,485,400. Numbers of epidemics were reduced during last five years due to early case detection, management and preventive measures like indoor residual spray (IRS) and impregnation of existing bed nets in epidemic areas.[1] Nowadays, different brands of LLINs were distributed in malaria prone areas and hard-to-reach areas by vector-borne diseases control programme (VBDC) MOH, Government societies, NGOs and INGOs in different parts of the country.

Malaria is one of the main mosquito-borne diseases transmitted by *Anopheles mosquitoes*. The mosquito *Culex quinquefasciatus* (Diptera: Culicidae) is the vector of filarial parasite *Wuchereria bancrofti* which causes bancroftion filariasis in humans.[2] *Cx. quinquefasciatus* is also a vector of West Nile virus[3] and Japanese encephalitis virus.[4] The mosquito *Aedes aegypti* (Diptera: Culicidae) transmits viral pathogens of humans, including yellow fever[5] and dengue[6] both of which can cause severe human morbidity and mortality.

Long-lasting insecticide treated nets (LLINs) have emerged as a potent tool in lowering morbidity and mortality of mosquito-borne diseases. In Myanmar, different brands of LLINs and WHO recommended Olyset net and especially PermaNet 2.0 (LLINs) have been widely distributed to reduce malaria transmission in endemic areas. PermaNet 2.0 with deltamethrin concentration at 55 mg/m^2 incorporated into a resin coating in the fibers at the factory were evaluated for bio-efficacy in Pakistan, Tanzania, India, Uganda, and a few other countries and reported to be functioning well even after repeated washing.[7-9]

Insecticide treated nets (ITNs) and LLINs led to a reduction of human-vector contact and diminish mosquito population[10] and also provided a physical barrier with high coverage levels that benefit the whole community.[11] However, efficacy of long-term insecticidal action of LLINs depends on the washing frequencies of the nets during the recommended period and duration of the time of unwashed used LLINs nets within or after the recommended period. Based on previous studies, it has been shown that exposure of climate, repeated washing and long-term use of LLINs can result in reduction of insecticidal efficacy.[12, 13]

Moreover, there is a lack of information about the impact of long-term use of unwashed LLINs nets on insecticidal action in Myanmar. Therefore, bio-efficacy of unwashed PermaNet 2.0 in relation to the period of nets against *Anopheles, Culex* and *Aedes* mosquitoes were investigated to provide information concerning retention capability of LLINs.

MATERIALS AND METHODS

Study place and study period

The study was conducted in the laboratory of Medical Entomology Research Division, Department of Medical Research (Lower Myanmar) from February 2013 to January 2014 (one year study). A laboratory-based descriptive study was done with laboratory-reared *Anopheles, Aedes* and *Culex* mosquitoes against brand-new and three different durations of unwashed PermaNet 2.0.

Mosquito collection

Anopheles mosquitoes from Mudon Township, Mon State and *Aedes* and *Culex* mosquitoes from north Dagon Township, Yangon Region were collected and identified by morphological methods according to standard procedure.[14-16] Collected mos-

quitoes were reared to F_1 generation at insectary of Medical Entomology Research Division in order to test the susceptibility status of mosquitoes against different durations of unwashed PermaNet 2.0 LLINs.

Preparation of mosquito net samples

(i) Two numbers each of brand-new and three different durations of unwashed PermaNet 2.0 long-lasting insecticidal nets (M/S Vestergaard Frandsen, Denmark, made of polyester netting material, Deltamethrin 55 mg/m^2, Factory product 2011) were used to test for bioefficacy against laboratory-reared three different species of mosquitoes.

(ii) Brand-new PermaNet 2.0 net was obtained from Vector Borne Diseases Control, Ministry of Health. Five years old unwashed used nets were collected from Magway and Bago Regions with the help of PhD students who did their work there for five years. Three years old nets were collected from Parasitology Research Division and one year old nets were collected from Medical Entomology Research Division, those utilized the mosquito nets in the malaria field surveys. After survey, the nets were folded in plastic bags and kept in the cardboard box in the laboratory. All collected nets were kept in individual black plastic bags in cardboard box at laboratory condition in Medical Entomology Research Laboratory till test.

(iii) Untreated standard polyester net purchased from local market was used as control.

Bioassay test

Bioassay test was conducted with net samples in three different durations of unwashed PermaNet 2.0 nets and untreated nets (as control) using WHO contact cone test method.[10] Plastic cones were fixed on five sites of each PermaNet 2.0 and untreated nets horizontally. After that, laboratory-reared 3-5 days old 5 mosquitoes of F_1 generation of *Anopheles, Aedes* and *Culex* mosquitoes, respectively, were exposed in attached cones for 3 minutes. After 3 minutes exposure, tested mosquitoes were transferred into holding paper cups with a net cover. Ten percent glucose cotton wool was supplied as food. Temperature and moisture were maintained by covering with water soaked damp towel. Knockdown effect was recorded after 60 minutes recovery period and subsequently, overall mortality rate was recorded after 24-hour post exposure period. Duplicate tests were done with remaining LLIN nets.

Insecticide impregnated paper for dose effect analysis testing

Deltamethrin (2.5 EC), was diluted from 0.1 g/l to 0.000000381469 g/l concentration by using serial dilution method and each individual dilution was impregnated on individual Whatman filter paper (3 mmx 15 cmx 11.6 cm) and dried in a shady and ventilated place. Dried insecticide treated papers were stored in black plastic bags in a cool and dry room till the susceptibility test. Five F1 generations of *Aedes, Culex* and *Anopheles* mosquitoes were exposed to the treated filter papers for one hour. After one hour exposure period, tested mosquitoes were transferred into holding tubes.

Ten percent glucose cotton wool was supplied as food and temperature and moisture were maintained by covering with water soaked damp towel. Overall mortality rate was recorded after 24-hour post exposure period.[17] Duplicate tests were done for dose effect analysis test.

Data analysis

The data were analyzed with SPSS software applying Student 't' test and ANOVA (Analysis of variance) test. Dose effect analysis was calculated according to Finney.[12]

RESULTS

Knockdown and mortality effect

All tested laboratory-reared 3-5 days old F1 generation of *An. dirus, Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes were found to have 100% knockdown against brand new PermaNet 2.0. *An. dirus* was found to

have 100% knockdown against one, three and five years duration of PermaNet 2.0. Next line *Ae. aegypti* and *Cx. quinquefasciatus* were found to have 96% and 90% knockdown against one year PermaNet 2.0 although bio-efficacy had declined after three to five years used nets without washing (Table 1).

Table 1. Percent knockdown of *Anopheles*, *Aedes* and *Culex* mosquitoes against different durations of unwashed PermaNet 2.0

Mosquitoes species	(Percent Knockdown) Different durations of unwashed PermaNet 2.0, No. (%)				
	Brand new	One year	Three years	Five years	Control*
An. dirus	50 (100)	50 (100)	50 (100)	50 (100)	0
Ae. aegypti	50 (100)	48 (96)	38 (76)	32 (64)	0
Cx. quinquefasciatus	50 (100)	45 (90)	35 (70)	30 (60)	0

*=Untreated net

Mortality rate

The bio-efficacy of brand-new, one- and three-year used unwashed PermaNet 2.0 nets were found to be 100% mortality and 86% mortality with five years duration of net against *An. dirus* (Fig. 1).

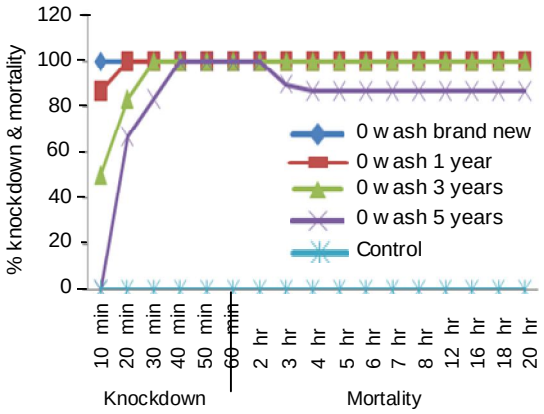

Fig. 1. Percentage knockdown and mortality of *An. dirus* within 24 hours against different duration of unwashed Perma-Net 2.0 long-lasting insecticide nets

Effective mortality of *Aedes* and *Culex* mosquitoes were found to be 100% for brand-new and 80-84% for one-year PermaNet 2.0. Three and five years Perma-Net 2.0 showed reduced bio-efficacy of both nets, i.e., 72% and 60% mortality for *Ae. aegypt*i and 64% and 52% mortality for *Cx. quinquefasciatus,* respectively (Table 2).

Table 2. Percent mortality of *Anopheles*, *Aedes* and *Culex* mosquitoes against different durations of unwashed PermaNet 2.0

Mosquitoes species	Different durations of unwashed PermaNet 2.0 Mortality [No. (%)]				
	Brand new	One year	Three years	Five years	Control*
An. dirus	50 (100)	50 (100)	50 (100)	43 (86)	0
Ae. aegypti	50 (100)	42 (96)	36 (76)	30 (60)	0
Cx. quinquefasciatus	50 (100)	40 (90)	32 (70)	26 (52)	0

*=Untreated net

Dose effect analysis

The results were analyzed by using probit regression analysis curve[18] to obtain the LC_{50} and LC_{90} values. The susceptibility of F1 generation of 3-5 days old laboratory-reared adult mosquitoes to deltamethrin is presented in Table 3.

Table 3. Toxicity effect of 95% confidential limits of corrected LC_{50} and LC_{90} values of the deltamethrin against *Ae. aegypti*, *Cx. quniquefasciatus* and *An. dirus* mosquitoes

Mosquitoes species	Corrected deltamethrin value				
	LC_{50}		LC_{90}		
	g/liter	Folds*	g/liter	Folds*	X^2
Ae. aegypti	0.000118	14.75	0.000435	11.87	1.2563
Cx. quinque-fasciatus	0.000256	32.00	0.000905	24.66	3.1480
An. dirus	0.000008	1	0.000037	1	0.7846

*=Against *An. dirus*

The study revealed that *An. dirus* was found to be the most susceptible mosquito to deltamethrin insecticide and their 95% confidence interval corrected LC_{50} value was 0.000008 g/l. Highest LC_{50} value 0.000256 g/l was observed against *Cx. quinquefasciatus* mosquito followed by *Ae. aegypti* 0.000118 g/l, respectively. Same trends of LC_{90} values were observed against all tested mosquitoes and *An. dirus*

was found to have lowest LC_{90} value (0.000037 g/l) and *Cx. quinquefasciatus* had highest LC_{90} value (0.000905 g/l). *Culex quinquefasciatus* was also found to have the highest tolerance rate, with corrected LC_{50} and LC_{90} values 32 fold and 24.66 folds higher when compared with those of *An. dirus*, followed by *Ae. aegypti* 14.75 fold and 11.87 fold, respectively (Table 3).

DISCUSSION

Anopheles dirus and *An. minimus* are main vectors for human malaria in Myanmar and Thailand,[19-21] although *Anopheles quadri-cmaculatus* is a vector of malaria in North America, and *An. culicifacies* and *An. stephinsii* in India.[22] Synthetic pesticides have been extensively used for mosquito control by either killing, preventing adult mosquitoes biting human beings or by killing mosquito larvae at the breeding sites of the vectors.[23] Nowadays, WHO recommended synthetic pesticides are deltamethrin, permethrin, alpha cypermethrin and lambda cyhalothrin and those have been extensively used for treatment to the net fibers in factory-made long-lasting insecticide nets (LLINs) or impregnation of mosquito nets in the field to prevent from mosquito bite.

The present study found high bio-efficacy of different durations of unwashed PermaNet 2.0 against *An. dirus, Ae. aegypti* and *Cx. quinquefasciatus*. The brand-new and one-year duration used PermaNet 2.0 were very effective to destroy all tested laboratory-reared 3-5 days old mosquitoes. *An. dirus* was found to have 100% knockdown against brand-new, one-, three- and five-year duration of unwashed PermaNet 2.0.

Regarding mortality, it was found 100% against brand-new, one-, three-year and 86% mortality against five-year duration of unwashed PermaNet 2.0 nets. The results agreed with those of Indian researchers working on unwashed PermaNet in India which found 100% mortality in both *An. minimus* and *An. dirus*.[24] In Western Kenya, 82.2% mortality of *An. gambia* was recorded after 2 years of field-used PermaNet.[25] A study found 80% mortality of *An. nunentovari* and *An. rangeli* after three years of field-use of PermaNet in Columbia.[11]

Pyrethroid insecticides are neurotoxins and share many characteristics with DDT including a negative temperature coefficient, a rapid knockdown effect followed by a lethal effect.[26] The knockdown effect is caused by effect on the peripheral nerves [27] and the lethal effect is due to an irreversible damage to both the peripheral and central neurons which occurs when poisoning is prolonged.[28]

Culex quinquefasciatus and *Ae. aegypti* mosquitoes were found to have 90-100% knockdown and 80-100% mortality against brand-new and one-year old PermaNet 2.0. There was no significant difference in knockdown and mortalities between species indicating that brand-new and one-year old PermaNet 2.0. are highly effective against all tested vector mosquitoes. A study in Vietnam reported that adulticidal activity of Olyset nets remained 100% up to 8 months on *Ae. Aegypti*.[29]

The bio-efficacy of 3- and 5-year old PermaNet 2.0 showed low mortality against *Culex* and *Aedes* mosquitoes, gradually declining to 60% knockdown and 52% mortality for *Cx. quinquefasciatus* and 64% knockdown and 60% mortality for *Ae. aegypti* mosquitoes. It may be due to the fact that *Cx. quinquefasciatus* and *Ae. aegypti* have some level of tolerability to pyrethroid insecticides than *An. dirus*. In 1991, there was a reduction in permethrin susceptibility in *An. gambiae* one year after introduction of small-scale pyrethroid treated nets in Kenya.[12] The resistance is mainly associated with target site in sensitivity arising from a single point mutation in the sodium channel gene, often referred to as knockdown resistance (kdr) characterized by leucine-phenylalanine mutation.[30]

In Myanmar, long-lasting insecticide treated nets (LLINs) and insecticide treated nets (ITNs) are widely distributed in malaria endemic areas as PermaNet 2.0 in Thanbyuzayet Township by IOM and VBDC, PermaNet 2.0 in Kamamaung Sub-township by three diseases fund (3DF), and deltamethrin treated nets in PyinOoLwin Township by NGOs.

The main reason for insecticide treated nets acceptability was effectiveness in killing mosquitoes and other insects.[31] The first time ITN and IRS intervention measures were undertaken in 10 villages which are remote, underserved, forested, hilly areas of Bago Yoma in 1995 and morbidity and mortality of malaria was significantly reduced in the study villages, more so with the insecticide treated bednet intervention.[32]

In malaria endemic area of Padaung Township, Bago Region, PermaNet nets are effective against the *Anopheles* vectors only for less than 6 months after distributing. Bioassay results indicated high knockdown rates within 60 minutes.[33] PermaNet were excellent as more than 90% of families in Zayethla area, Padaung Township used LLINs (PermaNet) throughout the year and significant reductions in parasite positive rate and the spleen rate of children highlighted the effectiveness of LLINs.[34]

The LC_{50} and LC_{90} values for *An. dirus* was lowest to deltamethrin compared to *Cx. quinquefasciatus*. It was found to have 32 and 24.66 fold higher tolerability to deltamethrin and *Ae. aegypti* was found to have 14.75 and 11.87 fold higher tolerability to deltamethrin than *An. dirus*. The development of resistance to various types of insecticides such as organochlorines, organophosphates and carbamates poses a serious threat to the conventional control measures for vectors, especially the mosquitoes.[35] At the same time many of these insecticides are egarded as environmental pollutants and create health hazards. Currently, synthetic pyrethroids (such as permethrin, deltamethrin and lambda cyhalothrin) are being used to control insect vectors because of their biodegradable nature, low mammalian toxicity without any harmful residual effect and higher efficacy against the target species and resistant vector population.[36] WHO gives full recommendation for Olyset Net (permethrin treated net) and an interim recommendation of PermaNet 2.0, Duranet, Net Protect and Interceptor for *Anopheles* mosquitoes to prevent mosquito bite and man-vector contact.[37]

This study concluded that brand-new and 1-, 3- and 5-year unwashed PermaNet 2.0 have best bio-efficacy against *An. dirus* and also brand-new and one-year PermaNet 2.0 have good bio-efficacy (effective mortality) against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. *An. dirus, Ae. aegypti* and *Cx. quinquefasciatus* are important vectors of vector-borne diseases in Myanmar. Generally *An. dirus* and *Cx. quinquefasciatus* are night biters and *Ae. aegypti* is a day biter. Therefore, the present study suggests to sleep in LLIN or ITN nets in day or night time. Bio-efficacy of long-term used PermaNet 2.0 declined from maximum to minimum after 3 years of using when tested with *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. This period is over the WHO recommended period of 2 years for unwashed LLINs and insecticides persist for 20 washes. *Aedes* and *Culex* mosquitoes may have developed pyrethroid tolerability or bio-efficacy of pyrethroid insecticides may have reduced in long-term using the LLINs.

Therefore, follow-up studies are needed to investigate the level of tolerability to pyrethroid in the mosquito population and to determine the residual properties of LLINs to support the mosquito-borne diseases control programme in Myanmar.

REFERENCES

1. Ministry of Health. *Health in Myanmar* 2011; 81-84.
2. Samuel PP, Arunachalam N, Hiriyan J, Thenmoxhi V, Gajanana A & Satyanarayana K. Host feeding pattern of *Culex quinquefasciatus*

Say and *Mansonia annulifera* (Theobald) (Diptera: Culicidae), The major vectors of filariasis in a rural area of south India. *Journal of Medical Entomology* 2004; 41: 442-446.

3. Godsey MS, Nasci JrR, Savage HM, Aspen S, King R, Powers AM, *et al*. West Nile virus-infected mosquitoes, Louisiana, 2002. *Emerging of Infectious Diseases* 2005; 11: 1399-1404.
4. Nitafpallana N, Apiwathnasorn C, Barbazan P, Leemingsawat S, Yoksan S & Gonzalez JP. First isolation of Japanese encephalitis virus from *Culex quinquefasciatus* in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 2005; 36: 875-978.
5. Aitken TH, Downs WG & Shope RE. *Aedes aegypti* strain fitness for yellow fever virus transmission. *American Journal of Tropical Medicine and Hygiene* 1977; 26: 985-989.
6. Degallier N, Herve JP, Travassos da Rosa AP & Sa GC. *Aedes aegypti* (L): Importance of its bioecology in the transmission of dengue and other arboviruses I. *Bulletin of Society of Pathology and Exot. Filiales* 1988; 81: 97-110.
7. Kroeger A, Skomand O, Phan QC & Boewond DT. Combined field and laboratory evaluation of a long-term impregnated bednet, PermaNet. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2004; 98:152-155.
8. World Health Organization. Review of Vectobac WG, PermaNet and Gokhilahts 5EC. Report of Seventh WHOPES working group meeting. (WHO/CDS/ WHOPES/ 2004.8: 29-57) WHO, Geneva, 2-4 December 2003.
9. Sreehari U, Mittal PK, Razdan RK, Ansari MA, Rizvi MMA & Dash AP. Efficacy of PermaNet 2.0 against *Anopheles culicifacies* and *Anopheles stephensi*, malaria vectors in India. *Journal of American Mosquito Control Association* 2007; 23: 220-223.
10. World Health Organization. Guideline for laboratory and field testing of long lasting insecticidal mosquito nets. WHO/ CDS/ WHOPES/ GCDPP/ 2005.
11. Gu W & Novak RJ. Predicting the impact of insecticide-treated bed nets on malaria transmission. *Malaria Journal* 2009; 8: 256.
12. Vulule JM, Beach RF, Atieli FK, Roberts JM, Mount DL & Mwangi RW. Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin impregnated bed-nets and curtains in western Kenya. *Medical Veterinary Entomology* 1994; 8(1): 71-75.
13. Rubaihayo J, Tukesiga E & Abaasa A. Reduced susceptibility to pyrethroid insecticide-treated nets by the malaria vector *Anopheles gambiae* s.l. in western Uganda. *Malaria Journal* 2008; 7: 92.
14. Harrison BA & Scanlon JE. Medical entomology studies II. The subgenus *Anopheles* in Thailand. *Contribution of American Entomological Institute* 1975; 12(1): 305.
15. Harrison BA. Medical entomology studies XIII. Myzomyia series of *Anopheles* (Cillia) in Thailand with emphasis on intraspecific variations (Diptera: Culicidae). *American Entomology Institute*1980; 17: 1-195.
16. Myo Paing, Thi Thi Naing, Sein Min & Zaw Myint. *Anopheline* mosquitoes of Myanmar III. *Anopheles (Cellia) Philippines* Ludlow 1902 and *Anopheles (Cellia) nivipes*. Theobald 1903 in Myanmar and their differentiating character. *Myanmar Health Sciences Research Journal* 1990; 2: 37-38.
17. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO informal Consultation. WHO/ CDC/ MAL/ 98.12. World Health Organization, 1998.
18. Finney DJ. Probit Analysis, Cambridge University press. Cambridge, United Kingdom, 1971.
19. Khin Maung Kyi. Malaria vectors in Burma: *Anopheles balabacensis balabacensis* Baisas, 1936. *Union of Burma Journal of Life Science* 1970; 3: 217-225.
20. Peyton EL & Harrison BA. *Anopheles* (*Cellia*) *dirus*, a new species of the Leucosphyrus group from Thailand (Diptera: Culicidae). *Mosquitoes System* 1979; 11: 40-52.
21. Peyton EL & Harrison BA. *Anopheles* (*Cellia*) *Takasagoensis morishita* 1946, an additional species in the *Balabacensis* complex of Southeast Asia (Diptera: Culicidae). Mosquitoes System 1980; 12: 335-347.
22. Sharma VP, Kalra NL, Patanayak S, Sharma RS & Orlav VS. Revised Report National Malaria Control, Malaria Research Centre, Delhi, 1995; 20.
23. Joshep CC, Ndoils MM, Malima RC & Nkuniya NHM. Larvicidal and mosquitocidal extracts, coumrin, isoflavonoids and petrocarpans from *Neorautanenia mitis. Transactions Royal Society of Tropical Medicine and Hygiene* 2004; 98: 451-5.
24. Prakash A, Bhattacharyya DR, Mohapatra PK, Gogoi P, Sarma DK, Bhattacharjee K, *et al*. Evaluation of PermaNet 2.0 mosquito bednets against mosquitoes, including *An. minimus* s.l. in India. *Southeast Asian Journal of Tropical Medicine and Public Health* 2009; 40(3): 449-457.
25. Lindblade KA, Dotson E, Hawley WA, *et al*. Evaluation of long-lasting nets after 2 years of household use. *Tropical Medicine International*

Health 2005; 10: 1141-1150.

