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Clinical profile of dengue haemorrhagic fever in adults in PyinOoLwin Hospital in 2009

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This study aimed to describe the clinical profile of dengue haemorrhagic fever that unusually occurred in adults in PyinOoLwin. A retrospective review of adult patients admitted to Medical Unit of 300-bed General Hospital, PyinOoLwin from July to December 2009 using preformed data collection sheets and patients' medical records was done. Sixty-three adult patients diagnosed to have dengue haemorrhagic fever (DHF) (n=46) and dengue shock syndrome (DSS) (n=17) were admitted to the hospital. The mean age of the patients was 22.5 years. The common presenting symptoms were: fever (100%), vomiting (69.8%), melaena (41.3%), abdominal pain (39.7%), haematemesis (33.3%), epistaxis (30.2%), gum bleeding (22.2%), body aches (22.2%), skin rash (20.6%), headache (12.7%), purpura (9.5%), haemoptysis (6.3%), ecchymosis (6.3%) and lethargy (6.3%). Seventeen patients (27%) presented with shock on admission. Mean day of onset of shock was 4.5 days. On physical examination, enlarged liver was found in 61.9% of the patients. Recovery rash was seen in 3.2% of the patients. One step rapid test for IgG & IgM antibodies for dengue virus infection was positive in 71.4% of the patients. At the time of admission, severe thrombocytopenia (platelet count <=20,000/mm^3) was present in 23.8% and haemoconcentration (haematocrit value ≥45%) was present in 49.2% of the patients. No patient died. Twenty-two of 63 patients progressed to severe dengue. Severe bleeding was found in 9.5% and hepatomegaly with enzyme abnormality was seen in 6.3% of the patients. Pleural effusion was found in 3.2% and ascites was detected in 1.6% of the patients. Univariate analysis revealed that the age and platelet count were significant predictors of severe dengue. There was significant correlation between thrombocytopenia and gum bleedings (r=-.323; p=0.01).

INTRODUCTION

Being the most rapidly emerging mosquito-borne viral infection among human in past five decades, the incidence of dengue fever has increased 30 times with increasing geographic expansion to new countries as well as from urban to rural areas, low land to hilly regions and from children to adult populations. More than 70% of the populations at risk for dengue worldwide live in the South-East Asia Region and Western Pacific Region. Dengue haemorrhagic fever (DHF) is a potentially lethal complication and a leading cause of hospitalization and death among children in most of Asian countries.[1]

In Myanmar, in 2007, the highest number of cases were reported in Ayeyawady, Kayin, Magway, Mandalay, Mon, Rakhine, Sagaing, Taninthayi and Yangon Divisions. The case-fatality rate was slightly above 1%.[1] In Mandalay Region, dengue infection among adult population has increased in recent years. Township reports of Mandalay Division stated that 91 of 803 DHF cases in 2007 and 485 of 4490 DHF cases in 2008 were adult patients (above 11 years of age).

Aung Sann Oo and Moe Kyaw Myint contributed equally to this study and are joint first authors.

In PyinOoLwin, one of the 31 townships of Mandalay Region, 26 patients were admitted to adult medical unit for DHF in 2008.[2] PyinOoLwin Township is situated at 3538 feet above sea level and it has temperate climate and DHF was seen sporadically.

An unusual event of dengue fever occurrence in adults started in the middle of 2009 in PyinOoLwin. From July to December 2009, a total of 114 adult patients were admitted to PyinOoLwin General Hospital for suspected dengue fever and 63 patients out of them were diagnosed as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

As the studies on adult DHF were relatively rare, the findings and results of this study will be valuable in management of adult dengue infection. This study was conducted to describe the clinical profile of dengue haemorrhagic fever in adults admitted to Medical Unit of 300-bed PyinOoLwin General Hospital during that period.

MATERIALS AND METHODS

This study was conducted at Medical Unit of 300-bed PyinOoLwin General Hospital in collaboration with Department of Medical Research (Upper Myanmar). From July to December 2009, 114 adults and 133 children with suspected dengue infection were admitted to PyinOoLwin 300-bed General Hospital. Sixty-three of 114 adults were diagnosed to have DHF/DSS. The criteria used to diagnose DHF included: an acute febrile illness within 2 to 7 days duration with haemorrhagic tendencies as evidenced by a positive tourniquet test (or) petechiae, purpura, ecchymosis (or) bleeding from mucosa, gastrointestinal tract and other locations plus evidence of plasma leakage and thrombocytopenia (platelet count $\leq$100,000/mm^3). The plasma leakage was operationally defined if the haematocrit value was either 45% and above or 20% drop or rise as compared to baseline.[3] The diagnosis of DSS needed an additional clinical criteria either hypotension or narrow pulse pressure. Those patients who developed dengue shock syndrome, fluid accumulation, severe bleeding and organ involvement (i.e. liver, nervous system, heart and other organs) were categorized as severe dengue infection. [1]

Forty-six of these 63 patients were diagnosed to have DHF and 17 patients fulfilled the criteria for the diagnosis of DSS. Twenty-two patients developed severe dengue infection. Retrospective review was done using preformed data collection sheets, medical records and laboratory reports.

A proper history taking and thorough physical examination were carried out on admission. Tourniquet tests (Hess Test) and haematocrit (PCV) measurement using capillary tubes and microcentrifuge were immediately done at bedside. Serial haematocrit values were assessed and recorded daily or hourly if required. Platelet counts were accurately obtained by Haemogram in all patients. The progress of clinical manifestations and laboratory findings were closely monitored. Liver function tests, chest radiograph and ultrasonography were also done in patients with suspected clinical features of liver insufficiency and fluid accumulations in the body cavities. One step rapid test for IgG and IgM antibodies for dengue virus infection (Standard Diagnostics Inc., Korea) was undertaken in all patients.

Treatment

Oral or intravenous fluid replacement therapy was given using oral rehydration salt (ORS), 5% dextrose, 0.9% dextrose saline, 0.9% normal saline and Ringer's lactate solution for isotonic dehydration as per the guideline set up by WHO. Plasma expander like Dextran 70, Gelofusin, platelet-rich plasma (PRP) and fresh whole blood were also used if necessary. Oral paracetamol was used as antipyretic. Analgesics such as diclofenac, ibuprofen, salicylates, other non-steroidal anti-inflammatory drugs and intramuscular injections were totally avoided.

Data analysis

The data were compiled, coded and entered into computer and analyzed using SPSS version 11.5 software. Paired t-test and independent sample t-test were used to compare means of two numerical variables. Univariate analysis was done to find out the association between a test variable and clinical outcome.

RESULTS

Among the 114 adult patients with suspected dengue infection, 63 with DHF/ DSS were studied. Mean age of the patients was 22.5 (SD 10.6) years with the age range of 12 to 54 years. Distribution of different age groups showed that 10 (15.9%), 26 (41.3%), 12 (19%) and 15 (23.8%) were distributed in 12 to 14 years group, 15 to 19 years group, 20 to 24 years group and above 24 years group, respectively. There were 25 males and 38 females. Male: female was 1:1.52. The mean duration of symptoms was 6.25 (SD 2.3) days. The mean duration of fever was 4.43 days (ranged from 2 to 7 days). The mean duration of hospital stay was 4.7 days (ranged from 2 to 10 days).

According to monthly distribution, 6 (9.5%), 20 (31.7%), 15 (23.8%), 12 (19%) and 10 (16%) patients were present in July, August, September, October and November, respectively. The highest number of patients was admitted in August.

Table 1 shows clinical manifestations: fever (100%), vomiting (69.8%), abdominal pain (39.7%), body aches (22.2%) and headache (12.7%) were the commonest presenting features. Gastrointestinal bleeding was the most common among haemorrhagic manifestations.

Melaena stool occurred in 41.3% and haematemesis as well as blood in vomitus occurred in 33.3% of the patients. Epistaxis and gum bleeding were found in 30.2% and 22.2% of these cases. Cutaneous purpura was seen in 9.5% of the patients.

Table 1. Clinical manifestation in 63 patients with DHF/DSS

Variable	Number (%)
Fever	63(100)
Vomiting	44(69.8)
Melaena	26(41.3)
Abdominal pain	25(39.7)
Haematemesis (including blood in vomitus)	21(33.3)
Epistaxis	19(30.2)
Gum bleeding	14(22.2)
Body aches	14(22.2)
Skin rash	13(20.6)
Headache	8(12.7)
Purpura	6(9.5)
Haemoptysis	4(6.3)
Ecchymosis	4(6.3)
Lethargy	4(6.3)
Recovery rash	2(3.2)
Haematuria	1(1.6)
Vaginal bleeding	1(1.6)
Conjunctival haemorrhage	1(1.6)
Shock	17(27.0)
Jaundice	5(7.9)
Hepatomegaly	39(61.9)
Splenomegaly	3(4.8)
Pleural effusion	2(3.2)
Ascites	1(1.6)

Haemoptysis was found in 6.3% and ecchymosis was also found in 6.3% of the patients. Haematuria, vaginal bleeding and conjunctival haemorrhage were seen in one patient (1.6%) each. Four patients were lethargic. Recovery rash was seen in 2 (3.2%) patients only. Tourniquet test was positive in 59 (93%) patients at the time of hospital admission. Enlarged liver was found out in 61.9% of the patients. Jaundice was encountered in 5 (7.9%). Splenomegaly was noted in 4.8% of the patients. Feature of plasma leakage such as pleural effusion and ascites were found in 3.2% and 1.6% of the patients, respectively.

Clinical examination (Table 2) showed mean pulse rate of 87.8 per minute (SD=14.4). Mean systolic blood pressure was 103.7 mmHg (SD=15.3) and mean diastolic blood pressure was 69.9 mmHg (SD=12.9). Seventeen patients (27%) presented with shock on admission. Mean day of onset of shock was 4.5 days. The mean respiratory rate at presentation was 22.5 per minute (SD=4.5).

Table 2. Clinical examination and laboratory findings in 63 patients with DHF/DSS

Variable	Mean (SD)
Systolic blood pressure mmHg (n=63)	103.7(15.3)
Diastolic blood pressure mmHg (n=63)	69.9(12.9)
Pulse rate /minute (n=63)	87.8(14.4)
Respiratory rate /minute (n=63)	22.5(4.5)
Haematocrit % (n=63)	43.5 (5.9)
Platelet count /mm^3 (n=63)	44888.9(27670.9)
Leucocyte count /mm^3 (n=14)	3367.9(1491.2)
Haemoglobin g/dl (n=29)	12.8(2.1)
Rapid test positive (IgG or M) (n=63)	45(71.4)
IgG positive	40(63.5%)
IgM positive	27(42.9%)
Liver function test	
Serum total bilirubin mg/dl (n=7)	4.7(11.1)
AST (IU/l) (n=7)	211(377)
ALT (IU/l) (n=7)	225(365)
ALP (IU/l) (n=7)	187(137)

The majority of patients had normal haemoglobin and haematocrit. Mean haematocrit value was 43.5% (SD 5.9). Haemoconcentration (haematocrit value $\geq$45%) was present in 49.2% of the patients on admission. Forty-three (75.4%) of the 57 patients had over 20% rise or drop in haematocrit value as compared to baseline. Mean haemoglobin value was 12.8 g/dl in 29 patients tested. All patients had thrombocytopenia with a mean platelet count of 44888.9/mm^3 (SD=27670.9). Severe thrombocytopenia (platelet count <=20,000/mm^3) was present in 23.8% of the patients. Leucopenia (<4000/ mm^3) was detected in 9 of the 14 patients whose total leucocyte count was available.

One step rapid test for IgG & M antibodies for dengue virus infection was positive in 71.4% of the patients. IgG antibody was demonstrated in 40 (63.5%) and IgM was positive in 27 (42.9%). Radiographic examination in 28 patients and ultrasonography in 17 patients were done to assess the presence of ascites, pleural effusion and organ involvement.

Liver function tests were done in 7 (11.1%) patients. Only one had hyperbilirubinaemia. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were raised in 1.6%, 4.8% and 6.3% of the patients, respectively. Twenty-two (34.9%) out of 63 patients progressed to severe dengue infection. Severe bleeding was found in 9.5% and hepatomegaly with hepatic enzyme abnormalities was seen in 6.3% of the patients. Pleural effusion was found in 3.2% and ascites was detected in 1.6% of the patients. Septicemia and disseminated intravascular coagulation were not found in the severe patients.

Table 3. Predictors of DHF/severe dengue

Variables	DHF (n=41)	Severe dengue (n=22)	P value
Age (year) Mean±SD	25.1±12.1	17.5±3.5	0.005
Hospital stay duration (day) Mean±SD	4±1.3	5.9±1.8	0
Respiratory rate /min Mean±SD	21.5±3.5	24.3±5.5	0.018
Platelet counts/mm^3 mean±SD	55244±26862	25590±16933	0

Univariate analysis (Table 3) revealed that the age, hospital stay duration, respiratory rate and platelet counts were significant predictors of clinical outcome that is severe dengue infection.

Table 4. Associations between thrombocytopenia and dependent variables.

Dependent variables	Pearson correlation	
	Coefficient (r)	P value
Gum bleeding	-0.323	0.01**
Hospital stay duration	-0.295	0.019*
Blood in vomitus	0.144	0.26
Ecchymosis	-0.101	0.431
Respiratory rate	-0.235	0.064

* = Correlation is significant at 0.05 level (2-tailed)
**= Correlation is significant at 0.01 level (2-tailed)

According to correlation analysis (Table 4), gum bleeding and duration of hospital stay were significantly correlated with platelet counts (thrombocytopenia).

DISCUSSION

This study revealed the clinical and laboratory manifestations of DHF in adult patients. Although only a small number of adult DHF was found in PyinOoLwin in

2008, an unusual rise of dengue fever occurred in 2009. Similar episodes were also noted in large cities like Yangon and Mandalay in 2007 and 2008. The outbreak of adult DHF in remote and smaller town like PyinOoLwin follows few years after that of the large cities.[4, 5]

In the present study, clinical presentation and laboratory parameters were demonstrated. Mean age of adult admitted patients was 22.5 years. The majority of the patients were young adults. Distribution of different age groups showed that 10 (15.9%), 26 (41.3%), 12 (19%) and 15 (23.8%) were distributed in 12 to 14 years group, 15 to 19 years group, 20 to 24 years group and above 24 years group, respectively. Similarly, the percentage of different age groups of adult DHF patients admitted to Yangon General Hospital in 2000-2008 revealed that 27.5%, 42.9%, 19% and 6% were in 12 to 14 years group, 15 to 19 years group, 20 to 24 years group and 25 to 29 years group, respectively. Therefore, this study showed that age distribution was not different from the findings of above study done in Yangon General Hospital.[4] A study done in Mandalay General Hospital revealed that the majority (49.5%) of the cases were seen in the month of July and August. This study also showed that most (31.7%) of the cases were found in the month of August. [5]

Studies in India revealed that the most common clinical presentations in adult patients were fever, headache, abdominal pain and diarrhea.[6, 7] Similarly, the present study showed that fever, vomiting and abdominal pain were most common symptoms. Vomiting was found in 44 (69.8%) adults as a common symptom in the present study. Gastrointestinal bleeding was common haemorrhagic manifestation in the studies done in India. [6, 7]

A study at Lashio (Myanmar) displayed that 11 out of 35 cases (31.4%) presented with melaena and/or haematemesis.[8] This study also revealed that melaena and haematemesis were found in 26 (41.3%) and 21 (33.3%) patients, respectively. Therefore, gastrointestinal bleeding was common haemorrhagic manifestation in adult in these regions. A study in Taiwan revealed that pleural effusion and ascites were found in 56.5% and 32.3% of the adult patients assessed with chest radiograph and ultrasonography.[9] In the present study, pleural effusion and ascites were encountered only in 3.2% and 1.6% of the patients. The present study showed that 22 of 63 cases developed severe dengue i.e., DSS, severe bleeding, hepatomegaly with enzyme abnormalities, pleural effusion and ascites. DSS was encountered in 17 of 22 severe cases. Therefore, dengue shock was frequently seen in the adult patients. This finding agreed with observations of the several reports in the outbreak in New Delhi, India in 1996. Those observations stated that DHF with overt shock in adults was not rare.[10]

Conclusion

Traditionally DHF was a disease primarily affecting children under 15 years of age. It could extend beyond this boundary and affect a wide range of ages in adults especially during outbreaks according to this study. It affected mostly young adults between the ages of 12 to 19 years. Gastrointestinal haemorrhage was not uncommon clinical manifestations in adults and dengue shock was also frequently seen. The WHO management guideline for dengue haemorrhagic fever is strongly practicable in treatment of DHF in adults.

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Bacteriological profile, drug sensitivity and virulence gene patterns of diarrheagenic *Escherichia coli* from diarrhea cases in Magway Township

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A total of 359 stool samples and 559 rectal swabs from patients with diarrhea admitted to Magway Regional Hospital from 2007 to 2009 were screened for diarrheagenic *Escherichia coli* (*Esch. coli*) using conventional laboratory methods, and polymerase chain reaction (PCR). Out of the 93 *Esch. coli* isolates, serotyping identified 28 enteropathogenic *Esch. coli* (EPEC), 13 enterotoxigenic *Esch. coli* (ETEC) and 8 enteroinvasive *Esch. coli* (EIEC). Forty-four isolates were nontypable. PCR assay showed only 5 EPEC, 8 ETEC and 8 enterohaemorrhagic *Esch. Coli* (EHEC) pathotype. The primers used in PCR assay were LTI-01 and LTI-02 for ETEC-labile toxin, ST-157 and ST-158 for ETEC-stable toxin, STX-01, STX-02, STX-101 and STX-102 for EHEC and *eae* 01 and *eae* 02 for EPEC. Enterotoxin production tests were performed only in 44 *Esch. coli* isolates. Latex agglutination test was done for verotoxin (VT) and LT, and ELISA for ST. Toxin assays showed 10 isolates produced VT, 22 isolates produced ST and only one isolate produced both VT and ST. The antibiotic susceptibility test showed that 71% of the isolates were sensitive to cefoperazone and 47%, 42%, 36%, 32% and 32% were sensitive to gentamicin, norfloxacin, flucloxacillin plus amoxicillin, chloramphenicol and amikacin, respectively. However, 90% and 82% of the diarrheagenic *Esch. coli* isolates were resistant to ampicillin and co-trimoxazole, respectively. This study pointed out that most of the isolates were susceptible to cefoperazone. In this study, the agreement between serotypes and the virulence genes detected by PCR was not significant. Therefore, serotyping alone is not considered a sufficient method for categorizing the diarrheagenic *Esch. coli* strains.

INTRODUCTION

Diarrhea remains a leading cause of morbidity and mortality worldwide, affecting mainly infants and, in Myanmar, it also constitutes a major health problem for adults. According to the National Health Plan (2006-2011)[1], it stands in the fourth position of priority ranking of identified diseases. The United Nation's Millennium Development Goal 4 (MDG 4) states that childhood mortality should be reduced by two thirds between 1990 and 2015. However, childhood diarrhea still claims nearly 2 million lives each year and remains responsible for 18% of all child deaths.[2] Therefore, more research is needed for childhood diarrhea to save as many children's lives as possible and to achieve the fourth Millennium Development Goal (MDG 4) target.

Rotavirus and diarrheagenic *Esch. coli* (DEC) are considered to be the most common of the many recognized enteropathogenic organisms.[3] In Vietnam, diarrheagenic *Esch. coli* is the second most common enteric pathogen with the prevalence of 22.5%.[4] In Myanmar, enterotoxigenic *Esch. coli* (ETEC) accounts for 27% of diarrhea cases with the highest peak (48%) in July.[5] DEC

was isolated from 16 stool samples among 60 stool samples of children admitted to the Pediatric Ward of North Okkalapa General Hospital.[6]

Esch. coli are members of the normal commensal flora that become pathogenic when they acquire virulence factor genes on plasmids, bacteriophages or pathogenicity islands.[7] *Escherichia coli* strains known to cause human diarrhea can be divided into at least five different categories. These are:

i. Enteropathogenic *Esch. coli* (EPEC)
ii. Enterotoxigenic *Esch. coli* (ETEC)
iii. Enterohaemorrhagic or verotoxin-producing *Esch. coli* or shiga toxin - producing *Esch. coli* (EHEC or VTEC or STEC)
iv Enteroinvasive *Esch. coli* (EIEC), and
v. Enteroaggregative *Esch. coli* (EAggEC)

The sixth category of *Esch. coli* termed diffusely adherent *Esch. coli* (DAEC), which is characterized by a pattern of diffused adherence to HEp-2 cells, has also been described.[8]

The emergence of multidrug-resistant diarrheagenic *Esch. coli* has been recognized as an important public health problem particularly among children in developing countries. There are reports worldwide on emergence of antibiotic resistance to ampicillin, tetracycline and trimethoprim-sulfamethoxazole from some regions like Kenya, Tanzania, Vietnam and the United States, where these classical antibiotics have been widely used but most of the isolated strains were found to be highly susceptible to quinolones.[9,4,10] The situation is also similar in Myanmar.[11]

Most of the studies on the role of *Esch. coli* as a cause of diarrhea in children in Myanmar have relied on conventional methods such as culture, biochemical tests, serotyping, tissue culture assay, Biken assay (precipitin test) for detection of heat labile enterotoxin (ETEC-LT) and ELISA. Although serogrouping is still used to define these pathogenic strains, it is now recognized that the serogroup is not well correlated with the presence of pathogenic factors.[12] There have not been any previous published studies on diarrheagenic *Esch. coli* in patients in Magway and antibiotic sensitivity profiles for currently prevailing strains need to be determined. In this study, PCR techniques, amplifying the *eae A* gene, LT and ST gene, and Verotoxin-1 and 2 (*stx*) gene, were used to detect EPEC, ETEC, and VTEC (EHEC), respectively, to evaluate the role of *Esch. coli* in the etiology of diarrhea in central Myanmar.

MATERIALS AND METHODS

A cross-sectional, hospital- and laboratory-based study was carried out from January 2007 to December 2009. All patients (both children and adults) with clinically diagnosed diarrhea and admitted to Magway Regional Hospital were selected. Informed consent was obtained from the patient or from the guardian if the subject was a child. Patients of any age and sex were recruited as subjects. Patients with other preexisting clinically diagnosed gastrointestinal diseases and with history of taking laxative within two days were excluded from the study.

All the 359 stool specimens and 559 rectal swabs from 918 patients with diarrhea were transported to the microbiological laboratory as soon as possible and then plated onto the MacConkey agar and incubated at 37°C overnight aerobically for culturing the enteric pathogens. Colonies from MacConkey agar were used for identification of bacterial pathogens. To study the bacteriological profile of diarrheagenic *Esch. coli*, further investigations were carried out by WHO standard microbiological methods such as colonial morphology, motility test, biochemical test, serotyping and toxin assay.[13] From the MacConkey agar, one colony of *Esch. coli* per stool sample was inoculated onto the nutrient agar slope in Bijou bottles as stock culture, incubated overnight at 37°C

and stored at room temperature. Stock cultures were transported to the Bacteriology Research Division, Department of Medical Research (Lower Myanmar) to perform antibiotic susceptibility test, serotyping, toxin assay and PCR.

