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Chemical composition of home-based fluids commonly used in Myanmar

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Rehydration measure should be instituted as early as possible when life threatening dehydration state due to diarrhoea or severe fever is suspected or detected. Home-based fluids are usually given under such circumstance and thus chemical compositions of syrups (n=9), soft drinks (n=5), fresh fruit juices (n=3) and plant decoctions (n=7) were analysed by using published methods and atomic absorption spectrophotometer. High concentrations of total sugars (8.67 to 34.87 g/100ml) and free reducing sugars, mainly as glucose and fructose (1.97 to 21.49 g/100ml) were detected in syrups and soft drinks. Potassium was found to be rich in plant decoctions (34.08 to 273.10 mg/100ml) and sodium in soups (140.0 to 475.0 mg/100g). Soft drinks were found to be acidic (pH = 2.76 to 3.63) whereas soups and plant decoctions have pH values 5.29 to 8.91. Colours used in syrups and soft drinks were found to be permitted dyes.

INTRODUCTION

Replacement therapy is needed when there is heavy loss of water, and electrolytes, as in prolonged fever, severe vomiting and diarrhoea [1]. Home-based fluids are usually given by household members, medical doctors and traditional practitioners under such circumstances.

Sugars and electrolytes had been determined in commercial fluids such as soft drinks and instantly prepared soups utilized in foreign countries [2]. Majority of Myanmar people use a variety of home-based fluids prepared traditionally. They are mainly syrups, fresh fruit juices, rice soups, and plant decoctions. Chemical properties regarding the concentrations of sugars, electrolytes, protein and pH values of the above fluids have not yet been determined. This paper presents the chemical composition of common Myanmar home-based fluids.

MATERIALS AND METHODS

Materials

Syrups, fruit juices and soups were purchased from shops in central Yangon. Plant decoctions were prepared according to the procedures usually used by traditional practitioners i.e. the weight of plant and water ratio is 1:4 which is boiled down to one quarter of its original amount [3].

Method

Free and total reducing sugars were determined by copper reduction method [4] and types of sugars and coloured substances present in the juices were investigated by thin layer chromatography (TLC) and paper chromatography (PC) techniques respectively [5,6]. Atomic absorption spectrophotometer (AAS) (model SP-9 PYE UNICAM) was used for determination of electrolytes. pH

values were measured by means of pH meter (model HM-20 B, HITACHI). Protein content of soup samples was detected by microkjeldahl method and total fat content of the moisture free soup samples was extracted with petroleum ether (60°-80°C) [7]. Carbohydrate percentage was calculated according to the method of difference [8]. Since syrups are commonly used after diluting five times to original, five times diluted samples were determined and other fluids such as soft drinks, soups and plant decoction were analysed as purchased or prepared.

RESULTS

Three types of sugar spots were detected when syrups and fruit juices were analysed by TLC. The spot having lower R_f (0.34) was found to be similar with standard sucrose. The upper spot (R_f = 0.52) was observed to be the same in colour (i.e. orange) with standard glucose when the plate was sprayed with p-aminobenzoic acid solution (PABA). However it showed the colour of the standard fructose spot when the plate was treated with naphthoresorcinol reagent. Thin layer chromatogram sprayed with naphthoresorcinol is shown in Figure 1.

All the data obtained from the analysis of three types of Myanmar home-based fluids such as syrups and fruit juices, soups, and plant decoctions were presented in Tables 1,2,3 respectively.

DISCUSSION

Sugars detected in the TLC study were identified as glucose, fructose and sucrose. Therefore, free reducing sugars represent mainly as the summation of two monosaccharides; glucose and fructose and total sugars represent

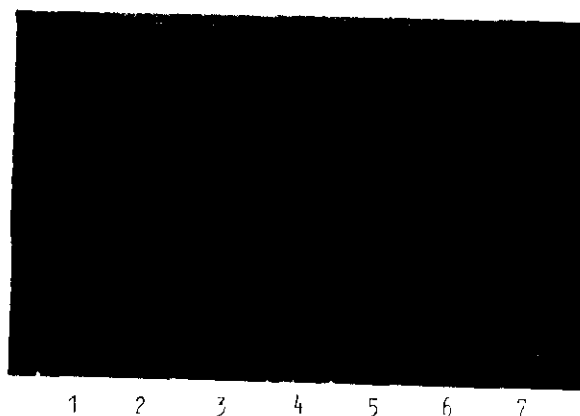


Fig. 1. TLC of sugars
1 = Standard fructose
2 = Standard glucose
3 = Standard sucrose
4 = Sparkling (Dagon)
5 = Barley (Dagon)
6 = Barley (Joker)
7 = Coca-Cola (Joker)

the summation of monosaccharides and sucrose. The overlapping of glucose and fructose spots in different solvent systems was confirmed by spraying with two reagents such as p-aminobenzoic acid and naphthoresorcinol solutions. These reagents gave distinct colours for glucose and fructose.

In three types of Myanmar home-based fluids, sugars are found to be rich in syrups, sodium in soups and potassium in plant decoctions (Tables 1,2,3). Slight acidic property of syrups (pH = approx. 2.92) could possibly be due to the addition of acidic preservatives in the original preparation. High concentrations of sodium in soups could be due to the common salt and monosodium glutamate. The particular type of Myanmar home-based fluids plant decoctions, showed the natural source of potassium.

Besides the main ingredients of oral rehydration salts (ORS), toxic element

Table 1. Sugars and mineral electrolytes of syrups and fresh fruit juices

	pH VALUE	FREE SUGAR g/100 ml	TOTAL SUGAR g/100 ml	MINERAL ELECTROLYTES (mg/100 ml)									
				Na	K	Ca	Mg	Fe	Zn	Mn	Cu		
<u>DRAGON PRODUCTS</u>													
5 times dilution of syrup (n = 3)	3.03 ±0.24	7.33 ±2.56	9.61 ±0.57	9.48 ±1.09	0.50 ±0.40	0.66 ±0.27	0.08 ±0.058	0.07 ±0.045	0.007 ±0.004	-	-	0.016 ±0.007	
Aerated soft drinks (n = 5)	3.20 ±0.32	6.72 ±2.49	14.07 ±1.71	12.22 ±6.44	1.70 ±0.40	1.47 ±0.64	0.37 ±0.14	0.046 ±0.013	0.012 ±0.006	0.012 ±0.008	0.012 ±0.008	0.02 ±0.008	
<u>MOONLIGHT PRODUCTS</u>													
5 times dilution of syrup (n = 1)	2.68	21.49	34.87	28.70	2.66	1.43	0.20	0.13	0.04	-	-	-	
<u>JOKER PRODUCTS</u>													
5 times dilution of syrup (n = 5)	2.76 ±0.13	13.76 ±1.03	19.02 ±0.48	13.45 ±6.17	2.95 ±0.82	2.10 ±1.27	0.29 ±0.33	0.09 ±0.03	0.01 ±0.003	0.014 ±0.017	0.002 ±0.001	0.002 ±0.001	
<u>FRESH FRUIT JUICES</u>													
Sugar cane juice	5.40	3.61	9.92	6.90	165.84	5.20	0.75	0.47	0.21	0.09	0.01	0.01	
Lime juice	3.00	0.38	14.77	23.30	17.56	5.03	0.65	0.12	0.01	0.02	0.003	0.003	
Tamarind solution	4.20	1.97	8.67	97.25	130.68	7.79	0.81	0.09	0.04	0.04	0.03	0.03	

Note: Values are $\bar{x} \pm S.E$ for numbers, n.

Table 2. Proteins, carbohydrates and mineral electrolytes of soups

	pH VALUE	MOISTURE	TOTAL FAT g/100g	PROTEIN g/100g	CARBOHY- DRATE g/100g	MINERAL ELECTROLYTES (mg/100g)							
						Na	K	Ca	Mg	Fe	Zn	Mn	Cu
Fish gruel rice ဆန်ပြုတ်(ငါး)	6.66	92.02	0.02	1.43	5.58	475.0	22.50	18.35	7.28	3.55	0.20	0.05	0.05
Duck gruel rice ဆန်ပြုတ်(ည)	6.46	91.79	0.03	1.31	5.91	295.0	18.86	22.96	7.35	6.57	0.48	0.11	0.06
Gruel rice ဆန်ပြုတ်	6.51	89.90	0.06	0.85	8.45	223.75	27.90	1.28	0.70	0.02	0.03	0.01	0.003
Rice soup ထမင်းရည်	5.67	99.20	0.06	0.34	0.05	12.80	31.30	0.90	0.70	0.01	0.05	0.04	0.12
Rice vermicellie chicken flavour မြွေပြုတ်(ကြက်)	6.49	88.90	0.08	2.82	7.53	260.0	27.82	3.31	4.19	0.96	0.89	0.02	0.08
Rice vermicellie duck flavour မြွေပြုတ်(ည)	6.53	88.13	0.09	3.12	8.07	140.0	34.37	3.06	4.70	1.47	0.94	0.09	0.09
Mutton soup ဆိတ်စွပ်ပြုတ်	6.25	86.50	7.27	2.08	3.09	295.0	57.08	3.44	6.65	1.70	0.53	0.03	0.07

Table 3. Sugars and mineral electrolytes of plant decoctions

	pH VALUE	FREE SUGAR g/100 ml	TOTAL SUGAR g/100 ml	MINERAL ELECTROLYTES (mg/100 ml)							
				Na	K	Ca	Mg	Fe	Zn	Mn	Cu
Piper betel Linn. ကုန်ရွက်ပြုတ်ရည်	4.67	0.18	0.31	7.50	194.30	0.50	0.83	0.39	0.06	0.27	0.01
Vitex trifolia Linn. ကြောင်ပန်ရွက်ပြုတ်ရည်	5.43	0.79	1.08	13.50	181.50	16.56	0.87	0.16	0.06	0.05	0.01
Mimosa pudica Linn. ထိကနန်း(ပုခင်ပါး)ပြုတ်ရည်	5.63	0.57	0.77	4.40	197.10	25.90	0.87	0.06	0.12	0.40	0.01
Adhatoda vasica Nees မုသားကြီးရွက်ပြုတ်ရည်	8.91	0.17	0.42	7.80	273.10	41.20	0.91	0.11	0.28	0.05	0.01
Ageratum conyzoides Linn. ဆိတ်သုံးရွက်(ခွေးသေးပန်း)ပြုတ်ရည်	5.61	0.33	0.53	12.90	187.60	45.90	0.92	0.12	0.15	0.10	0.02
Desmodium triquetrum DC. လောက်သေရွက်ပြုတ်ရည်	5.29	0.42	1.09	15.20	147.00	7.10	0.87	1.48	0.13	0.62	0.01
Zingiber officinale Rescoe ဂျင်းတက်ပြုတ်ရည်	6.96	0.13	0.23	6.10	174.80	0.40	0.82	0.12	0.09	0.47	0.01
Plain tea လွက်ရည်(အခါး)	5.97	-	-	1.90	34.08	0.11	0.74	0.01	0.02	0.39	0.004
Black coffe ကော်ဖီကြမ်း	6.23	-	7.45	2.20	49.90	4.14	0.78	0.02	0.01	0.01	-
Jaggery syrup ထန်လွက်ရည်	5.84	10.49	18.57	46.40	47.40	6.50	0.80	0.09	0.02	0.02	0.02
ဆားမီးဖုတ်ဖျော်ရည်	9.92	-	-	323.33	28.25	5.46	0.85	0.85	0.01	0.01	0.01

lead (Pb) was also determined in all samples but found to be negative. The coloured substances present in the juices were also investigated by paper chromatography using authentic markers and found to be permitted dyes such as tertrazine (yellow), carmosine (red) and amaranth (pink red).

Home-based fluids may play an important role in the replacement therapy since they contain essential electrolytes (Na and K), protein and sugars. It may not be recommended to be used as a therapeutic measure for severe dehydrated state where the electrolyte balance is critical. But jaggery salt solution or plant decoction laced with sugars, starch and common salt which is an example of home-based fluids that could be used as an alternative ORS solution. Therefore with reference to the analytical data, the clinical usefulness of home-based fluids for diarrhoea, severe fever, or any kinds of ailments needs further assessment.

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Variation in biological properties of venoms from different localities of Ayeyarwady Division

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Variation in biological properties and SDS-PAGE electrophoresis pattern of venoms from 5 localities (Nyaungdon, Danuphyu, Daedaye, Pyapon and Pantanaw) were studied according to the WHO recommended techniques. Snakes from each locality were grouped into 2 groups (<90 cm and >90 cm). Among mature (>90 cm) snakes, potent defibrinogenating, phospholipase, capillary permeability increasing activities (CPI) and lethality were observed in Daedaye venom, necrotic in Pantanaw, haemorrhagic in Pyapon and coagulant in Nyaungdone. Immature snake (<90 cm) of Danuphyu venom possessed potent CPI with poor coagulant activities and Nyaungdone venom was the least necrotic among immature snakes. SDS-PAGE electrophoresis of venoms showed quantitative and qualitative changes of protein bands. In general biological properties of venoms of mature and immature snakes from the same locality are comparable except for a few differences.

