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Hormone levels in Russell's viper bite follow-up patients Saw Naing, Tin Nu Swe, Mg Mg Thwin & Myo Khin	1
Histopathological severity of antral gastritis in relationship to <i>Helicobacter pylori</i> colonisation Than Than Htwe, Myo Khin, Kyaw Hla, Than Than Swe, Tin Nu Swe, Khin Myat Tun & Thida Kyaw	5
Clinical trial to determine the hypouricaemic potential of <i>Zizyphus jujuba</i> kernel Win Aung, Aye Kyaw, Myo Lwin, Khin Pyone Kyi, Myat Myat Uhn Khin & Than Swe	10
Screening for and utilization of contraceptive methods among clients at family planning clinic of Central Women's Hospital, Yangon Than Than Tin, Thein Hlaing, San Shwe, Maung Maung Toe, San San Myint & Myint Myint Shwe	15
Serum fibrin(-ogen) degradation products (FDP) in toxemia of pregnancy Ne Win, Moe San & Than Than Tin	23
Shigellosis among children attending the paediatric wards of hospitals in suburban areas Mar Mar Nyein, Than Saw, Htay Htay Win, Thein Aung, Saw Aung Mya Thein, Aye Than, Tin Aye & Myint Lwin	27
Effect of various cleansing materials on decontamination of hands Khin Nwe Oo, Cho Yin Win & Myat Thida	33
Post-mortem histological and dissecting microscopic appearance of the proximal jejunum of Myanmar children Than Than Htwe, Soe Soe, Myo Khin, S. Kyaw Hla, Win Myat Aye, Ni Win, Wynn Wynn Kyaw & Aye Myint Swe	37
Role of amniotic fluid index assessment in postdate pregnancy Thin Thin Hla, Khin Swe Tin, Than Than Tin, Khin Saw Naing & Khin Ma Lay	40
Comparison of <i>Plasmodium falciparum</i> infectivity to <i>Anopheles dirus</i> D using direct and indirect blood feeding methods Myat Myat Thu, W. Tun Lin, Than Than Swe & Ye Thue	45

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Hormone levels in Russell's viper bite follow-up patients

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Studies of clinical features of hypopituitarism and/or any endocrine deficiency and hormone levels were carried out in systemic envenomed Russell's viper bite victims of more than one year duration. Eighteen follow-up patients with history of post viper bite ranging from one to seven years were studied. Serum cortisol were assayed in all but serum ACTH, HGH and urinary cortisol and HGH were made in four patients. Eight were found to have clinical evidence of endocrine deficiency and their hormone levels were on the low side, although statistically not significant. Symptomless patients also have abnormal pituitary function. Chronic ill-health with vague clinical presentation in post viper bite cases should always raise the possibility of chronic pituitary dysfunction.

INTRODUCTION

Systemic envenoming by Russell's viper produces a complex clinical syndrome, which may include defibrination [1,2], spontaneous systemic bleeding, hypotension, acute renal failure [3,4,5,6] and pituitary failure [7,8].

Chronic anterior pituitary insufficiency in 3 adults thought to have been bitten by Russell's viper (*Vipera russelli russelli*) in Southern India was reported in 1976 [9]. All 3 patients had systemic bleeding, hypotension and renal failure and symptoms of anterior pituitary deficiency developed as early as a month after the bite.

In Myanmar Russell's viper bite is responsible for both major morbidity and mortality. Postmortem evidence of haemorrhagic necrosis of the pituitary in victims of Russell's viper bite has also been cited [10]. We attempted to study both clinical features of pituitary dysfunction and/or any endocrine deficiency and hormonal abnormalities in systemic

envenomed Russell's viper bite victims of more than one year duration.

PATIENTS AND METHODS

Patients

During the rice harvesting season (October to December) of 1990 to 1992, we investigated pituitary and other endocrine deficiencies both clinically and biochemically at the Clinical Research Unit (Snakebite), Thayawady Township Hospital, 80 km north of Yangon. All the viper bite victims, who were previously treated at the Clinical Research Unit (Snakebite), Thayawady Township Hospital, were requested to come for follow-up studies. Only 18 cases consented to take part in the study. They were requested to stay in the hospital for 48 hours for clinical examination and sample collection. Thorough history taking and clinical examination especially in the context of detecting hypopituitarism and other endocrine deficiencies were carried out as per proforma by the project medical officers.

Subjects with past history of endocrine diseases prior to viper bite, pregnancy, recent history of any form of hormonal therapy including contraceptives (oral and parenteral) and high potency vitamins were excluded from the study.

Collection of samples

For hormonal study, 4 ml of venous blood was drawn from the subjects at 9 am and 12 midnight for two consecutive days. The samples were centrifuged at 2500 rpm for 15 minutes and the supernatant serum was stored at -20 degree centigrade until analyzed at the Department of Biochemistry.

Urine specimens (9 pm to 9 am) were collected in a container containing sodium azide. A 10 milliliter aliquot urine was collected after recording the temperature and were stored at -20 degree centigrade until analyzed. A protein load (2 eggs) was given on the second night prior to the collection of the second urine specimen.

Laboratory methods

Circulating hormone levels were measured by using commercial radio-immunoassay kit "ACTH RIA kit" (Diagnostic Products Corporation, Los Angeles, USA), a sensitivity of 8 uIU/ml and cortisol RIA kit, a sensitivity of 27.6 nmol/l and cross reactivity of 37% with prednisolone and "Human Growth Hormone IRMA kit" (North East Thame Region Immunoassay Unit, England) a sensitivity of 0.2 mU/l without any cross reactivity. These kits were also used for measurement of overnight urinary cortisol and HGH levels.

RESULTS

Eighteen follow-up patients (17 males and 1 female) participated in the study. Out of which, 8 patients were 7 years, 3 (5 years), 2 (2

years) and 5 (1 year) after the Russell's viper bite. Their ages ranged from 22 to 53 years. Serum cortisol was determined in all 18 cases whereas serum ACTH, serum HGH, urinary cortisol and HGH were determined in four patients only.

Eight had clinical evidence suggestive of endocrine insufficiency of failure. Presenting symptoms and signs are shown in Table 1. Out of these

Table 1. Clinical evidence of endocrine insufficiency in 8 Russell's viper bite follow-up patients

Presenting clinical features	Number of patients (%)
1. Easy fatigability	6 (33%)
2. Weakness/tiredness	6 (33%)
3. Fainting attack	5 (28%)
4. Lack of inertia	4 (22%)
5. Cold intolerance	3 (17%)
6. Physical and mental slowness	3 (17%)
7. Smooth and soft skin	3 (17%)
8. Impotence	2 (11%)
9. Cold and thickened skin	2 (11%)
10. Hypotension	2 (11%)
11. Hair loss (axillary & pubic hairs)	2 (11%)
12. Pallor	2 (11%)
13. Hoarseness of voice	1 (6%)

eight patients, six had suffered from systemic envenomation with evidence of spontaneous systemic bleeding, three from acute renal failure and two needed peritoneal dialysis. Although four were complicated by profound hypotension during their illness only one of them later presented with clinical evidence of endocrine abnormalities.

The mean serum cortisol levels of 8 patients were 406.2 and 177.8 nmol/l at 9 am and 12 midnight of Day 1; 401.4 and 102 nmol/l at 9 am and 12 midnight of Day 2. When compared to mean serum cortisol levels of 21 controls (ie. 355.9 nmol/l at 9

am, Day 1 and 307.9 nmol/l at 9 am Day 2), it was found to be higher in morning values of 8 patients in both day 1 and 2. The mean serum ACTH and HGH measured in 4 follow-up patients were also found to be higher as compared to controls (data not shown).

Overnight urinary cortisol and HGH levels

The overnight urinary cortisol levels were low in four patients. One patient with the features of endocrine insufficiency had lower urinary cortisol level on the second night even after protein loading. Urinary HGH levels were low in 3 patients, where one of them had overt features of hypopituitarism, their urinary HGH levels failed to rise even after protein loading.

DISCUSSION

This study shows that eight out of eighteen follow-up patients, who apparently recovered from systemic envenoming are having both clinical and hormonal evidence of chronic pituitary dysfunction. Acute and chronic pituitary dysfunction following envenoming by Russell's viper bite in Myanmar was previously reported [7].

It was also observed that pituitary is a favoured site for fibrin deposition in patients with DIC and pituitary glands from patients with proven Russell's viper bite showed engorged blood vessels and fibrin was often demonstrated with specific stain [10]. However, the special susceptibility of pituitary to Russell's viper venom is still unclear. The gland is also vulnerable to reduced perfusion pressure and hypopituitarism has been described in shock [10].

Thus, acute and chronic pituitary insufficiency should be recognized as potential sequelae of significant

envenoming by the Burmese Russell's viper (Daboia russelli siamensis) and furthermore our findings would suggest that victims may require longterm hormone replacement. In conclusion, chronic ill health with vague clinical presentations in individuals, who apparently recovered from Russell's viper bites should always raise the possibility of chronic pituitary dysfunction.

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Histopathological severity of antral gastritis in relationship to Helicobacter pylori colonisation

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The association between *Helicobacter pylori* (HP) colonisation in gastric antrum and the degree of severity and activity of gastritis and the concomitant relationship with gastric atrophy and intestinal metaplastic changes were studied. A total of 204 pieces of endoscopic biopsy tissues were collected from the outpatient endoscopy unit of New Yangon General Hospital in 1994. Routine Haematoxylin and Eosin stain as well as special stains such as modified Giemsa and Carbol fuchsin stains were used for bacterial identification and quantitative analysis. A significant correlation (P value <0.001) was obtained between density of HP colonisation and severity of gastritis as well as its activity (P value <0.0001). However, no significant degree of atrophic changes and intestinal metaplastic changes were noted in these biopsies. A continuation for a better understanding of the pathogenesis of HP and its consequences is necessary.

INTRODUCTION

Many research works have been done to date concerning quantitative association between HP and the severity of gastritis. Most have been focussed on antral mucosa. Previous reports of quantitative relations involving HP have indicated that increased amount of the bacteria was associated with more abundant infiltration of neutrophil granulocytes together with mononuclear inflammatory cells and it was mentioned as active chronic gastritis [1,2,3]. However, Rollason and Fiocca had mentioned that there was no association between bacterial quantity and severity or type of gastritis [4,5]. Research into the relationship between bacterial quantities and glandular atrophy or intestinal metaplasia is rare [6,7]. In order to reveal an association between the amount of HP and degree and activity of gastritis and the relationship with

gastric atrophy and intestinal metaplasia, the following study was undertaken.

MATERIALS AND METHODS

Patients

A total of 204 pieces of biopsy were collected from endoscopic unit of New Yangon General Hospital in 1994. These are from patients who had undergone biopsy for the investigation of upper gastrointestinal tract pathology. A written consent was taken before undergoing biopsy. Patients with active bleeding, history of bleeding tendency and malignancy were excluded from the study.

Histopathological methods

The biopsy tissues were fixed in formalin and stained with Haematoxylin and Eosin. For grading of gastri-

tis and its activity, the degree of gastritis was estimated on a five point scale according to Bayerdorffer (annex 1) [3]. For the mucosa to be classified as normal, the existence of only occasional lymphocytes and plasma cells was allowed. In addition, the content of neutrophil granulocytes, eosinophils and mononuclear infiltrates were estimated in grades indicating a spectrum from grade 0-5, to identify the degree of activity of gastritis. A minor modification was done to the original grading, to simplify statistical analysis. Gastritis grades 2 + 3 were combined and categorised as 'mild', grades 4 + 5 were combined as 'moderate'. Similarly, gastritis activity grades 2 + 3 were grouped as 'low activity' and grades 4 + 5 as 'high activity'.

A semiquantative measure of foveolar hyperplasia, gastric atrophy and intestinal metaplasia was done in vertically sectioned parts of the specimen and they were graded as normal, mild and moderate.

Modified Giemsa stain and Carbol fuchsin stain were done for the identification and quantitation of bacterial colonisation. The two special stains were used simultaneously to countercheck and minimise false negative results.

Sections were interpreted by one pathologist only (TTH), who was unaware of the clinical or the endoscopic findings, to reduce bias and observer variation. Special stained sections were searched for bacterial colonisation by the same pathologist without having information on the results of H & E section. The density of bacterial colonisation was also graded according to Bayerdorffer [3]. However, for statistical analysis, only Grade 1 was categorised as HP negative and the remainder as HP positive.

Statistics

The results were evaluated statisti-

cally, using Student's "t" test for significant level.

RESULTS

The results obtained are shown in the table below.

Out of 204 biopsy pieces 127 (62.3%) were HP positive in different and 77 (37.7%) were negative. Among 127 HP positive 117 cases (92.1%) had moderate to dense inflammatory cells infiltration indicating significant degree of chronic gastritis (P value 0.001). Concerning the activity of gastritis, only 7 (5.5%) out of 127 HP positive cases were without neutrophilic infiltration and the remaining 120 (94.5%) cases were found out to have a significant degree of active gastritis (P value <0.0001).

