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Potency assay of antivenom: Failure of Indian (serum institute) antivenom to neutralise Russell's viper (*Daboia russelli siamensis*) venom of Myanmar

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Neutralisation of biological properties of Russell's viper (*Daboia russelli siamensis*) venom of Tharyarwady by a monospecific antivenom of Myanmar (DN 86608B ex. 4/92) and a polyspecific antivenom of Serum Institute of India (Sii) (batch 109 exp. 4/96) was carried out according to WHO standard tests of neutralising activity. Neutralising potency of Myanmar antivenom was superior to the Indian antivenom and the latter required 16-126 times more antivenom than the former in neutralising haemorrhagic, necrotic, lethality, defibrinogenating and capillary permeability increasing activities of the venom. In immunodiffusion and immunoblotting experiments fewer bands were detected in both. It is concluded that the Indian antivenom (Sii) will be less effective in treating Russell's viper bite cases of Myanmar.

INTRODUCTION

Monospecific and bispecific (Russell's viper and *Naja kaouthia*) antivenoms manufactured by Myanmar Pharmaceutical Factory (MPF) are used for treating Russell's viper bite cases. Recently, polyspecific antivenom of Serum Institute of India (Sii) was used for treating Russell's viper bite cases in some township hospitals of Myanmar. However, its potency has not been tested. There were reports on poor clinical efficacy of some antivenoms when used in areas distant from the sources of immunising venom [1]. This report concerns the laboratory assessment of potency of the Indian (Sii) and MPF monospecific antivenom in neutralising Russell's viper venom of Myanmar.

MATERIALS AND METHODS

Neutralisation of biological properties of Russell's viper venom (RVV) (Tharyarwady) such as lethality, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary perme-

ability increasing activities with a monospecific antivenom (batch no. DN 86608B exp. 4/92) manufactured by MPF and a polyspecific antivenom of Serum Institute of India (Sii) (batch no. 109 exp. 4/96) was carried out according to WHO standard tests of neutralising activity [2]. Briefly, a fixed amount of venom was mixed with a variable dilution of antivenom, incubated at 37°C for 30 min and then injected into laboratory animals. The end point was taken as the minimum amount of antivenom required to completely neutralise the biological effects of venom. Immunodiffusion and immunoblotting using the two antivenoms were also carried out following standard methods. Details of the methods have already been described [3].

RESULTS

Results of neutralisation of biological properties of venom by the two antivenoms are shown in Table 1. Neutralising potency of the MPF antivenom was superior to the Indian

Table 1. Results of neutralisation tests of the Indian (Sii) (batch no. 109) and MPF antivenom (batch no. DN 86608B)

Source of antivenom	5LD50 iv 23.4 ug/mouse ul	10MCD 2.512 ug ul	3MHD 126.5 ug/rat ul	3MND 119.4 ug/rat ul	5MDD 12 ug/mouse ul	100MCPID 0.724 ug ul
MPF ASV DN 86608B	2.5	0.063	2.5	2.5	0.63	2.5
Indian ASV B. no. 109	100.0	100.0	80	80	80	40

LD50 iv = lethality

MCD = Minimum coagulant dose

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MDD = Minimum defibrinogenating dose

MCPID = Minimum capillary permeability increasing dose

antivenom. The latter required 16-40 times more antivenom than the former to neutralise haemorrhagic, necrotic, lethality and capillary permeability increasing activity and 126 times more antivenom to neutralise defibrinogenating activity of the venom. It failed to neutralise procoagulant activity of the venom *in vitro* test system (Table 1). Fewer bands were recognised by the Indian antivenom in immunodiffusion when tested against Daydaye, Kungyangon and Tharyarwady venoms (Figure 1). The Indian antivenom failed to pick up small molecular weight bands in immunoblotting experiment (Figure 2).

DISCUSSION

The current practice of potency assay of antivenom is mainly based on testing antilethality potency only. Here we have an opportunity to test the potency of the Indian and MPF antivenoms according to WHO standard tests of neutralising activity. According to the manufacturer's instructions, 1 ml of the MPF antivenom can neutralise 2 mg of RVV and that of the Indian is 0.6 mg for *Vipera russelli*. However, almost neat Indian antivenom was needed to neutralise all the biological properties of the

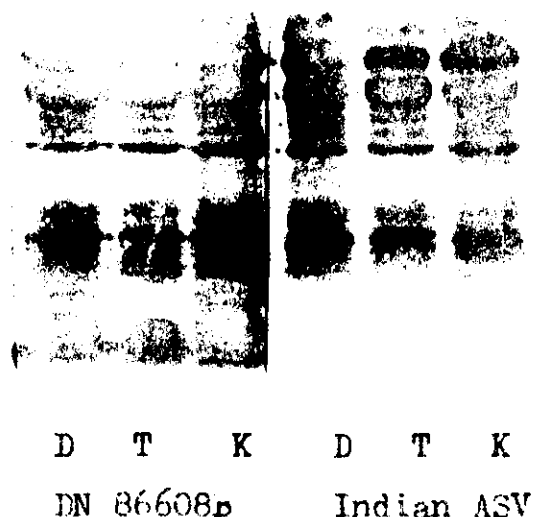


Fig. 1. A picture of immunodiffusion showing precipitin bands developed between Russell's viper venoms of (D) Daydaye, (K) Kungyangon and (I) Tharyarwady with MPF antivenom (Batch no. DN 86608B) and Indian Antivenom (batch no. 109)

venom indicating its poor efficacy. Geographical variation of venom composition of RVV [3-5] and subspecies differences may explain poor performance of the Indian antivenom.

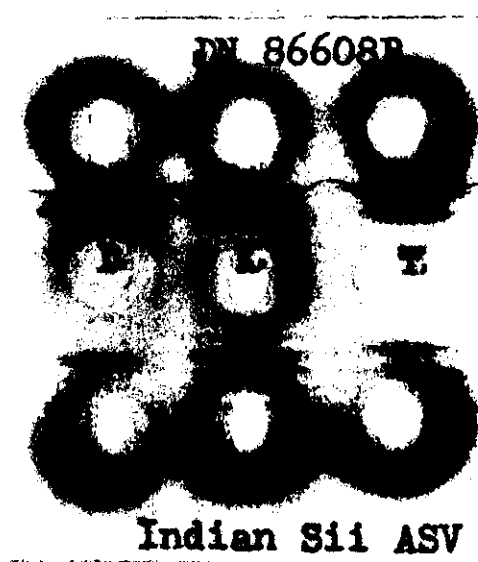


Fig. 2. A picture of immunoblot developed between Russell's viper venoms of Daydaye (D), Kungyangon (K) and Iharyarwady (I) and the Indian (Sii) (batch no. 109) and MPF (batch no. DN 866068) antivenoms

This observation was in agreement with the finding that poor clinical efficacy of some antivenoms was observed when used in areas distant from the sources of immunising venom [1]. Variation in neutralising potency of MPF antivenom against venoms from different localities of Myanmar has been observed [6]. It is concluded that the Indian (Sii) antivenom will be less effective in treating Russell's viper bite cases of Myanmar.

ACKNOWLEDGEMENTS

We are grateful to Dr. Than Aung, Divisional Health Director of Ayeyawady Division for providing the Indian antivenoms.

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Antibacterial properties of essential oils from six medicinal plants

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The essential oils obtained from six medicinal plants namely Vitex sp. (Kyaung-ban), Zingiber officinale (gyn), Cymbopogon citratus (sabalín), Curcuma longa (nanwin), Piper nigrum (ngayokekoun) and Coleus aromaticus (ziyarywethtu) were tested on 20 strains of bacteria. The bacteria comprised of 9 strains of Escherichia coli, 8 strains of Salmonella, and one strain each of Proteus morganii, Staphylococcus aureus, and Shigella sonnei. It was observed that Cymbopogon citratus and Coleus aromaticus were active on most bacteria tested.

INTRODUCTION

Plants and their products appear to have been used in the treatment of infectious diseases from very long time, even before the discovery of microorganisms. The systematic investigation of higher plants for antibiotic substances was studied by Osborn in 1943 [1] who screened 2300 species from 106 families against Staphylococcus aureus and Escherichia coli. Since then many investigators surveyed on higher plants from their respective countries to obtain new drugs for chemotherapy. The search is still carried out and recently, there were some reports on plants which possessed antimicrobial activity [2-6]. A screening programme which enabled the detection of antimicrobial activity in extracts of higher plants is important. Most of the parts of plants used in this study were edible and were available locally. They were also used in some traditional medicine for general purposes. The main aim is to know whether they possess the antibacterial activity.

MATERIALS AND METHODS

Plants used for general screening are

shown in Table 1. Screening of antibacterial activity of essential oil was performed by agar disc diffusion technique [7].

The bacterial strains tested were: E. coli (ATCC25922), E. coli (STLT/HI-3), E. coli (LT/536-2), E. coli (ST/568-3), E. coli (EAEC/N10/83), E. coli (EHEC/Sumon 10-1), E. coli (EPEC, 0125/WT493), E. coli (VTI/J116-3), E. coli (VT2/N272-3), Proteus morganii (Biken), Salmonella derby (WT683), S. enteritidis (WT685), S. krefeld (WT345), S. paratyphi B (WT684), S. seftenberg (WT347), S. stanley (WT346), S. typhimurium (WT593), S. typhi (Biken), Staphylococcus aureus (WS/1) and Shigella sonnei (WT113).

The essential oils were obtained by distilling the dried powder of plant materials with water in a volatile oil apparatus. Distillate was collected in a graduated tube and obtained as mixture with water after complete distillation. Water-oil mixture was partitioned with petroleum ether and essential oil was obtained by removing the solvent ether. The content of the essential oil is expressed as a percentage volume/weight on the basis of dried powder materials.

Table 1. Plants used to test for antibacterial activity (essential oils)

Sr.No.	Plants	Family	Parts used
1	<u>Coleus aromaticus</u> Benth. (Ziyarywethtu)	Libiatae (Lamiaceae)	leaf
2	<u>Curcuma longa</u> Koenig non L. (Nanwin)	Zingiberaceae	rhizome
3	<u>Cymbopogon citratus</u> (DC) Stapf. (Sabalin)	Gramineae	whole plant
4	<u>Piper nigrum</u> Linn. (Ngayokkoug)	Piperaceae	fruit
5	<u>Vitex</u> sp. (Kyaungban)	Verbenaceae	leaf
6	<u>Zingiber officinale</u> Roscoe. (Gyin)	Zingiberaceae	rhizome

RESULTS

The yield of essential oil of plants ranges from 0.04 to 3.3 per cent (V/W) (Table 2).

Table 2. Yield of essential oil of plants

Name of plants	Yield % (v/w)
<u>Vitex</u> sp. (Kyaungban)	1.0
<u>Zingiber officinale</u> (Gyin)	1.7
<u>Curcuma longa</u> (Nanwin)	3.3
<u>Piper nigrum</u> (Ngayokekaung)	1.7
<u>Cymbopogon citratus</u> (Sabalin)	0.8
<u>Coleus aromaticus</u> (Ziyaywethtu)	0.04

v/w = volume/weight

The essential oils of Cymbopogon citratus inhibited almost all the tested bacteria with the zone size ranges from 9 mm to 26 mm (Table 3). The plant Coleus aromaticus also inhibited 17 out of 20 bacteria tested and the zone size ranges from 14 to 19 mm. It was not active on 2 strains of Escherichia coli, [one strain of E. coli (STLT) and one strain of E. coli (VT1)] and one strain of Salmonella typhimurium.

It was also observed that there was a small zone of inhibition on E. coli (STLT), E. coli (EAEC) and E. coli (EHEC) by Zingiber officinale. The plant Curcuma longa also showed a small inhibition on Salmonella typhi

Table 3. Antibacterial activity of essential oils from plants tested (disc diam = 8 mm)

Bacteria strains	1	2	3	4	5	6
	Zone of inhibition (mm)					
1. <u>Escherichia coli</u>	-	-	13	-	-	19
<u>E. coli</u> (STLT)	-	9	13	-	-	-
<u>E. coli</u> (LT)	-	-	14	-	-	15
<u>E. coli</u> (ST)	-	-	14	-	-	14
<u>E. coli</u> (EAEC)	-	9	13	-	-	14
<u>E. coli</u> (EHEC)	-	9	13	-	10	14
<u>E. coli</u> (EPEC, O125)	-	-	26	-	-	14
<u>E. coli</u> (VT1)	-	-	9	-	-	-
<u>E. coli</u> (VT2)	-	-	13	-	9	14
2. <u>Proteus morganii</u>	-	-	12	-	-	15
3. <u>Salmonella derby</u>	-	-	12	-	-	15
<u>S. enteritidis</u>	-	-	9	-	-	14
<u>S. krefeld</u>	-	-	15	-	-	14
<u>S. paratyphi B</u>	-	-	11	-	-	15
<u>S. seftenberg</u>	-	-	13	-	-	15
<u>S. stanley</u>	-	-	11	-	-	15
<u>S. typhimurium</u>	-	-	12	-	-	-
<u>S. typhi</u>	-	-	19	9	10	15
4. <u>Staphylococcus aureus</u>	-	-	12	9	-	15
5. <u>Shigella sonnei</u>	-	-	13	-	-	15

- 1 = Vitex sp. (Kyaungban)
 2 = Zingiber officinale (Gyin)
 3 = Cymbopogon citratus (Sabalin)
 4 = Curcuma longa (Nanwin)
 5 = Piper nigrum (Ngayokekaung)
 6 = Coleus aromaticus (Ziyarywethtu)

and Staphylococcus aureus. Moreover, the plant Piper nigrum also showed a small inhibition on E. coli (EHEC),

E. coli (VT2) and S. typhi.

DISCUSSION

It was claimed by Trease and Evans [8] that very often the isolated constituents of a plant may not give the same biological effect as the plant extract itself, which might probably be due to the complex nature of the plant extract.