INTRODUCTION

Geographical variation of venom properties and composition has been reported including Russell's viper of Myanmar [1,2,3,4]. However there are very few reports on such studies in Myanmar except one reported by us recently. It is high time than further studies on venoms from divisions where most Russell's viper bites occurred will help in better understanding of pathophysiology of envenoming from different localities of Myanmar and will provide useful information for antivenom manufacturer. In this communication, biological properties of venoms from different localities of Ayeyarwady division were studied.

MATERIALS AND METHODS

Snakes from 5 localities (Fig. 1) (Nyaungdon, Danuphyu, Daedaye, Pyapon and Pantanaw) of Ayeyarwady division, bought at Myanma Pharmaceutical Industry were milked and

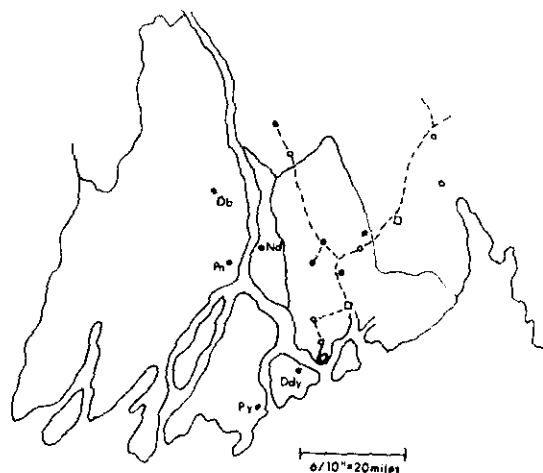


Fig. 1. A map of 5 localities of Ayeyarwady division where venoms were available for study. Db=Danuphyu, Nd=Nyaungdone, Pn=Pantanaw, Py=Pyapon and Ddy=Daedaye

lyophilised individually. The length of the snake and the colour of venom were noted. Venoms were pooled into 2 groups, immature (<90 cm) and mature (>90 cm). Immature snakes were not available for study

from Pantanaw and Pyapon.

Biological properties of venom such as coagulant (MCD), haemorrhagic (MHD), necrotic (MND), defibrinogenating (MDD) were studied according to WHO recommended techniques [5]. Approximate LD₅₀ i.v. [6]. Capillary permeability increasing (CPI) [7], phospholipase activity (MIHD) [8] and SDS-PAGE electrophoresis of 12% separating gel were also carried out.

RESULTS

Biological properties of venoms from 5 localities were shown in Table 1, 2 and 3.

Immature Nyaungdone venom possess potent lethality, defibrinogenating, coagulant and very weak necrotic activities whereas Danuphyu venom has potent CPI with weak coagulant activities (Table 1).

Among mature venom, potent lethality, defibrinogenating, CPI, phospholipase activities were observed in Daedaye venom, haemorrhagic in Pyapon, necrotic in Pantanaw and coagulant in Nyaungdone. Weak coagulant, defibrinogenating activities in Danuphyu and phospholipase activity in Pantanaw were also observed (Table 2). Venom properties of mature and immature snakes from the same locality was comparable except for few properties like very weak MND in immature and potent MCD in mature Nyaungdone venom, potent CPI in immature Danuphyu venom and potent MDD, CPI and PLA₂ in mature Daedaye venom (Table 3).

The colour of venom of both mature and immature snakes varies from pale yellow to yellow except immature venom of Danuphyu which is white, SDS-PAGE electrophoresis of immature and mature venoms from different localities showed qualitative and quantitative difference in protein bands (Fig. 2).

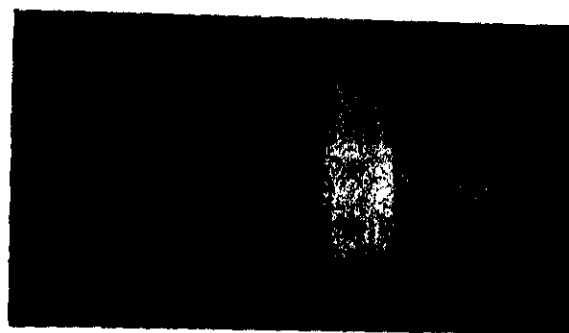


Fig. 2. SDS-PAGE electrophoresis of venoms from different localities of Ayeyarwady division using 12% separating gel. Lane 1-5 are mature venoms of Nyaungdone, Danuphyu, Daedaye, Pyapon and Pantanaw respectively; Lane 7-9 are immature venoms of Nyaungdone, Danuphyu and Daedaye respectively.

DISCUSSION

Preliminary study of venoms from 4 localities of Myanmar by our group showed length and geographical variation of biological properties of venom [4]. Such variation in toxicity of other venoms with geographical origin has been reported [1,2,3]. In clinical field practice, two populations of snake were responsible for bites during harvesting season in Tharyarwady [9]. Therefore, biological properties of venom were studied on 2 groups of snake, immature (<90 cm) and mature (>90 cm) based on the observation that 96% (51/55) pregnant vipers have a total length of >90 cm (unpublished observation). It is interesting to note that 92% (23/25) of immature venom of Danuphyu is white compared to 64% (131/204) of immature venom (unpublished observation) and has potent CPI but weak coagulant, defibrinogenating activity and lethality whereas Nyaungdone venom is least necrotic. Geographical variation of venom may be responsible for the colour difference. Individual variation in colour of venoms of immature and mature snakes was also observed. In general venoms of

Table 1. Biological properties of immature venoms of 3 localities of Ayeyarwady division

Locality	Length	No. snake	LD ₅₀ Approx: ug/mouse ± 1 SD	MHD ug/rat	MND ug/rat	MDD ug/mouse	MCD ug/ml	MCPID ug/ml	MIHD ug/ml
Daedaye	61-90	7	3.50 ± 1.96	42.17	29.85	3.33	0.159	0.016	3.16
Nyaungdon	61-90	17	2.87 ± 1.47	36.14	60.26	2.40	0.1	0.018	3.16
Danuphyu	71-90	14	4.42 ± 1.19	30.90	30.90	4.20	1.995	0.004	2.63

Table 2. Biological properties of mature venoms of 5 localities of Ayeyarwady division

Locality	Length	No. snake	LD ₅₀ Approx: ug/mouse ± 1 SD	MHD ug/rat	MND ug/rat	MDD ug/mouse	MCD ug/ml	MCPID ug/ml	MIHD ug/ml
Daedaye	91-120	7	2.86 ± 1.42	28.18	27.86	1.78	0.135	0.009	1.82
Nyaungdone	91-120	18	3.30 ± 2.35	46.77	32.36	2.40	0.033	0.015	2.63
Danuphyu	91-120	14	6.38 ± 0.98	30.20	27.54	5.10	2.291	0.018	2.51
Pyapon	91-130	4	6.44 ± 0.54	23.99	40.27	2	0.26	0.020	6.92
Pantanaw	91-110	4	5.39 ± 1.03	32.73	20.42	3	0.070	0.063	9.12

Table 3. Biological properties of immature and mature venoms of 3 localities of Ayeyarwady division

Locality	Length	No. snake	LD ₅₀ Approx: ug/mouse ± 1 SD	MHD ug/rat	MND ug/rat	MDD ug/mouse	MCD ug/ml	MCPID ug/ml	MIHD ug/ml
Daedaye	61-90	7	3.50 ± 1.96	42.17	29.85	3.33	0.159	0.016	3.16
	91-120	7	2.86 ± 1.42	28.18	27.86	1.78	0.135	0.009	1.82
Nyaungdone	61-90	17	2.87 ± 1.47	36.14	60.16	2.40	0.1	0.018	3.16
	91-120	18	3.30 ± 2.35	46.77	32.36	2.40	0.033	0.015	2.63
Danuphyu	71-90	14	4.42 ± 1.19	30.90	30.90	4.20	1.995	0.004	2.63
	91-120	14	6.38 ± 0.98	30.90	27.54	5.10	2.291	0.018	2.51

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MDD = Minimum defibrinogenating dose

MCD = Minimum coagulant dose

MCPID = Minimum capillary permeability increasing dose

MIHD = Minimum indirect haemolytic dose

immature and mature snakes from the same locality have comparable except for a few differences (see result). Mature venom of Daedaye is the most potent among Ayeyarwady division, however, it is less potent than Daik-Oo in lethality, haemorrhagic and CPI activities [4]. Although Daedaye and Kungyankone are not far from each other, marked variation in biological properties of venom was observed in contrast to our previous observation made where venoms from closed localities have comparable properties [4]. It is hoped that these findings will be useful for antivenoms manufacturer as well as clinicians who are treating Russell's viper bite cases in Ayeyarwady division).

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**Establishment of staphylococcal clumping test and detection of
normal level of fibrin(-ogen) degradation products in Myanmar
(Staphylococcal clumping test and normal FDP level in Myanmar)**

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Staphylococcal clumping test was established using a coagulase positive strain of staphylococci. 10 mg/ml of bacterial cell suspension was used as the optimum concentration for detecting 2 ug/ml of fibrinogen reference solution. The sensitivity of our method is of 2 ug/ml of fibrinogen. Using the staphylococcal clumping test, 150 samples of healthy blood donors were tested. The normal fibrin(-ogen) degradation product levels of Myanmar ranged from zero to 10 ug/ml. The results obtained from the staphylococcal clumping test were comparable to those obtained from Thrombo-Wellco latex agglutination test (Wellcome Diagnostics).

INTRODUCTION

The increased level of circulating fibrin(-ogen) degradation products (FDP) is found in disseminated intravascular coagulation (DIC) as a result of secondary or compensatory fibrinolysis. It is used as one of the sensitive indicators of DIC.

There are many commercially available kits for detecting FDP. However it can be carried out only in few places because they are very expensive and moreover they have to be purchased from foreign companies, using our limited resources of foreign currency. Therefore, locally prepared FDP assay kit should be established to save foreign currency and to make it available to more places in the country.

On the other hand, many workers (Mertens et al., 1969; Hawiger et al., 1970) have studied the FDP level in the serum of normal subjects using various techniques. Hence it is

essential to determine the FDP levels of normal Myanmar subjects using our established Staphylococcal Clumping Test (SCT) method.

MATERIALS AND METHODS

Staphylococcus aureus

Stock culture of coagulase positive strain of *S. aureus* was obtained from National Health Laboratory (NHL). It was rechecked for coagulase positivity by slide agglutination test (Cruickshank, 1970) using normal human plasma.

Subculture of *S. aureus* from stock culture was done on a trypticase soya agar (TSA) plate using the standard technique and the confirmation of *S. aureus* was done accordingly (Cruickshank, 1970).

Preparation of staphylococcal clumping factor

Preparation of staphylococcal clumping

factor (SCF) was done according to Hawiger et al. (1970). The optimum concentration of staphylococcal clumping factor was determined by using various concentrations of lyophilized bacterial powder (5 mg, 10 mg and 15 mg in 1 ml of distilled water) tested against different concentrations of fibrinogen reference solution (80 ug/vial from Sigma Company). The trizma-albumin solution (Sigma Company) was used for diluting fibrinogen reference. By using the optimum concentration of SCF, the lowest concentration of fibrinogen solution was also measured.

Subjects

Blood samples of 150 healthy blood donors (male 145, female 5, age 18 to 50 years) from blood bank at Yangon General Hospital and sera of 60 Russell's viper bite victims from Thayarwady township hospital were tested. Two millilitres of blood collected from each donor were added to respective test tubes, containing 0.1 ml of thrombin (100 unit/ml) and 0.05 ml of 100 gm EACA. It was mixed immediately by inverting the tube gently several times and then placed in a water bath at 37°C for half an hour. At the end of the incubation period, firm clots formed were removed gently with application stick. The tubes were then centrifuged at 3,000 rpm for 10 minutes. The supernatant sera was transferred into another tube and then stored at -20°C till tested.

The serum were diluted with trizma saline buffer (TSB) tested according to Thrombo-Wellco latex agglutination assay.

Recording and reading of results

Positive = Clumping of bacterial cell suspensions within 2 minutes

Negative = Uniform milky suspension.
No clumping.

The end point of the test is the highest dilution of serum showing clumping of cells. FDP is expressed as fibrinogen equivalent (ug/ml) by multiplying the dilution factor of TEST end point by the level of fibrinogen in the reference solution (2 ug/ml).

