

MYANMAR

Volume 12, No. 1-3, 2000

HEALTH SCIENCES

DEPARTMENT OF MEDICAL RESEARCH

(Lower Myanmar)

No. 5, Ziwaka Road

Yangon 11191, Myanmar

RESEARCH JOURNAL



CONTENTS

- Green pit viper (*Trimeresurus erythrurus*) bites in Myanmar: clinical features, venom antigen levels and development of natural venom antibodies.....1
Tun Pe, Aye Aye Myint & Nu Nu Aung
- Russell's viper (*Daboia russelii siamensis*) toxoid: variation in biological properties of stored toxoid.....7
Aye Aye Myint, Tun Pe & Kyi May Htwe
- Reproductive tract infections among gynaecological patients at Central Women's Hospital (CWH, OPD).....10
Mar Mar Nyein, Katherine Ba Thike, Hla Hla Win, Wah Wah Aung, Moh Moh Win, Khin Thet Wai, & Y.F. Ngeow
- Cost analysis of hospitalized children with two common diseases (acute respiratory tract infections and diarrhoea) in Yangon Children's Hospital.....14
Aung Kyaw Zaw, Than Nu Shwe, Mya Mya Yee, Yi Yi Myint & Soe Thein
- Fertility-related attitudes and behaviour of married men in Kyaunk-tan Township.....21
Le Le Win, San Shwe, Khin Myint Wai & Nyo Aung
- Antimicrobial susceptibility testing of *Shigella* species and enteropathogenic *Escherichia coli* (EPEC) isolated from dysenteric cases of children in Yangon Children's Hospital during 199625
Mar Mar Nyein & Khin Myat Tun
- Antibiotics susceptibility pattern of *Vibrio cholerae* O1, O139, *Salmonella typhi* and *Escherichia coli*30
Mar Mar Nyein, Wah Wah Aung, Mi Mi Htwe, Tin Ohn & Than Nu Shwe
- The role of lepro-agglutination test for serodiagnosis of leprosy in Myanmar.....36
Khin Nwe Oo, Nwe Nwe Yin, Hauk Kyng, Kyaw Nyunt Sein & Kyaw Kyaw

A double blind comparative trial of two dosage regimens of artesunate suppository in combinations with oral mefloquine in severe falciparum malaria	41
<i>Marlar Than, Zay Soe, Aung Zaw Oo, Aye Yu Soe, Kyi Kyi Tin, Khin Nyo & Myat Phone Kyaw</i>	
Low prevalence of lactose malabsorption and lactose intolerance in Myanmar top athletes	48
<i>Ye Tint Lwin, Myo Khin, Tin Oo, Ni Win, Than Than Lwin, San Kun, Mi Mi Nwe., Soe Min Thein, Tin Nu Swe & Hla Pe</i>	
Short report: ABO blood groups and susceptibility to malaria	52
<i>Khin Saw Aye, Soe Soe, Aung Htay & Than Swe</i>	
Short report: Bacterial quality of water from Kanbauk areas during 1997	54
<i>Mar Mar Nyein, Myint Oo, Sein Hla Kyaw, Nyan Moe, Thuzar Myint, Aye Aye Myint & Eldin Pedecoulas Philippe</i>	

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Green pit viper (*Trimeresurus erythrurus*) bites in Myanmar: clinical features, venom antigen levels and development of natural venom antibodies

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Green pit viper (*Trimeresurus erythrurus*) bite is common in Myanmar and only few bites have been reported. Clinical features, venom antigen levels and development of venom antibodies in four green pit viper bites were presented. Pain and local swelling started early after the bite and spread to involve more than half of the bitten limb in 2-3 days and subsided on 5 days after the bite. Incoagulability of blood was observed as early as 1.15 to 2.40 h after the bite. One systemic envenomed case presented with vomiting, abdominal colic, sweating, hypotension and shock. Thrombocytopenia, neutrophilic leucocytosis, anaemia and raised blood urea were present for a week. Initial venom antigen levels ranged from 47 to 103 ng/ml. Antigen clearance depends on "venom hours". The pattern of antibody response following the bite was short lived in nature. Monospecific Russell's viper antivenom has no effect on correcting coagulation defect.

INTRODUCTION

Green pit viper (*Trimeresurus erythrurus*) bite occurred throughout Myanmar and has an incidence of 4% bites per year and a fatality rate of 0.5% [1]. Although it carries very low mortality rate, because of non availability of specific antivenom, incoagulable blood remains for many days and resulting in loss of many working days. Although its bite is common, there were few reported cases [2]. Venom antigen levels and development of natural venom antibody following bites have never been reported.

MATERIALS AND METHODS

The studies were conducted at the Yangon General Hospital, Yangon and at Thayarwady hospital, 80 Km north of Yangon. Clinical data were recorded on standard proforma. The extent of local swelling was graded from 1 to 6 [3]. A simple whole blood clotting test was performed according to the method

described by Warrell *et al.* [4]. Clot quality was assessed according to Reid *et al.* [5]. Platelet count, total and differential white cells counts, blood urea were carried out by the standard methods.

Venom antigen and antibody detection

An enzyme immunoassay technique (EIA) was used for detection of venom antigen and venom antibody [6-7]. For controls of antigen assay, 103 normal healthy adults with the same socioeconomic status and the snake bite victims but who had no recollection of snake bite were screened for venom antigen by EIA. The mean absorbance value was 0.004 ± 0.01 (1SD). An absorbance value of 0.014 was used as a cut-off point. The minimum level of venom detection was 10 ng/ml and the coefficient of variation of the test was 10-12%. The specificity of rabbit antivenom was checked against *N. kaouthia* (monocellate cobra), *B. fasciatus* (banded-krait), *Ophiophagus hannah* (king cobra) and *Daboia russelli siamensis* (Russell's viper) venoms.

Table. Clinical details of four *T. erythrurus* bite cases

Case	1	2	3	4
Age/sex	24 female	50 male	27 male	60 male
Site of bite	Dorsum right hand	Back left leg	Dorsum left thumb	Dorsum left hand
Non clot detected	2.45h	2.30h	2.45h	93h
lasted	3 days (D/C)	26 days	9 days (D/C)	6 days
Venom levels	20h-85ng/ml 44h-18ng/ml 68h-nil	19h-103ng/ml	245h-47ng/ml 1045h-12.5ng/ml 18h-nil	NA
Local swelling	Elbow D2	Mid thigh D2	Mid arm D2	Mid arm D3
Clot quality	ND	ND	Grade V-D 1-3 Grade IV-D 4-6 Grade III-D 7-9	

D/C = discharge from the hospital
NA = not available

ND = not done
D = day

For antibody detection, the same controls were screened for development of venom IgG and IgM antibodies. The mean optical density of the controls for IgG antibody was 0.0016 ± 0.003 (cut-off point = 0.0046) and for IgM antibody was 0.002 ± 0.005 (cut-off point = 0.007).

RESULTS

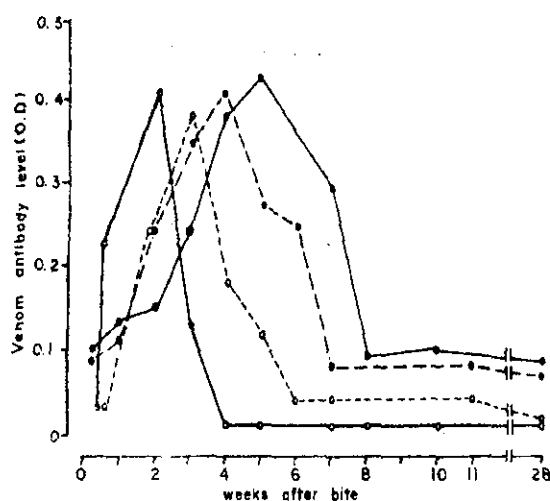
Clinical details of the four *Trimeresurus erythrurus* bite victims are shown in the table. Clinical features of one case will be presented.

A 50 yr old man from Bahan Township, Yangon was bitten by a unknown snake at the back of left leg on 14 September 1990 at 1920 h and was admitted to the Yangon General Hospital. Fang marks measured 20 mm apart. Fifteen minutes following bite, he felt severe pain at the site of bite and heaviness of the bitten limb. There was no bleeding, no bruising nor blister. Local swelling which rapidly spread to mid thigh on day 2. His blood pressure was 110/70 mmHg and a pulse rate of 100/minute. Clotting test on admission was normal. Two and half hour after the bite, he complained

of severe pain at the site of bite and abdomen, vomiting, profuse sweating with a blood pressure of 50/0 mmHg and a heart rate of 100/min. Clotting time was prolonged. Forty mls of monospecific Russell's viper antivenom with pressure agents were given to combat shock. There was no urine output since the onset of shock until blood pressure was restored to normal after 12 h. A bottle of fresh blood was infused on day 4. His blood remained incoagulable until 26 days after the bite. He was discharged from the hospital on the following day.

His platelet count was low up to 11 days after the bite (range $18-98 \times 10^9/L$) and platelet count returned to normal range thereafter. He had mild neutrophilic leucocytosis (10-17,000/cmm). His blood pressure was stable following recovery from shock. Blood urea rose to 16.8 nmol/L and returned to 5 nmol/L on day 11 after the bite. Haemoglobin level started to drop on day 7 (10 gm%) and returned to normal level (14g%) in 19 days after the bite. The earliest sample available for screening venom antigen at 19 h after the bite was 103 ng/ml. Subsequent samples were not

available until 4 days after the bite where no venom antigen was detected. Development of venom IgG and IgM antibodies following the bite were shown in the figure.



○ IgG and • IgM, — case 1 and - - case 2.

Fig. Venom IgG and IgM antibody levels of two *T. erythrurus* bite patients

DISCUSSION

An extensive literature review on green pit viper bites has been carried out by Hutton *et al.* [8]. Green pit viper bite (*T. erythrurus*) is common in Myanmar. Most believe that it bite leads to massive local swelling only and do not bother to seek medical treatment. No one brought the responsible snake for identification since the green pit viper (Mwe-sein) is known to every body in Myanmar. Its true incidence of bite has been underestimated. Of seven species known to be inhibited in Myanmar [9] (*T. albolabris*, *T. erythrurus*, *T. jardonii*, *T. mucrosquamatus*, *T. popeiorum*, *T. purpureomaculatus* and *T. stejnegeri*) *T. erythrurus* is the most frequently encountered species and was responsible for most bites. Previous reported cases from Myanmar [10] was also believed to be due to *T. erythrurus* bites.

Clinical features

Pain and local swelling started early after the bite and spread to involve more than half the bitten limb, reached maximum in 3 days

after the bite and took 5 days to subside after the bite. A similar features were observed following *T. albolabris* bite [8]. Local blister and necrosis were not observed.

The patient (No. 2) developed vomiting, abdomen colic, sweating and shock 2.40 h after the bite. Development of shock in a child and in two cases of *T. albolabris* bite had been reported [8,11]. Hypotension and shock may be due to extravasation of plasma and fluid into the bitten swollen limb. The same patient failed to produce urine for 12 h following onset of shock which was revived after 12 h but blood urea remained high for a week. It may be secondary to hypotension or complex mediated renal injury following infusion of equine Russell's viper antivenom. Renal failure following green pit viper bite is rare but has been reported earlier [12]. Shock and renal failure are common features of Russell's viper bite cases in Myanmar [13].

Haemostatic abnormalities

Like other *Trimeresurus* species, bites usually result in massive local swelling and development of incoagulable blood (for literature review see Hutton *et al.*, [8]). Incoagulable blood was detected as early as 1.15-2.40 h after the bite. Three out of four of our cases had incoagulable blood on admission. Late development of incoagulable blood in 93 h after the bite was observed in one case (No. 4). A similar late onset of incoagulable blood 9-47 h after admission in *T. albolabris* bites had been reported [8]. Late onset of development of incoagulable blood following bites by other vipers whose venom cause defibrination, *Echis carinatus* [4], *C. rhodostoma* [14] and *D. russelii siamensis* [6,15] has been reported. This lag phase probably represent the period before plasma fibrinogen level has fallen to a critical level. The short lived defibrination state suggested that the amount of venom injected into the body may be small. In an earlier report of the

green snake bite with no antivenom in Myanmar [10], blood incoagulability persisted for 11 days whereas in ours it persisted for as long as 26 days.

Detail haemostatic abnormalities following *T. erythrurus* bite has not been carried out. The coagulability induced by green pit viper (*T. albolabris*) has been studied *in vitro* and in envenomed patients [11, 16-19]. They have demonstrated that disturbances are consistent with the activity of a thrombin-like procoagulant, increased fibrinolytic activity and direct aggregation of platelet. Thrombocytopenia was found in 25% of the bitten children [16] and a higher thrombocytopenia was observed in adult patients [20]. In our case (No. 2) thrombocytopenia persisted for a week following the bite, accompanied by neutrophil leucocytosis and anaemia.

Study of clot quality following the bite in case (No. 3) indicated that there was a slow restoration of clot quality taking 9 days to convert grade V to III clot although venom antigen could not be detected in 18 h onwards. The clot quality assessment suggested that stability of the clot remains abnormal indicating the state of imbalance between thrombin-like procoagulant and synthesis of fibrinogen. The rise in fibrinogen levels following green pit viper bite was slow (72 h) compared to that seen following treatment of bites by *C. rhodostoma* (12-24 h) [8] where the latter toxin induced more extensive tissue damage which probably presents a greater stimulus to fibrinogen production than small wounds resulting from *T. albolabris* bites. Since clot restoration time following *T. erythrurus* bite takes days and weeks, it will be wise to monitor haemostatic defects using more specific and quantitative tests in future.

Venom antigen levels

Circulating venom antigen was detected in all 3 tested cases. It has been shown that circulating venom antigen could be detected in 39% of 59 patients envenomed by *T.*

albolabris and only 24% had incoagulable blood at the time of presentation [21]. Of 20 *T. erythrurus* bites studied [2], 65% showed no evidence of envenoming.

Venom antigen clearance time probably depends on the "venom hours" which is the product of initial venom concentration in ng/ml and the time elapsed between bite and admission to the hospital [8]. Venom antigen clearance time of case [1] (venom hours 1700) took 68 h compared to that of case [3] (venom hr 129.25) where early clearance of antigen was observed (18 h).

Venom antibody levels

The pattern of IgG and IgM antibody responses of two cases (case 1 & 2) was similar where IgM antibody response peak at 2-3 wks and declined to low level in 4-6 wks after the bite. IgG antibody response peaked 2 wks later than that of IgM and remained detectable up to 28 wks after the bite. The pattern of antibody response of the two *T. erythrurus* bite patients resembles those of the King cobra (*Ophiophagus hannah*) [22] and *Bothrops jararaca* (jararaca) bites [23]; unlike that seen in cases of *Daboia russelii siamensis* bites [7] where a slow peaking of antibody response was observed.

Antivenom treatment

Antivenom has a definite role in early restoration of normal blood clotting. Since monospecific green pit viper antivenom is not available in Myanmar, most cases were kept on observation. Some were treated with Russell's viper antivenom because of prolonged clotting time. Earlier studies [10] found that *Vipera russelii* antivenom, epsilon-aminocaproic acid and fibrinogen were not effective in correcting clotting defect.

Complications

In Mandalay, upper Myanmar, a patient died with intra abdominal haemorrhage after being massaged (Sann-Mya, personal

communication). In our case (No. 2) blood remained incoagulable for 4 wks resulting in loss of many working days. Ecchymosis was observed at the site of injections.

ACKNOWLEDGEMENTS

We are grateful to Professor Mya Mya Win and staff of renal unit, Yangon General Hospital and staff of Clinical Snakebite Unit, Department of Medical Research for collection of samples and providing data of the patients.

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Russell's viper (*Daboia russelii siamensis*) toxoid: variation in biological properties of stored toxoid

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Biological properties of formalin-treated Russell's viper venom toxoid stored at +4°C was monitored by using the WHO recommended methods at 2,3,4,6,10 and 12 months following preparation. Formal toxoiding of the venom resulted in reduction of 15- fold in lethal, 4- fold in coagulant, 22- fold in defibrinogenating, 6- fold in haemorrhagic and 10- fold in necrotic activities. A 44- fold reduction in capillary permeability increasing activity of the toxoid was observed from 4 months onwards. Further two-fold reduction in haemorrhagic and necrotic activities of the toxoid was noted from 6 months onwards. Decrease in number of protein bands in SDS-PAGE electrophoresis was observed when the toxoid aged. Such variation in biological properties of stored toxoid will influence the quality of antibody produced and hence the protective immunity of the immunised subjects.

INTRODUCTION

Russell's viper bite is an occupational hazard of our farmers. In 1997, there were 8000 bites with 500 deaths [1]. In spite of antivenom therapy, mortality rate remains high. Prophylactic active immunisation of the population at risk with Russell's viper (RV) toxoid will be helpful in reducing mortality rate. Effect of storage temperature on stability of RV toxoid had been studied [2]. Monitoring of biological properties of toxoid on storage was not studied in the earlier reports on formalin-treated Russell's viper toxoid [3-4]. Reversion of formalin linkage with venom in Russell's viper toxoid stored at 4° and 37°C after three months has been reported [5]. The objective of this study was to monitor biological properties of Russell's viper toxoid during storage at 4°C.