It was reported by Onawunmi and Ogunlana [9] that the essential oil of lemon grass Cymbopogon citratus possessed rapid bactericidal activity against gram negative and gram positive bacteria and the latter being more susceptible.

It was well known that several essential oils exerted a low degree of antibacterial action in vitro. Since the vapours of several essential oils were reported to exert an antimicrobial action by Maruzzella & Sicurella, [10], it is possible that the essential oils may have a therapeutic application as inhalants, supportive if not curative, in the treatment of pulmonary infections.

Low et al. [11] studied the antibacterial action of the essential oils of some Australian Myrtaceae with special references to the activity of chromatographic fractions of oil of Eucalyptus citriodora and showed that the oil exerted its antimicrobial activity through the synergistic action of citranellal and citronellol.

Gundidza et al. [12] also tested the antimicrobial activity of the essential oil from Eucalyptus maidenii and reported that it was effected on Klebsiella pneumoniae, Brocothrix thermosphacta, Citrobacter freundii, Serratia marcescens, Leuconostoc cremovis, Falvobacterium suaveolens, Clostridium sporogenes, Yersinia enterocolitica, Acinetobacter calcoaceticus, Brevibacterium linens, Aeromonas hydrophila and Bacillus subtilis.

It was also effective as an antifungal activity on Candida albicans, Aspergillus orchaceus, Aspergillus niger, Aspergillus parasiticus, Penicillium citrinum, Chaetomium and Aspergillus flavus.

In our study the bacteria strains tested were isolated from patients suffering from diarrhoea, dysentery or wound sepsis to know how the plants effect on the bacteria from the clinical sources. Thus, it was noted that the sensitivity varies within the strains by judging the size of zones of inhibition. As reported by Onawunmi and Ogunlana [9], the plant Cymbopogon citratus oil inhibited most of the tested bacteria in our study. In our medicinal use, the plant oils were used most extensively in therapeutic application as inhalant, supportive or as local application in various diseases or as preservative in some medicinal mixtures.

It is concluded that the essential oil could owe its antibacterial activity to that of its major component acting singly or in combination with other minor components present in it. Thus, it might be due to fact that the essential oil of plants varies in their activities on various strains of bacteria. The oil extracts from Cymbopogon citratus and Coleus aromaticus were found to be most promising and further clinical studies should be continued.

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The effect of antimalarials on reticulocyte count of patients with falciparum malaria

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One hundred and eleven adult patients who attended North Okkalapa General Hospital for uncomplicated falciparum malaria were chosen for the study. They were treated with dihydroartemisinin (Cetexin), quinine, artesunate tablets and artemether injection. Fifty nine adult male and female without malaria were chosen as controls. They were treated with sulfadoxine-pyrimethamine, quinine and artesunate. Thick and thin blood films were taken from the patients daily and were stained and counted for malaria parasites. Packed cell volume (PCV), WBC count and reticulocyte count were also done. Among the patients treated with drugs, there was a significant fall in the mean reticulocyte count (number concentration and number fraction) at day 3. But the reticulocytes were raised back to normal at days 7 and 14. There was no significant changes in the mean packed cell volume (PCV) and mean level of total WBC count of the patients in all 4 groups. Among normal controls treated with artesunate tablets, there was also a significant fall in the mean reticulocyte count at day 3 but the mean reticulocytes count was also raised back to normal at days 7 and 14. Among normal controls treated with quinine and sulfadoxine-pyrimethamine tablets, there was no significant fall in the mean reticulocyte count. And there was no significant changes in PCV and WBC count in all 3 groups of controls. The fall in reticulocyte counts can be due to part of the disease malaria as well as to the drugs including quinine and not singly to artemisinin therapy alone.

INTRODUCTION

There is no question about the value of artemisinin compounds on falciparum malaria. But the safety of the artemisinin group of drugs has been in question because the early preclinical and clinical studies were insufficient to satisfy international drug regulatory standards and requirements. This has necessitated repetition and augmentation of studies to validate the earlier findings. Acute toxicity studies in animals indicated that artemisinin and its derivatives have higher LD₅₀s and better chemotherapeutic indices than chloroquine. In animal studies cardiotoxicity was

described following administration of high doses of the drugs. Recent studies in rats and dogs have demonstrated neurotoxicity following administration of artemether or arteether at dosage regimens higher than those employed in humans. Following regimens of 10 mg/kg/day x 8 days, transient neurological defects occurred in dogs, while the dosage of 20 mg/kg/day produced a progressive neurological syndrome and death in most of the animals. The data suggest a dose-response relationship [1, 2].

But few adverse effects have been reported in patients monitored during

clinical trials of artemisinin or its derivatives. Transient first-degree heart block was documented in one patient administered with artesunate, and in the patients receiving artemether at the standard dosage. In some studies, up to 25% of volunteers receiving artesunate, artemether, or artemisinin have reported brief episodes of drug-induced fever. Tenesmus, abdominal pain and diarrhoea were reported in a minority (<6%) of patients administered with artemisinin suppositories. The toxicity following the use of artemisinin compounds was reviewed by Hien and WHO Informal Consultation Meeting [3, 1].

One of the possible complications following the use of artemisinin compounds is the fall in reticulocyte count. In volunteers, there was a dose-related decrease in reticulocyte counts for 4 days after artesunate or artemether at doses exceeding 4 mg/kg/per day for 3 days [4,5]. A low reticulocyte count was observed in 9 of 76 patients (11.8%) on day 3 and in 8 of 76 patients on day 7; it returned to normal by day 14. In patients using dihydroartemisinin tablets, a low reticulocyte count was observed in 29 of 162 patients (17.9%) on day 3 and in 3 of 162 patients (0.2%) on day 7 [6]. Hence this study was conducted.

Objective

To find out if there is a fall in reticulocyte level among adult falciparum malaria patients treated with different antimalarials commonly used in Myanmar.

PATIENTS AND METHODS

One hundred and eleven patients who attended North Okkalapa General Hospital for uncomplicated falciparum malaria were chosen for the study. They consisted of adult males and

females with Plasmodium falciparum infection, ages between 18 and 60 years with parasite density less than 200,000/cu mm and free from complications that can be attributed to malaria. They were treated with the following drugs.

- (1) Thirty one patients were treated with dihydroartemisinin (Cotexin tablets) prepared from Beijing COTEC New Technology Corp. Dose 120 mg at the first day followed by 60 mg per day for another six days. Total 480 mg given in seven days.
- (2) Thirty patients were treated with quinine tablets (Myanmar Pharmaceutical Factory) 600 mg three times per day for seven days.
- (3) Twenty five patients were treated with artesunate tablets (Guilin Pharmaceutical Works) 50 mg twice daily for five days.
- (4) Twenty five patients were treated with artemether injection (Kuming Pharmaceutical Factory). Dose 160 mg initially followed by 80 mg 12 hourly for another four doses.

Fifty nine adult male and female with no malaria (attendants of the patients) were included in the study as controls. To these (a) thirty were treated with sulfadoxine-pyrimethamine (Marcopharma Laboratories, Copenhagen, Denmark), three tablets (each containing sulfadoxine-pyrimethamine 500 mg and pyrimethamine 25 mg). (b) fifteen were treated with quinine tablets 600 mg three times a day for seven days as the patients and (c) fourteen were treated with artesunate tablets as the patients.

Patients with Plasmodium vivax, mixed malaria infection and patients with complications were not included in the study.

Thick and thin film were taken from the patients daily and were stained

and counted for malaria parasites according to WHO.

Packed cell volume (PCV), WBC count and reticulocyte count were done at days 0, 3, 7, and 14.

RESULTS

Among falciparum malaria patients treated with dihydroartemisinin (Cotecxin) tablets, artesunate tablet, artemether injection and quinine tablets, there was a transient fall in the mean reticulocyte counts (both number concentration and number fraction) at day 3. But the reticulocytes were raised back to normal at days 7 and 14 (Tables 1 & 2, Figs. 1 & 2).

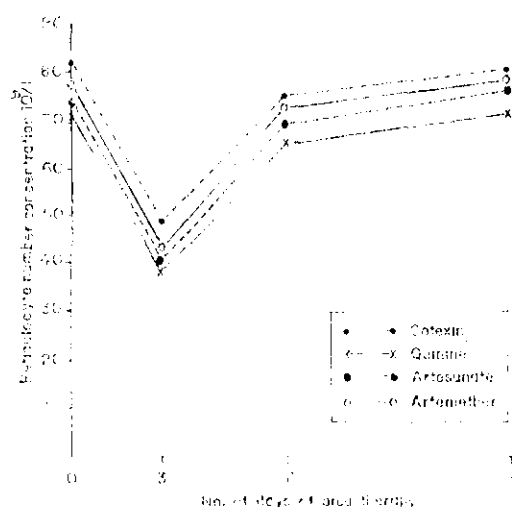


Fig. 1. Reticulocyte number concentration of patients treated with different antimalarials

There were also no significant changes in the mean packed cell volume (PCV) and mean level of total WBC count of the patients in all 4 groups.

Among normal controls treated with artesunate tablets, there was also a significant fall in the mean level of reticulocyte count (number concentra-

tion and number fraction) at day-3 but mean reticulocyte count was raised back to normal at days 7 and 14.

Among normal controls treated with quinine and sulfadoxine-pyrimethamine tablets, there was no significant fall in the mean reticulocyte count (number concentration and number fraction) at day 3.

There was also no significant changes in PCV and WBC count in all 3 groups of controls.

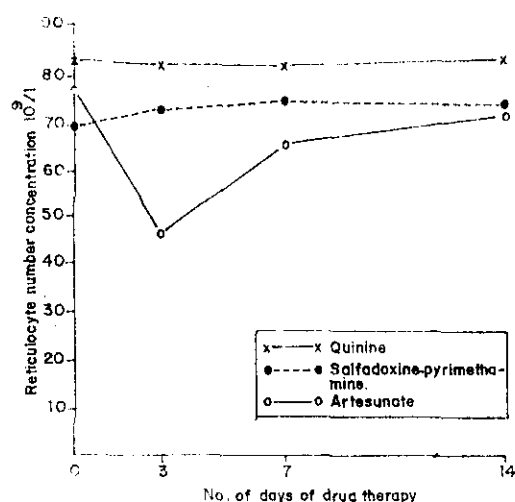


Fig. 2. Reticulocyte number concentration of normal controls treated with different antimalarials

DISCUSSION

In this study three artemisinin compounds, artemether injection, artesunate tablet and dihydroartemisinin tablets were tested for the study. They were extracted from some plant Qinghao or *Artemisia annua* L. These drugs have common action and proved to be effective to severe malaria and also for multi drug resistant falciparum malaria [1]. In this study, they also showed similar

Table 1. Reticulocyte count concentration of patients treated with different antimalarial drugs

Name of the drugs	Number of patients	Day-0		Day-3		Day-7		Day-14	
		mean	SD	mean	SD	mean	SD	mean	SD
Artesunate	25	79.70±17.97		44.24±13.11		74.38±16.95		80.57±17.27	
Artemether	25	76.57±14.68		41.21±10.29		70.44±12.41		77.22±13.85	
Quinine	30	73.68±25.08		38.28±13.79		66.06±22.92		72.47±24.24	
Cotecxin	31	84.28±16.62		49.33±14.56		77.23±13.91		82.74±13.36	

Difference between Day-0 and Day-3 sample is significant $P < .05$

Difference between Day-0 and Day-7 sample is not significant $P > .05$

Difference between Day-0 and Day -14 sample is not significant $P > .05$

Table 2. Reticulocyte count concentration of normal controls treated with different anti-malarial drugs

Name of the drugs	Number of patients	Day-0		Day-3		Day-7		Day-14	
		mean	SD	mean	SD	mean	SD	mean	SD
Artesunate*	14	76.44±16.88		47.64±12.82		63.84±14.18		74.89±16.63	
Quinine**	15	82.68±14.94		81.29±14.06		80.10±14.02		81.78±14.86	
Sulfadoxine** pyrimethamine	30	71.01±13.59		72.87±12.62		72.78±13.91		71.91±14.09	

*Difference between Day-0 and Day-3 sample is significant $P < .05$

*Difference between Day-0 and Day-7 sample is not significant $P > .05$

*Difference between Day-0 and Day-14 sample is not significant $P > .05$

**Difference between Day-0 and Day-3 sample is not significant $P > .05$

**Difference between Day-0 and Day-7 sample is not significant $P > .05$

**Difference between Day-0 and Day-14 sample is not significant $P > .05$

action on reticulocyte count. Hence, these three drugs will be discussed as a single drug group belonging to artemisinin.