Calculation

FDP = Dilution factor of TEST end point x Concentration of fibrinogen (ug/ml) showing comparable intensity of clumping

Example:

$$\begin{aligned} \text{FDP} &= 5 \times 2 \\ &= 10 \text{ ug/ml} \end{aligned}$$

In order to evaluate the sensitivity and specificity of SCT, Thrombo-Wellco latex agglutination test (TWT) was used as the reference test and all the results of SCT were compared with those obtained with the Thrombo-Wellcotest.

RESULTS

Table 1 shows the bacterial cell suspension tested with varying dilution of fibrinogen reference solution. The highest dilution of fibrinogen solution at which clumping was found to be 1:4 dilution corresponding to 2 ug/ml fibrinogen consistently. Therefore the sensitivity of the method was 2 ug/ml of fibrinogen. The concentration of 5 mg/ml, 10 mg/ml and 20 mg/ml bacterial cell suspension were found to give clumping reaction within 2 minutes with 2 ug/ml fibrinogen. However only the latter 2 concentrations gave strong reaction. Hence, 10 mg/ml and 20 mg/ml of bacterial suspensions were taken as optimum concentration. In the subse-

Table 1. Bacterial cell suspension tested with varying dilution of fibrinogen reference solution

Bacterial cell suspension concentration	Fibrinogen solution concentration					Control (TSB)
	8 ug/ml	4 ug/ml	2 ug/ml	1 ug/ml	0.5 ug/ml	
5 mg/ml	+	+	+	-	-	-
10 mg/ml	++	++	++	-	-	-
20 mg/ml	++	++	++	-	-	-

quent tests, the bacterial cell suspension of 10 mg/ml was chosen to be used.

The summary of serum FDP levels of 150 normal donors is shown in table 2. 95% of subjects had serum FDP levels of 0-<1 ug/ml and only 5% had >2-<10 ug/ml. Table 3 shows the comparative serum FDP results of Russell's viper bites patients. Each sample was tested with both SCT and TWT and same results were obtained.

Table 2. Summary of FDP results of 150 normal sera

	FDP (ug/ml)	No. of subjects	% of total
Normal sera	0 - <2	143	95%
	>2 - <10	7	5%

DISCUSSION

The presence of FDP in blood gives more definite evidence of an intravascular proteolytic episode than the conventional "fibrinolytic activity" methods (Merskey et al., 1966). There are several methods available for measuring FDP. Among them the currently and commonly used methods are the tanned red cell haemagglutination inhibition immunoassay (TRCHII), Thrombo-Wellco latex agglutination test and SCT. Based on these three methods,

Table 3. FDP results of snake bite sample tested with SCT and TWT

Number of RV cases	Test	The highest serum dilution at which clumping was found	Results (ug/ml)
3	TWT	1/5	>10<20
	SCT	1/5	>10<20
7	TWT	1/10	>20<40
	SCT	1/10	>20<40
13	TWT	1/20	>40<80
	SCT	1/20	>40<80
7	TWT	1/40	>80<160
	SCT	1/40	>80<160
7	TWT	1/80	>160<320
	SCT	1/80	>160<320
2	TWT	1/160	>320<640
	SCT	1/160	>320<640
11	TWT	1/320	>640<1280
	SCT	1/320	>640<1280
10	TWT	1/640	>1280<2560
	SCT	1/640	>1280<2560

different types of kits are available.

The sensitivity of one test differed from the other. TRCHII is sensitive to 0.6-1.25 ug (fibrinogen equivalent) FDP per ml whereas Thrombo-Wellco latex agglutination test is sensitive to 2 ug (fibrinogen equivalent) per ml. Regarding SCT, Hawiger and co-workers (1970) found that it was very sensitive for estimating FDP and the lower limit of sensitivity of their method being 0.04 ug per ml. This is contrary to our test sensitivity of

2 ug/ml. This may be due to the difference in the type of fibrinogen used as a standard.

The results of our study showed that 10 mg/ml of bacterial cell suspension can detect FDP in minimum concentration of 2 ug/ml. Using this concentration of bacterial cell suspension, out of 150 blood donors, the neat sera of 7 subjects showed weak positive clumping reaction. It was corresponding to FDP levels of greater than 2 ug/ml and less than 10 ug/mg. The remaining 143 cases showed no clumping reaction which corresponds to FDP levels of less than 2 ug/ml. The results obtained from SCT were comparable to those obtained from Thrombo-Wellco latex agglutination test.

The normal value of FDP in our subjects measured with SCT was in agreement with those of some workers (Allington et al., 1967, Mertens et al., 1969). The differences in the FDP levels of normal human subjects studied by different workers may be due to differences in the technique, age and sex. However, irrespective of these differences, FDP levels of normal sera ranged from a value of zero to less than 10 ug/ml in general.

Therefore, the results of our study indicated that our established SCT was as sensitive and as reliable as the commercially available Thrombo-Wellco latex agglutination test kit from Wellcome Diagnostics.

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The drug sensitivity patterns of Plasmodium falciparum in Myanmar
(1990)

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During 1990, sensitivity of P. falciparum to antimalarials was conducted in Mawlamyaing, Lashio and Seik Phyu townships. There were 40 successful in vitro tests in Mawlamyaing, 39 in Lashio, 26 in Seik Phyu township. Chloroquine was found to be highly resistant (95 to 100%) in Mawlamyaing and Lashio areas but 31% sensitivity was detected in Seik Phyu township. Amodiaquine was found to be much more sensitive (38 to 67%) than chloroquine. Effective concentration (EC₅₀) for chloroquine varies from 7.4 to 8.4 μ mol/L and EC₅₀ for amodiaquine varies from 0.58 to 1.6 μ mol/L. In all the areas studied sulfadoxine/pyrimethamine sensitivity was seen in 65 to 80%. All the isolates from the three areas were 100% sensitive to mefloquine and were also highly sensitive (93 to 100%) to quinine. EC₅₀ of quinine varies from 1.35 to 2.7 μ mol/L and EC₅₀ of mefloquine varies from 1.5 to 2.2 μ mol/L in the areas.

INTRODUCTION

The emergence and wide spread of resistant of P. falciparum to four aminoquinolines, sulphadoxine/pyrimethamine and quinine has been well documented in Myanmar [1,2,3]. The P. falciparum malaria patients resistant to antimalarials were also reported in hospital based study [4].

Multidrug resistance is becoming a major public health concern, especially in where population movement of non-immune to malaria endemic areas has increased during the years.

The in vivo sensitivity of P. falciparum to antimalarials involve many host factors, such as absorption and metabolism of drugs and host immunity. So the in vitro sensitivity method was chosen to find out the true nature of the sensitivity of P. falciparum isolates to antimalarials in

three areas of Myanmar. This study was conducted in Mawlamyaing, Lashio and Seik Phyu townships for the drug sensitivity patterns of P. falciparum in Myanmar during July to November 1990.

Study area and population

The study was conducted in Mawlamyaing, Lashio and Seik Phyu townships. During July to November 1990.

The screening of malaria infection was carried out among the population living in the respective areas. Population of 600 to 1300 were screened to meet the selection of In vitro criteria of WHO [5].

Preparation of blood films

Thick blood films were made from the deep prick of the tip of the patient's finger. The films were

quickly dried and stained with 10% Giemsa solution for 10 mins.

Selection criteria

The *P. falciparum* infected patients with 500 to 40,000 asexual parasites per μ l of blood was included in the study. The patients without the history of taking antimalarial prior 14 days of the study were included in the study.

In vitro drug testing

In vitro, drug testing was done according to the Rickmann microtechnique (1978) [6] using the precharged plates of chloroquine, quinine, amodiaquine, sulphadoxine/pyrimethamine and mefloquine which were provided by WHO. From each patient, 0.5 ml venous blood was collected with 1 ml syringe and mixed with 4.5 ml RPMI 1640 medium previously buffered with 25 m mol/L HEPES and 32 m mol/L NaHCO_3 . Aliquots (50 μ l) of blood-mixture were pipetted into the wells of the microtitration plates which were dosed with various concentrations of chloroquine, amodiaquine, mefloquine and quinine.

For assessment of resistance to sulfadoxine/pyrimethamine, the blood was mixed with RPMI 1640 medium containing lower concentrations of p-aminobenzoic acid (PABA) and folic acid [5]. Final concentrations of pyrimethamine were from 0.0125 to 12.5 μ mol/L blood with a constant ratio of pyrimethamine to sulfadoxine of 1:80. Control wells without any drug were always included in all assays.

The test plates were placed in a candle jar and incubated at 37.5°C for 26 to 30 hours. At the end of incubation, thick blood smears were prepared and stained with Giemsa. For each drug concentration tested, the number of schizonts per 200 asexual parasites was determined. Isolates with a schizont maturation (above 3 nuclei) of less than 10% in

the control wells were not used for evaluation.

RESULTS

There were 4 isolates from Mawlamyaing 39 isolates from Lashio and 26 isolates from Kazunma area successfully carried out for the in vitro tests. The response pattern to chloroquine from the three areas was shown in table 1(a).

Chloroquine resistant rate of *P. falciparum* isolates from the three areas varies from the highest resistant rate of 100% in Mawlamyaing to lowest rate 69% in Kazunma area.

The in vitro sensitivity of *P. falciparum* to amodiaquine was shown in table 1(b). The *P. falciparum* isolates were significantly more sensitive to amodiaquine than chloroquine in all the areas studied. The amodiaquine resistant rates of the three areas varied from the highest resistant rate of 62% in Lashio to lowest resistant rate of 32% in Mawlamyaing was detected.

The *P. falciparum* isolates were moderately resistant to sulfadoxine/pyrimethamine in all the three areas. The *P. falciparum* isolates were 86% sensitive to sulfadoxine/pyrimethamine in Kazunma, 73% in Mawlamyaing and 65% sensitive in Lashio (Table 2a).

P. falciparum isolates from the three areas were highly sensitive to quinine with a sensitivity rate of 93% to 100% (Table 2b). The mefloquine was found to be 100% sensitive in all the areas studied (Table 3a).

Drug resistance was assumed if schizont maturation was observed at 1.6 μ mol chloroquine to 0.4 μ mol amodiaquine/L and 3.2 μ mol mefloquine/L and 51.2 μ mol quinine/L, while resistance to sulfadoxine/pyrimethamine was considered if there was less than 90%

Table 1(a). The in vitro sensitivity of P. falciparum isolates to chloroquine

Dilution of drug (p mole/well)	Cumulative Number (%) inhibition of isolates		
	Mawlamyaing (n=40)	Lashio (n=39)	Seik Phyu (n=26)
1	0/40 (0%)	0/39 (0%)	0/26 (0%)
2	0/40 (0%)	0/39 (0%)	0/26 (0%)
4	0/40 (0%)	0/39 (0%)	0/26 (0%)
8	0/40 (0%)	2/39 (5.13%)	8/26 (31%)
16	0/40 (0%)	6/39 (15.4%)	12/26 (46%)
32	11/40 (27.5%)	18/39 (46.2%)	17/26 (65%)
64	23/40 (57.5%)	26/39 (66.7%)	23/26 (88%)

Underlined showed the cut off point for sensitivity

Table 1(b). The in vitro sensitivity of P. falciparum isolates to amodiaquine

Dilution of drug (p mole/well)	Cumulative Number (%) inhibition of isolates		
	Mawlamyaing (n=40)	Lashio (n=40)	Seik Phyu (n=18)
0.25	0/40 (0%)	0/40 (0%)	0/18 (0%)
0.5	0/40 (0%)	0/40 (0%)	0/18 (0%)
1.0	3/40 (7.5%)	5/40 (12.5%)	1/18 (6%)
2.0	15/40 (37.5%)	27/40 (67.5%)	10/18 (56%)
4.0	22/40 (55%)	31/40 (77.5%)	12/18 (67%)
8.0	30/40 (75%)	37/40 (92.5%)	13/18 (72%)
16.0	35/40 (87.5%)	40/40 (100%)	18/18 (100%)

Underlined showed the cut off point for sensitivity

Table 2(a). The in vitro sensitivity of P. falciparum isolates to sulphadoxine/pyrimethamine

Dilution of drug (p mole/well)	Cumulative Number (%) inhibition of isolates		
	Mawlamyaing (n=40)	Lashio (n=40)	Seik Phyu (n=28)
10	0/40 (0%)	0/40 (0%)	0/28 (0%)
30	0/40 (0%)	0/40 (0%)	0/28 (0%)
100	8/40 (20%)	6/40 (15%)	6/28 (21%)
300	29/40 (72.5%)	26/40 (65%)	24/28 (86%)
1000	29/40 (72.5%)	29/40 (72.5%)	25/28 (89%)
3000	34/40 (85%)	33/40 (82.5%)	26/28 (93%)
10000	38/40 (95%)	36/40 (90%)	28/28 (100%)