Antibiotic susceptibility testing

Antibiotic susceptibility test was done by using the modified Kirby-Bauer disc diffusion method and the Clinical and Laboratory Standards Institute (CLSI) zone size interpretation was used to identify susceptible and resistant *Esch. coli* isolates.[14, 15] Antibiotic discs (Hi-media) containing amikacin (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), norfloxacin (10 μg), gentamicin (10 μg), cefoperazone (30 μg), flumox (flucloxacillin+amoxicillin) (30 μg) were used. *Esch. coli* (ATCC 25922) was used as standard strain for quality control of disc diffusion test.

Serotyping

Serotyping was performed by slide agglutination test using four known polyvalent antisera, followed by 32 relevant monovalent antisera (Denka Seiken, Tokyo, Japan). The polyvalent and monovalent antisera used for serotyping are shown below.

For EPEC I, monovalent antisera included O26 K60, O44 K74, O55 K59, O111 K58, O119 K69, O125 K70, O127 K63 and O142 K+ whereas for EPEC II, O1 K51, O86 K61, O86 K62, O114 K90, O126 K71, O128 K67, O146 K89 and O157 K+ were used. The monovalent antisera used for EIEC were O28 K73, O112 K66, O124 K72, O136 K78, O143 KX1, O144 KX2, O152 K+ and O164 K+ and for ETEC, O6 K15, O8 K25, O8 K40, O25 K+, O27 K+ O78 K80, O148 K+, and O159 K+ were used.

Toxin detection

Esch. coli isolate to be tested was inoculated into 2 ml of sterile casein hydrolysate-yeast extract (CA-YE) broth and incubated, with continuous and vigorous shaking, at 37°C for 18 to 24 hours. After incubation, the cultured broth was centrifuged at 3,000 rpm for 30 minutes at 4°C and the supernatant was used as the test sample. Heat stable enterotoxin (ST) was detected in culture filtrate by *Esch. coli* ST EIA, TD 700 (Oxoid). VET-RPLA, TD 0920, (Oxoid) and VTEC–RPLA Kit, TD 960 (Oxoid) were used for detection of heat-labile enterotoxin (LT) and verotoxin (VT), respectively.

Polymerase chain reaction (PCR)

PCR was performed using boiled lysates from overnight-grown bacteria colonies as DNA template. The primers used for PCR are shown in Table 1.

Table 1. Detection of enterotoxigenic, enterohaemorrhagic and enteropathogenic *Esch. coli* by specific primers

Category	Target	Nucleotide sequence (5' ⟶ 3')	Amplicon size (bp)
ETEC -LT	LTI-01	fp:CAAGCTTGGAGAGAAGAACCC	203
	LTI-02	rp: TCATCCCGAATTCTGTTATAT	
ETEC -ST	ST-157	fp: TTTTCTTTCTGTATTATCTT	191
	ST-158	rp: ATTACAACACAATTCACAGC	
EHEC	STX-01	fp: ATCAGTCGTCACTCACTGGT	113
	STX-02	rp: CTGCTGTCACAGTGACAAA	
EHEC	STX-101	fp: CAACACTGGATGATCTCAG	349
	STX-102	rp: CCCCCTCAACTGCTAATA	
EPEC	EPEC-*eae*01	fp: GCTTAGTGCTGGTTTAGGAT	488
	EPEC-*eae*02	rp:TCGCCGTTCAGAGATCGC	

fp= forward primer, rp=reverse primer
eae=Esch. coli attaching and effacing
LT = heat-labile toxin, ST=heat-stable toxin
STX= Shiga toxin, bp=base pair

The reaction mixtures were amplified in a DNA thermalcycler (PCR Sprint Thermalcycler, Thermo Electron Corporation, USA). The thermocycling conditions were as follows: 94°C for one minute, 94°C for 30 seconds, 49°C for one minute, and 72°C for one minute, for 30 cycles. The amplified product was analyzed by electrophoresis in 1.5% (wt/vol) agarose gel using ethidium bromide staining. The DNA bands were visualized in UV light transilluminator. Control strains used in all PCR experiments were supplied by the Okayama University, Japan.

Data analysis

Data entry was done in Excel spread sheet and then transferred into SPSS format for analysis. Age of cases was regrouped into two groups (i.e. up to 12 years and above 12 years). Distribution of serotypes and PCR types of *Esch. coli* were cross-tabulated across two age groups. Significance was tested by chi square statistics. A 'p' value of <0.05 was considered statistically significant.

Ethical consideration

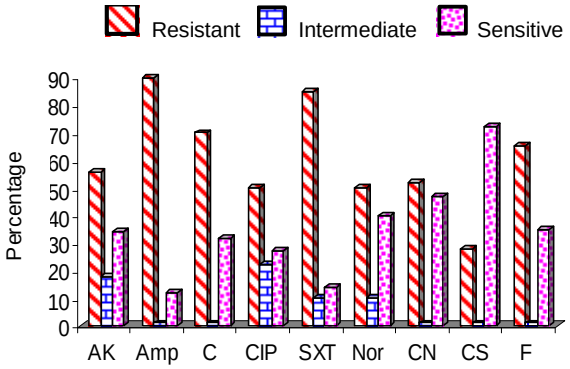
Approval was obtained from the Research and Ethical Committee, University of Medicine 1, Yangon.

RESULTS

Out of 93 diarrheagenic *Esch. coli* isolates, 66 isolates were isolated from stool samples and 27 from rectal swabs.

Antimicrobial susceptibility pattern of isolated diarrheagenic Esch. coli

The sensitivity pattern of amikacin, ampicillin, chloramphenicol, ciprofloxacin, co-trimoxazole, norfloxacin, gentamicin, cefoperazone and flucloxacillin+amoxicillin were 32%, 10%, 32%, 27%, 10%, 42%, 47%, 71% and 36%, respectively. Most of the DEC strains were resistant to ampicillin and co-trimoxazole. It was found that most sensitive antibiotic was cefoperazone which showed 71% sensitivity (Fig. 1).



Ak = Amikacin
Amp = Ampicillin
C = Chloramphenicol
CIP = Ciprofloxacin
F = Flucloxacillin plus Amoxicillin
SXT = Co-trimoxazole
Nor = Norfloxacin
CN = Gentamycin
CS = Cefoperazone

Fig. 1. Antibiograms of isolated diarrheagenic *Escherichia coli*

Serotyping of the isolated diarrheagenic Escherichia coli

Some 30.1% (28 of 93) EPEC serogroups, 8.6% (8 of 93) EIEC serogroups, 14% (13 of 93) ETEC serogroups were identified. The rest 47.3% (44 of 93 cases) were not serotypable due to inavailability of antigen (Fig. 2).

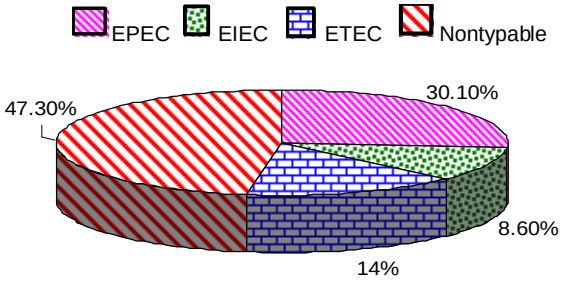


Fig. 2. Frequency distribution of serogroups of isolated diarrheagenic *Escherichia coli*

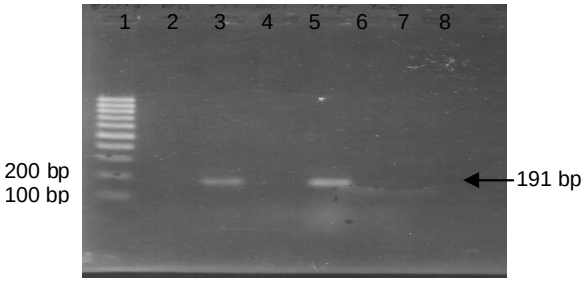
It was found that 11 EPEC serotypes (O44, O55, O111, O119, O125, O127, O142, O1, O86, O114 and O157); 6 ETEC serotypes (O6, O8 K25, O8 K40, O25, O27 and O148) and 6 EIEC serotypes (O28, O112, O124, O136, O152 and O164) were prevalent in Magway Township.

Toxin detection in diarrheagenic Esch. coli

There were 32 toxin-producing strains out of 44 isolates. Ten isolates produced VT, 21 isolates produced ST and one isolate produced both VT and ST. It was found that only 6 isolates were found to be ETEC strains among 21 ST-producing isolates, the other 4 were EIEC and 11 were EPEC strains.

Categorization of diarrheagenic Esch. coli according to virulence genes by PCR method

Only ST genes were detected for ETEC in 8 isolates out of 93 isolates accounting of 8.6% and there was no isolate positive for LT genes (Fig. 3). Eight *Esch. coli* isolates (8.6%) showed s*tx*-2 (VT) genes for EHEC and 5 *Esch. coli* isolates (5.38%) were positive for *eae* genes encoded for EPEC by PCR. Most of the *Esch. coli* isolates was found to be not typable with PCR (77.4%) because the primers for EIEC genes, for EAEC genes and for *bfp* genes of atypical



Lane 1 - 100 bp DNA ladder
Lane 2 - negative control
Lane 3 - positive control showing ETEC-ST gene at 191 bp
Lane 4 - sample 1
Lane 5 - sample 2 showing ETEC-ST gene at 191 bp
Lane 6, 7 & 8 - sample 3, 4 and 5

Fig. 3. An agarose gel stained with 0.5% ethidium bromide showing *Esch. coli* ETEC- ST DNA at 191 bp

EPEC were not available in Myanmar at the time of study. Comparing the two age groups (up to 12 years and above 12 years), all the PCR typable isolates were more common among children than adults.

Table 2. Agreement of serotyping and PCR typing

Serotype	PCR type				Total
	Non-typable	EPEC	ETEC	EHEC	
Non-typable	**33**	0	5	6	44
EPEC	21	**5**	2	0	28
ETEC	10	0	**1**	2	13
EIEC	8	0	0	0	8
Total	72	5	8	8	93

Total agree typing was (33+5+1=39) out of 93, thus 41.9%. Total disagreement will be (54/93) 58.1%

Agreement between serotyping and PCR typing

Thirty-three diarrheagenic *Esch. coli* isolates were non-typable by both serotyping and PCR typing, but 5 EPEC strains and 1 ETEC strain were identified by both serotyping and PCR typing. Therefore, serotyping results agreed with that of the PCR only in 39 isolates (33+5+1) out of 93 isolates (41.9%) and the other remaining 54 serotypes were not in concordance with the results of PCR. This result pointed out that the serogroups of *Esch. coli* were not likely to correspond with the virulence genes detected by PCR (Table 2).

DISCUSSION

In the present study, *Esch. coli* was isolated from 93 cases out of 918 samples, therefore the prevalent rate of diarrheagenic *Esch. coli* (DEC) was 10.1%. In a hospital-based study in Vietnam, the prevalence of DEC was 22.5%.[16] The prevalence rate was higher than that of the present study. In Myanmar, EPEC isolation rate was 10.91% in 2002, which was similar to the prevalence rate of the present study.[17] Classes of antimicrobial agents commonly used included: β-lactams, aminoglycosides, macrolides, tetracyclines, and quinolones. Reports from Myanmar and from other countries such as Kenya, Japan, Tanzania, and from Vietnam showed that there was high resistance of *Esch. coli* to ampicillin, tetracycline, co-trimoxazole and chloram-phenicol but they were still highly susceptible to quinolones.[11, 9, 4, & 10]

The results in the present study showed that only ampicillin and co-trimoxazole were resistant and cefoperazone was shown to be the highest susceptible drug to DEC. Cefoperazone was the drug of choice for treatment of diarrheagenic *Esch. coli* in Magway at the time of study, but other drugs still have some degree of sensitivity. This might be due to the fact that most of the people in Magway had less exposure to these drugs.

Forty-four *Esch. coli* isolates (47.3%) were not serotypable with antisera in this study. Apart from nontypable serogroup, the most common serogroup was EPEC followed by ETEC and EIEC. This finding was in concordance with that of Afset who stated that there was considerable variation in serotypes and almost half of the strains were O serogroup non-typable in Norway.[18] Another reason why the non-typable serogroups are more prevalent than other serogroups is that EAEC and EHEC antisera were not available for testing in this study. The serogroups detected in this study were similar to the serogroups reported by other researchers from Myanmar.[19, 6 & 11]

These serogroups (O26, O55, O114, O119, O125, O126, and O127) have been reported to be representative serogroups of typical EPEC. There were reports that typical EPEC is often isolated in developing countries, but rarely in industrialized countries. Atypical EPEC seems to be a more important cause of diarrhea in industrialized countries.[18] In Vietnam and Thailand, the frequency of isolation of typical EPEC has been reduced recently, and the isolation of atypical EPEC has increased.[16, 19] However in Myanmar, the frequency of isolation of typical EPEC still remains high.

In the present study, out of 32 enterotoxin-producing *Esch. coli* isolates, 21 isolates (65.6%) produced ST toxin, 10 isolates (31.2%) produced VT toxin and only 1 isolate (3.1%) produced both VT and ST. However, correlation between ETEC pathotypes and ST toxin production was present in only 28.6% of ST toxin-producing strains (6 of 21). EIEC serogroup was detected in 19% (4 of 21) of ST toxin-producing strains and EPEC serogroup was detected in 52.4% (11 of 21) of ST toxin-producing strains. Similar results were obtained in childhood diarrhea in Yangon.[6] Two out of 10 VT-producing strains (20%) were identified as EHEC strain in PCR assay and were compatible with the toxin assay. However, the other remaining 8 VT-producing strains were not typable due to the inavailability of antisera for EHEC in Myanmar. Serotyping alone is not sufficient to determine the pathotype of diarrheagenic *Esch. coli*, and toxin assays and PCR assays are becoming increasingly more important to determine the pathogenic type of *Esch. coli.*

In the present study, only ST genes were detected for ETEC. For EHEC, STX-01 and STX-02 genes representing *stx*-2 genes were identified. Only the *eae* gene was detected in the EPEC strains. In a study three atypical EPEC strains were isolated from 47 diarrheagenic *Esch. coli*.[11] However, there was no *bfp* gene to differentiate the typical and atypical EPEC strains in the present study.

Twenty-one isolates (22.6%) were shown to express virulence genes (ST, *stx* and *eae* genes) in this study. In one study carried out in Myanmar, virulence genes of diarrheagenic *Esch. coli* were obtained from (21.7%) of the *Esch. coli* isolates which is similar to the findings of the present study.[11] The *stx* gene was not detected in 8 VT toxin-producing isolates. The absence of *stx* gene in the isolates could be due to the fact that *stx* gene is bacteriophage coded and the isolate would have lost the gene during preservation.[20]

In conclusion, diarrheal infection caused by *Esch. coli* is common in Magway with occasional outbreaks. The characterization of diarrheagenic *Esch. coli* strains showed a high level of resistance to commonly used antimicrobials for treatment of diarrhea such as ampicillin and co-trimoxazol and the drug of choice was found to be cefoperazone. The common classical serotypes of EPEC, ETEC and EIEC were found in Magway. In addition, the virulence genes of ETEC, EHEC and EPEC were also detected. To correctly identify and categorize diarrheagenic *Esch. coli* strains, the organisms must be differentiated from nonpathogenic members of the normal flora.

However, in this study there was no correlation between serotypes and virulence genes as reported in other studies. This study highlights the value of detection of virulence genes to identify the pathogenic strains of *Esch. coli* in diarrhea even in the absence of serotyping.

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Vark learning style of medical students of University of Medicine (Magway)

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Learners are intrinsically different and have different learning styles. To identify the learning styles of medical students of University of Medicine, Magway, the VARK questionnaire were administered to a total of 559 medical students; 182 first year medical students, 196 third year medical students and 181 final part II medical students of University of Medicine, Magway. This study was performed at the Department of Microbiology, University of Medicine, Magway in March 2010. Of the study group (559 medical students of different grades), 17.9% (100 students) preferred unimodal learning style and 82.1% (459 students) preferred multimodal learning style. Students from all 3 grades utilized multimodal learning style more commonly than a single mode. Among them, bimodel learning style was used by most of the students (41.2%, n=189) followed by trimodality (32%, n=147). Only 26.8% (123 students) of the 559 medical students of different grades utilized all four senses (VARK). Among unimodal preferences, reading/writing learning style was found to be most commonly used modality followed by auditory learning style, kinesthetic learning style and then visual learning style (R=38, A=32, K=22,V=8). In students with multimodal learning style, reading/writing learning style was found to be commonly used modality followed by auditory learning style, visual learning style and kinesthetic learning style (AR-37%, VR-16.7%, RK-15.5%, VA-11.6%, AK-11.6%, ARK-27.9%, VRK-19.7%, VAK-10.5% and VAR-14.5%) in different combinations. Usage of kinesthetic learning style was slightly higher than that of visual learning style in unimodal learning style. In contrast to it, the usage of visual learning style was slightly higher than kinesthetic learning style among the students with multimodality preference.

INTRODUCTION

There are many thoughts and theories about individual learning styles; Dunn and Dunn, Joseph Renzulli, Howard Gardner, Jung, the Myers-Briggs Type indicator instrument and Kersley's Temperament Sorter, David Kolb and Anthony Gregorc's Type Delineator learning modalities. Learning strengths may also be classified as sensory: Learning Style Inventory (modality), Visual, Auditory, Kinesthetic & Tactile; Perceptual: Hemispheric Dominance, Differences Between Left and Right Hemisphere, Hemispheric Dominance Inventory; Cognitive Information-processing: Kolb's Learning Styles model (David Kolb's perception vs. processing), Learning Style Inventory (active/ reflective; sensing/ Intuitive; visual/ verbal; sequential/ golbal); personality type: Myers Briggs Type Indicator Instrument - The Use of Learning Style Innovations to Improve Retention, Personality Type Summary - Descriptions of four personality types, Center of Psychology Resources - Personality, The Keirsey Temperament Sorter II; Personal Talents: The Multiple Intelligence Inventory, Using Multiple Intelligences, Gardner's Seven Types of Intelligence, Seven Styles of Learning or situational. Honey and

Mumford's inventory is composed of 80 tick box questions, the results of which give the learner an indication of their preferred learning style; Activist, Reflector, Theorist or Pragmatist. Further research has expanded the knowledge in this area.[1, 2]

One of the most common and widely-used categorizations of the various types of learning styles is Fleming's VARK model: visual learners; auditory learners; reading/ writing-preference learners; kinesthetic learners or tactile learners which expanded upon earlier Neuro-linguistic programming (VAK) models.[3, 4]

Visual Learners learn through seeing. These learners need to see the teacher's body language and facial expression to fully understand the content of a lesson. In classroom, they benefit from teachers who use visual displays including: diagrams, illustrated text books, overhead transparencies, videos, flipcharts and hand-outs. During a lecture or classroom discussion, visual learners often prefer to take detailed notes to absorb the information.

Auditory Learners learn by talking things through and listening to what others have to say. They interpret the underlying meanings of speech through listening to tone of voice, pitch, speed and other nuances. They benefit from information obtained from verbal lectures and discussions. Written information may have meaning when it is heard. These learners often learn through reading text aloud and using a tape recorder.

Read-write Learners prefer printed words and texts. They also prefer lists, glossaries, textbooks, lecture notes, or handouts.

Tactile/Kinesthetic Persons learn best through a hands-on approach, activity and exploration.[5]

Different students exhibit unique strengths, talents and/or weaknesses. There are researches that showed significantly higher learning gains for college students when instructional strategies/resources compliment student learning styles.[6] Therefore, a variety of learning approaches must be provided in every classroom. Understanding the different ways that students learn, interact with and process information can help modify the way of teaching so that all students have an equal opportunity to succeed. The present study is aimed to identify the learning styles of medical students of University of Medicine, Magway.

MATERIALS AND METHODS

To determine the preferred mode(s) of learning, English version of the VARK questionnaire for young was administered at the end of the first semester (March, 2010) to 182 first year medical students, 196 third year medical students and 181 final part II medical students of University of Medicine, Magway. This study was performed at the Department of Microbiology, University of Medicine, Magway in March, 2010.

The VARK questionnaire (http://www.vark-learn.com; http://www.vark-learn.com/page.asp, questionnaire) was used (only 16 questions).[7]

Administering the questionnaire

When instructing the students to fill in the questionnaire they were told to make a selection (a, b, c or d) for each question, but they can omit a question or they can choose more than one option if they want to. Information on the meaning of words in the questionnaire and additional contextual or situational information were not given before they choose their answers as it may bias responses to the questions. They can choose more than one response if they think the context is not clear. Before they complete the questionnaire they were informed that the results indicate their preferences but are not necessarily their strengths. This reduces the anxiety for respondents who may express the view that the questionnaire *says* they are not good readers or not visually strong.

RESULTS

Eighty-two percent (459) of the study group (559 medical students of different grades), preferred multimodal learning style and 17.9% (100) preferred unimodal learning style. Among 100 students who preferred unimodal learning styles, 8 students (8%) showed preference for visual, 32 students (32%) auditory, 38 students (38%) reading/ writing, and 22 students (22%) kinesthetic mode (Fig. 1).

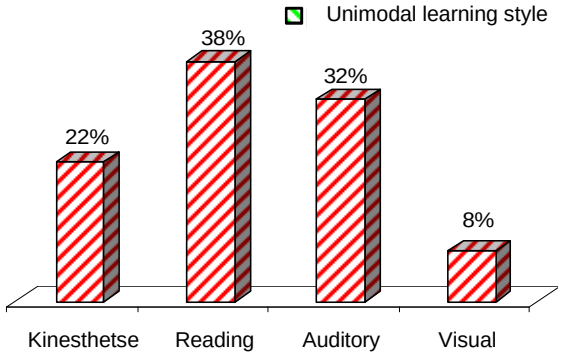


Fig. 1. Percentage distribution of unimodal learning style

Among the 459 students who preferred multimodal learning style, some students showed preference for two modes (bimodal, 41.2% 189 students), some for three modes (trimodal 32%, 147 students) and some for four modes (quadrimodal 26.8%, 123 students) (Fig. 2).