26. Miller TA. Mechanisms of resistance to pyrethroids on insects. *Parasitology Today* 1988; 4: 58-113.
27. Miller TA & Salgado VL. The mode of action of pyrethroids on insects. In: *The Pyrethroid insecticides*, edited by: Leahey JP. Taylor & Francis Ltd., London, 1985.
28. Gammon D & Casida JE. Pyrethroids of the most potent class antagonize GAMA action at the cryfish neuromuscular junction. *Neuroscience letter* 1983; S40: 163-178.
29. Nguyen HT, Tien TV, Tien NC, Ninh TU & Hoa NT. The effect of Olyset Net screen to control the vector of dengue fever in Vietnam. *Dengue Bulletin* 1996; 10: 87-91.
30. Martinez-T-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, *et al*. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s. s. *Insect Molecular Biology* 1998; 7: 179-184.
31. Hla Soe Tint, Kyaw Ko Ko Htet, Myitzu Tin Oung, Kay Thwe Thwe Maung, Yadanar Aung, Kyaw Thu Soe, *et al*. Community acceptance on insecticide treated bed-nets in selected rural communities. *Myanmar Health Sciences Research Journal* 2011; 23(2): 116-122.
32. Tun Lin W, Nay Soe Maung, Soe Aung, Sein Min, Sein Thaung, Maung Maung Mya, *et al*. Evaluation on the impact of two entomological intervention measures in a malaria endemic forested, hilly area of Myanmar. *Malaria Research finding Book Myanmar* 1990-2000; 65-66.
33. Tun Lin W, Saw Lwin, Sein Min, Yan Naung Maung, Sein Thaung, Thaung Hlaing, *et al*. Evaluation on the results of long-lasting insecticide treated bed nets on malaria morbidity in a malaria endemic area of Myanmar. *Myanmar Health Research Congress Programme and Abstract* 2004; 32.
34. Yan Naung Maung Maung, Pe Than Htun, Sein Min, Sein Thaung, Saw Lwin & Tun Lin W. Long lasting insecticidal nets (LLINs): A simple effective personal protective measure for malaria prevention. *Myanmar Health Research Congress Programme and Abstract* 2008; 58.
35. Singh Karam V & Bansal SK. Susceptibility of *Culex quinquefasciatus*, the vector of lymphatic filariasis, to few conventional and newer insecticides in different parts of Rajasthan. *Annals of Arid Zones* 2001; 40: 79-84.
36. Rahman SJ. Role of synthetic pyrethroids in vector control. *Journal of Communicable Diseases* 1989; 21: 333-338.
37. World Health Organization. Long-lasting insecticidal nets for malaria prevention: A manual for malaria programme managers. Trial edition. Global Malaria Programme, World Health Organization, 2007, Geneva. Available from: URL: http://www.who.int/ malaria/docs/ itn/ LLIN manual.pdf. accessed 17 November 2008.

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Role of Polymerase Chain Reaction in the Diagnosis of Tuberculous Pleural Effusion

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Diagnosis of tuberculous pleural effusion (TPE) is based on the demonstration of tubercle bacilli in the pleural fluid or that of caseous tubercle by histopathological examination. However, to reach a definitive diagnosis of tuberculous pleural effusion is still a challenge because of its nonspecific clinical presentations and inefficiency of conventional laboratory methods due to its paucibacillary nature. The aim of this study was to identify the role of polymerase chain reaction (PCR) method in the diagnosis of TPE. A total of 47 patients with clinically suspected TPE were subjected for pleural aspiration and closed pleural biopsy. Routine examination, cytological and PCR analysis of *mycobacterium* in pleural fluid were performed. Histopathological examination of pleural biopsy tissue was also done. Pleural fluid AFB staining and cytology showed no definitive diagnosis of TPE in all cases. Twenty-four cases (51.5%) reached the definitive diagnosis of tuberculosis by histopathology of pleural tissue while the remaining 23 cases (48.9%) were diagnosed as chronic nonspecific pleuritis. PCR analysis of pleural fluid revealed that 33 cases (70.2%) were positive and 14 cases (29.8%) were negative for tuberculosis. According to Receiver Operating Characteristic (ROC) curve, protein concentration and lymphocyte count at the levels of ≥5100 mg/dl and 85% were the best cut-off values where the sensitivities and specificities of TPE were 81.8%, 71.4% and 75.8%, 71.4%, respectively. Association and 95% confident interval between histopathology and PCR analysis showed sensitivity 69.7%, specificity 92.9%, positive predictive value 95.8% and negative predictive value 56.5%. PCR analysis of pleural fluid has a gain over conventional methods. This study pointed out that pleural tissue histopathology in combination with total protein concentration and lymphocyte count can be a useful tool in diagnosis of TPE.

Key words: PCR, Tuberculous pleural effusion

INTRODUCTION

Tuberculosis is a major alarming health problem in developing countries. In 2012, there were estimated 8.6 million incident cases and about 1.3 million people died of tuberculosis.[1] Myanmar is one of the 22 TB high burden countries and 27 MDR-TB high burden countries in the world and it is estimated that 1.5% of population become infected with tuberculosis every year, out of which about 130,000 people progress to develop tuberculosis. Also, TB is the third most leading cause of mortality in Myanmar.[2] Tuberculous pleural effusion is a common complication of primary tuberculosis and

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occurs in about 30% of pleural effusions.[3] Usually, patients present with constitutional symptoms, pleuritic chest pain, dyspnoea, and non-productive cough.

According to World Health Organization (WHO), diagnosis of extrapulmonary tuberculosis should be based on at least one specimen with confirmed *Mycobacterium tuberculosis* or histological or clinical evidence consistent with tuberculosis.[4] So, diagnosis of tuberculous pleural effusion depends on the demonstration of the presence of tubercle bacilli in the pleural fluid or the demonstration of caseous tubercle in the pleura by histopathological examination.

The main barrier to direct microscopy of tuberculous pleural effusion is due to the paucibacillary nature of pleural fluid. So, stained acid fast bacilli by Ziehl-Neelsen method is rarely positive and can be found only in 5-10% of cases.[5] Culture is the gold standard for definitive diagnosis of tuberculosis not only in isolation of organisms but also in determination of drug sensitivity, even in smear negative specimens. However, because of slow growing characteristics of mycobacteria, it takes about 6-8 weeks to reach the diagnosis.

Regarding the cytopathology, direct involvment of the pleural cavities may result in a major diagnostic dilemma because of a marked proliferation of mesothelial cells in sheets and clusters, features commonly observed in malignant mesothelioma and in metastatic cancer. In general, the specific diagnosis of tuberculosis cannot be made on the basis of fluid cytology.[6]

Histopathological examination of pleural tissue gives definitive diagnosis of tuberculous pleuritis. However, the biopsy samples are taken from such a minute portion of the vast area of pleural surface and many of the lesions affecting the pleura tend to be focally disturbed so the chance of missing diagnostic tissue is great. Because of the shortcomings of conventional methods, polymerase chain reaction analysis is becoming widely used as a rapid diagnostic technique for diagnosis of tuberculosis. It can detect fragments of microbial DNA, even killed bacilli, in small clinical specimens and the detection time is also reduced. It is expected that PCR method on pleural fluid specimens will be a useful tool in diagnosis of TPE and can be used as an alternative when other conventional procedures fail to give a definitive diagnosis.

MATERIALS AND METHODS

This cross-sectional, descriptive study comprised 47 patients with clinically suspected tuberculous pleural effusion, who had been admitted to Medical Wards of North Okkalapa General Hospital, Thingangyun Sanpya Hospital and Yangon General Hospital, Yangon Division, Myanmar between August 2011 and October 2012.

Informed consent was received from each patient. This study was approved by the Ethics and Research Committee of University of Medicine 2. All pleural fluid and pleural biopsies from patients with clinically suspected tuberculous pleural effusion were included. Those patients with (i) transudative effusion according to pleural fluid routine examination (as tuberculous pleural fluid is exudative in nature), (ii) pleural effusion who had already been receiving antituberculous therapy, including treatment failure and defaulter, and (iii) known cases of pleural effusion due to pneumonia, heart failure, nephrotic syndrome and malignant disease were excluded.

Method

This study was carried out on the pleural fluid and tissue biopsies taken from clinically suspected tuberculous pleural effusion already submitted to the histopathology laboratories of respective hospitals. Pleural tissue biopsy was processed and stained with routine haematoxyline and eosin (H&E). Five milliliters of pleural fluid sample were used for pleural fluid routine examination and Ziehl-Neelsen staining of acid-fast bacilli. Another 5 ml of pleural fluid were

used for cytology. And then, 5 ml of pleural fluid were stored at -20°C in an eppendorf tube to proceed to PCR at Immunology Research Division, Department of Medical Research (Lower Myanmar).

DNA extraction was performed according to manufacturer's instruction by Qiagen DNA extraction mini kit. The primers used for the amplification of a conserved repetitive sequence in the *M. tuberculosis* DNA (IS 6110) were Forward (5' CCT GCG AGC GTA GGC GTC GG3') and Reverse (5' CTC GTC CAG CGC CGC TTC GG3') which amplified a specific 123-base pair (bp) product in the PCR.

A typical PCR reaction contained a mixture of 10 x buffer 2 µl, 2.5 mM dNTP 2 µl, 10 µM primer 1(IS6110-F) 1 µl, 10 µM primer 2 (IS6110–R) 1 µl, *Taq polymerase* (5 U/1 µl) 0.2 µl, distilled water 12.8 µl and DNA sample 1 µl. Each set of the PCR reaction contained a positive control containing DNA extracted from TB bacilli that had been isolated in the laboratory and a negative control, containing the same amount of distilled water. The thermal cycle was programmed for 35 cycles with initial denaturation for 1 minute at 98°C for 1 cycles, denaturation at 98°C for 5 seconds, annealing at 55°C for 5 seconds, and extension at 72°C for 20 seconds and then, 72°C for 2 minutes for final extension. The amplified PCR products were detected by gel electrophoresis using 2% agarose gel with ethidium bromide, and a 123-bp amplified band was visualized on an ultraviolet transilluminator and photographed by polaroid camera.

RESULTS

Age and gender

Among the 47 patients, the age of the study population ranged from 18-70 years with the mean age of 41.79±13.19 years. The most common age group was 31-40 years, i.e, 15 cases (31.9%) of study population followed by 21-30 years 10 cases (21.3%). Males accounted for 32 cases (68%) and 15 cases (32%) were females.

Clinical manifestation

Common clinical presentations were dyspnoea 38 cases (80.9%), cough 37 cases (78.7%), fever 35 cases (74.5%), weight loss 30 cases (63.8%), night sweat 28 cases (59.6%) and chest pain 23 cases (48.9%). Haemoptysis was present in only 5 cases (10.6%).

Biochemical, cytological examination and Ziehl-Neelsen staining

There were 31 cases (65.95%) with protein concentration more than 5000 mg/dl and 16 cases (34.04%) had less than ≤5000 mg/dl of protein. Forty-two cases (89.36%) had lymphocyte count more than 50% and 5 cases (10.64%) had less than 50% lymphocyte count in pleural fluid. In the present study, Ziehl-Neelsen stain method of pleural fluid was used to detect the presence of acid fast bacilli. There were no positive findings in all cases. Also, cytological features showed only chronic non-specific pleuritis.

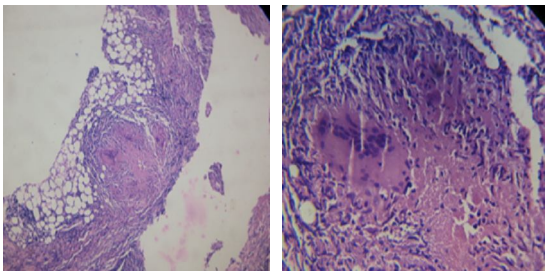


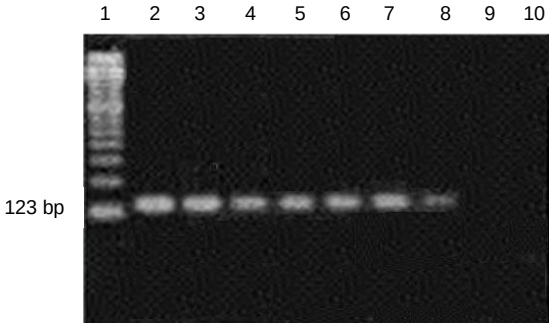
Fig. 1. Histological feature of tubercle of pleural biopsy tissue (H and E staining)

Histological features of pleural biopsy in clinically suspected tuberculous pleural effusion

Among 47 patients, 24 cases (51.5%) were diagnosed as tuberculous pleuritis by histological method while 23 cases (48.9%) were chronic non-specific pleuritis (Fig. 1).

Polymerase chain reaction (PCR) analysis of pleural fluid in cases with clinically suspected tuberculous pleural effusion

Thirty-three cases (70.21%) were confirmed as tuberculous pleural effusion by PCR after detection compared with known positive control and 14 cases (29.79%) were PCR negative compared with known negative control (Fig. 2).

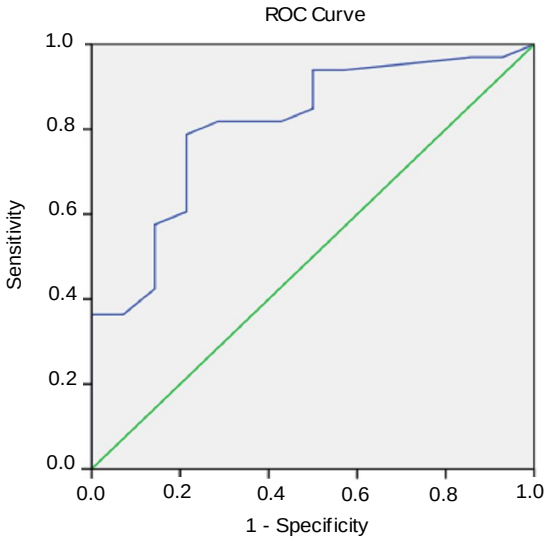


Lane 1 = 100 bp ladder
Lane 2 = positive control
Lane 3-8 = positive tests (123 bp)
Lane 9 = negative test
Lane 10 = negative control

Fig. 2. Interpretation of PCR results by agarose gel electrophoresis

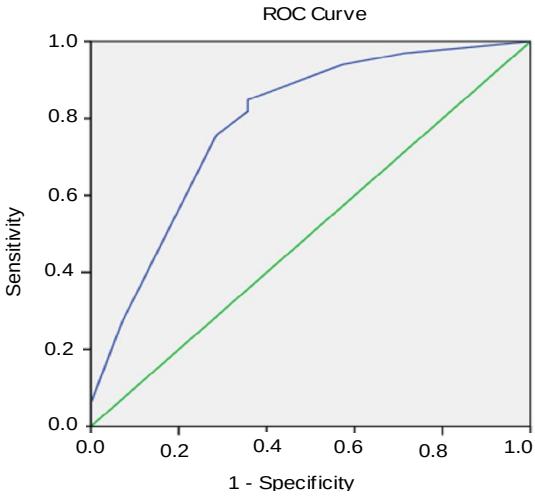
Prediction of TPE by total protein and lymphocyte count as risk factors

According to ROC curve analyses, the curves lie significantly above the 45 degree line of unity. The areas under the ROC curve were 0.81 and 0.788 denoting good accuracy of the test. The best diagnostic cut-off point for total protein level was ≥5100 mg/dl with the sensitivity of 81.8% and specificity of 71.4% (Fig. 3) and that for lymphocyte count was 85% with the sensitivity of 75.8% and specificity of 71.4% (Fig. 4).



Diagonal segments are produced by ties

Fig. 3. Total protein level in diagnosis of tuberculous pleural effusion



Diagonal segments are produced by ties

Fig. 4. Lymphocyte count in diagnosis of tuberculous pleural effusion

Association between histopathology of pleural tissue and PCR analysis

Out of 24 cases of histologically confirmed cases, 23 cases (95.83%) were PCR positive. Among 23 cases of histologically proven chronic non-specific pleuritis, 10 cases (43.47%) showed positive tuberculous pleuritis by PCR. The sensitivity, specificity, positive predictive value and negative predictive value were 69.7%, 92.9%, 95.8% and 56.5%, respectively.

DISCUSSION

Regarding age factor, the commonest age group belonged to 31-40 years which represented 15 cases (31.9%) of study population which was consistent with a three-year study at Chest Medical Ward, Yangon General Hospital, Myanmar, that included 108 patients with tuberculous pleural effusion which showed that the commonest age was 31-40 years.[7]

So, tuberculosis still prevails in the young and productive population. Clinical presentations in the study cases were mostly non-specific and did not positively contribute to the definitive diagnosis of tuberculous pleural effusion.

In the present study, among the 33 tuberculous pleuritis patients confirmed by PCR, total protein concentration >5000 mg/dl was found in 27 cases (81.8%) and ≤5000 mg/dl in 6 cases (18.2%). This is also consistent with a study of 254 patients at Spanish University Hospital with tuberculous pleuritis in which showed 98.8% of patients had high total protein concentration of more than 5000 mg/dl.[8]

In another study of 237 patients in Spain with tuberculous pleurisy, more than 50% of leucocytes were lymphocytes and the mean value was 77±19.9% (mean±SD).[9] This finding was not much different from the present study in which pleural fluid having lymphocyte count of more than 50% was found in 42 cases (89.36%), where the mean value was 81.55±1.52% (mean±SD).

Although pleural fluid smear for acid fast bacilli were done with Ziehl-Neelson stain, none were positive in this study. It may be due to the paucibacillary nature and low sensitivity of AFB, i.e, only 5-10% according to literature[5] and may also be attributed to the small sample size. The researchers did not have the chance to do pleural fluid AFB culture in this study. This procedure takes about 6-8 weeks to reach the diagnosis and has limited sensitivity.

In the present study, the cytological features of pleural fluid revealed predominance of lymphocytes and few mesothelial cells. These findings are representatives of a variety of other inflammatory or reactive processes and were reported as chronic non-specific inflammatory cytology of pleural fluid. Pleura fluid cytology is usually not specific for diagnosis of tuberculous pleurisy. Cytology is more useful for the diagnosis of malignancy than tuberculosis. Pleural biopsy can give the definitive diagnosis of tuberculous pleural effusion by the presence of caseating tubercles replete with epithelioid cells, mature lymphocytes, Langhan's giant cells and fibroblasts.[10]

However, biopsy samples are taken from such a minute portion of the vast area of pleural surface. So, the chance of missing diagnostic tissue is great. In this study, histology of pleural tissue gave definitive diagnosis of TB in 24 cases (72.7%) out of the 33 cases which were later confirmed by PCR as tuberculous pleural effusion. The rest were diagnosed as chronic non-specific pleuritis on biopsy.

The sensitivity and specificity of PCR in diagnosis of TPE varies, depending on the genomic sequence amplified and the procedure used during the extraction of the DNA. In this study, IS 6110 sequence was targeted to confirm the diagnosis of tuberculous pleurisy by PCR on pleural fluid and 33 cases (70.2%) were confirmed and 14 cases (29.8%) were PCR negative. So, the positivity rate of PCR in pleural fluid did not much differ from previous studies in other countries.[11-13]

Out of the 24 cases of histologically confirmed cases, 23 cases (95.83%) were PCR positive. Among the 23 cases of histologically diagnosed as chronic non-specific pleuritis, 10 cases (43.47%) showed positive as tuberculous pleuritis by PCR which showed an advantage over the conventional diagnostic method. Sensitivity, specificity, positive predictive value and negative predictive value of pleural tissue histology were 69.7%, 92.9%, 95.8% and 56.5%, respectively.

Therefore, reliable sensitivity and good positive predictive value in this method for diagnosis of tuberculous pleural effusion recommends that it can be used in areas where prevalence for the disease is high. However, the sensitivity of pleural biopsy varies from study to study.

Conclusion

In Myanmar, tuberculosis and malignancy are the two most common etiologies of pleural effusion. This study introduced and applied the PCR assay targeted against insertion sequence IS6110 of *Mycobacterium tuberculosis* in pleural fluid and 70.2% lead to a confirmed diagnosis of tuberculous pleural effusion. The use of PCR assay has an advantage over conventional methods such as AFB staining of

pleural fluid and histopathology of pleural biopsy tissue. Regarding age factor, young ages with pleural effusion are more likely to have tuberculosis.

Pleural fluid characteristics may help diagnose the tuberculous etiology. According to this study, protein concentration ≥5100 mg/dl and lymphocyte count >85%, when used in combination, may be used as an indicator of tuberculous pleural effusion.

Pleural tissue histopathology, which can give definitive diagnosis, may be useful as an ultimate procedure where facility and patient condition are favourable. PCR assay of pleural fluid can be employed for a more rapid, specific and reliable diagnosis. It should be considered in a setting where other conventional methods fail to diagnose a highly suspected tuberculous pleural effusion patient.

On the other hand, the use of PCR assays for every clinically suspected tuberculous pleuritis case may not be feasible as it is expensive and, also technical procedure may be demanding. Therefore, in areas where PCR assay is unavailable, pleural fluid protein concentration (≥5100 mg/dl) and lymphocyte count (>85%) can be used as an alternative tool in combination with or supplementary to pleural tissue histopathology.