MATERIALS AND METHODS

Toxoiding of crude Russell's viper (*D.r.siamensis*) venom (batch 90)

(Myanmar pharmaceutical Factory) (MPF) was carried out by stepwise formalinisation method [4,6]. The final preparation containing 2 mg protein/ml and aluminium phosphate was aliquoted and stored at 4°C. Biological properties of the toxoid such as lethality (LD₅₀ iv), haemorrhagic (MHD), necrotic (MND), coagulant (MCD), defibrinogenating (MDD) and capillary permeability increasing activities (MCPID) were monitored according to the WHO recommended methods [7] at 2, 3, 4, 6, 10 and 12 month following preparation. Immunogenicity of the toxoid was compared with the crude venom using 1% agarose gel immnuodiffusion plate [8]. SDS-PAGE electrophoresis of the unreduced toxoid was run on 12% separating gel [9]. Details of the methods have been already described [10].

Two Japanese white hybrid rabbits (Laboratory animal services, Department of Medical Research) weighing 2.5 kg were immunised with three doses of monthly injection of the toxoid aged 1, 2 and 3 months respectively. Neutralisation of the

biological activities of the venom by the rabbit serum collected at six week following three injections of the toxoid and a monospecific antivenom (MPF), batch DM90680E (expiry January 1996) were tested [7]. The latter was tested before it's expiry date. Briefly, various amounts of antivenom were mixed with a fixed amount of venom, incubated at 37°C for 30 min and then injected into the rats or mice accordingly. ED₅₀ neutralising dose of the antivenom was estimated as the amount of antivenom which will neutralise 50% of the activity of venom.

RESULTS

Biological properties of the toxoid of varying ages were shown in figure 1.

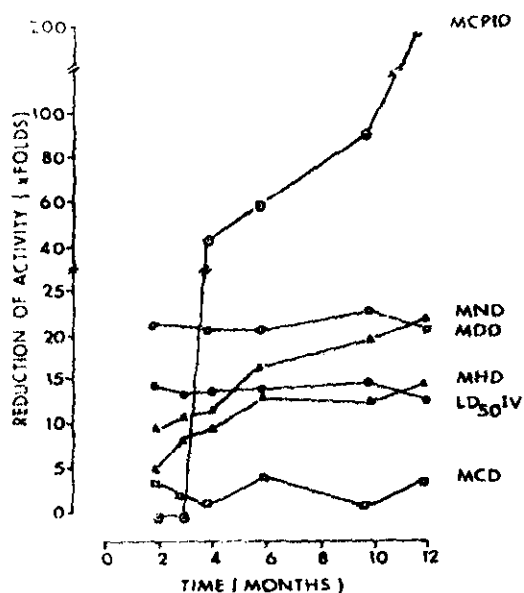


Fig.1. Biological properties of the Russell's viper toxoid of varying ages.

LD₅₀ iv = 50% lethality
MDD = minimum defibrinogenating dose
MCD = minimum coagulant dose
MHD = minimum haemorrhagic dose
MND = minimum necrotic dose
MCPID = minimum capillary permeability increasing dose

Reduction in 15 fold LD₅₀, 4 fold MCD, 22 fold MDD, 6 fold MHD and 10 fold MND was observed at two-month-old toxoid. No

decrease in capillary permeability increasing activity was noted until four months following preparation where a reduction of 44-fold was observed. Further two-fold reduction in haemorrhagic and necrotic activities of toxoid was noted from 6 months onwards. Coagulant activity fluctuated during storage. Lethality and haemorrhagic activities remained stable throughout a year of storage.

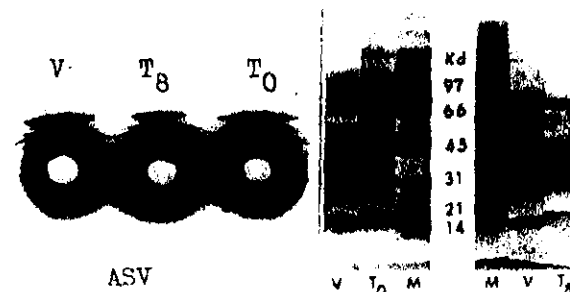


Fig. 2. Immunodiffusion (left) and SDS-PAGE electrophoresis (right) of the Russell's viper toxoid of varying ages.

V = venom
T0 = freshly toxoid
T8 = 8-month old toxoid
M = markers
ASV = antivenom

Quantitative decrease in number of precipitin and protein bands in SDD-PAGE electrophoresis and immunodiffusion was observed when the toxoid aged (fig. 2). Neutralising efficacy of the toxoid immunised rabbit serum is: 40ul for 1MLD, 80ul for 1MHD, and 1MND, 20ul for 1MDD, 5ul for 1MCD and 1.25ul for 1MCPID and that of the monospecific antivenom is 1.5ul for 1MLD and 1MHD, 3.12ul for 1MND and 1MDD, 0.02ul for 1MCD and 1.5ul for 1MCPID.

DISCUSSION

Formal toxoiding leads to decrease in toxicity far more greater than that produced by gamma irradiation (100 Krad) of venom (3-fold LD₅₀, 1.75-fold MHD and 4.5-fold MND) [11]. The rabbit serum raised with the former had less venom neutralising efficacy than that of antivenom raised with

the latter where 100ul of rabbit antivenom will neutralise 5LD₅₀, 8MHD or 8MND. However the latter antivenom had less neutralising effect on PLA [11] or may ever devoid of anti protease or PLA activity [12]. It is interesting to note the MCPI activity of the venom was not affected until four months of storage. It was supported by venom neutralisation study of toxoid immunised serum. The capillary permeability increasing activity is exclusively seen in Russell's viper (*D.r. siamensis*) bite cases of Myanmar and its presence signifies a bad prognosis. Further 44-folds decrease in the CPI activity and two-fold reduction in haemorrhagic and necrotic activities of the aged toxoid will further weaken the neutralising properties of the antivenom raised. It is expected that rabbit antivenom raised with formal toxoid will be far less potent than commercial monospecific antivenom, however it may confer some degree of protective immunity when snakebite occurs. Four to 22-folds reduction in biological activities of the toxoid at two months of storage calls for use of either freshly prepared toxoid or toxoid stored up to 2 months should be used in active prophylactic immunisation against Russell's viper bite.

Further reduction in biological activities of the stored toxoid will make it less suitable for use as an immunogen. Means to improve stability of toxoid on storage should be sought for.

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Reproductive tract infections among gynaecological patients at Central Women's Hospital (CWH, OPD)

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Ninety cases of women with vaginal discharge attending the Out-Patients Department (OPD) of Central Women's Hospital (CWH), Yangon were subjected for the isolation of pathogens during March and April, 1999. These subjects comprised of 39 cases with vaginitis and/or cervicitis; 41 cases with Pelvic Inflammatory Diseases (PID) and 10 cases with other infections. Their ages ranged from 19 to 78 years. They were from various sectors of Yangon (72 cases) and from its outskirts (8 cases). Pathogens were isolated from 28.21% of the cases with vaginitis and/or cervicitis; 24.39% with PID and 20% of the cases with other infections. *Neisseria gonorrhoea* was isolated from two cases (2.22%), one with Pelvic Inflammatory Disease (PID) and another with vaginosis. These strains were sensitive to cefaclor and amikacin. *Staphylococcus aureus* was isolated from three cases (3.33%) and these strains were sensitive to augmentin, carbenicillin, cephalothin and vancomycin; *Candida albicans* isolated from eight cases (8.88%); *Trichomonas vaginalis* isolated from three cases (3.33%), *Chlamydia* antigen was detected from 17.95% of the cases by Clear View kits. Clue cells were detected from 25 cases (27.50%). Bacterial vaginosis was found to be the commonest organism and then followed by *Chlamydia*.

INTRODUCTION

The Sexually Transmitted Diseases (STDs) represent a major public health problem in developing countries. Failure to diagnose and treat infections such as gonorrhoea, *Chlamydia* and syphilis can have deleterious effects on pregnancy and the newborn (such as miscarriage, prematurity, congenital and neonatal infections). Other implications particularly in women are Pelvic Inflammatory Diseases (PID), ectopic pregnancy, infertility and cervical cancer. Sexually Transmitted Disease is common in various countries [1]. The World Health Organization estimates that every year there are 333 million of new cases of STDs

around the world. They have far reaching health, social and economic consequences for many countries. According to the National Health Plan (NHP) of Myanmar (1993-1996), STDs ranked as the sixth priority health problem out of 39 diseases [2]. Existing data on reproductive tract infections among Myanmar women are somehow limited both in quality and in scope.

Objectives

The objectives are to explore the distribution of Chlamydial, gonococcal, *Candida* and *Trichomonas* infections among the women attended at Yangon Women's

Hospital with a note to antibiotic susceptibility of isolated pathogens.

MATERIALS AND METHODS

Subjects

A total of 90 patients with vaginal discharge attending the Out-Patients Department of Central Women's Hospital, Yangon were tested. These include 39 cases with vaginitis and/ or cervicitis; 41 cases with PID and 10 cases with other infections. Their ages ranged from 19 to 78 years.

Study period

It lasted from 4-3-99 to 30-4-99.

Collection of specimens

Endocervical swabs were taken in normal saline soaked swab sticks and brought to Bacteriology Research Division in Amies transport media. Simultaneously, the vaginal swabs were taken in normal saline. It was

transported in cold chain from the hospital to Bacteriology Research Division, DMR [3].

Identification of pathogenic organisms;

Wet mount and gram stained preparations were done for examination of red blood cells, epithelial cells, clue cells, yeasts and *Trichomonas*. Chocolate agar and Thayer Martin agar were used for culturing of *Neisseria gonorrhoea*. Sabouraud dextrose agar was used for culturing of *Candida albicans* and confirmed by germ tube test. *Chlamydia* antigen was detected by Clear View and CELISA test kits.

Antibiotic susceptibility test

It was done by Kirby-Bauer method [4].

RESULTS

Out of 90 cases tested, 23 cases (25.56%) were isolated with pathogens. These subjects were from 33 different places, out of which, pathogens were isolated from the subjects from Thaketa, Hlaingtharyar,

Table 1. Pathogens isolated from different areas

Areas	Tested	Chlam.	C. alb.	Tricho.	Staph.	N. gon.	Total
Thaketa	10	1	3	1			5 (50.00)
Hlaingtharyar	7	1	2				3 (42.86)
Dagon (N & S)	7	1	1	1			3 (42.86)
Dala, Dawpon	10	1	1			2	4 (40.00)
Ahlone	6	2			1		3 (50.00)
Tamwe	4				1		1 (25.00)
Kamayut, Insein	7	1		1	1		3 (42.86)
Lapputta	1		1				1
Others	38						Nil
Total	90	7	8	3	3	2	23

Figures in parenthesis denote percentages

Table 2. Pathogens isolated from different age groups

Areas	Tested	Chlam*.	C. alb.	Tricho.	Staph.	N. gon.	Total
19-20	4		1 (25.0)			1 (25.0)	2 (50.0)
21-30	31	2/14 (14.29)	5 (16.13)				7 (22.58)
31-40	27	5/14 (35.71)	2 (7.41)	1 (3.70)	2 (7.41)		7 (25.93)
41-50	13			1 (7.69)			2 (15.39)
51-60	10			1 (10)	1 (10)	1 (10)	3 (30)
61-80	5						0
Total	90	7/39 (17.95)	8 (8.88)	3 (2.22)	3 (3.33)	2 (2.22)	23 (25.56)

Figures in parenthesis denote percentages *tested on 39 cases

Dagon, Dala, Dawpon, Ahlone, Tamwe, Kamayut, Insein and Lapputa Townships (Table 1.)

Pathogens isolated from different age groups are shown in Table 2. It was found that 50.00%, 22.58%, 33.33%, 15.39% and 30% were obtained from the age groups of 19-20; 21-30; 31-40; 41-50 and 51-60 years respectively.

Table 3. Pathogens from cases with sexual transmitted diseases

Sr. No.	Pathogens	No. cases	%
1	<i>Chlamydia trachomatis</i>	7	17.95
2	<i>Candida albicans</i>	8	8.88
3	<i>Trichomonas vaginalis</i>	3	3.33
4	<i>Staphylococcus aureus</i>	3	3.33
5	<i>Neisseria gonorrhoea</i>	2	2.22
CLUE CELLS		25	27.78

Table 4. Clue cells from different age groups

Age group	Tested	Clue cells	%
19-20	4	1	25.00
21-30	31	9	29.03
31-40	27	7	25.93
41-50	13	3	23.08
51-60	10	4	40.00
61-80	5	1	20.00
Total	90	25	27.78

Different types of pathogens from the tested cases are shown in Table 3. *Neisseria gonorrhoea* was isolated from two cases (2.2%), one with Pelvic Inflammatory Diseases (PID) and another with vaginitis. These strains were sensitive to cefaclor and amikacin and they were resistant to streptomycin, septrin and tetracycline. *Staphylococcus aureus* was isolated from three cases (3.3%) and these strains were sensitive to augmentin, carbenicillin, cephalothin and vancomycin; resistant to penicillin and erythromycin. *Candida albicans* was isolated from eight cases (9.9%), *Trichomonas vaginalis* isolated from three cases (3.3%). *Chlamydia* antigen was detected from seven out of 39 cases tested (17.95%). Clue cells were detected from 25 cases (27.8%). However, *Chlamydia* antigen

was not found by CELLSA. Clue cells from different age groups are shown in Table 4. It was found that 40 percent of the cases were obtained from the age group of 51-60 years, with markedly increased than other age groups.

DISCUSSION

The introduction of oral contraceptives and intrauterine devices in the 1960s was followed by changes in human behavior as well as a marked increased in sexually transmitted diseases with a significant increase in the proportion of the population between the ages of 15 to 29 years. However, some organisms that are part of the flora and are normally present in very low numbers may increase sufficiently in number to cause symptoms. Infections may extend to the upper genital tract, involving the uterus, fallopian tubes, ovaries, or abdominal cavity (PID).

Role of bacterial vaginitis in pelvic inflammatory disease was reported by Sweet, 1995 [5]; and PID is a frequent infection in sexually active women and results in adverse sequelae, including tubal-factor infertility and ectopic pregnancy. Joesoef and Schmid, 1995 [6] reviewed the data on the treatment of bacterial vaginitis, published from 1989 through 1992 and suggests that oral metronidazole, 500 mg twice daily for seven days is the preferred treatment for bacterial vaginitis. Klebanoff *et al.*, 1995 [7] reported on the outcome of the vaginal infections and prematurity study, which were colonized with group B streptococci. Korn *et al.*, 1995 [8] also studied on plasma cell endometritis in women with symptomatic bacterial vaginitis. Singh *et al.*, 1995 [9], reported the clinical presentation of gynaecologic infections among Indian women. Papanicolaou stained vaginal smears and gram stained smears were reported by Platz-Christensen *et al.* [10]. The amino tests as a simple, rapid, inexpensive method for diagnosing bacterial vaginitis was also

introduced by Sonnex [11]. Anaerobes are also associated [12]. In our study the pathogens were isolated more (50%) in the age group of 19-20 than other age groups and not much decrease in the age group of 31-40 with the isolation rate of 25.93%. *Chlamydia* infection seems to be more prominent (17.95%) then followed by *Candida* infection (8.88%). The CLEAR VIEW test kit is so simple to detect the antigen and it might be very useful to study in the field. The STD Control Programme in our country will have to educate on prevention of STD, for interruption of STD transmission and prevention of complications sequelae as well as prevention of HIV/ AIDS in accordance with WHO recommendation [13]. The importance of STD in Paediatric gonococcal infection was also reported [14]. Thus, further laboratory diagnosis for STDs need to be carried out with antibiotic susceptibilities testing in different socioeconomic status in our community.

ACKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to Dr. U Soe Thein, Deputy Director-General, DMR, without his advice, this project will not be completed. To Dr. P. J. Rowe for advising and encouragements for the project and to Professor Y. F. Ngeow for supplying the CLEAR VIEW kits. Last, but not the least to the Superintendent of the CWH and staff of OPD, Yangon.

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Cost analysis of hospitalized children with two common diseases (acute respiratory tract infections and diarrhoea) in Yangon children's Hospital

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The cost of hospitalization of (477) patients admitted to YCH for two common diseases (viz: ARI 282 cases and diarrhoea 195 cases) were determined using semi-structured questionnaire and face-to-face interview. The main aim of the study was to reveal the direct and indirect costs incurred by the parents/families during the children's illness and the cost contributed by the government so that the findings might help in future policy implication. The mean cost of hospitalization of each child was 1705 Kyats and the median cost was 1350 Kyats. The government contributed about 36% of the cost of hospitalization. The cost borne by the family amounted to 64% of the total cost, of which only 13% were for medicine and investigations. The cost of hospitalization amounted to about 24.7% of their monthly income. It was noted that majority of the cases could be effectively treated at the hospitals in their local area. Despite having some extent of financial difficulty, more than 80% of the families were willing to pay for the hospital cost although 18% did not want to contribute anything at all. This study highlighted that: (a) public awareness regarding the availability of effective health care of the two diseases at their respective local hospitals should be promoted (This will reduce the unnecessary financial burden imposed on the families due to travel and food expenses if they attend YCH) (b) an interim appropriate local health care financial support mechanism should be considered for the poor patients attending tertiary hospitals before nationwide comprehensive health care financial support system is established.

INTRODUCTION

Since independence, the government had provided free medical services to every person living in the country. However, lately, it was realized that government funding alone was not sufficient to fully finance both preventive and curative care including special care services.