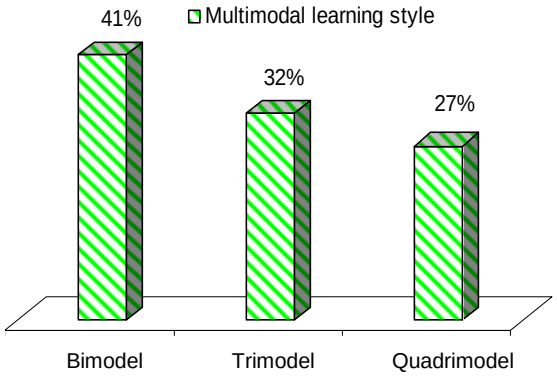
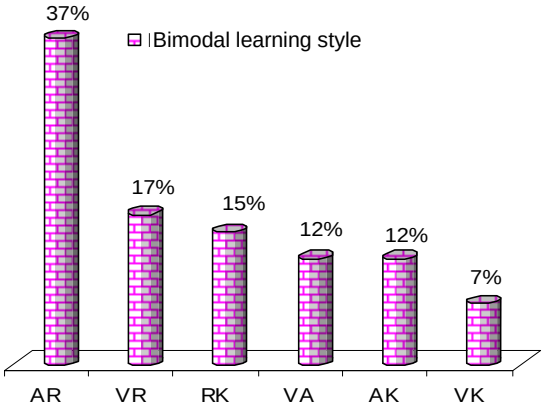


Fig. 2. Percentage distribution of multimodal learning style

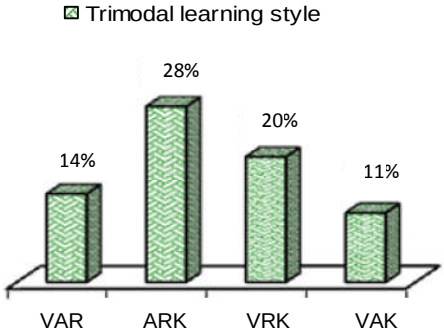
Of the 189 students who preferred bimodal learning style, 37% used auditory and reading/writing mode (AR), 16.9% visual and reading/writing mode (VR) and 15.3% reading/writing and kinesthetics mode (RK). Each of 11.6% of students preferred visual and auditory mode (VA) and auditory and kinesthetics mode (AK). Only 7.4% preferred visual and kinesthetics mode (VK) of learning (Fig. 3).



AR=Auditory and reading/writing mode
VR=Visual and reading/writing mode
RK=Reading/writing and kinesthetics mode
VA=Visual and auditory mode
AK=Auditory and kinesthetics mode
VK=Visual and kinesthetics mode

Fig. 3. Percentage distribution of bimodal learning style

For trimodal learning style, some students have preference on visual, auditory and reading/ writing mode (VAR-14.1%), some on auditory, reading/ writing and kinesthetics mode (ARK-27.9%), some visual, reading/ writing and kinesthetics mode (VRK-19.7%),and some visual, auditory and kinesthetics mode (VAK-10.8%) (Fig. 4).



VAR=Visual, auditory and reading/ writing mode
ARK=Auditory, reading/ writing and kinesthetics mode
VRK=Visual, reading/ writing and kinesthetics mode
VAK=Visual, auditory and kinesthetics mode

Fig. 4. Percentage distribution of trimodal learning style

DISCUSSION

The capacity to solve problems, to analyze and classify data, to gather evidence about solutions and to apply and test theories is very important in learning in the sciences. With the advancement in information technology and qualified research, the knowledge based in science becomes expanding too fast. Students have to cover all aspects of scientific knowledge within the duration of a university course. Emphasis on developing higher order cognitive skills of university science students is also growing.

Each student's learning process is different; some people learn better by listening, some by watching, or learn best by reading printed materials and others by doing. Students learn more in a manner compatible with their own learning preference. Utilization of different learning methods is more effective than using one method only. Knowledge on the ways they learn and process information will help develop effective instructional strategies and methods which make effective learning.[8]

In this study, learning style among 559 medical students of first year, third year and final part II were multimodal learning style (82.1%, n=449) and unimodal learning style (17.9%, n=100). Percentage utilization of multimodal learning style was greater than that of unimodal learning style among all three grades.

Students who preferred unimodal learning styles utilize reading/writing, auditory, kinesthetic and visual modes in decreasing frequency (R-38%, 38 students; A-32%, 32 students; K-22%, 22 students; V-8%, 8 students). Reading/writing learners benefits from write out important information again and again, read notes silently, organize any diagrams into statements, rewrite the ideas and principles in other words, make flashcards of words and concepts that need to be memorized, important points or outlines of the lecture material.[9]

Auditory learners tend to benefit most from traditional teaching techniques, lecture, lecture discussion because they have the ability to retain and learn new information through the process of listening and discussing. Tapes, audio, lectures, discussion, debate, games, questions and answer sections are effective for Auditory learners. Reading aloud, interviewing, debating, participating on a panel and giving oral reports, discussion, questions, answers, debate and verbal activities can be used to engage and support an auditory learner and so will enhance the auditory learner's classroom experience.[10]

Kinesthetic learners prefer doing, manipulation and moving to strengthen short and long-term memory. Role play, practical class, drama, things to build, drawing, playing board games, making diagrams, making models, movement, sports and physical games, tactile experience or hands on experience, field trips, visiting museums, studying with others, setting up experiments, using memory games, using flash cards to memorize promote deep learning for kinesthetic learners. They will do best in answering definitions, fill-ins and multiple choice.[11, 12]

Things written down in a handout, text or on the overhead such as drawing, maps, pictures, diagrams, demonstrations, gaph, chart, flow diagrams, display, computer graphics, cartoons and film will benefit visual learners who prefer to see. Visual learners as well as kinesthetic learners will value to-do lists, assignment logs, and written notes.[13]

In the present study, bimodal modes (41.2% of multimodal learning style) was the most commonly used mode followed by trimodal (32%) and then quadrimodal learning styles (27%).

Although different combination of reading, visual, auditory and kinesthetic modes were seen in different grades most frequently utilizing combinations were auditory reading (AR-37%), visual reading (VR-16.9%),

kinesthetic reading (KR-15.3%) and visual and auditory mode (VA) and auditory and kinesthetics (AK) mode (11.6% each).

Auditory, reading/writing and kinesthetics mode (ARK-27.9%) was found to be most frequently used mode combination in trimodal style. Visual, reading/writing and kinesthetics mode (VRK-19.7%) was the second most frequently used mode followed by visual, auditory and reading/writing mode (VAR-14.1%) and visual, auditory and kinesthetics mode (VAK-10.8%).

There will be problems if instruction that exposes only one mode of learning to the students with a diversity of age, experience, culture, ethnicity and learning preference. Therefore, it is important to provide a variety of learning to meet for the educational needs of all students to motivate and improve preformance of them and also make students to adapt to other modes of learning in addition to preferred mode. Methods that intentionally combine information processing across learning styles may have the greatest potential for supporting academic success for more students.

Reading aloud, lecture outline, written assignment can be used for reading writing learners. Replacing words with symbols or initials, translating concepts into pictures and diagrams, underline or highlighting notes or textbooks with different colors, practicing turning visuals back into words, and making flashcards of key information with words, symbols, and diagrams should be used for the Visual Learning Style.

Learning strategies for the Aural Learning Style include attend lectures and tutorials, discuss topics with teachers and other students, put summarized notes on tape and listen to them, join a study group, tape record lectures, and when recalling information or solving problems, talk out loud.[9, 13]

Learning strategies for the Kinesthetic/ Tactile Learning Style include sit near the instructor in classroom situations, read out loud from textbook and notes, copy key points onto large writing surfaces (i.e. chalkboard or easel board), copy key points using word processing software, listen to audiotapes of your notes while exercising, take in information through field trips, laboratories, trial and error, exhibits, collections, and hands-on examples summary, recall experiments and role-play, and use pictures and photographs that illustrate an idea.[9]

Visual learners remember things best by seeing something written. Things written down in a handout, text or on the overhead such as drawing, maps, pictures, diagrams, demonstrations, gaph, chart, flow diagrams, display, computer graphics, cartoons and film will benefit visual learners who prefer to see. Visual learners as well as kinesthetic learners will value to-do lists, assignment logs, and written note.[13]

Reading/writing, auditory, kinesthetic modes were commonly used modes in both unimodal and multimodal styles in the present study. Teaching technique that involves a variety of different methods in order to accommodate every student's unique learning style is most successful teaching technique. The techniques that engage students, catch students' attention and interest, stimulate students' mental activity and the techniques are more feasible, new and exciting make effective for students to learn.

Although currently using teaching methods including lectures, tutorial and practical demonstration can cover reading/writing, auditory, kinesthetic modes users, university teachers must take the initiative in designing new tasks/strategies whereby students engage in different forms of learning.

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A study of lipid profile in rheumatoid arthritis

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The aim of this study was to evaluate significance of lipid profile in rheumatoid arthritis patients. Forty rheumatoid arthritis patients and forty of age- and sex-matched healthy volunteers were included in the study. Serum lipid profile (total cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein) was determined using spectrophotometer. Disease activity was assessed by using disease activity score (DAS-28). Rheumatoid arthritis patients had mean total cholesterol level of 189.27±41.60 mg/dl, triglyceride level of 134.07±37.05 mg/dl, low-density lipoprotein level of 117.35±34.61 mg/dl, and high-density lipoprotein level of 45.02±7.79 mg/dl. Normal healthy volunteers had mean total cholesterol level of 177.53 ±26.67 mg/dl, triglyceride level of 76.69±24.82 mg/dl, low-density lipoprotein level of 100.87±23.49 mg/dl, and high-density lipoprotein level of 61.03±9.34 mg/dl. Serum triglyceride and low-density lipoprotein were significantly high in rheumatoid arthritis ($p<0.01$ and $p<0.05$, respectively) whereas serum high-density lipoprotein level was significantly low in rheumatoid arthritis ($p<0.01$). Mean atherogenic index of rheumatoid arthritis was 4.30±1.05 while that of normal healthy volunteers was 3.00±0.74 and the difference was statistically significant ($p<0.01$). Lipid profile pattern was not significantly and strongly correlated with disease activity score. Rheumatoid arthritis is associated with adverse lipid profile.

INTRODUCTION

Rheumatoid arthritis is a chronic systemic inflammatory disease of unknown etiology that is characterized by its pattern of diarthroidal joint involvement.[1] The arthritis is symmetrical. It may be remitting, but if not properly managed and treated, may lead to deformity and destruction of joints due to erosion of cartilage and bone. It affects about 1% of the world's population.[2] In Myanmar, rheumatoid arthritis is not uncommon, comprising 29.6% patients attending out-patient department of the Rheumatology Clinic, Yangon General Hospital. The prevalence of rheumatoid arthritis in Myanmar was 1.3% in the year 2004. [3]

Rheumatoid arthritis causes significant morbidity as a result of synovial inflammation, joint destruction and associated disability.[4] Rheumatoid arthritis patients have an increased mortality (standardized mortality rate 1.4-3.0) and die on average 2.5 years earlier in community-based studies and up to 18 years earlier in hospital-based cohorts than the general population.[5] Cardiovascular disease accounts for 35% to 50% of excess mortality in rheumatoid arthritis patients.[6]

This increased cardiovascular risk in rheumatoid arthritis patients could be caused by an increased prevalence of known risk factors for cardiovascular disease such as dyslipidemia, diabetes mellitus, hypertension, body mass index, physical fitness, and smoking habits; rheumatoid arthritis itself either the underlying inflamematory process or decreased functional capacity; and undertreatment of cardiovascular disease as a comorbid condition in patients with rheumatoid arthritis.

Approximately 50% of atherosclerotic coronary artery disease in the community occurs in the absence of traditional risk factors.[7] The ratio of total cholesterol and high-density lipoprotein cholesterol, an atherogenic index, is an important prognostic marker for cardiovascular disease.

Disease activity in rheumatoid arthritis is an expression of a cascade of immunological and inflammatory reaction, probably initiated by an unknown stimulus, and perpetuated for unknown reasons. Some clinical symptoms and signs are used to assess disease activity, e.g. the number of swollen and tender joints,[8] graded, ungraded or weighted joint indices[9], pain and fatigue [10], duration of morning stiffness and different scores for functional decline.[11]

The patient´s own global assessment of the disease activity is sometimes added. Laboratory markers of disease activity are for instance acute phase proteins and erythrocyte sedimentation rate (ESR). In some instances, clinical and laboratory markers for disease activities are combined, including the patient´s global assessment (PGA) of disease activity, into compound indices of disease activity, e.g. disease activity score (DAS).[12] DAS is useful and valid in assessing disease activity and it is the use of composite indices summarizing the information for various parameters in a single indicator. There is a modified DAS based on the number of painful joints (NPJ28) and swollen joints (NSJ28).

Hence, rheumatoid arthritis, one of the autoimmune diseases, is associated with accelerated vascular risk resulting in early mortality and excess morbidity. The risk of cardiovascular event is doubled in rheumatoid arthritis patients irrespective of traditional risk factors, and is frequently silent and subclinical.[13] Lipid profile in rheumatoid arthritis patients has not yet been studied in Myanmar. This study will evaluate lipid profile, a predictor of cardiovascular events, in rheumatoid arthritis.

MATERIALS AND METHODS

Forty rheumatoid arthritis patients who attended the Rheumatology Clinic at Yangon General Hospital and Rheumatology Clinic at Shwegontaing Specialist Centre were investigated. All patients fulfilled the American College of Rheumatology (ACR) 1987 criteria for rheumatoid arthritis.[14] Only those who gave informed consent were involved in the study.

Subjects with hypertension, diabetes mellitus, other medical diseases that will affect lipid profile and disease activity of rheumatoid arthritis, those taking drugs affecting lipid metabolism, those taking last main meal less than 6 hours on the day of blood sample collection, and the current smokers were excluded. Forty age- and sex-matched normal healthy volunteers were also included in the study. All the participants in the study were completed questionnaires regarding demographic data, diet pattern and last meal, medical history, drug history and smoking history. Disease duration and chest pain were also recorded for rheumatoid arthritis patients. Body mass index was calculated from body weight and height. NPJ, NSJ, and PGA were assessed by the Rheumatologist to calculate DAS28. ESR was determined by Westergren's method, and lipid profile was determined by enzymatic methods using semiautomatic spectrophotometer (Humalyzer 3000).

All samples were collected with code numbers and analyzed batch by batch in duplicate. Disease activity for the 28 joint indices score was calculated by using the formula[8] ; $DAS28=0.56\ (NPJ28)^{1/2}+ 0.28\ (NSJ28)^{1/2}+ 0.70\ (\ln ESR) + 0.014\ (PGA)$. The score for DAS28 can range from 0 to 10.

Data analysis was done by using the Statistical Package for Social Sciences (SPSS) software version 11.5. Standard statistical methods were applied for the calculation of mean, standard deviation and standard error. Student's "t" test was

applied to calculate the significance of difference between the means on 95% confidence interval of each parameter. Evaluation was done at the probability level of less than 0.05. Pearson's correlation coefficient (r) was calculated to assess the relationship between lipid levels and DAS28.

RESULTS

Basic parameters

Mean age of forty rheumatoid arthritis patients in the study was 53.88±12.40 years and that of forty normal healthy volunteers was 53.78±12.92 years. Four males and thirty-six females rheumatoid arthritis patients were involved in the study. Out of forty rheumatoid arthritis patients, eight patients (20%) had disease duration less than six months i.e., very early disease and thirty-two patients (80%) had disease for more than six months. Mean duration of disease was 49.30±66.72 months.

Total cholesterol levels

Mean total cholesterol level of rheumatoid arthritis patients was 189.27±41.60 mg/dl and that of normal healthy volunteers was 177.53±26.67 mg/dl and the difference was not statistically significant (p=0.098).

Triglyceride levels

Mean triglyceride level of rheumatoid arthritis patients was 134.07±37.05 mg/dl and that of normal healthy volunteers was 76.69±24.82 mg/dl and the difference was statistically significant (p=0.001).

LDL cholesterol levels

Mean LDL cholesterol level of rheumatoid arthritis patients was 117.35±34.61 mg/dl and that of normal healthy volunteers was 100.87±23.49 mg/dl and the difference was statistically significant (p=0.004).

HDL cholesterol levels

Mean HDL cholesterol level of rheumatoid arthritis patients was 45.02±7.79 mg/dl and that of normal healthy volunteers was 61.03±9.34 mg/dl and the difference was statistically significant (p=0.001).

Atherogenic index

Mean atherogenic index of rheumatoid arthritis patients was 4.30±1.05 and that of normal healthy volunteers was 3.00±0.74 and the difference was statistically significant (p=0.001) (Fig. 1).

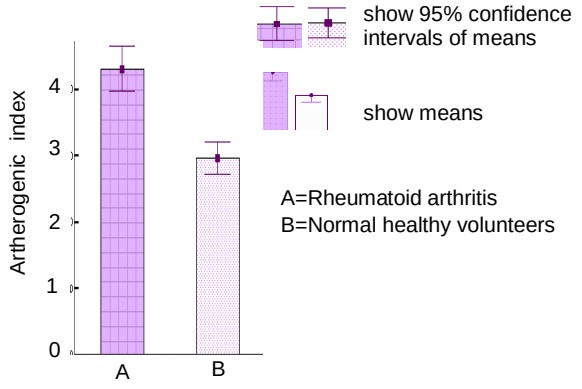

Fig. 1. Mean atherogenic indices of rheumatoid arthritis patients and normal healthy volunteers

Correlation between plasma lipid levels and DAS28 in rheumatoid arthritis

Correlation between plasma total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol levels and DAS28 in rheumatoid arthritis patients are shown in Table 1.

Table 1. Correlation between lipid profile and DAS28 in rheumatoid arthritis patients

	Correlation coefficient (r) with DAS28	Level of significance (p)
Plasma total cholesterol	0.29	0.07
Plasma triglyceride	0.27	0.09
Plasma LDL cholesterol	0.19	0.24
Plasma HDL cholesterol	-0.42	0.01*

*denotes statistically significant

DISCUSSION

Rheumatoid arthritis is associated with an excess mortality from cardiovascular disease, and this may be related to an atherogenic lipid profile. Plasma lipid profile of forty rheumatoid arthritis patients and forty age- and sex-matched normal healthy volunteers were studied. Mean total cholesterol level of rheumatoid arthritis patients was slightly higher than that of

normal healthy volunteers but not statistically significant in the study.

Mean triglyceride level was significantly higher in rheumatoid arthritis patients than in normal healthy volunteers. Mean plasma LDL cholesterol level was significantly higher in patients with rheumatoid arthritis than in normal healthy volunteers. Mean plasma HDL cholesterol in rheumatoid arthritis was lower than that in normal healthy volunteers and it was statistically significant. Atherogenic index was significantly higher in patients with rheumatoid arthritis than that in normal healthy volunteers.

There was no significant correlation between DAS28 and plasma total cholesterol, plasma triglyceride and plasma LDL cholesterol levels. But there was a significant weak negative correlation between DAS28 and plasma HDL cholesterol level.

The lipid profile of patients with rheumatoid arthritis has been evaluated in several studies. Some of these studies have reported lower levels of total cholesterol and HDL cholesterol in active and/or untreated disease than in the general population.[15, 16] However, other studies have not shown significantly different lipid levels from those observed in the healthy population[17] and others refer to an overall reduction in all lipid sub-fractions in cases of active disease.[18, 19] The discrepancies in the lipid levels observed in the various studies could be attributed to the size of the samples, the type of the study (prospective or cross-sectional), differences in the disease type (established or early), differences in the disease activity, or to differences in the studied population.

The dyslipidemia pattern observed in the study is highly atherogenic. Increased levels of total cholesterol, LDL cholesterol, triglyceride, and decreased HDL cholesterol level are associated with an increased incidence of cardiovascular disease in the general population. Low HDL cholesterol is a strong predictor of cardiovascular events. Atherogenic index, the ratio of total cholesterol/HDL cholesterol, is an important prognostic cardiovascular risk factor.

Mechanisms underlying the lipid pattern in rheumatoid arthritis include effects of cytokines at adipose tissue to increase free fatty acid release, at the liver to increase free fatty acid and triglyceride synthesis, at the vascular endothelium to reduce lipoprotein lipase activity, the principal catabolic enzyme for triglyceride-rich lipids. High triglyceride levels reduce HDL cholesterol by virtue of neutral lipid exchange, and this same process promotes synthesis of small, dense LDL. Increased LDL cholesterol level might be due to an increased level of secretory group IIa phospholipase A2, an acute phase protein and an independent cardiovascular risk factor. Atherosclerosis starts when LDL infiltrates the artery wall and is oxidized by reactive oxygen species to oxidized LDL. Oxidized LDL leads to phospholipid release, activating endothelial cells, thereby initiating an inflammatory process which leads to the formation of foam cells and subsequently fatty streaks. HDL exerts its antiatherogenic role by protecting LDL from oxidation in addition to the inhibition of the expression of adhesion molecules and its role in the reverse cholesterol transport.

Rheumatoid arthritis is associated with dyslipidemia. Tight disease control might lower cardiovascular risk in rheumatoid arthritis patients and it may also have some beneficial effects on the lipid profile and thereby reduce cardiovascular mortality in rheumatoid arthritis patients.

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Study on presence of macrophages in cervical cancer using immunohistochemistry

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It was a cross-sectional, hospital- and laboratory-based descriptive study aimed to study the presence of macrophages in cervical cancer using immunohistochemistry. A total of 55 formalin-fixed, paraffin-embedded cervical cancer tissues were collected from Pathology Department, CWH from January, 2009 to December, 2009. Routine haematoxyline and eosin stain was performed to confirm the diagnosis. Among them, 12 cases (21.8%) were diagnosed as well differentiated squamous cell carcinoma (SCC), 23 cases (41.8%) were moderately differentiated SCC and the rest 20 cases (36.4%) were poorly differentiated SCC. Then, immunohistochemical stain for macrophage marker (CD68) was done. High intensity of CD68 was found in 4 cases (33%) of WDSCC, 10 (43%) of MDSCC and 7 (35%) of PDSCC; moderate intensity in 8 cases (67%) of WDSCC, 8 cases (35%) of MDSCC and 9 cases (45%) of PDSCC; low intensity in 5 cases (22%) of MDSCC and 4 cases (20%) of PDSCC. The correlation of CD68 intensity with the staging of cancer cervix was also examined. High and moderate intensity was mainly found in advanced stages (Stage III and IV). In this study, we concluded that there was no significant correlation between the intensity of macrophage marker (CD68) with the histological grade (p=0.358) and clinical staging (p=0.201) in cancer cervix. The findings agreed with those of other studies done in squamous cell carcinoma cervix which highlighted that the beneficial role of macrophages in cellular immunity may be opposed by the elaboration of growth factors in the vicinity of neoplastic cells.