Underlined showed the cut off point for sensitivity

Table 2(b). The in vitro sensitivity of P. falciparum isolates to quinine

Dilution of drug (p mole/well)	Cumulative Number (%) inhibition of isolates		
	Mawlamyaing (n=39)	Lashio (n=40)	Seik Phyu (n=24)
4	0/39 (0%)	0/40 (0%)	0/24 (0%)
8	0/39 (0%)	0/40 (0%)	0/24 (0%)
16	0/39 (0%)	0/40 (0%)	2/24 (8%)
32	8/39 (21%)	8/40 (20%)	10/24 (42%)
64	15/39 (38%)	15/40 (37.5%)	15/24 (63%)
128	20/39 (51%)	27/40 (67.5%)	22/24 (92%)
<u>256</u>	<u>37/39 (95%)</u>	<u>37/40 (92.5%)</u>	<u>24/24 (100%)</u>

Underlined showed the cut off point for sensitivity

Table 3(a). The in vitro sensitivity of P. falciparum isolates to mefloquine

Dilution of drug (p mole/well)	Cumulative Number (%) inhibition of isolates		
	Mawlamyaing (n=39)	Lashio (n=40)	Seik Phyu (n=21)
2	0/39 (0%)	1/40 (2.5%)	6/21 (29%)
4	17/39 (44%)	15/40 (37.5%)	15/21 (71%)
8	34/39 (87%)	33/40 (82.5%)	20/21 (95%)
<u>16</u>	<u>39/39 (100%)</u>	<u>40/40 (100%)</u>	<u>21/21 (100%)</u>
32	39/39 (100%)	40/40 (100%)	21/21 (100%)
64	39/39 (100%)	40/40 (100%)	21/21 (100%)
128	39/39 (100%)	40/40 (100%)	21/21 (100%)

Underlined showed the cut off point for sensitivity

Table 3(b). Effective concentration (EC μ mol/L of blood) of the antimalarials in P. falciparum isolates calculated by probit analysis

	Mawlamyaing		Lashio		Kazunma		Total	
	EC ₅₀	EC ₉₉	EC ₅₀	EC ₉₉	EC ₅₀	EC ₉₉	EC ₅₀	EC ₉₉
CHLOROQUINE	1.05	8.4	0.72	7.4	0.63	7.4	0.74	8.4
AMODIAQUINE	0.15	16.	0.1	0.6	0.13	1.5	0.13	1.7
QUININE	0.24	2.5	0.26	2.7	0.14	1.15	0.2	2.4
MEFLOQUINE	0.27	1.6	0.12	2.2	0.05	1.5	0.18	1.9

schizont inhibition at a concentration of 60 u mol sulfadoxine 0.75 u mol/L of blood. The results were statistically evaluated using log-dose response probit analysis.

DISCUSSION

Mawlamyaing township is situated in the southern part of Myanmar with a high rate of population movement with Thailand border areas. Lashio township being in the Eastern area also has high population movement with Chinese border areas. However the Seik Phyu township is in the middle part of Myanmar with least population movement.

The parasite rate, at the time of screening were 12% in Seik Phyu and 17% in Mawlamyaing and in Lashio townships.

P. falciparum isolates from Mawlamyaing and Lashio areas were found to be completely resistant (95% to 100%) to chloroquine. However 31% of the *P. falciparum* isolates of the Seik Phyu areas were found to be still sensitive to chloroquine.

Amodiaquine was found to be a better drug than chloroquine in the areas studied. Though *P. falciparum* of the Mawlamyaing and Lashio townships were almost completely resistant to chloroquine, the isolates were 33% to 63% still sensitive to amodiaquine. Also the *P. falciparum* isolates from Seik Phyu township area were 44% sensitive to amodiaquine in compare to that of 69% sensitive to chloroquine. The *in vivo* study carried out in Tharyarwady Hospital also showed that amodiaquine has a better efficacy than the chloroquine on *P. falciparum* infected patients [3].

Though there were 80% resistant rate ($R_1 + R_2$) for chloroquine, only 58.6% resistant rate to amodiaquine was detected. However a similar pattern of resistance of *P. falciparum*

isolates to amodiaquine and chloroquine had been reported in a study in Acre, Brazil [4]. The discrepancy in results might be due to the differences in the *P. falciparum* population.

The EC₅₀ and EC₉₉ levels for quinine of the three areas were 0.2 and 2.4 u mol/L of blood respectively. Also the *in vitro* sensitivity of *P. falciparum* isolates to quinine in the three areas showed that 93 to 100% of the isolates were inhibited by quinine concentration below the generally accepted threshold of 5.12 u mol/L for *in vitro* resistant cut off point. The results tally with that of the *in vivo* study carried out in Yangon [1], where 91.42% of the *P. falciparum* infected patients were cured by quinine treatment [1]. However higher rate of quinine resistance have been reported [3] and [2].

The difference in the *in vivo* results may be due to the variation in place, time and the sample population.

Mefloquine is found to be highly sensitive in all the areas studied with 100% of the isolates gave schizont inhibition at 3.2 u mol/L (The EC₅₀ EC₉₉ were 0.18 and 1.9 u mol/L of blood respectively) (Table 3b). The study is in agreement with the study [1], carried out on 45 patients with mefloquine where 100% of them were cured [1].

In the test system for measuring sensitivity to sulfadoxine/pyrimethamine, schizonts were considered to be matured only if they had at least eight nuclei of normal appearance. The cut off point was taken at 3.75 u mol/L [4]. The *P. falciparum* isolates from the three areas were found to be 65% to 86% sensitive.

However 66% of the *P. falciparum* infected patients were reported to be resistant to sulfadoxine/pyrimethamine in a hospital based study [3].

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The efficacy of a bolus dose of Russell's viper antivenom in threatening viper bite cases

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Serum venom levels before and after administration of an intravenous bolus dose of 40 ml lyophilised monospecific antivenom manufactured by Myanma Pharmaceutical Factory in nine Russell's viper bite cases were measured by enzyme immunoassay (EIA) technique. In 3 out of 9 cases studied, venom antigen was still detectable in blood for more than 2h after therapy. Therefore, a single 40 ml bolus dose of antivenom is not sufficient to completely neutralise circulating venom in some cases of Russell's viper bite victims.

INTRODUCTION

The prime objective of treatment of snake bite victims with antivenom is to achieve prompt neutralisation of circulating venom so as to prevent ill effects produced by systemic envenoming. The antivenom used should be potent and adequate quantity must be administered as early as possible following bite. The neutralising dose of antivenom depends on the amount of venom deposited, route of administration and the kinetics of venom clearance in the body. In the absence of these information, a 40 ml of antivenom is given to all patients who give a history of Russell's viper bite with a normal or incoagulable blood at the time of admission. Failure of complete neutralisation of venom in the blood of Russell's viper bite victims treated with a single dose of 40 ml liquid antivenom (1) prompted us to study the efficacy of a bolus dose of 40 ml lyophilised antivenom in Russell's viper bite victims.

MATERIALS AND METHODS

A total of 9 patients bitten by *Vipera russelli*, confirmed retrospectively by immunodiagnosis, were studied mainly

in the Insein Civil Hospital from December 1982 to June 1983. Only one case from Kyauktaga Hospital was included in the study. A single batch of lyophilised monovalent Russell's viper antivenom (ASV) manufactured by the Myanma Pharmaceutical Factory, batch no. DF 82487 expiry 16-7-87 was used in treating all cases from the Insein Civil Hospital. These were stored in the cupboard at +40°C in Insein hospital and at room temperature in Kyauktaga hospital.

A standard dose of 40 ml lyophilised antivenom was administered intravenously immediately before removal of tourniquets to all patients. The patient from Kyauktaga hospital received an additional 20 ml antivenom after 4 h of the initial antivenom therapy because of the persistence of nonclotting state.

Serum samples were collected on admission and at 2,4,6,12,36 and 48 h following antivenom and were stored at +4°C or in an ice box where no cold storage facility is available and were despatched to the Department of Medical Research as soon as sample collection was completed i.e. after 48 h. Serum venom levels were assayed by enzyme immu-

noassay technique (EIA) [1]. Clotting time was done according to the method described by Warrell *et al.* [2].

RESULTS

Clinical Features

Of 9 cases studied, all patients have local swelling on admission, the increase in circumference of the bitten limb compared to control limb ranged from 0.5 cm to 5 cm. Blister developed in two cases resulted from initial treatment of the wound. Local lymphadenopathy was detected in 3 cases. The age ranged from 16 yr to 53 yr with male to female ratio of 2:1. Eight out of nine cases were bitten below ankle, toe (3), heel (2), dorsum of foot (2) and one at the ankle. Only one case was bitten above ankle.

Seven out of 9 cases studied have initial incoagulable blood indicating systemic envenoming. Five out of 7 patients presented with spontaneous haemorrhages such as haematuria (4), conjunctival haemorrhages (4), haemoptysis (3), bleeding from venipuncture site (3), haematemesis (1) and malena (1). Subconjunctival oedema (2/5) and periorbital oedema (1/5) were also observed. The case from Kyauktaga hospital developed bilateral swelling of the parotid glands 2 days after admission and lasted for 48 h. Two out of 7 systemic cases presented with hypotension; one has a blood pressure of 90/60 mmHg and the other with shock of 40 mmHg by palpation and 3 cases were fatal.

Serum venom levels before and after antivenom

Initial serum venom levels ranged from 18.5 ng to 175 ng/ml. Interval between time of bite and initiation of treatment ranged from 1.30 h to 48 h. It was found that in 3 out of 9 cases where the initial venom levels were 90 ng/ml or more, treatment with a single bolus dose of 40

ml lyophilised antivenom failed to completely neutralise the venom antigen in 2 h following therapy (Fig). Non clotting blood persisted as long as 18 h after antivenom therapy in a case whose initial serum venom level was 175 ng/ml.

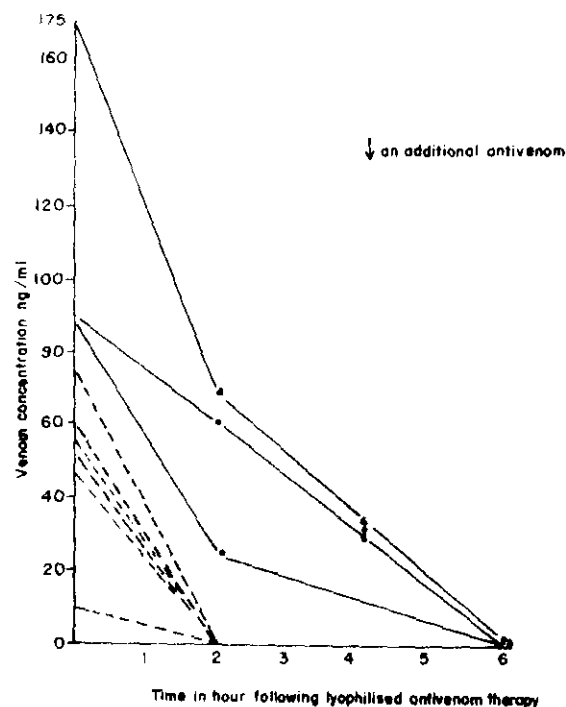


Figure: Venom antigen levels following 40 ml lyophilised monospecific antivenoms.

DISCUSSION

The routine practice of antivenom therapy in snake bite is administration of 40 ml of antivenom to all patients who gave a convincing history of Russell's viper bite with a normal or non clotting blood at the time of admission. This antivenom dose is based on the fact that 1/10 of the average yield of *Vipera palaestinae* (800 mg) is injected per bite. Therefore 40 ml of antivenom will be able to neutralise 80 mg of venom since 1 ml of antivenom can neutralise 2 mg of venom according to pigeon protection test (Myanma Pharmaceu-

tical Factory phamplet). Since a bolus dose of 40 ml antivenom failed to neutralise a venom level of 90 ng/ml and above in 3 out of 9 cases the dose of antivenom for routine use should be reassessed. Trinca (3) emphasised that the dose of antivenom calculated on the average yield of venom obtained through milking may not be adequate in some cases because snake may injected more venom than the average yield of venom. Moreover the initial dose of antivenom based on in vitro mouse protection test was found to be misleading in predicting the performance of antivenom in patients (4). It may be concluded that 40 ml of antivenom is not sufficient to neutralise circulating venom in 2 hr in some cases of human Russell's viper bite cases.

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Detection of Entamoeba histolytica antigen in stool samples by enzyme-linked immunosorbent assay using monoclonal antibodies

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An enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies, was applied for the detection of Entamoeba histolytica antigen in stool samples obtained from 148 patients with gastrointestinal symptoms, and the results are compared with microscopic findings. Ninety nine positives by microscopy generally had high ELISA OD values. Ninety one stool samples of asymptomatic cyst passers were also investigated by ELISA, and most were found to be positive. Although false positives were observed in both symptomatic and asymptomatic cases, the ELISA appears to be useful for the detection of amoebic antigen(s). However, our results suggest that both immunological methods and microscopic examination are needed for an accurate diagnosis of intestinal amoebiasis.