In this study, a significantly lower level of reticulocyte was recorded among falciparum malaria patients treated with artemisinin compounds as well as among those treated with quinine. Hence the temporary fall in reticulocyte level of the patients can be due to both the drugs (artemisinin or quinine) or to part of the disease process.

Among the controls, who were free from malaria, the controls who were treated with quinine, chloroquine (sulphadoxine-pyrimethamine) had no fall in reticulocyte level. Only those

controls who were treated with artesunate tablets had temporary fall in reticulocyte level. Hence we can conclude that the temporary fall in reticulocyte count can be due to both artemisinin therapy as well as to the disease itself.

In this study, among the patients treated with dihydroartemisinin had a fall in reticulocyte level at day 3. But this reticulocyte level was raised back to normal at day 7, while the patients are on dihydroartemisinin. Hence although both the disease as well as artemisinin compounds can cause the fall in reticulocyte, the disease itself may be more responsible for the fall in reticulocytes.

In this study, although there was

temporary fall in reticulocyte level of the patients, there was no fall in WBC count, as well as in PCV level of the patients. Hence, we may conclude that though artemisinin compounds can cause temporary fall in reticulocyte, there was no major bone marrow depression and cannot be put into the list of drugs that can cause bone marrow depression. There may be only a temporary arrest of reticulocyte production.

In literature, over 2 millions malaria cases are estimated to have been treated with artemisinin or its derivatives. Patients with aplastic anaemia has not been recorded [1]. Hence, artemisinin-group of drug should not be included in the list of the drugs that can cause bone marrow depression in therapeutic dosage. The fall in reticulocyte level is only temporary. This point is not to hinder the utilization of artemisinin compounds.

Among patients with blood loss, anaemia and iron deficiency anaemia, adequate therapy is followed by an increase in reticulocyte count which usually commences at the 4th day [8]. In this study, the reticulocyte levels were normal at day 7.

Hence, we can conclude that treatment of the disease can cause the improvement in the production of reticulocytes.

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Vibrios from fishes and their antibiotic sensitivity pattern

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The people of our community eat fishes and prawns as cooked ones. But, sometimes, we eat preserved fishes (ie: after salting, souring or drying). There are no reports on the types of vibrios that are prevalent in the fishes and prawns of our food. The study was conducted in Yangon between September 1993 and June 1994. The fish samples including raw and preserved ones were bought from Pazundaung market in Yangon Division. A total of 144 samples were studied. *Vibrio* species were isolated from 24% (35 of 144 samples) of all fish samples. They were isolated more from sea than river fishes and also from raw than preserved samples of all fishes. *V. cholerae* non-01 were isolated more than *V. fluvialis* from all fish samples. All of the isolates were sensitive to tetracycline, chloramphenicol, ampicillin and septrin.

INTRODUCTION

Diarrhoea is one of the major health problems in Myanmar. Food and water have been considered to be the main vehicle of infection in gastrointestinal diseases. Contamination of food and water is of great importance from epidemiological point of view. Several species of vibrios have been isolated from our natural waters of Yangon [1].

In the USA, patients frequently had a history of consumption of molluscs, especially raw oysters, within the 48 hours preceding the onset of diarrhoea which is caused by *V. cholerae* non-01 [2]. *V. mimicus* gastroenteritis could be transmitted by raw oyster consumption. Other vibrio species in which raw oysters had been implicated as vehicles of transmission included non-01 *V. cholerae*, *V. vulnificus* and *V. hollisae* [3].

The people of our community eat fishes and prawns as cooked ones. But, sometimes, we eat preserved ones after salting, souring and drying

of these fishes.

There are no reports on the types of vibrios that are prevalent in the fishes of our food and thus it was studied. The antibiotics sensitivity pattern of these isolated vibrios was also examined.

MATERIALS AND METHODS

Sample collection

The study was conducted in Yangon between September 1993 and June 1994. The fish samples including raw and preserved ones were bought from Pazundaung market in Yangon Division. They were collected in sterile plastic bags. They were transported to the laboratory without ice and processed within one hour after arrival. A total of 144 samples were studied.

Bacteriological examination

Twenty five gm of fish samples including raw and preserved ones were weighed and inoculated into

225 ml of alkaline peptone water (APW). It was incubated at 37°C for 6 hours, subcultured onto thiosulphate citrate bile-salt sucrose (TCBS) media and these APW flasks were again incubated into 10 ml of APW tubes and above procedure was repeated. It was done for three successive days until suspected vibrio organisms were isolated on TCBS.

Then the isolated vibrio species were identified by using classical biochemical tests according to standard methods [4]. Serotyping with polyvalent *V. cholerae* O1 antisera on *V. cholerae* strains and with *V. cholerae* O139 antisera on *V. cholerae* non-O1 strains was done.

Antimicrobial susceptibility

The antibiotic sensitivity test was performed on Mueller-Hinton agar by agar disk diffusion method [5]. The antibiotic disks used were: ampicillin, chloramphenicol, sulphonamethoxazole and tetracycline.

RESULTS

Vibrio species were isolated from 24% (35 of 144 samples) of all fishes. It was isolated from 22% (21 of 96 samples) and 28% (14 of 48 samples) of river and sea fishes respectively. It was isolated more from sea fishes (Table 1).

It was isolated from 37% (17 of 46 samples) and 8% (4 of 50 samples) of raw and preserved samples of river fishes respectively and from 45% (11 of 24 samples) and 12% (3 of 24 samples) of raw and preserved samples of sea fishes respectively. Thus it was isolated more from raw samples than preserved samples of all fishes.

V. cholerae non-O1 and *V. fluvialis* were isolated as 74% and 26% of all fish samples from which vibrio species were isolated. *V. cholerae* non-O1

Table 1. *Vibrio* species isolation of all fish samples

Types of fish examined	No. of samples examined	No. of samples positive	Percent positive
All river fishes	96	21	22
All sea fishes	48	14	28
Raw river fishes	46	17	37
Preserved river fishes	50	4	8
Raw sea fishes	24	11	45
Preserved sea fishes	24	3	12

was isolated more than *V. cholerae* from all fish samples (Fig. 1).

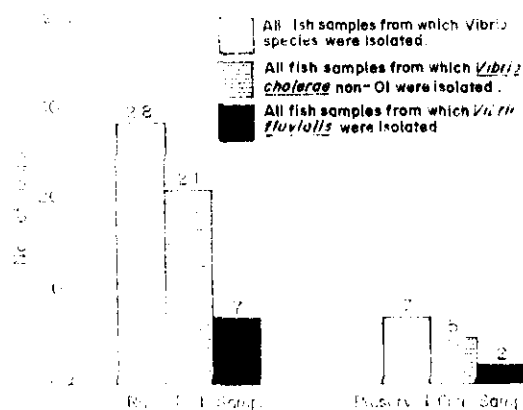


Fig. 1. Types of vibrio species isolated from all fish samples

All of the isolates were sensitive to tetracycline, chloramphenicol, ampicillin and septrin.

DISCUSSION

The role of environmental vibrios in the epidemiology of cholera is not clearly understood. Some workers

believe that the aquatic reservoir is probably the source for primary transmission of these infections [6].

In this study vibrio species were isolated more from sea fishes than river fishes. This finding is not surprising as vibrios can grow well and multiply in water which contains some amount of sodium chloride [7].

In preservation of fishes the people used salting, souring and drying processes in our community. Vibrios were isolated more from raw fish samples than preserved ones. This is because most of the vibrio species cannot grow well in large amount of salt concentration (salting process), at acidic pH (souring process) and in drying condition (drying process) [7]. Thus preserved fishes are safer than raw ones for consumption to avoid vibriosis.

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The anti-ulcerogenic activity of Plantago major Linn.

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Plantago major Linn. (Ahkyaw-baung-tahtaung; Mann-sote ywet; Htaung-Khaung-pwa; Sai-kyaw-gyi; Phar-kyaw-ywet) has long been used in official folk medicine for various purposes. Ethnobotanic field survey in Russia has shown that P. major (Plantaginaceae) can be used in the treatment of acid-peptic gastritis. So the plant was investigated for the anti-peptic ulcer activity by the previously standardized method using cimetidine. The aqueous extract of air-dried leaves of the plant was found to have a significant anti-ulcerogenic activity against aspirin-induced ulceration in in-vivo rat model ($p < 0.005$). The reduced ulcer severity was seen by the ulcer index of 8.4 ± 1.0 in the treated group when compared to the ulcer index of 20.6 ± 3.5 in the untreated group, showing a 59 per cent healing activity of the ulcers.

INTRODUCTION

An extract from P. major leaves had been reported to contain the polysaccharides or pectin which had been suggested for its therapeutic effectiveness in the treatment of peptic and duodenal ulcers [1-3]. Thus a large number of natural products has been shown to possess antiulcerogenic activity. The plant has long been used in official folk medicine for various purposes. Ethnobotanic field survey in Russia has shown that the plant has been used in the treatment of acid gastritis and peptic ulcers [4-6]. The purpose of this study was to confirm the claimed anti-ulcer activity of P. major grown in Myanmar.

MATERIALS AND METHOD

Preparation of the extract

The plant material used in this study was collected at the blooming stage from PyinOoLwin. The voucher specimen is deposited at the herbarium of the Pharmacology Research Division, Department of Medical Research,

Yangon, Myanmar. The habitat sketch of the plant (Fig. 1) had been botanically studied [7].

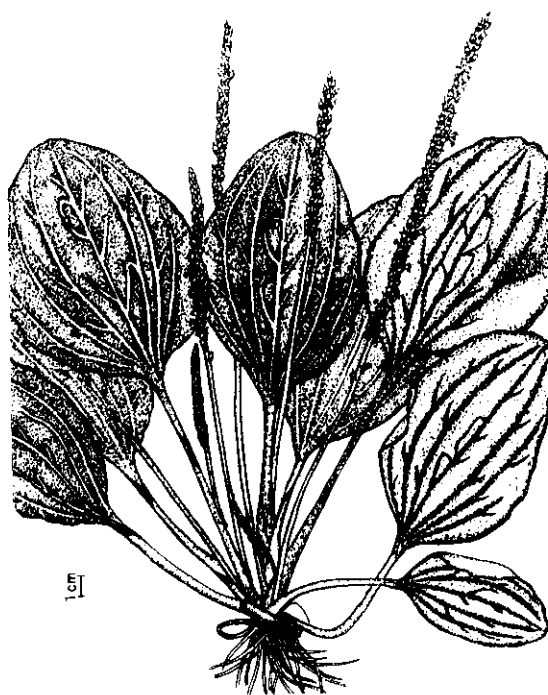


Fig. 1. Habitat sketch of Plantago major Linn. mature plant with inflorescence

The extract was prepared according to the method of Aye Than et al. [8]. Crude aqueous extracts were prepared by refluxing 50 g of air-dried leaf powder in a round-bottom flask in a boiling water bath using 500 ml of distilled water for about 6 hrs. It was filtered using cheese cloth and the filtrate was evaporated to dryness in a boiling water bath. The aqueous extract obtained was dissolved in 1% methyl cellulose before administration to respective animals.

Test for anti-ulcer activity

Normal white Wistar adult rats (150-200 g) of both sexes were used. They were fasted for 24 hr before the commencement of experiments. Water was allowed *ad libitum* to the animals. The adult albino rats were divided into groups of 5-10 each. Ulcers and erosions were induced in the stomachs of fasted rats by acetyl salicylic acid (aspirin) (Sigma Ltd., Poole, Dorset, London). In this study we have defined an ulcer as a penetration of the glandular mucosa. Preliminary experiments established that the oral administration of an aqueous aspirin suspension to rats previously fasted for 24 hr produced incidence of gastric ulceration.

