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Contraceptive prevalence survey in rural Hlegu <i>Mya Mya & Si Lay Khaing</i>	...	...	...	...	81
Bio-availability study of tetracycline <i>S.J. Tha, Aye Than & Saw Han</i>	...	...	...	...	86
<u>Aeromonas</u> and <u>Plesiomonas</u> strains isolated from diarrhoea and dysentery cases <i>Phyu Phyu Win & Tin Aye</i>	...	...	...	...	90
Epidemiology of sports injury in Myanmar athletes <i>San Shwe, Than Toe, Thein Hlaing, Ni Ni Win, Tin Mg Mg & Moe Thida</i>					96
The effect of cobra (<u>Naja naja</u>) venom on blood coagulation/ fibrinolysis system and on red blood cells <i>Khin Ei Han, Than Than, Myat Mon Oo & Ni Win</i>	...	...	...	...	100
Non-01 <u>Vibrio cholerae</u> isolation from flies, cooked children's foods, drinking water, currency notes and vegetables in Yangon <i>Khin Nwe Oo, Aung Myo Han & Tin Aye</i>	...	...	...	...	105
The effect of quinine and quinidine on patients with highly parasitised falciparum malaria (A double blind study) <i>Tin Shwe, Pe Than Myint, Cho Cho Myint & Tin Aung</i>	...	...	...	...	110
ELISA HTLV-III antibody reactivity of Myanmar patients infected with <u>P. falciparum</u> malaria (Short Report) <i>Pe Than Myint, Tin Shwe, Than Swe & Khin Pyone Kyi</i>	...	...	...	...	116
Lepromin reaction in children of parents with leprosy <i>Tin Shwe</i>	...	...	...	...	118
The importance of frequent PCV monitoring and timely fresh blood transfusion in the management of severe falciparum malaria (Short Report) <i>Tin Shwe & Pe Than Myint</i>	...	...	...	...	121

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Contraceptive prevalence survey in rural Hlegu

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A study on the prevalence of contraceptive use in Myanmar females (Phase II Rural) was undertaken in 26 villages of Hlegu Township. Two thousand five hundred ever married females of age 15 to 49 years were interviewed using the same questionnaire as in the urban phase. It was found that 84.24% of these women possessed knowledge regarding contraceptives. Oral contraception was the most recognised knowledgeable type, followed by the injectable. Sterilization is the third recognised method. The total ever users were 27.96% and current users are 10%. Oral pills were used by 22.50%, injectable by 9.53% and sterilization by 2.9% of knowledgeable persons. The main reason for not using contraceptives is fearfulness of the side effects (32.96%). Other causes were found to be desirous of more children, lack of knowledge and reluctance to interfere with nature. The most preferred method for future use was female sterilization (23.32%).

INTRODUCTION

This study followed the urban phase of the Contraceptive Prevalence Survey performed in Mandalay [1]. Because of transfer of the two authors from Mandalay to Yangon, the second phase of the study was carried out in the rural areas of Yangon division and not Mandalay. Great care was taken to select the survey site to be truly rural.

The objectives of this study being the same as that of the urban were as follows: to determine the knowledge, attitudes and practices about contraception in females living in a rural community in Yangon Division and to determine the availability of contraceptive methods.

MATERIALS AND METHODS

Twenty six villages in Hlegu Town-

ship were selected until 2500 ever married females between the age of 15 to 49 years were obtained.

Study method

An interview - administered questionnaire was used as the survey instrument. It contained 75 items regarding identification, socio-economic status, reproductive behavior and intentions, knowledge of fertility regulation methods, current and past contraceptive use, availability of contraceptive methods, reasons for not using contraceptives and preferred type of contraception use in future. Data were collected by project medical officer and staff of rural health centres and Aung San San-pya Health Training School from 1st November 1988 to 31st March 1990. Data were analysed with computer.

RESULTS

Personal characteristics

The age range stretched from 15 to 49 years. The largest distribution being seen between the age of 30 and 34 years (22.16%), followed by the 25 to 29 years of age (21.92%). The ages of 15 to 19 years were seen as the smallest group (4.8%). The mean age in this study was 30.67 years.

Among the interviewees, 12.6% had never attended school. Most of the women (71.36%) studied only up to the primary school level. Women who attended the middle school and high school were 11.8% and 3.8% respectively. In the rural community, only 0.48% of women could study up to the higher educational level. In this study, 49.2% of respondents were employed. They worked in the paddy fields and cutting wood in the Yoma.

Regarding the pregnancy state, 14.6% were pregnant and 83.92% were not pregnant at the time of interview. A few number of women (1.4%) were not sure whether they were pregnant at the time of interview.

Among the respondents, women having no children were 8.8%, one child 16.5%, two children 18.01%, three children 15.16%, four children 11.88% and those having five and above children were 29.5%. The mean parity for rural dwellers was 3.4.

Knowledge

In this survey, knowledge of various methods of contraception and of available sources were studied and shown in Figure 1.

Attitudes

Among the 2500 subjects, 67.4% agreed to space their family; 22.76% did not agree because they were afraid of

side effects, desirous to get more children and reluctant to interfere with nature, 1.08% want to have children depending upon conditions and 8.76% were undecided about spacing for their families. Regarding the questionnaire "number of children desire", only 0.48% wanted no children; 3.8% one child; 14.88% two children; 35.4% three children; 16.12% four children, 25.64% wanted five or more children and 3.86% answered that they wanted children as many as possible. The mean number of children desired by rural dwellers being 3.76.

Practices

The percentage of respondents with knowledge of contraception, and that of respondents practising contraception among the knowledgeable persons are shown in Table 1.

Although 84.24% of women possess knowledges concerning contraception, percentage of usage was 27.96% for ever users and 10% for current users. The causes of not using contraceptives were fearfulness of side effects (32.96%), desirous of more children (19.92%), lack of knowledge (13.56%) and reluctance to interfere with nature (10.24%). Non-usage due to financial problem in practising contraception was faced by 7.72% and 5.44% did not practise because of lactation or absence of husband from home for some reasons.

Sterilization was the most preferable method chosen (23.32%), followed by oral pills (18.44%) and injectables (15.2%).

DISCUSSION

Contraceptive prevalence study in the rural areas of Hlegu township had revealed that 84.24% of the women were knowledgeable of one or more contraceptive techniques. This

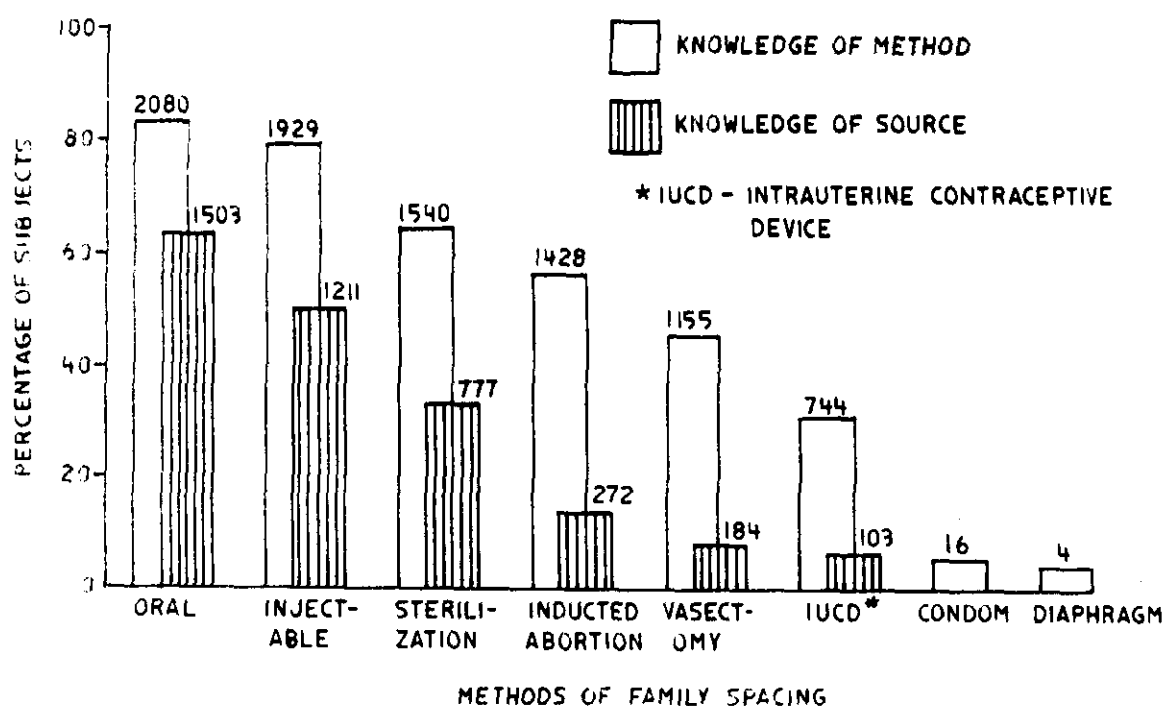


Fig 1. Comparison between knowledge about methods and sources of contraception

Table 1. Percentage of respondents (n=2500) having knowledge about various methods of contraception and the percentage of subjects practised contraception among those who have knowledge

No.	Method	Subjects having knowledge	Subjects Practised	Percentage	
				Subjects having knowledge	Subjects practised by knowledgeable person
1	Oral	2080	468	83.2	22.50
2	Injectable	1929	184	77.16	9.53
3	Sterilization	1540	45	61.6	2.9
4	Induced abortion	1428	23	57.12	1.6
5	Vasectomy	1155	3	46.2	0.25
6	Intrauterine contraceptive device	744	24	29.76	3.22
7	Rhythm	9	-	0.36	-
8	Withdrawals	3	-	0.12	-
9	Condom	16	-	0.64	-
10	Diaphragm	4	-	0.16	-
11	Others	76	8	3.04	10.53

figure is 13% lower than that of the urban population of Mandalay [1].

The actual difference in usage of contraceptives between the urban (20.88%) and rural (10%) areas may be due to difference in social and economic characteristics, such as women's education, husband's education and urban residence which influence successful use of contraceptives [2]. Both these prevalences are very much lower than those of Thailand in 1978 whence the contraceptive usage rate was 51% both in urban and non-urban population [3], but much higher than that in Rwanda which was 3-4% for 1988 [4]. In 1987 the contraceptive prevalence was increased to 68% in Thailand [5].