INTRODUCTION

Human infections with Entamoeba histolytica are widely distributed throughout the world. The parasite causes an enormous burden of illness and death from colitis and hepatic abscesses. It is estimated that about 10% of the world's population carry the parasite, although a much smaller percentage suffers from disease [1]. The prevalence rate of amoebiasis was shown to range from 3.6 to 47.4% in Eastern Asia [2]. In Myanmar, epidemiological data from two different areas indicated that amoebic dysentery and liver abscess are common: it was estimated that 0.3 to 7.2% of the population in rural areas carried the parasite [3,4]. In 994 stool samples obtained from gastroenteritis patients, identifiable trophozoites or cysts were found in 596 (89.2%) [5].

The diagnosis of intestinal amoebiasis usually depends on demonstration of the organism in faeces through serial stool examinations by persons with adequate expertise. However, there

are some inherent limitations in microscopic diagnosis, i.e., periodicity [5] and variation in daily numbers [6] of parasites discharged into the stool. To overcome these difficulties, serological techniques for detection of infection have been developed, including counterimmunoelectrophoresis (CIE), immunofluorescent assay (IFA), and enzyme-linked immunosorbent assay (ELISA). These serological methods are very useful for diagnosing asymptomatic cases and evaluating the prevalence of invasive infections [7]. Recently, ELISA techniques have been applied to detect the organism in human faces [8,9].

In this study, the applicability and efficacy of ELISA using monoclonal antibodies (MAb's) against E. histolytica trophozoites, were compared with the diagnosis of the parasite by microscopy.

MATERIALS AND METHODS

Stool samples

Stool samples were collected from

148 patients, with diarrhoea or dysentery, hospitalized in four different hospitals in Yangon during the period between 1987 and 1989. Direct smears of these samples were examined by microscopy. In addition, 91 stool samples from asymptomatic individuals admitted to other hospitals were collected and were examined by direct smear method also formal-ether concentration method [10].

Preparation of test samples

0.5 g of faecal material from each individual were suspended in 5 ml of 0.05 M phosphate buffered saline (PBS, pH 7.2) and kept at -70°C overnight. After thawing, the stool suspensions were centrifuged at 10,000 x g for 20 min, and the supernatant was used for the ELISA test. The sensitivity of the ELISA test was measured using homogenates of the HK-9 strain of *E. histolytica* as antigen. The organisms were cultured axenically in BI-S-33 medium, as described by Diamond (1968) [11]. The parasites were harvested, washed with PBS, and homogenated by ultrasonification. The protein concentration of the amoebic antigen suspension was measured by the procedure of Lowry et al. [12].

Monoclonal antibodies

The MAb's EHK82, EHK142 and EHK153, produced against trophozoites of the HK-9 strain of *E. histolytica*, and MAb's EHM83 and EHM110, produced against the HM1:IMSS strain, were obtained as previously described by Tachibana et al. [13].

Rabbit polyclonal antibodies against E. histolytica

Polyclonal antibodies were produced against the HM-1:IMSS strain of *E. histolytica* as follows; a rabbit was inoculated with a homogenate of cultured trophozoites in Freund's complete adjuvant. Six weeks later, the animal was exsanguinated, and the serum collected.

ELISA procedure

The assay was performed according to Matsuda et al. [14]. One hundred μ l of MAb solution, diluted to 10 μ g of protein per ml with 0.01 M carbonate buffer (pH 9.6), were added to each well of a flat-bottomed microtiter plate for ELISA (Sumitomo Bakelite, Tokyo). The plate was incubated at 37°C for 2 hours, then kept at 4°C until used. All wells were washed with PBS containing 0.05% Tween-20 (PBS-T). One hundred μ l of the stool suspension to be tested was then added to each well and incubated at 37°C for 1 hour. After washing the plate with PBS-T three times, 100 μ l of the anti-*E. histolytica* rabbit serum, adjusted to 10 μ g/ml by PBS-T containing 1% bovine serum albumin (BSA-T), was added to each well and the plate was incubated at 37°C for 1 hour. After another washing, as described above, 100 μ l of peroxidase-labelled anti-rabbit IgG (heavy and light chain specific, Cappel) diluted 1:1,000 by BSA-T, was added to each well and the plate was incubated at 37°C for 1 hour. After washing, 100 μ l of the substrate, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (ABTS), was added to each well and incubated at room temperature for 1 hour. After addition of 25 μ l of the stopping solution, 1.25% sodium fluoride, the absorbance of the contents of each well was measured at 405 nm by an ELISA reader (Myreader 7, Model ER-7, Sanko Junyaku).

RESULTS

The five MAb's used in the ELISA were tested by the serial dilution of antigen prepared from cultured trophozoites of the HK-9 strain of *E. histolytica*. All the MAb's were found to have a high degree of detectability down to 1.48 μ g/ml of antigen (Fig. 1). When the antigen was diluted to 0.14 μ g protein per ml,

the OD readings turned negative. Because MAb EHM-110 appeared to be the most reactive (Fig. 1), and hence it was used in this assay.

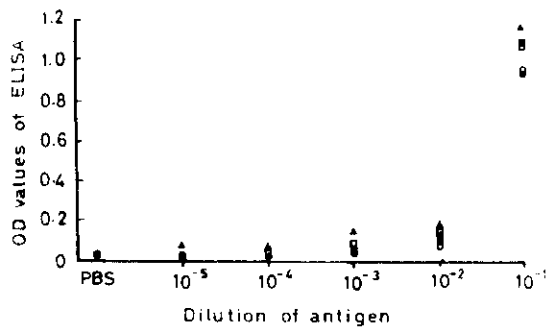


Fig. 1. Relationship between antigen concentration and OD values of ELISA. ● EHK 82, ○ EHK 142, ■ EHK 153, ▲ EHM 83, △ EHM 110. The protein concentration of the original antigen solution is 148 ug/ml and the number of *E. histolytica* trophozoites is 30×10^5 ml. PBS did not contain amoebic antigen.

As shown in Fig. 2, one hundred and forty eight stool samples obtained from symptomatic patients were tested by ELISA. *E. histolytica* trophozoites or cysts were detected in 99 samples by microscopic examination, but in 49 samples amoebae were not found. With the ELISA, it was observed that microscopically-positive samples showed higher OD values than the negative controls. However, high OD values were also observed in some microscopically-negative samples as well.

Stool samples from 91 individuals without intestinal symptoms were also tested by ELISA. These samples were divided into four groups according to the microscopic results: *E. histolytica* cyst-positive (11), *Entamoeba coli* cyst-positive (7), *Entamoeba* cyst-like forms-positive (5), and amoebae-negative (68). Trophozoites of *E. histolytica* were not

found in any of these samples. The results are shown in Fig. 3. Most of the *E. histolytica* cyst-positive specimens were shown to be positive

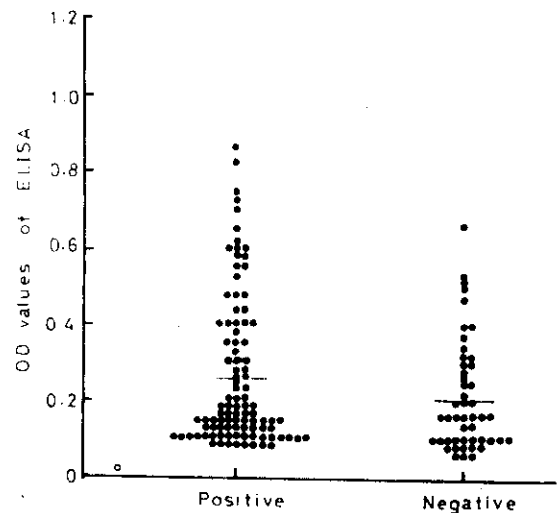


Fig. 2. ELISA values of microscopic-positive and negative stool samples. Bars indicate mean values and represent arbitrarily chosen cut-off points for interpreting the ELISA readings as positive or negative.

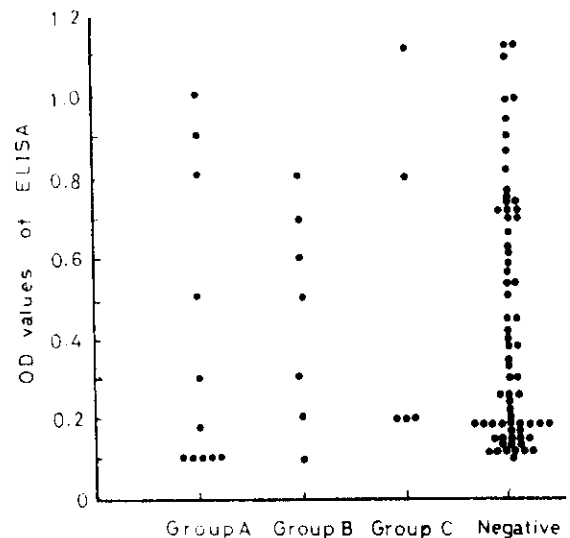


Fig. 3. ELISA values of stool samples obtained from 91 asymptomatic individuals Group A (positive), passing *E. histolytica* cysts; Group B (positive) passing *E. coli* cysts, and Group C (positive), discharging *Entamoeba* Cyst-like forms.

by ELISA. Samples containing *E. coli* cysts and *Entamoeba*-like cysts also gave high OD values. In the microscopic negatives, most of the samples were negative by ELISA, but a small number of specimens showed high OD values.

DISCUSSION

Immunological methods for the detection in faecal material of antigens of some pathogens causing intestinal infections, for example, rotavirus [15], *Salmonella* spp. [16,17], and bacterial toxins [18], have been reported. In parasitic diseases, Green [19] reported the detection of *Giardia lamblia* antigen in the stool using ELISA. Several studies have also been reported on the detection of *E. histolytica* antigen in the stool [8, 9,20]. These reports discussed some of the problems in immunological diagnosis.

In the present study, we applied ELISA using MAb to detect amoebic antigens in the stool of symptomatic and asymptomatic patients. The level of antigen detection was 1.48 ug of protein per ml, which corresponded to about 2.8 organisms/ml. As described in Fig. 2 and 3, high OD values were also observed in some microscopic negatives, and low values in some microscopic positives. These results are probably due to inherent limitations in the microscopic examination. The concordance positive ELISA results are likely due to the higher sensitivity of the ELISA test over microscopic examination. Unger et al. (1985) [8] reported that the sensitivity of antigen detection by ELISA can be as high as one organism per microscopic field. It therefore seems that a single microscopic examination, even after faecal concentration, is not reliable enough for diagnosis. On the other hand, our results demonstrate that there are some limitations in immunological methods. The low OD values in some samples with organisms indicate

that the sensitivity of the immunological method depends on specific amoebic antigens. It may also depend on the viability of the organisms, because some samples were obtained after treatment. Green (1986) [19] stated that in giardiasis, faecal antigens decreased with time after treatment with niridazole. Therefore, the false negative reactions seen in the ELISA indicate that the sensitivity of the assay is related to the amount of antigen in the stool. Grundy et al. (1987) [9] reported that ELISA using MAb appears promising for the detection of *E. histolytica* antigen because of its high sensitivity and specificity. Our results were found to be in agreement with their findings.

In Myanmar, about 4% of the population is infected with *E. histolytica*, and almost 90% of the infected individuals also suffer from other intestinal parasitic infections [3,4]. For appropriate treatment and epidemiological control, it is necessary to differentiate *E. histolytica* from other intestinal parasites. Although over 90% of amoebic infections are usually asymptomatic, amoebic antigens or soluble amoebic products can be present in the intestinal tract of both asymptomatic and symptomatic individuals [21]. The detection of amoebic antigens in the stool may be very useful for the diagnosis of amoebic infection. The ELISA using MAb, could have detected a number of microscopic-negative cases because of its high sensitivity. Therefore, we would like to advocate that both microscopic examination and ELISA should be applied for the diagnosis of intestinal amoebic infection, especially if there is only one opportunity for a time to test the sample.

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Access to family planning services through general practitioners

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In Myanmar, private medicine now plays a significant role in delivery of health care as well as the provision of family planning services. Thirty nine general practitioners, practicing in Sangyaung township, Yangon Division were inquired to determine the present situation of the family planning services provided by them. The majority (94.9%) realised the importance of the family planning services and believed that, they could help people for birth spacing. The findings will be helpful for the future planning of Family Planning Services in Myanmar.