No significant degree of gastric atrophy and intestinal metaplasia was noted in HP positive cases. Thirty-four biopsy specimen could not be evaluated for the purpose because of technical difficulties in interpretations.

DISCUSSION

In this study, we observed that the amount of HP in the antral mucosa was strongly associated with moderate severity of chronic gastritis and particularly with active chronic gastritis, since a *significant neutrophilic infiltration* was identified. This finding was in support to those of Steer, Loffeld and Karttunen [1,2,6]. The use of grading by Bayerdorffer [3] was strongly helpful to assess the degree and severity of chronic gastritis and its activity.

Regarding gastric atrophy, a six-year interval study by Kekki [8] had mentioned that (1) the appearance of HP colonisation occurred concomitantly with appearance of gastritis; and (2) the development of antrum atrophic gastritis occurred with concomitant

Table. Severity of gastritis in relationship to *H. pylori* colonisation

	HP (neg.)	HP (pos.)
1. Severity of gastritis*		
Normal (Grade 1)	11	1
Mild (Grade 2 + 3)	50	10
Moderate (Grade 4 + 5)	16	117
2. Activity of gastritis**		
Normal (Grade 1)	65	7
Low activity (Grade 2 + 3)	12	86
High activity (Grade 4 + 5)	-	34
3. Degree of atrophy ^a		
Normal	65	104
Mild	6	21
Moderate	0	2
4. Degree of intestinal metaplasia ^b		
Normal	60	121
Mild	9	10
Moderate	2	2
Total number of cases	77	127

n = 204

* p value <0.001

** p value <0.0001

a + b = 34 biopsy specimen could not be evaluated because of technical difficulties i.e. biopsies too small, too superficial, or not vertically sectioned.

diminution and eventual disappearance of HP in the antral side. In contrast, Karttunen [6] suggested that slight and moderate glandular atrophy were significantly more common in cases with abundant HP. In our study, no statistically significant degree of gastric atrophy was noted. However, a long-term follow-up and repeat biopsy would be necessary to have a better conclusion.

Study done by Shousha [7] revealed a contrasted finding between two patient populations concerning the relationship between intestinal metaplasia and HP

colonisation. Although Yemeni patients had a significantly lower prevalence of HP than British patients, the former had a significantly lower prevalence of intestinal metaplasia. They suggested that the presence of other factors, possibly genetic, control the development of intestinal metaplasia and the possibility of gastric carcinoma in HP positive patients [9]. In our study as well, we did not find any significant correlation between HP positivity and intestinal metaplasia. A personal communication with Dr. Thomas J. Borody (Centre for Digestive Diseases, Sydney, Australia) had suggested that investigation on a single bite of biopsy specimen was not sufficient enough to give a conclusive remark, as focal or patchy areas of intestinal metaplastic changes as well as focal colonisation could occur in the same individual at the same time.

In summary, we concluded that although the degree of gastritis and gastric activity correlated well with the positivity and density of HP colonisation, there was no direct correlation between bacterial density and mucosal atrophy or intestinal metaplasia. A better understanding for this correlation would be obtained if biopsy could be taken from multiple sites and other parameters of investigations like clinical endoscopic feature of the mucosa, ¹⁴C-urea breath test and CLO test could be analysed and assessed simultaneously.

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Annex 1. Grade of gastritis, *H. pylori* colonisation, and neutrophil infiltration

Grade	Gastritis	HP	Activity
1. none	No lymphocytes in the lamina propria	No HP detectable	No neutrophil granulocytes
2. minimal	Isolated lymphocytes and plasma cells in the upper lamina propria	Isolated focal HP	Single isolated neutrophils in the lamina propria
3. low	Uniform, slight infiltration with lymphocytes and plasma cells	Few evenly distributed HP	Few neutrophils in the lamina propria with no leukopedesis in the region of the gastric pits
4. medium	Moderately dense infiltration of the upper lamina propria by lymphocytes and plasma cells	Moderately dense colonisation	Moderate numbers of neutrophils in the lamina propria with moderate leukopedesis in the region of the gastric pits
5. high	Very dense lymphoplasmocellular infiltration of the lamina propria	Very dense HP colonisation	Abundant neutrophils in the lamina propria with pronounced leukopedesis and formation of leukocyte "plugs" in the pits

(Ref: Bayerdorffer et al., 1992)

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Clinical trial to determine the hypouricaemic potential of Zizyphus jujuba kernel

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Clinical trial to determine the therapeutic efficacy of a traditional herbal drug, Zizyphus jujuba kernel reputed for anti-gout action was carried out on 20 healthy volunteers. Using a cross-over design, the subjects were divided into two groups. Group I received standard hypouricaemic drug, allopurinol and Group II was administered Z. jujuba kernel for 14 days. After a wash-out period of another 7 days, the drugs were crossed over between the two groups in which Group I was administered Z. jujuba kernel and Group II was administered allopurinol for further 14 days again. Blood and urine samples of these subjects were taken at 5 days' intervals throughout the study period for determination of uric acid, creatinine and liver function tests. It was found that Z. jujuba shows no abnormalities on kidney and liver function tests. It also possesses neither hypouricaemic nor uricosuric actions.

INTRODUCTION

In Myanmar, most of the people living in rural areas rely on traditional drugs and herbal remedies. Zizyphus jujuba kernel (Zee Sei Asan in Myanmar name) i.e. softer, inner edible part of a fruit-stone (Fig. 1) is

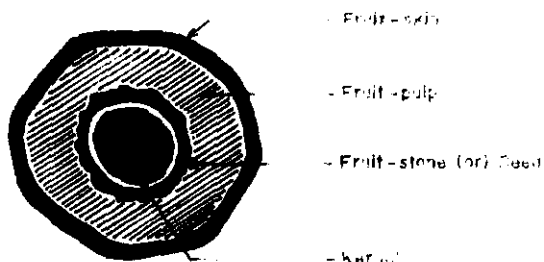


Fig. 1. Different components of Z. jujuba fruit

reputed for use in treatment of gout (Dular in Myanmar name) which is not an uncommon disease in Myanmar. However, anti-gout efficacy of this herbal medicine has never been investigated scientifically for specific hypouricaemic action and its adverse effects on human have not yet been

reported. This study has been conducted with an attempt to find out the hypouricaemic action of Z. jujuba and its toxic effects on kidney and liver on normal healthy volunteers.

EXPERIMENTAL DESIGN AND METHODOLOGY

Subjects

Twenty normal healthy subjects of both sexes and age between 25-55 years were selected. They were clinically free from heart, lung, liver, kidney, endocrine and connective tissue diseases. Pregnant women and lactating mothers were excluded from this study. The subjects were explained about the nature and purpose of this study and the right to withdraw at any stage without prejudice. Only those subjects who gave voluntary informed consents were allowed to participate in the study. All subjects were requested to abstain from smoking, alcohol drinking, excessive exercise, eating foods rich in purine (i.e. pancreas, liver, kidney, brain, heart

of animal origins, small fatty fish, fish roe, meat extract) [1] throughout the study period.

Drugs

Kernels of Z. jujuba were obtained from Department of Traditional Medicine. A standard hypouricaemic drug, allopurinol (100 mg tablet size), product of Roussel Laboratories Ltd., England was purchased locally.

Study design

The trial was a randomized 2-way cross-over design, in which the hypouricaemic efficacy of the standard hypouricaemic drug, allopurinol and Z. jujuba were evaluated on the same subjects. Twenty subjects were randomly divided into two equal groups; Group I in which allopurinol was administered first for 14 days followed successively by a wash-out period of 7 days and Z. jujuba administration for another 14 days; and Group II in which Z. jujuba was administered first followed by wash-out period and allopurinol administration (i.e. *vice versa* to Group I). Testing of these two groups was simultaneously carried out in this study. Prior to administration of the drugs, detailed history taking, thorough clinical examination and ECG check-up were performed on all subjects. Five cc of blood and 24-hour urine sample of each subject were collected at day 0 and day 5 to obtain baseline data. Then, the subjects were administered allopurinol in dosage of 100 mg three times a day or Z. jujuba in 10 seeds three times a day as mentioned in the above-schedule. The blood and urine samples were collected at every 5-day interval throughout the study till the 5th day after cessation of last drug administration.

Methods

Serum and urine uric acid were determined by the standard method of Brown [2]. In addition, serum and urine creatinine were assayed by

alkaline picrate method [3]. Liver function tests were also determined and glomerular filtration rate (GFR) was calculated from serum creatinine concentration using the method of Hull [4]. Statistical analysis of data were performed by using Student's unpaired 't' test.

RESULTS

Biometric data of the subjects of Group I and Group II who participated in this clinical trial are depicted in Table 1. No statistically significant

Table 1. Biometric data of the subjects in two test groups

	Group I	Group II	Significance
*Age (years)	28.8±2.1 (25-55)	39.9±1.5 (29-55)	NS
Sex (M:F)	4:6	5:5	NS
*Weight (kg)	49.6±1.3 (41-63)	50.8±2.1 (40-68.8)	NS
*Height (cm)	155.3±4.4 (145-186)	156.2±1.9 (140-169)	NS

*Each value represents mean ± SEM with range in parenthesis

NS means not significant at 5% probability level

In Group I, allopurinol was administered in "Test 1" period followed by Z. jujuba in "Test 2" period

In Group II, Z. jujuba was administered in "Test 1" period followed by allopurinol in "Test 2" period

differences were observed in age, sex, weight and height of the subjects between the two groups, thus showing validity of our trial design.

As shown in Figure 2, serum uric acid levels dropped significantly in subjects of Group I under administration of allopurinol whereas apparently no change was observed in subjects of Group II under Z. jujuba administration. When the subjects were crossed-over, serum uric acid levels of the subjects of Group I under Z.

jujuba administration showed little changes from pre-test values while the subjects of Group II under allopurinol administration showed significant decrease in serum uric acid levels.

Regarding the urinary uric acid excretion per 24 hours, Figure 3 illustrates

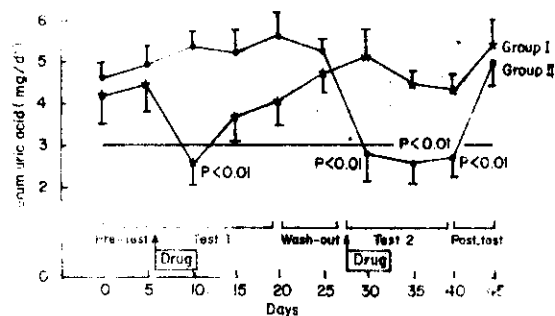


Fig. 2. Serum uric acid levels in two groups of subjects
Each point represents the mean $\pm$ SEM ($n = 10$)
The shaded area indicates the normal range

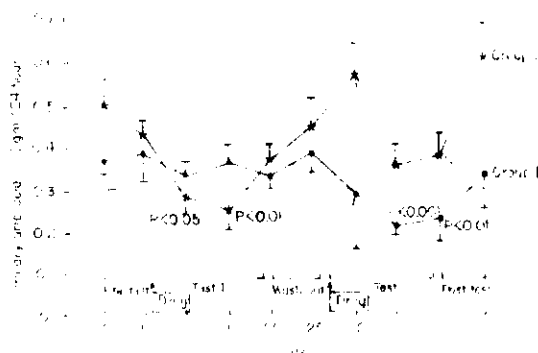


Fig. 3. Urinary uric acid levels in two groups of subjects
Each point represents mean $\pm$ SEM ($n = 10$). The shaded area indicates the normal range

that urinary uric acid excretion was significantly reduced in subjects under administration of allopurinol in both groups, coinciding with the low concentrations of serum uric acid in that same test period. However, there was apparently no change in urinary excretion of uric acid in those subjects under administration

of Z. jujuba in both groups.

The results of renal function tests (i.e. serum creatinine and GFR), and liver function tests (SGPT, SGOT and alkaline phosphatase) of the subjects in both groups were found to be within normal ranges throughout the study period (Figure 4 & 5).