Reproductive profile of pregnant women of Myaungtaga and Yegyaw village tracts in 1981 [6] also showed that the actual contraceptive practice was 9.92% which is almost the same as the present study 9 years later. The two sites quoted are not far from the present study area and comparison as mainly rural may be accepted. The fact that within 9 years the contraceptive practice has not altered augurs a rapid increase in population in these areas.

Results of knowledge of different contraceptives follow the same pattern as in the urban study where oral pills was the most popularly known method followed by the injectables, tubectomy, induced abortion, IUCD and others: but at a lower level of percentage. Regarding the knowledge of sources of contraception the results are similar for both urban and rural but at a smaller percentage for the latter. The real difference in the practice of contraception was particularly evident in the use of condom and diaphragm where they were never ever utilized in the rural areas.

Analysing the gaps in the knowledge

and practice, in different methods of contraception is alarming because there is a huge difference between the two. The reasons for not using contraceptive were almost the same. Contraceptive use in future follows a similar pattern in both urban and rural sites which is: sterilization first then comes oral pills and injectables. Intrauterine contraceptive device seems less known in the rural areas than in the urban ones.

Spacing attitudes are less favourable in the rural than the urban places because the percentage of respondents that did not agree for family spacing was higher in rural (23%) than urban (12%) areas.

ACKNOWLEDGEMENTS

Health staff of Hlegu Township and Aung San San-pya health training school for helping in data collection; Dr. U Aung Tun Thet, Director General, Department of Planning and Statistics, Ministry of Health for allowing the use of computer; Dr. Daw San San Myint and computer staff for helping the data entry and analysis and to all the women who patiently answered the questionnaire.

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Bio-availability study of tetracycline

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Bio-availability of tetracycline hydrochloride capsules, 500 mg orally administered, was studied on 24 healthy adult Myanmar, aged 16 to 40 years. The peak tetracycline serum concentration of 1.42 ± 0.24 $\mu\text{g/dl}$ was attained in 4 hours, with a gradual fall to 0.16 ± 0.04 $\mu\text{g/dl}$ at 24 hours. Myanmar females, when compared to their male counterparts, showed a poorer ability to absorb the drug and had a shorter drug half-life in accordance with their obviously greater drug elimination. Nevertheless, there were no significant differences between males and females regarding the remaining major pharmacokinetic parameters such as, the time at which maximum concentration of drug in serum occurred, apparent volume of drug distribution, and systemic blood clearance of the drug. The clinical implication is thus discussed.

INTRODUCTION

The bio-availability of drugs as most commonly administered by oral route is the very initially important step in the man-drug encounter as stated by Clark & Smith, [1]. Other than factors which might influence variation in drug bio-availability, an inherent genetic factor might result in its altered pattern which has never been studied among Myanmar even though it was reported for the Westerners by several authors like Brice & Hammer, [2]; Blair, Barnes, Wildner & Murray, [3] and Barr, Gerbracht, Letcher, *et al.* [4]. An example of tetracycline bio-availability has been reported among these populations (Neuvonen, Gothoni, Hackman *et al.* [5]; Neuvonen & Turakka, [6]; Neuvonen, Pentilla, Ross *et al.* [7]) and it therefore might be practical in carrying out the bio-availability of tetracycline in Myanmar subjects, the majority of whom have experienced this particular broad-spectrum antibiotics almost soon after its introduction into the armamentarium of therapeutics.

MATERIALS AND METHODS

Tetracycline capsules, 500 mg (Myanmar Pharmaceutical Factory) were used.

(1) Subject selection

24 healthy subjects of both sexes, aged 16-48 years, were selected on the basis of a satisfactory history and medical examination. The study protocol was approved by the ethical committee and each subject gave a written informed consent, as mentioned by Winter *et al.* [8]; WHO Europublication, [9]; Thaw Zin, [10] and Thaw Zin *et al.*, [11], a sample size of 24, 12 in each sex group were included.

(2) Experimental design

Twenty-four healthy adult Myanmar, equally divided among the sexes, with age range of 16-48 years and body-weight of 48 ± 2 kg participated in the study. Subjects were not allowed to take any form of tetracycline for one week prior to the study, and no other drugs were administered during the study

period. Following an overnight fast of about 10 hours, the drug was ingested in the morning at 8:00 a.m. The dosage employed for each subject was two capsules (500 mg) of tetracycline hydrochloride (Myanmar Pharmaceutical Factory) administered orally with about 250 ml of water, as has been tested by Neuvonen & Turakka [6]. No food was allowed for at least the next two hours. Blood samples (5 ml) were taken from a forearm vein into vacutainers (Becton Dickinson, USA) containing no anticoagulant at various time intervals, namely immediately before and at 2, 4, 8 and 24 hours after dosing. Serum was separated and deep-frozen until assayed, not longer than 40 hours. Serum tetracycline concentration of all samples were assayed in duplicate microbiologically by the agar plate method. Time-course of the effect of tetracycline on serum concentration was determined and the pharmacokinetics of tetracycline in Myanmar, evaluated.

(3) Analytical methods

The samples were assayed for serum tetracycline levels by the agar plate method according to the United States Pharmacopoeia, [12]. All samples were determined in duplicate microbiologically.

(4) Pharmacokinetic analysis

The serum concentration-time data of healthy volunteers were plotted on a semilogarithmic graph paper and calculations were carried out to determine the best least-squares fit of the individual curves to a monocompartmental model. The resultant absorption and elimination rate constants and their half-lives obtained were used to calculate the T_{max} and C_{max} using standard formulae as stated by Curry, [13].

(5) Statistical consideration

Statistical analysis of group data was performed by unpaired Student's 't' test.

RESULTS

Following the administration of tetracycline, 500 mg/50 kg, a peak tetracycline serum concentration of 1.42 ± 0.24 $\mu\text{g/dl}$ was obtained at 4 hours, after which there was gradual fall to 0.16 ± 0.04 $\mu\text{g/dl}$ at 24 hours (Fig. 1). Cardinal tetracycline

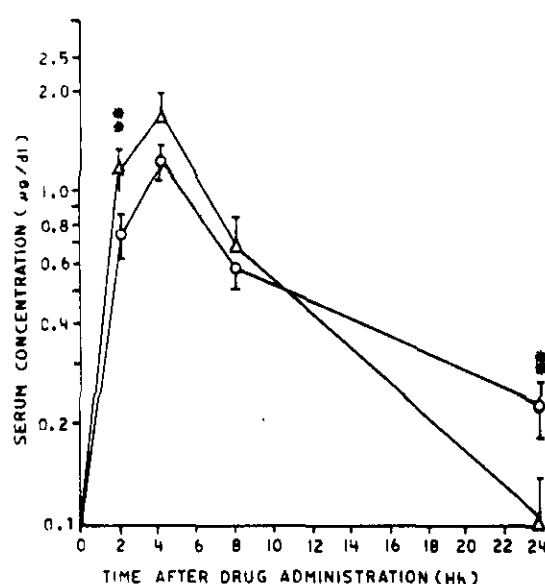


Fig. 1. Time-course of serum concentration of tetracycline administered orally in Myanmar subjects

Each point represents the means of 12 subjects and vertical bars indicate standard errors of the means. The groups tested were on 12 females (O) and on 12 males (Δ). Difference in levels * of statistical significance between female and male values are denoted as: $P < 0.025$.

pharmacokinetic indices on the 24 healthy adult Myanmar is shown in Table 1. The Myanmar females tested were younger in age and had a lesser body-weight ($p < 0.05$) than those of

Table 1. Serum parameters and pharmacokinetics of Tetracycline between male and female Myanmar subjects

Parameters	Male n=12	Female n=12	Difference p
Age range (yr)	20-48	16-35	
Body weight (kg)	51±2	44±2	0.05
Elimination half-life, $t_{1/2}$, in hr	7.3±0.8	4.2±0.4	0.005
Maximum concentration, C_{max} , in µg/dl	1.4±0.2	2.5±0.3	0.005
Time of maximum concentration T_{max} , in hr	2.8±0.3	2.9±0.2	N.S.
Apparent volume of distribution, aVd , in l/kg	5.8±0.6	4.4±0.8	N.S.
Systemic clearance, Cl_s , in ml min ⁻¹ kg	9.4±1.4	14.9±3.9	N.S.
Elimination rate constant in hr ⁻¹	0.1143±0.02	0.1854±0.03	0.025
Percentage absorbed in mg	71.99±3.3	57.10±4.0	0.005

N.S. = Not significantly different at the level of $p < 0.05$

their male counterpart. These women showed a poorer ability to absorb the drug and had a shorter drug half-life when compared with that of their men counterpart. Also, the drug elimination constant of the female was significantly greater than that of the male. However, there were no significant differences between males and females regarding the remaining major pharmacokinetic parameters such as, the time at which maximum concentration of drug in serum occurred, apparent volume of drug distribution, and systemic blood clearance of the drug.

DISCUSSION

The usual clinical dose of 500 mg of tetracycline orally had been previously studied by bio-availability in the Westerners by Neuvonen, *et al.* [7] and other workers where the values ranged from 60-80% was reported by Barza & Scheife [14] and Bennett, Mickelwait, Barrett, *et al.* [15]. The present study showed an equivalent bio-availability of tetracycline in Myanmar males (72 ± 3%) whereas a poorer ability was observed among

the females. It could be due to the varied consuming habits of Myanmar women which in the long run might have adapted the histology of the intestinal mucosal lining and thus resulting in interfering with drug absorption. At first glance the significant poor absorptions of Myanmar women might cause some anxiety towards its therapeutic concentration. However it could be noticed that there is a proportional lesser body weight of females to that of the males counterpart in relation to their poorer absorption, the ratio proportionately around 1.2-1.3. Therefore the administration of the same adult dose to either sex could attain equivalent therapeutic concentration in either sex. However, the pharmacokinetic parameters of tetracycline, namely greater elimination constant together with a shorter elimination half-life of Myanmar women, suggest the requirement to apply shorter time interval of less than 6 hours in the administering of tetracycline to this particular sex. Myanmar males could be administered on an equal footing with the Caucasians, on the other hand. In conclusion, the bio-availability of drug in Myanmar women could not affect the

therapeutic concentration of the drug, but it is necessary to shorten the time interval of drug administration, specifically in the case of tetracycline.