REFERENCES

1. World Health Organization (WHO). *Global Tuberculosis Report* 2013; 1.
2. Ministry of Health. *Health in Myanmar* 2009; 107.
3. Ferrer JS, Munoz XG, Orriols RM, Light RW & Morell FB. Evolution of idiopathic pleural effusion. *Chest Journal* 1996; 109: 1508-13.
4. World Health Organization. *Treatment of Tuberculosis Guideline* 2010; 25.
5. Escudero BC, Garcia CM & Cuesta CB. Cytologic and bacteriologic analyzes of fluid and pleural biopsy with cop's needle. *Archives of Internal Medicine* 1990; 150: 1190-94.
6. Koss LG & Melamed MR. Effusions in the absence of cancer. In: *Koss's Diagnostic Cytology and Its Histopathologic Bases*, 5th ed. Leopold GK, Myron R. Melamed. Lippincott Williams and Wilkins 2006; 922-46.
7. Zay Soe. Value of pleural fluid adenosine deaminase in the diagnosis of tuberculous pleural effusion. *Thesis*, PhD (Respiratory Medicine). University of Medicine 1, 2005.
8. Valdes L, Alvarez D, Jose' ES, Penela P, Valle JM, Pazos JMG, Suarez J & Pose A. Tuberculous pleurisy a study of 254 patients. *Archives of Internal Medicine* 1998; 158: 2017-21.
9. Valdes L, Alvarez D, San Jose E, Juanatey RGJ, Pose A, Valle JM, Salgueiro M & Suarez RRJ. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusion in young patients in a region of high prevalence of tuberculosis. *Thorax* 1995; 50: 600-603.
10. Rosai J. Lungs and pleura. In: *Rosai and Ackerman's Surgical Pathology*, 9th ed 2004; 1(6): 359-424.
11. Maurya AK, Kant S, Kushwaha RAS & Nag VL. The advantage of using IS6110-PCR *vs.* BACTEC culture for rapid detection of *Mycobacterium tuberculosis* from pleural fluid in northern India. *Bioscience Trends* 2011; 5(4): 159-164.
12. Hasaneen NA, Zaki ME, Shalaby HM & El-Morsi AS. Polymerase chain reaction of pleural biopsy is a rapid and sensitive method for the diagnosis of tuberculous pleural effusion. *Chest Journal* 2003; 124: 2105-2111.
13. de Lassence A, Denise L & Catherine P. Detection of mycobacteiral DNA in the pleural fluid from patients with tuberculous pleurisy by means of the polymerase chain reaction: Comparison of two protocols. *Thorax* 1992; 47: 265-269.

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Bioequivalence Study of Metronidazole Tablets in Myanmar Healthy Volunteers

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The use of generic drugs, which are safe, effective and lower cost alternatives to innovator, has been steadily increasing internationally. Metronidazole, one of the essential medicines, is widely used as an anti-protozoal and antibacterial drug. This study was performed to compare the bioavailability of generic metronidazole with that of innovator by using serum concentration for determining bioequivalence between them. Bioequivalence of generic and innovator metronidazole were assessed by single dose pharmacokinetic study in fourteen healthy human volunteers by using bioavailability parameters, such as peak serum drug concentration (C_{max}), time to reach peak concentration (T_{max}) and area under the serum drug concentration curve (AUC). A cross-over design with one-week wash-out period under fasting conditions was performed. Blood samples were taken up to 24 hours after drug administration and serum concentrations were determined by UV-Vis spectrophotometer. Bioavailability parameters were analyzed by WinNonlin version 6.3. $AUC_{(0\text{-}24\ hr)}$ were 92.96±6.41 *vs.* 89.43±5.08 µg hr/ml, C_{max} were 11.46±1.72 *vs.* 9.88±0.93 µg/mL and T_{max} were 2.0 *vs.* 1.5 hours for generic and innovator metronidazole, respectively. The 90% confidence intervals for the generic to innovator ratio of mean $AUC_{(0\text{-}24\ hr)}$ and C_{max} were 0.96(0.92-1.01) and 0.87(0.82-0.92), respectively, and they were within the accepted bioequivalent range of 0.8-1.25 described in ASEAN guideline. Therefore, generic metronidazole was bioequivalent to that of innovator and can be used cost effectively and interchangeably because it has similar efficacy and is considerably less expensive.

Key words: Metronidazole, Bioavailability, Bioequivalence

INTRODUCTION

Over the years, the prescription of generic drugs has increased from 19% in 1984 to 60-70% in 2009 and they are cost effective alternatives for the brand name drugs.[1] Generic pharmaceutical products need to conform to the same standards of quality, efficacy and safety as required of the innovator product and they must have demonstrated bioequivalence with the innovator brand.[2] Bioavailability and bioequivalence studies provide important information that ensures the availability of safe and effective medicines to patients and practitioners. Bioavailability is defined as the rate and extent by which the active moiety becomes available at the site of action.[3] The area under the concentration *versus* time curve (AUC) serves as the extent of absorption, the time to reach the peak concentration (T_{max}) reflects the rate of absorption, while the peak concentration (C_{max}) reflects both the extent and the rate of absorption.[4]

Metronidazole is an antimicrobial drug that is used to treat protozoal and anaerobic bacterial infections. It is one of the essential medicines and still the cornerstone for the management of anaerobic infections world-

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wide. Metronidazole is clinically effective in trichomoniasis, amoebiasis, and giardiasis, as well as in a variety of infections caused by obligate anaerobic bacteria.[5]

The aim of certain antibiotic therapies should be not only clinical success but also the prevention of antimicrobial resistance in future. Since the susceptibility of *Helicobacter pylori*, and certain other anaerobic organisms to the metronidazole is decreasing,[6, 7] the use of generic metronidazole in Myanmar must be assured good efficacy and safety.

Therefore, the bioequivalence study of locally available generic metronidazole was compared with that of innovator to assure for clinicians and patients whether two brands of the same drug (between generic and innovator brands) would result in similar efficacy or not.

MATERIALS AND METHODS

Study design was a cross-over, comparative study with one-week washout period under fasting condition. Two drugs (reference and test) were used. They are -

- Reference drug: Flagyl 400 mg tablet, each film-coated tablet containing metronidazole BP 400 mg, manufactured by Sanofi Aventis, France.
- Test drug: Nidazyl 400 mg tablet, each film-coated tablet containing metronidazole BP 400 mg, manufactured by Orion Pharma Ltd, Dhaka, Bangladesh.

Study sites were Department of Pharmacology, Department of Biochemistry, and Common Research Laboratory, University of Medicine (Mandalay).

Subjects

Fourteen subjects of both sexes (8 males and 6 females) were recruited in this bioequivalence study. Their mean age was 25.71±5.13 years (range, 19-35). The average body weight was 58.47±8.58 kg (range, 48-72.6), the average height was 1.64±0.1 m (range, 1.52-1.8) and BMI was 19.1-23 kgm^{-2}. The subjects were non-smoker, non-alcoholic. The volunteers were asked not to take any medication (including indigenous medicine) for at least one week prior to, and through-out the study.

Normal healthy persons were generally determined by history taking, clinical examination, and laboratory investigation including random blood sugar, urea, creatinine, alanine aminotransferase, asparate aminotransferase, and alkaline phosphatase levels. Subjects with allergic reaction to metronidazole as well as those with pregnancy were excluded. Blood pressure, pulse rate and respiratory rate were measured before the drug administration and at the end of study day. Metronidazole was well tolerated by all subjects. No subject was dropped out before the completion of the study period.

Randomization of the subjects and drug administration

On the study day, each subject received a single oral dose of metronidazole either generic (test) or innovator (reference) brand as shown in Table 1 with a glass (200 ml) of drinking water after overnight fasting. Hot drink or juice was provided 2 hrs after drug administration. A standardized lunch (fried noodle with chicken egg) was provided to all the subjects 4 hrs after drug administration. It was done identically for two periods of the study.

Table 1. Schematic table for cross-over design

Sequence	1st period	Washout period	2nd period
Sequence 1	Reference	One week	Test
Sequence 2	Test		Reference

Blood sampling

A cannula was inserted into a peripheral vein of forearm under the aseptic condition. Blood 5 ml was drawn just before (0.0 hr) and at 0.25, 0.5, 1, 1.5, 2, 4, 6 and 24 hrs after administration of the drugs. The collected blood samples were stood for 30-60 minutes and then centrifuged at 2000 rpm for 5 minutes. The separated

serum samples were labeled and stored carefully at minus 20 degree Celsius (-20°C) until analyzed.

Metronidazole level determination by UV-Visible spectrophotometer

The concentrations of metronidazole in the serum samples were determined with UV-Visible spectrophotometer according to the modified method of Wearley & Anthony.[8] The method was validated as the recommendations of ASEAN for the parameters like linearity, recovery, precision, accuracy, and stability of the analyte.[9] Standard solution of metronidazole was prepared by mixing 10 mg metronidazole standard powder in 10 ml of methanol to obtain 10 ml solution to get 1 mg/ml.

From that stock solution, 1, 5, 10, 20, 40, 80 and 100 µg/ml of metronidazole standard solutions were prepared by diluting with pooled serum. Extraction of metronidazole from the blood samples was carried out by mixing serum sample of 0.5 ml with 5 ml of 0.1 N sulphuric acid in methanol and vortexed for 30 seconds. And then, it was centrifuged for 15 minutes at 6000 rpm. The supernatants were separated and analyzed spectrophotometrically at 323 nm for metronidazole against serum blank containing no drug. Each serum concentration was determined in duplicate and calculated from the standard curve obtained from the same day. Quantitative serum metronidazole determination was done by using UV-Visible spectrophotometer within two weeks after collection of the sample.

Pharmacokinetics data analysis

Serum concentration time curves were drawn for each individual. Comparison between the absorption rates was carried out regarding both C_{max}, T_{max} and $AUC_{(0-\ 24\ hr)}$. The Phoenix 64/WinNonlin version 6.3 (Pharsight-a Certara™ Company, USA) was used for noncompartmental pharmacokinetic analysis. T_{max} and C_{max} were directly obtained from the serum concentration time data. The $AUC_{(0-\ 24\ hr)}$ was calculated using trapezoidal rule. The elimination rate constant (K_{el}) was estimated by linear regression from the points describing the elimination phase in a log-linear plot. Half-life ($T_{1/2}$) was derived from this rate constant ($T_{1/2}=0.693/K_{el}$).

Statistical analysis

Bioequivalence of two brands was assessed by means of an analysis of variance (ANOVA) and calculating 90% confidence interval (CI) of the ratio of test and reference. ANOVA for cross-over design was used to assess the effect of treatment, period, sequence, and subject nested in sequence.

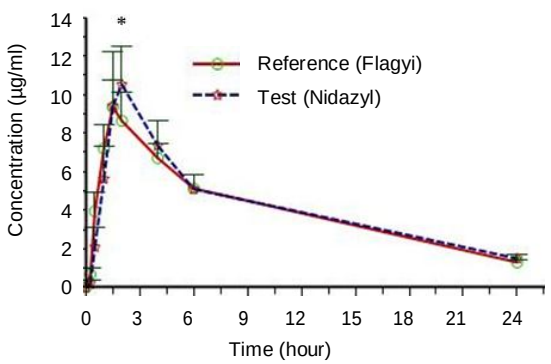
For maximum of serum concentration C_{max} and area under the serum concentration time curve from time zero to last time $AUC_{(0-24\ hr)}$, natural logarithms transformed values were used, however, time to reach maximum serum concentration T_{max} was carried out on untransformed data. The 90% confidence interval of the ratio of the test and reference (T/R) was calculated. The statistical significance of pharmacokinetic parameters and bioequivalence limits were determined by using SPSS statistical software version 20.

Ethical consideration

The volunteers were informed about the aims of the study, risks, benefits, procedures, and as well as their rights. Each volunteer signed an informed consent document before entering the study. The volunteers were allowed to withdraw from participation in the study at any time as they wish. The study protocol and the consent form were approved by the Postgraduate Board of University of Medicine (Mandalay).

RESULTS

Both brands of metronidazole (generic and innovator) were readily absorbed from gastrointestinal tract and it was measurable at the first sampling time (0.25 hr). The mean serum concentration-time curve of generic and innovator to the fourteen volunteers are shown in Fig. 1.



*=p<0.05 between generic and innovator metronidazole for C_{max}

Fig. 1. The mean serum concentration-time curve with SD bar after 400 mg single dose administration of generic (test) and innovator (reference) metronidazole (n=14)

Comparison of pharmacokinetic parameters of generic and innovator metronidazole are shown in Table 2.

Table 2. Comparison of pharmacokinetic parameters of generic and innovator metronidazole (n=14)

Pharmacokinetic parameter	Test (T)	Reference (R)	P values	Mean ratio (T/R)	90% confidence interval
C_{max} (µg/ml)	11.46 ±1.65	9.88 ±0.90	0.006	0.87	0.82-0.92
$AUC_{(0-24\ hr)}$ (hr. µg/mL)	92.96 ±6.17	89.43 ±4.90	0.117	0.96	0.92-1.01
T_{max} (hr)	1.75 ±0.31	1.68 ±0.24	0.519		
K_{el} (hr)	0.07 ±0.01	0.08 ±0.01	0.08		
$T_{1/2}$ (hr)	8.65 ±0.71	9.78 ±2.10	0.07		

Mean±SD values of T_{max} were 1.75±0.33 and 1.68±0.25 hr for generic and innovator, respectively. Mean T_{max} of the generic was slightly longer than that of the innovator but it did not reach the statistically significant level (p=0.52).

Mean±SD values of $AUC_{(0-24\ hr)}$ of generic and innovator were 92.96±6.41 and 89.43± 5.08 hr. µg/ml, respectively. Mean $AUC_{(0-24\ hr)}$ of generic metronidazole was slightly higher than that of innovator but it did not reveal significant difference (p=0.12). Mean±SD values of C_{max} were 11.46±1.72 and 9.88± 0.93 µg/ml for the generic and the innovator, respectively. Mean C_{max} of generic metronidazole was higher than that of innovator and significantly different (p=0.006).

The 90% confidence interval for logarithm transformation data of the ratio of mean of C_{max} was 0.87 (0.82-0.92) and $AUC_{(0-24\ hr)}$ was 0.96 (0.92-1.00), respectively. It indicated that these values are entirely within the bioequivalence acceptance criteria of 0.80-1.25.[10-12]

DISCUSSION

In this study, bioequivalence of two brands of metronidazole was performed in Myanmar healthy volunteers of both sexes. The drugs used in this study were generic as a test and innovator as a reference. The modified method of Wearley & Anthony was used for determination of serum concentration of metronidazole. The calibration curves were linear over the range of 1-100 µg/ml (r^2=0.998) by using linear regression analysis. Recovery was between 88% and 108.4% at low (1 µg/ml), medium (10 µg/ml) and high (100 µg/ml) concentrations.

Precision of method was in acceptable limit with coefficient of variation not exceeding 15% for both intra-day and inter-day precision. The stability of metronidazole serum samples storage at -20°C was 6 weeks. The serum sample of 0.5 ml was needed and it is economical, technically suitable for routine analysis by using UV-Visible spectrophotometer. Therefore, this method is appropriate for the analysis of metronidazole in serum samples.

A bioequivalence study is basically a comparative bioavailability study designed to establish equivalence between test and reference products.[10] The main objective of bioequivalence study is to assure the efficacy and safety of generic formulations. T_{max}, $AUC_{(0-24\ hr)}$ and C_{max} were the main target parameters in order to assess possible

bioequivalence between the two brands. T_{max} value of metronidazole tablet was described within the range of 0.25-4 hrs.[4, 5]

In this study, T_{max} of generic metronidazole was within the range of 1-2 hr, while that of innovator was 1.5 to 2 hrs. Therefore, T_{max} of these two brands was not different and consistent with the accepted range. Since T_{max} reflects the rate of absorption of drugs, those of the two brands of metronidazole would be the same.

The area under the concentration versus time curve (AUC) is an important parameter that can be considered as a representative of the total amount of drug absorbed following administration of a single dose of a drug. AUC ascertains the calculated dose that actually delivers the serum drug level required for its therapeutic effect. The fraction of the administered dose reaching the systemic circulation is termed bio-availability and AUC is commonly used to measure the extent of bioavailability.

In this study, $AUC_{(0\text{-}24\ hr)}$ of these two brands was not significantly different and comparable to the reported values of 70-110 hr. µg/ml in healthy volunteers.[13-15] Since $AUC_{(0\text{-}24\ hr)}$ of generic and innovator were similar, their concentration at the site of action and their effectiveness would be the same. The 90% confidence interval for natural logarithms (Ln) transformation data of the ratio of mean of $AUC_{(0\text{-}24\ hr)}$ was 0.96(0.92-1.01) and it reaches within the acceptable bioequivalence criteria of 0.80-1.25.

The peak serum concentration (C_{max}) indicates that drug is sufficiently absorbed to provide therapeutic response. C_{max} is directly proportionate to the dose of the drug administered and the fraction of drug being absorbed. Change in C_{max} holds for the potential for change in clinical effect. It also provides more persistent information about efficacious or toxic blood levels since it is the highest blood concentration that would be encountered in a given dose. Single concentrations, especially highest concentrations like C_{max}, generally have larger variation than AUC because C_{max} is usually determined by only one time point.[16] The absorption of drug may be altered by physicochemical nature of drug factors e.g., particle size, salt form, crystal polymorphism, coatings, used of dis-integrants and the presence of excipients can influence the case of disintegration, dissolution and, therefore, alter the rate of absorption.[17]

In this study, C_{max} of generic and innovator was significantly different and it may be due to the differences in physicochemical nature of drug factors. Since C_{max} of the generic was high enough to fulfill the primary goal of antibiotic therapy with sufficient concentration, it had good efficacy. The previous studies reported that C_{max} of metro-nidazole was ranging from 7 to 12 µg/ml in healthy volunteers.[13-15]

Therefore, C_{max} of the present study was in agreement with the previous ones and it might be assured the safety as them. The 90% confidence interval for natural logarithms (Ln) transformation data of the ratio of mean of C_{max} was 0.87 (0.82-0.92) and it also reaches within the acceptable bioequivalence criteria of 0.80-1.25.

The 90% confidence interval for the ratio of mean of C_{max}, and $AUC_{(0\text{-}24\ hr)}$ were 0.87 (0.82-0.92), and 0.96 (0.91-1.01), respec-tively, and these values are within the acceptable bioequivalence criteria of 0.80-1.25. Therefore, the generic metronidazole is bioequivalent to that of innovator.

Conclusion

Based on this study, it can be concluded that generic metronidazole is bioequivalent to that of the innovator. Since they are bioequivalent, their effectiveness will also be the same and can be interchangeable in clinical practice. Therefore, generic metro-nidazole can be used cost effectively because it is considerably less expensive than that of innovator and has similar efficacy.

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REFERENCES

1. Bapuji AT, Ravikiran HLV, Nagesh M, Syedba S, Ramaraju D, Reddy CRJ, *et al*. Bioequivalence testing: Industry perspective. *Journal of Bioequivalence and Bioavailability* 2010; 2(5): 98-101.
2. Tamboli AM, Todkar P, Zope P & Sayyad FJ. An overview on bioequivalence: Regulatory consideration for generic drug products. *Journal of Bioequivalence and Bioavailability* 2010; 2(4): 86-92.
3. Wesch R. Absolute and relative bioavailability. In: *Drug Discovery and Evaluation: Methods in Clinical Pharmacology,* Ed: Vogel HG, Maas J & Gebauer A, Springer, London 2011; 173.
4. Holford NHG. Pharmacokinetics and pharmacodynamic: Rational dosing and the time course of drug action. In: *Basic and Clinical Pharmacology,* 12th ed. Ed: Katzung BG, Masters SB & Trevor AJ, Singapore 2012; 43-44.
5. Phillips MA & Stanley SL. Chemotherapy of protozoal infections. In: *Goodman & Gilman's Pharmalogical Basis of Therapeutics,* 12th ed. Ed: Brunton LL, Chabner BA & Knollmann BC. The McGraw-Hill Companies, USA 2011; 1428-1430.
6. Lofmark S, Edlund C & Nord CE. Metronidazole is still drug of choice for treatment of anaerobic infections. *Clinical Infectious Diseases* 2010; 50 (S1): 16-23.
7. Wallace JL & Sharkey KA. Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. In: *Goodman & Gilman's Pharmalogical Basis of Therapeutics,* 12th ed. Ed: Brunton LL, Chabner BA & Knollmann BC. The McGraw-Hill Companies, USA 2011; 1320-1321.
8. Wearley LL & Anthony GD. Metronidazole. In: *Analytical Profiles of Drug Substances,* Ed: Florey K, Academic Press, New York, USA 1976; 329-340.
9. ASEAN. Guidelines for the validation of analytical procedure. Available from URL: http:// www. hsa.gov.sg/ publish/ etc/medialib/ hsa_ library/ health_ products_ regulation/ western_ medicines/ files_guidelines. Par.25302. File.dat/ ACTR_Guide-lines for Validation of Analytical Procedures.pdf, accessed 1 February 2012.
10. ASEAN (2004). ASEAN guidelines for the conduct of the bioavailability and bioequivalence study. Available from URL: http:// www.fda.gov.ph/ACTDA CTR/ACTR 4ASEAN GUIDELINES BABE.pdf, accessed 12 February 2012.
11. European Medicines Agency (2010). Guideline on the investigation of bioequivalence. Available from URL: http://www.ema.europa.eu, accessed 4 February 2012.
12. United States Food and Drug Administration. Guidance for industry bioavailability and bioequivalence studies for orally administered drug products: General considerations 2003; 1-21.
13. Lamp KC, Freeman CD, Klutman NE & Lacy MK. Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. *Clinical Pharmacokinetics* 1999; 36(5): 353-373.
14. Kolawole JA & Ameh IU. Chronopharmacokinetics of metronidazole in healthy human volunteers. *Journal of Pharmacy and Bioresoures* 2004; 1(1): 29-34.
15. Ashiq B, Usman M, Ashraf1 M, Omer O, Khokhar MI & Hassan SS. Comparative pharmacokinetics of metronidazole in healthy volunteers and in patients suffering from amoebiasis. *Pakistan Journal of Pharmacology* 2011; 24 (1 & 2): 41-46.
16. Pétricoul O, Cosson V, Fuseau E & Marchand M. Population models for drug absorption and enterohepatic recycling. In: *Pharmacometrics: The Science of Quantitative Pharmacology,* Ed: Ette EI, Williams PJ, John Wiley & Sons, Inc., Publication, USA 2007; 345-382.
17. Clark MA, Finkel R, Rey JA & Whalen K. Pharmacokinetics. In: *Lippincott's Illustrated Reviews: Pharmacology,* 5th ed. Ed: Harvey RA, Lippincott Williams & Wilkins, Philadelphia 2012; 8-10.