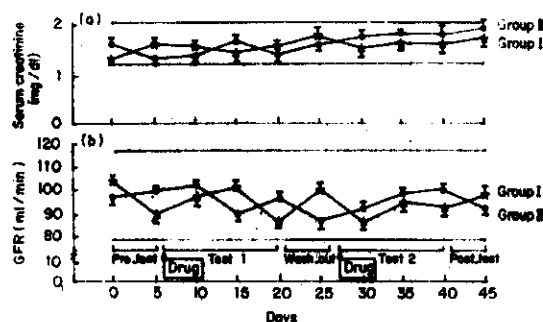


Fig. 4. Renal function tests (a) serum creatinine concentrations and (b) GFR in the two groups of subjects
Each point represents the mean $\pm$ SEM ($n = 10$). The shaded area indicates the normal range

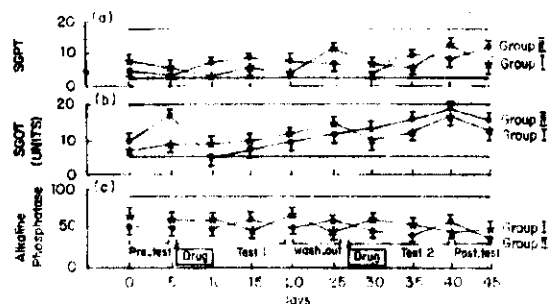


Fig. 5. Liver function tests (a) SGPT (b) SGOT and (c) alkaline phosphatase in the two groups of subjects
Each point represents the mean $\pm$ SEM ($n = 10$). The shaded area indicates the normal range

DISCUSSION

Z. jujuba is one of the plants traditionally used as Chinese medicinal herb [5]. Peptide alkaloids have been isolated from the stem bark of Z. jujuba [6]. A prostaglandin I_2 , known to be potent natural inhibitor of

platelet aggregation and a powerful vasodilator has been extracted from the leaves of Z. jujuba [7]. From the fruit of Z. jujuba, cGMP, known to have an antagonistic action to cAMP in the regulation of cellular activity in animal tissues, has been identified [8]. Although no literature is available, it is reputed that Z. jujuba kernel has been used as a traditional herbal drug in the treatment of gout in our country. Gout is a disease characterized by recurrent attacks of acute pain and swelling, at first affecting only one joint, usually the metatarso-phalangeal joint of the big toe, later becoming polyarticular. It is the result of an inborn error of purine metabolism producing high serum uric acid concentration in chronic cases [1]. In the present study, standard anti-gout drug, allopurinol showed hypouricaemic effect, confirming its inhibitory action on uric acid synthesis rather than uricosuric effect [9]. However, Z. jujuba kernel was found to have no effect on uric acid metabolism, showing neither hypouricaemic nor uricosuric actions. In addition, it showed no acute toxicity on kidneys and liver. This is consistent with the findings of Aye-Than who observed no acute toxicity of Z. jujuba kernel in animal models [10]. It is also well known that people in the middle Myanmar have been eating Z. jujuba kernel as snacks without showing any toxicity. Therefore, reputed action of Z. jujuba kernel on gout in the community is not based on its action with respect to uric acid metabolism. It may be due to the analgesic, sedative or anti-inflammatory effects which were not tested in the present study. The other possible explanation for the joint pain and other symptoms relieved with administration of Z. jujuba kernel, as reputed in the community, may be due to its action on other rheumatic diseases rather than on gout.

ACKNOWLEDGEMENTS

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Screening for and utilization of contraceptive methods among clients at family planning clinic of Central Women's Hospital, Yangon

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One thousand one hundred and thirty two clients attending the family planning clinic of Central Women's Hospital, Yangon during a 16-months period, beginning in May 1995 were screened according to a set of eligibility criteria, counselled on suitable method(s) among the 4 methods, viz. combined oral contraceptive pills (COC), depot-medroxyprogesterone acetate (DMPA), intra-uterine contraceptive device (IUCD) and condoms, and offered the method of informed choice. Among the clients, 904 (79.85%) were fit for COC. Of these, 9.4% chose COCs, whereas 58.9% chose DMPA, 28.3% IUCDs and 3.3% were past users. Similarly 1088 (96.1%) were fit for DMPAs. Of these, 55.33% chose DMPAs, whereas 30.69% chose IUCDs, 9.1% COCs and 4.9% condoms. Of these 44 clients who were unfit for DMPA, 59.1% had been past users. Likewise, 942 (83.2%) were fit for IUCD. Of these, 36.2% chose IUCDs, whereas 50.8% chose DMPAs, 7.6% COCs and 5.3% condoms. Of those 190 who were unfit for IUCD, 1.6% had been past users. Those who were unfit were reassessed to fulfil the wishes of users. In all, DMPA was the most utilized method (54.2%), followed by IUCD (31.3%), COC (9.0%) and condoms (5.5%). The utilization of IUCD was increased 6 times higher than that in 1990 before provision of contraceptive services by public sector. The creation of such services in the country will definitely improve the optimum contraceptive mix required for a family planning programme.

INTRODUCTION

The National Population Policy emphasises the provision of quality birth spacing services for married couples and the reduction of the total fertility rate to replacement level in the whole country by the year 2000 [1]. Since 1992, implementation of birth spacing project has been started and at present has covered 44 townships with a target of 79 by 1997 [2], resulting in provision of contraceptives to 650,000 currently married women [3]. Comprehensive proforma and effective individualized counselling are areas which call for technical expertise, patience and experience. From the past studies about the knowledge, attitude and practice on

contraception, it was found that there were considerable variations of contraceptive practice among the currently married women [4]. This is because of lack of method choice and mix, and certain aspect of birth spacing services. For instance, in 1990, the proportion of contraceptive method users were 24% for pills, 18% for injectables, 5% for IUCDs, 1% for condoms and 52% for other methods [5]. According to WHO, the choice of contraceptive methods available to clients through a family planning programme resulting in optimum contraceptive method mix is an important determinant of programme success [6].

Therefore, this study has been under-

taken with a view to screen for optimum method with a set of eligibility criteria and to determine the utilization of various contraceptive methods after informed choice among clients attending the family planning clinic of Central Women's Hospital, Yangon, a highly specialized urban centre of the 3 centres chosen for contraceptive study [7].

METHODOLOGY

Study area

This study is part of the UNFPA funded MYA/94/PO1 project on "Contraceptive Acceptability" in three areas, urban, semi-urban and rural. The urban study is being carried out in Central Women's Hospital, family planning clinic.

Study subjects and methods

One thousand one hundred and thirty two clients attending the family planning clinic of Central Women's Hospital, Yangon during a 16-month period, beginning in May 1995 were screened according to a set of eligibility criteria (Table 1), counselled on suitable method(s) and offered the method of informed choice. The four methods offered were combined oral contraceptive pills (COC), depot-medroxyprogesterone acetate (DMPA), copper intrauterine contraceptive device (IUCD) and condoms. COC, injectables and condoms were dispensed after informed choice by the project nurses and IUCDs by medical officers.

Analysis

Analysis is confined to initial phase of the study at CWH only. Data were checked for consistency, entered into computer and analysed by Epi-info 6.

The screening results were classified into fit and unfit categories according to each method of contraception (COC, injectables, IUCD). From these a second step analysis was

Table 1. Screening for Hormonals & IUCD

Screening for hormonals (Code 1 for Yes and 2 for No)

1. Over 35 years old ☐
2. Heavy smoker ☐
3. Epilepsy ☐
4. Breast lump ☐
5. Severe varicose veins ☐
6. Hypertension ☐
7. Diabetes mellitus ☐
8. Migraine ☐
9. Cerebro vascular accident (CVA) ☐
10. Rheumatic heart disease ☐
11. Thrombophlebitis/deep vein thrombosis/pulmonary embolism ☐
12. Congenital hyperlipidemia ☐
13. Jaundice within 6 months or during pregnancy ☐
14. Abnormal vaginal bleeding ☐
15. IHD or severe chest pain ☐
16. Unusual shortness of breath after exercise/heavy effort ☐
(If yes to any of the above, avoid COC) (If yes to any from No. 10 to No. 16, avoid injectables)

Screening for IUCD (Code 1 for Yes and 2 for No)

17. Childless ☐
18. Anaemia ☐
19. Uterine scars ☐
20. Severe dysmenorrhoea ☐
21. Abnormal vaginal bleeding ☐
22. Gynaecological malignancy ☐
23. Acute pelvic inflammatory disease/STD ☐
24. Ectopic pregnancy/gestation ☐
(If yes to any of the above, avoid IUCD)

performed for the method finally chosen by the clients. Those belong-

ing to the unfit category for a particular method, after re-assessment by the specialist in charge of the Unit and found to be relatively safe to use the insisted method were also analysed by method finally chosen.

Analysis was also carried out for socio-demographic characteristics according to method chosen.

RESULTS

(a) Screening for COC and acceptors of methods (Table 2)

Among the 1132 clients, 904 (79.85%) were fit for COC. Of these, 9.4% chose COCs, whereas 59% chose DMPA, 28.3% IUCDs and 3.3% condoms. Of 228 who were unfit according to the proforma for COC, 17 (7.5%) were given COC after re-assessment by gynecologist, 81 (35.5%) accepted injectable, 98 (43%) IUCD and 32 (14%) condoms. In addition, 50% of those unfit for COC were past COC users.

(b) Screening for injectable (DMPA) and acceptors of methods (Table 2)

One thousand and eighty eight clients (96.1%) were fit for DMPAs. Of these, 55.3% chose DMPAs, whereas 30.7% chose IUCDs, 9.1% COCs and 4.9% condoms. Of 44 who were unfit according to the proforma for injectables, 12 (27.3%) were given injectables after re-assessment, 3 (6.8%) accepted COC, 20 (45.5%) IUCD and 9 (20.5%) condoms. Among those unfit for DMPA, 59.1% had been past users.

(c) Screening for IUCD and acceptors of methods (Table 2)

Nine hundred and forty two clients (83.2%) were fit for IUCD. Of these, 36.2% chose IUCDs, whereas 50.8% chose DMPAs, 7.6% COCs and 5.3% condoms. Out of 190 who were unfit according to the proforma for IUCD, 13 (6.8%) were inserted IUCD

after re-assessment, whereas 30 (15.8%) accepted COC, 135 (71%) injectable and 12 (6.3%) condoms. Among those unfit for IUCD, 1.6% had been past users.

Over all, DMPA was the most utilized method (54.2%), followed by IUCD (31.3%), COC (9.0%) and condoms (5.5%).

Table (3) denotes the frequency of clients fit and unfit according to eligibility criteria to one or two or all methods. Nine clients were excluded from the study due to pregnancy. Therefore there was a deficit of 9 clients when analysis was finally performed on method accepted.

(d) Demographic features of method acceptors

Among all the clients, the age range was 17 to 48 years with the highest percentage among 25-29 year age group and the overall mean of 28.7 years. The mean age among the users of the 3 female contraceptives were similar but lower than those women whose husbands used the male condoms (Table 4). Age at first marriage ranged from 13 to 40 years with the overall mean of 21.7 years and mode 18 years (Table 4). Husbands' ages ranged from 17 years to 56 years with the overall mean of 32 years and mode of 30 years. Here also, the mean age of condom users among the husband was higher than those men whose wives used the female contraceptives.

As regards educational level, the majority in COC and condom group had university education and the majority in injectable and IUCD groups had completed primary education (Table 5).

Across all methods, most husbands were unskilled labourers and most of the wives were dependents, except in the condom group, where most husbands belonged to professional and

Table 2. Pattern of contraceptive use by clients after screening at Central Women's Hospital

Eligibility criteria for		Method accepted								Total	
		COC (%)		Injectable		IUCD		Condom		N = 1132	
		n1 = 102		n2 = 614		n3 = 354		n4 = 62		No.	%
		No.	%	No.	%	No.	%	No.	%	No.	%
COC	Fit	85	9.4	533	59.0	256	28.3	30	3.3	904	100
	Unfit*	17	7.5	81	35.5	98	43.0	32	14.0	228	100
Injectable	Fit	99	9.1	602	55.3	334	30.7	53	4.9	1088	100
	Unfit*	3	6.8	12	27.5	20	45.5	9	20.5	44	100
IUCD	Fit	72	7.6	479	50.8	341	36.2	50	5.3	942	100
	Unfit*	30	15.8	135	71.1	13	6.8	12	6.3	190	100
Total		102	9.0	614	54.2	354	31.3	62	5.5	1132	100

*Those regarded as unfit according to eligibility criteria were re-assessed by gynaecologists and offered the method if the client insisted and the advantages outweighed the minimal risks (category 2) [8]

Table 3. Frequency distribution of clients fit or unfit for one, two or all methods

Method	Fit	Unfit	Total
Injectable	1097 (96.1%)	44 (3.9%)	1141
COC	909 (79.7%)	232 (20.3%)	1141
Injectable + COC	909 (79.7%)	232 (20.3%)	1141
IUCD	95 (83.3%)	190 (16.7%)	1141
Injectable + COC + IUCD	759 (66.5%)	382 (33.5%)	1141

managerial levels.

DISCUSSION

According to the consensus reached at WHO office in Geneva, quality family planning services depend on the following factors. These include information on consistent and correct use, technical competence, counselling and ongoing support by providers, accessibility, acceptability and affordability of services to ensure ongoing of care and availability of methods [8].

The eligibility criteria for various methods have been classified into four categories as follows:-

Category 1. A condition for which there is no restriction for the use of the contraceptive method.

Category 2. A condition where the advantages of using the method generally outweigh the theoretical or proven risks.

Category 3. A condition where the theoretical proven risks usually outweigh the advantage of using the method.

Category 4. A condition which represents an unacceptable health risk if the contraceptive method is used.

The summary of eligibility criteria for contraceptive methods by category (indications and contra indications) is reproduced in Table 5.