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Aeromonas and Plesiomonas strains isolated from diarrhoea
and dysentery cases

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The study was carried out to determine the frequency and importance of Aeromonas and Plesiomonas shigelloides in pathogenesis of gastroenteritis. Stool samples were collected from the diarrhoea and dysentery patients attended to the Infectious Diseases Hospital as well as from the outpatient clinics during August, 1990 to June, 1991. Isolation and identification of the pathogens was processed by standard biochemical test described by Cowen (1974) and according to the criteria outlined by von Graevenitz (1985). Of 124 patients studied, Aeromonas spp. were isolated from 5 (4.03%) and Plesiomonas shigelloides from 2 (1.61%). It is evident that Aeromonas and Plesiomonas spp. are not an uncommon aetiological agents of acute gastroenteritis in Myanmar. So, it is anticipated to study on these bacteria to expand the knowledge and to provide an information on the epidemiological control of the disease caused by these bacteria.

INTRODUCTION

Aeromonas and Plesiomonas strains have been recognized for some time as causative agents of human disease, but few clinical data have been made available. Generally, it has been recognized that these bacteria are opportunistic pathogens in the immunocompromised host and persons with underlying malignancy. Some authors [1,2] have proposed Aeromonas and Plesiomonas spp. as causes of acute diarrhoea and dysentery cases, but others [3] have been unable to ascribe any clinical significance to these bacteria. Their importance as agents of gastroenteritis is, therefore, still controversial and subject to continuing investigation in many parts of the world.

In recent years, reports of the isolation of Aeromonas spp. and Plesiomonas shigelloides from patients with diarrhoea and dysentery in different parts of the world have

increased. This development has no doubt increased the level of awareness of their potential pathogenic role and geographical spread. Due to lack of awareness of their pathogenic potential in human and a failure to recognize these organisms in the laboratory, little information has been made available in Myanmar. So, it is hoped that isolation and identification of Aeromonas and Plesiomonas spp. would greatly expand the knowledge of the frequency of these bacteria and their importance as enteric pathogens in Myanmar.

MATERIALS AND METHODS

Patients and sources of isolate: The study included 124 patients with diarrhoea and dysentery diseases. Stool samples were collected from patients attending to the Infectious Diseases Hospital as well as from the outpatient clinics during August, 1990 to June, 1991. Diarr-

hoea in this study was defined as three or more loose stools a day and dysentery was defined as frequent passing of stools with blood and or mucous per day.

Other data obtained on each patient included age, sex, general medical history and accompanying clinical features.

Isolation and identification of Aeromonas and Plesiomonas spp.: Freshly collected stool samples were plated on to MacConkey Agar (MA), Salmonella-Shigella Agar (SS) and Thiosulfate Citrate Bile Sucrose Agar (TCBS) (Fig. 1). Cultures were incubated at 37° Celcius for 18-24 h after which non-lectose fermenting colonies were screened for oxidase production [4].

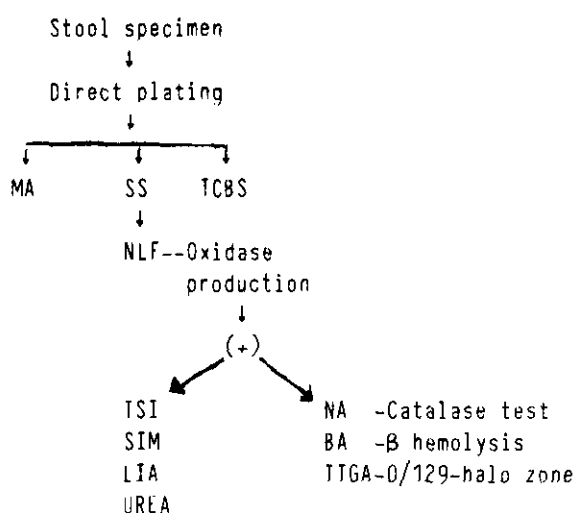


Fig. 1. Schematic drawing of the diagram showing isolation and identification of clinical specimens

Oxidase-positive colonies were subcultured on to Nutrient Agar plates (NA) (BBL) for catalase test, Blood Agar plates (BA) (BBL) for β hemolytic production and Taurocholate Tellurite Gelatin Agar Plates (TTGA) for the growth of colonies surrounded by halo or clear zones. The suspicious colonies from

these agar plates were later subjected to a short set of biochemical tests, and further confirmed as *Aeromonas* spp. or *Plesiomonas shigelloides* by standard biochemical tests [5] and according to the criteria outlined by von Graevenitz, 1985 [6].

RESULTS

Of 124 patients with diarrhoea and dysentery processed in this study, *Aeromonas* spp. were isolated from 5 (4.03%) and *Plesiomonas shigelloides* from 2 (1.61%), *Shigella* spp. from 4 (3.2%), *Salmonella* spp. from 2 (1.61%), of the cases and the rest were non-pathogenic *Escherichia coli*. *Vibrio cholerae* was not isolated from any of the specimens processed. Table 1 & 2 show the results of the biochemical characters of *Aeromonas* and *Plesiomonas* spp. obtained by the standard tests [6].

In this study, clinical features of diarrhoea and dysentery associated with *Aeromonas* and *Plesiomonas* spp. were similar to those of gastroenteritis caused by other enteropathogens. There were abdominal pain in 2 (100%), nausea and vomiting in 1 (50%) and no dehydration in 2 of the patients associated with *Plesiomonas shigelloides* infection. Similarly, there was abdominal pain in 3 (60%), nausea and vomiting in 4 (80%), and mild degree of dehydration in 1 (20%) of 5 patients from whom *Aeromonas* spp. were isolated.

Out of 124 cases, 82 cases (66.1%) were diarrhoea and the rest were dysentery cases (33.9%). Most cases of *Aeromonas* spp. were isolated from male dysentery patients (60%) and *Plesiomonas* spp. were isolated mostly from male dysentery cases. Furthermore, disease due to these

Table 1. Preliminary identification of Aeromonas and Plesiomonas spp.

Tests	<u>Aeromonas</u>					<u>Plesiomonas</u>	
	1	2	3	4	5	1	2
1. Oxidase	+	+	+	+	+	+	+
2. Catalase	+	+	+	+	+	+	+
3. String test	-	-	-	-	-	-	-
4. Growth on TCBS	-	-	-	-	-	-	-
5. Growth on TTGA (halo)	+	+	+	+	+	+	+
6. Growth on ISI	K/AG	K/AG	K/AG	K/AG	K/AG	K/A	K/A
7. Growth on LIA	K/A	K/A	K/A	K/A	K/A	K/A	K/A
8. Urease production	-	-	-	-	-	-	-
9. Growth on SIM							
Sulphide production	-	-	-	-	-	-	-
Indole production	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+
10. Salt tolerance test							
1% NaCl	+	+	+	+	+	+	+
3% NaCl	+	+	+	+	+	+	+
8% NaCl	-	-	-	-	-	-	-
10% NaCl	-	-	-	-	-	-	-

Key: K/AG alkaline slant, acid butt with gas production
 K/A alkaline slant, acid butt

Table 2. Biochemical characterization of Aeromonas and Plesiomonas spp.

Tests	<u>Aeromonas</u>					<u>Plesiomonas</u>	
	1	2	3	4	5	1	2
1. OF test	+	+	+	+	+	+	+
2. Citrate utilization	+	+	+	+	+	-	-
3. V P test	+	+	+	+	+	-	-
4. Growth in KCN	+	+	+	+	+	-	-
5. O/129 sensitivity	R	R	R	R	R	S	S
6. Sugar fermentation							
Lactose	-	-	-	-	-	-	-
Sucrose	A	A	A	A	A	-	-
Mannitol	A	A	A	A	A	-	-
Inositol	-	-	-	-	-	A	A
Arabinose	-	-	-	-	-	-	-
Salicine	-	-	-	-	-	-	-
Glucose	AG	AG	AG	AG	AG	A	A
7. Decarboxylation							
Control	-	-	-	-	-	-	-
Lysine	-	-	-	-	-	+	+
Arginine	+	+	+	+	+	+	+
Ornithine	-	-	-	-	-	+	+

Note: R, resistant; S, sensitive; A, acid production; G, gas production

two organisms affected all age groups as shown in Table 3.

Table 3. Distribution of cases of diarrhoea and dysentery associated with Aeromonas spp. and P. shigelloides

Age (years)	<u>Aeromonas</u> spp.		<u>P. shigelloides</u>	
	Male	Female	Male	Female
0-10	1	-	-	-
11-20	-	1	-	-
21-30	-	-	1	-
31-40	1	-	-	-
41-50	1	-	1	-
51-	-	1	-	-
Total	3(60%)	2(40%)	2(100%)	0(0%)

DISCUSSION

According to the results, it is evident that Aeromonas and Plesiomonas shigelloides are not an uncommon aetiological agents of diarrhoea and dysentery in our country, Myanmar. The study has shown that these bacteria are found in association with dysentery more often than in diarrhoea cases. This may be the main cause of Aeromonas and Plesiomonas gastroenteritis being misdiagnosed as bacillary or amoebic dysentery without proper investigation.

The clinical features of gastroenteritis associated with Aeromonas and Plesiomonas spp. consists of two syndromes namely, rice-watery like diarrhoea and dysentery. Accompanying symptoms like abdominal pain, nausea and vomiting, dehydration, and fever also are encountered. These features were not distinct from those of gastroenteritis due to recognized enteropathogens like Shigella, Salmonella and Escherichia coli. So, the results of this study strongly suggest the aetiological

role for both organisms. All the 5 Aeromonas strains isolated in this study were Aeromonas hydrophilia according to their biochemical characters. There are 4 species classified under the genus Aeromonas. Out of these species, Aeromonas sobria and Aeromonas hydrophilia are the main species associated with gastroenteritis in man [7]. So the results of this study is in agreement with the experience of other researchers [8,9,10].

Similar studies in other parts of the world have reported an isolation rate of less than 1 per cent for Aeromonas spp. in normal subjects [11,12,13]. Arai et al. [14] also found Plesiomonas shigelloides in the faeces of only 3 (0.01%) of 38454 normal subjects examined in Japan. However, there was no differences in isolation of these organisms from normal controls and patients with diarrhoea in Thailand [15]. But another study in Thailand have shown that Aeromonas spp. were isolated from diarrhoeic patients significantly more often than from the normal controls. Therefore, it is suggested that these divergent results may be related to geographical location, season of collection, and the microbial media used for isolation.