INTRODUCTION

Cervical cancer is a major cause of morbidity and mortality in women. Globally, it is also the second most common form of female cancer.[1] In Myanmar, it topped the list of the leading causes of female cancer morbidity and mortality according to Yangon Cancer Registry, 2008.[2] The pathogenesis of cancer cervix is influenced by many etiological factors such as sexual behaviour, cigarette smoking, oral contraceptives, immunosuppression, dietary factors and infection like HPV and HSV.[3, 4]

Nowadays, the role of different tumour markers (eg. SCCA in squamous cell carcinoma cervix), oncogenes, tumour associated antigens and cell cycle related antigens in the diagnosis and prognosis of cervical cancer has been studied by using immunological and molecular techniques.

CD68 antigen is a 110kD intracellular transmembrane glycoprotein which is highly expressed in human monocytes including tissue macrophages and was identified to be a member of a group of haemopoietic molecules including CD43 and CD34 and the lymphnode ligand for L-selectin GlyCAM-1. The antigen plays a role in endocytosis and/or lysosomal traffic. The function of CD68 antigen is unknown but the lysosomal glycoproteins are the major components and may protect the membranes from attack by hydrolases. CD68 is expressed intracellularly in cyto-

plasmic granules but can also be detected in smaller amount at the surface of cells.[5]

The presence of macrophages as well as other inflammatory cells has been noted in many of tumours including cervical cancer. Intratumoural macrophages/monocytes also induce energy to cytokine therapy and cause apoptosis in natural killer (NK) cells and T cells.[6] In tumors, macrophages can exhibit a different phenotype and thus contribute to tumor growth, invasiveness, metastasis, local immunoregulation and angiogenesis. In several different tumors, the accumulation of macrophages, which sometimes is the main component of the inflammatory infiltrate, is associated with the worst prognosis as, for example, in breast and ovarian carcinoma.[7]

It has been described that cervical cancer cells also express macrophage attractants, including monocyte chemoattractant protein-1 (CCL2), macrophage colony stimulating factor-1 (CSF-1) and vascular endothelial growth factor (VEGF), not only locally but also identifiable in the peripheral blood.[8, 9] However, data are conflicting on the role of macrophages in cervical carcinogenesis. So the aim of study was to find out the presence of macrophages in the cervical cancer. We expect that the results from this study can show whether there is any association between the clinicopathological parameters, especially grading and staging of the tumour with intensity of CD68 marker and thus help in predicting the prognosis of cervical cancer.

MATERIALS AND METHODS

A hospital- and laboratory-based cross-sectional study was performed on 55 leftover formalin-fixed, paraffin-embedded cervical cancer tissues which were collected from Pathology Department, CWH from January, 2009 to December, 2009.

Routine haematoxylin and eosin stain

Formalin-fixed, paraffin-embedded tissue samples of uterine cervical cancers were cut at 4 μm, with the first section stained with routine haematoxylin and eosin stain for confirmation of diagnosis and determination of histological type and grading.

Immunohistochemistry

Subsequent sections of formalin-fixed, paraffin-embedded tissue of uterine cervical cancers were cut and dried overnight at 37˚C on a silanized slide. Samples were deparaffinized in xylene at room temperature, washed with a graded ethanol/water mixture, and then with distilled water. The samples for CD68 antigens were soaked in a citrate buffer and then microwaved at 100˚C for 10 minutes. The protocol for DAKO LSAB2 Kit Peroxidase (DAKO) was followed for each sample.

In the described procedures, mouse antihuman macrophage CD68 (DAKO) was used at dilutions of 1:50 as the first antibodies for 30 minutes. The addition of the first antibody, mouse antihuman macrophage CD68 was omitted in the protocols for negative control. Subsequently, biotinylated secondary antibody, goat anti-mouse, was applied for 30 minutes followed by streptavidin-peroxidase for 10 minutes. Immunoreactive complexes were detected using diaminobenzidine chromogen exposure for 5 minutes. Finally, slides were counter-stained with haematoxylin for 5 minutes, washed in distilled water, dehydrated in graded ethanol, cleared with xylene and mounted.

Assessment of staining intensity of expression of CD68 marker

Scoring system (adapted from Fisher, *et al*, 1994)

Strong dark cytoplasmic staining that is easily visible with a low power objective and involves >50% of cells	High Intensity
Moderate focal darkly staining areas, (<50% of cells) or moderate cytoplasmic staining of >50% of cells	Moderate Intensity
Weak focal moderate staining in <50% of cells, or pale cytoplasmic staining in any proportion of cells not easily seen under a low power	Low Intensity
Negative tumours that show none of the above	Negative

Data analysis

Statistical software of SPSS-11 version was used for association, correlation and significance.

RESULTS

In the present study, a total of 55 patients with carcinoma cervix were confirmed diagnosis and histological typing and grading assessed by using routine H & E stain. All cases were confirmed as invasive squamous cell carcinoma. Among them, 12 cases (21.8%) were diagnosed as well differentiated squamous cell carcinoma (SCC), 23 cases (41.8%) were moderately differentiated SCC and the remaining 20 cases (36.4%) were poorly differentiated SCC (Table 1).

Table 1. Histological grades of total 55 patients of invasive squamous cell carcinoma

Histological grade of SCC	No. of patients (%)
Well differentiated SCC	12 (21.8)
Moderately differentiated SCC	23 (41.8)
Poorly differentiated SCC	20 (36.4)
Total	55 (100)

Clinical staging of total 55 cases of cervical cancer according to FIGO clinical staging is shown in Table 2.

Table 2. Clinical staging of 55 cases of carcinoma cervix

Clinical staging of cervical cancer	No. of patients (%)
Stage Ia	2 (3.6)
Stage Ib	2 (3.6)
Stage II	7 (12.7)
Stage III	32 (58.2)
Stage IV	12 (21.8)
Total	55 (100)

Because an association of macrophages with cancer cervix has been suggested, we have studied intensity of CD68 marker for macrophage in cervical tissue, in both stroma and epithelium from carcinoma cervix. In the present series of 55 patients, high intensity of CD68 marker was found in 4 cases (33%) of WDSCC, 10 (43%) of MDSCC and 7 (35%) of PDSCC; moderate intensity in 8 cases (67%) of WDSCC,

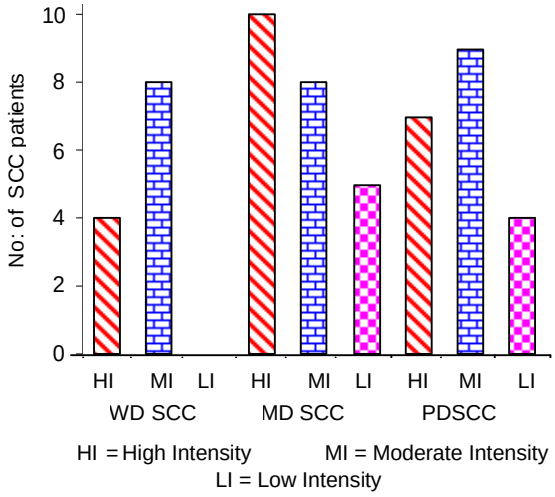


Fig.1. CD68 expression in 55 cases of different histological grades of squamous cell carcinoma cervix

8 cases (35%) of MDSCC and 9 cases (45%) of PDSCC; low intensity in 5 cases (22%) of MDSCC and 4 cases (20%) of PDSCC. There was no significant correlation between CD 68 expression and different histological grades of squamous cell carcinoma cervix (p=0.358) (Fig. 1).

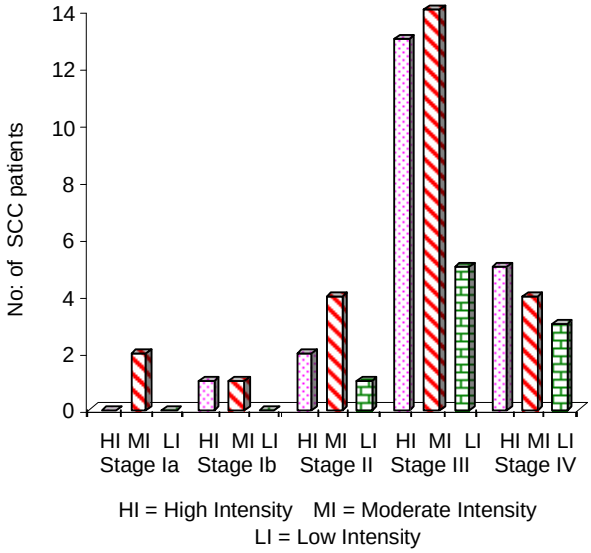
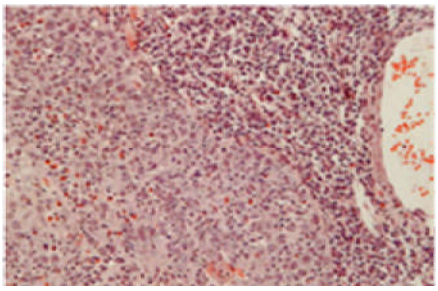


Fig. 2. CD68 expression in 55 cases of different clinical stages of squamous cell carcinoma cervix according to FIGO staging

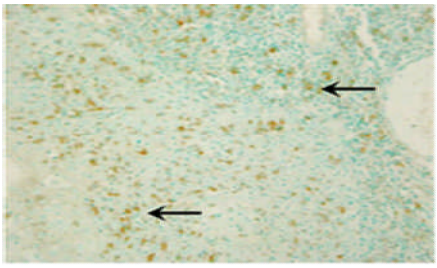
Intensity of expression of CD68 marker for macrophages was also studied in different clinical staging according to FIGO classification as shown in Fig. 2.

In relation to the clinical staging, the intensity of CD68 expression also showed no significance correlation with all clinical stages of cervical cancer (Stage I to Stage IV) (p=0.201).

(a)



(b)



Note: The presence of macrophages (black arrows)

Fig. 3 a & b. CD68 in squamous cell carcinoma with H&E staining (a) and IHC staining (b), (magnification × 200)

CD68 in squamous cell carcinoma with H & E staining and IHC staining are shown in Fig. 3 a & b. Intense inflammation and high count of macrophages were seen in the epithelium and in the stroma.

DISCUSSION

In this study, total 55 cases of carcinoma cervix were examined and all were found to be squamous cell carcinoma. So this study could not evaluate the expression of CD68, a marker for presence of macrophages in different histological types of carcinoma cervix.

Regarding the presence of macrophages in carcinoma cervix, CD68 expression was seen in 55/55 (100%) of all different histological grades of SCC and all clinical stages according to FIGO staging. The study revealed that there was a prominent macrophage component in the tumor-associated inflammatory infiltrate in squamous cell carcinomas of the cervix which was in agreement with other studies done for precancerous lesions of cervical cancer and cancer cervix.[10]

Furthermore, CD68 is a well established marker of macrophages and, in our study, its antibody clearly identified macrophages, with an intense cytoplasm staining. We also examined the staining intensity to evaluate the correlation between intensity of macrophage marker and different histological grades. However, it was found that presence of a prominent infiltrate of macrophages did not correlate with tumor grade. This finding is similar to that of a study in which there was no correlation between infiltrate of macrophages and tumour grade or with histologic lymph node status.[12]

In relation to the clinical staging, the intensity of CD68 expression also showed no significant correlation with all clinical stages of cervical cancer (Stage I to Stage IV). But high and moderate intensity was mainly found in advanced stages. The result was not consistent with a study in which there was a strong negative correlation with tumour stage.[11]

Conclusion

This study reflects that the mainstay of tumour diagnosis and grading rests on histological interpretation of haematoxylin and eosin stained cervical tissue. Although the role of immunohistochemistry in diagnosis and prognosis of several different tumours has been studied and widely used nowadays, we find that there was no association or correlation between the clinicopathological parameters especially grading and staging of the tumour and presence of macrophages which might help in predicting the prognosis of cervical cancer. The findings agreed with other studies done in squamous cell carcinoma of the cervix which highlighted that the beneficial role of macrophages in cellular immunity may be opposed by the

elaboration of growth factors in the vicinity of neoplastic cells.[12]

Because of the small sample size, it was not enough to evaluate macrophage intensity in the different histological types. Further studies with larger scale data are necessary to demonstrate whether this association is present or not. Staining intensity of expression of CD68 marker may also be needed to modify or other cell counting method used to get more definite assessment.

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Hepatitis B surface antigen subtypes in Yangon, Myanmar

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Blood samples were collected from 362 subjects attending the Liver Unit, Yangon General Hospital and the Hepatitis Carrier Clinic, Department of Medical Research (Lower Myanmar), Yangon during the period of 2000 to 2002. In all subjects, HBsAg positivity was confirmed by in-house ELISA test kit. Counter immunoelectrophoresis was carried out for determination of HBsAg titer. One hundred and three samples (103) showing strong HBsAg positivity by CIEP method (titer 1:10) were selected for subtyping by ELISA method. The most prevalent subtype among the study population was *adr* (93.2%), followed by *adw* (4.85%) and *ayw* (1.94%).

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem and it has been estimated by the World Health Organization that 2,000 million people (one third of the world's population) have been infected worldwide. Of these, more than 300 million are chronically infected carriers. Of the carriers, 25% are at risk of serious illness and eventual death from cirrhosis or hepatocellular carcinoma. (HCC).[1] In Myanmar, it has been estimated that 12% of the population carry HBV and 28/100,000 population could develop HCC.[2]

Hepatitis B virus is a member of the Hepadnavirus family and consists of virions which are 42nm in diameter and possesses an isometric nucleocapsid or core of 27nm in diameter, surrounded by an outer coat approximately 4nm thick. The protein of the virion core is termed as the surface antigen or hepatitis B surface antigen (HBsAg) which is sometimes extended as a tubular tail on one side of the virus particle.[3] Hepatitis B surface antigen (HBsAg) is a serologic marker on the surface of HBV. It can be detected in high levels in serum during acute or chronic hepatitis. HBsAg can be detected in an infected person's blood on the average of 4 weeks (ranged 1-9 weeks) after exposure to the virus.[3]

HBsAg contains a group-reactive *a* determinant shared by all strains of HBV and two pairs of mutually exclusive, subtype-specific determinants, *d* or *y* [4] and *w* or *r* [5] which are used for the identification of subtypes. Thus, there are at least four major groups into which HBsAg can be classified: *adw*, *adr*, *ayw* and *ayr*.[6,7] However, additional determinants designated the sub-determinants of *w* (*w1 to w4)* have allowed four serotypes of *ayw (ayw1, ayw2, ayw3, ayw4)* and two serotypes of *adw (adw2, adw4).* The *q* determinant was initially described as being present on all subtypes apart from *adw4.* Later, absence of *q* was demonstrated in some *adr* subtype containing sera. Thus, *adr strains* are described as *adrq+ or adrq-*.[8] The classification of HBsAg now includes nine main subtypes, namely, *ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+ and adrq-*.[9] The HBsAg subtypes show a distinct geographical distribution. The subtype *ayw* is predominant in a broad geographical zone

extending from West Asia, through Iran and Pakistan to India. Subtype *adw* is predominant in Europe, Australia and the Americas, while *adr* is prevalent in Southeast Asia and the Far East. Subtype *ayr* is extremely rare.[10]

HBV infection is endemic in Myanmar, and studies carried out among different population groups revealed HBsAg carrier rate of 10-12%.[11] In spite of the high percentage of HBsAg-positive individuals in Myanmar, no data exist on the hepatitis B subtypes prevalent in the country. The present study was carried out to determine the hepatitis B subtypes of HBsAg carriers from Yangon.

MATERIALS AND METHODS

Samples

Blood samples were collected from 362 subjects attending the Liver Unit, Yangon General Hospital and the Hepatitis Carrier Clinic, Department of Medical Research (Lower Myanmar), Yangon during the period of 2000 to 2002. In all subjects, HBsAg positivity was also done by in-house ELISA test kit. All sera samples were stored at -20°C until tested.

Counter immunoelectrophoresis (CIEP) method for determination of HBsAg titer

Counter immunoelectrophoresis was carried out for determination of HBsAg titer by the method of Kelkar, *et al*[12] with a slight modification. Agarose (Qbiogene, USA) gel 1.33% was prepared by adding 0.399g of agarose to 30 ml of Tris-Cl (Tris-Base, CalBiotech, Germany), which was heated in a microwave oven for 1 minute. It was cooled to 80℃. The agarose solution was poured onto a glass slide (3mm in thickness) and let to solidify. Five sets of two 3-mm-in-diameter wells at 5mm distance were punched in the gel. Twenty microliters of each of the 362 serum samples were added to the wells on the left side of the set. Twenty microliters of anti-HBs was dispensed to wells on the right side of the glass slide. The serum samples and the anti-HBs were subjected to electrophoresis placing the serum samples on the cathode side and anti-HBs on the anode side, in Tris-Cl buffer, 120mM, pH 8.6, for 1 hour at 120V. sera samples with the CIEP titer of 1:10 were selected for subtyping by ELISA method.

Subtyping of HBsAg by ELISA method

One hundred and three samples (103) showing strong HBsAg positivity by CIEP method were selected from 362 samples for subtyping. The subtype was determined by the method of Laperche, *et al.* with slight modifications. Three monoclonal antibodies against the immunodeterminants, *d*, *y* and *r*, anti-*d* and anti-*y* were provided by the Shin Heung College, Korea. Anti-*r* monoclonal antibody was purchased from the Institute of Immunology, Japan. Sandwich ELISA procedure with type-specific monoclonal antibodies was used for the subtyping. The microtiter plate (Nunc-Immuno-Plate, Apogent, Denmark) wells were coated with 100 *ng* per 100 µl of purified goat anti-HBs (gift from Green Cross Corp., Korea) in 100 mM bicarbonate coating buffer, pH 9.6. The plate was incubated at 4^{o}C overnight and washed 5 times with phosphate-buffered saline containing 0.5% Tween 20 (PBST). The plate was blocked with 10% skim milk (Difco, USA) for two hours. Each sample was diluted 500 times in PBS. One hundred microliters of sample were added to 6 wells (3 wells per sample and duplicated) and incubated at 37^{o}C for two hours. The plates were washed 5 times with PBST. One hundred microliters of each diluted immunodeterminant-specific monoclonal antibodies (400 µg/ml) were added into the each corresponding well and incubated for one hour at 37^{o}C. After washing 3 times with PBST, 100 µl of 5000 xdiluted horse-radish peroxidase-conjugated anti-mouse IgG (Sigma, USA) were added to the wells and incubated for one hour at 37°C. The plates were washed 3 times with PBST. One hundred microliters of chromogen-substrate solution containing

tetra-methyl-benzidine and hydrogen peroxide (Pierce, USA) was added to each well. The color development was stopped after 30-minute incubation at room temperature by adding 100 µl of 2N sulphuric acid. The absorbance was measured at 450nm wavelength by using the ELISA reader (Thermo-max, Molecular Devices, USA). Sample wells with an absorbance equal to or higher than 0.500 were considered as positive.

Interpretation of hepatitis B surface antigen subtypes

Samples reactive to anti-*d* and anti-*r* were interpreted as subtype *adr*. Those reactive to anti-*d* but not reactive to anti-*r* were determined as subtype *adw*. Samples reactive to anti-*y* and anti-*r* were determined as subtype *ayr*. Those reactive to anti-*y* and not reactive to anti-*r* were determined as subtype *ayw*.

RESULTS

The distribution of HBsAg subtypes are shown in Fig. 1. Out of 103 samples, 93.2% (96 of 103) were of *adr* subtype, while 4.85% (5 of 103) are subtype *adw*, and 1.94% (2 of 103) belongs to subtype *ayw*. In this study, subtype *ayr* was not detected.

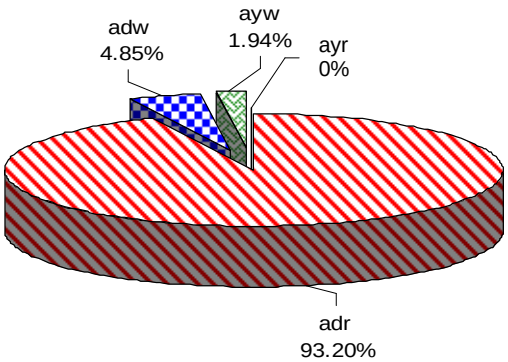

Fig. 1. Distribution of hepatitis B subtypes in Yangon

DISCUSSION

The genetic diversity of the world-wide circulating HBV strains has been extensively studied by different investigators. The genotypes and subtypes identification of a HBV strain can be useful in tracing the origin of infection or in epidemiological investigation of outbreaks of hepatitis. It is of importance to note that the viral genotype and subtype may play a role in the clinical outcome of interferon (INF-alpha) therapy.

Traditionally, subtyping of HBsAg has been performed by agarose gel immune-diffusion method. This technique, though convenient in establishing the identity, or the lack of identity, of the antigenic determinants, is not very sensitive and it has not always been possible to characterize the subtypic specificities in materials of known HBsAg positivity. Serological methods developed for subtype identification have been described by several investigators.

Some of them use polyclonal anti-sera which were able to distinguish major HBsAg subtypes. Anti-HBs monoclonal antibodies with restricted specificities thus represent a valuable tool for HBsAg sub-typing. This procedure can be easily used in large epidemiologic studies or hepatitis B transmission studies. The development of monoclonal antibodies with new anti-HBs specificities could further improve the HBV subtyping and could avoid the use of polymerase chain reaction.[12]

Different studies had shown that the human HBV strains can be segregated into eight genotypes A-H and nine subtypes of HBsAg known as *ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+ and adrq-*.[9, 13] Subtype *adw* predominates in northern Europe, America and Australia and is also found in Africa and Asia. Subtype *ayw* is found in the Mediterranean region, Eastern Europe, northern and western Africa, the near East and the Indian subcontinent. In the Far East, *adr* predominates. But the rarer *ayr* occasionally may be found in Japan and Papua New Guinea.[14] The indigenous strains of HBV from northern Europe and North America can be expected to be *adw*, strains from the Pacific, northern China and Korea to be *adr*, strains from Middle East and

south-east Europe to be *ayw* and from the southern Far East to be *adw* or *adr*.[15]

There was correlation between genotypes and HBsAg subtype and that HBsAg subtypes *adw* and *ayw* were prevalent in HBV genotype A, subtypes *adw* and *ayw* in genotype B, subtypes *adr*, *adw* and *ayr* in genotype C, subtype *ayw* in genotype D, subtype *ayw* in genotype E and subtype *adw* in genotype F.[16]

In this study, the most prevalent HBsAg subtype among the HBsAg-positive subjects in Yangon was *adr* (93.2%) and similar to the subtype distribution in Thailand where determination of HBsAg subtypes on 654 sera samples from Bangkok and other provinces in the central region showed that 86% was of *adr* and 12% of *adw* in Bangkok area.[17] In contrast, HBsAg subtyping on 78 sera samples in Surabaya, Indonesia elicited that 90% were of subtype *adw* and 10% of subtype *adr*.[18]

In another study, subtyping of 201 HBsAg carriers in Singapore had demonstrated that 65.7% were of subtype *adw*, 30.8% of subtype *adr* and 3.5% of subtype *ayr* and *ay*[19] while subtyping of 190 HBsAg positive sera from Kuala Lumpur and other areas of Malaysia, showed that 44% was of subtype *adr*, 29% of *adw* and 27% of *ayw*. The subtype distribution in Kuala Lumpur varied among the two main ethnic groups, the Malay and the Chinese. Subtype *adr* predominated in the Malays (61%), while the *adw* (51%) was detected predominately among the Chinese.[20]

Although the most prominent HBsAg subtype in Myanmar is *adr* and similar to the most common subtype in Thailand and among the Malay population in Malaysia, it is of interest that the subtype distribution is different from the subtypes prevalent in Singapore (*adw*), Indonesia (*adw*) and India (*ayw*).[21]

In the present study, although we could identify the most prevalent subtype in the study population as an initial step, HBsAg subtyping was carried out only on the hepatitis B surface antigen carriers residing in Yangon city and did not represent the whole country. Further studies are needed to elucidate the HBsAg subtype distribution in different areas of the country and among the various ethnic minorities.