The present study conforms with these criteria. Those belonging to category 2, 3 were assessed by OB/GYN specialist and offered the opti-

Table 4. Method acceptors by age

Age group	Method accepted								Total	
	COC		Injectable		IUCD		Condom		N = 1132	
	n1 = 102		n2 = 614		n3 = 354		n4 = 62			
	No.	%	No.	%	No.	%	No.	%	No.	%
Client age										
15 to 19	5	4.9	35	5.7	7	1.9	0		47	4.2
20 to 24	18	17.6	156	25.4	64	18.1	9	14.5	247	21.8
25 to 29	41	40.2	207	33.7	121	34.2	9	14.5	378	33.4
30 to 34	22	21.6	135	22.0	85	24.0	18	29.0	260	22.9
35 to 39	12	11.8	57	9.3	56	15.8	16	25.8	141	12.5
40 to 44	3	2.9	23	3.7	19	5.4	8	13.0	53	4.7
45 to 49	1	1.0	1	0.2	2	0.6	2	3.2	6	0.5
Mean $\pm$ SD	28.6 $\pm$ 5.8		27.8 $\pm$ 5.7		27.6 $\pm$ 5.7		33.0 $\pm$ 5.7		28.7 $\pm$ 5.9	
Client at first marriage										
13 to 19	37	36.3	248	40.4	147	41.5	18	29.0	450	39.8
20 to 24	26	25.5	203	33.1	124	35.0	19	30.7	372	32.9
25 to 29	27	26.5	122	19.9	70	19.8	16	25.8	235	20.8
30 to 34	10	9.8	35	5.7	11	3.1	7	11.3	63	5.5
35 to 40	2	1.9	6	0.9	2	0.6	2	3.2	12	1.0
Mean $\pm$ SD	22.7 $\pm$ 5.1		21.7 $\pm$ 4.6		21.2 $\pm$ 4.3		23.6 $\pm$ 5.3		21.8 $\pm$ 4.6	
Husband										
17 to 24	12	11.8	95	15.5	28	7.9	4	6.5	139	12.3
25 to 29	27	26.5	180	29.3	75	21.2	9	14.5	291	25.7
30 to 34	31	30.4	164	26.7	110	31.0	16	25.8	321	28.4
35 to 39	19	18.6	102	16.6	82	23.2	12	19.4	215	18.9
40 to 44	7	6.9	52	8.5	41	11.6	11	17.7	111	9.8
45 to 49	4	3.9	20	3.3	12	3.4	7	11.3	43	3.8
50 to 56	2	1.9	1	0.1	6	1.7	3	4.8	12	1.1
Mean $\pm$ SD	31.9 $\pm$ 6.8		31.0 $\pm$ 6.6		33.1 $\pm$ 6.4		36.1 $\pm$ 7.6		32.0 $\pm$ 6.7	

mum method after weighing the risks and benefits.

The preference of injectables by the majority of Myanmar may be due to the fact that the method is health-personnel dependent, only once in 3 month, not related to memory, i.e. convenient, do not have to remember to take daily (less requirement for client compliance), not invasive (i.e. IUCD), and not male-related. However, the attitude of males was found to be favourable from a past study [9].

After proper screening and counselling at the family planning clinic with the best available facilities available at the central level, the proportion of IUCD utilization among the clients (31.3%) in this study is 6 times higher than that of national average (5%) for 1990 [5] when there was no government-sponsored family planning clinics in the country. The increase in IUCD use as well as in the utilization of injectables would therefore occur in the country when such services could be provided in hospitals

Table 5. Method accepted by education and occupation

	Method accepted								Total	
	COC		Injectable		IUCD		Condom		n = 1132	
	n1 = 102		n2 = 614		n3 = 354		n4 = 62			
	No.	%	No.	%	No.	%	No.	%	No.	%
Client education										
- Illiterate	2	4.2	29	4.7	13	3.7	4	6.5	48	4.2
- Read and write	12	7.1	101	16.5	50	14.1	7	11.3	170	15.0
- Primary school	20	4.9	256	41.6	121	34.2	12	19.4	409	35.1
- Middle school	29	11.2	132	21.5	88	24.9	11	17.7	260	23.0
- High school	7	8.9	39	6.4	29	8.1	4	6.5	79	7.0
- University	32	19.3	57	9.3	53	15.0	24	38.7	166	14.7
Client occupation										
- Professional	16	15.7	31	5.0	25	7.1	7	11.3	79	7.0
- White collar	9	8.8	32	5.2	27	7.6	8	13.0	76	6.7
- Skilled, semi-skilled, armed forces	4	3.9	17	2.8	13	3.7	3	4.8	37	3.3
- Unskilled	11	10.8	95	15.5	44	12.4	3	4.8	153	13.5
- Dependent	62	60.8	439	71.5	245	69.2	47	66.1	787	69.5
Husband occupation										
- Professional	31	13.4	100	16.3	74	20.9	19	30.7	224	19.8
- White collar	16	15.6	93	15.1	47	13.3	10	16.1	166	14.7
- Skilled, semi-skilled, armed forces	21	20.6	136	22.2	107	30.2	14	22.6	278	24.6
- Unskilled	29	28.4	259	42.2	115	32.5	17	27.4	420	37.1
- Dependent	5	5.0	26	4.2	11	3.1	2	3.2	44	3.9
Total	102	100	614	100	354	100	62	100	1132	100

having obstetric and gynaecological services. In addition, the increase in use of IUCD is highly desirable for those married women who wish to limit child birth after having delivered sufficient numbers of children as permanent method by female sterilization is not easily permitted and may be inconvenient for the families.

In this study cohort, the married women, on average, started to use COC and DMPA after 6 years, IUCD after 8 years and condom, after 10 years of marriage (Table 4). The wide gap between the start of marriage and contraceptive use may be due to lack of provision of family planning service by public sector, lack of information about contraceptive use or inaccessibility to private

sector.

In our clinic, the majority of COC and condom users were more educated and their husbands were of social higher class than injectables and IUCD users.

The low utilization of condom would be much improved if couple attendance could be encouraged. The major barrier seems to be the inconvenience on the husband's part, who, as this study has brought out, were mostly hard earned bread-winners of the families.

The side effects of the contraceptives, the patterns of switching and the reasons, use and non-use continuation and the reasons will be published later after completing of the project.

Summary of contraceptive methods and category

	Category 1	Category 2	Category 3	Category 4
Breastfeeding	Condom, Inj.			
Post abortion	All method			
Age	Condom, Inj; IUCD		COC(>35yrs + light smoker)	
Hypertension	Condom/IUCD	Injectables	COC(History +)	
Diabetes mellitus			Injectable (vascular/renal/ retinal involve- ment)	COC(vascular/ involvement/ retinopathy)
DVT/Pulmonary embolism	Condom/IUCD/Inj.			COC
IHD/CVA	Condom/IUCD		Injectable	COC
Hyperlipidaemias	Condom/IUCD	Injectables	COC	
Valvular heart disease		Injectables (if complicated)		COC (if compli- cated)
Migraine			Injectable	COC
Vaginal bleeding	Condom, COC			Inj; IUCD (if suspicious)
Breast disease	Condom, IUCD			COC, Inj. (Cancer +)
Cervical cancer/CIN	Condom	COC, Inj. (CIN) (Cancer)		IUCD (Cancer)
PID/STDs	Condom, COC, Inj.			IUCD
Liver disease	Inj; IUCD, Condom		COC (Biliary disease)	COC (Viral hepatitis*/ Cirrhosis/ tumours)

COC = Combined Oral Contraceptive
Inj. = Injectables

IUCD = Intrauterine contraceptive device
* = Special diseases

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Serum fibrin(-ogen) degradation products (FDP) in toxæmia of pregnancy

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The relationship between the levels of FDP in the serum, blood pressure and the severity of other features of toxæmia of pregnancy were studied in 42 patients with pregnancy-associated hypertension and/or pre-eclampsia (PE). Serum FDP levels were found to be elevated in 88% of cases but not related to the severity of the clinical manifestations of toxæmia in pregnancy. It was elevated in cases with normal systolic blood pressure as well as in cases with no proteinuria. Subsequent serum FDP concentrations fell to normal along with the treatment in all cases. Serum FDP determination was suggested to include in investigation of severe cases of PE as a sensitive index for the diagnosis as well as for the assessment of response to treatment.

INTRODUCTION

It is widely accepted that PE is accompanied by a marked increase in FDP and fibrin monomer complexes [1]. Disseminated intravascular coagulation (DIC) is responsible for many of the manifestation of PE and eclampsia [2]. Toxæmia of pregnancy is an obstetric complication of unknown aetiology associated with fibrin deposition in the subendothelium of spiral arteries [3,4]. It is considered to be important in the pathogenesis of some clinical manifestations, especially proteinuria and hypertension. This is disputed by some investigators. It is difficult to know whether DIC is cause or effect [5,6]. But it is irrefutable that extensive haemostatic changes including increased fibrinolysis exist in PE. The present study is undertaken not to argue whether there is DIC or not, either cause or effect, but to assess the relationship between serum FDP level and features of toxæmia of pregnancy such as hypertension, proteinuria and generalised oedema.

PATIENTS AND METHODS

Pre-eclampsia was diagnosed by observing both (a) persistent hypertension or a sudden rise of blood pressure (BP) and (b) proteinuria and/or generalised oedema during the last 4 months of pregnancy. Forty two pregnant mothers with PE, admitted to CWH, were included in this study. Serum FDP levels were determined twice, one each on admission and before discharge by using locally developed FDP test kit [7,8]. Clinical data such as BP, proteinuria and oedema were obtained from patients' clinical charts by the permission of medical officer in-charge concerned. Thirty-five samples of blood collected from age and gestation matched normal pregnant mothers were also included in the study as controls.

RESULTS

Out of 42 cases only 5 cases were found to have serum FDP level within normal range ($>2<10\mu\text{g/ml}$) on admis-

ssion. Remaining 37 cases had increased FDP level ranging >10<160ug/ml. Serum FDP level fell to normal during treatment and before discharge in all cases.

FDP and BP

Table 1. Systolic BP and serum FDP titre

Systolic BP mmHg	Total cases	FDP conc. (ug/ml)				
		<10	>10 <20	>20 <40	>40 <80	>80
<140	8	-	3	3	1	1
140-160	15	5	2	6	2	-
>160-180	11	-	4	4	1	2
>180-200	5	-	1	3	1	-
>200	3	-	1	-	2	-
	42	5	11	16	7	3

Table 2. Diastolic BP and serum FDP titre

Diastolic BP mmHg	Total cases	FDP conc. (ug/ml)				
		<10	>10 <20	>20 <40	>40 <80	>80
90-100	23	5	5	10	2	1
>100-110	12	-	3	5	2	2
>110-120	4	-	2	1	1	-
>120	3	-	1	-	2	-
	42	5	11	16	7	3

Thirty four cases had systolic BP equal or >140mmHg. Eight cases had systolic BP <140mmHg and 15 cases had 140-160mmHg. Out of these 23 cases with mild systolic hypertension normal FDP was found in 5 cases but increased in remaining 18 cases.

Out of 19 cases of systolic BP >160 mmHg (i.e. moderate to severe systolic hypertension) no cases were found to have FDP level within normal range (Table 1). The difference is statistically significant (Fisher Exact test, $p=0.1$).

All 42 cases had diastolic BP 90mmHg

or more. Twenty three cases had diastolic BP 90-100mmHg where FDP was within normal range in 5 cases (21.8%) and 18 cases had increased FDP level. All of 19 cases having diastolic BP >100mmHg had increased serum FDP levels (Table 2). But this difference was not significant statistically (Fisher Exact test $p=0.5$). So increased serum FDP level was more strongly associated with high diastolic BP than with high systolic BP.

FDP and proteinuria

Table 3. Proteinuria and serum FDP titre

Proteinuria	Total cases	FDP conc. (ug/ml)				
		<10	>10 <20	>20 <40	>40 <80	>80
Clear	14	4	1	4	3	2
Trace	6	-	3	2	1	-
+	11	1	5	4	-	1
++	4	-	1	2	1	-
+++	5	-	1	4	-	-
Solid	2	-	-	-	2	-
Total	42	5	11	16	7	3

Out of 28 cases with proteinuria from trace to solid, all cases except in 1 case, had increased serum FDP level. Out of 14 cases with no proteinuria, 10 cases showed increased FDP concentration (71.5%) (Table 3). Serum FDP elevation seemed to precede proteinuria in PE. Proteinuria was found to be correlated not significantly with increase in serum FDP level ($r=0.12$). But proteinuria was significantly associated with increased serum FDP concentration (Fisher Exact test: 2 tailed p -value = 0.14).

FDP and oedema

Oedema is present in all 42 cases ranging from mild, moderate to severe. Serum FDP is increased in 37 cases. No correlation was observed between the two.

Controls

Out of 35 cases of controls, age and gestation matched, the serum FDP levels distributed as follows:

Serum FDP conc. (ug/ml)	No. of cases
<2	15
2-10	16
>10-<20	4
>20	Nil
Total	35

DISCUSSION

PE continues to be an important problem in developed and developing countries. It is one of the commonest causes of maternal deaths and is associated with increased risk of abruptio placentae, DIC, acute renal failure and cerebral haemorrhage. In addition, PE reduces uteroplacental perfusion which places the foetus at high risk of intrauterine growth retardation, preterm death and perinatal mortality [9].