The underlying principle was to screen the inhibitory action on peptic ulcer formation, using the method of Best et al. [9]. Following 24-hr fasting, each individual rat weighing (150-200 g) was administered orally with either the test aqueous extract (3 g/kg) or no drug (1% methyl cellulose 10 ml/kg) [10, 11]. Each treatment group comprised of 5-10 animals. One hour later, the ulcerogenic agent acetylsalicylic acid (aspirin) was administered orally to every animal, 300 mg/kg. At the end of 3-hr period of drug interaction, each animal was sacrificed by initial ether inhalation followed by cervical venes-section. A mid-line abdominal incision was made to extricate the stomach,

which was cut open along the greater curvature and the mucosa was examined on a visual basis and under a dissecting microscope. The number of ulcers and the severity and extent of gastric damage were scored, the latter marked from 1 + (2 mm); 2 + (3 mm) and 3 + (4 mm) respectively. The ulcer index (UI) was determined for each group by using the following formula.

$$UI = \frac{A_u}{A_t} + U + S$$

where A_u = percentage of animals showing ulcer

A_t = number of animals tested

U = average number of ulcer per stomach

S = average severity of ulcers

Chemical analysis of the aqueous extract

Qualitative identification tests of the chemical constituents present in the tested sample were conducted according to the standard procedure as stated in Trease and Evans [12], Pharmacopoeia [13] and Ikhiri et al. [14].

Evaluation of acute toxicity

Toxicity of the aqueous extract was evaluated by the method of Litchfield and Wilcoxon [15]. An attempt to determine the media lethal dose (LD₅₀) of each extract was made using 3 groups of 10 mice each. Group 1, 2 and 3 were treated orally with 1, 2 and 3 g/kg respectively. The animals were observed for 7 days.

RESULTS

Qualitative test for the aqueous extract

The yield of water soluble extract from the dried leaf powder was 13.3 g/100 g. Qualitative identification tests on the water soluble extract

showed that alkaloid, saponin and triterpine steroid were absent whereas amino acid, flavonoid, tannins and glycoside were present in the aqueous extract of the plant.

Toxicity determination (LD₅₀)

It was found not toxic up to 3 gm/kg orally in each acute toxicity study. The median lethal dose (LD₅₀) for each aqueous extract on mice was greater than 3 g/kg.

Effect of aqueous extract on aspirin-induced ulcer formation in rats

The aqueous extract showed anti-ulcer property. It markedly reduced the formation of gastric ulcers resulting in ulcer index of 8.4 ± 1.0 , compared to 20.6 ± 3.5 of the negative control group whereas ulcer severity of the control group was 35.2 ± 13.5 and that of the test group was 5 ± 1.9 as shown in Fig. 2. Therefore it was noted that the percentage inhibition of ulcer index was 59 per cent and percentage inhibition for ulcer severity was 86 per cent.

DISCUSSION

The results presented in this paper demonstrate that aqueous extract of dried powdered leaves of *P. major* has a healing effect on the ulcers induced by aspirin in rats. The results obtained were comparable to those seen with the clinically established anti-ulcer drug cimetidine [16]. It was observed that the reference drug markedly reduced the formation of gastric ulcers resulting in ulcer index of 13.0 and ulcer severity of 3 ± 1 . The percentage inhibition of ulcer index was 37 per cent and percentage inhibition for ulcer severity was 92 per cent [16]. The fact that the aqueous extract dosage employed were over 30 times higher due to the fact that they are basically nonrefined. Regarding the

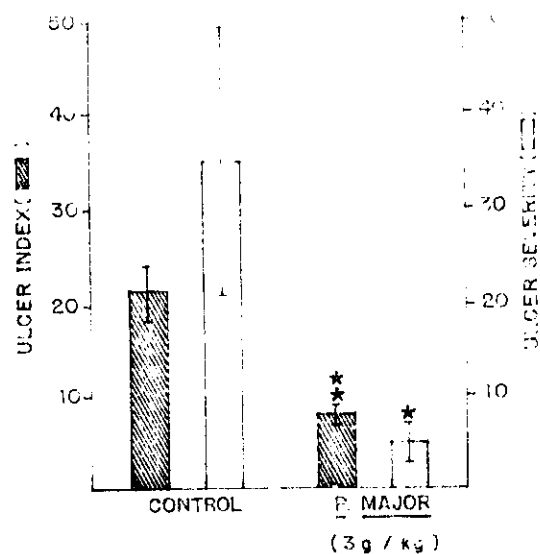


Fig. 2. Oral anti-ulcer activity of *Plantago major* aqueous extract on aspirin-induced peptic ulcer in rats. Each column represents the mean $\pm$ SEM response of 5 animals per group (5 groups), which were administered with single dose of aqueous extract or no drug.

* $p < 0.05$

* $p < 0.005$ (Significantly different from that of control group tested)

average severity of ulcer, there were significant reduction in ulcer severity when compared to that of the control group. From the present study it would be difficult to comment on the mechanism of anti-ulcerogenic action and the active principle of this aqueous extract of the plant. The mechanism underlying this effect is not evident from the data available. Further investigations are needed, such as studying its effect on serum calcium, gastric acid secretion and intestinal motility in the present ulcer model as well as in another experimentally induced ulcer model.

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**Drug resistance pattern in sputum positive treatment failure cases
admitted to Aung San TB Hospital**

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One hundred and thirty five sputum positive failure patients admitted to Aung San Tuberculosis Hospital during October 1994 to January 1995 were studied for drug resistance pattern, probable associated clinical, social, demographic factors and sputum negative rate on retreatment. Ninety three cases were able to proceed resistance testing to isoniazid, rifampicin and streptomycin. The result revealed resistant to one drug (31.2%); resistant to 2 drugs (37.7%) and resistant to 3 drugs (15.1%). Multidrug resistant cases were 22 (23.7%). No significant association was observed between drug resistance and cavity lung lesions, number of drugs previously exposed. An analysis of risk factor for drug resistance revealed no significant differences between age, sex, race, ethnic groups, area of localisation. Alcoholism, tobacco abuse, drug users, overcrowding in living conditions were common demographic characteristics. Inappropriate administration of drugs is commonly observed in patients. Sputum negative rate by short-course chemotherapy and 12-month regimen at the end of initial 3 months were 54.7% and 38% respectively.

INTRODUCTION

Tuberculosis is still a major health problem of worldwide dimensions, even more than a century after Robert Koch's discovery of TB bacilli.

Myanmar is one of the high risk countries for tuberculosis. It stands as a second prioritised health problem in 4th National Health Plan and also a second leading cause of death in 1990. Tuberculosis accounted for 9.4% of all death in 1991.

Nowadays resistance to major drugs used for tuberculosis also becomes a growing problem in developing world especially in middle income countries, including Myanmar. Drug resistance has direct consequences not only for individual patient but also for tuberculosis control in general. Patients

with untreatable antituberculosis drug resistant disease become the vector for additional generation of drug resistant tuberculosis, spreading resistant infection primarily to their children.

Several reasons have been cited for the failure of tuberculosis treatment, achieved among them being drug resistance of TB bacilli. Noncompliance, a notoriously worldwide problem magnifies their failure of treatment. Acquired drug resistance rate was 41% in US (1980) and 57.6% in Saudi Arabia (1988) [1]. It was reported to be increased in these patients with cavitary lesions in chest radiography, extrapulmonary tuberculosis among children, drug abuse, homeless, divorced and unemployed [2,3].

We present here our experience in treating sputum positive failure cases to determine the rate and pattern of drug resistance among them and to find out the possible risk factors to become a failure case.

PATIENTS AND METHODS

One thirty five patients with sputum positive failure, aged above 10 years admitted to Aung San TB Hospital during 1st October 1994 to 30th January 1995 were studied.

Relevant history taking and physical examination were carried out on all patients. Previous history of anti-tuberculosis treatment, compliance of treatment, social factors like alcohol drinking, drug abuse, educational status, marital status, nature of jobs and reasons for nonadherence to treatment were also included. All these cases were taken chest X ray to see the nature and extent of lesions and cavitations etc.

Ziehl Neelsen technique was used for staining in direct sputum smear examinations. Culture for AFB was done on 3% Ogawa media at 37°C for 6-8 weeks and watch for pigment formation and growth. Positive cultures were proceeded for sensitivity tests to streptomycin, isoniazid and rifampicin. Subcultures were done on 1% Ogawa drug media at 37°C using absolute concentration method and wait for one month for the results [4].

The results obtained were reported as follows:-

Sensitive

When growth is seen on drug-free medium, with no visible growth on all drug containing media.

Resistant

When growth is seen on all drug-free and drug containing media.

Relatively sensitive

When small amount of growth is seen on lowest concentration of drug media of 0.1 meg/ml isoniazid or 20 meg/ml streptomycin but no growth on drug media of control strain.

Relatively resistant

When equal or almost equal growth with drug-free media is seen on drug containing media of 0.1 meg/ml isoniazid or 20 meg/ml streptomycin or 10 meg/ml of rifampicin.

Overall report

Relatively sensitive and sensitive cases will be reported as sensitive. Relatively resistant and resistant cases will be reported as resistant [4].

RESULTS

One hundred and thirty five cases of sputum positive failure cases were studied. Positive cultures for *Mycobacterium tuberculosis* were isolated in 105 patients. Resistance testing to isoniazid, streptomycin and rifampicin were carried out in 93 cases. Sensitivity result revealed resistant to one drug in 29 (31.2%) cases, 2 drugs in 35 (37.7%) cases, 3 drugs in 14 (15.1%) cases and sensitive to all drugs in 15 (16.1%) cases (Fig. 1). Multi drug resistance was found in 22 (23.7%) cases.

Seventy nine (91%) cases among drug resistant and 14 (93%) cases among drug sensitive patients were found to have cavitations in chest radiography.

The relationship between development of drug resistance and number of drugs previously exposed, shown in Table 1 revealed no association ($P > 0.1$).

Seventy one per cent of the patients gave past history of non adherence to treatment, of which 66% and 81%

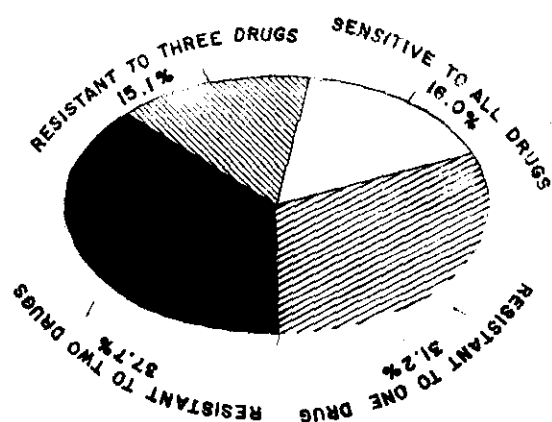


Fig. 1. Resistance testing to isoniazid, streptomycin and rifampicin in 93 patients

Table 1. Association between development of drug resistance and previous exposure to drugs

No. of drugs exposed	No. of positive culture	Sensitive to all 3 drugs (H,R,S)	No. of resistant cases
3 drugs + less	42	8	34 (80.95%)
4 drugs	18	1	17 (94.4%)
5 drugs + more	33	6	27 (81.8%)
Total no. of patients	93	15	78

$P > 0.1$, Not significant

Evaluation of data was performed by Chi Square test

H = Isoniazid; R = Rifampicin; S = Streptomycin

were from rifampicin and non rifampicin containing regimens respectively. Reasons for non adherence were multiple and are shown in Table 2.

Eighty-three per cent of the cases with rifampicin containing treatment had taken medicine after meals; 4

Table 2. Reasons for non-adherence to treatment

Reasons	No. of cases (%)
1. Economic reasons	36 (37.5%)
2. Feeling well	32 (33.33%)
3. Inconvenience to collect drugs	28 (29.16%)
4. Forgetfulness	7 (7.29%)
5. Side effects (Giddiness, pain due to injection etc.)	7 (7.29%)
6. Did not like to take drugs	5 (5.2%)
7. Did not understand the importance of drugs	4 (4.16%)

(.5%) cases had taken one drug at a time, 7 (7.9%) cases had taken in divided dose and 2 (2.3%) took drug in low dosage.

The association between failure cases and social factors revealed alcohol abuse in 68 (50%) cases; 3 (2.2%) were drug users; 120 (88.8%) were educated up to primary, middle or high school and illiterate consisted only of 3%. 71 (53%) cases have family income of 3000 kyats per month.

Out of 135 failure cases, 53 patients were given short-course chemotherapy for initial intensive 3 months. Sputum conversion was obtained in 29 (55%) patients by short-course chemotherapy compared to 38% by 12-month regimen. Case fatality rate was 17.8%.

DISCUSSION

Tuberculosis is still a major health problem of worldwide dimensions and remains the biggest killer in the world as a single pathogen even more than 40 years after the introduction of anti-tuberculosis chemotherapy.