Acute Kidney Injury in Children with Dengue Haemorrhagic Fever Admitted to Yangon Children's Hospital

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Tropical acute febrile illnesses including dengue fever are a common cause of acute kidney injury (AKI) in developing countries. This study aimed to evaluate the proportion and to categorize the severity of AKI using pRIFLE (paediatric Risk, Injury, Failure, Loss of kidney function, End stage renal disease) criteria, and to identify the risk factors for development of AKI in children with dengue haemorrhagic fever. It was a cross-sectional descriptive study conducted at medical wards of Yangon Children's Hospital (YCH). Urine output was observed and serum creatinine was tested within 24 hours after admission and daily before hospital discharge. Ninety-four children, 46 boys (48.9%) and 48 girls (51.1%) were recruited. Their age ranged from 2 to 14 years and mean age was 7.34±2.88 years. Twelve out of 94 patients (12.76%) were classified as having AKI, in which 10 patients were in risk group and 2 patients were in injury group according to pRIFLE criteria. Mean of the lowest GFR was 59.27±9.32 ml/min/1.73 m^2 in children with AKI and 119.85±35.21 ml/min/1.73m^2 in children with non-AKI ($p<0.0001$). AKI was more common in children ≤5 years age group ($p=0.001$). Bleeding manifestation was found significantly in AKI group ($p=0.04$). Children with AKI had longer hospital stay than those with non-AKI (3.50±0.67 days *vs.* 2.77±0.77 days) ($p=0.01$). All the cases had returned to normal renal function within one day by conservative treatment. This study highlighted that risk of AKI should be considered in paediatric dengue patients especially in younger children.

Key words: Acute kidney injury, AKI, Dengue, Dengue haemorrhagic fever, Children

INTRODUCTION

Acute kidney injury (AKI), previously known as acute renal failure, represents a significant and devastating problem in clinical practice.[1] It is defined as an abrupt or rapid decline in glomerular filtration rate (GFR) usually accompanied by a rise in serum creatinine and blood urea nitrogen.[2] Tropical acute febrile illnesses including dengue fever are a common cause of acute kidney injury in developing countries.[3] WHO currently estimates that 2.5 billion people (two fifths of the world's population) are now at risk from dengue and there may be 50 million dengue infections worldwide every year. Today dengue affects most Asian countries and has become a leading cause of hospitalization and death among children in the region.[4]

Dengue is a day-biting mosquito-borne (*Aedes aegypti*) infection caused by various strains of dengue virus. On the basis of climatic factors**,** Myanmar is included in Tropical monsoon and Equatorial Climatic Zone where *Aedes aegypti* is widespread in both urban and rural areas. Therefore, transmission is extended and dengue epidemics occur in 3-5 year cycles, associated with high morbidity in children.

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In 2006, Myanmar reported 6% of total dengue cases in the South East Asia region.[5] Dengue illness covers a broad spectrum of clinical manifestations as undifferentiated fever, dengue fever, dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).[6] DHF is a severe form of the disease characterized by fever, haemorrhagic phenomena, thrombocytopenia and evidence of plasma leakage.[7] Multifactorial causes can lead to AKI in DHF.[8, 9]

A study in adults using RIFLE criteria to detect AKI in tropical acute febrile illnesses found more than a third of cases with dengue infection have AKI.[3] Patients with AKI in DHF were found out to have high mortality in both adult and paediatric population.[10, 11] However, incidence of AKI with DHF in children has been unclear because pediatric AKI studies are limited and most studies in dengue infection with AKI were only case reports or retrospective review of medical records.

The previous studies used variable definition of AKI in dengue virus infection. Pediatric modified RIFLE criteria (pRIFLE) have been validated in children and appear to be quite promising for better characterization of AKI and can potentially pick up from mild to severe cases. It standardizes the definition of AKI based on the estimated creatinine clearance or on the urinary output reduction, in a body weight per hour basis.[12] This study aimed to assess the proportion and severity of AKI in children with DHF by using pRIFLE criteria.

MATERIALS AND METHODS

This study was a cross-sectional descriptive study conducted at medical wards of Yangon Children's Hospital. Children aged 2-12 years hospitalized with suspected dengue viral infection were included in the study. Those with chronic renal disease or critically ill conditions were excluded from the study. Diagnosis of DHF was made in cases with 3 to 7 days of fever by SD BIOLINE Duo (Standard Diagnostic Inc.) strip within the first 24 hours of hospitalization. Patients with positive IgM or NS1 with negative IgG was considered as primary dengue and patient with positive IgG with or without IgM or NS1 positivity was considered as secondary dengue infection according to the manufacturer's instruction. The severity of DHF (Grade I-IV) was in accordance with WHO criteria (1997).

The included patients were monitored during their stay in hospital. Daily urine output was observed and serum creatinine was tested within 24 hrs after admission and daily before hospital discharge. Serum creatinine was measured at Clinical Research Division, DMR (LM) using the modified Jaffe's kinetic alkaline picrate method. The estimated creatinine clearance (GFR) was calculated according to Schwartz formula.[11] The baseline GFR was assumed as the normal clearance value of 100 ml/min/1.73 m^2 as reference.[10] The pRIFLE class was determined based on the lowest level of either the Glomerular Filtration Rate (GFR) criteria or urine output criteria.

Categorization of AKI according to Paediatric-modified RIFLE (pRIFLE) criteria[10]

	Estimated CCI	Urine output
Risk	eCCI decrease by 25%	<0.5 ml/kg/hr for 8 hr
Injury	eCCI decrease by 50%	<0.5 ml/kg/hr for 16 hr
Failure	eCCI decrease by 75% eCCI<35 ml/min/1.73 m^2	<0.3 ml/kg/hr for 24 hr or Anuric for 12 hr
Loss	Persistent failure >4 weeks	
End stage	End-stage renal disease (persistent failure >3 months)	

eCCI=Estimated creatinine clearance

Statistical analysis

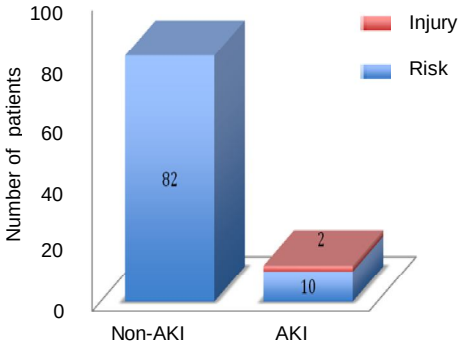
The data were subjected to statistical analysis using the SPSS 16.0 software package. Chi-square test, Fisher's exact test or Student 't' test were used as appropriate to compare the distributions of data between AKI and non-AKI groups. Statistical significance was considered if p values <0.05.

Ethical consideration

This study was approved by the Institutional Ethical Review Committee of DMR (LM).

RESULTS

Ninety-four children, 46 boys (48.9%) and 48 girls (51.1%) were recruited. Their age ranged from 2 to 14 years and mean age was 7.34±2.88 years. Twelve out of 94 patients (12.7%) were classified as having AKI, in which 10(10.6%) were in risk group and 2(2.1%) was in injury group according to pRIFLE criteria (Fig. 1).



AKI=Acute Kidney Injury
Non-AKI=Non-Acute Kidney Injury

Fig. 1. Proportion and severity of AKI according to pRIFLE criteria

Children with AKI were significantly younger than those without AKI (p=0.001). Mean of the lowest GFR was 59.27± 9.32 ml/min/1.73 m^2 in children with AKI and 119.85±35.21 ml/min/1.73 m^2 in children with non-AKI (p<0.0001). Children with AKI had longer hospital stay than those with non-AKI (3.50±0.67 days *vs.* 2.77±0.77 days) (p=0.01) (Table 1).

Table 1. Characteristics of children with dengue haemorrhagic fever

Characteristics	AKI (n=82) (mean±SD)	Non-AKI (n=12) (mean± SD)	P value
Age (year)	4.7±1.5	7.7±2.8	0.001*
Lowest pulse pressure (mmHg)	22.5±11.3	25.8±6.0	0.12
Lowest platelet count (/µl)	62.5±23.9	57.5±26.2	0.53
Highest haematocrit (%)	43.1±3.7	42.5±5.4	0.72
Lowest glomeruloar filtration rate (ml/min/1.73 m^2)	59.3±9.3	119.8±35.2	<0.0001*
Length of hospital stay (days)	3.5±0.6	2.7±0.7	0.001*

*=Statistically significant

All 12 cases of AKI were children with secondary dengue infection. AKI is more common in children ≤5 years compared to >5 years age group (p=0.001) (Table 2).

Table 2. Characteristics of AKI and non-AKI in univariate analysis

Characteristics	AKI (n=12) No. (%)	Non-AKI (n=82) No. (%)	P value
Age (year)			0.001*
2-5	8(66.7)	18(22.0)	
>5	4(33.3)	64(78.0)	
Sex			0.6
Male	5(41.7)	41(50)	
Female	7(58.3)	41(50)	
Final diagnosis			0.7
DHF	7(58.7)	52(64.2)	
DSS	5(41.3)	29(35.8)	
Type of DHF			0.3
Primary	0(0)	12(14.6)	
Secondary	12(100)	70(85.4)	

AKI=Acute kidney injury, DHF=Dengue haemorhagic fever, DSS=Dengue shock syndrome, *=Statistically significant

There is no significant difference in clinical presentations between AKI and non-AKI except bleeding manifestations which was found significantly in children with AKI (p=0.04) (Table 3).

Table 3. Clinical presentations of children with dengue haemorrhagic fever

Presentations	AKI (n=12) No. (%)	Non-AKI (n=82) No. (%)	P value
Headache	2(16.7)	23(28.0)	0.5
Retro-orbital pain	0(0)	4(4.9)	1.0
Nausea/ vomiting	7(58.3)	49(59.8)	0.9
Rash	1(8.3)	9(11.0)	1.0
Abdominal pain	8(66.7)	50(61.0)	0.7
Sore throat	0(0)	8(9.8)	0.6
Lethargy	10(83.3)	52(62.2)	0.1
Myalgia	3(25.0)	23(28.0)	1.0
Arthragia	1(8.3)	9(11.0)	1.0
Red eyes	1(8.3)	5(6.1)	0.5
Bleeding manifestations	6(50.0)	19(23.2)	0.04*

*=Statistically significant

Among DHF with AKI, 3(25.0%), 8(66.7%) and 1(8.3%) of children developed AKI in 5, 6 and 7 days of fever, respectively. Nine children (75%) developed AKI in the first 24 hours and 3 children (25%) developed AKI within 48 hours after admission to hospital.

DISCUSSION

Myanmar is a country having frequent endemics of dengue viral infection.[5] Since Dengue fever is a leading cause of hospitalization in children, a lot of research on clinical and laboratory features have been conducted in this country. However, occurrence of AKI in dengue infection has not been studied much and was neglected as it was considered as a rare complication.

The proportion of AKI in this study (12.7%) was comparable with some adult studies. By using Acute Kidney Injury Network (AKIN) criteria, AKI was present in 13.3% in Pakistani people[14] and 10.8% in Indian adults with dengue viral infection.[15] Incidence of renal failure in dengue fever was 16% on the basis of estimated GFR.[16] A study using RIFLE criteria to detect AKI in adults with tropical acute febrile illnesses found more than a third of cases with dengue infection have AKI.[3]

AKI studies are rare in children with dengue fever and most are retrospective in nature by assessing increase serum creatinine level. One study found 1.6% of ARF among 617 children with DHF in Cambodia.[17] A retro-spective study of medical records reported 0.9% of AKI in Thai children.[9] That study defined AKI as a rapid elevation of serum creatinine >2 mg/dl. The variable incidence and severity of AKI in dengue viral infection might be due to the use of different selection criteria for defining AKI. In children, serum creatinine changes with age, gender, and muscle mass and it does not reflect the renal function alone.[18]

In this study, paediatric patients-modified RIFLE version (pRIFLE) which based on the estimated creatinine clearance was used to provide a more relevant description of renal function. AKI definition should be standardized so that results can be compared across studies. When increase in serum creatinine level was used, it might have under-recognized the stage 1 of RIFLE which accounted for most of the patients in this study. Serial measurement of estimated GFR during the illness is also needed to ascertain the renal function in DHF. All children with DHF or DSS at the time of diagnosis had normal GFR calculated by Schwartz formula.[9] In our study, serial GFR was done and 75% of AKI were detected within 24 hours and 25% of AKI were detected within 48 hours of hospitalization in children with DHF. In this study, all cases of AKI were diagnosed between 5 to 7 days of fever and most of them developed in the 6th day of fever. These days are the critical period in dengue hemorrhagic fever and proper medical care is needed to avoid complications.

More than 80% of children with AKI had the mildest category in risk group of pRIFLE and all the patients in this study returned to have normal GFR after 1 day. In a study, serum creatinine returned to normal between 1 to 48 days in survived DHF patients with acute renal failure.[9] Among multifactorial causes, hypovolemia, which is assumed as the main cause of AKI in DHF, decreases cardiac output which in turn will reduce renal blood flow and affect GFR. [19, 20]

In our study setting, careful fluid therapy was given to hospitalized children with DHF by oral or intravenous route and all patients with AKI recovered fully and returned their normal GFR after 1 day. It was in concordance with a study in which all the adult dengue cases with acute renal failure recovered fully by conservative treatment only.[16] Therefore, physician's awareness and early initiation of careful supportive management for DHF is important to avoid further morbidity associated with AKI.

Paediatric AKI studies of hospitalized children demonstrated that children with AKI were significantly younger than those without AKI.[21] Children with DHF were also in line with that finding in this study. Contrast to children, AKI was more common in older age group in adults with dengue infection with the higher percentage of the underlying comorbid diseases like hypertension, diabetes, etc.[22] Gastrointestinal

bleeding was a significant variable found in adult DHF patients with acute renal failure.[11] Similar to this, bleeding manifestations were significantly higher in children with AKI compared to those with non-AKI in our study. This finding was not strange because bleeding was already identified as one of the severe manifestations that complicates the outcome of dengue.[23]

Dengue can cause an enormous economic burden in many countries. In our study, patients with AKI was found out to have longer hospital stay. It was in concordance with a study in which AKI was an independent predictor for length of hospitalization.[14]

The high mortality rate was reported in patients with AKI and DHF in previous studies. As the highest mortality rates, the retrospective studies of Thai children and Taiwan adults with DHF and AKI reported as 64% and 60%, respectively.[9, 12] In our study, there was no fatal case. This may be due to the difference between the characteristics of the study participants. About 70% of DHF with AKI had oliguric AKI and had higher mortality rate than non-oliguric AKI.[9]

In this study, there were no oliguric AKI cases and respond well to fluid replacement therapy. Oliguric AKI can precipitate to respiratory failure and may lead to death after fluid resuscitation due to fluid overload. Studies also described that the high mortality was primarily caused by the profound shock leading not only to acute renal failure but also to other major complications. Our data were based only on DHF and DSS patients who respond to standard therapy in medical wards, therefore, severely ill patients with deterioration who required Intensive Unit care were not included in the study. The exclusion of critically ill children with DHF in ICU can underestimate the incidence of AKI and its severity and this fact was the limitation of our study.

Conclusion

This study highlighted that risk of AKI should be considered in paediatric dengue patients especially in younger children. Further studies should also be conducted in critically ill children with DHF not responding to standard therapies and requiring Intensive Unit care.

REFERENCES

1. Mak RH. Acute kidney injury in children: The dawn of a new era. *Pediatric Nephrology* 2008; 23: 2147-2149.
2. Bhimma R. Newer insights into acute renal failure in children. Last Updated: 1st November 2009 Volume 6 Issue 11 Art # 57, Available from: URL: http://www. pediatriconcall.com/ fordoctor/ diseasesandcondition/ PEDIATRIC_ NEPH-ROLOGY/acute_renal_failure.asp.
3. Basu G, Chrispal A, Boorugu H, Gopinath KG, Chandy S, Prakash JAJ, *et al*. Acute kidney injury in tropical acute febrile illness in a tertiary care centre-RIFLE criteria validation. *Nephrology Dialysis and Transplant* 2007.
4. WHO, Dengue and dengue haemorrhagic fever. Fact sheet N°117, March 2009. Available from: URL: http:// www. who.int/ mediacentre/ factsheets/ fs117/en/print.html.
5. WHO, Dengue/DHF, situation of dengue/ hengue haemorrhagic fever in the South-East Asia Region. Last update: 14 August 2006, Available from: URL: http://www. searo.who. int/en/ Section10/Section 332_1104.html.
6. Guzman MG & Gustavo K. Dengue: An update. *Lancet Infectious Diseases* 2002; 2*:* 33-42.
7. Lima EQ, Gorayeb FS, Zanon JR, Nogueira ML, Ramalho HJ & Burdmann EA. Dengue haemorrhagic fever-induced acute kidney injury without hypotension, haemolysis or rhabdomyolysis. *Nephrology Dialysis and Transplant* 2007; 22: 3322-3326.
8. Abboud O. Tropical Acute Renal Failure. Available from: URL: http://www.uninet.edu/ cin2003/ conf/ aboud/aboud.html.
9. Rachmadi D, Djauharie EA, Hilmanto D & Sekarwana N. Renal function in childhood dengue haemorrhagic fever. *Majalah Kedokteran Bandung* 2011; 43(2S): 44S-47S.
10. Laoprasopwattana K, Pruekprasert P, Dissaneewate P, Geater A & Vachvanichsanong P. Outcome of dengue hemorrhagic fever-caused acute kidney injury in Thai children. *Journal of Pediatrics* August 2010; 157(2): 303-9.

11. Lee IK, Liu JW & Yang KD. Clinical characteristics, risk factors, and outcomes in adults experiencing dengue haemorrhagic fever complicated with acute renal failure. *American Journal of Tropical Medicine and Hygiene* 2009; 80(4), 651-655.
12. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS & Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. *Kidney International* 2007; 71: 1028-1035.
13. Schwartz GJ, Brion LP & Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. *Pediatric Clinics of North America* 1987; 34(3): 571-90.
14. Muhammad AM Khalil, Sarfaraz Sarwar, Muhammad A Chaudry, BailaMaqbool, Zarghoona Khalil, Jackson Tan, *et al*. Acute kidney injury in dengue virus infection. *Journal of Clinical Kidney* 2012; 5: 390-394.
15. Mehra N, Patel A, Abraham G, Reddy YN & Reddy YN. Acute kidney injury in dengue fever using acute kidney injury network criteria: Incidence and risk factors. *Tropical Doctor* July 2012; 42(3): 160-2.
16. Jain PK, Sharma AK, Agarwal N, Siddiqui MZ, Pawal P, Gaba R, *et al*. A prospective clinical study of incidence of hepatorenal and hematological complications in dengue fever and management of symptomatic bleed in Bundelkhand region of Northern India with fresh whole blood. *Journal of Infectious Diseases and Immunity* July 2011; 3(7): 24-133.
17. Mendez A & Gonzalez G. Dengue haemorrhagic fever in children: Ten years of clinical experience. *Biomedica* 2003; 23: 180-193.
18. Zappitelli M, Parikh CR, Akcan-Arikan A, Washburn KK, Moffett BS & Goldstein SL. Ascertainment and epidemiology of acute kidney injury varies with definition interpretation. *Clinical Journal of the American Society of Nephrology* 2008; 3: 948-954.
19. Nair VR, Unnikrishnan D, Satish B & Sahadulla MI. Case report acute renal failure in dengue fever in the absence of bleeding manifestations or shock. *Infectious Disease in Clinical Practice* 2005; 13: 142-3.
20. Vogt VA & Avner ED. Renal failure. In: *Nelson Textbook of Paediatrics*, Behrman RE & Kleigeman RM, Arvin AM eds. 17th ed. Philadelphia: WB Saunders Co. 2003; 1767-71.
21. Mehta P, Sinha A, Sami A, Hari P, Kalaivani M, Gulati A, *et al*. Incidence of acute kidney injury in hospitalized children. *Indian Pediatr*ics 2012; 49: 537-542.
22. Kuo MC, Lu PL, Chang JM, Lin MY, Tsai JJ, Chen YH, *et al*. Impact of renal failure on the outcome of dengue viral infection. *Clinical Journal of the American Society of Nephrology* 2008; 3: 1350-1356.
23. Shivbalan S, Anandnathan K, Balasubramanian S, Datta M & Amalraj E. Predictors of spontaneous bleeding in dengue. *Indian Journal of Pediatric*s 2004; 71(1): 33-36.