PE often is superimposed upon hypertensive disease. In this study 5 cases gave the history of hypertension previously, before this pregnancy. Hypertensive disorder in pregnancy seems to cause a state of chronic DIC where increased FDP could be observed [5]. In PE and eclampsia, DIC often remains low-grade and organ specific to the renal and placental circulation; however in about 10-15% of women the process becomes systemic and fulminant [10,11]. Extensive haemostatic changes have been observed in women with PE. These include activation of the coagulation system, platelet consumption and an altered fibrinolytic response [12]. This study also reveals that serum FDP levels are increased in 88% of PE but not a single case present with bleeding tendencies clinically. FDP reflect mild degree of coagulation activation. Serum FDP determination should be suggested

to include in investigation of severe PE as a sensitive index.

A better association is found with diastolic BP rather than systolic BP because all cases with normal systolic BP are found to have increased FDP level. All the cases under this study are having increased diastolic BP ranging from 90mmHg to >120mmHg.

One of the major haemostatic abnormalities which accompany PE is deposition of fibrin in the microcirculation [3,4] which subsequently leads to glomerular damage followed by proteinuria and hypertension. In this study there are 10 cases with increased FDP in serum without proteinuria, (71% i.e. out of total 14 cases without proteinuria). This finding may indirectly indicate that elevation of serum FDP precedes proteinuria which result from glomerular damage by fibrin deposition in the glomeruli. Hence a generalised process of slow intravascular coagulation apparently constitutes the primary factor responsible for the lesions in glomeruli in toxemia of pregnancy [10,11].

Subsequent serum FDP determination before discharge shows the level within the normal range in all cases under this study. So serum FDP determination will be useful as a good laboratory parameter for the assessment of cases under treatment in PE.

A prospective longitudinal study should be done to define which is the earliest marker in PE, either hypertension, proteinuria or increasing level of serum FDP. A cut-off point with increased serum FDP level should be established for the occurrence of PE or for the early diagnosis of PE in pregnant mothers who are visiting antenatal care unit for registration and for regular check-ups.

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Shigellosis among children attending the paediatric wards of hospitals in suburban areas

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A total of 55 cases of children with dysentery admitted to the Defence Services Obstetric, Gynaecology and Children Hospital and North Okkalapa General Hospital were included in this study. It was found that 49.1 per cent of the children were suffering from shigella bacillary dysentery. The *Shigella* spp. isolated were: *Shigella flexneri* (44.4 per cent), *Shigella dysenteriae* (29.6 per cent), *Shigella boydii* (22.2 per cent) and *Shigella sonnei* (3.7 per cent). The distribution of *Shigella* spp. among the different age groups were 42.9 per cent in the age group of less than 6 months, 40 per cent in 6 to 12 months, 55 per cent in 13 to 24 months and 66.7 per cent in 25 to 60 months. Antibiotic susceptibility test revealed that these shigellae were resistant to ampicillin (84 per cent), chloramphenicol (76 per cent), streptomycin (84 per cent), tetracycline (64 per cent) and septrin (72 per cent); were fully sensitive to amikacin, gentamycin, netilmicin, norfloxacin and were less sensitive to cephalothin (76 per cent) and furazolidone (72 per cent).

INTRODUCTION

Shigellosis is a common and serious disease, especially in infants and children of developing countries [1-5]. Dysentery, in contrast to watery diarrhoea, inhibits the growth of infants and children [6,7]. It is estimated that at least 140 million cases and almost 600,000 deaths occur annually due to shigellosis in young children under the age of 5 years all over the world [8]. The organism is found everywhere in the world, but it is most common in developing countries where poor sanitation and overcrowding facilitate transmission from person to person. In Myanmar shigellosis is also very common [9]. Shigellae of all groups

have acquired resistance to many antibiotics, making treatment difficult and expensive [10,11]. The present study is to determine the prevalence of various shigella serotypes as well as their antibiotic susceptibility patterns in dysenteric children admitted to two hospitals in Yangon.

MATERIALS AND METHODS

Fifty five cases of children admitted to the two hospitals (Defence Services Obstetric, Gynaecology and Children Hospital and North Okkalapa General Hospital) during May to December 1994 were included in this study. The study subjects were children under 12 years of age of both sexes.

Out of 55 cases tested 41.8 per cent of children had already been treated with one or more antibiotics or with antiamoebic drugs. Stool samples were tested within 2 hours for amoebic identification. Rectal swabs as well as direct plating onto *Salmonella* Shigella agar and MacConkey agar were tested bacteriologically as described previously [12]. Identification of bacteria was done according to the standard protocol of World Health Organization [13]. Serotyping of shigella was done by using the antisera obtained from Denka Seiken Co. Ltd., Japan; salmonella identification was done by using the antisera from Wellcome Reagents Limited, USA; *Escherichia coli* was detected by using the antisera prepared locally [14,15]. Antibiotic susceptibility testing was done by using the discs obtained from BBL Microbiology Systems (Becton Dickinson and Co.) products, USA. The method described by Kirby & Bauer was used for antibiotic susceptibility test [16].

RESULTS

Shigella species were isolated from 27 patients (49.1 per cent) of all the cases tested; *Shigella flexneri* from 12 patients (44.4 per cent), *Shigella dysenteriae* from 8 patients (29.6 per cent), *Shigella boydii* from 6 patients (22.2 per cent), and *Shigella sonnei* from one patient (3.7 per cent) (Figure 1).

Out of 7 cases with less than 6 months of age, shigella was isolated from 42.9 per cent. Shigella was isolated from 40 per cent of the cases among 7 to 12 months old infants and only 16.7 per cent of cases in those over the age of 60 months. The higher percentages of shigella (55 per cent and 66.7 per cent) were isolated from the middle age groups (13 to 24 months and 25 to 60 months) (Table 1).

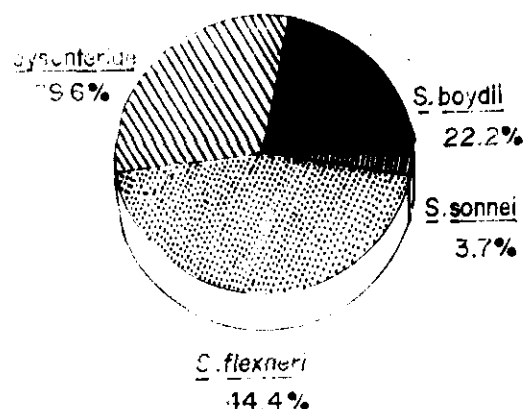


fig. 1. Distribution of shigellae among children with dysentery

The other associated pathogens isolated from the 55 dysentery cases are shown in Table 2. It was found that shigella alone was isolated from 14 cases; *Shigella* species in association with *Entamoeba histolytica* were isolated from 12 cases. Two were isolated in combination with *Salmonella* species, one case was isolated together with *Staphylococcus aureus*. Four cases were isolated with Enteropathogenic *Escherichia coli* of O148 serotype (2 cases); O125 (1 case) and O26 (1 case). Of these EPEC one was associated with *Shigella* species and one with *Entamoeba histolytica*. However, no Enterotoxigenic *Escherichia coli* was isolated from these children.

Table 3 shows the association of shigella species with *Entamoeba histolytica* and distribution of *Shigella* species. It was found that both *Shigella* species and *Entamoeba histolytica* were isolated from 12 cases. *Entamoeba histolytica* was also associated with *Salmonella* species, *Aeromonas hydrophilia*, or *Escherichia coli* O125 in 3 cases.

The resistance patterns of *Shigella* are shown in Table 4. *Shigella* species were highly resistant (72 per cent to 84 per cent) to ampicillin, tetracycline, sulfisoxazole, chloramphenicol

Table 1. Isolation of shigellae from different age groups of children with dysentery

Age group (months)	Tested cases	<u>S. boydii</u>	<u>S. dysenteriae</u>	<u>S. flexneri</u>	<u>S. sonnei</u>	All shigellae
0-6	7	1	1	1	0	3 (42.9)
7-12	10	2	1	1	0	4 (40.0)
13-24	20	0	3	8	0	11 (55.0)
25-60	12	2	3	2	1	8 (66.7)
over 60	6	1	0	0	0	1 (16.7)
All age groups	55	6 (10.9)	8 (14.6)	12 (21.8)	1 (1.8)	

Figures in parentheses denote percentages

Table 2. Bacterial pathogens isolated from children with dysentery

Bacterial pathogens	Number of cases isolated	Percentage
<u>Shigella</u> species	14	25.5
<u>Shigella</u> spp. + <u>Entamoeba histolytica</u>	12	21.8
<u>Shigella</u> spp. + EPEC	1	1.8
<u>Entamoeba histolytica</u>	4	7.3
<u>Entamoeba histolytica</u> + EPEC	1	1.8
<u>Entamoeba histolytica</u> + <u>Salmonella</u> spp.	1	1.8
<u>Entamoeba histolytica</u> + <u>Aeromonas hydrophilia</u>	1	1.8
Enteropathogenic <u>Escherichia coli</u>	2	3.6
<u>Klebsiella aerogenes</u>	2	3.6
<u>Salmonella</u> species	2	3.6
<u>Staphylococcus aureus</u>	1	1.8
No pathogens	14	25.5
Total number of cases	55	100.0

EPEC: Enteropathogenic Escherichia coli

Table 3. The association of Shigella species with Entamoeba histolytica

<u>Shigella</u> species	Number of cases
<u>Shigella boydii</u>	1
<u>Shigella flexneri</u>	7
<u>Shigella dysenteriae</u>	3
<u>Shigella sonnei</u>	1
Total	12

and septrin; moderately resistant (52 per cent to 64 per cent) to tetracycline and augmentin; least resistant (16 per cent to 24 per cent) to cephalothin and furazolidone.

Shigella flexneri was totally resistant to ampicillin, chloramphenicol, streptomy-

cin and tetracycline. Shigella dysenteriae was also 62 per cent to 80 per cent resistant; sulfisoxazole (80 per cent); ampicillin and septrin (75 per cent); augmentin (66.7 per cent); and to chloramphenicol, streptomycin and tetracycline (62.5 per cent) (Figure 2).

Shigella dysenteriae, S. flexneri and S. boydii were 100, 76 and 50 per cent sensitive respectively to cephalothin. However they were 62.5, 90 and 50 per cent sensitive respectively to the drug furazolidone (Figure 3).

DISCUSSION

A study by Panigrahi et al. [17]

Table 4. Resistance pattern of shigellae in percentages

Antibiotics	<i>S. boydii</i> n = 6	<i>S. dysenteriae</i> n = 8	<i>S. flexneri</i> n = 10	All shigellae n = 24
Ampicillin (Am10)	83.3	75.0	100	84.0
Augmentin (Amc300)	16.7	66.7	80.0	52.0
Chloramphenicol (C30)	66.7	62.5	100	76.0
Cephalothin (Cf30)	50.0	37.5	0	24.0
Furazolidone (Fr50)	33.3	25.0	0	16.0
Streptomycin (S10)	100	62.5	100	84.0
Tetracycline (Te30)	16.7	62.5	100	64.0
Sulfisoxazole (G25)	75.0	80.0	80.0	78.9
Septrin (SXT)	66.7	75.0	80.0	72.0

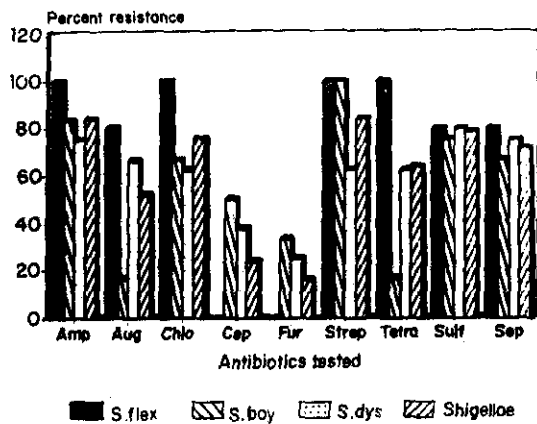


Fig. 2. Resistance pattern of shigellae among children with dysentery

Amp: Ampicillin Strep: Streptomycin
 Aug: Augmentin Tetra: Tetracycline
 Chlo: Chloramphenicol Sulf: Sulfisoxazole
 Fur: Furazolidone Sep: Septrin

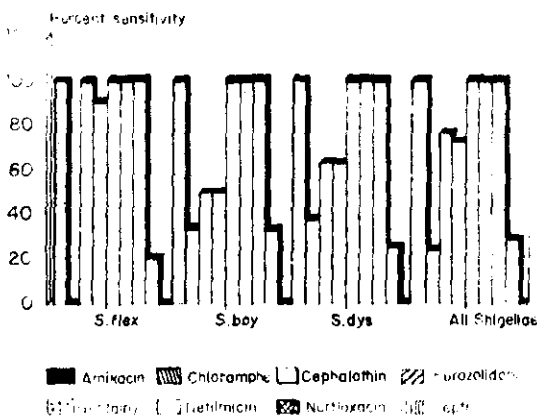


Fig. 3. Sensitive pattern of shigellae among children with dysentery

showed 6.0 per cent of shigellae were isolated from 12709 faecal specimens out of which *Shigella flexneri* was 55 per cent and *Shigella dysenteriae* was 29.7 per cent. They also reported that 15.7 per cent of children under 5 years of age were positive for shigellae. Shigellosis is an endemic disease in India [18,19]. These pathogens cause outbreaks of disease among individuals who have lowered resistance [20]. Recently, the increase in infections caused by *Shigella flexneri* has been traced to the high attack rate of this disease in patients with Acquired Immune Deficiency Syndrome (AIDS) [21]. The incidence of enteric bacterial diseases has been reduced in countries that have achieved sanitary control of drinking water, food, and sewage disposal. In our study a total of 49.1 per cent of shigellae were isolated from children; *Shigella flexneri* 44.4 per cent, *Shigella dysenteriae* 29.6 per cent and *S. boydii* 22.2 per cent.