Acquired drug resistance rate was

84% in sputum positive failure cases admitted to Aung San TB Hospital which was comparable to 82% among out-patients attended to Union Tuberculosis Institute [5]. This arises usually as a result of non adherence to recommended regimens or faulty prescribing. Most of the reasons for non adherence to treatment according to our study (Table 1) are correctable by repeated health education and reassurance given to the patients.

Nowadays, resistance to major drugs used for tuberculosis becomes a growing problem in developing world especially in middle income countries, including Myanmar. Drug resistance has direct consequences not only for individual patient but also for tuberculosis control in general. Multidrug resistance rate according to our study was 23.7%. There is no association between development of drug resistance and number of drugs previously exposed (Table 1). On the contrary, multi drug resistance was reported to be higher in patients who had previously received therapy by others [6]. Many such patients may have a history of non compliance. Age, sex, area of residence localisation of tuberculosis according to our study were found to have no association with development of drug resistance. This was in agreement with others [6,7]. For the achievement of cure, not only the regularity of treatment by pharmacological and biological factors like taking all drugs in an adequate regimen, dosage and empty stomach are also important. Wrong habit in taking drugs as revealed from our study may be one of the factors for becoming nonadherent to treatment.

From our study, sputum conversion rate at the end of 3 months by directly observed short-course chemotherapy (i.e 54.7%) was found to be higher as compared to 38% by intermittent supervised 12-month regimen. Preliminary results from a United

States Public Health Service trial indicated a relapse rate at 6-month follow-up among outpatients who took isoniazid and rifampicin daily for 6 months from initial treatment of sputum positive pulmonary tuberculosis [8]. This is a higher relapse rate than from the same regimen in East Africa [8] and illustrates that the efficacy of a regimen will vary with the program condition and populations in which it is used. Since the sputum conversion rate resulting from short-course chemotherapy was found to give a better response as compared to 12-month supervised regimen at the end of 3 months, the former regimen should be recommended for routinely treatment and the latter is best reserved for patients who are likely to be noncompliant if left unsupervised. This will save the cost involved in supervising the regimen, and also the inconvenience for the patients to make several weekly trips to the clinics.

Case fatality of 17.8% from our study is almost the same as that of WHO estimation (i.e 15%). It was reported that the presence of AIDS, increasing age (among patients without documented AIDS) and the presence of drug resistance (among patients with AIDS) were the most important predictions of high mortality [6]. According to him case fatality rate was 91% among patients with AIDS who were infected with organism resistant to both isoniazid and rifampicin.

In conclusion, it is very important to give adequate treatment (adequate in regimen, dosage, and duration of treatment) and repeated health education in new cases to prevent emergence of drugs resistant tuberculosis in the community and to achieve the goal of 85% cure rate by the year 2000.

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**Giardia lamblia infection in children attending diarrhoeal unit of
Yangon Children Hospital**

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A hospital-based study was conducted in Yangon from June to November 1994 to examine the importance of a protozoal pathogen Giardia lamblia as one of the causal organisms in acute diarrhoeal disease in children. This study was also carried out to observe the effectiveness of metronidazole on them. The stool samples were collected from diarrhoeal unit of Yangon Children Hospital. A total of 308 stool samples were examined. Giardia lamblia cysts (or) trophozoites were examined under ordinary microscope. If they were present in the stool, the patient was treated with metronidazole and the stool was rechecked again. The results were discussed in this study.

INTRODUCTION

Acute diarrhoeal disease is an important cause of childhood morbidity and mortality in developing countries. Bacterial enteropathogens such as *Shigella* spp., *Salmonella* spp. and enteroinvasive *Escherichia coli* are important etiological agents of invasive diarrhoea in children [1]. There is little information on the percentage of protozoa, particularly Giardia lamblia as a cause of acute diarrhoea in children in our community. Therefore a hospital-based study was conducted to examine the importance of the protozoal pathogen Giardia lamblia as a cause of acute diarrhoeal disease in children.

A number of effective agents including quinacrine, nitromidazoles, metronidazole, tinidazole and furazolidone are available for treatment of patients with giardiasis [2].

Thus the objective of this study was to investigate the infection rate of intestinal Giardia lamblia among diarrhoea patients attending diarrhoeal

unit of Yangon Children Hospital. This study was also carried out to observe the effectiveness of metronidazole on these patients.

MATERIALS AND METHODS

Stool collection

The study was conducted in Yangon from June to November 1994. The stool samples were collected from diarrhoeal unit of Yangon Children Hospital. They were collected on every week day, throughout the study. The fresh stool samples were collected in sterile plastic bags. They were recorded in a separate form. They were transported to the laboratory without ice, and processed within one hour after arrival. A total of 308 stool samples were examined.

Selection of subjects

The subjects were selected from the children of any age and sex with acute diarrhoea who came to diarrhoeal unit, Yangon Children Hospital to get the treatment. The patients

who did not respond to the treatment from this unit and admitted to ward of Yangon Children Hospital were also included. However, the patients who were referred to Infectious Diseases Hospital, Yangon were excluded. The consent from their parents (or) guardians were also asked to get the rectal swabs from them. If the patients did not respond to the treatment with metronidazole they would be stopped from the study.

Microscopic examination

Using a sterile Pasteur pipette a small portion of stool was taken and mixed with a drop of normal saline solution and another with 2% Lugol's iodine solution. The faecal smears were then made and the cover-slips were placed over these smears. The preparations were examined under ordinary microscope.

Treatment given to Giardia lamblia positive cases

When *Giardia lamblia* trophozoite (or) cyst was seen under microscope, this case was informed to pediatrician of diarrhoeal unit of Yangon Children Hospital. The patient was treated with metronidazole (Myanmar Pharmaceutical Factory) by pediatrician concerned with recommended dosage.

Response of metronidazole therapy

The stool samples were examined for presence of *Giardia lamblia* trophozoite or cyst on day 0 and day 7 of the treatment and day 14 also. The clinical features of the patients were also recorded daily.

RESULTS

The stool samples were collected from the diarrhoeal unit of Yangon Children Hospital, from June to November 1994. Altogether 308 stool samples were collected from patients having loose motions. *Giardia lamblia*

trophozoites (or) cysts were present in 30 stool samples among the diarrhoea cases. The intestinal giardiasis occurred in all ages and most commonly in the age group of 1.2 year (Fig 1).

All of the 30 intestinal giardiasis cases were cured by metronidazole except one case. He was treated again with tinidazole and was cured. Thus in this study 96.7% of the cases were sensitive to metronidazole therapy. One out of 30 cases (3.3%) showed unresponsiveness to the metronidazole therapy.

DISCUSSION

Giardiasis is the most common protozoal infection of the intestinal tract and is found world wide especially in temperate and tropical locations [3]. Prevalence varies between 2% and 5% in the industrialized world and up to 20-30% in the developing world. *Giardia lamblia* can cause acute and chronic diarrhoea with intestinal malabsorption and may be responsible for retarded growth and development in children [4]. In our study it was positive in 30 cases (nearly about 10%) of 308 cases of acute diarrhoea attending diarrhoeal unit of Yangon Children Hospital. Giardiasis is most common in the youngest age group of 2-4 year old (Fig. 1).

In a study of Southern India, giardiasis was reported to be uncommon under 6 months (2.1%), but occurred in 8-10% of all other age groups [5]. The rate of *Giardia* infection progressively increased in these age groups, because they were not yet toilet trained and hence were at highest risk for contaminative faecal transmission [6].

In this study, 96.7% of the cases were effective by the treatment with metronidazole. Thus metronidazole is still the drug of choice for treatment of giardiasis in our community. One out of 30 cases did not respond to

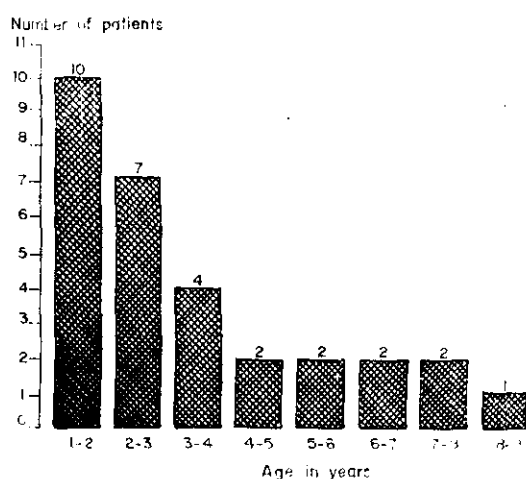


Fig. 1. The age distribution of giardiasis among children of Diarrhoeal Unit of Yangon Children Hospital (June to November 1994)

metronidazole treatment. Since blood levels of metronidazole were not measured in the cases, it is not known whether the unresponsiveness may be due to either drug failure or true resistance to the drug.

Metronidazole has a broad spectrum activity against anaerobic bacteria and protozoans. In the 30 cases of acute diarrhoea *G. lamblia* cysts were found in fresh stool samples. The patients were treated with metronidazole alone and when stool samples were checked again *G. lamblia* cyst was negative and these cases had also clinical improvement. Thus in these cases *G. lamblia* may be the possible causal agent of acute diarrhoea. Further studies are needed to

do on culturing of these positive stool samples and also immunodiagnostic methods on giardiasis in our community.

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Breath methane excretion status in children with lactose malabsorption

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We carried out a study to determine breath methane excretion status in relation to lactose absorption in children. The lactose absorption status of 125 children aged 1 to 12 years was defined using the hydrogen breath test using lactose test meal (2 g/kg, maximum 50 g). Lactose malabsorption was detected in 16.7% of children aged 1-2.9 years, the prevalence increasing with age to 39.5% of 3-5.9 year-olds and 88.5% of 6-8.9 years and reaching 91.7% of 9-11.9 year-old children. We measured breath methane excretion status in 69 children (56 lactose-malabsorbers and 13 lactose-absorbers) and found significantly higher breath methane excretion in lactose-malabsorbers.

INTRODUCTION

Lactase deficiency with resultant lactose malabsorption is prevalent in many parts of the world, especially in populations of non-Caucasian origin and studies from Asia reported a high prevalence of lactose malabsorption among children and adults, with the condition having an early onset [1-6].

In both clinical and field conditions breath hydrogen analysis has been widely used in evaluating lactose absorption. It is as accurate as other tolerance tests in identifying sugar malabsorption and has an advantage of being non-invasive [7]. Recently, an increase in breath methane excretion after ingesting lactose has been shown in children with lactose intolerance [8]. Methanogenic bacteria produced methane in the left colon by reduction of hydrogen and carbon dioxide. An increased carbohydrate load may provide increased hydrogen and carbon dioxide production and this increases substrate for methanogenic bacteria. The present study was carried out to investigate the prevalence of lactose malabsorption in Myanmar children

and to study the methane excretion in relation to lactose absorption status.

SUBJECTS AND METHODS

The study was conducted on 125 children, 65 boys and 60 girls, between one and 12 years of age from North Okkalapa Township in Yangon. All were apparently healthy, had not taken any antibiotics and had no gastrointestinal diseases during the previous two weeks. The parents of the children were informed of the nature of the study and informed consents were taken.

After an overnight fast, each child was given lactose (2 g/kg, maximum 50 g) in 10% aqueous solution (maximum 250 ml). From all subjects, breath samples for determination of hydrogen concentration were taken immediately before giving lactose, and thereafter every 30 minutes for 3 hours. We collected extra breath samples for the determination of methane concentration from 69 children. We had described our method for collecting breath samples [9]. The children were asked to breath

once into a collection bag. To ensure that we obtained end-expiratory breath samples, we used T-valve breath sampling plastic bags which allow expired air from the dead space to be discarded. The samples were immediately transferred to 30 ml air-tight plastic syringes, and were taken to the Department of Medical Research where breath gas (hydrogen, oxygen and methane) measurements were made within 24 hours. The children were monitored up to 48 hours after the lactose meal for evidence of symptoms of lactose intolerance.

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

Analysis of hydrogen, oxygen and methane in breath samples

Analysis of hydrogen and oxygen in the breath samples and normalization of hydrogen concentrations were carried out as previously described [9]. Two gas chromatographs (model 12 Microlyzer, Quintron Instruments Co., Milwaukee) were used for measuring hydrogen in breath samples. A portable oxygen analyzer (Oxywarn 100 I, Draeger Instrument Division, Blyth, Northumberland, Australia) was used to measure oxygen.

Methane was measured with a Model 16 Microlyzer equipped with a molecular sieve chromatographic column (Quintron Instrument Co., Inc., Milwaukee, WI 53215). Dry air was used as the carrier gas at a flow rate of 35 ml per minute. The chromatograph was calibrated with a methane reference mixture in com-

pressed air (Quingas, Quintron Instrument Co.). The smallest detectable concentration of methane was 2 microlitres per litre, with a linear accuracy response range of 2 to 200 microlitres per litre.