Aeromonas and Plesiomonas spp. have been recognized for some time as causative agents of human diseases [11] and recently several outbreaks of these bacterial infections have been reported from various countries. The bulk of Aeromonas and Plesiomonas strains have been isolated from stools of diarrhoeic patients living in subtropical and tropical areas, Japan, Australia, and the United States. Plesiomonas shigelloides have been reported as one of the agents causing Traveler's diarrhoea. Unfortunately,

little information has been made available in Myanmar. Suggested reasons for this are, lack of awareness of their potential human pathogenicity and a failure to recognize these organisms in the laboratory. It is also due to the fact that the patients are being treated by themselves without proper investigation and the mild nature of the illness itself. In conclusion, the results of this study strongly suggest the aetiological role for both organisms, particularly in dysentery cases.

Although this is the first report indicating the frequency and the importance of Aeromonas and Plesiomonas spp. causing gastroenteritis in Myanmar, more extensive and detailed studies will be required to understand more about the clinical spectrum and to expand the knowledge regarding the prevalence of these organisms in different seasons of Myanmar. By that mean, an epidemiological study can be facilitated to provide informations on the control of diseases caused by these bacteria.

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EPIDEMIOLOGY OF SPORTS INJURY IN MYANMAR ATHLETES

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An epidemiological study of sports injury was conducted in early part of 1990. Records of five different federations, namely track and field, foot ball, volley ball, boxing and weight lifting from outpatients department of the Aung San Stadium, were collected for 1985, 1986, and 1987. An interview study was also carried out in 116 Myanmar selected athletes from the above five federations, to determine the possible causal factors of sports injury. Three year average incidence rate of sports injury in five federations was 5.5 per 100 athletes. Common sites of injury, major types of injury, type of treatment, health seeking pattern, causes of sports injury were described, and discussed.

INTRODUCTION

Exercise, physical activity and sports always carry a risk of injury but most injuries are minor and preventable [1]. Nowadays, little is known about the problem of the sports injury in Myanmar due to lack of information. Thus the scarcity of the information and the need for prevention of sports injuries prompted us to undertake an epidemiological study to elucidate the magnitude of sports injury in Myanmar athletes. The specific objectives were: (a) to elicit the incidence of sports injuries in different federations of sports; and (b) to determine the causes of sport injuries in different sports federations.

MATERIALS AND METHODS

There are two components of the study: a record study and an interview study.

The record study consisted of collections of all available records and returns of sports injury for a period of 3 years, from 1985 to 1987

from the out-patient department (OPD) of the Aung San Stadium during March and April of 1990. Variables on identification of the person, type and site of injury, date of injury occurrence, and type and duration of treatment were extracted. A total of 1035 records for 248 athletes from different types of sports viz, Track and Field, Volley Ball, Foot Ball, Boxing and Weight lifting were analysed. In addition, the number of athletic group by each federation for the year 1986 were referred for estimation of incidence rate.

The interview study was conducted on 116 athletes from the above five federations in May through August, 1990, by using a set proforma including causal factors of the injuries. The coaches were also included in this study to check the information provided by the athletes in each federation.

RESULTS

Of the 248 injured athletes, 81% were males, and 19% were females.

Incidence rate

Three-year average incidence rate for the five federations was 5.5% and the sport-specific incidence rate of injury by federations were 7.1% among Volley Ball, 6.3% among Track and Field, 5.6% among Foot Ball, 2.3% among Weight lifting and 2.2% among Boxing athletes (Table 1).

Table 1. 3-year average incidence rate of sports injury by federation (1985-1987)

Type of sports	M	F	Total
Track & field	6.6 (136)*	5.9 (107)	6.3 (243)
Volley Ball	7.7 (205)	6.4 (152)	7.1 (357)
Foot Ball	5.6 (651)	-	5.6 (651)
Boxing	2.2 (137)	-	2.2 (137)
Weight lifting	2.3 (118)	-	2.3 (118)
Total	5.3 (1247)	6.2 (259)	5.5 (1506)

*Total no. of athletes for the year 1986 are shown in parentheses

Type of injury

The major type of injury was strain 45.9%, followed by sprain 30.9%, contusion 15.7% and back pain 3.4%. The other category including fractures and dislocation constituted only 1.4%. the distribution of the type of injury by federation is shown in Table 2.

Site of injury

The major site affected was seen in lower extremities i.e. about 60% in both the record and interview

studies. However, 24% of athletes had knee injuries in the record study and 25.8% had injured ankles in the interview study. Head injury was rarely noted in the records but 9.7% of athletes gave minor injuries on heads and 5% of athletes complained of low back pain on interviewing them.

Occurrence of repeated injuries

The mean proportion of repeated injuries among male athletes in the five federations during the 3 years was 3.5 and the corresponding value for females was 6.9.

Causes of injury

The causes of injuries revealed from the interview study were due to accidents (65%), fall (16%) and other causes like lack of required facilities and not doing enough warming up exercises (11%).

Type of treatment

From the record study, most of the injured athletes had treatment with ultra-sound (US)/US under water (48.8%) followed by short wave (SW) therapy (16.4%). A combination of SW, US and electrical stimulation (ES) constituted 11.1% and 5.9% respectively. The application of exercises, traction, ice pack together with ES or US were observed as other forms of treatment (Table 3).

From the interview study, the majority of the respondents took self treatment by applying liniment, massage, constituting about 32.2%, followed by treatment at the Stadium (Table 4).

Duration of treatment

Duration of treatment in the records

Table 2. Percent distribution of sports injury, by federation and injury type

Type of injury	Track and Field	Volley Ball	Foot Ball	Boxing	Weight Lifting	Total
Sprain	3.9 (40)*	11.5 (119)	13.1 (136)	0.7 (8)	1.2 (12)	30.4 (315)
Strain	12.2 (126)	15.9 (165)	15.7 (163)	0.9 (9)	1.3 (13)	46.0 (476)
Back pain						
Low back pain	0.4 (4)	1.4 (15)	1.2 (12)	0.1 (1)	0.3 (3)	3.4 (35)
Contusion (Blunt injury)	4.5 (47)	3.6 (37)	7.4 (77)	0.1 (1)	0.1 (1)	15.7 (163)
Tendonitis	0.5 (5)	0.4 (4)	0.5 (5)	-	-	1.4 (14)
Skin splint	0.6 (6)	0.9 (9)	0.1 (1)	0.1 (1)	-	1.7 (17)
Others	0.3 (3)	0.4 (4)	0.2 (2)	0.6 (6)	-	1.4 (15)
Total	22.3 (231)	34.1 (353)	38.3 (396)	2.5 (26)	2.8 (29)	100 (1035)

* Total no. of injuries in parentheses

Table 3. Distribution of sports injury by type of treatment (Record Study)

Type of treatment	No.	%
Ultrasound (US)	496	48.8
& US under water		
US & Electrical stimulation (ES)	118	11.6
US & Wax/SW/Ice pack	72	7.1
Short Wave (SW)	167	16.4
SW & Wax/ES	72	7.1
ES	17	1.7
Wax	50	4.9
Others (Exercise/Traction)	24	2.4
Sub total	1016	
Unknown	19	
Total	1035	100.0

revealed that 85.5% of athlete had taken 5 to 9 days and the mean was 4.9 days. While by interview, 66.1%

Table 4. Distribution of sports injury by types of treatment (Interview Study)

Type of treatment	No.	%
Self treatment	20	32.2
Treatment taken by MO at Stadium	13	20.9
Hospital treatment	2	3.2
Traditional Medical Practitioner	6	9.7
Chinese Medical Practitioner	4	6.5
Treatment by Specialist	4	6.5
More than one type of treatment	13	21.0
Total	62	100.0

respondents took treatment at least 1 to 4 weeks and only 19.4% were totally cured within a week.

DISCUSSION

This study provide the magnitude of the problem of sports injury in

Myanmar athletes to some extent. Common injuries in different type of sports were strain, sprain, contusions and back pain which are minor injuries. Fractures, dislocations, heel stress and skin splint were occasionally found in this study. We found that more injuries were observed in the Track & Field, Foot Ball and Volley Ball. There was a low occurrence of injury among Boxing and Weight lifting athletes. This may be due to lack of notification of these sports injuries or the athletes might have taken good precaution measures which were protective devices like wearing of head gear among boxing players while playing. This indicates the importance of having good facilities for each type of sports.

There were not much severe injuries seen in the record or by interview, and most of them were minor and preventable. However, it is noted that 5% of the athletes had repeated injuries of 10 times and more within the three years and females were more prone to develop repeated injuries. The determinants were accident, fall, lack of necessary facilities etc. This was confirmed by interview that pushing by other athletes (accident), fall due to uneven road, lack of appropriate facilities, e.g. walking shoes, running shoes, shin pad etc. were some of the causes for injury occurrence.

Most of the athletes treated themselves and some athletes consulted Chinese Medical and Traditional Medicine Practitioner first or after self treatment (Table 4). It is desirable that those with seemingly

severe injuries should consult general practitioners, specialists or hospital directly rather than Chinese Medical and Traditional Medicine Practitioners so as to avoid unnecessary complications.

In conclusion, it is highly recommended that further epidemiologic studies on injuries should be undertaken not only confined to selected athletes but also to other groups, like school children, other sports club teams etc.

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THE EFFECT OF COBRA (Naja naja) VENOM ON BLOOD
COAGULATION/FIBRINOLYSIS SYSTEM
AND ON RED BLOOD CELLS

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The effects of cobra (Naja naja) venom on blood coagulation, fibrinolysis and red blood cells were studied in vitro. Cobra venom had dose dependent inhibition effect on both intrinsic and extrinsic coagulation pathways. It also had inhibitive/destructive effect on the individual clotting factors V, VII and X but no effect on the action of thrombin on fibrinogen. However, cobra venom had neither fibrinolytic nor fibrinogenolytic effect even at high venom concentration (10mg/ml). Furthermore, cobra venom had direct haemolytic effect shown by increased red cell fragility and raised plasma haemoglobin level.

INTRODUCTION

There are several poisonous snakes prevalent in Myanmar Naing-Ngan. Among them Russell's viper and cobra bites are responsible for most of the snake bite cases. According to health statistic report [1] Russell's viper and cobra bites make up about 82-90% and 5.2% of all snake bite cases in Myanmar.

Cobra bite though not as common as Russell's viper bite is important because of the high mortality in its victims. The main causes of death in cobra bite are respiratory paralysis and cardiac arrest due to a potent curare like effect. Besides these neurotoxic and cardiotoxic actions, cobra venom has anticoagulant [2] and haemolytic actions [3].