Most of the strains of shigellae became resistant to various commonly used antibiotics [22,23]. Gebre-Yohannes [24] reported the changing patterns of drug resistance in *Shigella flexneri* serotypes from 1978 to 1982. Although the multiple drug resistance was not common during 1978 to 1981 study, the sudden increase of multiple drug resistance in *Shigella flexneri* isolates of 1982 was

related to an increasing incidence of serotype 2. In our study the multiple drug resistance was common especially with the common easily available antibiotics. However, they were found to be sensitive to newer modern drugs. In the previous report [24] they claimed that trimethoprim-sulphamethoxazole was recommended as a drug of choice. However our results showed shigellae were 28 per cent sensitive to it and 72 per cent sensitive to furazolidone.

This study indicates that, it is required to continue to study the changing pattern of serotypes in the population and drug sensitivity. The explosive onset of drug resistance is probably due to plasmid mediated transfer of genetic information coding for multiple drug resistance. The present indiscriminate use of antibiotics is partially responsible for the above situation, as selection pressure for resistance strains is highest where antibiotics are most intensively used. Community interest clearly demands that every safeguard, compatible with efficient use of antibiotics, be taken to delay the emergence of drug resistant strains.

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Effect of various cleansing materials on decontamination of hands

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In Myanmar, many people routinely use bar soaps such as Shwe-war and carbolic for hand washing. In this study the effectiveness of hand decontamination by using the natural products such as kin-mun-thee, neem leaves, ashes and locally available soap such as sand soap powder, kin-mun-thee soap, neem-seed-oil soap, Myingyan wooden soap and thet-thar detergent was observed. The study was carried out in the Bacteriology laboratory on 66 subjects from June to November 1996. The hand swabs were collected before and after hand washing with different cleansing materials and faecal coliform count was tested by using multiple tube method. Faecal coliform count was reduced in using carbolic, kin-mun-thee, neem-seed-oil and Myingyan wooden bar soap. It was also reduced when using the natural products such as sand soap powder, ashes from kitchen, liquid of boiled neem leaves and liquid of boiled kin-mun-thee.

INTRODUCTION

Diarrhoea is one of the major health problems in children in Myanmar. It occurs most frequently during the rainy season and is caused mostly by infections due to enteric bacterial pathogens [1]. Transmission due to contaminated hands is one of the important routes by which diarrhoea pathogens spread. However, thorough hand-washing with soap and water was found to be effective in decontaminating the hands [2]. Aung Myo Han and Thein Hlaing reported a 30 per cent reduction in diarrhoeal diseases, among children under five years old as a result of intervention to hand-washing [3].

In Myanmar, people usually use bar soaps such as Shwe-war, Carbolic etc. for hand-washing. Some people use other bar soaps such as neem seed oil bar soap which contains neem-seed-oil and kin-mun-thee bar soap which contains kin-mun-thee. Neem-seed (*Azadirachta indica*) and kin-mun-thee (*Sapindus rarak*) are the natural products in our community. Thus the effectiveness of hand-washing

by these natural products was studied. There are some studies on the effectiveness of hand-washing with ashes. Sand soap powder is also the natural product which is available in the middle part of Myanmar. Many people use this soap powder from the ancient time in our community. Thus the effectiveness of hand-washing with ashes and sand soap powder was also studied.

MATERIALS AND METHODS

Study place

The study was carried out in Bacteriology Research Division, Department of Medical Research.

Study period

It was done from June to November 1996.

Methodology

To determine the degree of hand decontamination by using bar soaps and naturally cleansing products, two hand swab samples were collected from each of the subject, one just before hand-washing and one after

washing hands with one type of bar soap or naturally available material. Swabs were collected using the modified method [4]. Briefly, the swab was removed from the sterile wrapping and was inserted into the tube containing sterile peptone water. After soaking it with peptone water, it was pressed against the tube to remove the excess solution so that it would be moist but not wet. It was then rubbed onto the dorsal and ventral surfaces, webs and creases of both hands in a systematic manner: four strokes on each portion of the surface. Two swabs were used, one for each hand. They were then replaced into peptone water tubes, and their terminal ends were broken off under sterile conditions. The tubes were then capped. The specimens were processed immediately in the laboratory to determine the faecal coliform (FC) count using the multiple tube method as described by the International Commission on Microbiological Specification for Foods [5]. Faecal contamination of hands was judged indirectly by the presence of *E. coli* (FC) which are the indicators of faecal contamination [6,7 & 8].

The hands were decontaminated by washing with soap or natural products and water using the modified method of Mackintosh and Hoffmann [9]. The hands were wetted under cool running water and soaked with soap for 5 sec, lathered for 5 sec and then rinsed until free of lather. Water was allowed to drip from the hands but they were not dried or allowed to touch anything until the hand swab was taken.

The cleansing materials which were used in this study for hand-washing were: Shwe-war soap, Carbollic soap, kin-mun-thee soap, neem-seed oil soap and Myingyan wooden bar soaps, Thet-thar detergent, sand soap powder, ashes from kitchen, ashes from rice coat, liquid of boiled neem-leaves

and liquid of boiled kin-mun-thee.

RESULTS

The degree of hand contamination before and after hand washing according to different types of locally manufactured soap bars used is shown in Table 1. Faecal coliform count

Table 1. The degree of hand contamination before and after hand-washing with different types of locally used soap bars

Different types of soap bars	Faecal coliform count (colony-forming units)	
	Before hand-washing	After hand-washing
Shwe-war bar soap	4	7
(n=6)	0	0
	4	23
Carbollic bar soap	0	0
(n=6)	460	9
	23	7
Thet-thar detergent	0	4
(n=6)	15	23
	9	23
Myingyan wooden soap	4600	2400
(n=6)	0	0
	0	0
Kin-mun-thee bar soap	460	0
(n=6)	0	0
	0	0
Neem-seed-oil bar soap	9	0
(n=6)	46	0
	0	0

was reduced in using Carbollic, kin-mun-thee, neem-seed-oil and Myingyan wooden bar soaps. It was not reduced in using Shwe-war and Thet-thar detergent.

The degree of hand contamination by using natural cleansing products used is shown in Table 2. Faecal coliform count was reduced in using sand soap powder, ashes from kitchen, liquid of boiled neem leaves and liquid of

Table 2. The degree of hand contamination before and after hand-washing with different types of natural cleansing products

Different types of natural cleansing products	Faecal coliform count (Colony-forming units)	
	Before hand-washing	After hand-washing
Sand soap powder (n=6)	4 23 0	0 0 0
Liquid of boiled Kin-mun-thee (n=6)	21 7 0	0 0 0
Liquid of boiled neem leaves (n=6)	1100 7 460	0 0 0
Ashes from kitchen (n=6)	460 0 0	0 0 0
Ashes from rice coats (n=6)	0 0 0	9 0 21

boiled kin-mun-thee. But it was not reduced in using ashes from rice coat.

DISCUSSION

In our community the mothers usually wash their hands properly with soap and water after the cleaning process of defaecation and it is the good habit. They need to wash their hands properly before preparing foods and feeding their children. In Myanmar, people usually use bar soaps to wash their hands. Some of the natural products are also effective in hand-washing. Some people use sand soap powder and it is available in the middle part of Myanmar such as Myingyan, Taungtha etc. Some use liquid of boiled neem leaves for bathing when they are infected with scabies in our community. People from Myingyan also produce Myingyan wooden soap and it is available in

our markets. Most of the people use liquid of boiled kin-mun-thee for hair washing and this liquid is available in our markets. There are also bar soaps which are named as neem-seed-oil and kin-mun-thee. Most of our people use Shwe-war and Carbolic bar soaps. Some use Thet-thar detergent now-a-days. Thus in this study, the effectiveness of hand washing was done by using the above cleansing products.

Faecal coliform count was reduced in using Carbolic, kin-mun-thee, neem-seed-oil and Myingyan wooden bar soap. But it was not reduced in using Shwe-war bar soap and Thet-thar detergent. It may be due to contamination of this bar soap on the surface. Thus the survival rate of faecal coliform on this bar soap needs to be studied.

In this study faecal coliform count was reduced when using the natural products such as sand soap powder, ashes from kitchen, liquid of boiled neem leaves and liquid of boiled kin-mun-thee. However, it was not reduced in using ashes of rice coat and it may be due to the fact that these ashes are originally contaminated.

Thus the natural cleansing products which we use in our community were found to be effective in hand-washing.

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Post-mortem histological and dissecting microscopic appearance of the proximal jejunum of Myanmar children

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A comparative morphological study of jejunal villi under the dissecting microscope and their histological changes under the light microscope was performed on 100 childhood necropsies from Yangon Children Hospital. They were from a-day-old neonate to twelve-year-old children. Four patterns of villi morphology were observed under the dissecting microscope: finger-like, tongue-like, leave-like and ridged villi. Histologically, inflammatory cells infiltration in the lamina propria and submucosa with vascular congestion of graded severity were observed. There is no correlation between villi morphology and histological changes. Tongue-like and leave-like villi could be regarded as normal for our children. Although histological examination of the post-mortem jejunal materials yields limited value, it could be of supportive or additive value if studied simultaneously with the dissecting microscopy.

INTRODUCTION

Gastroenteritis is a common clinical problem in children with high morbidity and mortality. A high incidence is found in children under five years of age. Sometimes, cases with septicemia of various causes also have gastrointestinal involvement. The value of examination of the biopsied gastrointestinal mucosa with dissecting microscope had been demonstrated [1-3]. The purpose of this paper is to compare the post-mortem dissecting microscopic and histological appearances of the proximal jejunum of Myanmar children and to correlate the morphological changes with the gastrointestinal problems.

MATERIALS AND METHODS

Jejunal specimens were obtained from 100 childhood necropsies from

Yangon Children's Hospital during May and August 1992. They were taken 50 cm away from the duodeno-jejunal junction within 12 hours of death. The specimens were divided into two pieces. One was allowed to stand in the running water for 24 hours and stained with ordinary ink and was examined under the dissecting microscope [3]. The appearances were recorded and photographed. The other piece of tissue was preserved in 10% buffered formalin and was embedded in paraffin, sectioned, stained with Haematoxylin and Eosin, using standard procedures for histological examination.

RESULTS

Cases were divided into 4 main groups depending upon their cause of death namely, gastroenteritis (G.E) (13), protein energy malnutrition (P.E.M.)

(16), septicemia (S) (38) and cases without apparent gastrointestinal problems (Non-GI) (33). Out of 100, only 18 had intact mucosa, 35 had partially detached mucosa and 47 had total detachment.

Appearances under the dissecting microscope

Four types of villi morphology were described [3] as follows:

1. Finger-like villi are narrow villi with parallel sides which may be short or long.
2. Tongue-like villi are broad-based villi with narrow tip.
3. Leaf-like villi are broad-based villi with rounded tip.
4. Ridged-villi are low flat villi.

These villi morphologies could be easily recognised under the dissecting microscope. It was the villous core rather than the surface mucosa that was observed. Tongue and leaf-like villi are seen in the majority (n=40) of the cases with GE, S and Non GI diseases, whereas ridged villi are predominantly seen in PEM (n=11). Only one case of finger-like villi was recorded in a-day-old neonate who died of septicemia.

Appearances under the light microscope

Tongue-like villi had a normal villous height and crypt depth and were lined by tall columnar epithelium with mild to moderate degree of both acute and chronic inflammatory infiltrates in the lamina propria and submucosa. These features were observed in cases with Non GI, GE and S cases. In S cases, in addition to above changes, vascular congestion was also noted.

The ridged villi that were seen mainly in PEM cases had a flat mucosa with marked inflammatory cells infiltration in the lamina propria and submucosa. Detachment of surface epithelium was not related to the interval between death of the patient and speci-

men collection.

DISCUSSION

Tongue and leaf-like villi seen in our cases were also observed by Chacko et al. [4] which were common in those children living in the tropical countries. According to Wood et al. [5] the broader villi were common in adults from the tropics than those from the temperate lands and were considered as evidences of tropical enteropathy. Changes in villi morphology were probably related to alteration in luminal environment, protein content of food, bacteria colonisation, viral and bacterial toxin content, dietary habit and worm infestation.