After 1988, with the change in the socioeconomic system, the Ministry of Health, under the guidance of National Health Policy, has introduced community cost sharing mechanisms in some hospitals [1]. Yangon Children's Hospital (YCH) introduced community cost sharing

mechanism in 1996. However, the services rendered by hospital staff, emergency treatment, routine treatment and care of the poor patients are still provided free of charge.

Some studies done in other countries suggested that hospitalized patients of lower socioeconomic status have longer stays and probably require more resources because of prolonged hospitalization [2,3].

On the other hand, people when they have to pay, they failed to appreciate the government contribution in terms of providing capital items and services in the hospitals.

This study attempted to find out the direct and indirect costs incurred by the parents during their children's illness, their financial source, their willingness to pay and the recurrent cost contributed by the government so that the results might help in future policy implication.

Aim

To estimate the proportion of cost borne by the parents and the proportion of cost contributed by the government during hospitalization of the children with the above two common diseases.

Specific objectives

- (A) to calculate the total amount of cost (direct & indirect) incurred by the parents/families during the period of hospitalization of their children with two common diseases, acute respiratory tract infections and diarrhoea
- (B) to calculate the amount of cost contributed by the government during the period of hospitalization
- (C) to determine the socio-economic background of these parents/families
- (D) to determine the duration of stay in hospital in relation to their socio-economic background
- (E) to evaluate the hospital cost and its socioeconomic implications
- (F) to find out the "willingness to pay" by the parents/families

MATERIALS AND METHODS

Study population

Parents/guardians of children who were admitted to Medical Unit III, YCH between 1st March and 31st August 1997 for acute respiratory tract infections (ARI) and diarrhoea and who were discharged on recovery, were included in this study.

Exclusion criteria

- Parents/guardians of children who expired
- Parents/guardians of children who were

admitted as private patients

- Parents/guardians who refuse to participate in the study

Data collection techniques

Face to face interview using a semi-structured questionnaire was conducted at the Medical Unit III of YCH. The purpose of the study and confidentiality of the data were explained to the respondents by the interviewer before the interview. The interview was conducted in a comfortable room, first time on the 2nd day of hospitalization and second time on the day of discharge so as to calculate the total costs.

Calculation of the cost of hospitalization

Cost contributed by the government was calculated by dividing the budget (money) allocated to the hospital (recurrent cost) for a year with the total number of patient-days for that year e.g. $9084450/126858=71.7$ Kyats per head per day. Capital cost was left behind because of technical difficulties in estimating the cost. The cost of medicine supplied to the patient "free of charge" was calculated according to the prices sold at the "cost-sharing" at that time. The cost of investigations was included under the government contribution if the investigations were carried out free of charge.

The cost incurred by the family was obtained by asking the mothers/attendants of their detailed daily expenses in connection with the hospitalization event.

Calculation of socioeconomic index

The socioeconomic status was determined by developing scales for father's education and occupation, mother's education and occupation, their housing condition, their source of water supply and sewage disposal. The socioeconomic index (SEI) [4] is the composite score and was made up of addition of these scores (annex 1). The scores ranged from 7 to 25. It was then

categorized into 3 grades. Those families who obtained scores less than 13 as SEI III, 13-14 as SEI II, 15 and above as SEI I.

RESULTS

A total of 477 parents/guardians of children admitted with acute respiratory tract infection (ARI) or diarrhoea was interviewed. Ninety four per cent of the attendants were mothers, and others were guardians or relatives.

Characteristics of the children

Nearly 85.5% of the children were under the age of 2 years. Male: female ratio was 1.07 to 1. Fifty one percent of the children were admitted for ARI and 49% for diarrhoea. The mean duration of illness prior to hospitalization was 3.5 days, 7.4% gave history of illness more than 7 days prior to admission. According to medical officer on duty, 13.3% were very ill at the time of admission, 80.3% were ill and only 6.7% were not ill at the time of admission.

Socioeconomic background

Most of the families came from extended families (43.8%). Nearly four per cent of the parents were widowed and 3.2% divorced. Sixty one per cent lived in huts, 26.6% lived in wooden houses, and 2.9% in semi-pucca buildings and only 9% lived in pucca buildings. Only 12.8% of the families have 24 hours water supply, 13.6% have artesian well, 34.8% have their own water sources (e.g. surface wells) and 38.8% has to rely on the public water source.

A few (3.2%) of the families have no latrines, 13.2% were still using surface latrines, majority used indirect pit latrines and only 9.9% used septic tank system.

Total income of a family ranged from no proper income (dependant) to Kyats 500,000/month. The mean income of a family was 12767 Kyats/month and median was 8600 Kyats/month. Fifteen per cent of

the families could be classified as SEI I (high social class), 20% as SEI II (middle social class) and 65% as SEI III (low social class).

Sixty two per cent of the families could not save any money from their monthly income, 15.7% could save less than 500 Kyats a month, 6.3% between 501 and 1000 Kyats a month and 15.9% could save more than 1000 Kyats a month. Majority (33.8%) of the families used their savings, 19.7% borrowed money from their friends, 1.5% of the families had to sell their goods, 3.5% had to pawn their properties for this event. Moreover, 39.4% of the families were in debt at the time of admission.

Parent's behavior and decision regarding hospitalization

The choice of the health care providers varied significantly between the different social class (table 1). Seventy five percent of the parents had faith in their health care providers and they never seek alternative treatment. However, 20% had changed the health care providers once and 3.2% twice, prior to admission mainly because they thought that their child did not improve with the treatment.

Forty three point five percent of the children were admitted according to doctor's advice. However, the mothers made their own decision in 49.8% of the cases. Two percents of the admissions were decided by grand parents and also by other health personnel. Three point five percent of the families admitted their children because it did not improve with GP treatment and another 3.9% were admitted because they could not afford GP treatment. Majority of the parents chose Yangon Children's Hospital because they believed they would have better medical services (48.5%). One percent said that it was more convenient for them to travel to YCH than to the local hospital, and only 0.4% said that they had already tried the local hospital services but they were not satisfied with the treatment.

Table 1. Relationship between socioeconomic status and health seeking patterns/disease severity

Factors	Socioeconomic status			Total n=452	P value
	*SEI I n= 66	SEI II n=91	SEI III n=295		
I. Health seeking patterns	(%)	(%)	(%)	(%)	0.00033
- General practitioner	48.5	49.5	53.5	52.0	(χ^2)
- Paediatrician	30.3	23.0	9.2	15.0	
- Traditional practitioner	3.0	6.5	6.1	5.8	
- Out-patient department	3.0	2.2	5.8	4.6	
- Others	1.5	4.4	10.8	8.2	
- Nil	13.7	14.4	14.6	14.4	
II. Disease severity	n=68	n=93	n=298	n=459	0.0008
- Mild	8.8	12.9	4.0	6.5	(χ^2)
- Moderate	82.4	82.8	79.2	80.4	
- Severe	8.8	4.3	16.8	13.1	
III. Willingness to pay	n=64	n=90	n=291	n=445	0.0001
- Yes	92.2	94.4	75.3	81.6	(χ^2)
- No	7.8	5.6	23.3	17.5	
- Others	0	0	1.4	0.9	
IV. Mean duration of stay in hospital	n=68	n=93	n=299	n=460	0.03**
- Days	5.12	5.69	6.19	6	(one way anova)

* SEI = Socioeconomic Index

** Significant different between SEI I & SEI III; Scheffe test

Fifteen percent of the cases did not come to the hospital soon after referral, because 8.7% hoped that their children would improve with time, 3.4% was due to financial reason and the others due to social reasons. But the parents really believed that their children needed hospital admission at the time. Delayed in hospitalization did not increase the duration of stay in hospital ($p=0.24$, χ^2) nor increase the hospital cost significantly in this study ($p=0.08$, χ^2).

Cost prior to hospitalization

Most of the families spent 20-2566 Kyats prior to admission, the mean value was 465 Kyats and median was 259.5 Kyats.

Cost of hospitalization

The average duration of stay in hospital of these children was 6 days, one day less than the average duration of stay of patients of all ages [5]. The total cost of hospitalization ranged from 293 to 13872 Kyats (table 2).

The proportions of cost incurred by patient's families and government were shown in table 3.

Table 2. Cost of hospitalization

Description	Range (Kyats)	Mean (Kyats)	Median (Kyats)
Total cost	293- 13827	1705.7	1350
Cost contributed by the government	97-3365	513.0	430.0
- services	71-2583	432.7	358.0
- medicine	0 -1193	70.3	36.0
- investigations	0 -265	10.5	0
Cost incurred by families	0-12433	1192.0	910.0
- medicine	0-3180	215.0	95.0
- investigations	0-600	37.0	0
- food	0-6226	426.7	270.0
- travel	0-8150	433.8	305.0
- others	0-790	65.4	40.0

The contribution of the government in terms of services, provision of some drugs and free investigations amounted to about 35.8% of the total cost (capital cost not included).

The direct cost of buying medicine and doing investigation amounted to 13% of the cost, whereas traveling expenses and expenses on food amounted to 25% and 22% respectively. In thirty six percent of the families, the government solely supplied medicines; in 58.9%, the government and

Table 3. Relationship between cost of hospitalization (Kyats) and residential areas and diseases

Determinants	Total cost	Cost of hospitalization							
		Government (%)				Family (%)			
		Service	Medicine & Investigation	Total	Medicine & Investigation	Food	Travel	Other	Total
I. Residential areas									
- All patients	1705	30.4	5.4	35.8	13.0	22.0	25.0	4.2	64.2
- Downtown area	1392	33.0	5.2	38.2	13.0	22.5	21.4	4.9	61.8
- Suburban area without specialist	1712	27.7	5.2	32.9	15.1	20.0	28.4	3.6	67.1
- Greater Yangon without specialist	1778	30.8	5.9	36.7	12.0	22.7	24.7	3.9	63.3
- *District without specialist	3666	26.2	4.8	31.0	8.1	33.0	25.1	2.8	69.0
II. **Diseases									
- ARI cases	1777	-	-	34.2	16.3	21.6	24.3	3.6	65.8
- Diarrhoea cases	1603	-	-	38.2	8.1	22.6	26.2	4.9	61.8

(p = 0.0000; one way anova); *(Significant different with the other groups; Scheffe test)

**p = 0.81; t-test

the families shared the cost of medicines. In 13.4%, investigation cost was borne solely by the government; in 7.7%, the cost of investigations was jointly shared by the families and the government.

Table 4. Disease severity and hospital cost

Hospital cost (Kyats)	Disease severity			Total
	Mild	Moderate	Severe	
<1350	24	200	15	239
1351-2280	5	118	21	144
>2281	3	65	26	94
Total	32	383	62	477

(p = 0.0000; χ^2)

Table 5. Total income and duration of stay in hospital

Total income in Kyats	Duration of stay in hospital			Total
	1-5 days	6-10 days	>10 days	
>15001	45	15	5	65
9001-15000	76	41	6	123
<9000	145	118	26	289
Total	266	174	37	477

(p = 0.022; χ^2)

The total cost was found to be significantly associated with the degree of severity on admission (table 4) and disease severity was significantly associated with socioeconomic status (table 1). The duration of stay in hospital was negatively correlated with socioeconomic status (table 1). Duration of stay was also associated with total income (table 5).

The cost of treating an ARI case was not significantly differed from treating a diarrhoea case (table 3) (t test; p = 0.81).

The average cost of hospitalization amounted to 24% of their total income. Nine percent of the families had to spend more than 50% of their monthly income and 5.4% more than their monthly income. A large majority of mothers (81.6%) were willing to pay for their children's medicines and investigation procedure. Naturally, "willingness to pay" varied significantly with their social status (table 1).

DISCUSSION

The present study is an exploratory study to determine the cost incurred by the patients

(user side) and the recurrent cost contributed by the government (provider side) in patients admitted to YCH for ARI and diarrhoea diseases. These two diseases were chosen, as they were the leading causes of morbidity of children through out the country. Moreover, WHO guidelines for the management of these two diseases have already been provided (through workshops and training programs) to most of the hospitals in the country through ARI programme and CDD programme. The study also aimed to have an insight into the socioeconomic implications faced by the patient's families to the introduction of community cost sharing mechanisms in the hospitals.

The government contributed about 36% of the cost of hospitalization of every patient. The cost borne by the family amounted to about 64% of the total cost of which only 13% were for medicines and investigations. Seventy percent of the children in this study did not need any investigation. This implies that these two diseases could be treated quite effectively even in 25-bedded hospital. A large proportion of the expense was spent on travel (25%) and on food (22%). This study also revealed that 27.7% of the families has specialist facilities in their local hospital. Most of the families were not aware of this facility, as only 0.4% of the families in this study had been to the hospital in the local area before coming to YCH. If these families sought health care at the hospitals in the local area a large proportion of the financial burden will be saved. A high proportion of malaria patients not attending the nearest service point was also observed in "Cost and performance of malaria surveillance" [6] study conducted in Thailand. Thus public awareness regarding the availability of effective health care at their respective local area hospitals for these common diseases should be promoted.

As expected hospital cost was related to severity (table 4) and severity was related to socioeconomic status (table 1). The mean

duration of stay in hospital was negatively related with the socioeconomic status. This implies that children from lower socioeconomic status were comparatively more ill on admission, they have to spend a much longer period in hospital and thus contributed to higher hospital cost. Epstein *et al.* [3] had also reported that patients from lower socioeconomic status had hospital stays 3-30% longer than patients from higher socioeconomic status and thus they require more resources. Fifteen percent of the patients did not come to the hospital soon after referral. Sixteen percent of the participants from Massachusetts's hospitals reported similar delay in obtaining their health care [2]. The reason was also very similar. Majority of the patients thought the problem would go away or was not serious enough. Myanmar families did not consider that their stay in hospital is their "lost time" they could spend in other matters. Not a single family complained of the time lost. Their only concern is to have the best services for their children.

In Mexico, the cost of medicine amounts to 16.6% of the income of low-income family for treating a diarrhoea patient. Traveling and accommodation expenses add to the financial burdens of families' [7]. In this study the cost of hospitalization amounted to about 24% of their monthly income. As the families in this study spent 82% of the family's monthly income on food and their children's education, the cost of hospitalization is quite a burden on such a family. Moreover, 39.4% of the families were in debts at the time of admission. 1.5% of the families had to sell their goods, 3.5% pawned their properties and 19.7% had to borrow money from their friends for this event. Even then, a large majority of mothers (81.6%) were willing to pay for their children's medicine and investigation procedures. Thus it is felt that an interim appropriate local health care financial support mechanism should be considered for the poor patients who need tertiary care before a nationwide comprehensive health

care financial support system could be developed.

It is hoped that the findings of this paper will, to some extent, contribute in efficient re-allocation of available resources to provide a better context of health care provision.

CONCLUSION

The government contributed thirty six percent of the cost of hospitalization. The cost borne by the family amounted to about 64% of the total cost of which only 13% were for medicine and investigations. A large proportion of the expense was spent on travel (25%) and on food (22%).

Effective service performance of the local hospitals should be made known to the public. Changes in health care behaviour of parents/guardians regarding the choice of hospitals should be made through health education activities if the cost of hospitalization is to be minimized.

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Annex 1. Calculation of Socioeconomic Index

	Items	Weight score
A.	Father's education	
	1. Illiterate	1
	2. 3R/Primary/Middle school education	2
	3. High school education	3
	4. Graduate	4
B.	Father's occupation	
	1. Professional	4
	2. Skilled	3
	3. Semi-skilled	2
	4. Unskilled	1
	5. Unemployed	0
C.	Mother's education	
	1. Illiterate	1
	2. 3R/Primary/Middle school education	2
	3. High school education	3
	4. Graduate	4
D.	Mother's occupation	
	1. Professional	4
	2. Skilled	3
	3. Semi-skilled	2
	4. Unskilled	1
	5. Unemployed	0
E.	Housing condition	
	1. Pucca	
	2. Semi-pucca	3
	3. Wooden house	2
	4. Hut	2
	5. Others	1
F.	Water supply	
	1. 24 hours running water	3
	2. Own artesian well	2
	3. Private water supply	2
	4. Public water source	1
	5. Others	1
G.	Type of latrine	
	1. Septic tank	3
	2. Indirect pit latrine	2
	3. Surface latrine	1
	4. None	1

Fertility-related attitudes and behaviour of married men in Kyaunk-tan Township

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A community-based study to explore the fertility-related attitudes and behaviour of married men was conducted in Kyauk-tan Township during 1997. A total of 169 married men of 18 years and above were included. It was observed that, regarding fertility regulation, husbands played the leading role in deciding to use the family planning method (FPM) (55.4%) and in taking responsibilities for obtaining information about FPM (57.2%). Furthermore they would like to discuss with their wives about the ideal number of children (59.8%) and FPM (77.1%). Their ideal number of children and desired children were found to be not much different (i.e. 3.3 and not more than 3). Although the majority (80.5%) said they knew the FPM, only 12 married men (7.1%) had used the method when having relation with their spouses, and condom was the most common method. On the other hand, 111 married men (65.7%) said their spouses had used pill or injection or IUD. During their married life, (34.3%) had tried to prevent from getting unplanned pregnancies, of which 15 had faced with such pregnancies. About 9% had experience of not having a child at the time of desire, among them 9 married men (60%) had consulted with either OG or other medical doctors. Regarding information on FPM for men and women, they said they had enough information (49.1%, 59.3%). As a whole, while the majority of married men from the study area were knowledgeable and willing to take responsibilities for FPM, the magnitude of their use of FPM was very low.