There are many studies of cobra bite relating to its potent neurotoxic and cardiotoxic effects [3,4,5]. However, only very few studies of

the effect of venom on blood coagulation/fibrinolysis system and on red blood cells (RBC) can be located [3,6,7]. This paper is concerned with in vitro effect of cobra (Naja naja) venom on individual clotting factors, fibrinolysis system and on RBC.

MATERIALS AND METHODS

Venom

The venom used was pooled crude venom (dried crystals) of captive snakes (Naja naja). It was purchased from Myanma Pharmaceutical Industry. It was weighed and freshly dissolved in 0.9% normal saline. This venom solution was further diluted in 0.9% saline to get desired concentrations.

Blood samples

For coagulation tests and factor assays pooled plasma of five normal

subjects were used. Same pooled plasma was used as control for each test. To note the reproducibility, these tests were done for three times on three different days and the mean of the values were taken.

Effect on coagulation system

Whole Blood Clotting Time (WBCT)

It is done according to the modification of standard method of Lee and White [8]. One ml of unanticoagulated whole blood was added into the series of tubes containing 0.1 ml of different concentration of cobra venom (or saline as control) at 37°C and clotting times were recorded.

Plasma Clotting Time (PCT)

0.2 ml of plasma was incubated with 0.1 ml different dilutions of venom (saline as control) at 37°C and the clotting times were noted.

Plasma Recalcification Time (PRT)

The tubes containing 0.2 ml of plasma, 0.1 ml of different concentrations of venom (saline as control) and 0.1 ml of 0.025 mM calcium chloride were incubated at 37°C and the clotting times were determined.

Prothrombin Time (PT), Thrombin Time (TT) and Activated Partial Thromboplastin Time (APTT)

All these tests were done according to standard methods [9] except instead of using 0.1 ml of plasma, 0.2 ml of plasma and venom solution mixture (i.e. 0.1 ml of plasma + 0.1 ml of various concentrations of venom or saline) was used in the system.

Effect on factors V, VII and X

Immediately after mixing 0.1 ml of citrated plasma with 0.1 ml of different dilutions of venom, the factor assays were performed using standard technique [9].

Effect on fibrinogen and fibrinolytic system

Effect on fibrinogen

The tubes containing 4.0 mg/ml of bovine fibrinogen (Diagnostic Reagents Ltd) and 0.1 ml of different concentrations of venom were incubated at 37°C. At intervals of 1, 2, 3 and 4 hours, 0.9 ml of bovine thrombin solution (Sigma) (5 units/ml in 0.025 mM calcium chloride) was added to clot the fibrinogen. Then the formed fibrin was quantitated by the dry clot weight method [9].

Effect on Euglobulin Clot Lysis Time (ECLT)

Euglobulin fraction was prepared according to Buckell's method [10]. After dissolving the euglobulin fraction in a borate solution, 0.1 ml of different dilutions of cobra venom (or saline as control) were added to the series of tubes and mixed thoroughly. These tubes were placed at 37°C and 0.5 ml of 0.025 mM calcium chloride was added to each tube. After clotting, the lysis times were recorded.

Effect on red blood corpuscles

0.5 ml of heparinized blood was put into tubes containing 0.05 ml of various concentrations of venom (or saline as control). These tubes were incubated at 37°C for 1 hour. Then the osmotic fragility test and plasma haemoglobin estimation were done according to Dacie and Lewis [11].

RESULTS

As shown in Table (1), cobra venom had no direct effect on PCT and TT. However, it had dose dependent inhibition effect on WBCT, PRT, PT and APTT.

Table 1. The effect of cobra venom on coagulation tests

Venom Dilutions	WBCT min	PT sec	TT sec	APTT sec	PRT min	PCT min
0 (10 mg/ml)	*NC	>120	23	>180	>10	>10
1:2	NC	>120	21	>180	>10	>10
1:4	NC	77	21	>180	>10	>10
1:8	NC	46	20	>180	>10	>10
1:16	8	37	20	>180	>10	>10
1:32	6	37	20	>180	>10	>10
1:64	5	29	20	60	>10	>10
1:128	5	24	21	53	>10	>10
1:256	5	22	20	48	7	>10
1:512	6	20	21	45	4	>10
1:1024	5	19	21	43	4	>10
1:2048	4	18	19	43	4	>10
1:4096	4	18	19	44	4	>10
Saline Control	4	18	20	44	3	>10

*NC = Not Clotted

On fibrinogen and fibrinolysis, cobra venom had no direct activation nor inhibition effect (Table 2 & 3).

Table (4) illustrates the results of the effect of cobra venom on factors V, VII and X. The inactivation of these factors were marked only at high concentrations of venom and were also dose dependent.

Figure (1) gives the effect of cobra venom on RBC. Osmotic fragility test showed significant difference between test and control in saline concentration of 4.5 g/l and 5.0 g/l. This difference was found at venom dilutions of 0 to 1:256. Similarly there was significant

Table 2. Effect of cobra venom on fibrinogen

Venom Dilutions	Fibrogen Level (g/l)			
	Incubation Time (Hr)			
	1	2	3	4
0 (10 mg/ml)	3.6	3.7	3.4	3.6
1:2	3.8	3.7	3.7	3.6
1:256	4.0	4.0	4.0	3.8
1:2048	4.0	4.0	3.8	3.6
1:4096	3.8	4.2	4.2	3.8
Saline Control	3.8	3.6	4.0	4.0

Table 3. Effect of cobra venom on euglobulin clot lysis time

Venom Dilutions	Euglobulin Clot Lysis Time Hr : min
0 (10 mg/ml)	(-)*
1:2	(-)
1:4	2 : 15
1:8	3 : 20
1:16	3 : 50
1:32	3 : 15
1:64	4 : 25
1:128	3 : 37
1:256	2 : 43
1:512	3 : 07
1:1024	3 : 30
1:2048	3 : 13
1:4096	2 : 53
Saline Control	2 : 53

*No firm clot was formed to determine lysis time. Only fine fibrin strands were precipitated out.

difference in plasma haemoglobin and these differences were also parallel to venom concentration.

DISCUSSION

The results of our study indicated that the Myanmar cobra venom has dose dependent inhibition effect on blood coagulation system. This

Table 4. The effect of cobra venom on factors V, VII and X

Venom Dilutions	Factor V%	Factor VII%	Factor X%
0 (10 mg/ml)	32	9	8
1:2	38	15	16
1:4	58	38	20
1:8	78	50	30
1:16	68	70	46
1:32	72	77	58
1:64	100	100	70
1:128	100	100	72
1:256	100	100	72
1:512	100	100	100
1:1024	100	100	100
1:2048	100	100	100
1:4096	100	100	100
Saline Control	100	100	100

evident by prolonged PT and APTT. Similar findings have been found by Mac Kay *et al.* (1969) [7].

Although cobra venom has inhibition effect on both coagulation pathways, it did not directly interfere the action of thrombin which converted the fibrinogen into fibrin. This was marked by TT which was done at very high concentration of venom, did not differ from TT of saline control.

In the study of some workers the cobra venom showed fibrinogenolytic activity which was increased with prolonged incubation time [7,12] and mild fibrinolytic activity [13]. However, in our experiment, there was neither fibrinogenolytic nor fibrinolytic activity even at higher concentration and in longer incubation time. This is similar to the report of Eagle (1937) [14].

inhibitory effect exerted both on intrinsic and extrinsic pathways as

Regarding the effect of cobra venom

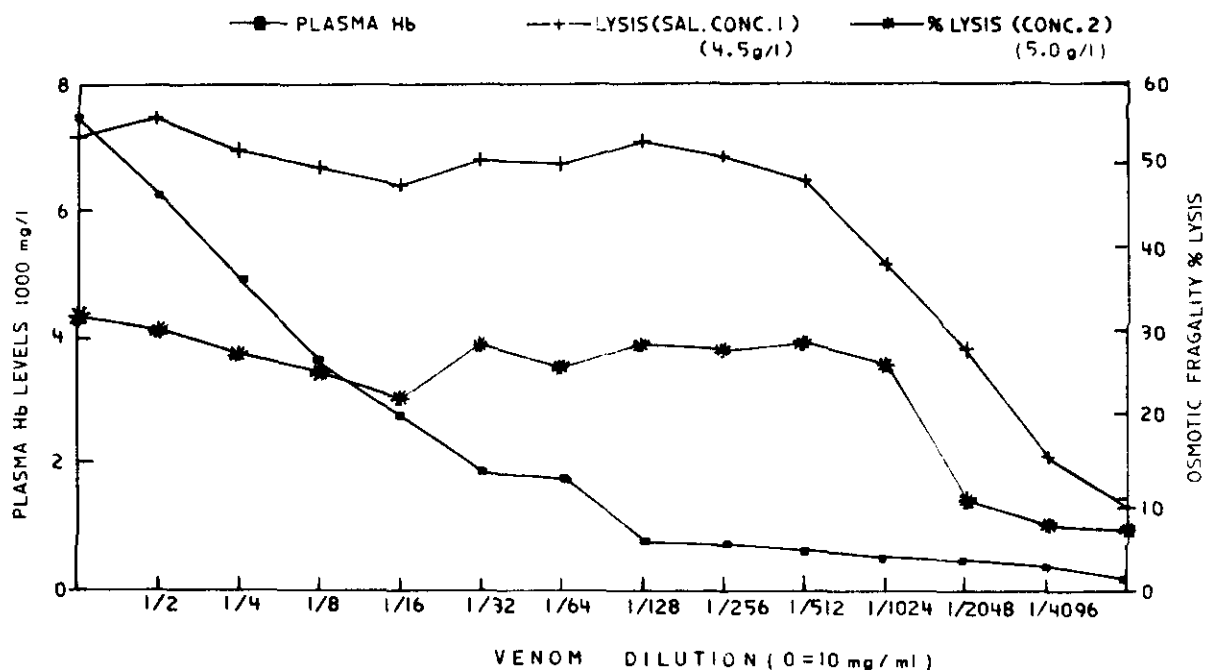


Fig. 1. Plasma Hb & % lysis of human RBC after one hour incubation with cobra venom

on the clotting factors, Lee (1971) [15] has reported that the cobra venom inactivated the factors V, VII, VIII, IX and other coagulation factors of platelet. In our experiment, this effect was seen only at higher concentration of venom and was more marked on factors VII and X than on factor V.

Thus the inhibition effect of cobra venom might be due to antithromboplastic activity together with the mild inhibitory effect on each individual coagulation factors.