Study from India had also shown that changes from finger-like villi were common in fetuses, but many subjects had leaf-like villi within the first month of life without apparent GI diseases [4]. Changes were acquired through the passage of food contents rather than due to racial differences. However, Walker-Smith in 1972 from Australia found finger-like villi in neonates as well as in children over 5 years of age [6]. In our study, only one case of finger-like villi was recorded in a-day-old neonate presenting with septicemia.

Burman [7] had discovered leaf-like villi and ridged-villi with increased cellular infiltration in kwashiorkor cases. A similar comparative study in children suffering from malabsorption and chronic diarrhoea was observed having broader villi [8,9]. In our cases, ridged-villi with intense inflammatory cells infiltration were observed in most cases with P.E.M. We would like to conclude that dissecting microscopy and histology should be done simultaneously and would be of great value if we could further proceed our study in biopsy tissues with different disease entities, like malabsorption, chronic diarrhoea and childhood GI malignancies. The clue for

diagnosis of coeliac disease and tropical sprue could also be obtained.

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Role of amniotic fluid index assessment in postdate pregnancy

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The objective was to determine the significance of amniotic fluid volume with regard to pregnancy outcome in postdate pregnancy with the amniotic fluid index (AFI) assessment and to define the optimal cut-off level where adverse pregnancy outcome can occur. Ninety-eight pregnant women under 35 years of age with over 40 weeks' completed gestational age and no other complications admitted to Central Women's Hospital during the study period of 6 months beginning in April 1994 were selected for AFI assessment by using real time linear array B-scan. Pregnancy outcomes were correlated with AFI in cases of delivery within 3 days of AFI assessment. Analysis revealed an inverse relationship between AFI and incidence of adverse pregnancy outcome and the optimal cut-off point was AFI 8.0 cm with sensitivity, specificity, positive and negative predictive value of 72.8%, 71%, 42% and 90% respectively for adverse fetal outcome. In conclusion, amniotic fluid volume assessment was an effective method in predicting adverse pregnancy outcome and AFI of 8.0 cm was shown statistically to be an appropriate cut-off level to identify the fetus at risk.

INTRODUCTION

Amniotic fluid assessment is a tool for evaluation of fetal well-being. Historically, amniotic fluid volume (AFV) has been measured using amniocentesis and dye or isotope dilution techniques [1]. In an observational study, Crowley [2] described a simple, non quantitative ultrasound method to assess amniotic fluid volume. Currently, the three ultrasound techniques used to evaluate amniotic fluid volume include (1) subjective assessment, (2) maximum vertical pocket and (3) amniotic fluid index (AFI).

A review of three techniques shows that the amniotic fluid index (AFI) originally described by Phelan et al. [3] and Rutherford et al. [4] is valid, and the subjective technique and the AFI technique appear to have good inter and intraobserver variability. Moore [5] showed that the AFI technique is superior to

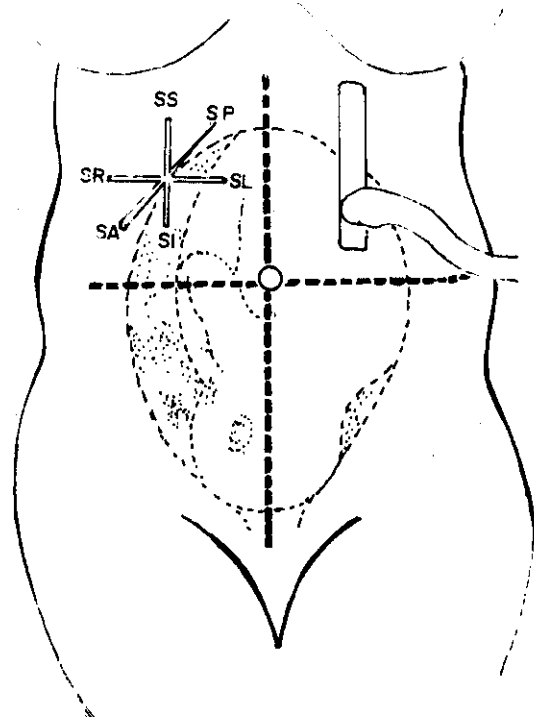


Figure. Ultrasonic four quadrant assessment of amniotic fluid volume

maximum vertical pocket measurement in screening for amniotic fluid abnormalities.

This study was therefore undertaken to determine the significance of amniotic fluid volume with regard to pregnancy outcome in postdate pregnancies.

Specific objectives were:

- (1) to compare the pregnancy outcomes in relation to levels of amniotic fluid index among the postdate pregnancies and
- (2) to define the optimal cutoff level where adverse pregnancy outcome can occur.

METHODOLOGY

Postdate pregnancies admitted to Central Women's Hospital during the study period of 6 months were selected for AFI assessment. The criteria for case selection were age below 35 years, singleton vertex pregnancy, more than 40 weeks completed gestational age and delivery within 3 days of amniotic fluid index assessment.

Those with known last menstrual period and previous regular cycles or ultrasound dating were included.

Patients with coexisting maternal disease or any other abnormal condition and complications of pregnancy were excluded because these conditions will prejudice the pregnancy outcomes.

By using real-time ultrasonography the amniotic fluid index measurement was performed as originally described by Phelan *et al.* [3].

The approach to assessing AFV began by first dividing the maternal abdomen in supine position into four quadrants. Using the umbilicus as one reference point, the uterus was divided into upper and lower halves. The linea nigra was used as the midline, with the uterus divided into right and left halves. The transducer head was

then placed on maternal abdomen along the longitudinal axis. With the transducer head perpendicular to the floor, the largest amniotic fluid pocket in each quadrant was identified. Then the vertical diameter of the largest pocket in each quadrant was measured. The numbers obtained from each quadrant were summed. The sum of four quadrant pocket depths was the amniotic fluid index (AFI), in centimeter, for each patient. When amniotic fluid index <10 cm was observed the average of three measurements was recorded to minimise the intraobserver variation encountered with relative oligohydramnios [6].

Pregnancy outcomes were assessed by fetal distress that required intervention, meconium passage, mode of delivery, Apgar score at 1 min, Ballard's scoring system [7] for gestational age, admission to Special Care Baby Unit (SCBU) for any reason and perinatal mortality.

RESULTS

During the study period, ninety-eight postdate pregnancies underwent ultrasonographic amniotic fluid index (AFI) assessment. The AF index measurements ranged from 3 to 16.5 cm in the study population. The mean AF index was 9.6 ± 2.95 cm. The Af index 0 to 5.0 cm (Group I) was found in 6 subjects (6.1%). The AF index 5.1 to 8.0 cm (Group II) was identified in 32 subjects (32.6%). Sixty subjects had AF index more than 8.0 cm (Group III) (61.2%). The pregnancy outcomes for 3 groups of patients are shown in Table 1.

Statistical significance was determined using 2x3 tables analysed by χ^2 analysis. Level of significance was set at the level of 0.05. Fetal distress occurred in 67% in Group I,

Table 1. Pregnancy outcomes in relation to AF index

Outcome	Amniotic fluid index						χ^2
	Group I		Group II		Group III		
	0-5.0 cm		5.1-8.0 cm		>8.0 cm		
	n=6		n=32		n=60		
	No.	%	No.	%	No.	%	
Intrapartum fetal distress	4	67.00	12	38.00	5	8.3	14.36
Meconium staining	4	67.00	12	38.00	5	8.3	14.36
Fetal distress that required intervention	4	67.00	9	28.12	2	3.33	15.64
Caesarean delivery	2	33.33	10	31.25	6	10.00	5.91
Low Apgar score <7 at 1 minute	1	16.66	10	31.25	1	1.66	15.06
Admission to Special Care Baby Unit (SCBU)	3	50.00	8	25.00	2	3.33	13.84
Ballard's maturity 42 weeks	3	50.00	12	38.00	7	11.66	8.35

38% ($\chi^2 = 14.36$, $p = <0.05$). The occurrence of meconium staining among the study population was 67% in Group I, 38% in Group II and 8.3% in Group III. AF index is significantly associated with meconium staining ($\chi^2 = 14.36$, $p = <0.05$). The requirement of intervention for fetal distress was 67% in Group I, 28% in Group II and 3.3% in Group III. AF index has significant association with requirement of intervention for fetal distress ($\chi^2 = 15.64$, $p = <0.05$). 3.33% of Group I, 31.25% of Group II and 10% of Group III were delivered by caesarean delivery. It was not significant statistically ($\chi^2 = 5.91$, $p = <0.05$). 16.6% of Group I, 31.25% of Group II and 1.66% of Group III had low Apgar scores <7 at 1 minute. There was significant association between low Apgar score and AF index level ($\chi^2 = 15.06$, $p = <0.05$). 50% of Group I, 25% of Group II and 3.33% of Group III required SCBU admission. Therefore, AF index is significantly associated with SCBU admission ($\chi^2 = 13.84$, $p = <0.05$). 50% of Group I, 38% of Group II and 11.66% of Group III had Ballard's maturity ≥ 42 weeks. Therefore, AF index had significant association with Ballard's maturity ($\chi^2 = 8.35$, $p = <0.05$).

As the study population are grouped based on their AF index levels, the

abnormal outcomes are compared between the group of subjects with AF index of ≤ 5.0 cm and all those with AF index above 5.0 cm. Similarly, the group with AF index of ≤ 8.0 cm is compared with those above 8.0 cm. The diagnostic values are computed. Comparison of these data indicates the higher negative predictive value using cut-off of AF index 8.0 cm. 90% of cases with AF index >8.0 cm will have normal outcomes with the false positive rate only 10%. Next to define the optimal cut-off level to predict adverse pregnancy outcomes, 3 levels were taken - AFI 8 cm as cut-off point A, AFI 7.5 cm as cut-off point B, and AFI 8.5 cm as cut-off point C. The pregnancy outcomes were compared between A + B (Table 2) and between A + C (Table 3). This comparison indicates the higher negative predictive value of AF index 8.0 cm among the different cut-off levels. Therefore, the optimal cut-off level to predict adverse pregnancy outcomes by AF index assessment is AF index of 8.0 cm for the study population.

DISCUSSION

The time required to perform a four-quadrant AF index is less than 5 minutes. If an ultrasound machine is currently available to an obstetric unit, there should be a relatively low

Table 2. Comparison of diagnostic values of cut-off 8.0 cm (A) and cut-off 7.5 cm (B)

Outcomes	Diagnostic values							
	Sensitivity		Specificity		Negative predictive value		Positive predictive value	
	A	B	A	B	A	B	A	B
Intrapartum fetal distress	76.19	61.90	71.43	81.82	91.67	88.73	42.10	48.15
Meconium staining	76.19	80.52	71.43	57.14	91.67	87.32	42.10	44.44
Intervention for fetal distress	86.67	78.31	70.00	60.00	96.66	91.54	34.21	33.33
1-minute Apgar score <7	91.66	75.00	68.60	79.00	98.33	95.77	28.94	33.33
SCBU admission	84.60	53.84	62.24	76.47	96.67	91.54	28.94	25.93
Ballard's maturity ≥ 42 weeks	68.18	50.00	69.74	78.94	88.34	84.50	39.47	40.74

Table 3. Comparison of diagnostic values of cut-off 8.0 cm (A) and cut-off 8.5 cm (C)

Outcomes	Diagnostic values							
	Sensitivity		Specificity		Negative predictive value		Positive predictive value	
	A	B	A	B	A	B	A	B
Intrapartum fetal distress	76.19	76.19	71.43	61.03	91.67	90.38	42.10	34.78
Meconium staining	76.19	76.19	71.43	61.03	91.67	90.38	42.10	34.78
Intervention for fetal distress	86.67	73.33	70.00	57.83	96.66	92.30	34.21	23.91
1-minute Apgar score <7	91.66	91.66	68.60	59.30	98.33	98.07	28.94	23.91
SCBU admission	84.60	76.92	62.24	57.64	96.67	94.23	28.94	21.73
Ballard's maturity ≥ 42 weeks	68.60	77.27	69.74	61.84	88.34	90.38	39.47	36.95

cost for this screening test. The interpretation of the results is also simple.

Although no one test can predict perinatal morbidity with 100% accuracy, any predictor of perinatal morbidity is clinically useful. The results of this study suggest that the AF index estimation provides clinically useful information.

The potentially high perinatal mortality and morbidity in postdate pregnancy make it difficult to justify a policy of non-intervention and selective

induction of labour without an appropriate method of fetal surveillance. The results of this study indicate that it can be used independently of any other biochemical or biophysical test to differentiate the normal from the abnormal pregnancy among the post-date population. At a time when non-selective obstetric intervention is being questioned, the application of AF index can accurately concentrate attention on the minority of post-date pregnancies where the fetus may be in danger.

As most of the centers in Myanmar do not use continuous FHR monitoring, the presence of a normal FHR tracing and AF index of >8 cm would be reassuring in such a setting. In contrast, an initially normal tracing with AF index <8 cm should suggest caution and consideration of continuous fetal monitoring because of the increased risk of perinatal morbidity.