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Antihyperglycemic activity and related chemical constituents of *Premna integrifolia* Linn. (*Taung-Tan-Gyi*)

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The aims of the study were to evaluate the antihyperglycemic activity of *Premna integrifolia* Linn. (*Taung-Tan-Gyi*) and to identify the chemical constituent(s) from active extracts. Using standard glibenclamide (4 mg/kg) as positive control, 70% EtOH extracts of various parts (leaves, stem barks and roots) (4 g/kg) were tested for antihyperglycemic activity on adrenaline-induced rat model. Blood glucose levels of rats at various time intervals were measured by glucometer. All extracts showed blood glucose lowering effect, with leaves extract possessing antihyperglycemic activity at 1 hr ($p<0.01$) while stem bark and root extracts at 2 hr ($p<0.005$) and 3 hr ($p<0.05$), respectively. The most active stem bark extract was further fractionated to chloroform-soluble and insoluble portions, and antihyperglycemic activity tested. Chloroform-insoluble fraction showed significant blood glucose lowering effect at the dose of 2 gm/kg (that is less than crude 70% EtOH extracts) at 2 hr ($p<0.05$) and 4 gm/kg dose (that is the same dose of crude 70% EtOH extracts) at 1 hr ($p<0.05$), 2 hr ($p<0.005$) and 3 hr ($p<0.05$), respectively. Column chromatographic separation of this active extract (chloroform-insoluble fraction of 70% EtOH extracts of stem bark) yielded and led to the isolation of a pure compound. The compound was chemically identified to be "aphelandrine" ($C_{28}H_{36}N_4O_4$) by means of UV, FT-IR, ^{13}CNMR, ^{1}HNMR, HMQC, HMBC, COSY and mass spectroscopy.

INTRODUCTION

During the past decade, traditional medicine has become a global interest. Current estimates suggested that, especially in developing countries a large proportion of the population relies mostly on traditional practitioners and medicinal plants to meet primary health care needs.[1] It is estimated that 80% of the world's population utilize traditional medicine for treatment of various diseases. Herbs are major remedies in traditional medicine, which is based on the use of roots, leaves, barks, seeds and flowers of plants.[2]

Diabetes mellitus is one of the six major priority diseases in Myanmar. Recently used drugs for diabetes mellitus include insulin and oral hypoglycemic drugs. These drugs have considerable side effects and toxicity. Even today, a large number of herbal drugs are being used for the treatment of diabetes mellitus in different regions of the world. Myanmar is rich in plants, many of which are known to have medicinal values. Since ancient times, Myanmar population has relied on household traditional remedies, which constituted mostly of herbal category.

Therefore, safe, scientific and systematic development of effective drugs is mandatory to ensure the safety use of herbal medicines. According to the literature search, *Premna integrifolia* Linn. is used as hypoglycemic, anticolic, antipyretic and cordial in India.[3]

In Myanmar, *Premna integrifolia* Linn. (*Taung-Tan-Gyi*) is widely available but has not been scientifically tested for hypoglycemic activity. Therefore, leaves, stem barks and roots of *Premna integrifolia* Linn. were evaluated to assess the antihyperglycemic activity and isolation of possible active phytoconstituents.

Objectives

- To evaluate the antihyperglycemic activity of crude extracts from various parts (leaves, stem barks and roots) of *Premna integrifolia* Linn.
- To isolate and identify secondary metabolite(s) from plant extracts with antihyperglycemic activity by modern spectroscopy.

MATERIALS AND METHODS

The plant samples were collected from Magway Division during summer season. Dry plant samples (leaves, stem barks and roots) were separately percolated in pet-ether (40-60ºC) for two weeks, and then filtrates were discarded. One hundred grams of defatted plant samples were extracted with 70% ethyl alcohol at 70°C by temperature control waterbath for about 5 hours. The filtrate was evaporated, remaining dried residue and stored in desiccators.

Fractionation of stem bark of Premna integrifolia Linn.

Stem bark of *Premna integrifolia* Linn. contains wax. Therefore, firstly, this stem bark was percolated with pet-ether (40-60°C) solvent for two weeks and then filtered. The filtrate containing wax was discarded and the residue plant sample was extracted with 70% EtOH by waterbath at 70°C. These 70% ethanolic extracts were fractionated into three parts by non-polar to polar series.

fraction-1= pet-ether-soluble fraction (non-polar compounds)
fraction-2= chloroform-soluble fraction (moderately polar compounds)
fraction-3= chloroform-insoluble fraction (polar compounds)

Pet-ether-soluble fraction (fraction 1) containing fat and wax was discarded. Pet-ether-insoluble portion was stirred with chloroform and pale yellow color solution was decanted into a conical flask and stirred until clear. Defatted chloroform-soluble fraction (fraction 2) was evaporated with air fan. The residue used for test sample was defatted chloroform-insoluble fraction (fraction 3). Chloroform-soluble (fraction 2) and chloroform-insoluble (fraction 3) fractions were characterized by UV, FT-IR spectrophotometer and phytoconstituents were investigated.[4]

Evaluation of antihyperglycemic activity of various parts of plant extracts

Antihyperglycemic activity of defatted 70% EtOH extracts of various parts of *Premna integrifolia* Linn. was tested on adrenaline-induced rat model and blood glucose levels were determined by gluco-meter. In order to determine the normal blood sugar level (50-75 mg/dL), both sexes of Wistar strain albino rats of body weight 180-230 gm, maintained with the standard laboratory diet were used.

The rats were fasted overnight (16-18 hrs) before the experiment, to ensure stable blood sugar level. Hypoglycemia was induced in albino rats by using sub-cutaneous injection of adrenaline tartrate B.P 0.8 mg/kg body weight, using the method of Gupta, *et al.*[5] Blood was drawn from the lateral tail vein of the rats and blood sugar levels were determined by glucometer (Elite XL,Bayer Corporation, USA) using glucocard test strip II. Food was withheld during the experiment for 18 hours.[6]

Seven fasted albino rats were administered orally of distilled water as control. After 45 minutes, hyperglycemia was induced by

using subcutaneous injection of adrenaline tartrate and blood sugar levels were determined at 0 hr and every hour up to 4 hr after injection of adrenaline tartrate. The rats were provided usual amount of food and water after the experiment. All the rats were allowed to rest for a week as the washout period. After the wash-out period, fasting blood glucose levels (0 hr) of all the rats were determined. The 70% ethanolic extracts of plant materials (leaves, stem barks and roots) 4 g/kg body weight or standard glibenclamide 4 mg/kg body weight was administered orally instead of distilled water. Adrenaline tartrate was injected subcutaneously as in control group. Blood glucose levels were taken at 1 hr, 2 hr, 3 hr and 4 hr after adrenaline injection. The mean blood glucose levels of control, test drug and standard glibenclamide-treated groups were then compared and analyzed by paired Student 't' test.

Evaluation of antihyperglycemic activity of crude fractions from stem bark

Antihyperglycemic activities of chloroform-soluble and chloroform-insoluble fractions of 70% EtOH extract of stem bark from *Premna integrifolia* Linn. were tested on adrenaline-induced rat model and blood glucose was determined by glucometer, with the same procedure of plant extract.

Isolation and identification of pure compound from active fraction

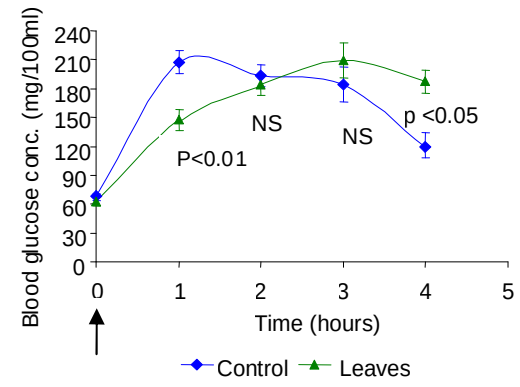
Isolation of secondary metabolites from chloroform-insoluble fraction of stem bark extracts was done by column chromatography using silica gel GF_{254} (70-230 mesh size). Chloroform-insoluble fraction (1 g) was chromatographed on silica gel (17 g) column (1.2 cmx47 cm), using chloroform and methanol mixture as eluent to yield 39 fractions, respectively. Every fraction was checked by thin layer chromatography (TLC) as the same solvent with different ratio. Pure compound was characterized by TLC with appropriate spray reagent[7, 8] and identified by UV, FT-IR, ^{1}HNMR, ^{13}CNMR, HMBC, HMQC, COSY, EI-mass spectrum.[9, 10]

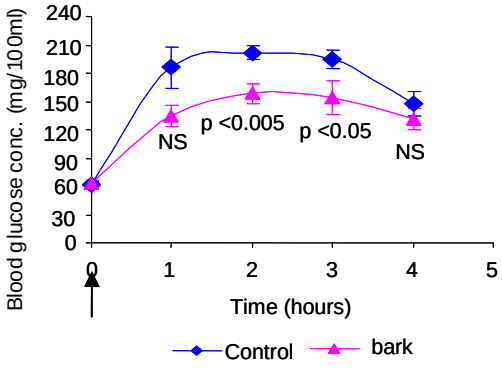
RESULTS AND DISCUSSION

Evaluation of antihyperglycemic activity of various parts of plant extracts

Defatted 70% EtOH extracts of leaves, stem bark and roots of *Premna integrifolia* Linn. 4 g/kg were tested for antihyperglycemic activity on adrenaline-induced rat model. Blood glucose levels of rats at various times were measured by glucometer. All extracts possessed blood glucose lowering effect, with leaves extract showing antihyperglycemic activity at 1 hr ($p<0.01$) while stem barks at 2 hr ($p<0.005$) and 3 hr ($p<0.05$) and root extract at 2 hr ($p<0.05$) and 3 hr ($p<0.005$), respectively.

Positive control using standard glibenclamide (4 mg/kg) was significantly inhibited the adrenaline-induced blood glucose level at 1 hr ($p<0.005$), 2 hr ($p<0.005$), 3 hr ($p<0.005$) and 4 hr ($p<0.05$), respectively, as shown in Fig.1.





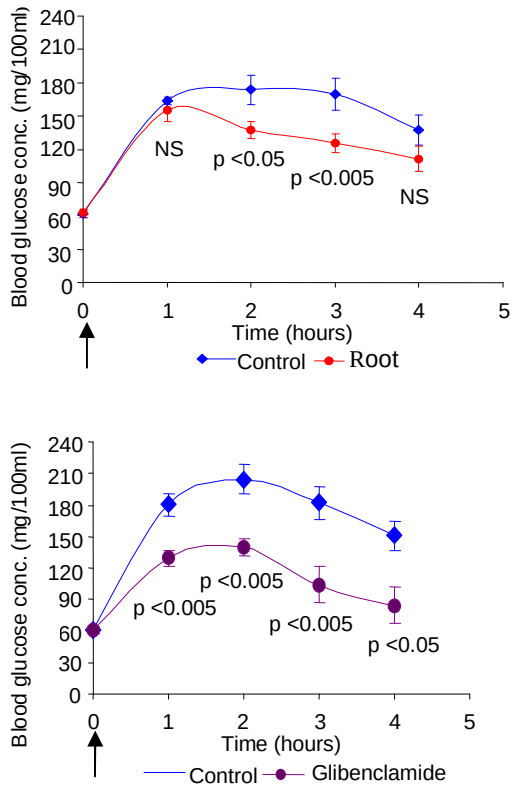


Fig. 1. Time course of the effect of various parts (leaves, stem bark and roots) of 70% EtOH extracts of *Premna integrifolia* Linn. on adrenaline-induced hyperglycemic rat model

The comparisons of percent inhibition of defatted 70% EtOH extract of leaves (4 g/kg), defatted 70% EtOH extract of stem barks (4 g/kg), defatted 70% EtOH extract of roots (4 g/kg), and standard glibenclamide (4 mg/kg) are shown in Table 1.

Table 1. Comparison of percent inhibition (Mean±SE) of antihyperglycemic effect of various parts of plant extracts and standard glibenclamide

Test samples	Percent inhibition of hypoglycemic effect			
	1 hr	2 hr	3 hr	4 hr
70% EtOH ext. of leaves (4 g/kg b.w)	38 ±10.1	29 ±12.4	28 ±15	138 ±53.3
70% EtOH ext. of stem barks (4 g/kg b.w)	41 ±13.6	31 ±5.8	30 ±9.8	21 ±12.2
70% EtOH ext. of roots (4 g/kg b.w)	8 ±8.5	33 ±10.7	41 ±7.3	2 ±4.7
Standard glibenclamide (4 mg/kg b.w)	42 ±5.8	45 ±5.7	61 ±8.9	74 ±12.7

In this study, significant antihyperglycemic effect was observed with various parts of plant extracts when tested on rat model. The stem bark and root extracts were more potent than leaves extract, and since stem bark is more easily available than roots, it was selected for isolation of secondary metabolite from active fraction with antihyperglycemic effect.

Characterization of fractions from stem bark

Phytochemical investigations of crude fractions from stem bark showed alkaloid, steroids/terpene in chloroform-soluble fraction (F2) and amino acid, alkaloid, flavornoid, polyphenol were found to be present in chloroform-insoluble fraction (F3). Characterization of 1% in EtOH solution of UV spectral data was 281 nm for chloroform-soluble fraction and 276 nm, 320 nm for chloroform-insoluble fraction. FT-IR spectral data were showed at 3487 cm^{-1}, 3353 cm^{-1}, 2947 cm^{-1},1643 cm^{-1}, 1442 cm^{-1}, 1350 cm^{-1}, 1257 cm^{-1}, 1172 cm^{-1}, 1006 cm^{-1} for chloroform-soluble fraction and 3549 cm^{-1}, 3524 cm^{-1}, 1632 cm^{-1}, 1350 cm^{-1} for chloroform-insoluble fraction.

Evaluation of antihyperglycemic activity of crude fractions from stem bark

The blood sugar lowering effect of chloroform-soluble fraction (fraction 2) was studied on adrenaline-induced hyperglycemic rat model. Chloroform-soluble fraction from defatted 70% ethanolic extract of stem bark (2 g/ kg) did not show significant antihyperglycemic activity on animal model when compared with that of control. Chloroform-insoluble fraction (fraction 3) showed significant antihyperglycemic activity at 2 g/kg dose (that is less than crude 70% EtOH extracts) at 2 hr ($p<0.05$) and 4 g/kg dose (that is same dose of crude 70% EtOH extracts) at 1 hr ($p<0.05$), 2 hr ($p<0.005$) and 3 hr ($p<0.05$), respectively (Fig. 2).

The comparison of % inhibition of chloroform-insoluble fraction (2 g/kg), chloroform-insoluble fraction (4 g/kg) and standard

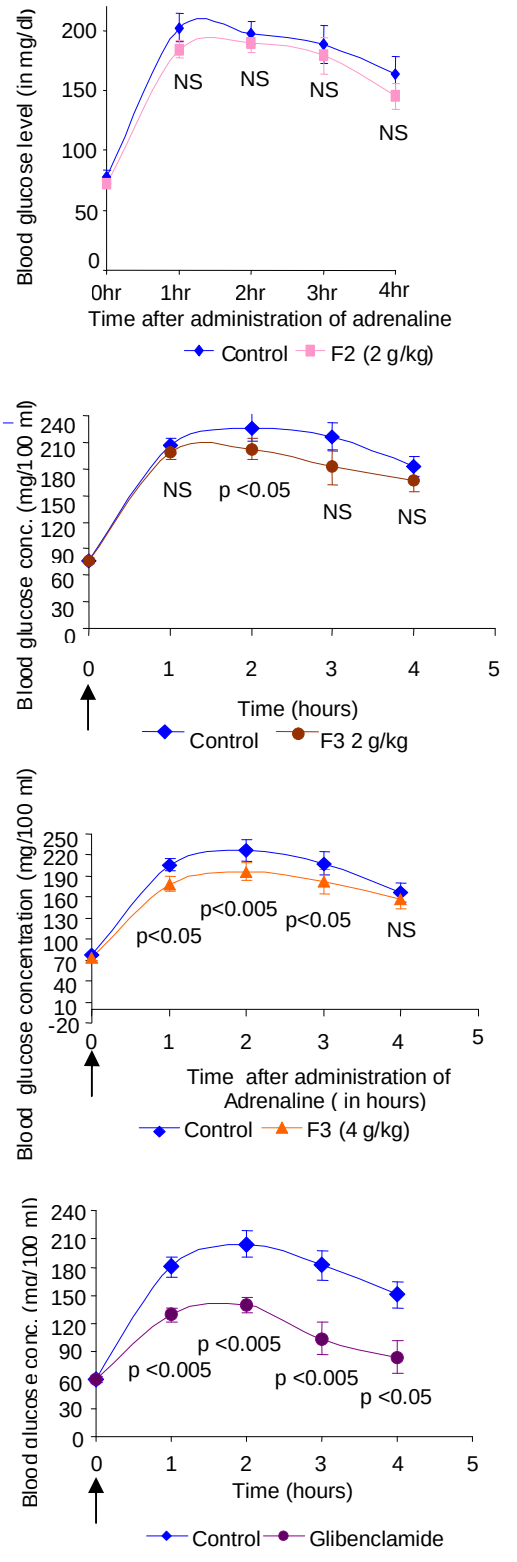


Fig. 2. Time course of the effect of defatted chloroform-soluble fraction and chloroform-insoluble fraction from 70% EtOH extract of stem bark of *Premna integrifolia* Linn. on adrenaline-induced hyperglycemic rat model

glibenclamide (4 mg/kg) is shown in Table 2. Since significant blood sugar lowering effect was observed with chloroform-insoluble fraction when tested on rat model, this fraction was selected for isolation of pure compound.

Table 2. Comparison of percent inhibition (Mean±SE) of blood sugar lowering effect of chloroform-insoluble fraction and standard glibenclamide

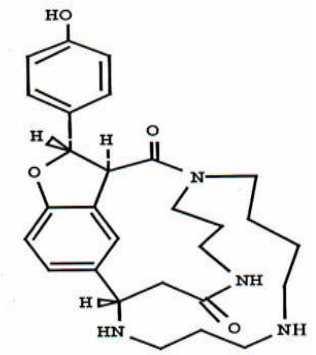
Test samples	Percent inhibition of antihyperglycemic effect			
	1 hr	2 hr	3 hr	4 hr
Defatted $CHCl_3$ -insoluble fraction of stem bark (2 g/kg)	3.5 ±2.7	10.2 ±3.3	15.7 ±7.8	8.9 ±4.3
Defatted $CHCl_3$ -insoluble fraction of stem bark (4 g/kg)	10.2 ±6.8	14.3 ±5.3	25.8 ±8.6	23.2 ±13.1
Standard glibenclamide (4 mg/kg)	42 ±5.8	45 ±5.7	61 ±8.9	74 ±12.7

Identification of pure compound from chloroform-insoluble fraction

Isolated pure compound 0.58 mg (0.005%) from chloroform-insoluble fraction of defatted 70% EtOH extract of stem bark was characterized by TLC using chloroform and methanol solvent system (R_f value=0.62). It showed orange color with Dragendorff reagent spray, violet-red color with ninhydrinbutanol-acetic acid reagent spray, black color with 10% ferric chloride reagent spray, and dark spot under UV-254 nm. UV spectrum showed 228 nm & 283 nm and it was identified by FT-IR spectrum, 3593.0 cm^{-1}, 3487.0 cm^{-1} for hydroxyl group (-OH stretching), 1739.8 cm^{-1} for (-CONH), 1657.3 cm^{-1} for (>N-CO) groups were present in its compound.

With TLC, UV spectrum, and FT-IR data in agreement with literature[9], the isolated compound may be assumed to be aphelandrine (alkaloid). According to ^{1}HNMR and ^{13}CNMR spectrums, isolated alkaloid compound contains thirty-two protons and twenty-eight carbons. EI-mass spectrum showed molecular mass of this isolated compound was 493 m/z. Moreover, it was confirmed by COSY, HMBC, HMQC

spectrums.[10] Thus, the isolated alkaloid compound was confirmed as aphelandrine ($C_{28}H_{36}N_4O_4$ (Fig. 3).



Aphelandrine ($C_{28}H_{36}N_4O_4$)

(Two macrocyclic spermine alkaloid)

Fig. 3. Chemical structure of aphelandrine compound

Conclusion

It was concluded that various parts (leaves, stem barks and roots) of *Premna integrifolia* Linn. (*Taung-Tan-Gyi*) possessed anti-hyperglycemic activity on rat model. The stem barks and roots extracts were more potent than leaves extracts. Chloroform-insoluble portion was more potent than chloroform-soluble portion of 70% EtOH extract from stem bark of *Premna integrifolia* Linn. regarding antihyper-glycemic activity on rat model.

ACKNOWLEDGEMENT

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Study on antihypertensive effect of Myanmar Traditional Medicine Formulation - Number 27

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Myanmar Traditional Medicine Formulation - Number 27 (*Pyilonechanthar Hsay*) has been used in hypertensive patients for many years at traditional clinics and hospitals. Objective of the study was to determine the antihypertensive effect of this medicine. A clinical trial was carried out on 30 mild to moderate hypertensive patients, Buddhist monks, attending the out-patient clinic of Sasana University, Mandalay, in September and October 2008. After washout period of 1 week, patients were treated orally with *Pyilonechanthar Hsay* crude powder tablet 2 grams three times a day daily for 4 weeks. Blood pressure was monitored daily by the observers. Self-measurement of blood pressure was performed in the morning and evening on last three days of both washout period and drug administration period. Laboratory investigations such as blood tests and electro-cardiography were done before and after the study. The results showed that reduction of blood pressure from baseline level was found during the drug administration period and maintained up to the last day. Significant reduction of mean blood pressure from 142/96 mmHg (baseline blood pressure) to 128/88 mmHg was observed. This drug decreased the systolic and diastolic blood pressures from baseline level by 14 mmHg (p=0.0000) and 8 mmHg (p=0.0002), respectively. Evening blood pressures of last three days of drug administration period were less than morning blood pressures of those days. No side effects were found except slight nausea and dizziness. Therefore, Myanmar Traditional Medicine Formulation - Number 27 showed antihypertensive effect on mild to moderate hypertensive patients with minimum side effects.