Concerning the effect on red blood cells, cobra venom has direct haemolytic effect as shown by increased osmotic fragility and raised plasma haemoglobin level. This haemolytic effects of cobra venom can be seen in some clinical cases [3].

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Non-01 Vibrio cholerae isolation from flies, cooked
children's foods, drinking water, currency notes
and vegetables in Yangon

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Non-01 V. cholerae isolation was done from flies, cooked children's food, drinking water, currency notes and vegetables in Yangon during 1984 to 1989. They were found in 45.7% of flies samples, 3.5% of cooked children's foods, 4.4% of drinking water samples, 5% of currency notes and 0.6% of vegetables samples. The strains of non-01 V. cholerae isolated from these sources shared identical biochemical reactions. They all were grown at both 37°C and 42°C and they were resistant to polymyxin B sulphate.

INTRODUCTION

Non-01 V. cholerae has been increasingly reported as a cause of diarrhoeal disease in Asia [1] and elsewhere [2]. It is found in both food and water and it is presumed that it is transmitted by these routes [3]. It is isolated from fly pools in yards, animal pens, bathrooms and kitchens [4].

Cooked children's foods and vegetables may be contaminated by flies and fingers and this is probably one of the important ways by which diarrhoeal pathogens including vibrios are transmitted. The paper money notes may be contaminated by fingers and may transmit to fingers of other people who handle them. Thus these contaminated money act as a potential vehicle for disseminating vibrios to susceptible persons [5]. This paper attempts to show the isolation of non-01 V. cholerae from flies, cooked children's foods, drinking water, currency notes and vegetables in

Yangon.

MATERIALS AND METHODS

(1) Collection of samples and their process for bacterial isolation

Flies were captured alive in cages from animal pens, bazaar, kitchens, latrines, refuse dumps and food stalls during September 1984 to August 1985 in Tamwe Township in Yangon. They were collected in sterile wide mouth tubes from the cages and anaesthetized by exposure to cold at 0°C. An approximate 10% suspension of a pool of 10 flies were made using 0.9% normal saline (N/S). Only one sample (a pool of 10 flies) was studied from one source on the study day, throughout the study period. Then vibrios spp. were tried to isolate from all the samples using alkaline peptone water (APW-pH 8.4) and thio-sulphate citrate bile sucrose (TCBS) medias.

Children's food samples were taken

in the mornings from their morning meals. They were collected by field workers in sterile containers after the child's feed had been prepared and was just about to be fed to the child. The study was conducted in North-Okkalapa Township in Yangon during July to September 1986. 10 gms of these samples was mixed with 90 ml of peptone water and homogenized employing the stomacher lab blender. They were incubated at 37°C overnight as pre-enrichment. The vibrio spp. was tried to isolate from all the samples.

Drinking water samples were collected from households using pond, municipal, shallow-well and tube-well users. The study was conducted in North-Okkalapa Township in Yangon during July to September 1986. They were collected from the water storage pots in sterile bottles using sterile spoons. 500 ml of water was filtered by membrane filtration method as described by WHO [6], the filters were inoculated into APW, incubated at 37°C for 6 hrs and subcultured onto TCBS media for vibrio spp.

Specimens of one kyat notes were randomly collected from vendors selling chicken, pork, beef, fish, prawn, fish paste, Myanmar snacks, vegetables and eggs during December 1986 to July 1987 in a local market in Mingalar-Taung-Nyunt Township in Yangon. They were collected in sterile containers in the mornings and transported to the laboratory for processing on the same day. They were put in 1% peptone water, preincubated at 37°C for 4 hours and the vibrios spp. was tried to isolate by standard procedures.

Vegetable samples such as coriander, leek, legume, cabbage, green chilli, morning glory, mimosa, leaves of lemon tree and small plants of beans

were bought from whole sale market as well as from small shops in Dagon Township during April to October 1989 in Yangon. They were collected in sterile plastic bags in the mornings and transported to the laboratory for processing on the same day. 10 gms of samples were mixed with 90 ml of 1% peptone water, preincubated at 37°C overnight and vibrios spp. was tried to isolate from all the samples.

(2) Identification of V. cholerae non-01

Vibrio like colonies on TCBS were identified by using ID-EB 20 test kit (Nissui Pharmaceutical Co. Ltd.). Salt tolerance test and growth test at 37°C and 42°C were done. Oxidase test was done with oxidase test paper. Gas from glucose and motility were known by using with triple sugar iron, lysine iron agar, urease and semi-solid indole motility biochemical agar tubes. Serological identification was done by slide agglutination with diagnostic V. cholerae 01 antisera produced by a company from Japan.

(3) Other tests

Polymyxin B susceptibility was tested by the plate method of Roy et al [7]. Samples 10 ul of cultures grown in Mueller-Hinton broth was spotted on nutrient agar plates supplemented with 0.5% (w/v) sodium chloride and 15 ugm polymyxin B sulphate/ml. Full confluent growth in the spots comparable to that of the same culture spotted on control plates with out antibiotic was scored as resistance. The presence of cholera toxin (CT) like toxin from the Vibrio cholerae non-01 isolates was detected by a reverse passive latex agglutination test (Denka Seiken Co. Ltd., Japan).

RESULTS

Non-O1 *V. cholerae* isolations from flies, cooked children's foods, drinking water, currency notes and vegetables are shown in Table 1.

Table 1. Isolation of non-O1 *V. cholerae* from different sources, Yangon, 1984-89

Sources	No. of samples examined	No. of non-O1 <i>V. cholerae</i> positive samples	No. of non-O1 <i>V. cholerae</i> positive samples
Flies (1984-85)	186	85*	45.7%
Cooked Children's foods (1986)	779	28**	3.5%
Drinking water (1986)	113	5**	4.4%
Currency notes (1986-87)	60	3	5%
Vegetables (1989)	90	6	6.6%

* 12 strains were CT-positive

** 3 strains each were CT-positive

They were found in 45.7% of flies samples, 3.5% of cooked children's foods, 4.4% of drinking water samples, 5% of currency notes and 0.6% of vegetables samples. Table 2 shows the biochemical characteristics of isolated non-O1 *V. cholerae*. They all were grown at both 37°C and 42°C and they were resistant to polymyxin B sulphate.

Of non-O1 isolates, 12 (out of 85) from flies samples, 3 each from cooked children's foods and water samples produced CT like toxic to be connected as CT like toxin.

DISCUSSION

The optimum temperature of *V. cholerae* non-O1 is 37°C. The other vibrios other than *V. cholerae* such as *V. furnissi*, *V. anguillarum* etc.

Table 2. Strains of non-O1 *V. cholerae* isolated from flies, cooked children's foods, drinking water, currency notes and vegetables shared identical biochemical reactions

Salt tolerance:	0% +	Carbohydrate fermentation
	6% -	glucose acid
	8% -	adonitol -
	10% -	inositol -
growth at 37°C	+	rhamnose -
42°C	+	rhamnose -
indole	+	sorbitol -
VP	+ ²	sucrose +
citrate utilization	+	mannose +
malonate	-	arabinose -
urease	-	Decarboxylation
oxidase	+	lysine +
motility	+	arginine -
ONPG	+	ornithine +
O-F (glucose)	F	resistance to
hydrogen sulphide	-	polymyxin B
phenyl pyruvic acid	-	sulphate +

(biochemical reactions were recorded after 37°C incubation overnight unless indicated)

+² = positive after two days

can't grow at 42°C [8]. *V. minicus* is susceptible to Polymyxin B [9]. Thus in our study we did the tests of growth at 42°C and resistance to polymyxin B sulphate. From these tests vibrios from our study could be differentiated from the other vibrios. The biochemical tests were identical to *V. cholerae*. They were not serotyped by *V. cholerae* O1 antisera. Thus they were proved to be in *V. cholerae* non-O1 strains.

From the results of Echeverria et al [4] and of ours, we know that flies can transmit non-O1 *V. cholerae*. It is clear that even though there were cooked foods, they could easily become contaminated with non-O1 *V. cholerae* through handling and using contaminated utensils. In our community both these factors may

probably facilitate contamination because the mothers usually feed children with their hands which are more or less contaminated and also the utensils that they use are most often contaminated [10]. Non-O1 V. cholerae can also multiply in cooked foods in a range of level up to 10^9 - 10^{10} /gm of foods/24 hrs [11].

Contamination of drinking water may result during collection and use. It may result from collecting drinking water with contaminated buckets, filtering it into the storage pots with dirty cloth filters and getting it from the storage containers with unclean contaminated hands [12].

Contaminated hands of vendors may transmit these organisms to currency paper notes and again contaminated on fingers of other people who handle these paper notes. The contamination of vegetables might have originated from the water used for irrigation, from manure or from the handlers of vegetables.

Cooked children's foods, drinking water, currency notes and vegetables may be contaminated by fingers and this is probably one of the important ways by which diarrhoeal pathogens including non-O1 V. cholerae are transmitted.

Non-O1 V. cholerae are readily destroyed by heat, thus raw foods which are contaminated with them should be safe to eat after normal cooking methods [11]. The hazard lies in the recontamination of cooked food and thus we have to take care not to get contamination of food by flies and to wash hands especially before food handling or eating.

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The effect of quinine and quinidine on patients with
highly parasitised falciparum malaria
(A double blind study)

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Sixty patients with high level of parasites in the blood (i.e., more than 2% of RBCs parasitised) were chosen for the study. They were paired in sex and associated complications as nearly as possible. The first group of patients was treated with drug (A) and the remaining with drug (B). Drugs (A) and drug (B) consisted of quinine or quinidine, (injection and tablets) which is unknown to investigators. 15 mg/kg quinine or quinidine was given as a loading dose infused over 4 hours followed by 2 doses of 7.5 mg/kg base also infused over 4 hours each at 8 hourly intervals. This was followed by oral therapy. The oral drugs were continued as 7.5 mg/kg base 3 times/day till day 7. The efficacy of the 2 drugs were compared in terms of mortality, development of complications parasite and fever clearance, time. All patients survived, significantly higher level of serum quinine was recorded, when compared to quinidine through out the study. Serum insulin of five pairs of patients and blood glucose level of 15 pairs of patients were within the normal range (in all patients) throughout the study period. Blood glucose level in patients treated with quinine is significantly lower than those treated with quinidine at the first 36 hours of treatment. Since the parasite clearance time, fever clearance time mortality rate and recrudescence rate between the 2 groups of patients were comparable, we conclude that quinidine is clinically equal but not more potent than quinine. It is probably more toxic because of more ECG changes. Quinidine may be used as alternative only if quinine is not available.