INTRODUCTION

Men's reproductive health concerned not only to their own, but their health status and behaviour also affected the women's reproductive health. Since the opinion of the men were dominant in decision-making process, they represented the important role in family, which included family planning practices, prevention and treatment of sexually transmitted diseases, infertility and so on and so forth. In many countries, men remained under-served by most reproductive health programme, since most family planning programmes were the female-to-female basis [1,2]. At the same time, the importance of more male participation and sharing of responsibility in family planning was stressed by Programme of Action of International

Conference of Population and Development [3]. It had stated that family planning could be achieved through contraception and *treatment of involuntary infertility – delaying of child bearing, using contraception, seeking treatment for infertility, interrupting of unwanted pregnancies and breast feeding* [4].

Information on how husbands' view of family planning differed from those of their spouses was limited. A few large-scale surveys done in Africa showed that husbands' attitudes had prevented many women from using family planning. It was also found that most men were interested in *contraception even when they and their partners were not using it* and many men wanted to know more about family planning. Younger husbands and wives

agreed that family interest and responsibility should be shared [5]. Although information and technical know-how regarding fertility regulation were not so difficult to access in our country, there was still lacking for men.

Thus role of men's attitudes and responsibilities taken for the fertility regulation should be explored for the future family planning strategies and programmes and our country was not exception to conduct such a study.

Objectives

The general objective was to explore the fertility-related attitudes and behaviour of the married men.

The specific objectives were to determine the married men's attitudes toward responsibility for family planning, to find out the involvement of married men in fertility-related decision making and practice and to explore the men's reproductive health information need and preferred source of information.

MATERIALS AND METHODS

A cross sectional study was conducted in Kyaunk-tan Township during 1997. The study subjects were recruited through random sampling of households with married men of 18 years and above. If there were more than one eligible subject in a household, only one subject was selected by random. After getting the consent forms, those who were involved in the study were interviewed by the pretested questionnaire, including socio-demographic variables, knowledge and practices of contraception, desired children, making decision to use family planning and source of information of contraceptions [6-8]. The trained interviewers did face-to-face interviewing.

FINDINGS

The study was planned to interview 200 married men of 18 years and above but only 169 married men completed the interview so the response rate was 84.5%. Mean age was 35.9 years with a range of 18 to 59 years. Among them majority had passed either middle level (23.7%) or higher-level education (37.9%). About 35% of them had own business and 32.5% were labourers and only 16% were government employee. Mean number of having children alive was found to be 2.6 with a range of 0 to 9 children (Table 1).

Table 1. Socio-demographic characteristics of respondents

Characteristics	No.	%
Age groups		
18-25	19	11.2
25-35	65	38.5
35-45	62	36.7
45-55	22	13.0
55+	1	0.6
Education		
Illiterate	2	1.2
Can read and write	18	10.7
Elementary level passed	32	18.9
Middle level passed	40	23.7
Higher level passed	64	37.9
College	13	7.7
Occupation		
Own business	59	34.9
Unskilled labour	55	32.5
Government employee	27	16.0
Employee at private business	23	13.6
Dependant	5	3.0

Knowledge about fertility regulation

Out of total subjects, 136 (80.5%) married men stated about knowledge of fertility regulation, of which, all had known about breast-feeding and only 6.6% heard about interrupting of unwanted pregnancies (Table 2).

Fertility-related attitudes

Regarding family planning method (FPM), majority decided to use it (55.4%) and taken responsibilities for obtaining information

about EPM (57.2%). They were also willing to take the responsibilities of family planning for a family (78%). When acquired about the means and ways of taking responsibilities, 131 married men agreed to take part in family planning. Among them, 39.7% said as a faithful husband, 24.4% would co-operate with authority concerned, 11.5% would explain about FPM to wife and 28.2% said they would involve by any means.

Table 2. Knowledge about fertility regulation

Knowledge	No.*	%
Breast feeding	136	100.0
Using of contraception	86	63.2
Seeking treatment for infertility	45	33.1
Delaying of child bearing	13	9.6
Interrupting unwanted pregnancies	9	6.6

*Some responses were multiple responses

As for a person who should decide the number of children in a family, about 60% thought a couple should decide together and only 1.8% of them said it should be by wife. Furthermore, they would like to discuss with their wives about the ideal number of children (59.8%) and about FPM (77.1%). It was observed that, their perception about ideal number of children and their desired number of children were not much different (i.e. 3.3 and not more than 3).

Behavior related to fertility regulation

The study population seemed to have knowledge about fertility regulation but only 7.1% practiced with their own choice i.e. condom. But utilization of contraceptives by wives was high. Some (65.7%) had said their spouses had used injection (53.2%) or pill (37.8%) or IUD (6.3%) respectively. Most of them mentioned that they had supported their wives for using FPM, i.e. 75.5%.

During their married life, although 58 (34.3%) had tried to prevent from the unplanned pregnancies by using condom

(0.6%) and by asking their spouses to use contraception (32.5%). Of which, 15 (8.9%) had faced with such pregnancies. Regarding situation of not getting a child when they really wanted, 8.9% had that experience for fertility regulation. Among them, 9 (60%) had consulted with either OG or other medical doctors. Eight of the married men (4.7%) said their wives had also tried to be pregnant but only 2 (25%) of them consulted with OG for that issue.

Information on family planning methods

Majority of them said existing available information about FPMs for both men and women were effective (60.4%, 62.7%). Regarding information of FPM used, they received from either health personnel (15.9% or health centre (14.8%) or GP 91.8%) or roadside shops (1.2%). Among them 83 (49.1%) and 99 (58.6%) revealed that they had enough information on FPMs for men and women and the sources of information were shown in Table 3.

Table 3. Source of information on FPMs for men and for women

Source	For men		For women	
	No. (n=83)*	%	No. (n=99)*	%
Health centre	31	37.4	46	46.5
Health personnel	30	34.1	40	40.4
GP	14	16.9	17	17.2
Book	17	20.5	17	17.2
Poster	17	20.5	9	9.1
Health talk	3	3.6	12	12.1
Radio	9	10.8	10	10.1
TV	26	31.3	19	19.2

*some responses were multiple responses

CONCLUSION

Unlike from African studies, Myanmar married men from the study area had optimistic attitudes toward fertility regulation. They took responsibilities for family planning and so also supported their spouses to use contraception. They favoured for a small family. Although the majority of

married men were knowledgeable and willing to take responsibilities for FPM, only few practiced it. Regarding information on family planning method, the existing information seemed to be effective for them and most information was obtained from health sector. The findings described that; Myanmar husbands were accepted as key persons in family planning. The gap between knowledge and practice still existed. So couple counseling, targeted education to needy ones should be promoted.

ACKNOWLEDGEMENT

We would like to express our gratitude to Director-General of Department of Medical Research for encouraging conducting the study and for the funding. We also like to thank Township Medical Officer of Kyauktan Township and the subjects who gave permissions for interviewing.

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Antimicrobial susceptibility testing of *Shigella* species and enteropathogenic *Escherichia coli* (EPEC) isolated from dysenteric cases of children in Yangon Children's Hospital during 1996

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A total of 56 cases of children admitted to Medical Wards of Yangon Children's Hospital during March to September 1996 with dysentery were tested for isolation of bacterial pathogens in a cross-sectional descriptive study using convenience-sampling method. Bacterial pathogens were isolated from 29 cases (51.79%) with *Shigella* species (35.71%) and enteropathogenic *Escherichia coli* (EPEC) (16.1%). The species of *Shigella* include *S. boydii* (25%), *S. dysenteriae* (10%) and *S. flexneri* (65%). The EPEC serotypes were O25K70, O26K60, O55K59, O112K66, O125K70, O126K71, O127K+ and O157K+. The antimicrobial susceptibility revealed that they were mostly susceptible to amikacin, gentamicin, kanamycin, minocycline, nalidixic acid, neomycin, netilmicin, norfloxacin and sisomicin. Most of them were resistant to ampicillin, chloramphenicol, streptomycin, tetracycline and trimethoprim/sulfamethoxazole.

INTRODUCTION

Dysenteric patients constitute 1.14% of total patients admitted to three medical units of Yangon Children's Hospital [1]. In endemic areas, shigellosis is a year-round disease, with peak incidence in the hot season. The incidence of shigellosis is highest in densely populated areas with unsafe or insufficient water supply and inadequate sanitation [2]. At the International Centre for Diarrhoeal Disease Research, Bangladesh, 65% of all episodes of shigellosis and 80% of all deaths from shigellosis occurred in children under five years old during 14 years. Among children, the risk of death from shigellosis was greatest in infants and in those who were severely malnourished [3]. Treatment with antibiotic has been shown to decrease the duration of dysentery and reduce the period of excretion of the organisms [4]. The changing pattern in the prevalence of *Shigella* strain with rising antimicrobial resistance stresses the need for

continuous surveillance of acute bacillary dysentery cases.

MATERIALS AND METHODS

A cross-sectional descriptive hospital based study using convenience-sampling method was conducted during May to December 1996 among 56 dysenteric children (male=39; female=17) in Yangon Children's Hospital. Their ages ranged from one month to 12 years who were passing loose stools with blood and/or mucus of more than 3 times per day were selected. Fresh stools were collected with two rectal swabs; one for direct plating onto MacConkey agar, Salmonella-Shigella agar and another in Cary Blair transport media. The specimens were sent to the Bacteriology Research Division within one to two hours.

Secondary enrichment media, Selenite F was also used simultaneously and then

incubated at 42°C overnight. Non lactose fermenting and lactose fermenting colonies of typical appearance were picked from MacConkey agar and Salmonella Shigella agar as suspected *Shigella* species and *Escherichia coli* species respectively [4].

Scrotyping was done by using antisera obtained from Denka Seikan, Japan; for *Shigella* species and *Escherichia coli* species.

Antibiotic susceptibility testing was done by using Muller-Hinton agar and broth as described by Kirby-Bauer method [5].

RESULTS

Pathogens isolated from different age groups

Pathogenic bacterial organisms isolated from 56 dysenteric cases attended at Yangon Children's Hospital are shown in Table 1. It was demonstrated that the pathogens were isolated from 6 cases (85.71%) in the age group of less than six months. Also, the pathogens were isolated from 14 cases (63.64%) out of 22 cases from the age group of 1-2 years. The pathogens were isolated from a total of 29 cases (51.79%) out of 56 cases tested.

Table 1. Percentages of pathogens isolation from different age groups of dysenteric patients

Age groups (months/ years)	No. of cases tested	Pathogens isolated	Percentages of isolation
<6 months	7	6	85.71
6 mth - 1 yr	4	0	0.00
1-2 yrs	22	14	63.64
2-3 yrs	9	5	55.56
3-4 yrs	2	1	50.00
4-5 yrs	4	1	25.00
5-12 yrs	8	2	25.00
Total	56	29	51.79

Distribution of Shigella species and enteropathogenic Escherichia coli (EPEC) from dysenteric cases of children

As shown in Table 2, it was noted that *Shigella* species and Enteropathogenic *Escherichia coli* (EPEC) were isolated from 20 cases (35.71%) and 9 cases (16.07%) respectively.

Table 2. Isolation of *Shigella* species and enteropathogenic *Escherichia coli* (EPEC) from dysenteric cases

Age groups (months /years)	Number of cases from which pathogens isolated						
	Tested		<i>Shigella</i>		EPEC		
	To- tal	M	F	M	F	M	F
< 6 months	7	6	1	4	1	1	0
6 mth-1 yr	4	3	1	0	0	0	0
1-2 yrs	22	16	6	8	2	1	3
2-3 yrs	9	7	2	2	0	2	1
3-4 yrs	2	1	1	0	1	0	0
4-5 yrs	4	4	0	0	0	1	0
5-12 yrs	8	5	3	0	2	0	0
Total	56	42	14	14	6	5	4
Percentages	100	75.0	25.0	70.0	30.0	55.6	44.4
Grand total		56		20 (35.71)		9 (16.07)	

Figures in parentheses denote percentages

Different types of Shigella species isolated from dysenteric cases

Out of 20 cases of *Shigella* species isolated; 5 cases (25%) were serotyped as *Shigella boydii*; 2 cases (10%) *Shigella dysenteriae* and 13 cases (65%) *Shigella flexneri* (Fig. 1).

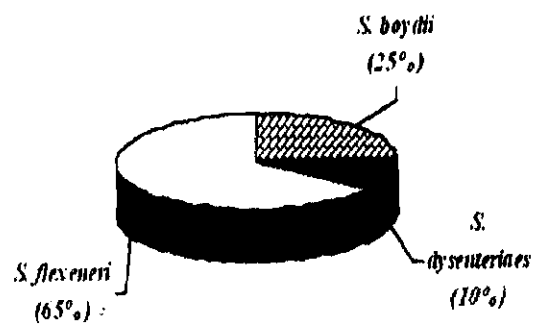


Fig.1. Different types of *Shigella* species isolated from dysenteric cases

Serotypes of enteropathogenic E. coli

The serotypes encountered in this study were two cases of O55K59; one case each of O25K70, O26K60, O112K66; O125K70; O126K71; O127K+; and O157K+ (Table 3).

Table 3. Serotypes of enteropathogenic *E. coli* (EPEC) isolates

Serotypes	Cases	Sex/Age/Type of stool/ Place
O25K70	1	Male, 1yr 5mths, mucous stool, Thaketa
O26K60	1	Male, 3yrs, mucous stool, Wakema
O55K59	2	Female, 3 yrs, bloody stool, Dala Male, 5 yrs, bloody stool, Kyaikwine
O112K66	1	Male, 2yrs 4mths, mucous stool, South Dagon
O125K70	1	Female, 1yr 8mths, mucous stool, South Dagon
O126K71	1	Female, 1yr 5mths, bloody stool, Mayangone
O127K+	1	Female, 1yr 4mths, watery stool, Thanlyin
O157K+	1	Male, 6mths, mucoid stool, Insein
Total	9	Male = 5 cases, Female = 4 cases

Antimicrobial susceptibility pattern of *Shigella* species isolated from dysenteric cases

Table 4 shows the susceptibility pattern and found that the isolated *Shigella* species were susceptible to netilmicin, norfloxacin and sisomycin (100%); amikacin (95%); nalidixic acid (90.9%); minocycline and neomycin (90%).

Antimicrobial susceptibility pattern of EPEC isolated from dysenteric cases

Table 5 shows the susceptibility pattern and found that EPEC were susceptible to amikacin and furazolidone (100%); nalidixic acid, netilmicin and tobramycin (75%); gentamicin (71.4%).

Resistance pattern of Shigella species and EPEC to different antimicrobial agents

As shown in Fig. 2, the comparison of resistance pattern of the two species tested revealed that EPEC were found to be more resistant than *Shigella* species. When tested with norfloxacin, all the isolated *Shigella* species were sensitive to it; however 71.4% were resistant in EPEC.

Table 4. Antimicrobial susceptibility pattern of *Shigella* species (n=20) isolates from dysenteric cases

Antimicrobial agents	Resistant	Intermediate	Moderately susceptible	Susceptible
Amikacin AN-30	1 (5.0)	0	0	19 (95.0)
Amoxicillin/Clavulanic acid AmC-300	2 (10.0)	0	7 (35.0)	11 (55.0)
Ampicillin AM-10	18 (90.0)	0	2 (10.0)	0
Carbenicillin CB-100	10 (50.0)	0	7 (35.0)	3 (15.0)
Cephalothin CF-30	6 (30.0)	0	1 (5.0)	13 (65.0)
Chlormphenicol C-30	16 (80.0)	2 (10.0)	0	2 (10.0)
Furazolidone* Fx	4 (20.0)	0	2 (10.0)	14 (70.0)
Gentamicin GM-10 (n=10)	0	2 (20.0)	0	8 (80.0)
Kanamycin K-30	0	3 (15.0)	0	17 (85.0)
Minocycline MI-30	0	2 (10.0)	0	18 (90.0)
Nalidixic acid NA-30 (n=11)	0	1 (9.1)	0	10 (90.9)
Neomycin N-30	0	2 (10.0)	0	18 (90.0)
Netilmicin NET-30	0	0	0	20 (100)
Norfloxacin NOR-10	0	0	0	20 (100)
Streptomycin S-10	16 (80.0)	2 (10.0)	0	2 (10.0)
Sisomycin SIS-10	0	0	0	20 (100)
Tetracycline Te-30	15 (75.0)	1 (5.0)	0	4 (20.0)
Trimethoprim/Sulfamethoxazole SXT	18 (90.0)	0	0	2 (10.0)

Figures in parentheses denote percentages

Table 5. Antimicrobial susceptibility pattern of enteropathogenic *Escherichia coli* (EPEC) (n=7) isolates from dysenteric cases

Antimicrobial agents	Resistant	Inter-mediate	Moderately susceptible	Sus-ceptible
Amikacin AN-30	0	0	0	7 (100)
Ampicillin AM-10	7 (100)	0	0	0
Carbenicillin CB-100	5 (71.4)	0	0	2 (28.6)
Cephalothin CF-30	3 (42.9)	0	2 (28.6)	2 (28.6)
Chlormphenicol C-30 (n=6)	5 (83.3)	0	0	1 (16.7)
Furazolidone* Fx	0	0	0	7 (100)
Gentamicin GM-10	2 (28.6)	0	0	5 (71.4)
Kanamycin K-30	4 (57.1)	0	0	3 (42.9)
Nalidixic acid NA-30 (n=4)	1 (25.0)	0	0	3 (75.0)
Netilmicin NET-30 (n=4)	1 (25.0)	0	0	3 (75.0)
Norfloracin NOR-10	5 (71.4)	1 (14.3)	0	1 (14.3)
Streptomycin S-10	6 (85.7)	0	0	1 (14.3)
Sisomycin SIS-10 (n=6)	2 (33.3)	0	1 (16.7)	1 (16.7)
Tetracycline Te-30	5 (71.4)	1 (14.3)	0	1 (14.3)
Tobramycin NN-10 (n=4)	1 (25.0)	0	0	3 (75.0)
Trimethoprim/ Sulfamethoxazole SXT	6 (85.7)	0	0	1 (14.3)