INTRODUCTION

Among the twelve health policy elements identified by the National Health Committee, population issue has been included as one important aspect [1]. With the increasing rate of population growth, it is expected that there would be the need for extension of social and health services as well as birth control as part of family planning [2]. At the moment, the Government health service operates the Medical Board for authorized sterilization, which affects only legally married women with obstetric and gynaecological and other medical problems. Postnatal clinics where birth control services can be obtained on request or when indicated medically are present at many hospitals, Central Women's Hospital (CWH) is one of them, for which most women from Sangyaung township can utilise it. Apart from these places, others who want family planning services have to seek the services elsewhere. Obviously, general practitioners (GP) therefore have become the major service providers for them. Now, private medicine in our country has come to play a significant role in the delivery

of health care, especially in the provision of curative care [3]. General practitioners' clinics are the major sources for contraceptives when compared to other private sources, such as private hospitals, drug stores and road side stalls [4]. The aims of the study were to determine the magnitude of family planning services provided by the GPs in Sangyaung township and their knowledge, attitudes and practices related to family planning services.

MATERIALS AND METHODS

Study area and population

The study was conducted in Sangyaung township, during March and April, 1991. This township was chosen randomly from Yangon city area. In the initial plan, all GPs (about 60) registered in Sangyaung township were to be included in the survey. Among them, only 49 GPs could be included in the survey because some had moved out of the area or the clinics were closed. Of them, only 39 returned the completed questionnaires and diaries, producing a response rate of 79.6%.

Study variables and data collection

Questionnaires and diaries were developed and pretested with 10 medical scientists from DMR, who were also practicing as GPs. After necessary modifications, the forms were used for the main study. All GPs practicing in Sangyaung township were requested to fill the self-administered questionnaires and to record diaries with regard to the family planning services rendered during a specified one month period. The questionnaires and diaries were collected at the end of one month.

The following variables were included in the self-administered questionnaire: GPs' awareness of the existence of Hospital OPDs for birth control in Yangon, the ideal child spacing interval, the period of protection covered by one injection of different injectable contraceptive, side effects of different contraceptives, willingness to be involved in the implementation of the National Family Planning Programme, approval of induced abortion under seven possible conditions, advice given on family planning methods and managing the side effects and methods prescribed.

RESULTS

In the study area, it was found that 36.1% of the GPs had been practicing for 6-10 years, 33.6% for 1-5 years and 16.7% for 11-15 years. The percentage of GPs practicing full time was greater than those practicing part time, 55.6% vs. 44.4%. All of them were found to be rendering family planning services to a lesser or greater extent.

Knowledge about family planning services

Fifty-four percent of GPs could identify the location of the CWH OPD for birth control (3 of them were aware of the existence of, but could not identify the location).

Seventy-four percent of GPs knew that the next pregnancy could be delayed by breast feeding and 100% stated that the ideal child spacing interval is at least 2 years. When asked which was the best time for female sterilization, 76.9% stated it was during the puerperium and 17.9% stated 3 months after delivery. When they were asked about the period covered by one injection for the different injectables, most commonly available at market, 57.6% answered correctly that it was 2 months for Norethisterone, 97.4% could give the right answer of 3 months for Medroxy Progesterone and 73.3% could give the correct answer of 1 month for Hydroxyprogesterone.

Attitudes toward family planning services

About 90% of GPs thought that family planning was important. Amongst them, 37.1% indicated socio-economic problems as the reason, 11.4% stated that it could reduce maternal mortality rate and perinatal mortality rate, another 11.4% stated that it contributed towards healthy families, and 8.6% said that it was to control the population. Although 31.4% approved of the idea of its importance, they could not give any reason.

When the GPs were asked about their willingness to be involved in the implementation of the National Family Planning Programme, 84.6% said they were willing to take part and 12.8% said they were not. Among those willing to take part, they were further asked to propose and name the activity that they would like to participate in, 21.2% said that they would be willing to take part in any way required and to give health education, 9.1% said they could participate through clinic activities and 6.1% said they could give counselling services.

Regarding their attitudes toward the permission for induced abortion, 56.4% agreed that it should be per-

mitted legally on medical grounds only; while 18% felt that it should not be allowed under any circumstances. Twenty-three percent of GPs had no idea on it, and 2.6% said that it should be permitted on a pregnant woman's request.

Most (68.9%) of them would approve induced abortion when childbearing would endanger a mother's life, and on the other hand, 76.9% would not approve even the couples agreed on it, despite the medical problems (Table 1).

Table 1. Attitudes of GPs toward approving abortion under different conditions

Condition	Approve	Do not approve	Don't know	No response
Baby would endanger the mother's life	34 (87.2)*	2 (5.1)	0 (0)	3 (7.7)
Baby would be born deformed	28 (71.2)	6 (15.4)	1 (2.6)	4 (10.3)
Mother became pregnant because she had been raped	23 (58.9)	8 (20.5)	4 (10.3)	4 (10.3)
Parents find difficulty to feed and clothe children properly	12 (30.8)	22 (56.4)	2 (5.1)	3 (7.7)
Mother is pregnant, but not married	6 (15.4)	22 (56.4)	6 (15.4)	5 (12.8)
Parents find it hard to get a college education for children	5 (12.8)	25 (64.1)	3 (7.7)	6 (15.4)
Couples agreed on it	1 (2.6)	30 (76.9)	3 (7.7)	5 (12.8)

*Percentages are shown in parentheses

Eighty seven percent of GPs were of the opinion that the Government should provide family planning services as part of the general health care services. Ninety five percent of GPs

also accepted that family planning counselling as part of their responsibilities.

Practices on family planning services

The findings were based on 29 GPs (74.4%) who returned their diaries. During the period under study, the proportion of family planning activities done by GPs was small, relative to other work, constituting 2.5% of all consultation visits, and the services provided were about 9 consultations per GP per month.

Eighty five percent of GPs gave suggestions in the selection of family planning method(s) and explained the use of, advantages, disadvantages and side effects of each method. Ninety percent had advised a couple to apply for sterilization when a woman had more than 4 children. Thirty six percent of GPs advised the patients to stop the method for side effects, and to adopt other contraceptive(s). Only 7.7% referred them to Obstetricians and Gynecologists (OGs). On the average, during the one month period, over 90% gave 3 monthly contraceptive injectable (Medroxy Progesterone), 75.9% prescribed and gave contraceptive pills, 68.9% gave 1 monthly contraceptive injectable (Hydroxyprogesterone-caproate), 34.5% gave 2 monthly contraceptive injectable (Norethisterone) and 31.03% counselled family planning (Table 2).

All the patients using the contraceptives were found to be married women except one engaged woman. Amongst those, the majority (85.2%) were self referrals, 3 were sent by their husbands, 2 by Obstetricians and Gynecologists (OGs) and one each by the township medical officer and nurse.

DISCUSSION

Almost all of the GPs were found to be rendering family planning services. Although the proportion of family planning services provided by

Table 2. Family planning activities carried out by GPs during one month

Family planning activities	No. of GPs (n = 29)	Percentage
Giving contraceptive injections		
- 1 monthly injection	20	68.9
- 2 monthly injection	10	34.5
- 3 monthly injection	27	93.1
Prescribing and giving contraceptive pills	22	75.9
Intra uterine device	1	3.5
Counselling	9	31.0

GPs was not much, they were willing to take part in it, and they accepted it as part of their responsibilities. Even though they were willing to take part, some of them were not sure how to take part in it. However, once family planning services are implemented in our country, GPs can be recruited as one of the manpower resources.

Most of the GPs had knowledge about the correct way of using the different types of injectables as prescribed. But it was also found that, some of them did not know the correct contraceptive duration of the specified injectable.

It is also quite interesting that, even the GPs suggested that the Government should provide family planning services as part of the general health care services, most of them stated that, induced abortion should be approved legally on medical grounds.

Since the majority of them proposed to take part in family planning services by giving health education and by participating in any way required, health education material/manual

concerned with contraception about family planning should be prepared for them, emphasizing on the correct way of using the contraceptive injections and management of side effects of the contraception.

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The serodiagnosis of amoebiasis by enzyme-linked immunosorbent assay

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We attempt to detect antibodies against Entamoeba histolytica in sera obtained from both healthy individuals and hospitalized patients by an enzyme-linked immunosorbent assay (ELISA). The limiting value for ELISA-positive was established on the basis of serologic results of 1869 healthy persons living in a non-endemic area (Japan). The ELISA then was applied to 61 patients in 5 hospitals in Myanmar who were suspected of having amoebiasis. The ELISA results were compared with those of stool examinations, and evaluated for sensitivity. Using the limiting value for the ELISA, there was an 83% agreement of positivity between the ELISA tests and microscopic examinations. Specific anti-E-histolytica IgM was observed in the sera of 44 patients; IgG in all of the 61 patients. Since high titers of specific IgM were not observed in both stool-positive and stool-negative patients, we judged that patients with early stages of amoebic infections were not included in this population. In our ELISA, the sera of the patients did not exhibit cross-reactions between antigens prepared from the HK-9 strain of E. histolytica and antigens prepared from Trichomonas vaginalis and Giardia lamblia. On the basis of these results, we suggest that it is necessary to do two or more different examinations in order to make a definite diagnosis of amoebic infection.

INTRODUCTION

Recently, serological tests have been found to be of great value both for diagnosing invasive amoebic disease and for assessing the prevalence of amoebiasis in a population [1]. The immunological tests used for the serodiagnosis of amoebiasis are as follows; indirect haemagglutination assay (IHA) [2], ouchterlony immunodiffusion (ID), immunofluorescent assay (IFA) [3], and enzyme-linked immunosorbent assay (ELISA) [4,5]. The sensitivity of these immunological examinations was claimed to be as high as 95 to 100% in amoebic liver abscess, and 85 to 95% in invasive intestinal amoebiasis [6].

In Myanmar, it is estimated that about 4% of the population is infected with E. histolytica by microscopic demonstration of the parasites in the

stool, and that almost 80-90% of the infected individuals have clinical symptoms [7,8]. In this study, the ELISA technique was applied to the detection of intestinal amoebic infection and its usefulness evaluated by comparison with the diagnostic results obtained by microscopy.

MATERIALS AND METHODS

Sera

61 serum samples were obtained from patients with diarrhoea or dysentery who had been admitted to 5 different hospitals in Yangon. E. histolytica trophozoites and/or cysts were microscopically detected in the stool samples of 46 of the 61 individuals. The other 15 individuals were diagnosed as negative. To establish a limiting value for ELISA-positive, serum samples were

obtained from 1869 healthy volunteers living in Japan.

Antigen for ELISA

Soluble antigens of *E. histolytica* were prepared from trophozoites of the HK-9 strain cultured axenically in Biosate-Iron-Serum 33 (BIS-33) medium [9]. After three days of culture, the organisms were cooled in an ice bath, and harvested by centrifugation at 1,000g for 5 min. The sediment was washed 3 times with 0.05M phosphate buffered saline (PBS) (pH 7.2). The packed trophozoites were then homogenized for 3 min with a sonicator (Bronson, Sonifier, Cell Disrupter 200). The disruption of the trophozoites was performed in an ice bath. After centrifugation at 10,000g for 20 min, the supernatant was used as the soluble antigen. The protein concentration of the soluble antigen was determined by the method of Lowry *et al.* [10], and adjusted to an appropriate concentration by 0.01M carbonate buffer (pH 9.6).

Procedure of ELISA

The procedure of ELISA was similar to that described by Matsuda *et al.* [11]. ELISA microtiter plates (Sumitomo Bakelite, Tokyo), with 96 flat-bottomed wells, were sensitized with 100 μ l of diluted soluble antigen at 37°C for 2 hr. The plates were then washed 3 times with 0.05M PBS containing 0.05% Tween 20 (PBS-T), following which 100 μ l of test or control serum, diluted 1:400 in PBS-T containing 1% bovine serum albumin (BSA-T), were added to each well. The plates were incubated at 37°C for 1 hr and washed 3 times with PBS-T. One hundred μ l of goat anti-human IgG conjugated to horseradish peroxidase (Cappel Co., Ltd.), diluted 1:1,000 with BSA-T, were added to each well. To observe specific IgM in the test sera, horseradish peroxidase-conjugated goat anti-human IgM, diluted 1:100, was used. The plates were again incubated

at 37°C for 1 hr. After washing 3 times with PBS-T, 100 μ l of substrate solution (0.3 mg/ml 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid in 0.1M citric buffer containing 0.0003% hydrogen peroxide), were added to all wells. After incubation of the plate at room temperature for 1 hr, the reaction was stopped by the addition of 25 μ l of 1.25% sodium fluoride solution. Optical density (OD) at 405 nm was measured by an ELISA Reader (My reader 7, Model ER-7, Sanko Junyaku, Tokyo). To check cross-reactivity to other protozoans, soluble antigens prepared from cultured trophozoites of *Trichomonas vaginalis* and *Giardia lamblia*, were used instead of the amoebic antigens, and their absorbance measured in the same manner as stated above.