Working definitions

Lactose malabsorption was defined as a peak hydrogen >10 parts per million (ppm) above baseline values in the 90-, 120-, 150-, or 180- minute samples after a lactose test meal. Nonhydrogen production was defined as a peak hydrogen <10 ppm above baseline values in the 90-, 120-, 150-, or 180- minute samples following lactulose. Peak hydrogen was calculated as the difference between the highest normalized breath-hydrogen value in any one of the breath samples collected 90- to 180-min after lactose or lactulose test meals, and the lowest normalized breath-hydrogen value in any one of the 0, 30, or 60 min samples. Methane excretion was defined as a reading of 3 ppm or greater.

Statistical analysis

Data analysis was performed with SPSS (Statistical Package for Social Scientists Ver 5.01, SPSS Corporation, Chicago, Ill., USA) on a Toshiba T3100e computer. Data are expressed as mean $\pm$ SEM. Comparisons between breath methane concentrations among groups (lactose-absorbers and lactose-malabsorbers) were made using Student's 't' test for unpaired samples (two tailed). Temporal differences in breath methane concentrations in relation to time of collection were assessed using Student's 't' test for paired data (two tailed). The distribution of lactose-absorbers and lactose-malabsorbers among different categories was analysed by Chi squared test. Proportion of methane excretors in relation to sampling time was analysed by Chi squared test for

trend [10]. Differences were considered significant if $p < 0.05$.

Ethical considerations

The study was approved by the Medical Ethical Committee of the Department of Medical Research, Yangon, Myanmar.

RESULTS

Age and sex prevalence

Of 125 children tested, 51 (40.8%) were lactose-absorbers, 66 (52.8%) were lactose-malabsorbers and 8 children (6.4%) were found to be non-hydrogen producers. There was no significant difference in the prevalence of lactose-malabsorbers according to sex (47.7% of boys and 58.3% of girls) and the data for both sexes were combined for all subsequent analyses. Table 1 shows age group specific prevalences of lactose-malabsorption. 16.7% of children under 3 years of age malabsorbed lactose and the percentage of lactose malabsorption increased with age and 91.7% of children aged 9-12 years were lactose-malabsorbers.

Table 1. Number of lactose-malabsorbers and lactose-absorbers in different age groups among 117 children aged 1 to 12 years

Age group	Lactose-malabsorber (%)	Lactose-absorber (%)	Total
1-2.9 y	4 (16.7%)	20 (83.3%)	24
3-5.9 y	17 (39.5%)	26 (60.5%)	43
6-8.9 y	23 (88.5%)	3 (11.5%)	26
9-11.9 y	22 (91.7%)	2 (8.3%)	24
All ages	66 (56.4%)	51 (43.6%)	117

None of the children under five had gastrointestinal symptoms such as abdominal pain, diarrhoea, nausea, vomiting, flatulence, borborygmi, but 21% of those older than 6 years of

age with lactose malabsorption experienced gastrointestinal symptoms.

Breath gas concentrations

Table 2 shows mean ($\pm$ SEM) concentrations of breath methane and increments from basal values at different sampling times in lactose-malabsorbers and lactose-absorbers. Lactose-malabsorbers had a significantly higher breath methane concentration than lactose-absorbers at 180-min sampling time. A significant increment in methane concentration from the basal values was observed in lactose-malabsorbers at 120-, 150-, and 180-min samples, but there were no significant increments of methane concentration at any time in lactose-absorbers.

Table 3 shows methane excretors (methane ≥ 3 ppm) and non-methane excretors at basal (0-min) to 180-min breath samples in both study groups. The percentage of methane excretors in lactose-malabsorbers increased with time after lactose ingestion with a significant trend ($p < 0.001$).

DISCUSSION

Studies on lactose malabsorption in children and adults from Asia reported a high prevalence of lactose malabsorption with an onset early in life. The present study confirmed that the overall prevalence of lactose malabsorption is high (56.4%) in Myanmar children and lactose malabsorption can be detected in 16.7% of children 1-3 years with the prevalence increasing sharply after 9 years of age to 91.7%. A previous study on Myanmar village children under five years of age also found 75% to be lactose-malabsorbers [11]. Also, a hospital based study using blood glucose levels, had demonstrated that 53% of 1-5 year old children and 92% of Myanmar adults to be lactose-malabsorbers [12]. Brown and his colleagues in

Table 2. Mean methane (CH₄) production by lactose-malabsorbers and lactose-absorbers at different sampling times following lactose test meal

Time (min)	Lactose-malabsorbers			Lactose-absorbers		
	CH ₄ ppm (Mean $\pm$ SEM)	* (Mean $\pm$ SEM)	N	CH ₄ ppm (Mean $\pm$ SEM)	* (Mean $\pm$ SEM)	N
0	2.69 $\pm$ 0.56	0	56	1.54 $\pm$ 0.70	0	13
30	2.32 $\pm$ 0.47	-0.46 $\pm$ 0.92	56	1.61 $\pm$ 0.59	0.08 $\pm$ 0.82	13
60	3.52 $\pm$ 0.63	1.89 $\pm$ 1.27	55	1.69 $\pm$ 0.82	0.15 $\pm$ 0.84	13
90	3.83 $\pm$ 0.64	1.56 $\pm$ 1.09	54	1.69 $\pm$ 0.71	0.15 $\pm$ 0.56	13
120	5.02 $\pm$ 0.76 ^a	2.57 $\pm$ 1.06	54	3.31 $\pm$ 1.31	1.77 $\pm$ 1.45	13
150	4.98 $\pm$ 0.74 ^a	2.44 $\pm$ 0.89	52	2.31 $\pm$ 0.91	0.77 $\pm$ 1.14	13
180	5.52 $\pm$ 0.73 ^{a,b}	2.88 $\pm$ 1.02	52	1.92 $\pm$ 1.03 ^b	0.38 $\pm$ 1.27	13

* = determined by subtracting the baseline value from subsequent CH₄ levels

a = significantly higher (p<0.05) as compared to basal (0 min) value

b = significantly different between lactose-malabsorbers and lactose-absorbers (p<0.05)

N = number of subjects

Table 3. Methane excretors (breath methane $\geq$ 3 ppm) and non-methane excretors at different sampling times in lactose-malabsorbers and lactose-absorbers

Time (min)	Lactose-malabsorber			Lactose-absorber		
	Methane excretor	Non-methane excretor	% of methane excretor	Methane excretor	Non-methane excretor	% of methane excretor
0	17	39	30.4	3	10	23.1
30	17	39	30.4	4	9	30.7
60	22	32	40.7	3	10	23.1
90	25	28	47.7	3	10	23.1
120	30	23	56.6	4	9	30.7
150	29	23	55.8	3	10	23.1
180	32	20	61.5	2	11	15.4

Chi squared test for trend

$\chi^2 = 20.17$

p < 0.001

Chi squared test for trend

$\chi^2 = 0.024$

p = not significant

their study on Bangladeshi children found a 20% prevalence of lactose malabsorption in well-nourished diarrhoea-free village children from 7 to 18 months of age, with a prevalence of 89% in those over 3 years of age. We found a much slower transition period, with a prevalence of 39.5% in 3 to 6 years, increasing to 88.5% in 6-9 years, reaching 91.7% at 9 to 12 years of age. Although we did not include children under one year of

age in the present study, lactose malabsorption in a 9-month-old Myanmar village child has been reported previously [11]. A study in Thailand also found 34% of children 1 month to 1 year of age with lactose malabsorption, and 59% of those from 1 to 2 years of age [3]. Studies from Jamaica [13] and Peru [14] also reported that 80% of children aged >3 years to be lactose-malabsorbers. By contrast, Jewish children studied

in Israel [15] had a much delayed onset: no lactose-malabsorbers was found in children less than 3 years of age. A much lower prevalence of lactose malabsorption was reported from Finland [16] where the prevalence of lactose malabsorption in adults is only 15% and only 4% in the 7- to 11- year age-group. We and other investigators [2,6] found no sex preponderance in lactose-malabsorbers.

Although Tadesse *et al.* [5] had reported that Hong Kong children with lactose malabsorption lack accompanying symptoms following lactose meal, we found gastrointestinal symptoms in 21% of lactose-malabsorbers over 6 years of age. However, none of the under six-year-old children had any complaints. There may be a gradual loss of lactase activity in our population as indirectly indicated by a gradual increase of the prevalence of lactose malabsorption with age.

In the present study, 4.7% of the enrolled subjects had breath hydrogen concentrations of ≥ 20 ppm before lactose ingestion. This high fasting hydrogen excretion is usually caused by previously ingested fermentable dietary substances. To overcome the problem of high fasting hydrogen values, peak excess hydrogen was calculated as the difference between the highest normalized breath-hydrogen value in any one of the breath samples at 90-, 120-, 150-, 180- mins, and the lowest normalized breath-hydrogen value in any one of the 0, 30, or 60 min.

In order to determine the temporal relationship between lactose malabsorption and breath methane excretion, the time course of breath methane excretion were measured in lactose-malabsorbers and lactose-absorbers up to 180 mins after lactose ingestion. We found that methane production increases significantly after 120

minutes in lactose-malabsorbers (Table 2). The trend of increment is also significant (Table 3). However, breath methane concentrations between lactose-malabsorbers and lactose-absorbers were significantly different only at 180 mins. Similar findings had been reported by Meadows *et al.* [17]. They found that methane excretion by lactose-malabsorbers rose significantly at 90 min and 120 min following the lactose load. However, they found that the percentage of methane producers (>1 ppm above ambient air) in lactose intolerant children is not significantly different from normal children.

Recently, Strocchi *et al.* [18] had reported that extension of collection time greatly enhanced the sensitivity and specificity of lactulose malabsorption. Extension of methane collection time following lactose load may discriminate the breath methane excretion status between lactose-malabsorbers and lactose-absorbers.

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**The cardiovascular responses to stress in Myanmar male subjects:
The influence of the family history of hypertension**

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The heart rate and arterial blood pressure responses to mental (Arithmetic test) and orthostatic (75° head - up tilt) stress were studied in apparently healthy young Myanmar male subjects (age 20-28 yrs). It was found that the heart rate responses (measured with computerized ECG system) to mental arithmetic and orthostatic stress in the group with family history of hypertension (FH + group) were significantly higher ($p < 0.05$) than those without the family history (FH - group). The blood pressure responses (indirect sphygmometry) to both stresses tended to be greater in the FH (+) group compared with those in the FH (-) group. It was concluded that the cardiac responses to mental and orthostatic stresses were greater in young Myanmar male subjects with family history of hypertension compared with those without.

INTRODUCTION

Various workers indicated that the principal difference between subjects with a family history of essential hypertension and those without was that the former group showed a greater heart rate [1,2] and blood pressure [1,3] responses to mental stress. A proportion of hypertensive patients and normotensive children of hypertensive parents exhibited an enhanced responsiveness of the autonomic nervous system; 56 per cent of subjects with family history of hypertension who showed the hyper-reactivity to mental stress progressed to established hypertension within one to four years [4]. This implies that mental stress test may provide some indications of development of hypertension in a normal group. In his review of the literature on cardiovascular response to stress, Herd [5] stated that of all the modifiers influencing the response to a psychological stressor, family history of hypertension is the one most likely to have an effect.

Tilting has often been used as a cardiovascular stress situation. Earlier studies [6,7] indicated that hypertensives differed from normotensives in their response to orthostatic stress, and one study [8] suggested that postural blood pressure changes may predict development of hypertension. Borderline hypertensives have been reported to show a greater total peripheral resistance response [9] or diastolic blood pressure response [8] to orthostatic stress compared with the normotensives. Horikoshi *et al.* [10], however, reported that changes in heart rate, blood pressure and plasma epinephrine levels in response to standing and mental stress test were not different in subjects with and without a parental history of hypertension. The objective of this study was to find out whether the cardiovascular responses to mental and orthostatic stress are influenced by the family history of hypertension

in Myanmar subjects.

MATERIALS AND METHODS

Subjects

Twenty-nine male medical students (age 20-23 years) and 33 another male medical students and male staff members of the Institute of Medicine 2 (age 20-28 years) participated in the mental stress test and orthostatic stress test respectively. History of hypertension present at least in one of the parents or grandparents was taken as the presence of family history of hypertension (FH +). Those with a present or past history of major systemic disease and those taking medications were excluded from the study. Informed consent was obtained from all subjects.

Methods

Blood pressure and heart rate determinations were carried out by a single experimenter so as to eliminate interobserver variability. Heart rate was read from the computerized ECG system (Cardiovit AT - 3) in orthostatic stress test; and in the mental stress test, from the sphygmogram obtained from a custom made model consisting of blood pressure cuff, the pressure transducer and chart recorder (WPA CQ 95). Blood pressure was measured indirectly over the right brachial artery using a mercury sphygmomanometer (Accoson) and the deflation rate was controlled in a linear fashion at 2 mmHg sec⁻¹. Phase I and V sounds were recorded as the systolic blood pressure (SBP) and diastolic blood pressure (DBP) respectively.