INTRODUCTION

Hypertension affects approximately one billion individual worldwide. Hypertension is the most common cardiovascular disorder affecting 20% of adult population world-wide. It is also an important public health problem of global dimensions, both in the developed and developing world. Many kinds of antihypertensive drugs are used by the hypertensive patients. Majorities are western drugs. Moreover, Myanmar traditional medicine and raw herbal plants are also used in Myanmar. Some patients do not take any western medicine because of high cost, intolerability and inaccessibility by community. Some people take self-care with diet restriction. Some asymptomatic cases do not take any medicine.[1]

Myanmar traditional medicines are easily available in locality with low cost. Ministry of Health encourages the utilization of Myanmar traditional antihypertensive drugs among the people. Therefore, doing the research studies concerning the efficacy and safety of those traditional antihypertensive drugs will be helpful for dissemination of safe, effective, locally prepared, cheap and easily available drugs in the public.

Varieties of Myanmar traditional antihypertensive drugs are used in traditional therapy. Among them, Myanmar Traditional Medicine Formulation - Number 27 (TMF 27), also called as "*Pyilonechanthar Hsay*" is the popular drug which has been used in the traditional clinics and hospitals for many years. *Pyilonechanthar Hsay* is a combination of 16 medicinal plants which are easily available in locality (Table 1).

Table 1. Ingredients of TMF 27

Scientific name	Myanmar name	g/100 g
Major ingredient		
Tinospora spp (stem)	Hsin donmanwei	33.3
Other ingredients		
Carallia brachiata (bark)	Maniawga	4.4
Cassia renigera (root)	Phwahbetkyee	4.4
Clerodendron phlomoides (stem)	Tapasay	4.4
Croton oblongifolius (root)	Thetyingyee	4.4
Dragea volubilis (root)	Gwaydauk	4.4
Holarrhena antidysenterica (bark)	Lethtokkyee	4.4
Jatropha multifida (stem)	Saymakhan	4.4
Oroxylun indicum (bark)	Kyaungshar	4.4
Plumbago rosea (stem)	Kantgyokni	4.4
Strychnos nux-vomica (root)	Khabounggye	4.4
Strychnos potatorum (stem)	Khaboungyaykyi	4.4
Unidentified (stem)	Thinwinpaukphyu	4.4
Unidentified (bark)	Katma	4.4
Unidentified (foot)	Hsaypale	4.4
Unidentified (stem)	Ngabyayyin	4.4

TMF 27 can be used in neurological disorders such as hemiparesis, epilepsy, hemiplegia, paraplegia, neuralgia, and parkinsonism and muscle weakness. It is also indicated for gastrointestinal disorders, for example, abdominal indigestion, constipation and haemorrhoids. Cardiovascular disorders such as oedema with sweating, improper distribution of heat and dyspnoea, are treated with the use of TMF 27 and other traditional medicines. It is recommended to use with the dose of 2 orally three times per day for adult.

According to the toxicological study done at the Department of Medical Research (Lower Myanmar), the median lethal dose (LD_{50}) on mice, rats and rabbits were greater than 4.8, 2.4 and 1.2 of kg, respectively.[2]

Main ingredient of the TMF 27 is *Tinospora cordifolia* species which is called in Myanmar as *Hsin donmanwei.* It is bitter, stomachic, antiperiodic and aphrodisiac. It is used as antipyretic, antiinflammatory, hypoglycemic, antihypertensive, antirheumatic, antiallergic, analgesic and hepatoprotective. Watery extract of *Hsin donmanwei* can decrease the arterial pressure and heart rate but increase ventricular contractibility.[3]

This study was aimed to demonstrate the efficacy and side effects of Myanmar Traditional Medicine Formulation - Number 27 (*Pyilonechanthar Hsay*) in 30 mild to moderate hypertensive patients.

MATERIALS AND METHODS

This study was a phase 1, clinical trial. It was conducted for 5 weeks covering the period from 2nd September to 9th October, 2008. The trial was done at the out-patient clinic of Sasana University which has close-relationship with Ziwitadana Specialist Hospital. A total of 30 participants, Buddhist monks, attending at the out-patient clinic of Sasana University, were enrolled into the study. The subjects were enrolled according to the following inclusion criteria.

- The patients of 30-59 years old,
- Patients with stage I and stage II hypertension according to JNC 7 criteria.[4] Stage I (mild): 140/90 to 159/99 millimeter mercury (mmHg), Stage II (moderate): 160/100 to 179/109 mmHg,
- The patients who were willing to participate.

The subjects were not enrolled according to the following exclusion criteria.

- The patients who were taking any antihypertensive drug within 14 days,
- The patients with other diseases such as diabetes, ischaemic heart disease and renal failure,

- The patients with severe hypertension (180/110 mmHg and above),
- The patients with secondary hypertension,
- The patients who had hypertension with end-organ damage e.g. heart failure, ischaemia and renal failure,
- The patients with white coat hypertension.

Screening of patients

All Buddhist monks living in the Sasana University were measured their blood pressure in three consecutive visits in three days. A total of 461 Buddhist monks were examined. During those days, the normal subjects and subjects with white coat hypertensions, that is hypertension occurred only in first few days due to psychological stress condition, were excluded.

The monks were measured the blood pressure at sitting position. The arm cuff was applied at the left arm and placed at the same level with the heart. Two blood pressure measuring apparatus were used. Those are international gold standard sphygmomanometer and self-measurement device, arm type digital blood pressure monitor (DBPM). The monks who had high blood pressure at both visits were enrolled into the study according to inclusion criteria. A total of 30 monks were enrolled into the study.

The investigations such as blood test for creatinine, blood test for sugar, electrocardiography (ECG), body weight measurement and height measurement were done to assess presence of complications of the hypertension, renal impairment, diabetes mellitus and ischaemic heart disease among the participants. The blood test for creatinine was done before and after the study period to assess the side effect of trial drug.

Methods of blood pressure measurement

In this study, two ways of blood pressure measurements were conducted. Those were self blood pressure measurement at home, i.e. the participants' private room and blood pressure measurements by observers. Blood pressure measuring apparatus used in the study were;

1. International gold standard sphygmomanometer - it is used for validation test for DBPM, and
2. Self-measurement device - arm type DBPM

Validation test for the digital blood pressure monitor

Blood pressure measuring apparatus used in the study were validated with international gold standard brand-new mercury sphygmomanometer. Systolic pressure must be recorded at the appearance of first Korotkoff sound and diastolic pressure must be recorded at the disappearance of fifth Korotkoff sound. Arm type DBPM were compared with international gold standard mercury sphygmomanometer by measuring in 10 numbers of people. Three measurements each with DBPM and mercury meter were done at both arms alternately with one minute interval in each individual. The measurement variations between DBPM and gold standard mercury sphygmomanometer, of less than 5 mmHg in more than 50% of paired readings are regarded as passed validation. The validated DBPM were used for the whole study period.

Washout period

A total of 30 hypertensive monks were observed for 7 days washout time without giving any antihypertensive drug. During the washout time, the baseline blood pressure was obtained by measuring the blood pressure every morning by observers. During the washout time the monks and their roommates were trained about the details of self-measurement instructions for at least half an hour. Then, self-measurement of blood pressure was done on last three days of washout period.[5]

Drug administration period

After the washout time, the patients had to take antihypertensive drug orally for

4 weeks. The observers directly gave the drugs to the monks with the dose of seven tablets (300 milligrams of *Pyilonechanthar Hsay* in one tablet) orally for three times a day, i.e. 6:00, 14:00 and 22:00 hour. The observers had to take daily morning blood pressure before meal. Self-measurement of blood pressure was also done again on last three days of drug administration period.[5]

Self Blood Pressure Measurement (SBPM)

SBPM was done by the monks themselves according to instructions and training. It was done at last three days of washout period and another last three day drug period as mentioned above. SBPM had to be performed after a period of 5-minute rest in sitting position. Measuring within 30 minutes after bath was avoided. It had to be done after micturation if bladder is full. It was done at left arm uniformly. The patients were instructed to keep arm at heart level and to keep arm steady during measurement. Device cuff might be at heart level on the arm. SBPM were performed with validated fully automated devices (Tensoval home blood pressure monitor, Hartmann Company, Germany).

It is brachial artery occluding device (arm type), memory equipped and can store the readings serially. The monks were trained how to inflate and deflate the cuff, how to store the readings into the memory of the device and explained about failed measurements and what the monitor will do.

SBPM were done in their private rooms for two times in a day, one time in the morning within 1 hour waking before taking drug and another in the evening within 16:00 hour to 18:00 hour. There were three measurements in each time. Each measurement was done at least one minute interval.[6-9]

Blood pressure measurements by observers

Blood pressures were also measured by trained observers with DBPM at every morning (9:00 to 10:00 hour) during wash-out period and drug period. It was measured on left arm at sitting position, after the 5-minute rest before administering the antihypertensive drug. Three blood pressure measurements were obtained with more than one minute apart at each clinical visit by trained and observers. In addition, monks were advised to avoid cigarette smoking, coffee/tea and exercise for at least 30 minutes before their BP measurement.

Ethical considerations and emergency response team

Ethical approval for conducting this study, which involved humans as study subjects, was obtained from Institutional Ethical Review Committee, Department of Medical Research (Upper Myanmar). Before study, informed consents were taken from all participants. Informed consents were written in Myanmar language.

Emergency response team was formed to respond the emergencies encountered among the participants during the study period.

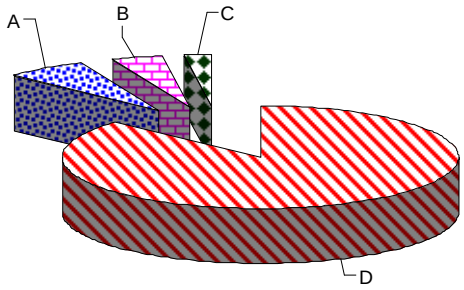
Data Management and analysis

Data entry was done using preformed 'SPSS Based Data Entry Form for Myanmar Traditional Anti-hypertensive Drugs' developed by Medical Statistics Division of Department of Medical Research (Upper Myanmar). Analysis was primarily done by using R 2.7.1 software. Daily mean systolic blood pressure, daily mean diastolic blood pressure and mean blood pressure reduction were analyzed.

RESULTS

A total of 461 monks were examined their blood pressures systematically. Out of 461 monks, 61 monks were investigated as mild to moderate hypertension. Eleven (2.4%) were screened out as white coat hypertension (WCH). Another 20 monks (4.3%) with hypertension were associated with other complications and noneligible criteria conditions. Therefore, 11 monks with WCH and 20 with complications were excluded.

As a result, a total of 30 monks (6.5%) with mild to moderate hypertensions were included into the study according to selection criteria (Fig.1).



A = Hypertension with complication 4.2%
B = Hypertension under study 6.5%
C = WCH 2.3%
D = Normal 87%

Fig. 1. Hypertension among the monks

General characteristics of the studied monks

General characteristics of the monks are different from inhabitant community people. They are single, male, religious, holy and spiritual individuals. All of them have their daily meals with same curries at the same time. There are two meals, breakfast and lunch, for the monks. Breakfast is taken at early morning (6:00 hour) and lunch is taken at 10:30 hour. In the evening, they have only light food such as juice, coffee and cake etc. Out of 30 participants, five monks (i.e. 16.67%) like salty diet. In contrast, only one prefers low salt diet.

Physical activities

Monks of the Sasana University have habits of taking regular exercise by walking for half an hour per day. They always go to the teaching hall by walking for hundreds of meter. Therefore, they have regular daily physical activities.

Psychological stress

Being devoted in the religious works, the Buddhist monks are almost free from psychological stress. They also take regular meditation everyday. Therefore, physical and psychological stresses were not affecting the existing hypertension of the monks in this study.

Mean of age, weight, height and body mass index

A total of 30 monks were participated in the study. All of them were Buddhist monks. Therefore they were male. Twenty-six monks (i.e. 86.67%) were Myanmar, three monks (i.e. 10%) were Shan and only one was Rakhine. Majority of the participants were students (i.e. 90%) and 10% were lecturers of Sasana University, Mandalay. The age of participants ranged from 30 to 53 years. Mean age was 36 years. The weight of participants ranged from 45 to 90 in kilograms, and mean body weight was 63 in kilograms. The height of participants ranged from 1.5 to 1.78 in meters, and mean height was 1.63 meter. Mean body mass index was 24. Body mass index of participants ranged from 15.7 to 30.92. BMI value of more than and equal to 30 indicates the higher body weight, i.e. obesity for height. Out of 30 participants, 10% were obese according to BMI value.

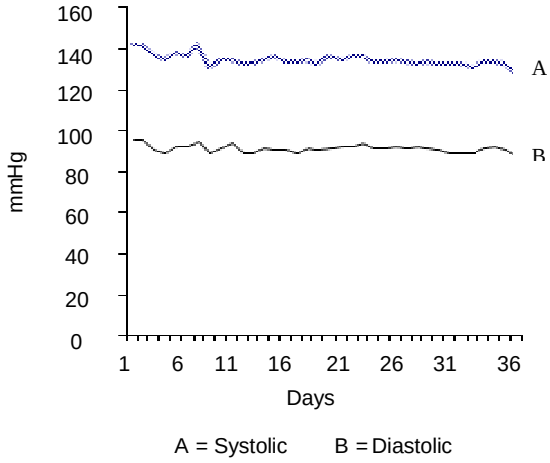


Fig. 2. Daily systolic and diastolic pressure monitoring

Daily blood pressure monitoring

In this study, 90% of the participants with high blood pressure touched to the normal blood pressure level and only 10% failed to touch the normal level during the study period. Fig. 2 shows the daily mean blood pressure monitoring of studied monks. Daily blood pressure was measured for 35 days by observers.

Mean blood pressure reduction

Before the study, mean blood pressure was 142.46/96.43 mmHg. TMF 27 was administered orally to participants on day 7. Mean reduction in sitting blood pressure levels at day 8, 14, 21, 28 and the end of the study were -7/-4, -8/-5, -6/-2, -9/-5 and -14/8, respectively. It is shown in Table 2. On day 35 of the study, mean blood pressure was 128.07/88 mmHg. Significant blood pressure reductions were observed. The systolic and diastolic blood pressure were decreased from baseline level by 14 mmHg (p=0.0000) and 8 mmHg (p=0.0002), respectively.

Table 2. Mean blood pressure level of patients before and after the trial

Days	Mean systolic blood pressure±SD (mmHg)	Blood pressure reduction & p-value	Mean diastolic blood pressure±SD (mmHg)	Blood pressure reduction & p-value
Before trial (Baseline blood pressure)				
Day 0	142±10.01		96±7.37	
After taking trial drug				
Day 8	135±13.04	-7 P=0.01*	92±13.4	-4 P=0.01*
Day 14	134±9.17	-8 P=0.001**	91±7.68	-5 P=0.006**
Day 21	136±10.29	-6 P=0.02*	94±8.91	-2 P= 0.01*
Day 28	133±7.89	-9 P=0.0001***	91±7.56	-5 P=0.006**
Day 35	128±9.73	-14 P=0.0000***	88±7.96	-8 P=0.0002***

Morning and evening blood pressure of washout period and drug period

According to self-measurement of blood pressure done by the monks, morning and evening blood pressures of both washout and drug administration period could be compared. Mean blood pressures in the mornings and evenings of day 4, 5 and 6, i.e. last three days of washout period, were 138/93 mmHg and 136/95 mmHg, respectively. However, mean blood pressures in the morning and evening of day 33, 34 and 35, i.e. last three days of drug administration period, were 133/91 mmHg and 128/88 mmHg, respectively. Therefore, evening blood pressures in drug administration period were less than morning blood pressures of those days. It indicates that the blood pressure become decreased in the evening because of the effect of drug taken orally in the morning.

Side effects

After the oral administration of the TMF 27 with the dose of 2 grams three times daily, only two numbers of monks suffered slight nausea and one monk experienced slight dizziness. Other organic and physiological side effects were not encountered according to the results of blood tests and ECG monitoring. The results of blood tests for sugar and creatinine show normal levels after the study period. Most of the participants said that they felt muscular and nervous relaxation after taking drug. Therefore, the trial drug produced only minimum side effects in 10% of the studied Buddhist monks.

DISCUSSION

It was the first clinical trial done in Buddhist monks of Sasana University (Mandalay) and it was conducted by the Department of Medical Research (Upper Myanmar) in collaboration with the University of Traditional Medicine and Myanmar Traditional Medicine Association, Mandalay. According to survey in certain areas in Myanmar, the prevalence of hypertension differed in different parts of the country. It was 22.8% in Gangaw, 13.7% in Inlay Lake area, 2.1% in Lay Daung Gan and 7.5% in Mandalay.[1] In this study, 10.8% of the monks were screened out as mild to moderate hypertension (not including WCH).

A study concerning traditional medicinal plant *(Ahkyawpaung-tahtaung)* on mild to moderate hypertensive patients done in Yangon showed that mean systolic and diastolic blood pressure reduction by 16 mmHg and 9 mmHg from baseline levels were seen at 4th week of trial.[10] On the last day of this study, mean blood pressure of the monks was 128.07/88 mmHg. Significant blood pressure reductions were observed. This trial drug decreased the

systolic and diastolic blood pressures from baseline level by 14 mmHg (p=0.0000) and 8 mmHg (p=0.0002), respectively.

At the end of the study, blood pressure of 90% of the studied monks touched to the normal blood pressure level. Moreover, Myanmar Traditional Medicine Formulation - Number 27 produced only minimum side effects in 10% of the studied Buddhist monks. In conclusion, Myanmar Traditional Medicine Formulation - Number 27 *(Pyilonechanthar Hsay)*, combination drug of sixteen herbal remedies, is found to be effective in treatment of patients with mild to moderate hypertension.

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Adherence to the recommended regimen of artemether-lumefantrine for treatment of uncomplicated falciparum malaria in Myanmar

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This study aimed to assess the adherence of six-dose regimen of artemether-lumefantrine (AL), the recommended artemisinin-based combination therapy in uncomplicated falciparum malaria patients in the community. Cross-sectional comparative study was conducted on 248 uncomplicated falciparum malaria patients at four townships in Mandalay Region. Thirty-three percent of the patients (95% CI=27.5-39.1) took the medicine with fatty food. Ten patients (4%, 95% CI=2.2-7.3) were classified as definitely non-adherent. Patients in the probably non-adherent category were 16 (6.5%, 95% CI=4-10.2). Reasons for non-adherence were 18 (69.2%, 95% CI=50-83.5) thought of being cured, 3 (11.5%, 95% CI=4-29) expected rapid cure, 3 (11.5%, 95% CI=4-29) feared of side effects and 2 (7.7%, 95% CI=2.1-24.1) shared the drug with another patient. Two hundred and twenty-two patients (89.5%, 95% CI=85.1-92.7) were probably adherent to the recommended regimen. The youngest age group had significantly higher adherence than the eldest age group had (p=0.02), but no significant differences were seen among other age groups. The background characteristics of patients or caregivers were not associated with probably adherence to recommended regimen of AL. The high level of adherence to AL found in our study is likely to be the result of the effectiveness of the health care programmes and the awareness of malaria among the community members. Miss perception toward the regimen was still present, nevertheless.

INTRODUCTION

Malaria is one of the priority diseases in Myanmar. About 70 percent of the populations are residing in malaria endemic areas. Malaria control programme aims to reduce malaria morbidity and mortality through vector control, quality diagnosis and early treatment with appropriate anti-malarials. Artemether-lumefantrine (AL) is the recommended artemisinin-based combination therapy (ACT) for treatment of uncomplicated falciparum malaria in Myanmar. In 2008, 358, 122 cases were treated with ACT. It is a six-dose regimen and each dose is best taken with fatty food. It is a very effective antimalarial.[1-3] After introduction of it into antimalarial treatment policy in 2002, malaria mortality declined dramatically. However, for long-term use of this ACT, adherence of patients has to be considered. Poor levels of compliance may decrease the cure rates and may precipitate the development of the drug resistance.[4]

In current practice, the *Plasmodium falciparum* positive patients are instructed to take the first dose of AL under observation of the practitioner at the clinic and they are supplied with the rest tablets and explained how and when to take at home. It is assumed that the drug is taken as prescribed. Levels of adherence and possible risk factors for non-adherence to this ACT are needed to know for the long-term successful

implementation of the malaria control programme.

There has been growing interest to study in drug effectiveness and patient adherence, particularly more on the former and recently increasing in the latter.[5] Some studies measure patient adherence by drug assays as a sole measure of adherence.[6, 7] Many studies assess adherence by self- or caregiver report and pill counts or container inspection.[8-15] One study measured the adherence by comparing the effectiveness of drugs taken with and without supervision .[16]

This study aimed to assess the adherence of recommended six-dose regimen of artemether-lumefantrine in uncomplicated falciparum malaria patients in the community.

MATERIALS AND METHODS

Operational definitions

Variables were operationally defined as follows: Definitely non-adherence: Patients who have remaining tablets; Probably non-adherence: Patients who have no remaining tablets and who do not report taking all doses at the correct time on the proposed day and in the correct amount; Probably adherence: Patients who show the blister pack without remaining tablets or the pack is missing and who report taking all doses at the correct time on the proposed day and in the correct amount.

Study design, participants and sample size

Cross-sectional comparative study was conducted on uncomplicated falciparum malaria patients who took a six-dose regimen of AL. Patients were selected according to the following criteria: history of fever within the last 48 hours and/or an axillary temperature >37.5℃, a blood smear or rapid diagnosis test positive for *P. falciparum* with no signs of complicated malaria or other severe disease and no household member previously participating in the study. According to the differences in number of tablets in each dosage, patients were selected in four age groups: less than 5 years old, 5-<9 years, 9-<14 years, and older than 14 years of age. Assuming an adherence of 80%, a precision of 10% and a type 1 error of 5%, 62 patients were selected in each age group.

Study area, data collection procedures, instruments used, quality control, confidentiality

The study was conducted at PyinOoLwin, Madaya, Singu and Thabeikkyin townships in Mandalay Region, one of the malaria endemic areas in Myanmar. Malaria control programme of Ministry of Health and two international NGOs, CESVI and Population Service International (PSI), cover the area for malaria prevention and control in those townships.