Figures in parentheses denote percentages

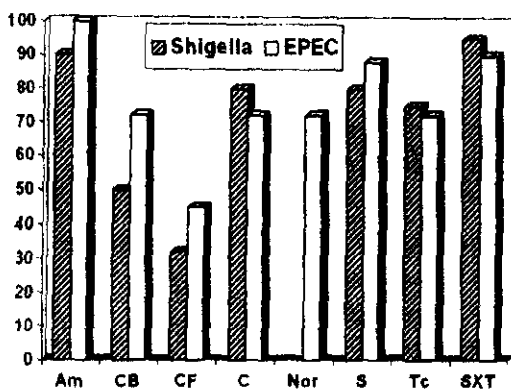


Fig. 2 Comparison of resistance to anti-microbial agents between *Shigella* species and enteropathogenic *Escherichia coli* (EPEC)

Am = Ampicillin Nor=Norfloracin
 CB = Carbenicillin S= Streptomycin
 CF = Cephalothin Tc= Tetracycline
 C = Chloramphenicol
 SXT = Trimethoprim/Sulphamethoxazole

DISCUSSION

According to the National Health Plan, (1996-2001), dysentery stood at fourth

position of the national priority diseases in Myanmar. In this study, from 5 out of 6 cases of less than 6 months old had isolation with children *Shigella* species (*S. flexneri* = 3 cases; *S. boydii* = 2 cases) and the remaining one with EPEC (O157K+). Among 56 cases tested, 39 cases were male (19 cases = 48.72% with pathogens) and 17 were female (10 cases = 58.82% with pathogens) and thus male preponderance was detected in this study. Peak admission of dysentery cases to the hospital was also noted during the rainy and summer seasons. During the study period, the mortality rate was found to be 10.71% and in these 6 cases; 2 cases by *Shigella boydii*, and one each with *Shigella dysenteriae*, *Shigella flexneri*, *Escherichia coli* O26K60 and O55K59 respectively. Out of 56 cases; 47 cases of attendees were staying in Yangon area (Mayangone, Mingalartaungnyunt, Yankin, Shwepyithar, Htaukkyant, Dala, Kyaikwaing, South Dagon, Thaketa, Mingaladon, Tamwe, Insein, Thuwunna, Latha, Kyimyindaing, South Okkalapa and Thanlyin) and the remaining 9 cases came from outskirts of Yangon area (Bago, Tonte, Nyaunglaybin, Wakema, Khayan, Loikaw

and Htantapin). Most of them used indirect, pits, direct pits or surface of latrines.

Antibiotics are powerful agents and effective in controlling infectious bacterial diseases. Since the 1940's they have saved millions of lives. But today in many parts of the world, people succumb to diseases that are no longer treatable. These resistance strains can emerge in any area and spread locally or crossing political borders, creating a worldwide problem. The introductions of enormous quantities of potent drugs into the environment during therapy and in animal feeds have challenged the entire prokaryotic world on our planet [6]. In this study, EPEC seems to be more resistant to different antimicrobial agents than *Shigella* strains. These two pathogens were highly resistant to ampicillin, chloramphenicol, streptomycin, tetracycline and trimethoprim/sulfamethoxazole. Thus the changing pattern in the prevalence of *Shigella* strain with rising antimicrobial resistance stresses the need for continuous surveillance of acute bacillary dysenteric cases.

ACKNOWLEDGEMENTS

The authors would like to expressed their sincere thanks to Director-General Professor Dr. Paing Soe and Deputy Director-General Dr. Soe Thein for their keen interest in research activities. To Director Dr. Tun Pe

for guidance in research activities. To the Superintendent, Professors and Medical Officers of the Medical Wards in Yangon Children's Hospital for their kind cooperation. Last but not the least to the staff of Bacteriology and Clinical Research Division, Department of Medical Research (Lower Myanmar).

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**Antibiotics susceptibility pattern of *Vibrio cholerae* O1, O139,
Salmonella typhi and *Escherichia coli***

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Bacterial pathogens isolated from clinical specimens were tested for susceptibility pattern to different antibiotics using agar disc diffusion technique. They were *Vibrio cholerae* O1 (21 cases) and *Vibrio cholerae* O139 (136 cases) from diarrhoea cases attending the Infectious Diseases Hospital during 29-7-98 to 29-10-98; *Salmonella typhi* (38 cases) and *Escherichia coli* (15 cases) from cases with high fever for more than five days admitted to Yangon Children's Hospital during 27-8-98 to 17-8-2000. *Vibrio cholerae* O1 were resistant to ampicillin (50%), chloramphenicol (28.6%), septrin (57.1%), tetracycline (57%); and sensitive to amikacin (85.7%), furazolidone (92.3%), nalidixic acid (73.7%), netilmicin (100%), norfloxacin (92.9%). *Vibrio cholerae* O139 were resistant to ampicillin (81.8%), chloramphenicol (34.6%), septrin (43.3%), tetracycline (43.8%); and sensitive to amikacin (90.5%), furazolidone (60.8%), nalidixic acid (74.6%), netilmicin (100%), norfloxacin (81.8%). *Salmonella typhi* were resistant to ampicillin (76.3%), chloramphenicol (79%), carbenicillin (74%), streptomycin (89.5%), septrin (79%), tetracycline (70.3%); and sensitive to amikacin (92.1%), cefaclor (54%), ceftriaxone (75%), cephalothin (63.2%), gentamicin (89.5%), nalidixic acid (83.3%), netilmicin (92.1%), norfloxacin (81.6%). *Escherichia coli* were resistant to ampicillin (66.7%), chloramphenicol (60%), cephalothin (80%), streptomycin (80%), septrin (60%); and sensitive to amikacin (86.7%), gentamicin (57.1%), nalidixic acid (54.5%), netilmicin (73.7%), and norfloxacin (58.3%).

INTRODUCTION

The extent of child and adolescent morbidity and mortality in the developing world was caused by acute respiratory infections, diarrhoea, measles, malaria or malnutrition; or a combination of these conditions. Everyday, millions of people seek health care for sick people, taking them to hospitals, health centres, pharmacists, community health care providers and traditional healers [1]. Bacterial infections cause all ages and both sexes. Infection is generally spread by way of air borne, vector borne, faecal oral, direct contact and sexual contact routes of transmission. Cholera,

typhoid and diarrhoea are commonly transmitted by faecal oral route. There is no doubt that the source of contamination is widely through faeces of convalescent carries or from patients. Infection occurs mostly in crowded communities with poor standards of hygiene. Antibiotics are powerful agents and effective in controlling infectious bacterial diseases. Since the 1940s, they have saved millions of lives. But today, in many parts of the world, people succumb to disease that is no longer treatable; as they developed resistance to antibiotics. Inappropriate use of antibiotics is also very common in this locality. This paper aims to identify the susceptibility

pattern of antibiotics to *Vibrio cholerae* O1 and O139, *Salmonella typhi* and *Escherichia coli*.

MATERIALS AND METHODS

Cases tested for antibiotics susceptibility

It includes 136 cases of *Vibrio cholerae* isolated from diarrhoea cases attended to Special Hospital (Waibagi, North Okkalapa); and 38 cases with *Salmonella typhi* and 16 cases of *Escherichia coli* isolated from children attended to Children's Hospital, Yangon for management of diarrhoea.

Isolation of bacterial pathogens and identification

Bacterial pathogens were isolated from clinical specimens by conventional bacteriological methods using Blood agar, MacConkey agar, *Salmonella*-*Shigella* agar, Nutrient agar and Thiosulphate Citrate Bile Sucrose agar for primary isolation and Selenite F and alkaline peptone water were also used for secondary enrichment [2].

Antibiotic Susceptibility Testing

It was done by agar disc diffusion technique using Mueller Hinton agar and broth as described [3].

RESULTS

Distribution of age and sex among tested cases isolated with different pathogens

A total of 212 isolates (108 males: 50.9% and 104 females: 49.1%) were tested for antibiotic susceptibility pattern. From Specialist Hospital, Waibagi, 21 isolates of *Vibrio cholerae* O1 (8 males: 38.1% and 13 females: 61.9%); of which 19 cases are under 35 years of age. Among 136 cases of *Vibrio cholerae* O139, the ages of 75 males (55.2%) and 61 females (44.9%) ranged from seven months to 80 years. From Yangon Children's Hospital, out of 55 cases tested, 39 isolates of *Salmonella typhi* were from 17 males (43.6%); and 22 females

(56.4%). The 16 cases of *Escherichia coli* were from 8 cases each of males and females (Table 1).

Table 1. Distribution of age and sex among tested cases from which different pathogens were isolated

(a) Infectious Diseases Hospital

Age groups (yr)	<i>Vibrio cholerae</i> (O139)			<i>Vibrio cholerae</i> (O1)		
	No. of Males	No. of Females	Total	No. of Males	No. of Females	Total
<5	5 (62.5)	3 (37.5)	8	0	2	2
6-15	11 (68.8)	5 (31.3)	16	1	3	4
16-25	12 (63.2)	7 (36.8)	19	4	5	9
26-35	17 (50)	17 (50)	34	2	2	4
36-45	10 (43.5)	13 (56.5)	23	-	-	-
46-55	14 (60.9)	9 (39.1)	23	-	-	-
56-65	5	0	0	-	1	1
66-75	1 (16.67)	5 (83.3)	6	-	-	-
76-80	0	2	2	1	-	1
Total	75 (55.2)	61 (44.9)	136	8 (38.1)	13 (61.9)	21
Grand total	157					

(b) Yangon Children's Hospital

Age groups (yr)	Isolated species	No. of Males	No. of Females	Total
<5 yrs	<i>Salmonella typhi</i>	17 (43.6)	22 (56.4)	39
<5yrs	<i>Escherichia coli</i>	8 (50)	8 (50)	16
Grand total -				55

Table 2. antibiotics susceptibility pattern of *Vibrio cholerae* O1

Antibiotics	Tested	Resistant	Inter-mediate	Moderately Susceptible	Susceptible
Amikacin AN-30	21	0	3 (14.3)	0	18 (85.7)
Ampicillin AM-10	20	10 (50)	0	5 (25)	5 (25)
Carbenicillin CB-100	12	7 (58.3)	0	0	5 (41.7)
Cephalothin CF-30	21	9 (42.9)	0	4 (19.1)	8 (38.1)
Chloramphenicol C-30	21	6 (28.6)	4 (19.1)	0	11 (52.4)
Furazolidone*	13	1 (7.7)	0	0	12 (92.3)
Gentamicin GM-10	21	1 (4.8)	0	0	20 (95.2)
Nalidixic acid NA-30	19	1 (5.3)	4 (21.1)	0	14 (73.7)
Netilmicin NET-30	21	0	0	0	21 (100)
Norfloxacin NOR-10	14	1 (7.1)	0	0	13 (92.9)
Streptomycin S-10	21	15 (71.4)	1 (4.8)	0	5 (23.8)
Tetracycline Te-30	21	12 (57.1)	3 (14.3)	0	6 (28.6)
Trimethoprim/ SXT Sulfamethoxazole	21	12 (57.1)	0	0	9 (42.9)

*Hi Media; others BBL products

Figures in parentheses denote percentages

Table 3. Antibiotics susceptibility pattern of *Vibrio cholerae* O139

Antibiotics	Tested	Resistant	Inter-mediate	Moderately Susceptible	Susceptible
Amikacin AN-30	136	3 (2.2)	10 (7.4)	0	123 (90.4)
Ampicillin AM-10	136	111 (81.6)	0	8 (5.9)	17 (12.5)
Carbenicillin CB-100	132	63 (47.7)	0	13 (9.9)	56 (42.4)
Cephalothin CF-30	136	73 (53.7)	0	23 (16.9)	40 (29.4)
Chloramphenicol C-30	136	47 (34.6)	15 (11.0)	0	74 (54.4)
Furazolidone*	79	26 (32.9)	5 (6.3)	0	48 (60.8)
Gentamicin GM-10	136	7 (5.2)	16 (11.8)	0	113 (83.1)
Nalidixic acid NA-30	126	16 (12.7)	16 (12.7)	0	94 (74.6)
Neomycin N-30	119	3 (2.5)	21 (17.7)	0	95 (79.8)
Netilmicin NET-30	136	0	0	0	136 (100)
Norfloxacin NOR-10	132	11 (8.3)	13 (9.8)	0	108 (81.8)
Streptomycin S-10	120	49 (40.8)	23 (19.2)	0	48 (40)
Tetracycline Te-30	136	59 (43.4)	0	22 (16.2)	55 (40.4)
Tobramycin NN-10	32	0	0	0	32 (100)
Trimethoprim/ SXT Sulfamethoxazole	136	57 (41.9)	0	14 (10.3)	65 (47.8)

Figures in parentheses denote percentages *Hi Media, others BBL products

Table 4. Antibiotics susceptibility pattern of *Salmonella typhi*

Antibiotics	Tested	Resistant	Inter-mediate	Moderately Susceptible	Susceptible
Amikacin AN-30	38	2 (5.3)	1 (2.6)	0	35 (92.1)
Ampicillin AM-10	38	29 (76.3)	0	1 (2.6)	8 (21.1)
Carbenicillin CB-100	38	28 (73.7)	0	2 (5.3)	8 (21.1)
Cefaclor* Cj-30	28	12 (42.9)	0	1 (3.6)	15 (53.6)
Ceftriaxone* Ci-30	16	3 (18.8)	0	1 (6.3)	12 (75.0)
Cephalothin CF-30	38	8 (21.1)	0	6 (15.8)	24 (63.2)
Chloramphenicol C-30	38	30 (79)	1 (2.6)	0	7 (18.4)
Furazolidone*	25	3 (12)	0	0	22 (88)
Gentamicin GM-10	38	3 (7.9)	1 (2.6)	0	34 (89.5)
Nalidixic acid NA-30	18	2 (11.1)	1 (2.6)	0	15 (83.3)
Netilmicin NET-30	38	1 (2.6)	2 (5.3)	0	35 (92.1)
Norfloxacin NOR-10	38	4 (10.5)	3 (7.9)	0	31 (81.6)
Streptomycin S-10	38	34 (89.5)	1 (2.6)	0	3 (7.9)
Tetracycline Te-30	37	26 (70.3)	0	0	11 (29.7)
Trimethoprim/ SXT	38	30 (79)	0	0	8 (21.1)
Sulfamethoxazole					

Figures in parentheses denote percentages *Hi Media, others BBL products

Antibiotic susceptibility pattern of Vibrio cholerae O1

Among 13 antibiotics tested, the most susceptible pattern was obtained in aminoglycoside group: netilmicin (100%), gentamicin (95.2%), amikacin (85.7%); and next to quinolones: norfloxacin (92.9%), and naladixic acid (73.7%) (Table 2).

Antibiotic susceptibility pattern of Vibrio cholerae O139

Among 15 antibiotics tested, the most susceptible pattern was obtained in aminoglycoside group: netilmicin (100%), amikacin (90.4%); gentamicin (83.1%), and neomycin (79.8%). They were susceptible to quinoline group: norfloxacin (81.8%), and naladixic acid (74.6%). They were also susceptible to tobramycin (100%) and furazolidone (60.8%) (Table 3).

Antibiotics susceptibility pattern of Salmonella typhi

Among 15 antibiotics tested, the most susceptible pattern was obtained in

aminoglycoside group: amikacin (92.1%), netilmicin (92.1%), gentamicin (89.5%). Then to quinolone group nalidixic acid (83.3%) and norfloxacin (81.6%). They were susceptible to beta-lactam group: ceftriaxone (75.0%), cephalothin (63.2%), cefaclor (53.6%). Also susceptible to furazolidone (88.0%) (Table 4).

Antibiotics susceptibility pattern of Escherichia coli

Among 15 antibiotics tested, the most susceptible pattern was obtained in aminoglycoside group amikacin (86.7%), netilmicin (73.3%), gentamicin (57.1%); to quinolone group norfloxacin (58.3%) and nalidixic acid (45.5%). They were less susceptible to beta-lactam group: ceftriaxone (44.4%), cefaclor (15.4%) and cephalothin (13.3%).

Resistance pattern of antibiotics to pathogens

As shown in Fig. 1, it shows that ampicillin was mostly resistant to *V. cholerae* O139

(81.6%), *S. typhi* (76.3%), *E. coli* (66.7%) and *V. cholerae* O1 (50%). Chloramphenicol was resistant to *S. typhi* (79.0%), *E. coli* (60.0%), *V. cholerae* O139 (34.6%) and *V. cholerae* O1 (28.6%). Carbenicillin was resistant to *S. typhi* (73.7%), *V. cholerae* O1 (58.3%), *V. cholerae* O139 (47.7%) and *E. coli* (46.7%). Streptomycin was resistant to *S. typhi* (89.5%), *E. coli* (80.0%), *V. cholerae* O1 (71.4%) and *V. cholerae* O139 (36.0%). Septrin (trimethoprim/sulfamethoxazole) was resistant to *S. typhi* (79.0%), *E. coli* (60.0%), *V. cholerae* O1 (57.1%) and *V. cholerae* O139 (43.3%). Tetracycline was resistant to *S. typhi* (70.3%), *V. cholerae* O1 (57.1%), *V. cholerae* O139 (43.8%) and *E. coli* (14.3%).