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Serum 25(OH)D_3, Calcium, Phosphorus Levels and Bone Mineral Density in Adult Women

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Vitamin D is important for bone health. Nowadays, there is a high incidence of low bone mineral density (BMD) in Myanmar postmenopausal women; therefore, its association with vitamin D status among Myanmar women needs to elucidate. This study was aimed to determine the serum 25(OH)D_3, calcium, phosphorus levels and BMD in adult women and the association between biochemical markers and BMD T score. A total of 120 women between 31-60 years of age were studied for their BMD T score and concentration of serum 25(OH)D_3, calcium and phosphorus. The subjects were categorized according to three age groups (31-40, 41-50 and 51-60 years) and by menstrual status (premenopausal and postmenopausal women). Among the three age groups, mean serum 25(OH)D_3 level (108.52±40.77, 113.48±46.57, 54.57±12.66 nmol/L) and BMD T score (-1.67 ±0.69, -1.93±0.64, -2.74±0.76) were found to be significantly decreased in the oldest group ($p<0.0001$) whereas serum phosphorus level was significantly increased in this group (3.81±0.47, 4.00±0.49, 4.27±0.65 mg/dl, $p<0.001$). Premenopausal women were found to have significantly higher level of serum 25(OH)D_3 (113.41±43.08 *vs*. 56.81±15 nmol/L, $p<0.0001$) and BMD T score (-1.73±0.67 *vs*. -2.76±0.66, $p<0.0001$) than those of postmenopausal women. But serum phosphorus level was significantly higher in postmenopausal women than in premenopausal women (3.89±0.49 *vs*. 4.26±0.62 mg/dl, $p<0.0001$). Serum calcium level was not statistically different ($p=0.062$) among three different age groups as well as between pre- and postmenopausal women ($p=0.245$). There was significant positive correlation between BMD T score and serum 25(OH)D_3 level ($r=0.645$, $p<0.05$) in the whole group. Thus, it can be concluded that vitamin D status of adult women is positively associated with their BMD.

Key words: Serum 25(OH)D_3, Calcium, Phosphorus, Bone mineral density, Adult women

INTRODUCTION

Bone is a dynamic structure made up of microscopic hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$] crystals of phosphates and calcium within a matrix of collagen. Old bone is constantly resorbed and new bone is formed. During aging, the rate of bone formation falls behind the rate of resorption, and there is generally a net loss of bone. Decreasing in bone mass may lead to increased vulnerability to fractures. The most common bone disease in women is osteoporosis. Women have a lifetime risk of osteoporotic fracture as high as one in three. World Health Organization estimated that the number of hip fracture worldwide increase to 6.3 million per year at the year 2050.[1]

In Myanmar, it was reported that 6.6% of subjects gave a history of bone fracture in their postmenopausal life in a community-based study.[2] In North Okkalarpa General Hospital (Orthopedic ward), 42.57% of admission cases of postmenopausal women

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were suffered osteoporotic-related fractures.[3] Osteoporotic-related fracture is also still high in Myanmar.

Vitamin D metabolites promote the absorption of calcium and phosphate from gastrointestinal tract (GIT) and retention of calcium and phosphate in the body, which enables normal mineralization of bone. In vitamin D deficiency state, calcium absorption from GIT is decreased. Low serum calcium stimulates the production of parathyroid hormone (PTH) which regulates calcium homeostasis by increasing the conversion of vitamin D to its active form in the kidney. Vitamin D in turn increases intestinal calcium absorption. Thus, in a state of vitamin D deficiency, calcium absorption from GIT is decreased.[4] Many researches over last decades reported that serum $25(OH)D_3$ is associated with bone mineral density (BMD).[5-7]

Since Myanmar is a tropical country, it could be assumed that Myanmar population would get enough sunlight for vitamin D synthesis. However, osteoporotic-related fracture is not uncommon in Myanmar women. Even so, there was limited information about vitamin D status of Myanmar women. Therefore, the present study was aimed to examine whether there is an association among the serum $25(OH)D_3$, serum calcium and serum phosphorus levels and bone mineral density in adult women.

The study was conducted to compare the serum $25(OH)D_3$, serum calcium, serum phosphorus levels and bone mineral density among adult women with different age and menstrual status, and to correlate the serum $25(OH)D_3$ level, serum calcium level, serum phosphorus level with bone mineral density of them.

MATERIALS AND METHODS

Subject

Type of study was cross-sectional analytical design. Sampling method is random sampling method. A total of 120 women between 31-60 years were selected. Eligible subjects were women aged between 31 to 60 years, had no history of renal disease and liver disease, and were not taking vitamin D supplementation, calcium supplementation, anticonvulsant, diuretics and steroid drugs. These subjects were asked about their age, menstrual status, and past history of fractures. Absence of menstrual period for 12 months was defined as menopause.

Bone mineral density measurement

Bone mineral density of heel bone was measured by ultrasonic bone densitometer (SONOST 3000, Osteosys, Korea). The result is reported in T score which is comparison between the bone density of the participant and that of a normal healthy young adult of the same sex.

Laboratory method

For the serum analysis, 5 cc of venous blood were taken without applying tourniquet to prevent haemolysis which could make error in serum phosphours level determination. Serum was separated and stored at -20°C for two months. Serum calcium and phosphorus were analyzed by spectrophotometer using reagent kits (Human, Germany). Serum calcium and phosphorus were determined at Laboratory of Department of Biochemistry, University of Medicine (Mandalay). Estimation of serum $25(OH)D_3$ was done by ELISA method using a kit purchased from Euroimmune, Germany and performed in Public Health Laboratory, Mandalay.

Statistical analysis

The subjects were categorized into three groups by age (31-40, 41-50, and 51-60 years age groups) and also two groups by menstrual status (premenopausal and postmenopausal women). Serum calcium, phosphorus, vitamin D level and BMD T score were mentioned as mean±standard deviation. The mean values of them among different age groups were analyzed by ANOVA. Differences in serum calcium, phosphorus, $25(OH)D_3$ and BMD T score between premenopausal women and

postmenopausal women were analyzed using unpaired 't' test. A value of $p \leq 0.05$ was considered as a significance. Pearson correlation coefficient was used to evaluate a correlation between serum calcium, phosphorus, 25(OH)D_3 and BMD T score in all participants.

Ethical approval

The study was approved by Post-graduate Academic Board Studies of University of Medicine (Mandalay).

RESULTS

Table 1 shows the mean±SD of the serum calcium, serum phosphorus, serum 25(OH)D_3 levels and BMD T score in the three different age groups (31-40 years, 41-50 years and 51-60 years).

Table 1. Comparison of serum calcium, serum phosphorus, serum 25(OH)D_3 levels and BMD T score among different age groups of women (mean±SD)

Parameters	Age (years) (n=40)			ANOVA (p)
	31-40	41-50	51-60	
Serum calcium (mmol/L)	2.23 ±0.19	2.23 ±0.18	2.12 ±0.26	0.062
Serum phosphorus (mg/dl)	3.81 ±0.47	4.00 ±0.49	4.27 ±0.65	<0.001*
Serum 25(OH)D3 (nmol/L)	108.52 ±40.77	113.48 ±46.57	54.57 ±12.66	<0.0001*
BMD (T score)	-1.67 ±0.69	-1.93 ±0.64	-2.74 ±0.76	<0.0001*

*=Significant differences among groups

Both mean serum calcium and phosphorus levels were within normal range. In this study, mean serum calcium level between the three different age groups was not statistically different (p=0.062). Serum phosphorus level between three age groups was significantly different (p<0.001). Serum 25(OH)D_3 was not significantly different between 31-40 years and 41-50 years age groups but it was lower in 51-60 years age group comparing with the other two groups (p<0.0001). BMD T score between three different age group was also significantly different, 51-60 years age group showed the lowest (p<0.0001) BMD T score.

The mean age of premenopausal and postmenopausal women were found to be 40.1±6.19 years and 54.28±3.65 years, respectively. Biochemical data are shown in Table 2.

Table 2. Comparison of age, serum calcium, serum phosphorus, serum 25(OH)D_3 levels and BMD T score in pre-menopausal and postmenopausal women (mean±SD)

Parameters	Menopausal women		p value
	Pre (n=75)	Post (n=45)	
Age (years)	40.1±6.19	54.28±3.65	<0.001*
Serum calcium (mmol/L)	2.21±0.19	2.17±0.26	< 0.245
Serum phosphorus (mg/dl)	3.89±0.49	4.26±0.62	<0.0001*
Serum 25(OH)D_3 (nmol/L)	113.41±43.08	56.81±15	<0.0001*
BMD (T score)	-1.73±0.67	-2.76±0.66	<0.0001*

*=Significant differences among groups

The mean serum calcium levels were not different between the two groups (p<0.245). Mean serum phosphorus level of premenopausal women was found to be significantly lower than that of post-menopausal women (p<0.000l). But pre-menopausal women have significantly higher level of mean serum 25(OH)D_3 and greater BMD T score as compared to those of postmenopausal women.

Table 3. Correlation of various blood parameters with bone mineral density

Blood parameters	Bone mineral density (T score)	
	r	p
Serum 25(OH)D_3 (nmol/L)		
Whole group	0.645	<0.01*
Premenopausal women	0.42	<0.0001*
Postmenopausal women	0.7	<0.0001*
Serum calcium (mmol/L)		
Whole group	0.21	<0.05*
Premenopausal women	0.18	<0.06
Postmenopausal women	0.19	<0.1
Serum phosphorus (mg/dl)		
Whole group	-0.25	<0.01*
Premenopausal women	-0.11	<0.1
Postmenopausal women	-0.03	<0.4

*=Significant differences among groups

There was positive correlation (r=0.645, p<0.01) between BMD T score and serum

$25(OH)D_3$ level in all subjects and the finding was statistically significant. Significant positive correlation between serum $25(OH)D_3$ level and BMD T score was found ($r=0.42$, $p=0.0001$) in premenopausal women but the correlation was stronger in postmenopausal women ($r=0.7$, $p=0.0001$). There was weak positive correlation between serum calcium level and BMD T score ($r=0.21$, $p<0.05$) and weak negative correlation between serum phosphorus level and BMD T score ($r= -0.25$, $p<0.01$) in the whole study group but both correlations were significant (Table 3).

DISCUSSION

Bone is a dynamic tissue with constant resorption and formation, permitting the maintenance of bone tissue, repairing damaged tissue and the homeostasis of the phosphocalcaemic metabolism. Approximately 5-10% of bone is renewed per year. Bone remodeling occurs throughout life. Up to the third decade, the balance is positive and the bone mass is at maximum during the third decade. However, age-related bone loss occurs around the fourth decade, resulting in a gradual decline of BMD.[8] This process is accelerated in females, especially during and up to 10 years postmenopausal period owing to possible oestrogen deficiency deriving the bone loss.[9] The development of bone disease in later life is related to the attainment of maximum peak bone mass and the maintenance of bone mass in adulthood.[10]

In the present study, BMD T score was found to be significantly decreased in accordance with increasing age (-1.67±0.68, -1.9±0.64 and -2.7±0.76 for 31-40 years, 41-50 years and 51-60 years, respectively) ($p<0.0001$, ANOVA). A study found that BMD measures in black women increased with increasing age up to 44 years, and then decline with advancing age. But in women of mixed race, increasing BMD was found up to 39 years and beyond this age group BMD decreased with advancing age.[11] Similarly, a study in Vietnamese adult women showed an increase of BMD up to fourth decade followed by a significant decrease after 45 years.[12] Therefore, it is evident that starting age of BMD declining might be influenced by genetic, environmental, physical activity and nutritional status.

In the present study, BMD was significantly lower in postmenopausal women than those of premenopausal women ($p<0.001$). Mean T-score of BMD in postmenopausal women was observed to be -2.7±0.66 and premenopausal women was -1.7±0.67. One study also reported that mean T score of BMD of postmenopausal women was significantly lower than that of premenopausal women (-3.0±0.54 and -1.42±1.11, respectively).[13] Therefore, these findings were consistent among studies of Myanmar population. A similar finding was also reported in Turkish population where T score of postmenopausal women is -2.8.[14] In conclusion, fall in oestrogen secretion after menopause might be one of the factors which can deteriorate BMD.

In the present study, the lowest mean serum $25(OH)D_3$ level was found in 51-60 years age group and postmenopausal women have significantly lower level of serum $25(OH)D_3$ than that of premenopausal women ($p<0.0001$). Therefore, it would be suggested that age profoundly influence the vitamin D status among adult women. The possible explanation for this finding is that precursor of $25(OH)D_3$ exists as 7 dehydrocholesterol in epidermal layer of skin and amount of this precursor decreases with increasing age and reduces as much as 70% at the age of 70.[15]

The other factors responsible for lower vitamin D status include poor dietary vitamin D intake, decreased exposure to sunlight due to latitude, season, time of day, atmospheric components, clothing, sunscreen use and skin pigmentation, obesity and several chronic illnesses.[16] In the study, participants were asked for their chronic illness, and measured BMI. However, the current study could not study about dietary

vitamin D intake, which is one of the reasons of vitamin D insufficiency. Further studies are required to understand the reason of lower serum vitamin D level in old age, particularly in Myanmar people.

Lower level of serum vitamin D level observed in older age group has further consequence in BMD. Normal serum $25(OH)D_3$ levels are important for calcium absorption efficiency in the gut.[17] An alteration in vitamin D status and/or a reduced synthesis of 1, 25-dihydroxyvitamin D predispose to decrease intestinal calcium absorption. Low serum calcium stimulates the production of parathyroid hormone which regulates calcium homeostasis by increasing the conversion of vitamin D to its active form.

Vitamin D, then, acts directly upon enterocytes to increase active transcellular transport of calcium. The impact of low serum calcium is reduced by release of calcium from bone in response to parathyroid hormone and 1, $25(OH)D_3$. As a result of homeostatic responses, vitamin D-deprived people maintain near normal serum calcium but display increased intestinal calcium absorption, increased bone resorption and progressive osteopenia.[18]

This is evident in the present study. The strength of correlation of serum vitamin D and BMD T score was strong ($r=0.42$, $p=0.0001$) for premenopausal women and it was stronger in postmenopausal women ($r=0.7$, $p=0.0001$). This data indicates that the vitamin D status is one of the important contributory factors in lower BMD of Myanmar women. A study showed that $25(OH)D_3$ insufficiency was a common risk factor for osteoporosis, associated with increased bone remodeling and low bone mass.[6]

In the study, mean serum calcium and phosphorus levels were within normal range. Moreover, serum calcium levels between three different age groups were not different. However, serum phosphorus level of older age group (51-60 years) was higher than those of other two groups. By comparison between premenopausal and postmenopausal women, serum phosphorus levels were found to be significantly lower in premenopausal women. Meanwhile, serum calcium levels were not different between two groups.

The findings were consistent with previous study of Myanmar female in which mean serum phosphorus level of menopausal women (4.27 ± 0.21 mg/dl) was significantly different ($p<0.05$) from that of premenopausal women (3.77 ± 0.11 mg/dl).[19] The change in serum calcium and phosphorus levels at older age group may be due to hormonal derangement at postmenopausal age.

When the correlation between BMD and serum calcium or phosphorus was determined, it was observed that there were weak positive correlation between serum calcium ($r=0.21$, $p<0.05$) but weak negative correlation between serum phosphorus with BMD T score ($r=-0.25$, $p<0.01$), respectively. This indicates that changes in BMD T score would be explained weakly by the observed serum levels of calcium and phosphorus. Similar finding is also observed in other study in which serum calcium and phosphorus levels did not have significant correlation with BMD in Iran postmenopausal women.[20] Serum calcium and phosphorus levels show the current calcium and phosphorus levels of an individual while BMD denotes bone mineral status over a period of time depending upon a variety of other factors.

There would be other factors contributing to the lower BMD in older age group or in the postmenopausal women of the present study. The amount of peak bone mass that is gained during childhood and adolescence is also an important preventive strategy for further bone loss and resistance to fracture.[10] Another important factor would be the effect of estrogens. Estrogen deficiency in postmenopausal groups potentiated the effect of PTH excess with lower vitamin D level.[21]

There are some limitations in the present study: the type of study is cross-sectional; the study did not analyze serum estrogen and serum parathyroid levels which play important contributions to low BMD. In addition, there is limited information for the participants' dietary intake of calcium, vitamin D and physical activity. Moreover, Dual energy X-ray absorptiometry (DEXA) is the gold standard diagnostic tool for osteoporosis but high cost and lack of portability restrict its uses. The current study used ultrasonic bone densitometer, which measures bone loss and predict risk for fracture, to measure the bone mineral density.

Conclusion

The present study explored the status of serum calcium, phosphorus, $25(OH)D_3$ level and BMD T score in adult women. Although mean serum calcium and phosphorus levels were within normal range, low BMD was seen in older women or postmenopausal women. Serum calcium and phosphorus levels were weak correlation with BMD in whole study group. In the present study, serum $25(OH)D_3$ showed positive correlation with BMD in all participants especially in postmenopausal women.

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REFERENCES

1. Delmas PD & Fraser M. Strong bones in later life: luxury or necessity? *Bulletin of World Health Organization* 1999; 77(5): 416-422.
2. Mya Thida. Review of the studies on women health after menopause in Myanmar. National Seminar on Women Health in Myanmar 2001; 184-191.
3. Ngwe Soe. Epidemiological study on osteoporosis related orthopaedic problems in postmenopausal women. *Thesis,* M.Med.Sc, University of Medicine 2, 1995.
4. Khaw KT, Sneyd MJ & Compston J. Bone density, parathyroid hormone and 25-hydroxy-vitamin D concentrations in middle aged women. *British Medical Journal* 1992; 305: 273-277.
5. Adami S, Bertoldo F, Braga V, Fracassi E, Gatti D, Gandolini G, *et al*. 25-hydroxy vitamin D levels in healthy premenopausal women: Association with bone turnover markers and bone mineral density. *Bone* 2009; 45: 423-426.
6. Mezquita-Raya P, Munoz-Torres M, Luna JDD, LunaV, Lopez-Rodriguez F, Torres-Vela E, *et al*. Relation between vitamin D insufficiency, Bone density, and bone metabolism in healthy postmenopausal women. *Journal of Bone and Mineral Research* 2001; 16(8): 1408-1415.
7. Kuchuk NO, Van Schoor NM, Pluijm SM, Chines A & Lips P. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: Global perspective. *Journal of Bone and Mineral Research* 2009; 24(4): 693-701.
8. Emaus N, Berntsen GK, Joakimsen RM & Fønnebø V. Longitudinal changes in forearm bone mineral density in women and men aged 25-44 years: The Tromso Study: A population-based study. *American Journal of Epidemiology* 2005; 162: 633-643.
9. Weitzmann NM & Pacifici R. Estrogen deficiency and bone loss: An inflammatory tale. *Journal of Clinical Investigation* 2006; 116: 1186-1194.
10. Slemenda CW, Hui SL, Longcope C, Wellman H & Johnston CC Jr. Predictors of bone mass in premenopausal women: A prospective study of clinical data using photon absorptiometry. *Annals of Internal Medicine*1990; 112: 96-101.
11. Constant D, Rosenberg L, Zhang Y, Cooper D, Kalla AA, Micklesfield L, *et al*. Quantitative ultrasound in relation to risk factors for low bone mineral density in South African premenopausal women. *Archives of Osteoporosis* 2009; 4(1-2): 55-65.
12. Vu TTH, *et al*. Determining the prevalence of osteoporosis and related factors using quantitative ultrasound in Vietnamese adult women. *American Journal of Epidemiology* 2005; 161: 824-830.
13. Ni Ni Lin. Serum C-telopeptide, serum estrogen level, BMD and OSTA score in premenopausal and postmenopausal women. *Thesis,* M.Med.Sc, University of Medicine 2, 2012.
14. Ceilker R & Arslan S. Comparison of serum insulin-like growth factor I and growth hormone levels in osteoporotic and non-osteoporotic

postmenopausal women. *Rheumatology International* 2000; 19(6): 205-208.

15. Holick MF. Vitamin D-new horizons for the 21st century. *The American Journal of Clinical Nutrition* 1994; 60: 619-30.
16. Tsiaras WG & Weinstock MA. Factor influencing vitamin D status. *Acta Dermato-Venereologica* 2011; 91: 115-124.
17. Heaney RP, Dowell MS, Hale CA & Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *Journal of the American College of Nutrition* 2003; 22: 142-6.
18. Bringhurst FR, Demay MB & Kronenberg HM. Homones and disorders of mineral metabolism. In: *Williams Textbook of Endocrinology*. 11th ed 2008; 1203-1268.
19. May Thazin. Calcium and phosphate homeostasis in apparently healthy Burmese subjects and in some clinical status. *Thesis*, M.Med.Sc, University of Medicine (Mandalay), 1989.
20. Nozila F, Alireza O, Soltanali M & Susan K. Assessment of association between serum biomarkers (calcium, phosphorous and parathyroid hormone (PTH)) of osteoporosis and bone mass density (BMD) in postmenopausal women in Iran, Tabriz. The 4th Pan Arab Osteoporosis Society Congress *2008*.
21. Ooms ME, Lips P, Roos JC, van der Vijgh WJ, Popp Snijders C, Bezemer PD, *et al*. Vitamin D status and sex hormone binding globulin: Determinants of bone turnover and BMD in elderly women. *Journal of Bone and Mineral Research* 1995; 10: 1177-1184.

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Predictive Model of Birth Weight Using Simple Anthropometric Measurements of Baby and Selected Maternal Characteristics

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This cross-sectional analytic study was conducted to develop a statistical model to predict birth weight based on anthropometric measurements of baby namely, thigh circumference (TC), calf circumference (CC), midupper arm circumference (MUAC) and head circumference (HC), and selected maternal characteristics such as age, gravida, height and inter-pregnancy interval in Central Women's Hospital (Mandalay) during 2008 and 2009. The subjects were 991 babies delivered during study period and their mothers. The anthropometric measurements were taken within 24 hours after delivery. Mean birth weight was 3.12±0.48 kg. The anthropometric measurements of babies were strongly correlated to his/her birth weight. The present study found out that maternal height and baby's sex, gestational age, TC, CC, MUAC and HC were significant predictors of birth weight. The final model containing these variables can explain about 84% of variation of baby's birth weight. The separate predictive models were also developed using each anthropometric measurement of a baby as a predictor. A predictive model using TC along with gestational age, sex and maternal height explains about 77% of baby's birth weight. A similar model containing CC, gestational age, sex and maternal height explains about 72% of birth weight of a baby. The MUAC along with gestational age, sex and maternal height can also explain about 71% of birth weight of a baby. A model containing HC, gestational age and maternal height can explain about 60% of baby's birth weight. These models can be used to predict birth weight of a baby in areas where there is no facility to measure birth weight.