Mental stress test

The subjects were requested not to take food and drinks in the morning of the test day. Each subject was given a glass of cold drink sweetened with one tablespoonful of sugar 15

minutes before the test. All tests were performed between 8 am and 9 am. The subjects had been previously familiarized with the laboratory setting. After the attachment of recording devices, they were seated in a semirecumbent position in a reclining chair. After a rest period of 10 minutes, baseline heart rate and blood pressure were determined. The subject was then asked to perform mental arithmetic test: randomized numbers (1 to 20) were orally presented to the subject, two numbers at first and then one at a time; the numbers were to be mentally added consecutively as rapidly as possible. The wrong answers were immediately corrected. Blood pressure and heart rate were recorded at 1 and 4 min, during the five-minute test period and at 1 and 5 min following the test.

Orthostatic stress test

These tests were carried out in the early afternoons. The subject was asked to lie on a tilt-table which is of weight bearing type with a foot-board and a leather strap so that no muscular effort would be required on the part of the subject to maintain the position. The table takes 5 sec to rotate to the head-up tilt with a 75° angle from the horizontal. After trial tiltings, the subject was allowed a 15 min rest period on the tilt-table in a horizontal position during which heart rate and blood pressure were recorded at 5-min intervals. The values at 15 min were taken as the supine resting blood pressure and heart rate. The subject was then passively tilted to 75° head-up position and the cardiovascular parameters were again recorded at 1st, 2nd, 5th and 10th minutes. The values at the last time point were taken as the orthostatic blood pressure and heart rate. The subject was then passively tilted back to the horizontal position and the cardiovascular parameters

recorded every 5 min for 15 min.

The responses were expressed as changes from the control values.

Statistical analysis

The results are presented as means $\pm$ SD. Changes from the control values were tested using the Student's paired t-test. Differences between the two groups were tested by Student's unpaired t-test. Significance level was set at P value of less than 0.05.

RESULTS

There were no significant differences in baseline heart rates and blood pressures between the FH+ and FH- groups (Table 1).

The heart rate response to mental stress was significantly greater ($p < 0.05$) in the FH+ group compared with those in FH- group (Figure 1).

Table 1. Baseline cardiovascular data (mean $\pm$ S.D.)

	Mental Stress Test		Postural Stress Test	
	FH(-)	FH(+)	FH(-)	FH(+)
Number of subjects	11	18	15	18
Systolic blood pressure (mmHg)	114.2 $\pm$ 10.9	112.1 $\pm$ 13.2	109.6 $\pm$ 9.9	111.7 $\pm$ 8.5
Diastolic blood pressure (mmHg)	63.3 $\pm$ 10.0	65.4 $\pm$ 8.7	57.9 $\pm$ 9.8	54.1 $\pm$ 11.8
Heart rate (beats/min)	74.4 $\pm$ 6.4	69.6 $\pm$ 13.1	77.7 $\pm$ 11.6	70.1 $\pm$ 10.1

The individual SBP and DBP responses are shown in figure 2. In the FH+ group, 50 per cent of subjects showed a high SBP response equal to or

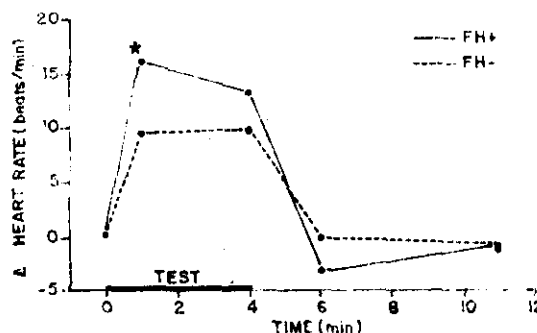


Fig. 1. Heart rate changes during mental arithmetic test in FH+ group ($n = 18$) and FH- group ($n = 11$)
*($P < 0.05$ between groups)

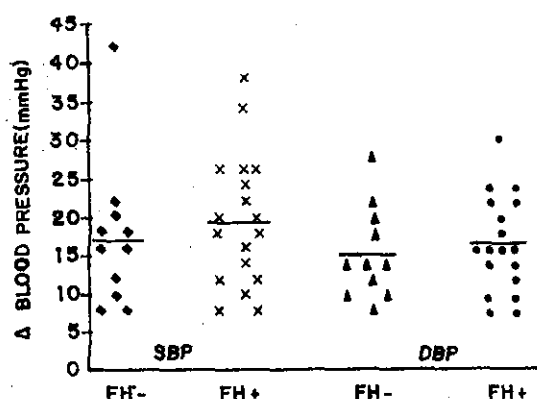


Fig. 2. Increases in systolic blood pressure, SBP (left panel) and diastolic blood pressure, DBP (right panel) during the first minute of mental arithmetic test in FH- and FH+ groups. Bars represent mean value

greater than 20 mmHg (high responders) whereas only 27 per cent in the FH- group exhibited the high SBP response.

The heart rate increments during orthostatic stress were significantly greater ($P < 0.05$) throughout in the FH+ group compared with those in the FH- group (Figure 3). The fall in SBP and the rise in DBP (and hence the fall in pulse pressure) in

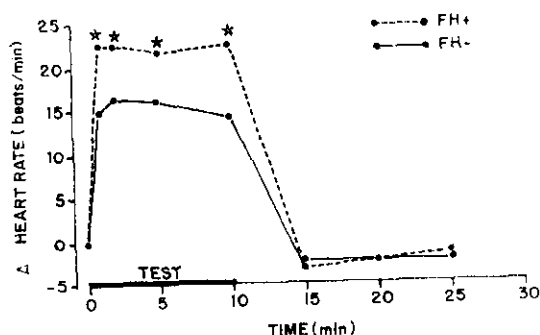


Fig. 3. Heart rate responses to 75° head-up tilt (duration of the tilt indicated by a thick bar) in FH+ group (n = 18) and FH- group (n = 15)
*(P<0.05 between groups)

response to the head-up tilt were also greater (though not statistically significant) in the FH+ group compared with those in the FH- group (Figure 4). Although postural hypotension (falls in SBP and DBP of more than 20 mmHg and 5 mmHg respectively) was not observed in any of the 33 subjects after head-up tilt, postural tachycardia (heart rate increase of more than 30 beats/min) was observed in 3 subjects with family history of hypertension. When the subjects were retilted back to the horizontal position, the cardiovascular responses become reversed: heart rate and DBP decreased while the SBP and pulse pressure increased. The fall in heart rate was also significantly greater (P<0.01) in the FH+ group than in FH- group (25.8 ± 10.5 vs 16.7 ± 7.3 and 25.0 ± 9.5 vs 16.7 ± 6.4 beats/min at 5th and 10th min respectively).

DISCUSSION

Many studies [1-3] have demonstrated that there is a small difference in cardiovascular responses to stress between normotensives with a family history of hypertension and those

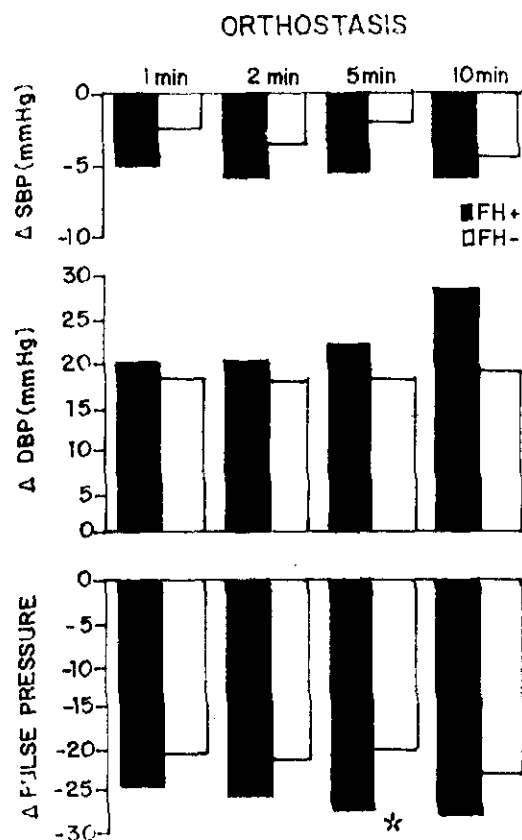


Fig. 4. Blood pressure responses to orthostasis (head-up tilt) in FH+ group (n = 18) and FH- group (n = 15)
SBP = Systolic blood pressure
DBP = Diastolic blood pressure

without such a history. We also found that in Myanmar male adults, the heart rate responses to psychological stress in the form of mental arithmetic in FH+ group were significantly greater than those in the age-matched FH- group; the blood pressure responses also tended to be greater in the FH+ group. It seems that those with the family history of hypertension triggered themselves to higher levels of activation in response to mental stress; the underlying mechanism, however, are not known.

In humans, the maintenance of blood

pressure on changing posture depends largely on the sympathetic nervous system. When the subject is tilted to head-up position, SBP falls and this stimulates the baroreflex resulting in increased heart rate and DBP. This compensation is completed within 18-30 seconds, after which the SBP returns to within 5 mmHg of the control value while heart rate and DBP continue to rise [11]. Although the general pattern of the cardiovascular responses to orthostasis is similar, a decline in SBP of more than 5 mmHg occurred on head-up tilt in Myanmar subjects with family history of hypertension. This could be interpreted as an inadequate response on the part of the sympathetic nervous system. The defect may also be on the afferent side of reflex response to changing position [12]. However, the range of SBP changes upon standing had been said to be considerably wide [13]. Moreover, marked heart rate and DBP responses to head-up tilt observed in FH+ group in the present study would rather imply a marked increase in efferent sympathetic activity.

Since enhanced heart rate responses to mental and physical (orthostatic) stresses were observed in Myanmar male adults with a family history of hypertension, it may be concluded that the genetic predisposition to hypertension may play a role in modifying the responsiveness to stress in Myanmar subjects.

ACKNOWLEDGEMENT

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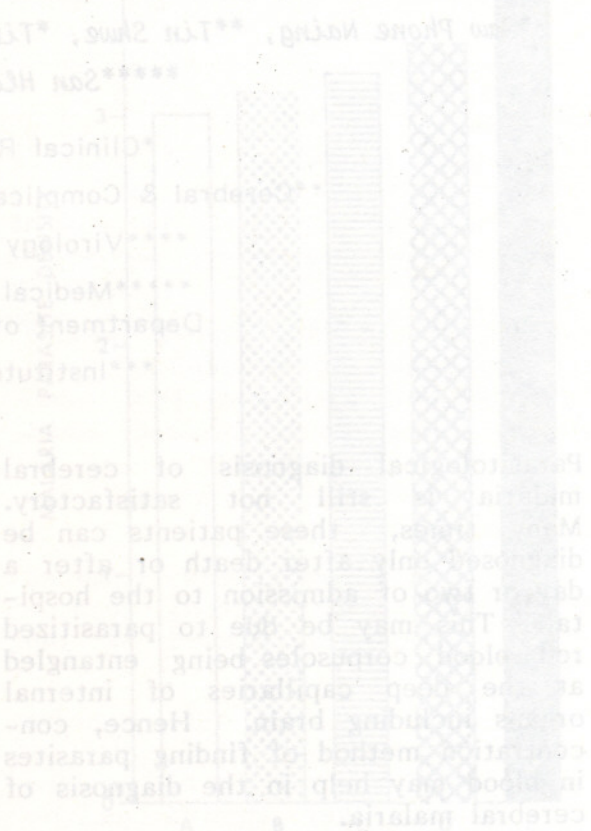
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Apert from standard method (taking thick and thin film) from the finger tips and staining with Giemsa, there are a number of published papers on the diagnosis of malaria: concentration method of detecting Plasmodium falciparum using microcentrifuge and heparinized capillary tubes [1], collecting blood from antecubital vein which yield a higher parasite count [2], acridine orange staining method [3], Polymerase Chain Reaction method [4], concentration method using Giemsa stain [5], Giemsa stain [6], Giemsa stain [7], rapid diagnostic test (RDT) [8], Giemsa stain [9], Giemsa stain [10], Giemsa stain [11], Giemsa stain [12], Giemsa stain [13], Giemsa stain [14], Giemsa stain [15], Giemsa stain [16], Giemsa stain [17], Giemsa stain [18], Giemsa stain [19], Giemsa stain [20], Giemsa stain [21], Giemsa stain [22], Giemsa stain [23], Giemsa stain [24], Giemsa stain [25], Giemsa stain [26], Giemsa stain [27], Giemsa stain [28], Giemsa stain [29], Giemsa stain [30], Giemsa stain [31], Giemsa stain [32], Giemsa stain [33], Giemsa stain [34], Giemsa stain [35], Giemsa stain [36], Giemsa stain [37], Giemsa stain [38], Giemsa stain [39], Giemsa stain [40], Giemsa stain [41], Giemsa stain [42], Giemsa stain [43], Giemsa stain [44], Giemsa stain [45], Giemsa stain [46], Giemsa stain [47], Giemsa stain [48], Giemsa stain [49], Giemsa stain [50], Giemsa stain [51], Giemsa stain [52], Giemsa stain [53], Giemsa stain [54], Giemsa stain [55], Giemsa stain [56], Giemsa stain [57], Giemsa stain [58], Giemsa stain [59], Giemsa stain [60], Giemsa stain [61], Giemsa stain [62], Giemsa stain [63], Giemsa stain [64], Giemsa stain [65], Giemsa stain [66], Giemsa stain [67], Giemsa stain [68], Giemsa stain [69], Giemsa stain [70], Giemsa stain [71], Giemsa stain [72], Giemsa stain [73], Giemsa stain [74], Giemsa stain [75], Giemsa stain [76], Giemsa stain [77], Giemsa stain [78], Giemsa stain [79], Giemsa stain [80], Giemsa stain [81], Giemsa stain [82], Giemsa stain [83], Giemsa stain [84], Giemsa stain [85], Giemsa stain [86], Giemsa stain [87], Giemsa stain [88], Giemsa stain [89], Giemsa stain [90], Giemsa stain [91], Giemsa stain [92], Giemsa stain [93], Giemsa stain [94], Giemsa stain [95], Giemsa stain [96], Giemsa stain [97], Giemsa stain [98], Giemsa stain [99], Giemsa stain [100].