INTRODUCTION

Both quinine and quinidine are the principal, alkaloids of cinchona, bark. Quinidine has been reported as safe and probably more effective than quinine in uncomplicated falciparum malaria. White et al. [1], Sunthrasamai et al. [2], Phillips et al. [3] had reported that quinidine is effective in severe falciparum malaria also.

The isolate of quinine resistant P. falciparum had also been proved to

be more sensitive to quinidine than quinine in vitro.

Dalacollette et al. [4] also observed that slow release quinidine was easier to use and cause less side effects than quinine.

There are also reports that mentioned quinidine as more potent and toxic than quinine [5,6,7].

But there is no study comparing the effect of quinidine and quinine on patients with complicated falciparum

malaria. Hence this study is needed.

PATIENTS AND METHODS

Out of the malaria patients who attended the Tharyarwady Civil Hospital during 1988-89 malaria season, 30 pairs of patients with high level of parasites in the blood (over 2% of RBCs parasitised) and who have no quinine in the urine were chosen for the study. They were associated with complications such as very high level of parasites in blood, anaemia, jaundice or renal dysfunction. Patients who were under 12 years, above 65 years, those with cerebral signs and symptoms and those with antimalarials in urine were not included in the study.

Clinical assessment and laboratory tests done to the patients.

All patients were admitted to the hospital for at least 7 days and were followed on days 14, 21, 28.

Body temperature was recorded every 6 hours until it is normal for 24 hours, 12 hourly thereafter.

Thick and thin blood films were drawn every 6 hours until negative from parasites for 24 hours, then daily up to day 7 and on days 14, 21 and 28.

Plasma for quinine and quinidine were collected from all patients at following hours of therapy. 0, 1, 2, 4, 6, 9, 12, 24, 36, 48 hour and days 3, 4 and 7.

Blood sugar was determined on fifteen pairs of patients who were free from hepatic and renal complications at following hours of treatment. 0, 1, 2, 4, 6, 9, 12, 24 hours and days 3, 4 and 7.

Plasma insulin was determined from five pairs of patients who were free from renal and hepatic impairment at following hours of treatment. 0, 4, 12, 24 hours and day 3.

Blood PCV, total WBC and urine RE and blood urea were done at days 0, 7 and 28.

Blood for G6PD was done at day 0.

Recording the signs and symptoms of drug toxicity such as nausea, vomiting, dizziness, diarrhoea, abdominal pain, skin rash and neuro-psychiatric changes was done daily. ECG monitoring was done on days 0, 3, 7 and 14.

Plasma quinine and quinidine were estimated by Benzene Extraction Method.

Plasma insulin was determined by Radio Immuno Assay Method using insulin RIA Kit (AMERSHAM).

Plasma sugar was determined by O-toluidine Method.

Treatment regimes

The patients were paired in sex and associated complications as nearly as possible.

The first group of patients were treated with drug (A) and second group of patients with drug (B). Drug (A) and drug (B) consisted of quinine or quinidine (injection and tablets), which is unknown to investigators. 15 mg/kg quinine or quinidine base was given as loading dose diluted with normal saline infused over 4 hours, followed by two doses 7.5 mg/kg base also infused over 4 hours at 8 hourly intervals. This was followed by oral therapy. The oral drug was continued as 7.5 mg/kg base (3) times per day, till 7 day. Quinine

and quinidine double blind tablets and injections provided by Dr. N.J. White, Oxford, Mahidol Research Programme, Thailand were used for the test.

The efficacy of drugs were compared in terms of mortality, development of complications, recrudescence rate, parasite and fever clearance time.

RESULTS

All the patients survived. The fever clearance time and parasite clearance time of the patients treated with quinine were 45.6 hours 56.6 hours and fever clearance time and parasite clearance time of patients treated with quinidine were 44.9 hours and 54.4 hours respectively. There was no significant differences between the two groups of patients. There was one patient with recrudescence before day 28 in each group of patients.

More ECG changes were recorded at day 3 and 7, on patients treated with quinidine when compared to quinine. This finding is requested in separate paper [11].

Significantly higher level of serum quinine was recorded when compared to quinidine though the two drugs were used at the same dosage at the same time ($p < 0.05$).

Among five patients treated with quinine there was a trend in level of insulin to rise at end of twenty-four hours. Among another five patients treated with quinidine there was a trend in level of insulin to fall at end of twenty-four hours with an initial rise at 12 hours. But the level of insulin in both groups of patients were within the normal range.

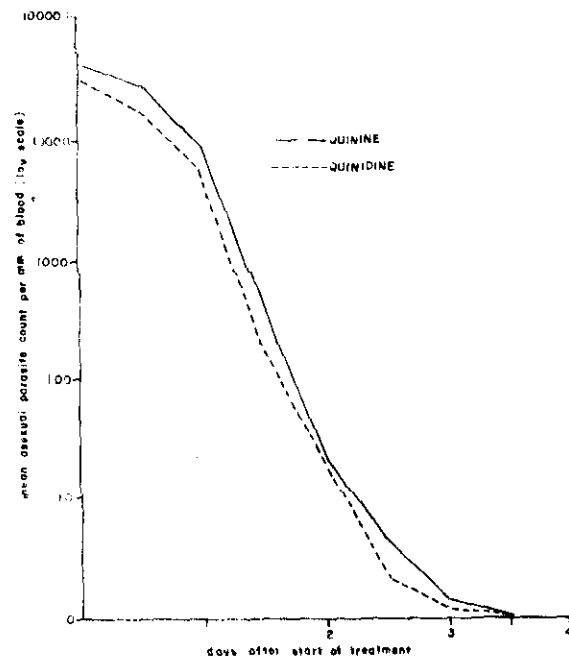


Fig. 1. Mean clearance of *P. falciparum* (asexual forms) from peripheral blood after treatment with quinine and quinidine $n = 30$ each

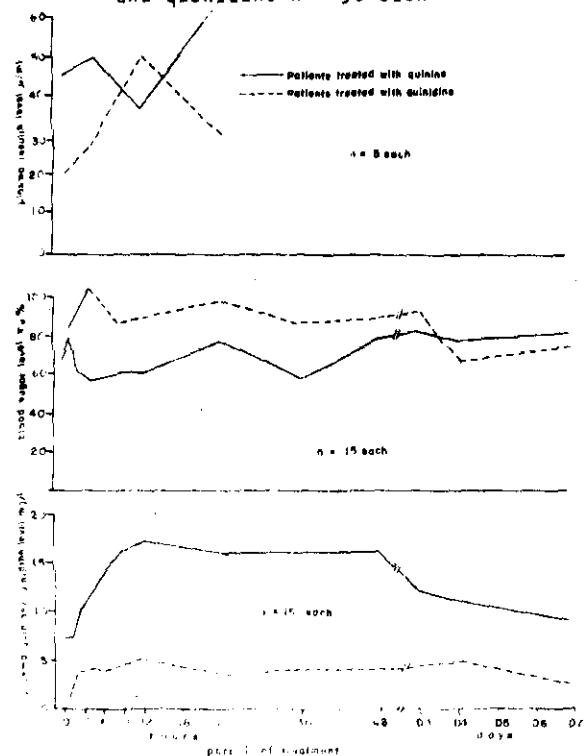


Fig. 2. Plasma level of quinine/quinidine, glucose and insulin in patients treated with quinine or quinidine

Blood glucose level in patients treated with quinine is significantly lower than quinidine from the 3rd hour till 36 hours of treatment ($p < 0.05$).

Patients with level of serum insulin above 65 u/ml and very low level of sugar (below 50 mg/dl) was not recorded among the patients during the whole period of drug therapy.

Summary of the results of two groups of patients treated with two different drugs using the same dosage at the same time

	Quinine	Quinidine
-Initial,	152,000	133,000
-Mean and S.D.	$\pm 353,000/\text{cumm}$	$\pm 361,000/\text{cumm}$
-No. of patients	30	30
-Sex ratio (male/female)	26:4	27:3
-Mean age	28.8 ± 9.6 yrs	34.3 ± 13.8 yrs
-Mean weight	47.3 ± 5.1 Kg	48.8 ± 15.7 Kg
-Hyperparasitaemia (more than 5%)	10%	11%
-PCV less than 30%	16 (2 need blood transfusion)	11 (4 need blood transfusion)
-Clinical jaundice	4	10
-Raised blood urea	1	2
-Initial hypoglycaemia	1	2
-Hyperpyrexia more than 105°F	1	1
-More than one complication	9	12
-Patients with recrudescence before day 28	1	1
-Minor ECG changes	14	28
-Signs and symptoms of cinchonism (nausea, vomiting, blurred vision, tinnitus, palpitation, dizziness, diarrhoea)	9	15

	Quinine	Quinidine
-Parasite clearance time	56.6 14.7	54.4 20.3
-Fever clearance time	45.6 20.7	44.9 22.4

The results are also shown in Figures (1), (2).

DISCUSSION

In this study, all patients survived. There was no significant difference in fever clearance time, parasite clearance time, recrudescence rate and mortality rate, between the two groups of patients treated with two different drugs with the same dosage and same route and method. At the same time symptoms of cinchonism which included dysphoria, tinnitus, headache nausea and occasional visual disturbance are seen in both groups of patients. Other signs and symptoms of toxicity were not recorded. Our finding are similar to the effects of parenteral quinidine on 14 patients with severe *P. falciparum* malaria from Thailand (Phillip *et al.*) [3]. They also found that the clinical and parasitological response to quinidine was comparable to quinine.

In this study ECG changes (lengthening of QT interval lengthening of PR interval and widening of R wave) were twice as common in patients treated with quinidine when compared to quinine. But patients with hypotension were not recorded. None of the patients had complaints relating to Cardiovascular System (CVS). None of the patients had bradycardia (less than 60 min) or arrhythmia in both groups of patients. Such high prevalence of changes in ECG following quinidine had also been previously reported (White *et al.* [1], Phillip *et al.* [3]).

Quinine is reported as a stimulant to islet cells for insulin secretion. White *et al.* [8] had shown the release of insulin in healthy subjects following quinine therapy. But in this study as well as in our previous experience with quinine, patients with very low level of blood glucose was not recorded during quinine therapy. In our experience patients with pregnancy, young age and those at the terminal stage of the disease are at times associated with hypoglycaemia with or without quinine therapy. Hypoglycaemia in untreated African children with severe malaria had also been reported (White *et al.*) [9]. Children patients with hypoglycaemia following chloroquine therapy had also been observed (Bamber *et al.*) [10]. Hence quinine or quinidine therapy may not cause hyperinsulinaemia and hypoglycaemia in Myanmar patients with highly parasitised falciparum malaria.