Key words: Predictive model, Birth weight, Anthropometric measurements

INTRODUCTION

Birth weight means weight of a baby at birth. It is a reliable indicator of not only health status of a mother, but also a baby's chance of survival and future health.[1] Therefore, birth weight of every newborn baby should be measured. However, it is estimated that more than half (58%) of children in developing countries are not weighed at birth.[2]

In Myanmar, only 40% of deliveries were attended by skilled persons, mainly mid-wives (MWs), 12.5% by auxiliary midwives (AMWs) and 7.5% by traditional birth-attendants (TBAs). Just over 20% of pregnant mothers delivered in a hospital or health centre. It was, therefore, uncertain whether birth weights of remaining babies were measured. The number of facilities with functioning basic essential obstetric care and comprehensive essential obstetric care was 8 per 500,000 population and 4 per 500,000 population, respectively.[3] In India, about 90% of deliveries were conducted by TBAs at home.[4] Therefore, weighing babies immediately after birth poses a considerable challenge and it was necessary to train and use the health workers for measurement of

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birth weight of newborn babies in the field situation. Simple anthropometric measurements of baby were used to predict birth weight in some studies done in various countries.[5-8]

So, this study was carried out to develop a predictive model for birth weight, based on simple anthropometric measurements of baby (TC, CC, MUAC & HC) and selected maternal characteristics such as age, gravida, height and inter-pregnancy interval that can be used by any person whether trained or untrained, in any part of Myanmar.

MATERIALS AND METHODS

A hospital-based, cross-sectional analytic design was used in this study. Altogether 991 babies delivered in Central Women's Hospital, Mandalay (CWH) during 2008 and 2009 and their mothers were recruited into this study after getting informed consent. The sample size requirement was calculated by using following formula:[9]

$n = [(z_\alpha + z_\beta)/C]^2+3$

(Where; z_β=The standard normal deviate for β, $C = 0.5 * \ln [(1 + r)/(1 - r)]$, r=Correlation coefficient)

The estimated correlation coefficients between birth weight, and thigh circumference, calf circumference, MUAC and head circumference were set at 0.6, 0.8, 0.7 and 0.6, respectively,[5-8] and a significant level at 95%, and power at 90%.

Observation and interview methods were utilized for data collection. Flexible, but non-stretchable measuring tape was used to get the thigh circumference, calf circumference, MUAC and head circumference of the babies. Pre-tested and pre-coded proforma was used to achieve the data on characteristics of mothers and their babies.

All newborns were measured their respective thigh circumferences (TC) at the juncture between right thigh and buttock, calf circumferences (CC) at maximum horizontal circumference of right leg, mid-upper arm circumferences (MUAC) at mid-point between tip of right shoulder and tip of right elbow and head circumferences (HC) at maximum transverse circumference within 24 hours of birth. All were measured in centimeter. Birth weight of these babies was measured in kilogram using Seca digital baby weighing scale (model 835). Normal birth weight was defined as birth weight between 2.5 kg and 4 kg.[10]

The data on general characteristics of mothers and their babies were collected by interviewing (to mothers) and reviewing their hospital records. Maternal height was measured in centimeter, at standing position on admission (or) just before discharged from hospital if it was not feasible to measure on admission because of being in labor. The mother was asked to remove shoes, heavy outer garments and hair ornaments before measuring height. She was also asked to stand straight with her back to the height rule and to look straight. The head piece of the stadiometer was lowered so that the hair was pressed flat in measuring her height. To avoid or lessen the measurement errors (i.e., instrumental bias and interviewer bias), only one (and well-trained) data collector who is a medical doctor and the same measuring tape were used. The length of the measuring tape was checked with the calibrated length rod at least once per month. To avoid the spread of infection among newborns through the measuring tape, it was disinfected according to the existing protocol of CWH.

The data were analyzed by using STATA 9 software. Pearson correlation was done to determine the relationship between anthropometric measurements and birth weight. Receiver Operating Characteristic (ROC) analysis was used to determine the optimal cut-off points of these measurements to predict normal birth weight. Multiple linear regression analysis was also utilized to develop a reliable and parsimonious predictive model for birth weight. During the process of building a model, forward step-

wise strategy and regression diagnostic procedures were applied, and outliers and influential subjects were identified and excluded. Then, the model was built again and so on. After getting the best fitted regression model to predict birth weight, validation process (i.e., reliability analysis) was done to ensure that the results are generalizable to the population and not specific to the sample used in estimation.

The study protocol was approved by the Ethical Committee of the University of Medicine (Mandalay). Written informed-consent was also obtained from mothers for participation in the study.

RESULTS

General and obstetric characteristics of mothers

Ages and heights of the mothers ranged from 15 to 47 years and 132.1 to 170 cm, respectively. Mean±SD values of age and height were 29±6.1 years and 153.6±6.1 cm, respectively. Most of them had passed primary school (34%), middle school (26%) and high school (17%). Only 3% were illiterate. Almost all (99.9%) were non-smokers. About half (44.9%) were primigravidae and 10% delivered within 2 years after delivering last child. Mean±SD birth interval was 56±32.1 months.

General characteristics and anthropometric measurements of babies

More than half (52.1%) were males. Mean±SD gestational age was 277±16.4 days. Pre-term, term and post-term deliveries were 9.8%, 78.5% and 11.7%, respectively. Their birth weight and anthropometric measurements are shown in Table 1.

Table 1. Birth weight and anthropometric measurements of babies

Variables	Lowest	Highest	Mean (SD)
Birth weight (kg)	0.9	5.0	3.1(0.5)
Thigh*	10	21.7	16.5(1.6)
Calf*	6.8	13.6	10.8(0.9)
Mid upper arm*	5.7	13.5	10.1(1)
Head*	23.7	37.5	33.4(1.5)

*=Circumference (cm)

There was strong correlation between birth weight and anthropometric measurements of baby. Receiver Operating Curve analysis also revealed that these measurements were very useful in predicting normal birth weight (Table 2).

Table 2. Correlation between birth weight, anthropometric measurements and Area Under the Curve (AUC) in predicting normal birth weight

Anthropometric measurements (Circumference)	Correlation coefficients (p value)	AUC (95%CI)
Thigh	0.82(<0.001)	0.96(0.94, 0.97)
Calf	0.81(<0.001)	0.96(0.94, 0.98)
Mid upper arm	0.80(<0.001)	0.96(0.94, 0.98)
Head	0.71(<0.001)	0.92(0.89, 0.95)

Proper cut-off values and diagnostic accuracy of anthropometric measurement in predicting "normal birth weight" are summarized in Table 3. Final predictive model for birth weight of a baby is shown in Table 4.

Table 3. Cut-off values and diagnostic accuracy of anthropometric measurements in predicting normal birth weight

Anthropometric measurements (Circumference)	Cut-off (cm)	Sensitivity (%)	Specificity (%)	PPV (%)	Correctly Classified (%)
Thigh	14.9-18.8	88.1	73.3	96.5	86.4
Calf	9.9-11.9	83.3	83.8	97.7	83.2
Mid upper arm	9.1-11.1	83.1	78.1	97.0	82.4
Head	32- 35	81.5	77.1	96.8	80.8

PPV=Positive predictive value

Table 4. Final model to predict birth weight of a baby

Variables	Coefficient	't' value	P value
Thigh circumference	0.1177881	10.1	<0.001
Calf circumference	0.0452062	2.2	0.030
Mid upper arm circumference	0.1041789	5.8	<0.001
Head circumference	0.0871093	12.5	<0.001
Gestational age	0.0017646	3.5	0.001
Sex	-0.0711183	-4.3	<0.001
Maternal height	0.0053474	4.1	<0.001
Constant	-4.5665160	-16.2	<0.001

R^2, Adjusted R^2 and MSE were 0.8395, 0.8376 and 0.1880, respectively. It meant that the final model could explain almost 84% of variance of birth weight. Regression equations to predict birth weight of a baby

using all anthropometric measurements and single measurement are as follows:

$Y=\beta_0+\beta_1 X_1+\beta_2 X_2+\beta_3 X_3+---------+\beta_k X_k$

Model (1) using all anthropometric measurements of a baby and mother

Birth weight=0.1177881(TC) +0.0452062(CC)+ 0.1041789(MUAC) + 0.0871093 (HC)+0.0017646(GA)-0.07111830 (S)*+0.0053474(MH)-4.5665160

Model (2) using mainly thigh circumference (TC) of a baby

Birth weight=0.2392766(TC)+0.0034745(GA)- 0.1591719(S)*+ 0.0065968(MH)- 2.746721

Model (3) using mainly calf circumference (CC) of a baby

Birth weight=0.3884975(CC) + 0.0043067(GA) -0.0790642(S)*+0.0068385(MH)- 3.286707

Model (4) using mainly mid-upper arm circumference (MUAC) of a baby

Birth weight=0.3750642(MUAC) + 0.0035274 (GA) - 0.1144479(S)*+0.0063086 (MH)-2.585819

Model (5) using mainly head circumference (HC) of a baby

Birth weight=0.2121929 (HC) + 0.004295(GA) +0.0086685(MH)-6.513948

(TC=Thigh circumference, CC=Calf circumference, MUAC=Mid upper arm circumference, HC=Head circumference, GA=Gestational age, MH=Maternal height, S=Sex, *=0 for male baby and 1 for female baby. Adjusted R^2 of model 1, 2, 3, 4 and 5 were 0.8376, 0.7663, 0.7188, 0.7127 and 0.5980, respectively.)

DISCUSSION

This study showed that the mean birth weight of babies born in CWH (Mandalay) was 3.12±0.48 kg. It was slightly larger than those reported in similar studies conducted in neighboring countries such as India,[4] Nepal,[11] Thai[8] and Vietnam[8] while those found in China,[8] Pakistan[8] and Singapore[8] were larger than that of the present study. This may be due to real difference, or differences in time period (i.e., year the study performed) or place (i.e., urban or rural) or socio-demographic characteristics of mothers or race. Thigh circumference, calf circumference, mid upper arm circumference and head circumference were strongly correlated to birth weight. These findings were consistent with those found in various similar studies.[7, 12, 13]

The present study developed parsimonious predictive models for birth weight based on anthropometric measurements of newborn. Two kinds of predictive models were developed; one included all anthropometric measurements namely, TC, CC, MUAC and HC, and the other contained each (anthropometric) measurement. There are some differences in predictors included in the predictive models developed by previous studies.[14, 15] This may be due to the use of different objectives or predictors. The differences in race or socio-economic conditions or nutritional status might also be responsible.

Although the model containing all anthropometric indicators could explain almost 84% of the variance of birth weight, the possible problem of multi-collinearity in the model as well as difficulty in routine practice should be taken into consideration.

Therefore, it is better to use predictive models that include single anthropometric indicator of baby as an explanatory variable. Among them, the model involving thigh circumference (TC) seems to be the best because of its explanatory power in estimating birth weight and the easiness in practice (i.e., measuring TC at the juncture between thigh and buttock).

Conclusion

These anthropometric measurements can be regarded as simple and reliable indicators that can be used to predict birth weight in areas where there is no facility (to measure birth weight). Among them, thigh circumference seems to be the best indicator.

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REFERENCES

1. World fit for children. Low birth weight, Available from: URL: http://www.childinfo. org/ areas/birthweight/LowBirthweight.htm, accessed 22 April 2007.
2. UNICEF/WHO. Low birth weight: Country, regional and global estimates, 2004.
3. Ministry of Health, Myanmar. *National Health Plan (2006-2011)*, 2006.
4. Kapoor SK, Kumar G, Pandav CS & Anand K. Incidence of low birth weight in rural ballab-garth, Haryana. *Indian Pediatrics* 2001; 38: 271-275.
5. Lawoyin TO. Validation and use of a simple device to identify low birth weight babies at birth. *African Journal of Medicine and Medical Sciences* 1998; 27(3-4): 143-145.
6. Bhat IA, Dhar GM, Shah GN, Neelofar K & Shehzada A. Efficiency of various anthro-pometric measurements in determining low birth weight babies. *Indian Journal of Maternal and Child Health* 1995; 6(2): 40-42.
7. Diamond I, McDonald J & Guidotti R. Use of a simple anthropometric measurement to predict birth weight: WHO collaborative study of birth weight surrogates. *Bulletin of the World Health Organization* 1993; 71(2): 157-163.
8. Naik BD, Kulmi AP & Aswar NR. Birth weight and anthropometry of newborns. *Indian Journal of Pediatrics* 2003; 70(2): 145-146.
9. Hulley SB, Cummings SR, Browner WS & Newman TB. Estimating sample size and power: The nitty-gritty. In: *Designing Clinical Research* Hiscock TY (ed) Lippincott Williams & Wilkins, Philadelphia, 2001; 65-95.
10. CDC. PNSS health indicators, 2004. Available from: URL:http://www.cdc.gov/pednss/what _is/ pnss_health_indicators.htm, accessed 22 April 2007.
11. Sreeramareddy CT, Chuni N, Patil R, Singh D & Shako B. *Anthropometric surrogates to identify low birth weight Nepalese newborns: A hospital-based study*, Available from: URL: http:// www. biomedcentral.com accessed 16 March 2009.
12. Taksande A, Vilhekar KY, Chaturvedi P, Gupta S & Deshmukh P. Predictor of low birth weight babies by anthropometry. *Journal of Tropical Pediatrics* 2007; 53(6): 420-423.
13. Samal GC & Swain AK. Calf circumference as an alternative to birth weight for identification of low birth weight babies. *Indian Pediatrics* 2001; 38: 275-277.
14. Keeley RD, Birchard A, Dickinson P, Steiner J, Dickinson LM, Rymer S, *et al.* Parental attitudes about a pregnancy predict birth weight in a low income population. *Annals of Family Medicine* 2004; 2: 145-149.
15. Kutty VR. Why low birth weight is still a problem in Kerala?: A prelaminary exploration. In: *Discussion paper*, Kerala Research Pro-gramme on Local Level Development, Centre for Development Studies, India, 2004.

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Insulin Receptor Substrate-1 Gene (G972R) Polymorphism and Insulin Resistance in Overweight and Obese Type 2 Diabetes Mellitus Patients

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The insulin receptor substrate-1 gene (IRS-1) gene has been considered a candidate for insulin resistance in type-2 diabetes and coronary artery disease. The most common IRS-1 variant, a glycine to arginine change at codon 972(G972R) is more prevalent among subjects who have features of insulin resistance syndrome associated with type 2 diabetes patients. The aim of the present study was to determine the insulin receptor substrate-1 gene (G972R) polymorphism and insulin resistance in overweight and obese type 2 diabetes mellitus patients. The genomic DNA of the subjects was amplified by polymerase chain reaction (PCR) and digested by restriction fragment length polymorphism (RFLP) with BstN1 used for codon 972. Fasting insulin and fasting glucose were determined and insulin resistance was evaluated using homeostasis model assessment index for insulin resistance (HOMA-IR). The prevalence of IRS-1(G972R) polymorphism was 19% in the study population, 18 patients were of heterozygous (A/G) genotype and only one patient was of homozygous (A/A) genotype. Remaining 81 patients were of wild type, (G/G) genotype. The percentage of patients with family history of diabetes mellitus was significantly higher in the G972R carrier group than in the non-carrier group. There was a significant association between family history of diabetes mellitus and IRS-1(G972R) polymorphism in the type 2 diabetic patients (p=0.02). Between IRS-1(G972R) carriers and non-carriers, there was no significant difference in insulin resistance. It was concluded that IRS-1(G972R) polymorphism might have a role in the genetic susceptibility of development of type 2 diabetes mellitus in those with family history. But, IRS-1(G972R) polymorphism does not significantly increase insulin resistance compared to the wild type individuals.

Key words: IRS-1gene, G972R, Polymorphism, T2DM

INTRODUCTION

Diabetes mellitus is now declared as a global epidemic.[1] World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes, according to 2005 figures.[2] This number is likely to be more than double by 2030 without intervention. According to WHO estimation, the prevalence of diabetes mellitus in Myanmar was 2.4% in 1995 and it will be 3.2% in the year 2025.[3]

Insulin receptor substrate-1 (IRS-1) occupies a key position in the insulin signaling pathway.[4] As IRS-1 is the first substrate in this cascade, an impaired IRS-1 function may result in a defect in insulin signaling.[5] Thus, genetic changes in IRS-1 may potentially contribute toward the development of insulin resistance, the most common of these being a glycine to arginine change

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at codon 972(G972R).[6] The prevalence of IRS-1(G972R) polymorphism was higher in T2DM especially in obese patients, and the prevalence of polymorphism is reported to be varied in various studies probably due to differences in genetics, race and ethnicity, etc.

There are also conflicting reports regarding the relationship between the IRS-1(G972R) polymorphism and insulin resistance, fasting plasma insulin and blood glucose control. Since, the prevalence of IRS-1(G972R) polymorphism in Myanmar might differ from other regions and the effect of polymorphism on insulin resistance in T2DM subjects is not yet reported, the prevalence of polymorphism and the association of polymorphism with insulin resistance were investigated in the present study.

The findings of the present study would highlight the genetic variation of polymorphism in T2DM among populations and show whether the IRS-1 variant has effect on the insulin resistance particularly in obese individuals.

MATERIALS AND METHODS

History taking and physical examination including anthropometric measurement were done and blood samples were collected from diabetic patients attending Diabetes Outpatients Department of YGH.

Fasting blood sugar, serum insulin were measured and polymerase chain reaction (PCR) and restriction fragment length plymorphism (RFLP) for IRS-1 gene were also done at Pathology Research Division, Department of Medical Research (Lower Myanmar). Data were analyzed by SPSS (version 16.0) statistical software. Overweight and obesity were defined according to WHO guideline (2006), overweight as BMI $\geq$25 kg/m^2, obesity as BMI $\geq$30 kg/m^2.

Data were presented as mean value± standard deviation (SD). Comparison between two means was done using Student's *'t'* test (unpaired) and the difference was considered significant when p value is <0.05. The disease association with proportions of sample variables was tested by 'Chi' square test with 95% confidence interval.

RESULTS

Among 100 overweight and obese T2DM, 81 patients were of homozygous (G/G) genotype, 18 patients were of heterozygous (G/A) and only one patient of homozygous (A/A) genotype. The allele frequencies of 'G' was 90% and that of 'A' was 10% (Table 1).

Table 1. Genotype distributions and allele frequencies for G972R mutation in IRS-1 gene in patients with overweight and obese type 2 diabetes mellitus

Variables	Obese	Overweight	Total (%)	Remark
Genotypes				NS
G/G	23	58	81(81)	
G/A	7	11	18(18)	
A/A	-	1	1(1)	
No. of patients	30	70	100	
Allele				NS
G	53	127	180(90)	
A	7	13	20(10)	
No. of patients	60	140	200	

NS=Not significant

Family history of diabetes mellitus between the G972R carrier and non-carrier groups

It was found that 10 out of 19 G972R carriers and 19 out of 81 non-carriers had family history of diabetes mellitus.

The proportion of patients with family history of diabetes was greater in the G972R carrier than in the non-carrier group (52% *vs.* 23%). The G972R polymorphism is significantly associated with family history of diabetes in the study groups (p=0.02) (Fig.1).

Insulin status of the study population

Mean value of insulin resistance (HOMA-IR) calculated from fasting blood sugar and fasting serum insulin was 6.28 (0.93-34.59) and β-cell function was 117.78 (7.12-540.91)% in the present study (Table 2).

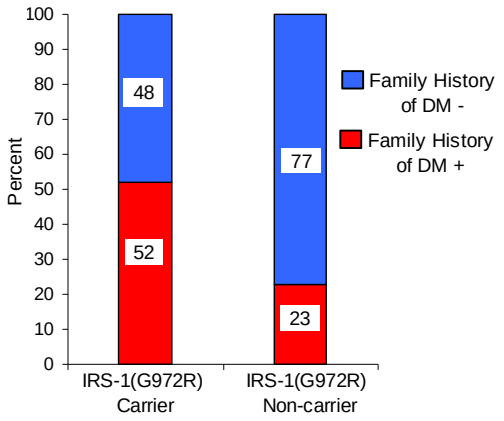

Fig. 1. Family history of diabetes mellitus in the study groups

Table 2. Insulin status of the study population

Clinical characteristics	Study group (n=100), Mean±SD
Fasting blood sugar (mmol/L)	8.25±3.38
Fasting serum insulin (µU/mL)	17.45±14.65
HOMA-IR	6.28±6.29
Log HOMA-IR	0.64±0.35
β-cell function (%)	117.78±121.27
Log β-cell function	1.83±0.48

Table 3. Insulin status of IRS-1 (G972R) carriers and non-carriers

Insulin status	Carrier (n=19)	Non-carrier (n=81)	Remark
Fasting blood sugar (mmol/L)	8.27±2.10	8.24±3.63	NS
Fasting serum insulin (µU/mL)	17.39±11.34	17.47±15.38	NS
Log fasting serum insulin	1.15±0.29	1.09±0.34	
HOMA-IR	6.43±4.63	6.25±6.65	
Log HOMA-IR	0.70±0.31	0.62±0.36	NS
β-cell function (%)	84.9±64.29	125.49±130.2	NS
Log β-cell function (%)	1.80±0.34	1.83±0.51	

NS=Not significant

Insulin status of the IRS-1 (G972R) carriers and non-carriers

There was no significant difference in FBS, FSI, HOMA-IR and β-cell function between IRS-1(G972R) carriers and non-carriers (Table 3).