RESULTS

To determine the limiting value in the ELISA, all OD readings obtained from the healthy volunteers were plotted. As shown in Fig. 1, the

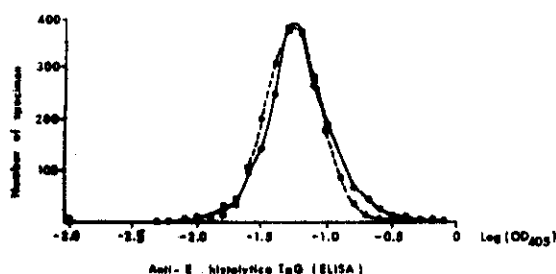


Fig. 1. Distribution of anti-*E. histolytica* IgG detected by ELISA in 1869 healthy Japanese volunteers. "●" indicates measured data and "○" is the calculated normal distribution.

distribution of the data was shown to be logarithmic-normal against logarithmic OD values. The mean logarithmic OD value and standard deviation (SD) was -1.287 and 0.293, respectively, calculated from the mean OD value at 405 nm (0.053). When the 95% tolerance limit (mean $\pm$ 2SD) was calculated from the

data, the negative controls were found to be localized between -1.873 and -0.701 in log (OD₄₀₅). Therefore, we determined that the limiting value for the ELISA-positive was -0.700 in log (OD₄₀₅), corresponding to 0.2 in OD₄₀₅.

A close relationship between the microscopic findings and the ELISA results was observed in the Myanmar patients, as shown in Fig. 2. Of the

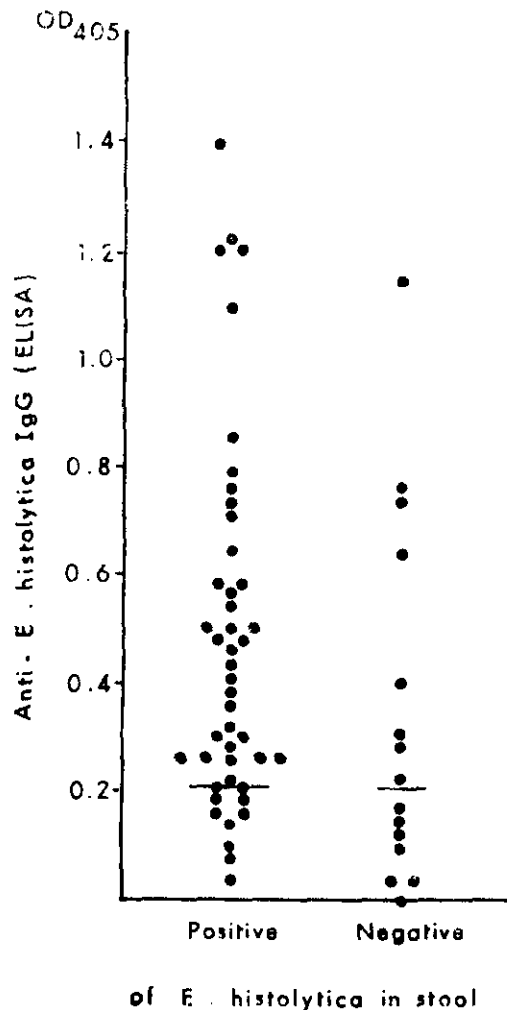


Fig. 2. ELISA values of microscopic-positive and negative serum samples from Myanmar patients: 0.495 ± 0.353 (Mean $\pm$ SD) in microscopic-positives; 0.338 ± 0.322 in microscopic-negatives. Bars indicate limiting values for interpreting the ELISA readings as either positive or negative.

46 individuals in whom amoebae were microscopically detected, 38 cases (82.6%) showed ELISA OD values over 0.2, and 8 cases (17.4%) were under 0.2. On the other hand, 8 of the 15 microscopic negative cases (53%), showed OD values higher than 0.2.

Anti-*E. histolytica* IgM was also measured by ELISA in the serum samples of 44 of the Myanmar patients; 31 microscopic positives and 13 negatives. It was observed that there was no significant difference in OD values between the microscopic-positive and negative group (Fig. 3).

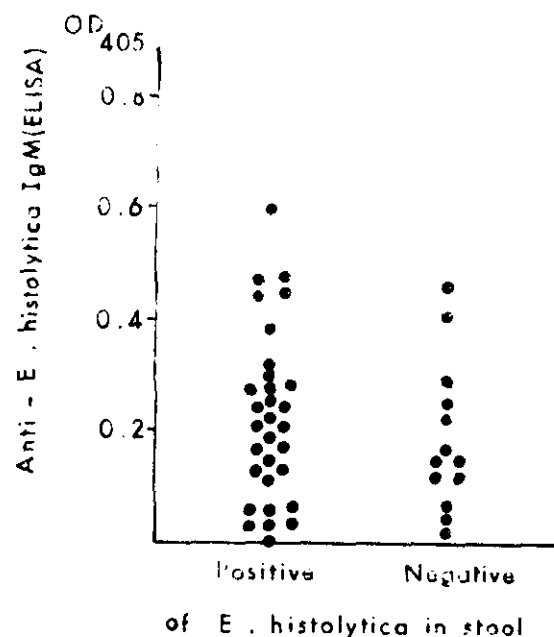


Fig. 3. Relationship between microscopic examinations and anti-*E. histolytica* IgM detected by ELISA: 0.203 ± 0.155 (Mean $\pm$ SD) in microscopic-positives; 0.159 ± 0.115 in microscopic-negatives.

To estimate the specificity of ELISA against *E. histolytica*, cross-reactivities were examined by using antigens of two other protozoans, *T. vaginalis* and *G. lamblia*. We tested for the presence of IgG antibodies against *T. vaginalis* and *G. lamblia* in all the serum samples obtained from the Myanmar patients. No correlation

was found between the IgG antibodies against either of the 2 protozoan parasites with the anti-*E. histolytica* IgG (correlation coefficients of $r=0.438$ and $r=0.104$, respectively), as shown in Figs. 4 and 5.

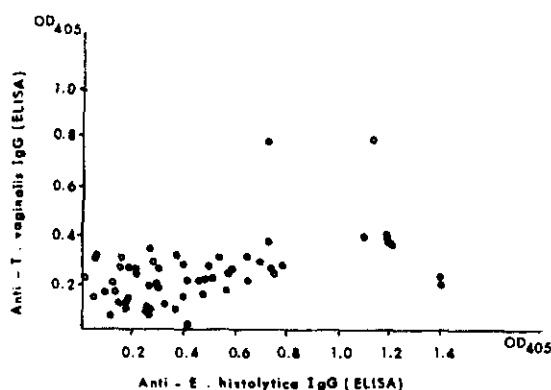


Fig. 4. Lack of correlation between anti-*E. histolytica* IgG and anti-*T. vaginalis* IgG antibodies detected by ELISA. Samples in which amoebae were detected by microscopy in the stool (●); microscopic-negatives (○). Correlation coefficient (r)=0.438.

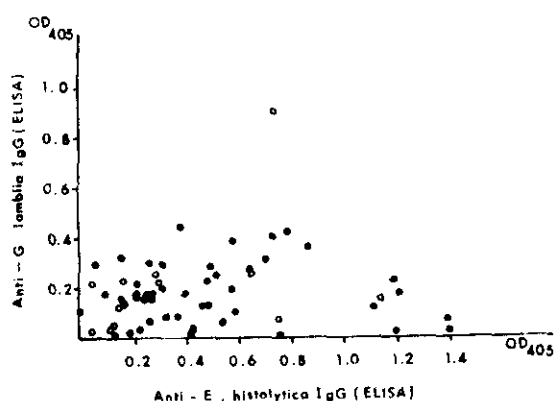


Fig. 5. Lack of correlation between anti-*E. histolytica* IgG and anti-*G. lamblia* IgG antibodies detected by ELISA. Samples in which amoebae were detected by microscopy in the stool (●); microscopic-negatives (○). Correlation coefficient (r)=0.104.

DISCUSSION

The serological detection of antibodies to *E. histolytica* is useful in diagnosis and in epidemiological surveys. It has been reported that circulating specific antibodies are detectable during the early stages of invasive amoebiasis by immunologic procedures [1]. Unfortunately, such tests have several problems and limitations. The tests are more sensitive and specific in cases of extraintestinal than intestinal amoebiasis, serological reactions being relatively insensitive in detecting cyst carriers [12,13]. Moreover, circulating antibodies persist long after treatment [1]. In this study, there was an 83% agreement of positivity between the microscopic diagnosis and the ELISA test. These results correspond with those of previous serologic studies [13-16]. Kagan [6] claimed that circulating antibodies were detected in 50-80% of patients with severe dysentery requiring hospitalization. It seems likely that they were cyst passers, and that their symptoms of diarrhoea or dysentery were due to other pathogens. On the other hand, some of the microscopic-negatives in our studies were shown to have high ELISA OD values. These individuals were suspected of having extraintestinal amoebiasis, and who were not excreting cysts or trophozoites in their stools. On the basis of the ELISA, we diagnosed 46 cases, including both microscopic-positives and negatives, as amoebiasis. For definite diagnosis of amoebic infection, we suggest that it is necessary to do two or more different tests, although the ELISA test was shown to be both specific and sensitive. Furthermore, to estimate an accurate prevalence rate in a epidemiologic survey, both stool examinations and serological tests must be done on all the participants.

The measurement of IgG antibodies is not useful to determine the period of infection with *E. histolytica*,

because the specific antibodies persist in human sera; IgG antibodies detectable by IHA have been shown to persist for up to 21 months [1]. It is well known that specific IgM antibodies develop only during the early stages of the infection. In the present study, high IgM values were not observed in either the microscopic-positive or negative groups. This may be due to the absence of early infections in the surveyed population.

We observed that there was no cross-reactivity of the amoebic ELISA against either T. vaginalis or G. lamblia. These results suggest that the ELISA is useful in epidemiological studies, especially to distinguish amoebiasis from other protozoan infections.

ACKNOWLEDGEMENT

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Applicability of WHO criteria by community health workers for diagnosis of acute lower respiratory infections

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Applicability of simple clinical signs (WHO, 1990 ARI classification) by community health workers (CHWs) for the diagnosis of Acute Lower Respiratory Infections (ALRI) especially pneumonia, were evaluated in out-patient department of Yangon Children's Hospital from November 1990 to February 1991. 944 under-five children presenting with Acute Respiratory Infection related symptoms of less than 7 days duration were included in the study. The percentage agreement of diagnosis status by CHWs with reference to project Medical Officers (gold standard) were Kappa 0.5126 ($p < 0.001$) in less than 2 months old infants and Kappa 0.5442 ($p < 0.001$) in children aged 2 months to 5 years. CHWs were able to detect important clinical signs like chest indrawing in percentage agreement of 85.1%. However, percentage agreement in respiratory rate was found to be 60.2% and there was incorrectness in counting the respiratory rate as the rate increased. Differences in the level of agreement on these important clinical signs misclassified the disease category resulting in low percentage agreement in discriminating the disease severity.

INTRODUCTION

Acute respiratory infection was a major priority problem that needed attention, in the global strategy on child survival revolution. Of the estimated 15 million deaths around the world each year in children under five years of age, 25%-30% are due to ARI and almost all of them are caused by pneumonia [1].

Also in Myanmar, ARI is one of the five leading causes of morbidity and mortality accounted for nearly 31% of all hospital deaths in children under-five [2] and demands a solution within the context of primary health programme.

WHO has developed accurate guidelines to enable differentiation of children with LRIs especially pneumonia, who can safely treated with antibiotics as out patients from those who require immediate referral, based on symptoms

and signs that can readily be assessed.

In this programme, peripheral health workers are trained using recognizable signs, such as increased respiratory rate and the presence of chest retractions, to detect pneumonia that requires treatment with antibiotics at home or referral to hospital. Several studies suggest that these signs are sensitive indicators of lower respiratory infection [3,4,5].

The present study was conducted to determine the applicability of WHO criteria for diagnosis of severe ARI cases (especially pneumonia) by community health workers and to determine the proportion of discriminating disease severity by CHWS with reference to the standard after a course of training for diagnosis of ARI at the primary health care level.

MATERIALS AND METHOD

Study area and population

The study was conducted at the out-patient department of Yangon Children's Hospital. Three medical doctors and 24 community health workers were recruited and they were trained for two-week duration during the middle of October 1990.

Training consisted of theoretical knowledge of ARI and practical demonstration of important clinical signs and symptoms like chest indrawing, stridor and counting of respiratory rate. Exercise on the assessment of knowledge and skill gained from this course was tested after the completion of each and every secession of the course.