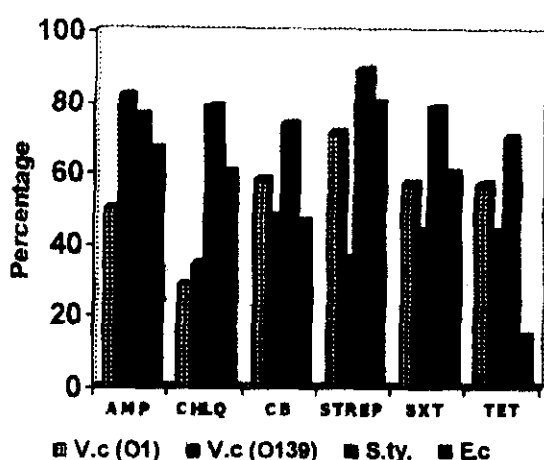


Fig. 1. Antibiotic resistance pattern of different pathogen

Resistance pattern of antibiotics to *Vibrio cholerae* O139 isolated from different age groups

Among the age group of seven months to less than five years, the resistant pattern of five antibiotics was found to be: ampicillin (87.5%), septrin (50%), chloramphenicol and tetracycline (12.5%) each. In the age group of 6-15 years *V. cholerae* O139 was resistant to ampicillin (75.0%), septrin and tetracycline (43.7%) each and chloramphenicol (31.3%). In the age group of 16-35 years, they were resistant to ampicillin (62.3%), tetracycline (50.9%), septrin (49.1%), chloramphenicol (32%) and norfloxacin (7.6%). In the age group 36-55

years, they were resistant to ampicillin (86.9%), tetracycline (47.8%), septrin (43.5%), chloramphenicol (41.3%), and norfloxacin (11.6%). In the age group of 56-80 years, they were resistant to ampicillin (100%), septrin (57.1%), chloramphenicol (38.5%), tetracycline (28.6%) and norfloxacin (8.3%) (Fig. 2).

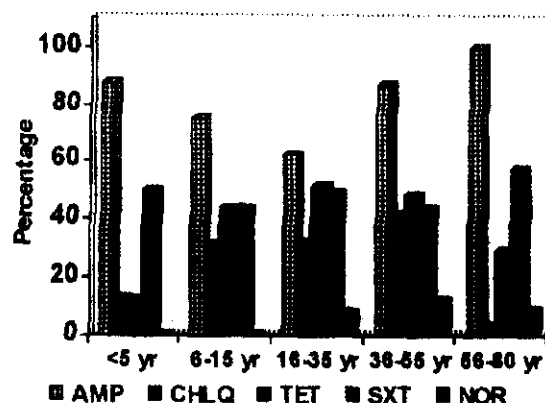


Fig. 2. Antibiotics resistance pattern of *Vibrio cholerae* O139 isolated from cases with different age groups

DISCUSSION

Escherichia coli is one of the most common causes of infection in humans and animals worldwide. It is associated with a wide variety of diseases, but a large degree of host and disease specificity exists for the different serotypes. Thus, certain pathogenic *E. coli* serotypes predominates as causes of intestinal infections in humans and different animal species. In contrast, extra intestinal infections are caused by *E. coli* serotypes, which are found as normal intestinal flora. Few studies on antimicrobial susceptibility patterns among O157 revealed that they were fully susceptible, even though resistance to ampicillin, streptomycin, sulphonamides and tetracycline [5]. In this study, it shows that most of these strains became resistance to conventional antibiotics usually used for various ailments. However, they were sensitive to quinolones and aminoglycosides. Thus, the spread of resistant strains to environment by various means should be always considered.

ACKNOWLEDGEMENTS

The authors would like to gratitude to Director-General Professor Paing Soe, Deputy Director-General Dr. Soe Thein and Director Dr. Tun Pe for their keen interests in research activities. To Superintendent of Yangon Children's Hospital and Specialist Hospitals and staff of Bacteriology Research Division, Department of Medical Research (Lower Myanmar).

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The role of lepro-agglutination test for serodiagnosis of leprosy in Myanmar

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If infectious cases of leprosy could be diagnosed for effective chemotherapy before they would manifest the disease, it would significantly prevent the transmission of infection from man to man and consequently would greatly help the leprosy eradication programme of the country. Therefore the role of lepro-agglutination test as a serodiagnostic assay of leprosy in our community was carried out in this study. It was carried out from August 1999 to January 2000. After getting informed consent, 2 ccs of blood were collected from 101 fresh leprosy cases from Central Special Skin Center, Yangon General Hospital, 178 contacts of above cases, 100 controls from National Blood Bank by using disposable needles and syringes. Sera were separated from clotted blood by centrifugation at 3000 rpm for 15 min and they were stored at -20°C before testing. Lepro-agglutination (LA) test and ELISA test were carried out on these sera samples. The sensitivity of multibacillary (MB) and paucibacillary cases of LA test was 81.13% and 56.25% respectively and the specificity for controls was 99% in this study. Both LA and ELISA tests were more sensitive in diagnosis of MB cases. Interruption of *M. leprae* transmission in areas of predominantly MB disease may be needed more due to the relatively greater number of cases acting as sources of infection before the disease clinically manifested. Therefore, in such areas there may be a role for PGL-1 serology and LA test in leprosy control.

INTRODUCTION

Early leprosy is doubtful early cases which do not satisfy the cardinal signs of leprosy and those who do not exhibit clinical symptoms but would manifest signs of leprosy at a future date (preclinical leprosy). One of the methods of preventing spread of the disease would be effective early diagnosis and treatment of these subjects at early (preclinical) stage itself (WHO, 1985). Hence, if the future infectious cases could be diagnosed for effective chemotherapy before they have manifest disease it would significantly prevent the transmission of infection from man to man and consequently would greatly help the leprosy eradication programme of the country.

Various workers have attempted serodiagnostic tests for leprosy on radio immunoassay, Indirect Immunofluorescence, enzyme linked immuno-sorbent assay, monoclonal antibody based assays, serum antibody competition tests etc. All the above tests are useful in detecting a small group. However they are practical and financially viable proposition as a screening test in the community.

M. leprae particle agglutination (MLPA) has been developed by Izumi *et al.* (1990) [1]. The test is based on agglutination of PGL-1 coated gelatin particles and gives results comparable with ELISA [2]. However, the test is not cost effective as the kits have to be imported at high cost.

Leprosy is in 8th position of our national health priority diseases (1993-1996). In National Health Policy, one of the strategy is to improve case detection rate. Thus one of the serodiagnostic tests for detection of early leprosy will be needed to carry out in our community.

In the study of Thawani *et al.* larger number of multibacillary (MB) sera were positive by Lepro-agglutination (LA) (86.75%) than by MLPA (45.12%) test [3]. Paucibacillary (PB) sera showed positivity of 16% in LA test and 24% in MLPA test. It can screen out as nearly as MLPA test in PB cases. This should be adequate for the purpose of screening individuals for leprosy.

LA test is useful as a diagnostic method as identifies cases. Therefore the role of LA test as a serodiagnostic test of leprosy in our community was carried out in this study. The results of LA test were also compared with the conventional method of PGL-1 ELISA test.

MATERIALS AND METHODS

The study was institutional and community based longitudinal study. It was carried out from August 1999 to January 2000. One hundred and one fresh leprosy cases attending the Central Special Skin Center, Yangon General Hospital were tested during this study period. After getting informed consent 2 ccs of blood were collected from them by using disposable needles and syringes. Our field workers also surveyed to their homes and 2 ccs of blood were collected from their contacts after getting informed consent. Control sera were collected from apparently healthy blood donors from National Blood Bank, Yangon. Sera were separated from clotted blood by centrifugation at 3000 rpm for 15 min and they were stored at -20°C before being tested.

Lepro-agglutination test

One volume of alcoholic solution of cardiolipin and lecithin (0.5 mg/ml) was rapidly diluted with 19 volumes of saline, then mixed with 5 volumes of kaolin suspension in water (1 mg/ml). After incubation at 37°C for 30 min, the mixture was diluted 1:2 with saline. The antigen suspension was prepared fresh on the day of testing. Sera were inactivated at 50°C for 30 min and doubling dilutions of the sera were made in saline up to 1:64 on all the cases and contacts. The test were performed in microtitre plates.

Microtitre plate method

Each well contained 50 µl of sera in corresponding dilution. To this 50 µl of antigen suspension was added. After mixing on a rotator for a few minutes, the plate was incubated at 37°C for 4 hours. Then agglutination reaction was recorded. The titre was expressed by the highest dilution of sera showing agglutination.

ELISA technique

ELISA microtitre plate (Fastec Microplate U, Fujirebio Inc.) with 96 U shaped wells were coated with 50 µl of 1 µg/ml concentration of NT-P-BSA (*M. leprae*) antigen and placed at 4°C for overnight. The antigen was diluted with carbonate-bicarbonate buffer to get the final concentration. Next day the solution was sucked out and the plates were then washed 3 times with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBST). 100 µl of blocking agent was added to each well and incubated at 37°C for 30 min. The solution was sucked out and dried. Fifty microlitre of test or control serum, diluted in 1:300 in dilution buffer was added to each well and incubated at 37°C for 1 hour. The plate was washed with PBST for 3 times again. 50 µl of goat anti human IgM conjugated to horse-radish peroxidase, diluted in 1:2000 with dilution buffer was added to each well. It was incubated at 37°C

Table 1. Dilution titre of LA test in leprosy cases, contacts and controls

Cases/Contacts	N	Total positive	Positivity in dilution of the serum						
			N	1:2	1:4	1:8	1:16	1:32	1:64
MB cases	53	43 (81.13)	0	6	4	0	4	9	30
PB cases	48	27 (56.25)	3	12	6	1	5	3	18
Contacts of MB	90	5 (5.56)	25	46	14	4	1	0	0
Contacts of PB	88	8 (9.09)	18	41	21	7	0	1	0
Controls	100	1 (1)	24	16	15	0	0	0	0

45 samples give negative results in controls

Figure in parenthesis = percentage

N = neat

Sensitivity for MB cases = 81.13%

Sensitivity for PB cases = 56.25%

Specificity for controls = 99% when using the cut-off point at 1:4

for 1 hour. The plate was then washed with PBST for 3 times again. 100 µl of substrate solution (ortho phenylene diamine) was added to each well and incubated at 37°C for 15 min. Then the color change was stopped by stopping solution. Instead of coating with antigen, the adjacent wells were coated with bovine serum albumin (BSA) and the ELISA test was carried out. OD (optical density) was measured by an ELISA reader with wave-length of 492 nm. OD of BSA well was subtracted from antigen coated well. Mean OD was calculated from duplicate results.

RESULTS

One hundred and one sera of fresh leprosy cases, 178 sera of contacts and 100 sera of

Table 2. Age distribution of leprosy cases and contacts

Age in year	MB cases No (%)	PB cases No (%)	MB contacts No (%)	PB contacts No (%)
1-10	1 (0)	3 (6.25)	16 (17.77)	18 (20.45)
11-20	14 (25.92)	5 (10.41)	28 (31.11)	22 (25)
21-30	15 (27.77)	8 (16.66)	12 (13.33)	21 (23.86)
31-40	11 (30.37)	8 (16.66)	15 (16.66)	6 (6.81)
41-50	6 (11.11)	10 (20.83)	5 (5.55)	10 (11.36)
51-60	5 (9.25)	12 (25)	7 (7.77)	6 (6.81)
>60	3 (5.55)	2 (4.16)	7 (7.77)	5 (5.68)

controls were tested in this study. Table 1 shows the agglutinin titres in sera of fresh leprosy cases, contacts and controls. Most of the cases were positive in titre of 1:32 and above and most contacts were positive in 1:4 and below (Fig. 1). This LA test gave the sensitivity of MB and PB cases as 81.13% and 56.25% respectively and the specificity for controls as 99% when using the cut-off point at 1:4 in this study.

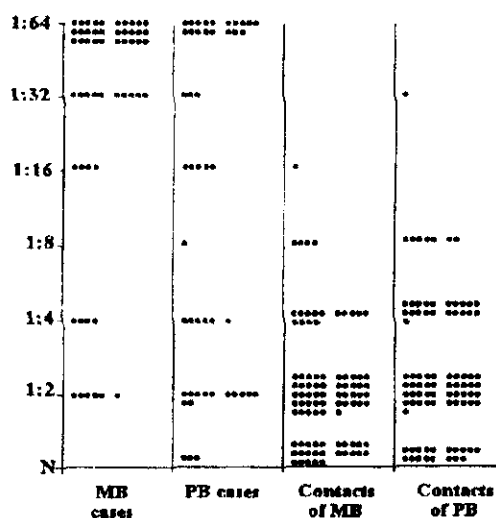


Fig. 1. Scattergram showing levels of lepro-agglutinin antibodies titre in different categories of leprosy cases and contacts

Table 2 shows that there were only very few cases of children under 14 years of age. The sex ratio in cases and contacts studied was shown in Table 3. There were male preponderance in both MB and PB cases. Table 4 shows the correlation between LA and IgM PGL-1 ELISA tests results and

both were more sensitive to diagnose MB cases.

Table 3. Sex distribution of cases and contacts

Sex	MB cases No (%)	PB cases No (%)	MB Contacts No (%)	PB Contacts No (%)
Male	31(57.4)	34(70.83)	38(42.22)	33(37.5)
Female	23(42.6)	14(29.16)	52(57.77)	55(62.5)

MB = Multibacillary

PB = Paucibacillary

Table 4. Correlation between LA and IgM PGL-1 ELISA tests results

Cases	N	Both positive	Both negative	LA+ve IgM+ve	LA+ve IgM+ve
Multibacillary	8	15 (53.57)	2 (7.14)	5 (17.86)	6 (21.43)
Paucibacillary	33	8 (24.24)	14 (42.42)	11 (18.03)	11 (18.03)
Both types	61		16 (26.23)		

Figure in parenthesis percentage

DISCUSSION

According to WHO (1985) [4], the number of *M. leprae*, infected individuals in our endemic area of leprosy are far greater than those with overt disease. A single test is also required for screening suspected cases of small centers, whose facilities for ELISA do not exist. This test is cheap and simple. Antibodies in leprosy sera were reacting with a mixture of cardiolipin and lecithin in 1:1 ratio. The antibodies in syphilis were on the other hand was affinity for the above constituents in 1:10 ratio. The using mixture of cardiolipin and lecithin in different ratios makes it possible to distinguish leprosy sera from syphilitic sera.

In our study there was male preponderance in both of the cases. It may be due to that the female patients would be afraid of showing their skin lesions mostly in hidden areas to the other people. Thus it is needed to take on doing active case finding on the female patients. There was also very few cases in children under 14 years of age in our community. It is also similar to the other

endemic countries because of MDT (multi drug therapy) to our community.

In this study most of the contacts were positive in titre of 1:4 and below. Four contacts of MB cases and 6 contacts of PB cases were positive in titre of 1:8. One contact of MB case was positive in 1:16 and 1 of PB case positive in 1:32. Thus it is known that there is infection in contacts of both MB and PB cases. It will be changed to overt clinical cases to these individuals and there will be continuous source of infection from these contacts.

The serological activities of the specific PGL-1 from *M. leprae* were examined by ELISA test against hyperimmune anti-*M. leprae* rabbit antiserum and sera from patients with leprosy and other mycobacterial diseases. High anti-PGL-1 immunoglobulin M antibodies were found in 23 of 24 (96%) of lepromatous leprosy patients on short-term chemotherapy and in 8 of 13 tuberculoid leprosy patients (62%). Sera from patients with TB or atypical mycobacterial infections were devoid of anti-PGL-1 activity. Thus PGL-1 is specific in serological activity [5]. In our study, LA test gave similar sensitivity on MB and PB cases.

Leprosy control by health education and administration of MDT to self reporting cases, might not be as successful with respect to a foreseeable interruption of *M. leprae* transmission in areas of predominantly MB disease as it may be in areas of predominantly PB disease due to the relatively greater number of cases acting as sources of infection before the disease clinically manifested or not is diagnosed and treated. Therefore, in at least such former areas there may be a role for PGL-1 serology and LA test in leprosy control.

ACKNOWLEDGEMENT

The authors would like to thank Technical Core Group of Leprosy Research for granting to carry out this research work.

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A double blind comparative trial of two dosage regimens of artesunate suppository in combinations with oral mefloquine in severe falciparum malaria

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To confirm the usefulness of artesunate suppositories (Plasmotrim™ rectocaps) in severe falciparum malaria, including patients in un-rousable coma, a prospective double blind randomized controlled study was carried out at the Clinical Research Unit (Malaria), DSGH, Mingaladon and at No. 1 Military Hospital (300-Bedded), Myitkyina, between May 1998 to April 2000. A total of 100 patients with severe falciparum malaria (WHO definition) with positive asexual parasitaemia in the peripheral blood film, were assigned to two dosage regimens. In regimen 1 - 55 patients received 1200 mg of artesunate (6 rectocaps) over 3 days. In regimen 2 - 45 patients received 800 mg of artesunate (4 rectocaps) over 3 days. Oral mefloquine 1250 mg in two divided doses was given on the fourth day in both regimens. Numbers of patients with cerebral malaria were 33 and 25 respectively in regimen 1 and 2.