Patient recruitment was carried out at the out-patient malaria clinics. All uncomplicated falciparum malaria patients who met the inclusion criteria were asked to participate in the study. They were given standardized treatment regimen and instructions as the routine procedures in malaria clinics. They received the weight-specific artemether-lumefantrine (Coartem ®) blister packs (10-14.9 kg: 1 tablet per dose; 15-24.9 kg: 2 tablets; 25-34.9 kg: 3 tablets; ≥35 kg: 4 tablets; Novartis Pharma AG, Basle, Switzerland). The packs contain pictures that explained the regimen schedule.

Health care providers also gave written instructions on the pack by local language. To be practical in the community, the dosage schedule was related to age of the patient (less than 5 years old: 1 tablet per dose; 5-<9 years old: 2 tablets; 9-<14 years old: 3 tables; ≥14 years old: 4 tablets). Timing of dosing was as follows: the first dose in front of the provider after the confirmation of uncomplicated falciparum malaria; the second dose on the first day at any time between 8 and 12 hours after the first dose; twice a day (morning and evening) on the second and third days.

Field survey teams visited the patients after the last dose should have been completed. A pre-tested questionnaire was used to assess adherence. Respondents were patients themselves if ≥14 years old or their caregivers if younger. There were basic socio-demographic information, open and structured questions in the questionnaire. They were requested to show the remaining tables and the blister packs, if available. Confidentiality was maintained in the collection, management and analysis of data.

Statistical analysis

Data were entered and analyzed by using SPSS 11.5 for window version. Three categories of adherence were analyzed as proportions and compared among age groups using a chi-square test. The association between adherence and independent variables (age, educational levels of the respondent, history of anti-malarial intake, presence/absence of fever on presentation were first analyzed in a univariate model using a chi-square test.

Ethical considerations

Verbally informed consent was obtained from adult patients and from parents or caregivers if the patient was less than 18 years old. This study was approved by the Institutional Ethical Review Committee of Department of Medical Research (Upper Myanmar).

RESULTS

The study was conducted at the highest malaria risk area in Mandalay Region. Study populations were forest-related workers such as gold miners, wood and bamboo cutters, and fishermen at the dam sides and farmers who work at the fields at the foothill areas.

According to the dosages taken by the patients, there were four groups: less than 5 years old, 5-<9 years old, 9-<14 years, and older than 14 years of age. Age distribution is shown in the Table 1. Most of the patients or caregivers were forest-related workers (44%) and farmers being second most (30.2%). Sixty-nine of the patients or caregivers had primary level of formal education (Table 1).

Table 1. Age, occupation and education distributions of patients/caregivers in each age group

Characteristics	Age group (years) (n=62 each, total=248) (%)				
	<5	≥5 to<9	≥9 to<14	≥14	Total
Age (year) (Mean±SE)	3.1 ±0.15	6.9 ±0.14	12 ±0.18	27.6 ±1.5	12.4 ±0.71
Occupation of patient/caregiver					
Forest-related worker	27 (43.5)	25 (40.3)	19 (30.6)	38 (61.3)	109 (44)
Farmer	15 (24.2)	20 (32.3)	26 (41.9)	14 (22.6)	75 (30.2)
Employee	1 (1.6)	1 (1.6)	4 (6.5)	0 (0)	6 (2.4)
Dependent	5 (8.1)	5 (8.1)	3 (4.8)	2 (3.2)	15 (6)
Retailer	3 (4.8)	5 (8.1)	8 (12.9)	1 (1.6)	17 (6.9)
Coolie	11 (17.7)	6 (9.7)	2 (3.2)	7 (11.3)	26 (10.5)
Education of patient/caregiver					
Illiterate	3 (4.8)	1 (1.6)	2 (3.2)	1 (1.6)	7 (2.8)
Primary school	49 (79)	53 (85.5)	44 (71)	25 (40.3)	171 (69)
Middle school	9 (14.5)	6 (9.7)	13 (21)	27 (43.5)	55 (22.2)
High school	0 (0)	1 (1.6)	1 (1.6)	8 (12.9)	10 (4)
University and graduate	1 (1.6)	1 (1.6)	2 (3.2)	1 (1.6)	5 (2)

Background characteristics of patients/ caregivers are shown in Table 2. Males accounted for 56.9%. About 40% of the patients had a family composed of more than 5 family members. Regarding previous antimalarial intake, 44.4% had ever taken some form of antimalarials. All the patients or caregivers reported that they or patients had fever at the time of presentation. They responded that health care providers explained how to take the medicine and they understood the instruction.

Thirty-three percent of the patients (95% CI=27.5-39.1) reported that they had taken the medicine with fatty food. Ten patients (4%, 95% CI=2.2-7.3) had tablets remaining in the blister pack and they were

Table 2. Background characteristics of patients/ caregivers

Characteristics	Age group (years) (n=62 each, total=248) (%)					
	<5	≥5 to<9	≥9 to <14	≥14	Total	P
Sex						
Male	40 (64.5)	26 (41.9)	35 (56.5)	40 (64.5)	141 (56.9)	0.035
Female	22 (35.5)	36 (58.1)	27 (43.5)	22 (35.5)	107 (43.1)	
Formal education of patient or caregiver						
At most primary	52 (83.9)	54 (87.1)	46 (74.2)	26 (41.9)	178 (71.8)	<0.001
At least middle	10 (16.1)	8 (12.9)	16 (25.8)	36 (58.1)	70 (28.2)	
Household size						
1-5	49 (79)	38 (61.3)	27 (43.5)	36 (58.1)	150 (60.5)	0.001
>5	13 (21)	24 (38.7)	35 (56.5)	26 (41.9)	98 (39.5)	
History of antimalarial intake						
Yes	6 (9.7)	23 (37.1)	32 (51.6)	49 (79)	110 (44.4)	<0.001
No	56 (90.3)	39 (62.9)	30 (48.4)	13 (21)	130 (55.6)	

classified as definitely non-adherent. Three patients, all were adults, took 4 tablets per dose three times a day with the expectation of rapid cure. Another three patients took lower than the instructed dose with the reason of fear of side effects. Ten patients did not complete the full course. So patients in the probably non-adherent category were 16 (6.5%, 95% CI=4-10.2). Reasons for non-adherence were: 18 (69.2%, 95% CI=50-83.5) thought of getting cured, 3 (11.5%, 95% CI=4-29) took more than prescribed dose with the expectation of rapid cure, 3 (11.5%, 95% CI=4-29) feared of side effects and 2 (7.7%, 95% CI=2.1-24.1) shared the drug with another patient. Two hundred and twenty-two patients (89.5%, 95% CI=85.1-92.7) were probably adherent to the recommended regimen. Percentages of probably adherent patients in each age category are shown in Table 3.

The youngest age group had significantly higher adherence than the eldest age group had (p=0.02) but no significant differences were seen among other age groups. There were no statistically significant association between the background characteristics of patients or caregivers and probably adherence to recommended regimen of AL in total or in each specific age group (Table 4).

Table 3. Patient/caretaker adherence to artemether-lumefantrine by self-report and blister pack inspection

Age group (years) (n=62)	Probably adherence duration (hour) n (%) (95% CI)				
	12	24	36	48	60
<5	62(100)	62(100)	62(100)	61(98.4) (91.4-99.7)	61(98.4) (91.4-99.7)
≥5 to<9	62(100)	62(100)	62(100)	59(95.2) (86.7-98.3)	56(90.3) (80.5-95.5)
≥9 to<14	62(100)	62(100)	62(100)	58(93.5) (84.6-97.5)	53(85.5) (74.7-92.2)
≥14	60(96.8) (88.9-99.1)	59(95.2) (86.7-98.3)	57(91.9) (82.5-96.5)	53(85.5) (74.7-92.2)	52(83.9) (72.8-91)
Total (n=248)	246(99.2) (97.1-99.8)	245(98.8) (96.5-99.6)	243(98) (95.4-99.1)	231(93.1) (89.3-95.7)	222(89.5) (85.1-92.7)

Table 4. Association between background characteristics of patients/caregivers and probably adherence to recommended regimen of artemether-lumefantrine

Characteristics	Age group (years), n (%)				
	<5	≥5 to <9	≥9 to <14	≥14	Total
Sex					
Male	40 (100)	23 (88.5)	31 (88.6)	32 (80)	126 (89.4)
Female	21 (95.5)	33 (91.7)	22 (81.5)	20 (90.9)	21 (95.5)
p	0.35	0.68	0.48	0.47	0.35
Formal education of patient or caregiver					
At most primary	51 (98.1)	48 (88.9)	38 (82.6)	22 (84.6)	159 (89.3)
At least middle	10 (100)	8 (100)	15 (93.8)	30 (83.3)	63 (90)
p	1.00	1.00	0.425	1.00	1.00
Household size					
1-5	48 (98)	33 (86.8)	24 (88.9)	28 (77.8)	133 (88.7)
>5	13 (100)	23 (95.8)	29 (82.9)	24 (92.3)	89 (90.8)
p	1.00	0.39	0.71	0.17	0.67
History of antimalarial intake					
Yes	6 (100)	22 (95.7)	27 (84.4)	42 (85.7)	97 (88.2)
No	55 (98.2)	34 (87.2)	26 (86.7)	10 (76.9)	55 (98.2)
p	1.00	0.39	1.00	0.42	0.54

DISCUSSION

Age-specific adherence rates were 98.4% (95% CI=91.4-99.7) in less than 5 years age group and 90.3% (95% CI=80.5-95.5) in five to nine years age group. Most of the under nine years old children were not the family members who stayed in the forest. They were children of farmers. Most of them had no history of previous malaria attacks. This fact may support the good adherence to the recommended regimen of the AL in these age groups. Most of the adult participants in our study were mobile gold mine workers. Children of 9 to 14 years old age group also stayed with their mobile parents in the forest and carried out light activities. Many of them had frequent malaria attacks.

This aspect may reflect the fewer adherences among them than that of the younger age groups. However, adherences among older age groups were also good, having 85.5% (95% CI=74.7-92.2) in nine to 14 years of age group and 83.9% (95% CI=72.8-91) in over 14 years of age group. Number of tablets per dose may affect the adherence of the patients. Patients younger than nine years had to take one to two tablets per dose and had better compliance than the older patients. The latter had to take three to four tablets per dose. However, statistically significant difference in adherence was seen between youngest and oldest age groups. In Uganda study, age group is not associated with adherence.[12]

Other explanatory factors associated with adherence could not be found in our study. A study in Bangladesh shows that adherence to AL is very high in both supervised and non-supervised treatment groups.[11] In a study in Uganda, the 90% of the patients are probably adherent and the rest 10% are definitely or probably non-adherent to AL.[12] By contrast to Bangladesh study, lack of formal education is the factor associated with non-adherence in that study. The study conducted on children in Sudan shows that 59.1% of the children are probably adherent, 22.6% are probably non-adherent and 18.3% are definitely non-adherent to AL.[13] With regard to other ACT, artesunate plus sulfadoxine-pyrimethamine, it is 60.0% certainly or probably non-adherent in Zambia and 10.4% and 25% non-adherent at 24 hours and 48 hours, respectively, in Tanzania.[14, 15]

The commonest reason for not completing the course of the recommended regimen was that the patient or caregiver thought of getting cure from malaria when the symptoms were improved on the third day. The remaining tablets might be shared to other patient or saved for the next attack or left them alone. Taking of higher than recommended dose with the expectation of rapid cure and taking of lower dose with the fear of side effects were also other problems of adherence. Even with taking of all doses at the correct time on the proposed day and in the correct amount, taking of each dose accompanied by a fatty meal so as to maximize lumefantrine absorption was low (33%, 95% CI=27.5-39.1).

The high level of adherence to AL found in our study is likely to be the result of the effectiveness of the health care programmes and the awareness of malaria among the community members. Miss perception toward the regimen was still present, nevertheless.

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Utilization pattern of traditional medicine in rural community in PyinOoLwin and Naungcho townships

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The study was conducted to find out utilization, diagnosis and treatment patterns of traditional medicine users in rural community. Community-based, cross-sectional descriptive study was done in rural area of PyinOoLwin and Naungcho townships. There were 1132 participants with the age ranged from one month to 93 years. Within last 12 months, 18% of the respondents did not use any type of medicine. Traditional medicine only and western medicine only were used by 13.5% and 20.7% of the respondents, respectively. About half (47.8%) of the respondents used both types. With regard to age group, traditional medicine only was used by 12.5%, 13.3%, 11.0%, 9.8%, 15% and 20% of infant, one to five years group, six to fourteen years group, 15-24 years group, 25-59 years group and above 60 years group, respectively. Above 60 years age group was the highest user (68.2%) of both traditional and western medicine. Female used traditional medicine more frequently than male. The most common health problem that the respondents have taken traditional medicine was gastrointestinal symptoms (26.9%), followed by fever (16.1%). Most frequently used form of the traditional drugs was powder form (61.1%). More than 90% of the respondents diagnosed their diseases and symptoms by themselves or relatives. Self prescription was also the majority (88.3%). Easy availability was the most common reason (49.6%) for using traditional medicine. While WHO and many countries are promoting the utilization of traditional medicine, our study might be a help for national health system of Myanmar.

INTRODUCTION

Traditional medicine (TM) has been existed in Myanmar since time immemorial and also has been playing an important role in provision of healthcare as a national and cultural heritage. The scope of TM is very wide in terms of its various methods of treaties, pharmaceuticals and herbal medicines with a variety of uses. TM is formulated with natural resources such as medicinal plants, medicinal animal products and minerals, in accordance with long experience in health care. Therefore, it is difficulty to synchronize and develop Myanmar TM in a systematic way. TM is widely practiced in Myanmar, partly as a supplement and partly as an alternate to modern or western medicine (WM). In Myanmar, TM has been widely used in daily practice both in public and private sectors. Community still relies on TM despite the WM has taken place in public health care.

TM includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness. Herbal medicine is also a type of medicine that uses roots, stems, leaves, flowers, or seeds of plants to improve health, prevent disease, and treat illness. TM was officially introduced as a branch of health services in 1972, under the Department of Health.

TM was upgraded as a separate department in August 1989. Under the Department of Traditional Medicine, there are 214 TM clinics, covering 17 states and divisions of the country.

Traditional medical practitioners are trained at the Institute of Traditional Medicine in Mandalay. The duration of training is two years with one year internship after completion of the training. It is a diploma course. The Institute has been upgraded and is now conferring Bachelor Degree with four years course.

Myanmar TM Practitioners Conference has been held annually with the lofty objectives of developing and standardizing Myanmar TM and of promoting public health care services. The TM law has been enacted in order to ensure that Myanmar TM is standardized and safe and to raise the standard, qualification and dignity of their profession in accord with the noble aims.

Traditional medicine hospitals, dispensaries and clinics have been opened the length and breadth of the nation as a mean to extend the public health care services of the TM sector. TM factories are emerging in the nation, as the state has been given encouragement for the advancement of the industry. Altogether 5794 TM practitioners were registered under the law. Altogether 3188 items of drugs were registered and 697 manufacturers were granted license at the end of 2006.[1]

Most of the people believe that TM is developing with greater momentum, seeing a lot of good results in many areas, and winning greater confidence and reliance of the people. The TM used in township traditional medical clinics and hospitals are provided by two factories owned by the department of Traditional Medicine, one in upper and another in lower Myanmar. Attempts have been made to integrate traditional and allopathic medicines in the management of some challenging diseases, such as pulmonary tuberculosis, diabetes mellitus, hypertension, stroke, bronchial asthma and malaria.

Many of drug shops in rural areas are selling TM and TM healers are also giving treatment with their own traditional drugs and traditional drugs from the drug shops in many parts of the country. People in rural community are lack of accessible and affordable to health facilities of WM and doctors. Therefore, they may take treatment with traditional healers in rural areas, especially chronic cases like hypertension, diabetes and chest infection.

In India, the medical systems that are truly Indian in origin and development are the Ayurveda and the Siddha systems. Ayurveda is practiced throughout India, but the Siddha systems are practiced in the tamil-speaking area of South India. Chinese medicine claims to be the world's first organized body of medical knowledge dating back to 2700 BC. It is based on two principles - the yang and the yin. The yang is believed to be an active masculine principle and the yin a negative feminine principle. The balance of these two opposing forces meant good health. Hygiene, dietetics, hydro-therapy, massage and drugs were used by the Chinese physicians. The Chinese systems of "bare-foot doctors" and acupuncture have attracted worldwide attention in recent years. In other countries like Egypt, Greek and Roman, traditional medicines are also famous and their medicines become foundations of modern medicine.[2]

Most of rural people in Myanmar believed and trusted in and rely on Myanmar TM. Public have given priority to TM naturally compounded from tubers and bulbs of herbal plants instead of western drugs because of high prices and side effects of the latter. It can be vividly seen that the TM and medical sciences of some eastern nations become popular in the world, deeply penetrating international community. The government has been endeavouring to improve the health standard of rural people by accelerating the health care services across the nation including rural areas.

At the same time, it is taking systematic measures for ensuring effective use of TM

on which the majority of rural people place trust. The State Peace and Development Council encourages and gives priorities in production and using the TM especially in the treatments of six common diseases such as hypertension, diabetes, tuberculosis, malaria, diarrhoea and dysentery. Now, it is time to carry out scientific research on Myanmar TM uses.

Most of people, both in urban and rural community, are using TM liberally. Theses drugs are easily available, readily affordable and relatively cheap, but they do not know the side effects and their consequences. Both TM and WM are being sold in stores and drug shops and these drugs may be prescribed by physicians or not. Many people, in the rural community, are using TM by themselves without instruction by traditional medical practitioners. Some traditional medical practitioners are trained in the traditional medical school but others are not. There are no special disciplines in TM and they may treat any diseases. Therefore, we should know whether people believe the effects of TM really or it is used as an alternative of WM which is expensive and they do not know what drugs to be used except some vitamins and antipyretics of WM. Most of drugs need prescription by physician or respective specialists. We conducted this study to find out the acceptance, utilization of TM which is formulated from medicinal plants, medicinal animal products and minerals, diagnosis and treatment patterns of TM users in rural community.

MATERIALS AND METHODS

Community-based, cross-sectional descriptive study was done at the rural area of the PyinOoLwin (Mandalay Division) and Naungcho (Northern Shan State) Townships. Study population was all individuals in selected rural areas of both townships. All individuals of any age and both sexes residing in rural areas more than three years and above were included in the study. Out of 397 villages in the rural areas of the PyinOoLwin and Naungcho Townships, 30 villages were selected by random sampling. There were 15 villages from each township. Again five households from each selected village were selected randomly. There were 75 households in each township. All the individuals residing in selected households were interviewed. A total of 1132 participants including 545 from PyinOoLwin Township and 587 from Naungcho Township were interviewed. Face-to-face interview was conducted by using pre-tested structured questionnaire including open-ended questions. The respondents were all individuals in selected households. In case of children under the age 15 years, parents or guardians were interviewed.

Data were checked for consistency. Data entry and data analysis was done by SPSS 11.5 for Windows. Mean and frequencies of variables such as age, sex, occupation, education and traditional medicine use were calculated.

Ethical issues

All the respondents were explained about the aims of the study prior to interview. Informed verbal consent of eligible respondents was obtained after making sure that the interviewee understood the study purpose. In the selected villages, respondents who cannot use Burmese language were explained by interpreter in their local language. Informed consent was also obtained from local authorities in respective village after explanation of the purpose of the study before conducting the survey.

RESULTS

Out of total 1132 participants, 48% (545/1132) were from PyinOoLwin and 52% (587/1132) from Naungcho townships. Among 30 villages, only in 4 villages (13.3%) there were no traditional healers and 7 villages (23.3%) and 19 villages (63.3%) had traditional healers in the villages and in nearby villages, respectively. Traditional drug shops were present in 27 villages (90.0%) and respondents from

3 villages (10.0%) said that traditional drugs were available in nearby villages. Regarding the presence of health care providers, one government doctor, two general practitioners, two nurses, two health assistants, two lady health visitors and 11 midwives were giving health care services in the study area.

Table 1. Background characteristics of the respondents (total=1132)

Characteristics	No.	%
Sex		
Male	542	47.9
Female	590	52.1
Race		
Shan	557	49.2
Bamar	448	39.6
Nepalese	77	6.8
Lee-su, Kachin and Chin	50	4.4
Education level		
Before school-going age	106	9.4
Illiterate	32	2.8
Read and write	94	8.3
Primary school level	484	42.8
Middle school level	220	19.4
High school level	141	12.5
University/graduate level	55	4.9
Occupation		
Before school-going age	106	9.4
Students	230	20.3
Dependents	106	9.4
Daily wage earners	27	2.4
Farmers	579	51.1
Government employees	16	1.4
Domestic jobs	14	1.2
Merchants	54	4.8

Age of the respondents ranged from one month to 93 years. Male accounted for 48% and female 52%. About 49% of the respondents were Shan race and others were Bamar (39.6%), Nepalese (6.8%) and Lee-su, Kachin and Chin (4.4%). Majority (97.6%) were Buddhists and the rest (2.4%) were Christians. Education level and occupation of the respondents are shown in Table 1.

Within last 12 months, only 18% of the respondents did not use any type of medicine. Traditional medicine was used by 13.5% of the respondents. Western medicine was used by 20.7% and both types of medicine was used by 47.8% of the respondents. Relationship between background characteristics and types of medicine used is shown in Table 2.