In this study, significantly lower level of glucose was recorded among the patients treated with quinine at the first 36 hours of drug therapy when compared to quinidine. Hence it is still advisable to monitor the level of glucose during quinine therapy.

In this study, using the same dosage and same route plasma level of quinine is significantly higher when compared to the patients treated with quinidine. The difference may be due to the difference in systemic clearance rate of the two drugs.

Since the parasites clearance time, fever clearance time, recrudescence rate and mortality of the 2 groups of patients are comparable, we conclude that quinidine is clinically equal but not more potent than quinine. It is probably more toxic because of more ECG changes.

Quinidine may be used as an alternative only if quinine is not available.

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SHORT REPORT

ELISA HTLV-III antibody reactivity of Myanmar patients infected with P. falciparum malaria

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A serological survey on 250 outpatients in rural Zaire showed that the prevalence of antibodies against HTLV-I, HTLV-II and HTLV-III as detected by Enzyme-linked-immunosorbent Assay (ELISA) correlated with levels of antibodies against P. falciparum [1].

Simooya et al [2] had also reported the relation between falciparum malaria and HIV seropositivity from Zambia. They suggested that many patients with HIV infection may be presenting with an illness clinically similar to malaria before AIDS related complexes or AIDS is recognisable. Hence this study, the possible association between ELISA HTLV-III antibody reactivity and P. falciparum malaria infection was studied.

73 samples of sera collected from in-patients of Thayarwady Civil Hospital with the following clinical groups were tested for ELISA HTLV-III antibody reactivity.

Group 1 - 30 patients with uncomplicated P. falciparum malaria.

Group 2 - 30 patients with highly parasitised falciparum

malaria (more than 5% of RBCs parasitised)

Group 3 - 13 patients with cerebral malaria.

All the samples were freshly collected and frozen at -20°C and sent to National Health Laboratory, Yangon within one month. Antibody reactivity to HTLV-III was tested by a competitive enzyme immunoassay using Wellcome Anti-HTLV-III kit.

All the 73 samples were seronegative for HTLV-III antibody.

Greenberg et al. [3] found that exposure to plasmodium does not result in HTLV-III/LAV seropositivity and HTLV-III antibodies are not strongly cross-reactive with malaria antigens. Their study was based on serum samples from 460 patients with previous plasmodium infection.

Facer et al. [4] had also screened sera for HTLV-III antibodies from 92 Gambian children under the age of 10. All the children were seronegative for HTLV-III antibody. In addition, 5 adult Caucasians with acute P. falciparum malaria were also seronegative for HTLV-III reactivity.

Nguyen Dinh et al. [5] had reported the absence of association between P. falciparum malaria and human immuno-deficiency virus infection in children from Zaire.

Recently Subramanyam [6] had examined 119 malaria sera from Orissa, all were negative for anti-HTLV-III antibody reactivity.

Hence there may be no association between ELISA HTLV-III antibody reactivity and P. falciparum malaria infection.

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Lepromin reaction in children of parents with leprosy

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Lepromin reaction in (65) apparently healthy children (age between 5 and 10 years) of parents with leprosy were studied. 30 children born of parents living in same village with no clinical leprosy were included in the study as controls. The mean diameter of lepromin reaction in children of parents with lepromatous leprosy is significantly reduced when compared to children of parents with tuberculoid leprosy, and those with no clinical leprosy.

INTRODUCTION

Reaction to lepromin which is the diluted suspension of leproma in saline is found to be negative in the majority of lepromatous leprosy patients, and positive in the majority of patients with tuberculoid leprosy. It shows the immunological status of the person to leprosy infection. The lepromin reaction among leprosy patients, healthy contacts, children etc. had been studied in detail and the literature had been reviewed by Sato and Nashimura (1967) [1]. But the possible hereditary nature of lepromin reaction was not studied before. Hence the lepromin reaction in children born of parents with lepromatous leprosy was compared with the children of parents with tuberculoid leprosy and those with no leprosy.

PATIENTS AND METHODS

All the primary school children belonging to Say-Yeik-Tha Village (The leprosy village close to leprosy Hospital, Htaukkyant, Yangon) of age five and ten years, with no clinical signs and symptoms of leprosy, and with no history of

BCG vaccination was studied. The children of healthy parents living in the same village and who were going to the same school was included in the study as controls.

Standard lepromin obtained from Dr. R.J.W. Rees (National Institute for Medical Research, England) was used for the test. 0.1 ml of lepromin was injected to the palmar aspect of right fore arm of each child using a tuberculin syringe and intra-dermal needle. The diameter of skin nodule following the lepromin injection was measured and recorded in between 4th and 5th weeks.

RESULTS

Out of thirty one children born of parents with lepromatous leprosy twenty one children showed no response to lepromin reaction, eight showed doubtful reaction, one showed one plus and one showed two plus reactions. Mean diameter of the lepromin reaction is 3.0 mm.

Out of thirty four children born of parents with tuberculoid leprosy, two children showed no response to lepromin reaction, three showed doubtful reaction, nine children

showed one plus and twenty children showed two plus reaction. the mean diameter of lepromin reaction is 8.4 mm.

Out of thirty children born of parents with no leprosy, all showed some degree of reaction to lepromin. Mean diameter of lepromin reaction is 7.9 mm.

The percentage of children born of parents with no leprosy, all showed some degree of reaction to lepromin. Mean diameter of lepromin reaction is 7.9 mm.

The percentage of children with no response to lepromin and the mean diameter of lepromin reaction between children born of parents with lepromatous leprosy is significantly different from the children born of parents with tuberculoid leprosy and those with no leprosy ($p < 0.001$). There is no difference between children born of parents with tuberculoid leprosy and the children born of parents with no leprosy. The details are given in Table (1).

DISCUSSION

Studies on twins had shown that there is familial predisposition in leprosy (Mohamed Ali and Ramnau Jam, 1964) [2]. Epidemiological studies had also shown that leprosy is common among blood relatives in particular among children, while infection among conjugal pairs (between husband and wife) are low despite their intimate relations (Sato and Nashimura, 1967) [1]. This may be interpreted as the blood relatives of leprosy patients inherit a constitutional weakness to leprosy infection and the resistance to leprosy infection is particularly low in childhood.

The theory of familial predisposition is also supported by the studies on the prevalence of leprosy in children of patients discharge from Htaukkyant Leprosy Hospital. The children of parents with lepromatous leprosy are more prone to have leprosy when compared to children of parents with non-lepromatous leprosy and those with

Table 1. Lepromin reaction in children born of parents with and without leprosy (Children ages 5-10 only)

Children group	No. of children studied	Lepromin negative cases	Lepromin positive cases			Mean diameter of lepromin reaction and standard deviation
			Doubtful <5 mm	Positive + 5-9 mm	Positive ++ 10 mm or more or ulceration	
1. Children born of parents with no leprosy	30	0*	3	10	17	7.9(3.1)*
2. Children born of parents with tuberculoid leprosy	34	2*	3	9	20	8.4(3.9)*
3. Children born of parents with lepromatous leprosy	31	21**	8	1	1	3.0(2.9)**

N.B. Difference between *and **statistically significant.

no leprosy though they are living in the same environment (Kyaw Lwin & Tin Shwe, 1973) [3].

In this study the percentage of children with no response to lepromin and the mean diameter of lepromin reaction between children born of parents with lepromatous leprosy is significantly different from these children born of parents with tuberculoid leprosy, and those with no leprosy, though these children are staying together around a leprosy hospital. This finding could be due to the fact that these children born of lepromatous leprosy parents inherit some genetical weakness to lepromin reaction.

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SHORT REPORT

The importance of frequent PCV monitoring and timely fresh blood transfusion in the management of severe falciparum malaria

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In our experience, cerebral malaria is not the only cause of death among patients with malaria. Low pack cell volume (PCV) was often associated with the time of death of patients. During 1987 we had a patient with PCV 31% and while preparing for the treatment, his PCV fell to 18% in two hours and he expired. We also had four patients with PCV 12%, 18%, 16% and 15% at time of admission to hospital. Hence we started following PCV of the patients with complicated malaria and fresh blood transfusion was given to all those with PCV less than 20%. This is the report of the first ten cases treated with method.

PATIENTS AND METHODS

The study was conducted at Tharyawady Civil Hospital during 1987 malaria season. (76) patients with severe falciparum malaria were chosen for the study. PCV was done daily on all patients and (6) hourly on (10) selected cases with low PCV. To those patients with PCV less than (20%) at any time during their stay in hospital, fresh blood (1-4) units

depending on the case was given within two hours.

RESULTS

Out of (76) patients with severe falciparum malaria (10) patients (8 males and 2 females) had low PCV (less than 20%) and 7 of these had more than 2% of RBCs parasitised with malaria parasites. These included 3 patients with more than 10% of RBCs parasitised. 2 of these were treated with quinine 4 with Artemether and 4 with Mefloquine Sulfadoxine Pyrimethamine (MSP). 2 patients had initial low level of PCV and 5 had fall in PCV during the first 2 days of admission. One patient each had fall in PCV on days 4, 7 and 11. The last patient was proved to be resistant to Mefloquine Sulfadoxine Pyrimethamine at R2 level. 6 out of 10 patients were G6PD deficient. All the patients showed dramatic clinical improvement soon after blood transfusion. They all recovered completely with no case fatality.

DISCUSSION

In this study, 10 out of 76 patients with complicated falciparum malaria had PCV less than 20% and need blood transfusion.

All these 10 patients treated by blood transfusion and antimalarial drugs recovered dramatically. They were the worst and most complicated cases admitted to hospital. In our previous experience, 8-30% mortality rate was expected from such patients. Hence timely blood transfusion therapy is definitely essential to routine treatment with antimalarial drugs.

Phillip *et al* (1986) also found anaemia as a universal feature of severe malaria. Approximately 30% of patients require blood transfusion as haematocrit falls below 20%. Hence, all possible facilities

should be pre-arranged for immediate blood transfusion in district hospitals at the malaria season.

In this study, only 2 patients had initial fall in PCV five patients had sharp fall during the first 2 days and one patient each had fall on days 4, 7 and 11. Hence it is advised to determine PCV daily on all patients with severe falciparum malaria while they are in hospital.

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