DISCUSSION

Demographic risk factors and IRS-1 gene (G972R) polymorphism

In the present study, the G972R polymorphism was observed in 19% of T2DM. The prevalence of the G972R polymorphism appears to be higher than other Western (Danish, Finnish, African, Turkish and American) and Asian (Japanese, Taiwanese and Indian) studies.[7-10]

IRS-1(G972R) polymorphism was detected in various age groups in different study population. G972R polymorphism was reported in younger T2DM cases (age range 18-32 years) in one study.[11] The polymorphism was also detected in later ages (54.5± 8.2 years) in another study.[12] In the present study, the mean age of G972R carrier was 56.89±11.4 years which was similar to that of two other studies (59±6 years).[12, 13]

The prevalence of polymorphism does not seem to vary in different age groups. One study[14] showed that the prevalence was 12.4% and age of the study population was 54.81±6 years whereas in another study,[15] the prevalence was 15.8% and age of the study population was 37.5±12.2 years. The prevalence was 1.8% and age of the study population was 31.38±11.7 years in a study[16] whereas the prevalence (4.2%) and age of the study population (53.7±15 years) were found in Japanese patients.[17]

The prevalence of G972R polymorphism does not seem to be related to BMI since the prevalence was quite high 15.8% in the study[15] in which BMI was only 22.14± 3.98 kg/m^2; yet it was only 4.2% in another study[17] with more or less similar BMI, 22.9±3.8 kg/m^2. In a meta-analysis study,[5] there was no association between BMI and G972R was reported among individuals with BMI less than 27 kg/m^2. However, it was found that a stronger association between the G972R and T2DM was reported among participants with BMI less than 23.1 kg/m^2 than among participants with BMI of at least 23.1 kg/m^2.[18]

The prevalence of G972R polymorphism in the present study was much higher than that reported in the Asian region. The BMI of other studies was found to be much lower than the present study (25.1±3.1 kg/m^2, 25.69±5.27 kg/m^2 *vs.* 28.35±4.36 kg/m^2).[16, 19]

However, the prevalence of G972R polymorphism in those studies was 1.1% and 1.8%, respectively and that of the present study was many folds higher, 19%. Thus, it seems that factors other than regional and ethnic difference might have a role in the prevalence of polymorphism since the percentage of polymorphism differs between studies carried out in two places on the population with comparable BMI range.

The percentage of G972R polymorphism in general population was 13% in a study[20] and 4% in the other.[6] In the above studies, the prevalence of G972R polymorphism in T2DM was 23% and 11%, respectively. In one combined analysis, it was reported that G972R substitution was present in 15% of 117 patients with T2DM and 7% of 94 normal subjects, indicating that the prevalence of G972R polymorphism was twice higher in diabetic patients than the normal subjects. These observations are consistent with the hypothesis that mutations in the IRS-1(G972R) gene contribute to the pathogenesis of T2DM in 10-20% of the population.[20]

Moreover, family history of diabetes played an important role in T2DM patients. In the present study, 29% of T2DM patients had family history of diabetes mellitus. Among them, 34% of the subjects were G972R carrier patients. It was reported that among 153 non-diabetic offspring with only one parent affected by T2DM, 17% of patients had G972R polymorphism.[21] Comparing with this study, the prevalence of G972R polymorphism was half less in non-diabetic with family history than diabetic with family history.

In the present study, the proportion of patients with family history of diabetes was greater in the G972R carrier than in the non-carrier group (52% *vs.* 23%) which was similar to the Mexican study (36% *vs.* 13%).[18] These findings suggested that diabetes risk might be higher in the G972R carrier with family history of diabetes.

IRS-1 genotypes and insulin status

In the present study, out of 100 overweight and obese individual, 18 patients were heterozygous (G/A) carrier and only one overweight patient was homozygous (A/A) carrier. In two studies,[12, 14] it was highlighted that the majority of the G972R polymorphism was of heterozygous (G/A) but there was no case of homozygous (A/A) carrier in T2DM patient in the Japanese study.[12]

The insulin and glucose status, and the severity of diabetes mellitus was found to be higher in the population with higher G972R polymorphism prevalence. In this study and also in African-Americans study,[14] although the age and BMI are comparable, FBS and FSI levels were found to be higher. That might also imply to normal subjects because it was reported in a study that two people who were of homozygous G972R(A/A) substitution showed impaired glucose tolerance and a moderate degree of insulin resistance.[12]

However, no significant differences in BMI, FBS and FSI level were observed between G972R carrier and non-carrier in the present study. It thus suggested that G972R polymorphism alone may not impair the insulin and glucose status but other genetic, environmental and life style factors would play a role in the development and progression of the disease. Therefore, analysis of polymorphism at site other than 972 in IRS-1 gene and finding out other genetic alterations and consideration of the risk factors seem to be required when attempt is made to determine the role of genetic polymorphism in the disease pathophysiology.

The G972R polymorphism has 2 forms: heterozygous (G/A) and homozygous (A/A). Although it is not known that whether genotypic difference has an effect on the insulin and glucose status, in the present study, one and only homozygous case was found to be insulin resistant whereas 13 out of 18 heterozygous cases were insulin

resistance. The allele frequencies of ˙G' was 90% and that of ˙A' was 10%. Neither the allelic frequency nor the genotypic frequency seems to be significantly different between the overweight and obese T2DM patients, further disapproving the notion of any relationship between BMI and gene polymorphism.

It was reported in a study that among the common polymorphisms of the IGF-1R, IRS-1 and IRS-2 genes, IRS-1 and IRS-2 genes did not show the conversion from IGT to T2DM, whereas IGF-1R may regulate the risk of developing T2DM.[22] Moreover, a study have analyzed the same two polymorphisms in diabetes subjects participating in the United Kingdom Prospective Diabetes Study (UKPDS) and found only an association between obesity and the beta-3-adrenergic receptor (beta-3-AR) polymorphism but not obesity and the IRS-1 polymorphism[23] but when both polymorphisms were present, there was a huge increase in the frequency of T2DM in Caucasian obese subjects.[24]

Conclusion

The prevalence of IRS-1G972R polymorphism was 19% in overweight and obese type 2 diabetes mellitus patients in the present study. In the presence of family history of diabetes mellitus, IRS-1(G972R) polymorphism is associated with the development of type 2 diabetes mellitus.

It was concluded that IRS-1(G972R) polymorphism might have a role in the genetic susceptibility of development of type 2 diabetes mellitus in those with family history. But IRS-1(G972R) polymorphism does not significantly increase insulin resistance compared to the wild type individuals. Genetic sequencing of IRS-1 and other genes in the insulin signaling pathway, and finding out the alteration in their genetic patterns would provide clues for the association of the site-specific polymorphisms of these genes with insulin resistance in type 2 diabetes mellitus.

REFERENCES

1. Alberti KGMM & Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine* 1998; 15: 539-553.
2. World Health Organization. Diabetes, Fact sheet No. 312, August 2011. Available from: URL: http://www. who.int/ mediacentre /factsheets/ fs 312/en/ accessed 30 October 2011.
3. World Health Organization, Country office for Myanmar. WHO country co-operation strategy (2008-2011) Myanmar. New Delhi, 2012: 120-121.
4. White MF & Kahn CR. The insulin signaling system. *Journal of Biological Chemistry* 1994; 269: 1-4.
5. Jellema A, Mensink RP, Kromhouta D, Saris WHM & Feskens EJM. Metabolic risk markers in an overweight and normal weight population with oversampling of carriers of the IRS-1 972 Arg-variant. *Atherosclerosis* 2003; 171: 75-81.
6. Almind K, Bjorbaek C, Vestergaard H, Hansen T, Eshwald SM & Perterson O. Amino acid polymorphism in insulin receptor substrate-1 in non-insulin dependent diabetes mellitus. *Lancet* 1993; 342: 828-832.
7. Bodhini D, Venkatesan R & Viswanathan M. Association study of IRS-1 gene polymorphisms with type 2 diabetes in south Indians. *Diabetes Technology and Therapeutics* 2011; 13(7): 767-772.
8. Hitman GA, Hawrami K & McCarthy MI, *et al*. Insulin receptor substrate-1 gene mutation in NIDDM: Implications for the study of polygenic disease. *Diabetologia* 1995; 38: 481-486.
9. Laakso M, Kekalainen MMP, Kuusisto J & Deeb SS. Insulin receptor substrate-I variants in non-insulin-dependent diabetes. *Journal of Clinical Investigation* 1994; 94: 1141-1146.
10. Mori H, Hashiramoto M & Kasuga M. Amino acid polymorphisms of the insulin receptor substrate-1 in Japanese noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 1995; 80: 2822-2826.
11. Clausen JO, Hansen T & Bjorbaek C, *et al*. Insulin resistance: Interaction between obesity and a common variant of insulin receptor substrate-1. *Lancet* 1995; 346: 397-402.
12. Yamada K, Yuan X, Ishiyama S, *et al*. Codon 972 polymorphism of the insulin receptor substrate-1 gene in impaired glucose tolerance

and late-onset NIDDM. *Diabetes Care* 1998; 21(5): 753-756.

13. Sigal RJ, Doria A, Warram JH & Krolewski AS. Codon 972 polymorphism in the insulin receptor substrate-l gene, obesity, and risk of noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 1996; 81(4): 1657-1659.
14. Lei HH, Coresh J, Shuldiner AR, Boerwinkle E & Brancati FL. Variants of the insulin receptor substrate-1 and fatty acid binding protein-2 genes and the risk of type 2 diabetes, obesity, and hyperinsulinemia in African-Americans: The atherosclerosis risk in communities study. *Diabetes* 1999; 48: 1868-72.
15. Orkunoglusuer FE, Mergen H, Bolu E & Ozata M. Molecular scanning for mutations in the insulin receptor substrate-1(IRS-1) gene in Turkish with type 2 diabetes mellitus. *Endocrine Journal* 2005; 52: 593-598.
16. Neena S, Achyut P, Jai P, Agarwal CG, Pant DC & Balraj M. Association of β_2-adrenergic receptor and insulin receptor substrate-1 polymorphism with obesity in a Northern Indian population. *Indian Journal of Human Genetics* 2008; 14(2): 48-54.
17. Ura S, Araki E, Kishikawa H, *et al*. Molecular scanning of the insulin receptor substrate-1 (IRS-1) gene in Japanese patients with NIDDM: Identification of five novel polymorphisms. *Diabetologia* 1996; 39: 600-608.
18. Burguete-Garcia AI, Miguel CP & Vicente MM. Association of Gly972Arg polymorphism of IRS-1 gene with type 2 diabetes mellitus in lean participants of a national health survey in Mexico: A candidate gene study. *Metabolism Clinical and Experimental* 2010; 59: 38-45.
19. Chaung LM, Chuen SL & Jih IY. No association between the G971R variant of the insulin receptor substrate-1 gene and NIDDM in Taiwanese population. *Diabetes Care* 1996; 19(5): 446-449.
20. Imai Y, Fusco A, Suzuki Y, *et al*. Variant sequences of insulin receptor substrate-1 in patients with non-insulin dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 1994; 70: 1655-1658.
21. Marini MA, Frontoni S, Mineo D, *et al*. The Arg972 variant in insulin receptor substrate-1 is associated with an atherogenic profile in offspring of type 2 diabetic patients. *Journal of Clinical Endocrinology and Metabolism* 2003; 88: 3368-3371.
22. Laukkanen O, Pihlajamaki J, Lindstrom J, *et al*. Common polymorphisms in the genes regulating the early insulin signalling pathway: Effects on weight change and the conversion from impaired glucose tolerance to type 2 diabetes. *Diabetologia* 2004; 47: 871-877.
23. Zhang Y, Stratton IM, Warren-Perry MG, Orho M, Groop L & Turner RC. UKPDS 19: Heterogeneity in NIDDM: Separate contributions of IRS-1 and beta-3-adrenergic-receptor mutations to insulin resistance and obesity respectively with no evidence for glycogen synthase gene mutations. *Diabetologia* 1996; 39: 1505-11.
24. Benecke H, Topak H, Muhlen VZ & Schuppert F. A study on the genetics of obesity: Influence of polymorphisms of the beta-3-adrenergic receptor and insulin receptor substrate-1 in relation to weight loss, waist to hip ratio and frequencies of common cardiovascular risk factors. *Experimental and Clinical Endocrinology and Diabetes* 2000; 108: 86-92.

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Incidence of Injury due to Road Traffic Accidents in Lashio Township, Northern Shan State

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Road traffic injury is a major public health problem with higher mortality, morbidity, and disability. Trauma due to road traffic accidents (RTA) is the leading cause of mortality in young people and one of the commonest causes of death overall. The paper aimed to study the incidence of injuries due to RTA among the patients admitted to Lashio General Hospital. It was a cross-sectional descriptive study conducted from August 2012 to July 2013. Among 3268 injured patients, 1462 cases (44.7%) were caused by RTA. Of these cases, 1031(70.5%) were males and 431(29.5%) were females. Mean age was 29±14.3 years. Majority of patients (76.7%) were motor-cycle users, and 187 patients (12.8%) rode car, 25(1.7%) tricycle, 6(0.4%) bicycle, 1(0.1%) cart and 1(0.1%) train. One hundred and twenty-one patients (8.3%) were only pedestrians. Nearly half of the patients, (42.5%) were injured at their lower limbs. It was followed by at head among 509 patients (34.8%), upper limbs 324 patients (22.2%), face 314(21.5%), chest 123(8.4%), abdomen 68(4.7%), back 43(2.9%), neck 23(1.6%) and perineum 12(0.8%). Blunt injury causing bruise and abrasion were observed in 876 patients (59.9%). It was followed by laceration 587 patients (40.2%), bone fracture 252(17.2%), crush injury 25(1.7%), penetration 11(0.8%), sharp incision 10(0.6%), joint dislocation 7(0.5%), burn 3(0.2%) and inhalational injury 1(0.1%). As a conclusion, head injury was second commonest injury in this study and deaths from head injury were observed in 55(70%) of all 79 deaths due to RTA ($p<0.001$). Neurosurgical management was the important role in care of head injury and Neuro-surgical specialty was needed for Lashio hospital.

Key words: Incidence, Injury, Road traffic accident, Vehicles, Outcomes

INTRODUCTION

Population is growing in everywhere and correspondingly vehicles are increasing on the roads of public areas. However, capacity of road is not enough for increasing road users and vehicles especially in low-income countries. Moreover, quality of road is crucial for road safety and injury prevention. Thus, road traffic injury continues as a major problem in public health management in low-income and middle-income countries. The health and risk transition has directed to noncommunicable diseases (NCDs) because of increasing morbidity, mortality and socio-economic losses. Injuries of both unintentional and intentional types are also included under the broader umbrella term of NCDs by some member countries.

Road traffic injury endures a major public health problem according to its higher mortality, morbidity, and disability. Trauma due to road traffic accidents (RTA) is the leading cause of mortality in young people and one of the commonest causes of death overall. Road traffic mortality rate is higher in low-income and middle-income countries

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(21.5 and 19.5 per 100,000 populations, respectively) than high-income countries (10.3 per 100,000). Globally, more than 1.2 million people died from road traffic injury each year and 20 to 50 million people are injured or disabled.[1]

In Myanmar, according to the WHO report by Department of Measurement and Health Information, December 2004, morbidity due to RTA was 519,000 among 2,274,000 with unintentional injuries due to all causes. Therefore, 23% of injuries were due to road accidents. Mortality report also showed 166,00 numbers of deaths were due to RTA among 555,00 who died with unintentional injuries in that year.[2]

Road traffic accidents mostly occurred in the traffic jam areas. Lashio Township has many road traffic jams because it has so many public interesting places and it occupies the Myanmar-China Border Trade road. Therefore, many vehicles like trucks, express buses and motor bikes are running across the township. Morbidity and mortality due to injuries caused by road traffic accidents are continuing in Lashio Township year by year.

The present study aimed to obtain incidence of injuries due to RTA, type of accident, pattern of injury, severity of injury, type of care, disability, death, possible related risk factors and demographic characteristics of these patients taking treatment at Lashio General Hospital. Hopefully, the study can provide great helps in road traffic injury prevention mainly by delivering more needful post traumatic care, such as chain of help for patients injured in road accidents, pre-hospital care, hospital care and rehabilitation.

MATERIALS AND METHODS

Hospital-based, one year cross-sectional descriptive study started from 1st Aug 2012 to 30th July 2013 was conducted at Lashio General Hospital. All of road traffic injured patients were registered at Out-patient Department, and then admitted to General Surgical Unit and Traumatology Unit.

Any crash or accident of motor vehicles, bicycles, train, boat and pedestrians occurred on road or off road in Lashio Township was defined as road traffic accident. Car driver, motorcyclist, pillion, passengers, pedestrians and others injured in RTA attended or were admitted to Out-patient Emergency Department, General-surgical and Traumatology units of Lashio General Hospital and they were defined as patients of RTA. Any patients died in RTA and brought dead to the hospital were defined as the brought dead in RTA. The patients admitted at Ophthalmology and Otolaryngology units were not included into the study.

Clinical conditions of patients were assessed by well-trained medical officers. Thorough history taking, physical examination, investigations with X-ray, ultrasound, bio-chemical laboratory tests and surgical and general treatment were done by medical officers in charge. Patients with minor trauma were treated at Out-patients Emergency Department and severe injured patients were admitted to respective units. Injury at limbs, bones and external parts of body were treated at Traumatology Unit.

There was no special Neurology Unit in Lashio General Hospital. Therefore, head, neck, chest, abdomen and internal organ injuries were treated at General Surgical Unit. General particulars of patient, mode of accident, type of vehicle, type of injury and site of injury were noted and administered to data collection form by trained observers who were medical officers. During stay in hospital and follow-up care, medical-care notes were also recorded and entered into the data form. The cases who died at accidents and were brought to the hospital were also included in the study. Postmortem examination and findings were noted at data form.

Data analysis

Data entry was done using SPSS 20.0 version. Statistical analysis was done with SPSS 20.0 version software and R 12.2.1 software. General particulars, mode of

accidents, type of injury, site of injury, type of vehicle used and treatment outcome of patients were analyzed with descriptive statistics. Odds of risk factors for injury were calculated with bi-variate analysis.

Ethical consideration

Ethical consideration in data collection concerning examination of patients, general particulars, findings of injuries and outcome of patients were reviewed at Institutional Review Board of Department of Medical Research (Upper Myanmar) and ethical clearance was obtained.

RESULTS

Among total 3268 injury patients admitted to Lashio General Hospital, nearly half, i.e., 44.7% injured due to RTA and 1806 (55.3%) injured due to non-RTA causes.

Table 1. Characteristics of the patients (n=1462)

Variables		Frequency (%)
Age (year)		
Mean±SD	29±14.3	
Mode	18	
Range	2 to 90	
Age group distribution		
<10 year		76(5.2)
11-20 year		446(30.5)
21-30 year		389(26.6)
31-40 year		260(17.8)
41-50 year		165(11.3)
>50 year		126(8.6)
Sex		
Male		1031(70.5)
Female		431(29.5)
Care unit distribution		
Out-patient		323(22.1)
Traumatology		500(34.2)
General surgical		639(43.7)
Road users		
Motor-cycle		1121(76.7)
Car		187(12.8)
Pedestrian		121(8.3)
Tricycle		25(1.7)
Bicycle		6(0.4)
Cart		1(0.1)
Train		1(0.1)

General characteristics of patients

Of these 1462 road traffic injured patients, 1031(70.5%) were males and 431(29.5%) were females. Mean age of patients was 29±14.3 years. The youngest was 2 and the eldest was 90 years. In age group distribution, patients with <10 years were 76(5.2%), 11-20 were 446(30.5%), 21-30 were 389(26.6%), 31-40 were 260(17.8%), 41-50 were 165(11.3%) and 51 and above were 126(8.6%) (Table 1).

Care unit distribution

Nearly half of the patients (43.7%) were treated at General Surgical Unit. One third of the patients (34.2%) took treatment at Traumatology Unit. The rests (22.1%) were cured at Out-patient emergency department.

Kinds of road users

Majority of patients (76.7%) were motor-cycle users. 187 patients (12.8%) rode car, 25(1.7%) tricycle, 6(0.4%) bicycle, 1(0.1%) cart and 1(0.1%) train. One hundred and twenty-one patients (8.3%) were only pedestrians (Table 1).

Monthly distribution of patients

According to monthly distribution, the most occurrences (11%) were observed in the month of February followed by 144 cases (9.8%) in October, 143 cases (9.8%) in May and so on (Fig. 1).

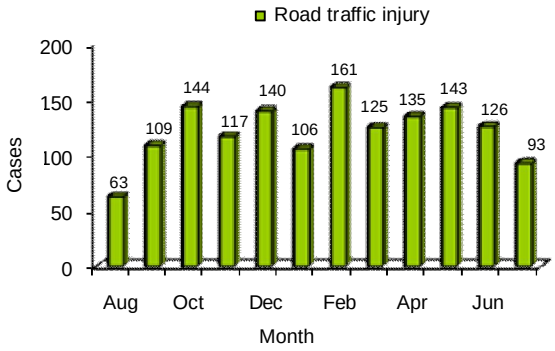

Fig. 1. Monthly distribution of patients

Distribution of patients per calendar days

According to calendar year 2012 and 2013, there were 118 numbers of official holidays and 247 numbers of non-holidays in a year round (from 1st August 2012 to 31st July 2013). Number of patients presented on holidays was 570. Therefore, number of patients per day was 4.8(570/118). Number of patients presented on non-holidays was 892 and number of patients per day was 3.6(892/247).

Admission time at hospital

Nearly half (48.7%, 712) of the patients came to the hospital evening time, i.e., 4 pm to midnight. Then, it was followed by 648(44.3%) and 102(7%) patients who arrived at the hospital in the daytime (8 am to 4 pm) and night time (12 pm to 8 am), respectively.

Parts of body injured

Nearly half of the patients, 42.5% were injured at lower limb in accidents. It was followed by head (34.8%), upper limb (22.2%) and so on (Table 2).

Types of injury

The commonest injury was blunt injury causing bruise and abrasion. It was observed in 59.9% (876) of patients. It was followed by laceration (40.2%), bone fracture (17.2%) and so on (Table 2).