Results: Parasite clearance times were 59.32 ± 27.5 and 50.4 ± 28.2 hours, fever clearance times were 34.6 ± 46.0 and 33.83 ± 39.44 hours, mortality rates were 7.2% and 2.2% and 28 day cure-rates were 100% and 91.1% respectively in regimen 1 and 2. Patients' compliance was satisfactory and there were no serious adverse effects.

Conclusion: Artesunate suppositories are well tolerated, rapidly clear parasites in severe falciparum malaria and are effective even at a low dosage of 800 mg given over 3 days. The addition of mefloquine ensures satisfactory 28-day cure-rates.

INTRODUCTION

Severe falciparum malaria carries a high mortality and most deaths occur in the community and the primary health care level and not in the wards and intensive care units of secondary and tertiary care hospitals. This is mostly due to the lack of diagnostic and treatment facilities at these lower health care levels. Delay in getting specific treatment is a proven major risk factor for deaths in severe falciparum malaria [1,2]. Treatment regimens, which are effective, safe and appropriate for

remote areas, for moving military troops, primary care hospitals, rural health clinics and even at the villages and households, need to be designed and tested.

It is generally agreed that a rapid decrease in initial parasitaemia is desirable to prevent the development of complications in falciparum malaria. Various studies on suppository forms of artemisinin derivatives have been made. Intravenous artesunate and artemisinin suppositories have been shown to be very effective in uncomplicated and severe falciparum malaria in China

since 1985 [3]. In Vietnam, artemisinin derivatives including the suppository form were reported to quickly reduce parasitaemia. The most interesting result reported was that the suppository formulation of artemisinin appeared to be as effective and safe as parenteral artesunate or artemether [4]. Other studies in Vietnam showed a mean 50% parasite clearance time (PC 50) of 6.2 hours and 100% parasite clearance time (PCT) of 25.8 hours by using artemisinin suppository in children with acute falciparum malaria [5] and a PC 50 of 9.7 hours and 100% PCT of 37.9 hours by using artemisinin suppository 2800 mg over 3 days with mefloquine 500 mg stat in adults with cerebral malaria [6]. In Thailand, sequential artesunate suppository 1600 mg and mefloquine 1250 mg was claimed to be safe and effective with 28 day cure-rate of 92% in severe falciparum malaria in 1995 [7]. In Ecuador, artesunate suppository 1400 mg and mefloquine 250 mg obtained a 28-day cure-rate of 97.5% in severe falciparum malaria [8].

The standard treatment for severe falciparum malaria in Myanmar is intravenous quinine dihydrochloride infusion. Intramuscular artemether in cerebral malaria is equally effective and more rapid in clearing parasitaemia than quinine [9]. It is now used as an alternative drug in severe falciparum malaria. In areas where severe falciparum malaria is a major problem and where facilities for basic parenteral administration (equipment and personnel) are not readily available, suppositories may be more practical and convenient.

A study to find out the tolerability and efficacy of artesunate suppositories in falciparum malaria was started by our group since 1994. The idea was to find a drug regimen for use in the frontier areas. It would have to be easy to administer without needing any trained personnel and also be able to rapidly and effectively clear *Plasmodium falciparum* parasites. The

suppository forms of artemisinin derivatives seemed to be the most likely candidates.

As the first phase of the study, the tolerability of these artesunate suppositories (PLASMOTRIMTM RECTOCAPS) was tried on 20 healthy volunteers and then on 30 uncomplicated falciparum malaria patients using the suppository 200 mg O.D. for three days as mono-therapy in 1994. 100% PCT was about 38 hours, but with high recrudescence (R1 = 50%) as in other artemisinin mono-therapy when given for less than 5-7 days. The suppositories were found to be as rapid as the other formulations of artemisinin derivatives in clearing parasitaemia and well tolerated and safe with no serious adverse effects [10].

The second phase was conducted in 1996 by using sequential artesunate suppository (800 or 1200 mg) and mefloquine oral (1250 mg) in 54 severe falciparum malaria patients (including seven cerebral malaria patients with Glasgow Coma Score more than 10). There were again a rapid clearance of parasites (mean 100% PCTs of 42 and 35 hours) and a satisfactory 28 day cure-rates of 84 and 92% (attributed to the sequential addition of mefloquine) and no mortality [11].

The present study including cerebral malaria patients with Glasgow Coma Score less than 10 was conducted not only at CRU (Malaria), DSGH, but also at a District Hospital. No. 1 Military Hospital (300-Bedded), Myitkyina, with the nursing orderlies inserting the suppositories, as the third phase.

Objectives

General objective

To find a safe, effective, practical and convenient drug formulation and regimen for use in areas where facilities for parenteral therapy are not available.

Specific objectives

1. To determine whether artesunate suppository can rapidly clear parasitaemia in severe falciparum malaria (including patients with Glasgow coma score less than 10).
2. To find out if a rapid parasite clearance can equally be achieved with a low dose of artesunate suppository 800 mg rather than 1200 mg given over three days.
3. to confirm that a sequential artesunate suppository plus oral mefloquine can achieve a satisfactory cure rate in severe falciparum malaria.
4. to verify that artesunate suppositories can easily be given by untrained personnel at the district hospital.

Sites of study

1. Clinical Research Unit (Malaria), Defense Services General Hospital, Mingaladon.
2. No. 1 Military Hospital (300-Bedded), Myitkyina.

Study period

May 1998 to April 2000

Study design

A prospective double blind randomized controlled study.

MATERIALS AND METHODS

Study population

100 severe falciparum malaria patients.

Inclusion criteria

1. Patients – both sexes
2. Age 15-50 years
3. Weight more than 35 Kg
4. Patients with clinical symptoms of malaria and peripheral blood film positive for asexual forms of *P. falciparum*.

5. Any one or more of the following manifestations of severe falciparum malaria
 - a. Cerebral malaria – Glasgow Coma Score between 3-14
 - b. Acute renal failure – Urine output of less than 400 mL in 24 hours in adults failing to improve after rehydration and blood urea and/or serum creatinine more than three times of normal.
 - c. Jaundice – serum bilirubin more than 50 $\mu\text{mol/L}$
 - d. Hyperparasitaemia – more than 250,000/ μL
 - e. Severe anaemia – normocytic anaemia with haemoglobin less than 7 gm/dL
 - f. Hyperpyrexia – oral temperature above 40°C or 104°F
 - g. Hypoglycaemia – whole blood glucose less than 2.2 mmol/L
 - h. Haemostatic abnormalities – spontaneous bleeding/DIC
 - i. Malaria haemoglobinuria (Black water fever)
 - j. Repeated generalized convulsions
 - k. Prostration, extreme weakness – cannot sit, stand or walk (with no obvious neurological explanation)
6. Informed (verbal and written) consent of patient or relative.

Exclusion criteria

1. Other concomitant illnesses
2. Diarrhoea and if suppository cannot be retained
3. Diseases of the rectum
4. Patients given rescue treatment

Procedure

Patients fulfilling the criteria were initially subjected to a thorough history and physical examination by one of our group. thick and thin peripheral blood films were taken. Giemsa's stained and asexual parasitic count was done (number of parasites per 400 white blood cells) by well-trained laboratory technicians. All patients were managed according to the WHO recommendations (1986) [12]. Blood pressure, axillary

temperature, pulse rate and Glasgow Coma score were measured at 4 hourly intervals until fever clearance and full recovery of consciousness and at 12 hourly thereafter. Patients were examined daily for symptoms and signs and also for adverse effects of the drug according to the prepared proforma.

Laboratory procedures

Giemsa's stained thick and thin blood smears were examined and parasite counts performed 6 hourly until negative for 24 hours and daily up to 3 consecutive negatives, then weekly up to Day 28 and whenever it was indicated e.g. reappearance of fever. Negative parasite count was considered when no parasites were seen in 200 oil immersion fields. Blood for complete picture, platelet count, serum bilirubin, plasma glucose, blood urea, plasma protein and urine for RE were done on Days 0, 7, 14, 21 and 28.

Chest X-ray, ECG and other investigations were done as necessary to exclude differential diagnoses and to detect complications depending on individual cases.

All the cases were supervised by the medical specialists in our team. Patients considered not to be satisfactorily responding to the treatment were to be given a rescue treatment of standard intravenous quinine according to the discretion of the attending physician.

Treatment regimens

Artesunate suppositories

- (PLASMOTRIMTM RECTOCAP 200 mg) Lot No. 9833101, Mepha Ltd., Aesch Basel, Switzerland (Exp. 10-2001)

Mefloquine tablet

- (MEPHAQUIN LACTAB 250 mg) Lot. No. 96758, Mepha Ltd., Aesch Basel, Switzerland (Exp. 07-2001)

REGIMEN 1 (AsR 1200 mg + M 1250 mg)
PlasmotrimTM rectocap

- 1 suppository at 0, 12, 24, 36, 48, 60 hours (total 1200 mg)

MephaquinTM LACTAB

- 3 tabs at 72 hours and 2 tabs at 84 hours (total 1250 mg)

REGIMEN 2 (AsR 800 mg + M 1250 mg)
PlasmotrimTM rectocap

- 1 suppository at 0, 12, 24, 48 hours (total 800 mg)

MephaquinTM lactab

- 3 tabs at 72 hours and 2 tabs at 84 hours (total 1250 mg)

Nursing orderlies were instructed to insert the suppositories. Patients were asked to lie in bed for one hour after administration of the rectocaps. The rectocaps were re-inserted if they were expelled within one hour. Patients who were not able to retain the rectocaps at all were to be excluded from the study. Likewise mefloquine administration was under supervision and re-administered if it was vomited within one hour of administration.

Randomization

the patients were randomly allotted by means of sealed envelopes containing the prepared packages of either of the two regimens.

Data analysis

All data were entered in the prepared proformas. Statistical evaluations comparing the two treatment regimens were done by computer using EPI-INFO software.

Withdrawals

One patient in Regimen 1 and three patients in Regimen 2 did not complete the 28 days follow up and were excluded from the study. One patient from Regimen 2 presenting with cerebral malaria (Glasgow Coma Score 9) needed rescue treatment with intravenous quinine at 24 hours and was also excluded. The patient survived. Replacements were made for the withdrawn patients so that the

total numbers of patients were 100 for the total Phase III trial.

The unequal numbers of patients in the two regimens were due to the fact that when Phase IIIa was stopped for a preliminary report it included 18 patients in Regimen 1 and 28 patients in Regimen 2. Phase IIIb was then continued with equal numbers in each regimen making up to a total of 45 and 55 in the two regimens.

Ethical consideration

Ethical clearance was given by the Ethical Committee of the Department of Medical Research, Ministry of Health and by the Ethical Committee of the Medical Research (MOH) and Development Committee Ministry of Defense (MOD).

RESULTS

One hundred patients evaluable for the total phase III trial included 94 males and 6 females. Twenty-six patients were from No. 1 Military Hospital (300-Bedded), Myitkyina - (13 in each regimen). All the rest were from DSGH, Mingaladon.

There were no patients who could not tolerate the rectocaps or mefloquine. The nursing orderlies had no difficulty in inserting the suppositories. No re-insertion of the rectocaps was needed.

This study concentrated on the early clearance of parasitaemia, but the usual parameters for assessing efficacy were also analyzed.

Table 1 shows that the sex distribution, mean age, initial body temperature and initial parasitaemia between the two groups were comparable with no statistically significant differences between them.

Table 2 shows the number of patients with different severe manifestations in the two groups.

Table 3 shows the mean Glasgow Coma Scores and times to regain consciousness of patients with cerebral malaria in the two groups. Times to regain consciousness in these patients were also satisfactory and comparable.

Table 1. Base-line characteristics of the patients in two groups

Characteristic	Regimen 1 (n = 55) AsR 1200 mg + M 1250 mg	Regimen 2 (n = 45) AsR 800 mg + M 1250 mg	'P'
Sex	52 : 3	42 : 3	
Male : Female			
Age (years) Mean (SD)	23 (7.8)	22.1 (7.1)	>0.5
Initial temperature(°C) Mean (SD)	39.2 (1.3)	38.8 (1.1)	>0.5
Initial parasit- aemia (/µl) Mean (SD)	20494 (28273.7)	19543 (44876.4)	>0.5

Table 2. Various presentations of severe malaria in two groups

Manifestations	Regimen 1 (n = 55) AsR 1200 mg + M 1250 mg		Regimen 2 (n = 45) AsR 800 mg + M 1250 mg	
	No.	%	No.	%
Cerebral malaria (GCS > 10)	26	47.3	17	37.8
Cerebral malaria (GCS < 10)	7	12.7	8	17.8
Jaundice	6	10.9	4	8.9
Anaemia (Hb < 7 gm/dL)	11	20	11	24.4
Hyperpyrexia (> 40°C)	3	5.4	2	4.4
Hyperparasitaemia (> 250,000/µL)	1	1.8	2	4.4
Malaria haemoglobinuria	1	1.8	1	2.2

Table 3. Glasgow Coma Score and time taken to regain full consciousness

	Day 0 Glasgow Coma Score Mean (SD)	Time to regain full consciousness (hours) Mean (SD)
Regimen 1	13.1 (2.6)	38.8 (34.3)
Regimen 2	11 (2.3)	40.22 (45.5)
'P'	>0.01	>0.5

Table 4 shows the therapeutic responses. There were no statistically significant differences between two groups.

Table 4. Therapeutic responses of the two groups

Response	Regimen 1 (n = 55)	Regimen 2 (n = 45)	'p'
FCT (hours)	34.6	33.8	>0.5
Mean (SD)	(46)	(39.4)	
PCT (hours)	59.3	50.4	>0.05
Mean (SD)	(27.5)	(28.2)	
28 day cure- rate No. (%)	55 (100)	41 (91.1)	>0.5
Mortality No. (%)	4 (7.2)	1 (2.2)	>0.1

FCT = Fever Clearance Time

PCT = Parasite Clearance Time

There were no serious adverse effects clinically except for slight dizziness in 14 cases (8 patients in Regimen 1 and 6 in Regimen 2). There were no remarkable adverse haematological, biochemical or ECG changes caused by the Plasmotrim-Mephaquin sequential regimens.

DISCUSSION

In South East Asia including Myanmar, where multi-drug resistance of *Plasmodium falciparum* is a major problem, the use of artemisinin derivatives will increase. Neurological adverse effects have been reported in animals given very high doses of these drugs, but this has not been apparent in the thousands of patients included thus far in clinical trials and in over a million people who have been treated with these drugs. Studies are now underway comparing the efficacy of different artemisinin derivatives and formulations. Suppositories of artemisinin derivatives may represent an important advance in the rural management of severe malaria.

The first phase of our study confirmed the tolerability and acceptability of the artesunate suppositories and its rapid parasite clearance time in uncomplicated

falciparum malaria. The second phase of the study proved its efficacy in severe falciparum malaria. The combination with mefloquine achieved the desired cure rate. Now, this third phase of the study proved its efficacy in deeply comatose cerebral malaria patients and confirmed that even a lower dose of 800 mg over three days was sufficient to clear parasitaemia within 40 to 60 hours.

CONCLUSION

This study confirmed the in-vivo efficacy of artesunate rectocaps in severe malaria in adults.

An artesunate suppository are well tolerated, rapidly clears parasites in severe falciparum malaria and are effective even at a low dosage of 800 mg given over 3 days. The addition of mefloquine ensures a satisfactory cure rate over 28 days. It is a simple and practical alternative that can be used in severe falciparum malaria in remote areas.

RECOMMENDATION

Studies comparing intra-muscular artemether, intra-venous artesunate and artesunate suppositories in severe falciparum malaria should continue, to obtain a larger number of patients.

In the mean time artesunate suppositories may be used as alternative to the standard parenteral anti-malarial therapy in places where facilities for injections are not available.

ACKNOWLEDGEMENTS

We are indebted to Brigadier General Mya Thein Han, Director of Medical Services, Ministry of Defense and to the Prof. Dr. Paing Soe, Director General, Department of Medical Research (Lower Myanmar), Ministry of Health for their encouragement

and support; to Colonel Tin Thein Lwin, Commanding Officer, DSGH, for his kind permission to carry out this work; to all the staff of the CRU (Malaria), DSGH, for their dedication and to all the patients.

This work was funded by Mapha Ltd., Aesch Basel, Switzerland.

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Low prevalence of lactose malabsorption and lactose intolerance in Myanmar top athletes

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Good nutrition is essential for success in athletes. Although milk is an easily available good source of nutrition, abdominal discomfort and diarrhoea due to milk and milk products in lactose intolerant athletes can hamper their athletic performance. The present study was carried out to identify those athletes who are intolerance to lactose by using milk breath hydrogen test and advice them to refrain from taking milk and milk products during competition to avoid ill effects. Out of 324 Myanmar top athletes from various sports disciplines selected for 18th SEA Games (Thailand, 1995) who were subjected to milk breath hydrogen test, 15 (4.63%) were found to be lactose intolerance and 81 (25%) turned out to be lactose malabsorbers. Lactose malabsorptions in Myanmar adults have been reported to be as high as 93%. The present study reveals a much lower prevalence of lactose malabsorption among athletic population in Myanmar.