Conclusion

The following conclusions can be drawn from the present study:

1. The AF index measurement is an acceptable alternative to current methods for determining AF volume. It is a rapid, simple and clinically valuable test for predicting the risk of adverse perinatal outcome.
2. An inverse relationship was observed between the amniotic fluid index and incidence of adverse pregnancy outcome. It deserves consideration as a screening tool for all postdate pregnancies.
3. High negative predictive value for various adverse pregnancy outcomes was observed at AF index of 8.0 cm. It was shown statistically to be an appropriate cut-off level to identify fetus at risk. The results of this study can be used as an additional parameter in management of postdate pregnancies.

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Comparison of Plasmodium falciparum infectivity to Anopheles dirus D using direct and indirect blood feeding methods

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Membrane feeding method (indirect blood feeding) using defibrinated blood has been established and was found to be as good as the direct feeding method with no significant differences in the production of oocysts and sporozoites in Anopheles dirus D ($p > 0.05$). However, successful feeding was greater in the direct feeding method (69.4%) when compared to the membrane feeding method (46.7%) ($p < 0.0001$). Based on 19 malaria patients who had >400 gametocytes per μ l of blood, the effect of antimalarial drugs on oocyst and sporozoite rates was also studied and it was found that mefloquine treated patients produced reduced infection and infectivity in An. dirus D than those who received antimalarial drugs other than mefloquine. Patients who had gametocytes and have been treated for causes other than malaria and who did not receive any antimalarial drugs were found to produce the highest infection and infectivity rates in An. dirus D ($p < 0.0001$).

INTRODUCTION

Transmission of malaria from the human host to anopheline vectors involves mosquito, parasite, human host and environment. Susceptibility to malaria parasites in anopheline species may differ and are generally influenced by several factors such as genetic constitution of mosquitoes, size of blood-meals, number of gametocytes as well as asexual parasitaemia in human host and seasonal variation in the infectivity of malaria patients to anophelines [1,2]. In addition, antimalarial drugs may also play an important role in mosquito infectivity after the mosquitoes had fed on antimalarial drug(s) treated patient's blood.

In order to conduct experimental infection studies with anopheline mosquitoes in the laboratory, we introduced the use of membrane feeding device utilising gametocyte positive blood which was defibrinated

immediately and this method was compared with direct blood feeding method. Along with the comparative infectivity study between the two methods, the effect of antimalarial drugs on mosquito infectivity was also studied and presented.

MATERIALS AND METHODS

Malaria patients

Male patients aged between 18-45 years who were admitted to No. 2 Military Hospital, with >400 gametocytes per μ l of blood and who volunteered were included in the study. Date of admission, age, Plasmodium species, status of antimalarial drugs used were recorded. Before the start of each experiment, an informed consent from each patient was obtained.

Based on the usage of antimalarial drug treatment by these patients, they were divided into three main

groups. Group I consisted of *An. dirus* D which fed on 8 patients who received mefloquine alone or in combination of with other antimalarial drugs (halofantrine, primaquine, chloroquine and artesunate). Group II consisted of *An. dirus* D which fed on 8 patients who received antimalarial drugs other than mefloquine (halofantrine, quinine, primaquine, quinine + tetracycline, chloroquine + primaquine, artesunate and artemether). Group III consisted of 3 patients with no antimalarial drug treatment.

Direct feeding on malaria patients

Three to five day-old 50-55 female *Anopheles dirus* D in each paper cup were allowed to bite on the forearm of each patient for 45 minutes. Mosquitoes were starved overnight before feeding.

Membrane feeding (indirect feeding)

From the same patient used in the direct feeding, 7 ml of blood was withdrawn intravenously using disposable syringe, defibrinated immediately and fed through an artificial membrane feeder for about an hour. Another lot of 50-55 female *An. dirus* with the same age was used. Artificial membrane feeding method was followed according to WHO manual [3] with some modifications. A parafilm ("M" lab film, American Can Co., Greenwich, CT, 06830) was used as membrane.

Defibrination of blood

Blood was placed in a conical flask containing small pieces of glass capillaries and the flask was rotated gently for about 10 minutes. Defibrinated blood was used within 5 hours.

Maintenance of mosquitoes

Blood fed females were sorted out and maintained in an insectary at 24°-29°C and 70%-80% relative humidity. Water only was supplied in the first 48 hours and later, 10% sugar solution daily.

Control mosquitoes

In every experiment, 50 females *An. dirus* D were fed on mice and kept as control. Daily mortality was recorded.

Oocysts and salivary gland sporozoites observation

On day 14 onwards up to day 20 both midguts and salivary glands were dissected and examined.

Data analysis

Data were analysed using the Epi-info and Stata software packages.

RESULTS

*Comparison between two types of blood feeding methods based on successful blood feeding, infection and infectivity of *An. dirus* D*

The results were based on mosquitoes infected from 10 gametocyte carriers. There were more blood fed females with the direct blood feeding than with the membrane feeding method. The results were significantly different ($\chi^2 = 58.2$, $df=1$, $p<0.0001$) (Table 1). However, no significant differences were observed in oocysts formation and salivary gland sporozoites formation in both methods (Table 2).

If one or both methods produce sporozoites in any experiment, then the presence of sporozoites in that particular experiment is regarded as "true positive" i.e. sporozoites will definitely be obtained regardless of the method used. In this way, in direct feeding, there were 5 true positives out of 7 experiments and in membrane feeding method, there were 6 true positives out of 7 experiments. Thus, membrane feeding method was as sensitive as the direct feeding method in the production of oocysts and sporozoites. The sensitivity for direct feeding and membrane feeding was 71.4% and 85.7% respectively.

Table 1. Comparison between two types of blood feeding methods by successful feeding of An. dirus D on 10 gametocyte carriers

Type of feeding	Number of <u>An. dirus</u>			% of successful feeding
	Having successful feeding	Without any feeding	Total	
Direct	376	166	542	69.4
Indirect (membrane feeding)	265	302	567	46.7
Total	641	468	1109	

$$\chi^2 = 58.2, df = 1, p < 0.0001$$

Table 2. Comparison between two types of blood feeding methods based on infected (oocyst rate) and infective An. dirus D (sporozoite rate) and based on 10 gametocyte carriers

(a) Oocyst rate

Type of feeding	Number of <u>An. dirus</u>			Oocyst rate
	With oocysts	Without any oocysts	Total	
Direct	96	188	284	33.8%
Indirect(membrane feeding)	50	143	193	25.9%
Total	146	331	477	

$$\chi^2 = 3.37, df = 1, p = 0.07$$

(b) Sporozoite rate

Type of feeding	Number of <u>An. dirus</u>			Sporozoite rate
	With sporozoites	Without any sporozoites	Total	
Direct	38	246	284	13.4%
Indirect (membrane feeding)	24	169	193	12.4%
Total	62	415	477	

$$\chi^2 = 0.09, df = 1, p = 0.76$$

The mean number of mosquitoes $\pm$ SD dissected in both direct and membrane feeding methods for each experiment was 47.7 ± 14.10 ; the mean number of mosquitoes with oocysts $\pm$ SD for each experiment was 14.6 ± 14.71 and the mean number of mosquitoes with sporozoites $\pm$ SD for each experiment was 6.2 ± 11.49 .

Effect of antimalarial drugs on mosquito infectivity

In group I, out of 8 patients, only 2 patients (25%) have blood which produced sporozoites in An. dirus D. In group II, 6 out of 8 patients (75%) and in group III, 3 out of 3 patients (100%) have blood which produced sporozoites in An. dirus D. A total of

Table 3. Comparison on the effect of antimalarial drugs on the infection (oocyst rate) and infectivity (sporozoite rate) of *An. dirus* D after feeding on three groups of 19 gametocyte carriers

(a) Oocyst rate

Group	Number of <i>An. dirus</i>			Oocyst rate
	With oocysts	Without any oocysts	Total	
I*	35	249	284	12.5%
II**	112	167	279	40.1%
III***	25	15	40	62.5%
Total	172	431	603	

$\chi^2 = 77.68$, $df = 2$, $p = <0.0001$

χ^2 for Linear trend: 77.20 (Odds ratio = 1, 4.77 and 11.86)

(b) Sporozoite rate

Group	Number of <i>An. dirus</i>			Sporozoite rate
	With sporozoites	Without any sporozoites	Total	
I*	2	282	284	0.7%
II**	61	218	279	21.9%
III***	17	23	40	42.5%
Total	80	523	603	

$\chi^2 = 86.58$, $df = 2$, $p = <0.0001$

χ^2 for Linear trend: 86.43 (Odds ratio = 1, 39.45 and 104.22)

* 8 patients treated with antimalarial drugs including mefloquine

** 8 patients treated with antimalarial drugs other than mefloquine

*** 3 patients not receiving antimalarial drugs

19 patients were obtained.

Lower infection and infectivity rates were obtained in mosquitoes from group I than in groups II and III. The results are significantly different (for oocysts: $\chi^2 = 77.68$, $df = 2$, $p < 0.0001$; for sporozoites: $\chi^2 = 86.58$, $df = 2$, $p < 0.0001$) (Table 3).

Days lapsed between antimalarial drug ingestion and blood feeding ranged between 3-34 days in group I and 1-10 days in group II.

Gametocyte counts and mosquito infection and infectivity

There was no correlation between gametocyte counts in the patients and percentage of mosquitoes with

either oocysts ($r=0.10$, $p>0.05$) or sporozoites ($r=0.16$, $p>0.05$) as determined by Kendall rank correlation. The mean $\pm$ SD gametocyte counts of these patients was 1306 ± 1234.5 per μ l of blood.

DISCUSSION

Most of the experimental infection studies in mosquitoes were conducted by feeding directly on the forearm of patients who volunteered [2,4,5], or feeding blood infected with cultured gametocytes through an artificial membrane feeder [6] or feeding mosquitoes with different natural isolates of malaria parasites from

patients through a water jacketed membrane feeder [7]. We introduced a membrane feeding method using gametocyte positive blood from patients which was defibrinated immediately and then fed on mosquitoes. Our method was quite as sensitive, reliable and applicable as the direct feeding method. The two methods showed no differences in mosquito infection and infectivity and therefore, the membrane feeding method (indirect feeding method) could be utilised as an alternative method to infect mosquitoes in the laboratory in future infection studies.

During the establishment of membrane feeding method, almost all the patients had already been treated with antimalarial drugs. Due to scarcity of gametocyte positive patients, all patients with gametocytes, no matter what antimalarial drugs had been used, had to be included in this study. Experiments were therefore, conducted with different batches of mosquitoes at different times, depending on the availability of gametocyte positive patients. Fortunately, all patients with gametocytes obtained were more than 400 gametocytes per μ l of blood. Sixteen were *Plasmodium falciparum* infected patients, 1 *P. vivax* and 2 were mixed infections.

Various antimalarial drugs including gametocidal drug primaquine had been used in No. 2 Military Hospital. Out of 19 patients studied, 4 primaquine treated patients were recorded in this study. The treatment doses and courses of primaquine were 15 mg OD x 14 days for *P. vivax* and 45 mg stat. for *P. falciparum* gametocytocidal activity. Positive results 5% (3/60) in mosquito salivary glands were obtained from mosquitoes fed on 2 primaquine treated patients' blood from group II. Days lapsed between primaquine treatment and mosquito blood feeding were 2 and 4 days for *P. falciparum* and *P. vivax* infections respectively. Although it

is a well-known fact that primaquine has gametocytocidal activity, *An. dirus* fed on 2 patients treated with this drug developed sporozoites. It must be noted that the terminal half life of chloroquine is 1-2 months, whereas primaquine is eliminated in hours [8]. Another factor to be considered is that various strains of *Plasmodium vivax* do show different susceptibilities to the action of primaquine in man [5]. We had no information on the vivax strain in one of the patients and the susceptibility status of the above 2 patients was unknown. In the inclusion criteria, because of the limited number of patients with gametocytes, patients were selected if they had gametocytes regardless of the antimalaria drugs taken prior to the experiments.

An unexpected finding with mefloquine was also obtained. For mosquitoes with oocysts, 94.3% (33/35), 45.5% (51/112) and 32.0% (8/25) failed to develop salivary gland sporozoites in group I, II and III respectively. The highest reduction rate was observed in mosquitoes which fed on mefloquine treated patients' blood. On the other hand, the fact that infection (oocyst rate) and infectivity (sporozoite rate) were highest in group III (those who received no antimalarial drugs) than in group I and II (those who received antimalarial drugs) indicated that the effect of antimalarial drug treatment plays an important role in the malaria parasite transmission cycle in mosquitoes. Although halofantrine and mefloquine are known to be blood schizontocides [10], reduction of infectivity may be due to sporontocidal activity of antimalarial drugs especially with mefloquine. Although the sample size is small (overall 19 patients and 8 patients for mefloquine) at least the above finding gives an indication of the reduction in infectivity due to mefloquine. Further studies need to be carried out for confirmation of

the sporontocidal activity of mefloquine.

For further infection studies in the laboratory, utilisation of patients' blood just prior to antimalarial drugs therapy is recommended for membrane feeding to obtain high sporozoite rates as well as for mass production of wild strain sporozoites for malaria vaccine trials.

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