SHORT REPORT

Comparison of the different methods of parasitological diagnosis on patients with cerebral malaria

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Parasitological diagnosis of cerebral malaria is still not satisfactory. Many times, these patients can be diagnosed only after death or after a day or two of admission to the hospital. This may be due to parasitized red blood corpuscles being entangled at the deep capillaries of internal organs including brain. Hence, concentration method of finding parasites in blood may help in the diagnosis of cerebral malaria.

Apart from standard method (taking thick and thin film) from the finger tips and staining with Giemsa, there are a number of published papers on the diagnosis of malaria: concentration method of detecting Plasmodium falciparum using microcentrifuge and heparinized capillary tubes [1], collecting blood from antecubital vein which yield a higher parasite count [2], acridine orange staining method [3], Polymerase Chain Reaction method [4], concentration method using Becton Dickinson QBC II centrifugal hematology analyzer [5], rapid diagnostic test (Parasight F test) using DIP-STICK TEST by Bacton-Dickinson Co., USA and chemiluminescent detection of repetitive DNA sequences of P. falciparum.

Out of these different methods some need special apparatus which are

often costly and are not applicable for the field conditions of the developing countries. Hence this study, using equipment that are easily available in the developing countries was carried out.

The objective of this study is to find out the most suitable method of finding malaria parasites in blood of patients with cerebral malaria at peripheral hospitals of developing countries.

Sixty-nine consecutive patients with cerebral malaria who attended the medical units of North Okkalapa General Hospital with signs and symptoms of cerebral malaria during 1995 malaria season were studied.

Blood samples were collected at the day of admission to the hospital and stained. Different methods of blood collection and staining were used as follows. (1) Routine method of collecting blood from the finger tips and stained with Giemsa. (2) Method of blood collecting from the cubital vein and stained with Giemsa. (3) The blood collected from the finger tips, concentrated using microcentrifuge and heparinized capillary tubes. The red blood corpuscles from the upper part of PCV tube just below the buffy coat were collected and stained with Giemsa. (4) The blood

collected from the antecubital vein, concentrated and stained as in (3), and (5) Blood collected from finger tip and stained with acridine orange.

Blood samples were collected all at the same time. Except the ones for acridine orange staining which need fluorescence microscope, the other blood samples were stained and parasites were detected at the same day.

Blood samples for acridine orange method were collected on glass slides, stored at (-4°C) and were stained and observed under fluorescence microscope at the end of every week according to method of Shute & Sudeman [6] and Mu Mu Sein Myint [3].

The presence of parasites in the blood samples were recorded according to Bruce-Chwatt scale and the number of parasites were also counted with relation to white cell count.

The blood samples were also collected from the different sites and the different methods as mentioned above to document the best method of blood collection and stained with acridine orange.

621 samples of blood collected from different sites of the body and stained with the different methods were studied.

There was no significant difference in the mean parasite count using different staining methods.

Similar result was also recorded when the parasite density using Bruce-Chwatt scale was determined for comparison. But, there was the trend for the other methods to show higher parasite density when compared with standard method of blood collection from the finger tip and stained with Giemsa (Figure 1).

But the 4 patients who were negative using standard method of blood collection and stained with Giemsa, showed positive parasite count with the

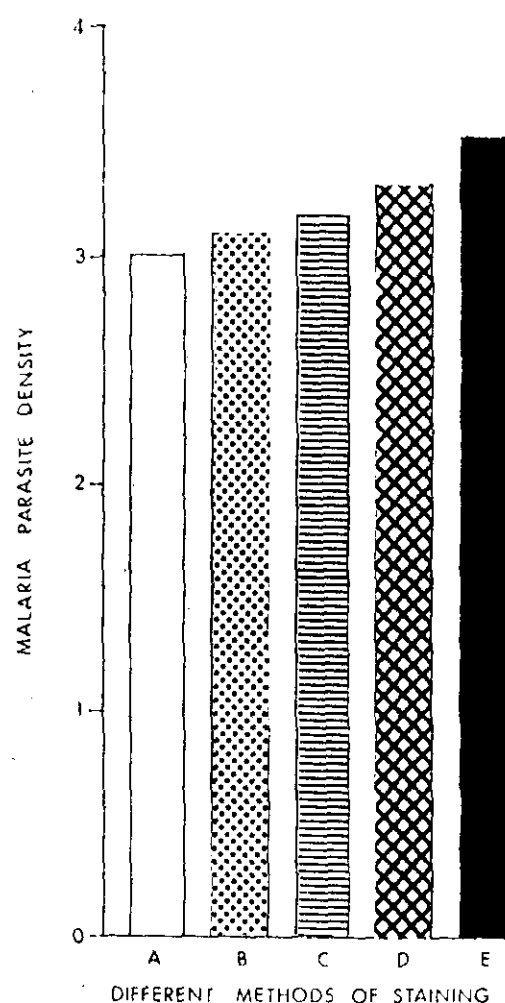


Fig. 1. Parasite density (Bruce-Chwatt Scale) using different staining methods

- (A) Finger tip (Giemsa)
- (B) Vein (Giemsa)
- (C) Finger tip/PCV (Giemsa)
- (D) Vein/PCV (Giemsa)
- (E) Finger tip (Acridine orange)

other four methods. The highest count was recorded from the sample of blood collected from finger tip and stained with acridine orange, though the sample size is small for statistical analysis (Table 1).

When parasite density (Bruce-Chwatt scale) was determined for comparison, similar results were also recorded.

When the different blood collection

Table 1. Mean parasite count and S.D among 4 patients (who are negative by routine finger tip blood collection and Giemsa staining method) using different staining methods

Methods	Patient's register no.				Parasite count (Mean and S.D)
	Sr. 3	Sr. 6	Sr. 63	Sr. 66	
Finger tip (Giemsa)	0	0	0	0	0
Vein (Giemsa)	11	16	135	202	91±81.058621
Finger tip/PCV (Giemsa)	25	7	226	436	173.5±174.23906
Vein/PCV (Giemsa)	30	31	350	516	231.75±209.63346
Finger tip (A.O)	163	266	516	941	471.5±299.92207

($P > 0.05$, ANOVA)

methods with acridine orange staining were compared, blood collected from the antecubital vein with PCV tubes showed higher mean parasite count per microliter. But there was no significant difference among the different methods.

Similar results were also recorded when mean parasite density (Bruce-Chwatt scale) was studied as the comparison.

Though the 4 patients were negative with the standard method of collecting blood from the finger tip and stained with Giemsa, they showed positive result with the other four methods of blood collection and stained with acridine orange.

When the mean parasites count of the blood samples of these 4 patients were examined for comparison, the highest was recorded among the samples of blood collected from antecubital vein and PCV tube. But the sample size is again small for statistical analysis.

In this study there was no statistical difference between the different staining methods and between different methods of blood collection. But, when patients who are negative with standard method of blood collection and stained with Giemsa are compared with all four other methods of staining, all four showed positive results. This is in accordance with the previous reports [1-3].

In the previous reports the method of staining with acridine orange had not been compared with the other methods. In this study acridine orange staining method showed positive rate as high as the sample of blood collected from antecubital vein, PCV tubes. Hence, we can conclude that fluorescence staining method is better than standard method.

In this study only four patients who are negative with standard staining method are compared with the other new methods. If more samples of blood who are negative with standard staining method are available and can be compared, we may be able to document which is the best method for the diagnosis of cerebral malaria. Based on the present finding, it may be concluded that the following methods of (1) blood collection from the antecubital vein, (2) blood collection with the PCV tube, (3) blood collection from the antecubital vein with PCV tubes and (4) acridine orange staining are superior to standard staining method.

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SHORT REPORT

The role of whole cell tube agglutination test in serodiagnosis of pulmonary tuberculosis

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A rapid, reliable and inexpensive serological test for the diagnosis of tuberculosis would have great applicability as a clinical, epidemiological and public health tool. Thus the use of simple whole cell tube agglutination test as a serodiagnostic test for pulmonary tuberculosis was studied.

Blood samples were collected from Union Tuberculosis Institute and from normal healthy persons attending Workers' Hospital for preemployment medical check up (as a control group). The control subjects were selected as follows: who had no signs and symptoms of pulmonary tuberculosis, had chest X-ray diagnosed as no active pulmonary tuberculosis, with or without past history of pulmonary tuberculosis and their age ranging from 16-65 years. Informed consents were obtained from all the patients and control subjects. Then sera were separated and stored at -20°C before being tested.

M. tuberculosis H37 Rv strain was cultured on 3% Ogawa medium and incubated at 37°C for 6-8 weeks. The selected colonies were picked up and suspended in 0.85% sterile saline. Four percent phenol was added and incubated at 37°C for 3 days. Non-viable tubercle bacilli were collected by treating with N sodium hydroxide and heated at 80°C for 30 mins. The cell suspension was washed 3 times with 0.3% normal saline. The antigen suspension was prepared by suspending the final deposit in 0.3% sterile saline giving a concentration

of 4 mg (net wt) of cells/ml [1].

Serial doubling dilution of sera was done upto 1:512 with 0.3% sterile saline. Equal volume of homogenous suspension of antigen was added to each tube and incubated in 37°C water bath for 2 hours and left for 30 mins at room temperature. The tubes were kept at 4°C for 20-22 hours. Cob-web agglutination was taken as positive and button appearance was taken as negative.

Sixty-seven blood samples were collected from test group 1 (AFB smear-positive). Twenty-five samples were obtained from test group 2 (AFB smear-negative). From the control group, 50 blood samples were collected. All of the patients from test group 1 and 2 had agglutinin titre of 1:128 and above. In the control group 45 out of 50 normal persons (90%) had agglutinin titres of 1:128 and below. Only 5 normal persons (10%) had agglutinin titre of above 1:128. The sensitivity (True positive/True negative + False positive x 100) of both test group 1 and 2 was 100% and the specificity (True negative/True negative + False positive x 100) of control group was 90% in this study. Since the vast majority of the serum samples taken from the normal control persons had titres of 1:128, it was decided that 1:128 should be adopted as the significant level in differentiating positive and negative tests.

In test group I with positive smears,

2 minimal lesions had a titre of 1:128. In moderately advanced cases, 14 had a titre of 1:256, 6 had 1:512 and 2 had 1:1024. A titre of 1:1024 was recorded in 43 far advanced cases. In test group II with negative smears, 15 minimal lesions had a titre of 1:128, 1:256 in one moderately advanced and 1:1024 in 5 moderately advanced and 4 far advanced cases.

Five controls had a titre of above 1:128. One suspected case had a titre of 1:512 not detected by miniature chest X-ray, 4 cases had a titre of 1:256, only two gave past history of pulmonary tuberculosis.

The agglutinin titres were compared during the successive follow-up visits in the patients from test group I. The majority of these patients had very high titres up to 1:1024 at all three visits. Agglutinin titres did not decline significantly after anti-tuberculosis treatment and might be due to long-lived antibody which was not related to improvement of the disease condition. Thus this test may not be useful to assess the prognosis of the disease.

The results from the present study indicate that the test is beneficial for screening of pulmonary tuberculosis (both AFB smear-positive and smear-negative cases). The other advantages are that it is economical, simple to perform and needs no special apparatus.

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