INTRODUCTION

Lactose, the principal carbohydrate found in milk, can be absorbed only when the lactose molecule is hydrolyzed by lactase into glucose and galactose. In human as well as in most suckling mammals, intestinal lactase activity falls gradually with commencement of weaning and the introduction of non-lactose containing foods in the diet. The ability to digest lactose decreases and the primary delayed onset lactase deficiency is well established by the age of 3-7 years in most human population. Racial and ethnic differences have been reported in lactose malabsorption and lactose intolerance [1]. Only about 10% of persons of European ancestry manifest malabsorption whereas Orientals in Asia generally show about 90% malabsorption [2]. However, within areas

where tolerance is generally low, isolated groups of individuals show high level of tolerance. Although the prevalence of lactose malabsorption in Myanmar adults has been reported to be 93% [3], the prevalence among Myanmar athletic population is still unknown.

Through the pioneering work by Calloway and associates, the breath hydrogen method has been used widely in the study of lactose intolerance [4]. Originally the dose has been 50 g lactose. This doses is unphysiological because it is equivalent to the amount of lactose in a litre of milk. The breath hydrogen testing has been demonstrated to detect the malabsorption of as little as 2 g of incompletely absorbed carbohydrate. The question regarding carbohydrate tolerance usually involve dietary issue and the use of

physiological dose of carbohydrate in their natural food forms become increasingly popular. Rosado and Solomons had shown that sensitivity and specificity of the breath hydrogen using 360 ml of whole milk for detecting lactose malabsorption was 80% and 100% respectively [5]. The present study was carried out to determine prevalence of lactose malabsorbers and lactose intolerance among Myanmar top athletes using milk breath hydrogen test.

MATERIAL AND METHODS

Subjects

The first study group consisted of 16 adults of both sexes (8 males and 8 females) between 20-50 years of age from the Department of Medical Research. The second group consisted of 324 Myanmar top athletes (175 males and 149 females) from 18 sport disciplines and events. Their ages ranged between 12-45 years. All were apparently healthy, had not taken any antibiotics and no gastrointestinal disease during previous 2 weeks. The informed verbal consent was taken from each subject. Smoking was prohibited during the test.

Lactose absorption test

After an overnight fast, each subject was given 360 ml of milk solution containing 46.8 gm of cow's milk powder, which contained 17.6 gm of lactose. Breath samples were taken immediately before given milk solution and every 30 minutes for 3 hours. To ensure that end-expiratory breath samples were obtained we used T-valve breath sampling plastic bag, which allows expired air from the dead space to be discarded. The samples were immediately transferred to 30 ml airtight plastic syringes. The hydrogen and oxygen collected breath samples were measured within 24 hours. The subjects were monitored up to 48 hours after the milk meal for evidence of symptoms of lactose intolerance.

Using the definitions described below, the subjects were classified as lactose-absorbers or lactose-malabsorbers. All lactose absorbers were re-tested within a week. They were given a standard test meal of 10 g lactose (15 ml Duphalacs; Duphar B.V. Holland) to determine their hydrogen-producing status and were classified as true lactose absorbers or non-hydrogen producers.

Analysis of hydrogen and oxygen in breath samples

A gas chromatograph (Model 12 Microlyzer, Quintron Instruments Co., Milwaukee) was used to measure hydrogen in the breath samples. A portable oxygen analyzer (Oxywarm 100 I, Draeger Instrument Division, Blyth, Northumberland, Australia) was used to measure oxygen in the breath samples.

Symptom score

The subjects were asked to record the presence or absence of the three symptoms: flatulence, meteorism and/or diarrhoea experienced during the test period.

Working definition

Lactose intolerance – was defined as any symptoms (flatulence, meteorism and/or diarrhoea) appear after the ingestion of a test dose of milk.

Lactose malabsorption – any peak hydrogen concentration greater than 20 ppm above baseline values in the 90-, 120-, 180- or 240- min samples after a milk meal.

RESULTS

Table 1 shows the prevalence of lactose malabsorption and lactose intolerance in Myanmar top athletes and control group. Myanmar top athletic population has lower prevalence of lactose malabsorption and lactose intolerance than control group (24.38% vs 68.75% and 4.63% vs 75% respectively).

Table 1. Percentages of lactose malabsorbers and lactose intolerance persons among control subjects and top athletes in Myanmar

Population	No. of persons	Lactose absorbers	Lactose malabsorbers		
			Lactose intolerance	Without intolerance	Total
Control subjects	16	5 (31.25%)	3 (18.75%)	8 (50%)	11 (68.75%)
Athletic population	324	243 (75.62%)	15 (4.63%)	66 (19.75%)	81 (24.38%)

Table 2 shows the comparison of the prevalence of lactose malabsorbers in Myanmar athletes and people from Southeast Asia and East Asia. Except Japanese, all people from Southeast Asia and East Asia showed high prevalence of lactose malabsorption. Myanmar athletes showed only 24.38% lactose malabsorption.

Table 2. Comparison of the prevalence of lactose malabsorbers in Myanmar top athletes, Myanmar general population and people from South East Asia

Study population	No. of persons in the study	Lactose malabsorbers	%	Reference
Thai	100	96	96	[2]
Chinese in Australia	30	27	100	[2]
Japanese	40	29	73	[2]
Indonesia	53	48	91	[2]
Vietnamese	6	6	100	[2]
Myanmar (1972)	31	29	93	[3]
Myanmar athletes (Present study)	324	81	24.38	

DISCUSSION

Being 100% specific and 100% sensitive the breath hydrogen test is the most valid indirect method of detecting lactose malabsorption. Rosado and Solomons had shown the milk breath hydrogen test to have a sensitivity of 80% and a specificity of

100% [4]. In the present study, the prevalence of lactose malabsorption among Myanmar top athletes was found to be 24.38% and that of lactose intolerance was only 4.63%. This finding was much different from lactose malabsorption (93%) and lactose intolerance (68%) rate among Myanmar general population previously reported [3]. They used blood glucose determination after lactose loading to detect lactase deficiency. Along with the Myanmar top athletes, 16 apparently healthy adults of both sexes (8 males and 8 females) between 20 to 50 years of age from the DMR took part in the present study. 68.78% of them were found to be lactose malabsorbers with the lactose intolerance of 18.75%. Since athletes involved in this study were coming from the same Myanmar population (Ethnic and Racial), the difference in the prevalence of lactose malabsorption and lactose intolerance may not be due to genetic cause. In our country, it is a tradition to take milk as a nutrient source for developing children and sick persons and most of athletes have habit of milk consumption. This finding may support the induction hypothesis, which was introduced by Bolin, Davis and their colleagues [7]. They suggested that ingestion of certain foodstuff containing lactose induces intestinal lactase activity. However, overwhelming evidence is that lactase is not inducible enzyme. Therefore, another possible explanation may be due to adaptation brought about by the effect of the unhydrolysed lactose on the pH and bacteria flora of colon [8]. Under these conditions, the fermentation of undigested lactose gives rise to organic acid. An acidic colonic environment is inhibitory to further

fermentation with consequent reduction in hydrogen production. Furthermore, the undigested lactose may result in the altered composition of colonic micro flora and presumably, a reduction of gas forming bacteria and increase in non-gas producing organisms. These mechanisms could ameliorate symptoms and improve tolerance to lactose.

ACKNOWLEDGEMENT

We would like to thank Director General of the Department of Medical Research for his encouragement and guidance for this study. Our heartfelt thanks are also extended to all the respondents of this study. The Department of Medical Research financially supported this work.

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SHORT REPORT

ABO blood groups and susceptibility to malaria

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The ability of a host to resist malaria infection is determined not only by immunological mechanisms but also by innate characteristics of the host. Innate resistance is genetically determined and as such exists without exposure to the parasite. Because of these factors, the host may completely resist an infection or reduce its severity. Among these genetic factors, the association between Duffy blood group determinants and *Plasmodium vivax* infection is well established [1]. Association between ABO blood groups and malaria infection was reported by many authors [2-5], but with conflicting results. Thus, ABO blood groups and malaria infection was examined in malaria endemic regions of Myanmar.

During September 1997 a cross-sectional study was carried out in four malaria endemic villages in Kanbauk village tract, Yephyu Township, Union of Myanmar. A total of 425 villagers from all four villages were tested for ABO blood groups and malaria parasites simultaneously.

Blood samples were taken by finger prick with sterile disposable lancets (Bayer Diagnostics). Both thick and thin blood films were stained with Giemsa's and examined under oil-immersion ($\times 10^3$) for malaria parasites. Thick films without visible parasites after screening 200 oil-fields were regarded as negative. Species identification was confirmed in thin films. ABO grouping was determined by slide agglutination test using anti-A and anti-B sera from Epiclone^R, CSL limited, Australia. Statistical analysis is calculated by the Student's 't' test.

The ABO blood group distribution was 22%, 35%, 38% and 5% for A, B, O and AB groups respectively. Their allotment are similar to other parts of the country [6]. The parasite positive rate in all persons irrespective of blood groups was 16% with *P. falciparum* and *P. vivax* accounting for 11% and 4% respectively. When the parasite positive rates and parasite species were compared with the ABO blood groups as in Table 1, there was no significant difference in regards to parasite positive rate

Table 1. Parasite positive rates and species in relation to ABO blood groups

SN	Blood groups	Total positive	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed	Significance
1	A	19/95 (20%)	19/95 (20%)	0/95 (0%)	0/95 (0%)	N.S.
2	B	21/148 (14%)	5/148 (3%)	13/148 (13%)	3/148 (2%)	N.S.
3	O	25/160 (16%)	21/160 (13%)	3/160 (3%)	1/106 (0.6%)	N.S.
4	AB	3/22 (14%)	2/22 (9%)	1/22 (1%)	0/22 (0%)	N.S.
5	All groups	68/425 (16%)	47/425 (11%)	17/425 (4%)	4/425 (0.9%)	N.S.

N.S. = not significant ($P > 0.05$)

and the ratio of *P. falciparum* and *P. vivax* ($P>0.05$) in all blood groups.

There are only limited data on the relationship between malaria and ABO blood group antigens. There were reports of no association between ABO blood group and susceptibility to falciparum malaria in West Africa [4,5] and in India [2]. However, Singh *et al.*, 1995 [3] found lower parasite positive rate in AB group and significantly lower frequency of *P. falciparum* infection among people with A and B groups.

Udomsangpetch *et al.*, 1993 [5] determined rosette formation of *P. falciparum* infected RBCs and formed rosettes better with blood group B and AB RBCs than with RBCs of other groups. They concluded that the nature of the receptors on normal RBC involved in the rosette formation may be related to ABO blood group antigens.

Our results revealed no significant difference in the parasite positive rates among different ABO blood groups. However, our study population consisted of heterogeneous people with differing malaria exposure, some living in low and others in moderate malaria endemicity. Thus it is advisable to study larger population who

live under the same malaria endemicity preferably in areas of hyperendemicity so that all the population has the chance of exposure to malaria.

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SHORT REPORT

Bacterial quality of water from Kanbauk areas during 1997

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Epidemic outbreak of shigellosis [1] and water-borne typhoid fever in Catalonia in 1994 [2] highlight the effect of contaminated water. This study aims to demonstrate the water quality, which is consumed by local people. It was carried out in rural area, Kanbauk (Dawe) that includes Eindayazar, Phaungdaw, Pyingyi, Hlegone and Ohnbinkwin quarters, from January and November 1997. A total of 107 water samples from Kanbauk, (Dawe) and 23 rectal swab samples of diarrhoea cases from Ohnbinkwin were tested. Presumptive test for water quality was done as described in the guidance for drinking water quality [3]. Isolation and identification of enteric bacteria from rectal swab samples was done

as described by WHO, 1981 [4].

Coliforms and faecal coliforms were positive in 70.59% and 11.76% respectively from the water samples of the well from Eindayazar in January. Moreover, the counts were positive in 100% and 82.61% respectively in November. These differences were significant ($p < 0.001$) between these two months. Coliforms and faecal coliforms were positive in 82.76% and 10.34% respectively from water samples of the wells from Phaungdaw in January 1997. Coliforms were positive in the water sample of three wells were and from one reservoir from Pyingyi (80%). All the five samples were free of faecal coliforms during

Table 1. Bacteriological quality of water from wells of Kanbauk

Place/study period	No. of wells Tested	Coliforms (C)	Faecal coliforms (FC)	C & FC
		Present	Present	Absent
Eindayazar, Jan., 1997	17	12 (70.6)	2 (11.8)	5 (29.4)
Eindayazar, Nov., 1997	23	23 (100)	19 (82.6)	Nil
Phaungdaw, Jan., 1997	29	24 (82.8)	3 (10.3)	5 (10.3)
Pyingyi, Nov., 1997	5	4 (80)	Nil	1 (20)
Hlegone, Nov., 1997	3	3 (100)	Nil	Nil
Ohnbinkwin, Nov., 1997 (Diarrhoea cases used)	11	11 (100)	9 (81.8)	Nil
Ohnbinkwin, Nov., 1997 (nondiarrhoeal cases used)	19	19 (100)	5 (26.3)	Nil

Figures in parentheses denote percentages

November 1997. All the water samples of the three wells tested in Hlegone were positive with coliforms and faecal coliforms were free in those samples in November 1997. All the water samples of the 30 wells tested were positive with coliforms in Ohnbinkwin. From the sample of the wells used by diarrhoea cases and non-diarrhoeal members, the faecal coliforms were present in 81.8% and 26.3% respectively ($p < 0.001$). All these results were shown in Table 1. *Vibrio cholerae* O1 was isolated from three cases of diarrhoea from Ohnbinkwin village.

The factors influence the level of contamination of water in hand dug wells were rainfall, number of households using the wells, amount of water usage daily and the distance of the well from the nearest kitchen or toilets. The installation of a simple rope-pump on family wells improves the quality and availability at a favourable cost/benefit ratio [5]. In our study, it was found that the wells used by the individual household members were less contaminated than the wells used by many households. It was also noted that wells with positive isolation of faecal coliforms were associated with those used by members of household of diarrhoea cases. During the study in Ohnbinkwin, a small spread of acute diarrhoea was occurred. The bacterial pathogen isolated was found to be *Vibrio cholerae* O1 (Ogawa). This strain was isolated from three diarrhoeic cases; one

adult (female: 32 years) and two children (one female: three years and one male: nine years). The faecal coliforms were positive in 81.8% are in the water samples of the wells used by non-diarrhoeal members. It had been shown that water plays an important vehicle in the role of transmission of enteric bacterial pathogens. Therefore, control measures should be undertaken to avoid contamination of water.

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INDEX TO VOLUME 12 (2000)

AUTHOR INDEX

A			Nyan Moe	-	54
Aung Htay	-	52	Nyo Aung	-	21
Aung Kyaw Zaw	-	14	P		
Aung Zaw Oo	-	41	Philippe, Eldin		
Aye Aye Myint	-	7, 54	Pedecoulas	-	54
Aye Yu Soe	-	41	S		
B			San Kun	-	48
Ba Thike, Katherine	-	10	San Shwe	-	21
H			Sein Hla Kyaw	-	54
Hauk Kyung	-	36	Soe Min Thein	-	48
Hla Hla Win	-	10	Soe Soe	-	52
Hla Pe	-	48	Soe Thein	-	14
K			T		
Khin Myat Tun	-	25	Than Nu Shwe	-	14
Khin Myint Wai	-	21	Than Nu Shwe	-	30
Khin Nwe Oo	-	36	Than Swe	-	52
Khin Nyo	-	41	Than Than Lwin	-	48
Khin Saw Aye	-	52	Thuzar Myint	-	54
Khin Thet Wai	-	10	Tin Nu Swe	-	48
Kyaw Kyaw	-	36	Tin Ohn	-	30
Kyaw Nyunt Sein	-	36	Tin Oo	-	48
Kyi Kyi Tin	-	41	Tun Pe	-	1, 7
Kyi May Htwe	-	7	W		
L			Wah Wah Aung	-	10, 30
Le Le Win	-	21	Y		
M			Ye Tint Lwin	-	8
Mar Mar Nyein	-	10, 25, 30, 54	Yi Yi Myint	-	14
Marlar Than	-	41	Z		
Mi Mi Htwe	-	30, 48	Zay Soc	-	41
Moh Moh Win	-	10			
Mya Mya Yee	-	14			
Myat Phone Kyaw	-	41			
Myint Oo	-	54			
Myo Khin	-	48			
N					
Ngeow, Y.F.	-	10			
Ni Win	-	48			
Nu Nu Aung	-	1			
Nwe Nwe Yin	-	36			

SUBJECT INDEX

A

ABO Blood Group System	-	52
Agglutination test	-	36
Antibiotics	-	30
Antimicrobial Drug Resistance	-	25
Antivenins	-	1

D

Diarrhoea	-	14
-----------	---	----

E

Escherichia coli	-	30
------------------	---	----

F

Fertility	-	21
-----------	---	----

I

Infections	-	10
------------	---	----

L

Lactose Intolerance	-	48
Lactose malabsorption		
<u>See</u>		
Lactose Intolerance		
Leprosy	-	36

M

Malaria	-	52
---------	---	----

Malaria, falciparum	-	41
---------------------	---	----

Mefloquine	-	41
------------	---	----

R

Reproductive tract infections <u>see</u>		
Infections		

R

Respiratory tract infection	-	14
Russell's viper	-	1, 7

S

Salmonella typhi	-	30
Serodiagnosis <u>see</u>		
Serologic Tests	-	36
Shigella dysenteriae	-	25

T

Toxoids	-	7
---------	---	---

V

Vibrio cholerae	-	30
Viper Venoms	-	1

W

Water-Microbiology	-	52
--------------------	---	----