Table 2. Parts of body injured and types of injury

Variables	Frequency (%)
Parts of body	
Lower limb	621(42.5)
Head	509(34.8)
Upper limb	324(22.2)
Face	314(21.5)
Chest	123(8.4)
Abdomen	68(4.7)
Back	43(2.9)
Neck	23(1.6)
Perineum	12(0.8)
Types of injury	
Abrasions/ bruise	876(59.9)
Lacerations	587(40.2)
Bone fracture	252(17.2)
Crushed injuries	25(1.7)
Penetrations	11(0.8)
Sharp incisions	10(0.6)
Joint dislocations	7(0.5)
Burns	3(0.2)
Inhalational injuries	1(0.1)

Outcome of patients

Among total 1462 patients presented at the hospital, 1339 patients were cured. Sixteen patients (1.1%) absconded from the hospital. Twenty-eight patients (1.9%) were referred to Mandalay General Hospital for further management. Among hospitalized patients, 43(2.9%) died during emergency intensive care. Thirty-six cases (2.5%) were brought dead. Therefore, total deaths due to RTA were 79 and the rests 1383 were alive (Table 3).

Table 3. Outcomes of the patients

Variables	Traumato-logy unit	General-surgical	Out-patient	Total
Cured	475	572	292	1339
Absconded	11	5	-	16
Referred	12	15	1	28
Died at hospital	2	41	-	43
Brought dead	-	-	36	36
Total	500	633	329	1462

Table 4. Bi-variate analysis

Variables	Died (Brought dead/die) (%)	Alive/ cured (%)	Total (%)	OR (95%CI)	P value
Injury					
Head	55 (10.8)	454 (89.2)	509 (100)	4.7 (2.9-7.7)	<0.001
Other	24 (2.5)	929 (97.5)	953 (100)	1 (Ref:)	
Total	79 (5.4)	1383 (94.6)	1462 (100)		
Vehicle					0.481
Motor-cycle	58 (5.2)	1063 (94.8)	1121 (100)	0.8 (0.5-1.4)	
Others	21 (6.2)	320 (93.8)	341 (100)		
Total	79 (5.4)	1383 (94.6)	1462 (100)	1 (Ref:)	
Age (yr), (mean)	34.2	28.7			<0.001

Bi-variate analysis

Chi squared test was performed by analyzing the odd ratio (OR) and 95% confident interval for bi-variate analysis. Deaths from head injury were observed in 10.8% (55/509) and death due to other injury were found in 2.5% (24/953) of all deaths (OR=4.7, 95% CI=2.86-7.76, p<0.001).

Among the all head injury cases, 79.4% (404/509) were motor-cycle user and 20.6% (105/509) were other vehicle users. Among the all deaths, 73.4% (58/79) were motor-cycle users and 26.6% (21/79) were other vehicle users (OR=0.8, 95% CI=0.52-1.36, p=0.481). Mean age of the death cases was 34.2±14.8 years and that of alive or cured cases was 28.7±14.2 years (p<0.001).

DISCUSSIONS

In this study, 70.5% were males and mean age of patients was 29±14.3 years. The commonest age groups were 11 to 20 and 21 to 30 years comprising 30.5% (446) and 26.6% (389) of patients, respectively. Similarly, the study done in PyinOoLwin revealed that 71.8% were males and mean age of patients was 29.8±13.8 years. The commonest age groups in that study were 11 to 20 and 21 to 30 years comprising 24.7% and 32.8% of patients, respectively.[3]

In present study, majority (76.7%) were motor-cycle users. The study in western Nepal in 2005 showed that 54% were motor-cycle users. The study in PyinOoLwin also revealed that motor-cycle users were observed in 66.1% of the patients.[4]

The present study revealed that the commonest injuries were found in lower limb and head comprising 42.5% and 34.8%, respectively. Study in Egypt (2004) showed that head injury was found in 60.9% of studied patients. The study of PyinOoLwin detected head injury in 48.3% of patients.[5] Head, face, neck and back injuries with possibly neurological involvements were observed in 34.8%, 21.5%, 2.9% and 1.6% of the patients, respectively. Because of no specific Neurology Unit was available in Lashio General Hospital, all of the neurological injuries were treated at General Surgical Unit. Therefore, General Surgical Unit was responsible for managing in 43.7% of the total patients. Even major neurosurgical interventions were done for severe head injury cases in General Surgical Unit.

Conclusion

Injury due to RTA mainly occurred in younger age groups. Education program for road traffic injury prevention should go to basic education school and university students. High rate of motor-cycle use, high rate of head injury among motor-cycle users and higher risk of death due to head injury highlights injury prevention measure like proper helmet wearing in motor-cycle users. Moreover, head injury was observed in 34.8% of the patients and it was responsible for 70% of all deaths. That's why, neuro-surgical management was the important role in care of head injury and Neurosurgical specialty was needed for Lashio hospital.

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REFERENCES

1. Global status report on road safety: Time for action. Geneva, World Health Organization, 2009. Available from: URL: www.who.int/violence-injury-prevention/road_safety_status/2009.
2. WHO report of morbidity and mortality in Myanmar by Department of Measurement and Health Information, December 2004.
3. Moe Kyaw Myint, Nay Win Myint, Aung Sann Oo, Kyaw Thu Soe, Hla Soe Tint & Kyaw Zin Thant. Incidence of injury due to road traffic accident in PyinOoLwin. Paper presented at International Conference in Medicine and Public Health (ICMPH 2012), Siriraj Hospital, Mahidol University, Bangkok Thailand, 2012.
4. Agnihotri AK & Joshi HS. Pattern of road traffic injuries: One year hospital-based study in Western Nepal. *International Journal of Injury Control and Safety Promotion* June 2006; 13(2): 128-130.
5. Farghaly A, El-Khayat1 R, Awad W & George S. Forensic Medicine and Clinical Toxicology, and Neurosurgery Departments, Faculty of Medicine, Assiut University, Assiut, Egypt; Head injuries in road traffic accidents. Available from: URL: http://www.icadts 2007. org/print/ poster102_ head injuries.pdf.

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Knowledge and Practice on MDR-TB Disease among MDR-TB Patients Attending Aung San MDR-TB Clinic (Yangon)

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A cross-sectional study was conducted to assess the knowledge and practice on MDR-TB disease among MDR-TB patients attending Aung San MDR-TB clinic (Yangon) in 2014. A total of 217 patients were interviewed with pre-tested structured questionnaire. Most of respondents were aged 24-44 years (59.5%), male (72.4%), middle and high school education level (60.8%) and employed (56.2%). Only 40% of the MDR-TB patients' bed rooms had proper lighting and cross ventilation and 40.6% got counseling and health education sessions at least 3 times before treatment. About 144(66.4%) had high knowledge score and proper practice. In this study, occupational status (p=0.02), number of counseling and health education sessions (p=0.012), getting IEC materials (p=0.029) were statistically significant association with knowledge level. The dealing of the health staff was statistically significant with the proper practice on MDR-TB among these patients (p=0.043). Knowledge and practice level were significantly associated with each other (p<0.001). Therefore, pre-treatment counseling and getting IEC material are essential to impart knowledge and dealing of the health staff influences on practice of MDR-TB patients. These could also enhance treatment adherence and can prevent transmission of infection in community through the patients.

Key words: Knowledge, Practice, MDR-TB

INTRODUCTION

Myanmar is one of the 27 multi-drug resistant tuberculosis (MDR-TB) high burden countries. The World Health Organization (WHO) estimates that 5,500 cases of MDR-TB occurred among notified pulmonary TB cases in Myanmar in 2011. MDR-TB among new and previously treated patients was 4% and 15.5%, respectively (2002-2003) and 4.2% and 10% of new and previously treated TB patients were MDR-TB, respectively (2007-2008).[1]

In the third drug resistant survey (DRS), MDR-TB among new and previously treated patients was 5% and 27%, respectively in 2012-2013.[2] MDR-TB is a man-made problem. It is costly, deadly, debilitating and a major threat to the current control strategies. Knowledge, attitude and practice of MDR-TB in MDR-TB patients play an important role in order to stop the spread of disease and decrease mortality and morbidity of the disease.

Therefore, it is necessary to explore the level of knowledge and practice on MDR-TB among MDR-TB patients under the Programmatic Management of Drug Resistant TB (PMDT). By doing this study, it can inform policy makers how to strengthen the PMDT in Myanmar, and to be helpful in curing the MDR-TB patients and stopping the spread of infection. The aim of the

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study was to assess the knowledge and practice on MDR-TB disease among MDR-TB patients at Aung San MDR-TB clinic (Yangon).

MATERIALS AND METHODS

Study design

It was a cross-sectional descriptive study.

Study population and area

MDR-TB patients who came to follow-up MDR-TB clinic at Aung San (Yangon) were included from September 2014 to December 2014. Inclusion criteria were MDR-TB patients under the PMDT, who gave informed consent, aged above 18 years and had received MDR-TB treatment for at least one month.

Sampling procedure

Systematic random sampling method was applied and sample size determination was as follows:

- Epi info 7 was used to calculate the sample size.
- Study population=1174 (registered MDR-TB patients from 1. 1. 2013 to 30. 9. 2014)
- Expected frequency=50% (expected proportion of knowledge and practice about MDR-TB among participants)
- Confidence limit (d)=6%
- Sample size (n)=217

Data collection methods and tools

Face-to-face interviews with MDR-TB patients were conducted by using pre-tested structured questionnaire.

Data management and analysis

Collected data were checked for consistency and completeness. Cleaned data were analyzed by using Statistical Software for Social Science (SPSS) version 16.0. Descriptive analysis was done.

Ethical consideration

This study was conducted through the permission of Ethical Board of University of Public Health (Yangon). In this study, researchers followed the principles and guidelines of ethical consideration for privacy, confidentiality and getting informed consent.

RESULTS

Socio-demographic characteristics

The detailed socio-demographic characteristics of MDR-TB patients are shown in Table 1.

Table 1. Socio-demographic characteristics of MDR-TB patients (n=217)

Socio-demographic characteristics	Number	Percent
Age (years)		
≤24	38	17.5
25-34	77	35.5
35-44	52	24.0
45-54	38	17.5
55-64	11	5.1
≥65	1	0.5
Sex		
Male	157	72.4
Female	60	27.6
Marital status		
Single	97	44.7
Married	111	51.2
Divorced	3	1.4
Widowed	5	2.3
Separated	1	0.5
Education		
Low (Illiterate, read/write and primary school level)	31	14.3
Medium (Middle and high school level)	132	60.8
High (University level)	54	24.9
Occupation		
Dependent	51	23.5
Manual/Unskilled laborers	42	19.4
Owned business	50	23.0
Government staff	24	11.1
Private employers	48	22.1
Other	2	0.9
Monthly family income (kyats)		
≤100000	36	16.6
100001-200000	101	46.5
200001-300000	57	26.3
300001-400000	9	4.1
>400000	14	6.5
Family size(persons per house)		
≤4	133	61.3
>4	84	38.7
History of TB in family		
Yes	46	21.2
No	171	78.8
History of MDR-TB in family		
Yes	9	4.1
No	208	95.9

Most of respondents were aged 24-44 years (59.5%), male (72.4%), middle and high school education level (60.8%) and employed (56.2%). Among 217, 46(21.1%) had history of TB and 9(4.1%) had history

of MDR TB in the family. Among 217 respondents, only 88 MDR-TB patients (40.6%) got 3 times and above counseling and health education sessions, 102(47%) got less than 3 times and 27(12.4%) did not get any.

Most of the respondents (76%) accessed Information, Education and Communication (IEC) material during diagnosis and the rest did not get them. Regarding the dealing of the health staff, 116 MDR-TB patients (53.5%) responded that the dealing of the most of the health staff was good, 96(44.2%) said it was fair, but only 5(2.3%) said, poor. Mean±SD of age was 39.39± 10.96, mean±SD of monthly family income (kyat) was 237,000±171,498 and mean±SD of family size (persons per house) was 4.3±2.13.

Knowledge on MDR-TB

Among 217 MDR-TB patients, 183(84.3%) answered the meaning of MDR-TB is the TB bacilli resistant to some anti-TB drugs and 2(0.9%) answered the same as non-drug resistant TB. Only 32 patients (14.7%) did not know the meaning of MDR-TB. Most of the patients 191(88%) answered the correct statement of its cause, resistant TB germ. The second most responding answer by the patients was strained job and 29 patients (13.4%) believed that alcohol drinking was the cause.

Concerning knowledge about different modes of transmission, most common answer (96%) was transmission through the air when TB patient is coughing or sneezing. The second most responding answer (24%) was the transmission of the disease through sharing of dishes. Other answers were 'touching' (3%), 'do not know' (3%) and 'others' (1%).

Regarding knowledge about different prevention strategies to MDR-TB transmission, 98% stated covering mouth and nose when coughing and sneezing and 25% used separate dishes. About 32 patients (15%) answered the proper disposal of sputum and only two patients (1%) and five patients (2%) answered the regular taking of drugs and the separate living.

Almost MDR-TB patients (99.5%) believed that the second line anti-TB drug could completely cure MDR-TB and only one patient (0.5%) thought that these drugs could not cure the disease. Among 217 patients, 210(96.8%) knew the correct duration of treatment course (20 to 24 months) and other patients answered the 6 months (0.5%), 12 months (1.4%) and some do not know the duration of treatment (1.4%).

Regarding knowledge of the drug side effects, 168 MDR-TB patients (77%) stated as the joint pain, 134 patients (64%) as deafness/hearing defect and 98(45%) as the nausea/vomiting/dizziness. Other answers were psychosis (40%), rash (17%), jaundice/liver disorder (14%), renal diseases (11%) and goiter (6%).

Concerning knowledge on risk person for getting MDR-TB, about one fourth of MDR-TB patients (27%) answered people living with HIV were more vulnerable to MDR-TB infection, 55 patients (25%) stated as the improper practice on anti-TB drugs taken was the risk factor and 43(20%) answered that the person with MDR-TB contact was the risk person. Other answers elicited by MDR-TB patients were heavy alcohol drinker (10%) and diabetes patients (5%). Nearly one-third of MDR-TB patients (30%) did not know the risk person for getting MDR-TB.

Almost all MDR-TB patients (99%) stated that the death was the consequence of MDR-TB if they did not take MDR-TB treatment correctly. Other consequences that were mentioned by MDR-TB patients were transmission of infection to other person (25%) and XDR-TB (2%). Most of the MDR-TB patients (96%) had knowledge on the monthly follow-up sputum examination throughout the treatment course and only (4%) did not know how to do sputum examination monthly. Nearly all of the MDR-TB patients 216(99.5%) answered that they would go to the health facility for

consultation about transfer form before travelling or moving to other township during their treatment.

There was a statistically significant association between occupational status, number of counseling and health education sessions, getting IEC materials and knowledge level. Educational level and the knowledge score were marginally significant in association. Other variables were not statistically associated with knowledge level (Table 2).

Table 2. Association between sociodemographic characteristics and knowledge on MDR-TB

Socio-demographic characteristics of respondents	Knowledge (n= 217)				p value
	High		Low		
	No.	%	No.	%	
Age group (years)					0.619
≤35	84	67.7	40	32.3	
>35	60	64.5	33	35.5	
Sex					0.366
Male	107	68.2	50	31.8	
Female	37	61.7	23	38.3	
Marital status					0.068
Married	80	72.1	31	27.9	
Others	64	60.4	42	39.6	
Education					0.052
Low (Illiterate, read/write and primary school level)	18	58.1	13	41.9	
Medium (Middle and high school level)	83	62.9	49	37.1	
High (University level)	43	79.6	11	20.4	
Occupation					0.02*
Unemployed	55	57.9	40	42.1	
Employed	89	73.0	33	27.0	
Monthly family income (Kyat)					0.078
≤ 200000 per month	85	62.0	52	38.0	
>200000 per month	59	73.8	21	26.2	
History of TB/ MDR-TB in family					0.604
Yes	32	69.6	14	30.4	
No	112	65.5	59	34.5	
Getting support during treatment	81	69.2	36	30.8	0.333
Yes	63	63.0	37	37.0	
No					
Number of counseling & health education sessions					0.012*
≥3 times	69	76.1	21	23.9	
<3 times	77	59.7	52	40.3	
Getting IEC materials					0.029*
Yes	116	70.3	49	29.7	
No	28	53.8	24	46.2	

*=Statistically significant at p=0.05 level

Practice of the MDR-TB patients on MDR-TB disease

Regarding time interval between starting TB symptom and going to the health facilities, 48.4% of MDR-TB patients went to the health facilities less than 3 weeks after suffering from TB symptom. Other 51.6% of MDR-TB patients went there only after 4 weeks. Among 217 MDR-TB patients, 204(94%) always covered their mouth and nose when coughing and sneezing. Only 13 MDR-TB patients (6%) did not always cover them. Out of 217 MDR-TB patients, 182(84%) used the condensed milk tin/ spittoon when they were spitting sputum. Other 35 patients (16%) spitted their sputum on the ground. Among 182 patients who used the condensed milk tin/spittoon, 173 patients (90%) covered it. Other nine patients (10%) did not. out of 182 patients, 124 (68%) disposed the sputum into latrine by using disinfectant. Other 26 patients (14%) disposed on the ground, 22(13%) buried in earth and 10(5%), to the drain.

Regarding the practice of sleeping, 209 patients (96.3%) did not sleep with family members under the same net and 100% of these patients answered that they would sleep separately with their family until getting cured treatment outcome. Another 8 patients (3.7%) slept with family members under the same bed net.

Concerning treatment seeking behavior of respondents if they get drugs side effect, 67.7% would go to the health facility, 31.8% would consult with their DOT provider (health staff) and 0.5% would treat by themselves. All of the MDR-TB patients (100%) always did monthly follow-up sputum examination during MDR-TB treatment.

Knowledge, practice scores and their association

Score of the respondents' knowledge and practice on MDR-TB was summed up by transforming it into knowledge and practice score. Mean, median and mode of the knowledge score were 11, minimum score was 5 and maximum score was 16. Low level of knowledge was decided as score less than 11 marks. The scores greater than and equal to 11 marks were regarded as high level of knowledge. So, the finding indicated that 144(66.4%) respondents got high score and 73(33.6%) got low score.

Regarding the practice score, mean, median and mode of the practice score were 8, 8 and 9, respectively. The minimum and maximum scores of the practice were 3 and 10, respectively. The practice score level more than and equal to 8 marks was marked as proper practice and score level less than 8 marks would be marked as improper practice. One hundred and forty-four MDR-TB patients (66.4%) did proper practice and 73(33.6%) did improper practice.

Table 3. Association between sociodemographic characteristics and practice on MDR-TB

Socio-demographic characteristics of respondents	Practice (n=217)				p value
	Proper		Improper		
	No.	%	No.	%	
Age group (year)					0.171
≤35	87	70.2	37	29.8	
>35	57	61.3	36	38.7	
Sex					0.483
Male	102	65.0	55	35.0	
Female	42	70.0	18	30.0	
Marital status					0.501
Married	76	68.5	35	31.5	
Others	68	64.2	38	35.8	
Education					0.356
Low (Illiterate, read/write and primary school level)	24	77.4	7	22.6	
Medium (Middle and high school level)	86	65.2	46	34.8	
High (University level)	34	63.0	20	37.0	
Occupation					0.554
Unemployed	61	64.2	34	35.8	
Employed	83	68.0	39	32.0	
Number of counseling & health education sessions					0.053
≥3 times	65	73.9	23	26.1	
<3 times	79	61.2	50	38.8	
Getting IEC materials					0.238
Yes	113	68.5	52	31.5	
No	31	59.6	21	40.4	
Getting supports during treatment					0.854
Yes	77	65.8	40	34.2	
No	67	67.0	33	33.0	
Patients' opinion on the dealing of the most of health staff					0.043*
Good	84	72.4	32	27.6	
Fair and Poor	60	59.4	41	40.6	

*=Statistically significant at p=0.05

As shown in Table 3, dealing of health staff was statistically associated with the practice of MDR-TB patients. Getting counseling and health education sessions was marginally significant with the practice of MDR-TB patients. Other variables were not statistically significant. There was a statistically significant association between knowledge level of respondents and practice on MDR-TB ($p<0.001$).

DISCUSSION

According to the guideline, MDR-TB patients must get counseling and health education sessions at least 3 times before treatment.[3] However, only 88 MDR-TB patients (40.6%) got them. And, most of the respondents (76%) accessed IEC materials during diagnosis and treatment and the rest (24%) could not access them. The findings indicated that NTP should give instructions to the health staff to deliver the counseling and health education sessions at least 3 times before treatment and deliver the IEC materials to every patient. If NTP can strengthen in these areas, the higher level of patients' knowledge on MDR-TB can be obtained.

In this study, most of the patients got the high knowledge and practice score. However, most of them did not know the detail information about different ways of preventing the transmission of disease to others and the consequences of MDR-TB if they did not take MDR-TB treatment correctly. Concerning the practice of MDR-TB patients, about 33% of them did not have proper practice on the disposal of the sputum. Therefore, health care providers need to emphasize in these areas during their health education sessions.

Moreover, dealing of the health staff can influence in the proper practice on MDR-TB among these patients. The patients wanted to obey the instructions of the proper practice behavior from the health staff during their treatment if they were treated patiently and friendly. This study revealed that there was a statistically significant association between knowledge and practice levels. Patients with higher knowledge expressed more proper practice on MDR-TB. The similar finding was found in the previous study conducted in Hlaingthaya among TB patients in 2009.[4]

Conclusion

Based on the findings from this study, pre-treatment counseling and getting IEC materials are essential to impart knowledge and dealing of the health staff influences on practice of MDR-TB patients. These could also enhance treatment adherence and can prevent transmission of infection in community through the patients.

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REFERENCES

1. NTP 2010. Annual TB Report, 2010. In: *National Tuberculosis Programme, Myanmar*.
2. NTP 2014. Third National drug resistant-TB survey. In: *National Tuberculosis Programme, Myanmar* (unpublished report).
3. NTP 2013. Guideline for the Management of multidrug-resistant tuberculosis (MDR-TB) in Myanmar, In*: National Tuberculosis Programme, Myanmar*.
4. Cho Cho San. Knowledge, attitude and practice of TB disease among pulmonary TB patients in Hlinethaya Township, 2009, *Thesis*, MPH University of Public Health, Yangon.

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