Table 2. Relationship between background characteristics and types of medicine used (n=1132)

	Types of medicine used within 12 months				
	Traditional	Western	Both	None	Total
	no.(%)	no.(%)	no.(%)	no.(%)	no.(%)
Age groups					
Infant	2 (12.5)	2 (12.5)	7 (43.8)	5 (31.3)	16 (100)
1-5 (yr)	12 (13.3)	25 (27.8)	42 (46.7)	11 (12.2)	90 (100)
6 -14 (yr)	23 (11.0)	62 (29.5)	64 (30.5)	61 (29.0)	210 (100)
15-24 (yr)	20 (9.8)	48 (23.4)	76 (37.1)	61 (29.8)	205 (100)
25- 59 (yr)	79 (15.0)	91 (17.3)	294 (55.9)	62 (11.8)	526 (100)
≥60 (yr)	17 (20.0)	6 (7.1)	58 (68.2)	4 (4.7)	85 (100)
Sex					
Male	68 (12.5)	136 (25.1)	223 (41.1)	115 (21.2)	252 (100)
Female	85 (14.4)	98 (16.6)	318 (53.9)	89 (15.1)	590 (100)
Race					
Shan	90 (16.2)	93 (16.7)	249 (44.7)	125 (22.4)	557 (100)
Bamar	52 (11.6)	95 (21.2)	253 (56.5)	48 (10.7)	448 (100)
Nepalese	5 (6.5)	30 (39.0)	22 (28.6)	20 (26.0)	77 (100)
Lee-su, Kachin& Chin	6 (12.0)	16 (32.0)	17 (34.0)	11 (22.0)	50 (100)
Education level					
Before school-going age	14 (13.2)	27 (25.5)	49 (46.2)	16 (15.1)	106 (100)
Illiterate	4 (12.5)	2 (6.3)	24 (75.0)	2 (6.3)	32 (100)
Read and write	15 (16.0)	12 (12.8)	54 (57.4)	13 (13.8)	94 (100)
Primary school level	64 (13.2)	109 (22.5)	212 (43.8)	99 (20.5)	484 (100)
Middle school level	39 (17.7)	38 (17.3)	98 (44.5)	45 (20.5)	220 (100)
High school level	13 (9.2)	38 (27.0)	72 (51.1)	18 (12.8)	526 (100)
University/ graduate level	4 (7.3)	8 (14.5)	32 (58.2)	11 (20.0)	55 (100)
Occupation					
Before school-going age	14 (13.2)	27 (25.5)	49 (46.2)	16 (15.1)	106 (100)
Students	4 (12.5)	2 (6.3)	24 (75.0)	2 (6.3)	32 (100)
Dependents	15 (16.0)	12 (12.8)	54 (57.4)	13 (13.8)	94 (100)
Daily wage earners	64 (13.2)	109 (22.5)	212 (43.8)	99 (20.5)	484 (100)
Farmers	39 (17.7)	38 (17.3)	98 (44.5)	45 (20.5)	220 (100)
Government employees	3 (18.8)	3 (18.8)	9 (56.3)	1 (6.3)	16 (100)
Domestic jobs	6 (42.9)	3 (21.4)	5 (35.7)	0 (0.0)	14 (100)
Merchants	4 (7.4)	6 (11.1)	37 (68.5)	7 (13.0)	54 (100)

In the community, diseases and symptoms of the diseases were treated with traditional medicine. Symptoms related to gastro-intestinal and biliary systems were most frequently treated with traditional medicine and it accounted for 26.9% of the total uses. Diseases and symptoms which were usually treated by traditional medicine are shown in Table 3.

Table 3. Diseases and symptoms treated by traditional medicine

Disease and symptoms related to the respective system	No. of respondents	%
Gastrointestinal and biliary system	187	26.9
Fever	112	16.1
Respiratory system	106	15.3
Musculoskeletal system	93	4
Central nervous system	44	6.3
Hypertension	40	5.8
Diarrhoea and dysentery	27	3.9
Malaria	24	3.5
Genital system	20	2.9
Cardiovascular system	15	2.2
Dental symptoms	8	1.2
Ulcer	6	0.9
Mucocutaneous system	6	0.9
Diabetes	2	0.3
Renal system	2	0.3
Cancer	1	0.1
Eye symptoms	1	0.1
Total	694	100

Most frequently used form of the traditional drugs during the study period was powder form (61.1%). Balm and lotion form accounted for 22.2% and, tablet and capsule form (10.4%). Other forms were liquid (2.6%), decoration, nasal syrup, home made remedy and injection (3.6 %).

More than 90% of the respondents diagnosed their diseases and symptoms by themselves or relatives. About 4% of the respondents were diagnosed by doctors but they used traditional medicine in addition to doctors' prescription. Only 2.2% of the respondents said that their diseases were diagnosed by traditional healers. Others were diagnosed by drug sellers (1.2%) and health staff (1.2%). About 90% of the respondents used traditional medicine by their own decision. Five percent of the respondents were prescribed by traditional healers and drug sellers. Regarding with reasons for using traditional medicine, about half of the respondents said that traditional medicine was easily available. Turst to traditional medicine was reported by 32.6% of the respondents. Other reasons were local language prescription (9.1%), cheap price (4.0%), willing to test effectiveness (3.0%), and friends and relatives' advice (1.7%).

DISCUSSION

The WHO estimated that 80% of the population in some Asian and African countries depended on TM for primary health care. Furthermore, in many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine.[2] In our study, 13.5% of the respondents used TM alone and another 47.8% used both TM and WM.

Prevalence of TM use among infants was 56.3% (9 out of 16); using TM alone 12.5% and both TM and WM 43.8%. More or less similar picture was seen in 1 to 5 years old children; using TM alone 13.3% and both TM and WM in 46.7%. Among old age population (60 years and above), 88.2% of the respondents used TM alone or with WM. Studies have shown that older participants used alternative and complementary medicine more commonly.[3, 4]

In our study, 53.6% of males used TM alone or with WM in comparison with 68.3% of female respondents. There was no association with gender and use of alternative and complementary medicine.[3-6]

With regard to education, 87.5% of illiterate used TM with or without WM while 65.5% of respondents with university or graduate level education were using TM. Educational attainment was not an associated factor with utilization.[6]

This study showed that TM is mostly used for symptomatic treatments, commonly in gastrointestinal symptoms, fever, symptoms related to musculoskeletal system and

respiratory system. Similar finding was found in other study.[6]

Most common forms of preparation were powder (61.1%) and balm or lotion (22.2%). Many remedies were locally and nationally distributed while some remedies were prepared at home. Self (or relative or friend) diagnosis was very common (91.6%) and so self prescription was 88.3%. Other studies showed similar findings.[7-9]

About 2% of the respondents said that doctor and health staffs prescribed TM. Doctor and nurses prescribed or referred for alternative and complementary medicine in Israel.[10]

In more than 90% of the villages, there were shops that sold TM. Therefore, about 50% of the respondents explained that they used TM because of easy availability. Trust to TM was a reason for use in 32.6% of the rural people. About 10% of the users explained that they could read the prescription in native language.

While the WHO and many national health systems are promoting the use of TM for health care, our study might be a help for national health system of Myanmar.

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IgG antibodies against measles among vaccinees

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The study was conducted in "Ka" and "Sa" wards of North Okkalapa Township in the year 2010. The vaccinees were enrolled by convenience sampling on August 29 and September 5. The participants and/or guardians who gave written informed consent were selected and a total of 51 documented measles vaccinees were obtained. The ethical committee of University of Medicine 2, Yangon approved the study. Serum samples collected from subjects who have had vaccine card were assayed for anti-measles Ig G using Genzyme Virotech ELISA test kit. The serological assay was done at Common Research Laboratory, University of Medicine 2. Of the 51 vaccinees, 23 were males and 28 were females, and the ages ranged from 1 to 20 years. Fourteen subjects were two-dose vaccinees, the remaining 37 were vaccinated only once. The overall seropositivity rate was 31 (61%), the borderline and seronegative results were 5 (10%) and 15 (29%), respectively. Eleven (78.57%) of two-dose and 20 (54.05%) of one-dose vaccinees were seropositive. The study groups were stratified into 5 age groups with the interval of 4 years. The highest (100%) was found in 13-16.9 and >17 years age group, the lowest (47.06%) seropositivity in 5-8.9 years age groups and the other two groups, 1-4.9 and 9-12.9 years age groups showed 66.67% and 62.5% seropositivity, respectively. In this study, the seropositivity rate did not differ significantly with respect to age and sex, whereas vaccinees who had received two doses of measles vaccine had increased association with seropositivity.

INTRODUCTION

Measles is a highly contagious viral disease of childhood. It can prove fatal and serious complication is highest in young children and adults. More than 20 million people are affected by measles each year. In 2007, there were 197,000 measles deaths globally, mostly in children under five years of age.[1] Of vaccine-preventable diseases, measles remains one of the most important causes of childhood morbidity and mortality in developing countries, fatality rate can still be as high as 15% most probably due to the lack of vaccination of many individuals in the population.[2]

The incidence of measles has dramatically reduced with introduction of effective vaccine. About 576 million children who live in high-risk countries were vaccinated against the disease during the period from 2000 to 2007 and as a result global measles deaths were decreased by 74%.[1] In Myanmar, a single dose immunization at the age of 9 months was started in 1987.[3] Expanded Programme of Immunization (EPI) provides measles immunization to 1.3 million children under 1 year every year. WHO estimated that Myanmar has 82% measles immunization coverage among one year old children in 2008 and, 68% and 84% in 1990 and 2000, respectively. There is no measles death in 2008.[4]

Mass Measles Immunization Campaigns (MMC) were carried out in 1995, 1997, 2002, 2003, 2004, 2006 and 2007 to give second opportunity to under five children by introducing second dose of measles vaccine.

MMC has been conducted for the whole country in phase manner since 2002. Unfortunately routine immunization was suspended for two months in late 2005 and early 2006 because of adverse event following immunization (AEFI). Comprehensive Strategies Package for Measles Control Campaign (CSPMC) including measles catch up campaign targeting 6 million children were conducted throughout the country during 2007 in three phases and 5.7 million children at 9 month to 5 years (94% of target children reached) were immunized against measles. In 2008, second dose of measles immunization at 18 month of age was introduced in routine immunization programme to ensure all children have second opportunity for measles vaccination.[3]

Elimination of measles requires the continued commitment to increase vaccine coverage level, the genetic analysis of circulating strains, and serosurveys of vaccinated individuals to establish the population at risk. Monitoring the cold chain and immunization safety, including injection safety and adverse events following immunization is vital for improving vaccination systems that aim to control and eliminate measles. Disease surveillance is also critical component of measles control and elimination efforts.

At the mortality reduction stage: it provides data for monitoring incidence and coverage in order to assess progress (i.e. decreasing incidence and increasing coverage), identifying the high-risk areas or areas with poor programme performance, describing the changing epidemiology of measles in terms of age, immunization status and the intervals between epidemics. At the low incidence or elimination stage: identifying high-risk population, determining when the next outbreak may occur due to accumulation of susceptible persons, and accelerating activities beforehand, and determining the high-risk area where measles virus is circulating or may circulate, assessing the performance of the surveillance system (e.g. reaction time for notification, specimen collection) in the detection of virus circulation or potential importation; using performance indicators to identify areas necessary to strengthen surveillance is required for measles control programme. Outbreak investigation and determination of factors attribute to outbreak such as failure to vaccinate, vaccine failure, and accumulation of susceptible persons are important for both stages.[5]

In general, surveillance lags behind vaccination efforts in most programmes for the controlof vaccine-preventable diseases. Effective vaccination strategies can quickly reduce disease incidence, whereas establishing a surveillance system takes time and changing surveillance practices is difficult. Countries should therefore develop and follow long-term measles control strategies providing a surveillance system that can respond to changes in the incidence of the disease.

Routine laboratory testing is not feasible and not done for all measles cases in Myanmar. Only serological confirmation of measles outbreak was done by measles IgM antibody ELISA testing at National Measles Laboratory (NML) of National Health Laboratory from 2001 to 2006. Isolation and identification of measles virus from cases was initiated in August 2006 and isolated measles viruses were sent to Regional Reference Laboratory (RRL), Thailand for genotyping.[6]

Reliable and sensitive laboratory methods are very important to accurately determine the antibody level and protection achieved after vaccination and the level of antibodies that persists in previously vaccinated person.[2] In my knowledge, serosurvey among Myanmar community has not done before, due to lack of facility. In this study, a total of 51 blood samples were collected from 1-20 years old population residing in “Ka” and “Sa” wards, North Okkalapa Township, Yangon to determine the measles-specific IgG by ELISA technique among vaccinated (single or booster) study population.

Objective

To determine measles-specific IgG among 1-20 years old vaccinated population and thereby exploring the status of herd immunity against measles among the study population.

MATERIALS AND METHODS

Cross-sectional descriptive study was done in 51 participants, ages ranged from 1-20 years, from Ka" and "Sa" wards of North Okkalapa Township. The vaccinees were enrolled by convenience sampling on August 29 and September 5 in the year 2010. The vaccination status of measles was obtained from vaccination cards. Written informed consent was taken from guardian of the subjects under 18 years of age and from the subjects over 18 years after explaining the purpose and procedure of this study. The ethical committee of University of Medicine 2, Yangon approved the study.

After proper disinfection and cleaning to the proposed skin, 2-3 ml of peripheral blood were collected using sterile disposable syringes and needles by venepuncture into sterile screw-capped bottles. Code number, name, age and date of specimen collection were recorded. The samples were allowed to clot at room temperature and then the serum was transferred aseptically to a sterile labeled 2 ml micro vial using transfer pipette, and stored in deep freezer (-20˚C) till tested.

The serological assay was done at the Common Research Laboratory, University of Medicine 2, Yangon. Stored serum was tested for measles-specific IgG by using Genzyme Virotech Measles ELISA IgG test kit. The ELISA test was performed according to the manufacturer's instruction.

Prediluted patient serum was added to wells coated with purified antigen. If measles-specific IgG antibody was present, it binds to the antigen. All unbound materials were washed away and the enzyme conjugate was added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate was washed off and substrate solution (TMB) was added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme (peroxidase). The reaction was stopped by adding the stopping solution. The ready to use controls served for a semiquantitative determination of specific IgG antibodies. The results were measured extinction (OD) at 450/620 nm (Reference Wavelength 620-690 nm) using ELISA Reader (Stat Fax-2100). Cut-off points and antibody index calculations were done according to manufacturers' recommendation to categorise seropositive (antibody index >11), borderline positive (antibody index 9 to 11) and seronegative (antibody index <9). Their concentrations were expressed in Virotech units (VE).

RESULTS

A total of 51 vaccinees, of which 23 were males and 28 were females, and the ages ranged from 1 to 20 years were included in this study. The subjects were grouped into 5 age groups with the interval of 4 years. Age distribution of study population is shown in Table 1.

Table 1. Distribution of study population by age group

Age group (year)	Number	%
1-4.9	24	47.05
5-8.9	17	33.33
9-12.9	8	15.68
13-16.9	1	1.9
>17	1	1.9
Total	51	100

The overall seropositivity rate was 31 (61%), the borderline and seronegative results were 5 (10%) and 15 (29%), respectively (Fig. 1). Seropositivity was highest in 13-16.9 and >17 years age groups (100%). The least seropositivity was found in 5-8.9 years age group (47.06%). Seropositivity in 1-4.9 and 9-12.9 years age groups were 66.66% and 62.5%, respectively. The highest

seronegativity was 41.18% in 5-8.9 years age group. Seronegativity in 1-4.9 and 9-12.9 years age groups were 25% and 37.5%, respectively. In 5-8.9 years age group, the highest borderline result was obtained (17.65%). Borderline result was found to be 8.33% in 1-4.9 years age group. No borderline result in 9-12.9, 13-16.9 and >17 years age groups (Fig. 1).

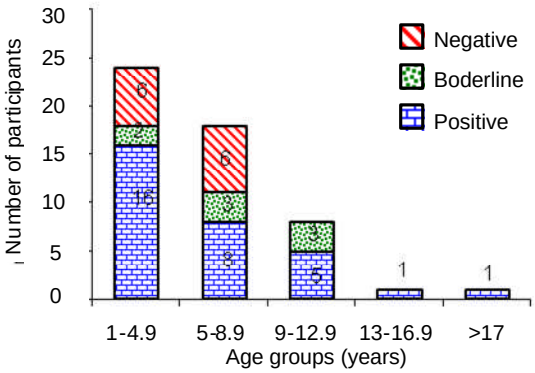

Fig. 1. Measles-specific IgG seroprevalence in different age groups

Among the seropositive cases, 60% each of the male and female participants were seropositive for measles Ab. There was no significant difference between male and female (p=1.000, 95% CI) (Fig. 2).

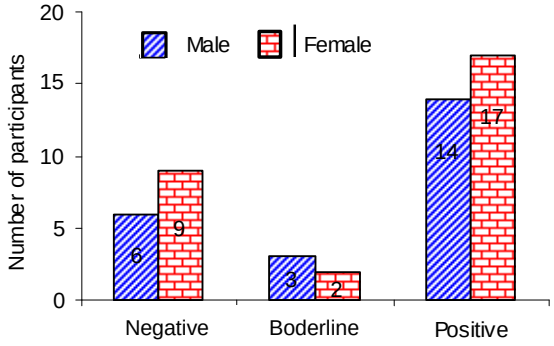

Fig. 2. Seroprevalence of measles-specific IgG by sex

Regarding the frequency of administration, fourteen subjects received two doses of measles vaccine, the remaining 37 were vaccinated only once. Eleven (78.57%) of two-dose and 20 (54.05%) of one-dose vaccinees were seropositive (Fig. 3). Among one-dose vaccinee, 12 (32.43%) were sero-negative and 5 (13.51%) were borderline.

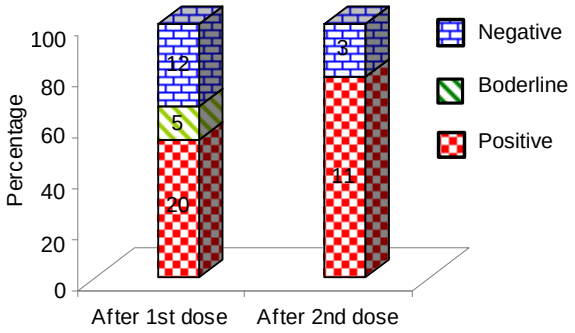

Fig. 3. Seroprevalence of the measles-specific IgG among different dose of vaccination

Of two-dose vaccinees, 3 (21.43%) were sero-negative and no borderline result. Measles antibody response was more increased after second dose than first dose. However, there was no significant difference (p=0.1798, 95% CI).

DISCUSSION

In Myanmar, a single dose measles vaccine at nine months of age is being administered, as recommended by the World Health Organization (WHO). According to local health data, measles vaccine coverage of North Okkalapa Township was 66.2%, 82% and 83% in 2007, 2008 and 2009, repectively. The health statistic report released by WHO in 2010 stated that immunization coverage of Myanmar children in 2008 was 82%. The probability of seroconversion and the amount of anti-bodies induced are determined by the persistence of maternal antibody and age of infant at the time of vaccination because of the relatively lower immunogenic respon-siveness to same antigens early in life. Vaccine efficacy is occasionally impaired in children under 1 year of age. Vaccine efficacy is 85% at 9 months of age and increases to 95% at 12 months of age and only 67% below 9 months of age.[7] Since vaccine is only 85% efficacious when given at 9 months of age, only 69.7% of the vacci-nated children in Myanmar will be estimated to develop vaccine-induced immunity.

In this study, seropositivity among the documented vaccinees was 61% which seemed to be reduced than that of estimated

coverage. There was increasing seropositivity with the increasing age except in 5-8.9 years age group. Many studies described that antibodies titers after vaccination are lower than those following natural infection, while other studies have found evidence of waning immunity in vaccinated children. These groups provide susceptibility of subclinical infection which can be possible source for transmission of measles. Waning of immunity is likely to increase population contribute to measles circulation.[8] Estimated secondary vaccine failure rate was approximately 5% at 10 to 15 years after immunization. When vaccine is given after 12 months, this may be lower.[7]

The mean duration of protection conferred by single dose vaccination is about 25 years. Eighty percent of vaccinated population would become susceptible to clinical or subclinical infection in the absence of exposure to wild type measles virus. The prevalence of susceptibility to clinical or subclinical reinfection in vaccinated population was estimated to be 19-31%.[9] In Iran, more than half of the case has occurred among 5-19 years old immunized children during several recent outbreaks which may be due to primary or secondary vaccine failure. Measles-specific antibodies titers after vaccination is lower than post natural infection, so vaccinated persons may gradually lose protection against measles.[8] So increasing seropositivity among the study population may be the result of exposure to wild type measles virus.

The seropositivity rate was not differed significantly with respect to age and sex. The seroprevalence was not effected by gender, socio-economic status and sibling size.[10] Many studies also described no significance in seropositivity among male and female.[11, 12]

A study done in Harare, Zimbabwe during 1988 outbreak showed immunization coverage of 83%. Even at that coverage, they have faced periodic epidemics and believed to be significant transmission of measles among community. Low herd immunity caused by accumulation of non-immunized children and vaccination failures may result in a pool of susceptible older children large enough to result in an outbreak.[13] That occurrence may explain current finding of endemic transmission of measles in our country even achieving measles vaccine coverage of 82%.

The seronegativity was found in 29% of the study population. The highest seronegativity was 41.18% in 5-8.9 years age group. Seronegativity in 1-4.9 and 9-12.9 years age groups were 25% and 37.5%, respectively. Even with vaccination coverage of 98% and seropositivity of more than 95%, measles outbreaks have been reported. The absence of antibodies in a high proportion (10.2-27.4%) of children aged 5-18 years together with overcrowded schools in Yeman would enough to cause effective measles spread.[14]

There are many factors contributing to the efficacy of the measles vaccine; vaccine factors such as improper production, transporting and storage, dosage and administration, and vaccinee factors such as genetic, demographic and environmental factors as well as to the immunocompetence status of individuals. There were many studies documented both stable and waning titers of vaccine-induced antibody and estimation of decay rate of vaccine-induced antibody titers. Studies in two different geographic regions estimated that the half life of measles antibody was 5.5 years in Taiwan and 12 years in Canada.[9, 15]

With regard to dose of vaccine, two-dose vaccinees showed increased seropositivity. This study showed that the two-dose schedule is successful in achieving high levels of immunity. Study in Urmia described that the average antibody titer was significantly higher in one-dose vaccinees than two-dose vaccinees due to boosting effect from repeated exposure to circulating wild virus resulting in subclinical reinfection. This group is most likely to support viral transmission in the absence of disease.[8]

Western Europe seroepidemiology study described that anti-measles antibodies are lower after the first years of vaccination and the levels increase by natural booster.[11] Even high level of vaccine coverage have not always prevented explosive school-based outbreak in Finland. There was unusual occurrence among two or three-dose vaccinees which may be due to poor cold chain or the impact of large measles inoculums by air borne transmission. Total protection might not be achievable because of highly contagious in nature of measles and vaccine failure, intense exposure resulted in high risk even among revaccinees.[16]

This finding points out that the need of nation-wide serosurvey to explore the status of herd immunity and to evaluate the in-effectiveness of immunization.

In the United States where measles have been eliminated since 2002 with effective vaccination strategies, 140 confirmed measles cases were reported in 2008, all of which were either imported or import related.[16] Most cases were younger than 20 years and 91% were unvaccinated or had an unknown vaccination status. Failure to immunize all children, primary vaccine failure and importation from endemic countries are the reasons why measles has not fully been eliminated from the United States.[17]

In this study, increased seropositivity was detected in two-dose vaccinees but it was not significant. This may be due to the limited number of the study participants.

Conclusion

Measles is one of the eradicable infections which can be prevented by effective vaccination. Lack of availability to check the antibody response after immunization is very important because 5% of the vaccinated persons were found to have no antibody response after 5-10 years. Therefore, 2-dose immunization schedules should be encouraged and measles antibody serosurveillance studies should be carried out to check the antibody response of the vaccinated persons after immunization.

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