The CHWs who took part in the study were from North Okkalapa township and they were assigned to work in out-patient department of YCH in turn. The CHWs were divided into three groups and each group was attached to one Project Medical Officer who acted as a gold standard.

All under five children attending the OPD of YCH with ARI related symptoms of less than one week duration, from November 1990 to February 1991 were included in the study. They were received by one of the three working groups, and the proformas were filled in by observing the symptoms and the history given by the accompanying person. CHWs performed a physical examination on each child and assessed the relevant clinical signs and counted the respiratory rate in one full minute using a 60 second timer and recorded their own findings in the separate proforma. No discussions were allowed during the time of examination. Based on the individual findings, CHWs categorized the patients according to WHO 1990 ARI classification. A project medical officer conducted the detailed clinical examination and filled in the separate clinical proforma and corresponding proformas filled in by each

CHWs were compiled and numbered serially.

The study population consisted of 944 under-five children of which 521 were male and 423 were female. 44 patients were under 2 months of age and 900 children were in the age range of 2 months to 5 years. They were examined a total of 2001 occasions by CHWs.

Data analysis

Weekly performance of variables on important clinical signs like chest indrawing, respiratory rate, wheezing, and accuracy of the classification of pneumonia status in different age groups by CHWs were presented as Pearson correlation coefficient. Percentage agreement of corresponding variable by CHWs with reference to project medical officer were determined. Agreement in respiratory rate was considered, if the rate measurement by CHW fell within the range of ± 5 counts per minute with reference to that of the project medical officer. The statistical calculations were performed by means of Kappa statistic interpreted as intraclass correlation coefficient [6].

RESULTS

The diagnosis agreement of Project Medical Officers vs CHWs on severity category of pneumonia in under 2 months old infants was Kappa (% agreement 70.33 chance = 34.90) = 0.5442 ($p < 0.001$) (Table 1) which is slightly higher than in 2 months to 5 years aged group showing Kappa (% agreement = 66.18 chance = 30.61) = 0.5126 ($p < 0.001$) (Table 2).

Regarding the sensitivity and specificity in recognition and diagnosis of pneumonia by CHWs in accordance with WHO (1990) ARI classification, it was shown that more sensitive in 2 months to 5 years age group and more specific in under 2 months old infants. The predictive value of positive and negative diagnosis were

Table 1. Diagnosis of pneumonia status by Project Medical Officer vs Community Health Workers in under 2 months

Medical Officer				
Disease category	No pneumonia	Severe pneumonia	Very severe disease	Total
C No pneumonia	32	4	8	44
H Severe pneumonia	2	10	11	23
W Very severe disease	2	-	22	24
Total	36	14	41	91

Chi-square (4 df) = 58.9788 ($p < 0.0001$)

Kappa (% agreement = 70.33 Chance = 34.90)
= 0.5442 ($p < 0.0001$)

Sensitivity = 78.2%

Specificity = 88.9%

Predictive value of positive diagnosis 91.5%

Predictive value of negative diagnosis 72.7%

91.4% and 72.2% in infants under 2 months and 81.5% and 78.8% respectively in 2 months to 5 years old children.

Percentage agreement on individual clinical features by CHWs in recognition of important clinical signs revealed that they were able to detect chest indrawing and wheezing (Table 3).

Fig. 1 shows the detailed analysis of percentage agreement of respiratory rate in different grades.

Analysis on weekly performance of CHWs in recognition of important clinical signs and fortnightly performance on accuracy of the classification of pneumonia status revealed that there was progressive improvement during the period of study.

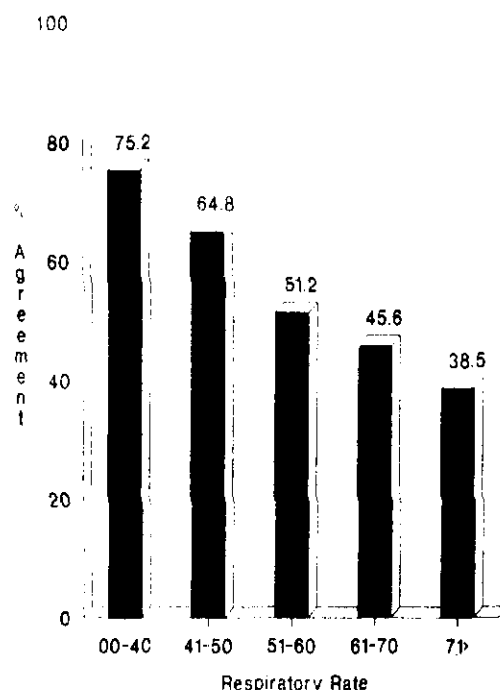


Fig. 1. Percent agreement of respiratory rate by CHWs with reference to project M.Os

DISCUSSION

In rural areas of the developing countries the case-fatality rate from LRI in children is most likely to be reduced if primary health care workers can identify the most serious form of ALRIs and deal with them appropriately.

In our findings every CHW were able to detect some important clinical signs such as wheezing and chest indrawing to a certain limit. Assessment of other clinical signs like stridor and respiratory rate were found to be unsatisfactory. In our series only two patients had stridor and CHWs failed to detect this sign. Instead, they made high proportion of false-positive in eliciting this sign. In view of the high proportion of false-positive diagnoses these children would have been wrongly classified as the severe disease category leading to unnecessary use of antimicrobial and no benefit on

Table 2. Diagnosis of pneumonia status by Project Medical Officer vs CHWs in 2 months to 5 years old children

Disease category	Medical Officer				Total
	No pneumonia	Pneumonia	Severe pneumonis	Very severe disease	
No pneumonia	650	138	51	6	825
Pneumonia	160	269	65	49	543
Severe pneumonia	32	44	187	67	330
Very severe disease	9	10	35	158	212
Total	851	461	318	280	1910

Chi-square (9 df) = 1678.4861 ($p < 0.0001$)

Kappa (% agreement = 66.18 Chance = 30.61) = 0.5126 ($p < 0.0001$)

Sensitivity 83.5%

Specificity 76.4%

Predictive value of positive diagnosis 81.5%

Predictive value of negative diagnosis 78.8%

Table 3. Percentage agreement of individual clinical features by Medical Officers vs CHWs

Symptom	Medical Officers vs Community Health Workers								
	Yes-Yes	Yes-No	No-Yes	No-No	Grand total	% agreement	K value	S.E.(K)	P value
1. Chest indrawing	434	200	98	1269	2001	85.1	0.6405	0.022	P<0.001
2. Wheezing	88	152	134	1627	2001	85.7	0.3003	0.0223	P<0.001
3. Stridor	0	2	100	1899	2001				

reducing of ALRI morbidity and mortality. The satisfactory results for chest indrawing may be due to the clear nature of the sign among the patients examined. Detailed analysis also revealed that there was incorrectness in counting the respiratory rate by CHWs as the rate increases. Variation in counting the respiratory rate and difference in the level of agreement of other clinical signs misclassified the disease category. These are the possible explanation for the reduction in percentage agreement in discriminating the disease severity.

The value of a sign in the clinical diagnosis of a condition is dependent on whether its presence, in conjunction with other signs, discriminates between disease, and on the consistency with which observers agree on its presence or absence. Shann *et al.* advocated that it is difficult for CHWs to count respiratory rate correctly, because when counting the respiratory rate the child should be awake but quiet, which can be difficult to achieve [4].

Gove and Kumar stated that for acute respiratory illness control programme the accurate measurement of respiratory rate in a calm children is the most important clinical observation in the detection of pneumonia. The study in Chandigarh, India, found that the health workers who counted the respiratory rate and examined for chest retractions could discriminate disease severity as well as a physician could [7].

WHO ARI management guidelines recommended three respiratory rate thresholds to define fast breathing; a respiratory rate of 60 or more in young infants (under 2 months); 50 or more in infants 2-11 months of age; and 40 or more in children 1-4 years old.

The ability of the health workers to decide whether or not a child's breathing is normal is the important steps in decision for management of

ARI in the community. The health workers do not need to make exact respiratory count but able to distinguish between normal and fast breathing is key element for success of ARI programme. If the breathing is fast the child should be given an antibiotic as soon as possible. This measure alone could prevent many deaths from pneumonia.

Agreement on the presence of individual signs is related to the frequency with which they are observed. In our study as the CHWs gained experience, their efficacy also increased as indicated by the progressive improvement in recognition of individual clinical features in successive weeks of study.

This study revealed that the CHWs could recognized the chest indrawing and wheezing easily but weakness in counting the respiratory rate and viriability in noting stridor directed the needs for better initial training in recognizing these simple clinical signs. Regular assessment and refresher course training in ARI case management would provide the increased efficacy in discriminating the disease severity, so as to have significant impact on reducing ALRI mortality.

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SHORT REPORT

Prevalence of *toxocara canis* among the dogs of Htaukkyant Leprosy Hospital

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Toxocariasis has been shown to be associated with a considerable proportion of the canine and human population of Great Britain, United States and other parts of the world. In Myanmar the prevalence of *Toxocara* in stool of dogs and cats had not been investigated yet. Htaukkyant Leprosy Hospital was chosen as the inmates living around the area were helpful to collect the stool specimens of the dogs.

All the dogs within the compound of Htaukkyant Leprosy Hospital were tied with ropes all at one night at the respective buildings of the patients. The stool specimens shed from all these dogs were collected on the next morning. The fresh stool specimens were examined by formal-ether concentration method, at the same day.

Out of sixty dogs only forty eight shed stool on the next morning. Of these twenty four specimens showed *Toxocara canis* eggs. Other eggs seen together with *toxocara* eggs were not identified.

The dogs were the ones kept by the patients as pets and they belong to the race *Canis familiaris* commonly found at Myanmar homes.

In this study fifty percent of the dogs had *toxocara* eggs in their stool. This finding is higher than these reported from England, Malta, Ibadan, Kampala Uganda, Dares-Salaam, Kenya Nairobi, and Masai where only 5.7-35.6% of the dogs had *toxocara* [1].

The eggs of these dogs worms when ingested can develop larvae in the intestine of human host, penetrate the bowel wall, and by direct migration or through the circulation reach

the various organs of the body.

Clinically the infection usually runs a chronic benign course as long as eighteen months. The severity of the infection varies with the number of larvae in the tissue and the immune or allergic state of the infected individual. Many infections are sub-clinical and the only salient finding is moderate and sustained eosinophilia, hepatomegaly, hyperglobinemia and probably a patchy pneumonia. Intermittant fever, leucocytosis primarily to the increase in eosinophilia, malaise, pallor, anorexia, failure to gain weight, muscle and joint pains, abdominal pain nausea and vomiting, neurologic disturbances, with convulsions and petit mal attacks may occur some cases. An impetiginous skin rash sometimes appear on the trunk and extremities. A nematod endophthalmitis clinically resembling retinoblastoma may be caused by the larvae migrating the orbit. This entity has been observed frequently in children [2].

The above signs and symptoms with persistent eosinophilia is often seen in patients belonging to all parts of Myanmar. Hence further study is suggested.

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SHORT REPORT

First paediatric AIDS case in Myanmar

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A four year old boy (NLA, Reg. no. 15178) from Mawlamyine was admitted to Medical Unit III of Yangon Children's Hospital on 23-8-93 for fever of one month duration, blindness of one and half month duration and loss of weight and appetite of three months duration.

Clinical examination on admission revealed that the child was very much emaciated and lethargic. His body weight was 9.5 Kg (ie: 53% of expected weight) and the height was 93 cm (ie: 93% of expected height). Both eyeballs were depressed and there was no light reaction of the pupils. The child had fever, oral thrush, marked hepatomegaly (5 cm below the 5th intercostal space), generalized lymphadenopathy and a few crepitations in the lungs.

Our first impression was that the child is suffering from chronic malnutrition with blindness due to vitamin A deficiency and the fever due to some intercurrent infection. However, the family social background history of the child didn't support our first provisional diagnosis. The parents were seem to be quite well off (the father is a driver and the mother a clerical staff) and according to the history the child was thriving well till April 1993. The boy was the 2nd offspring, pregnancy was uneventful and the birth weight was 7 lbs. Because of neonatal jaundice he was treated with phototherapy and blood transfusion was given on 7th day because of anaemia (ie: in April 1989). The blood was supplied by the hospital and they didn't know who the donor was. The child had frequent attacks of diarrhoea since the age of one and half years and the paediatrician they consulted told

that the child had hepatomegaly but they didn't take it seriously as the child was growing well. However, later on, as the child started losing weight they consulted with a liver specialist in April 1993. As the child developed impaired vision they consulted a neurophysician one and half months later. The child was also seen by an opthalmic surgeon and was diagnosed as having exudative intraocular inflammation of obscure aetiology. Because of the deteriorating condition of the child, the child was referred to Yangon Children's Hospital by a paediatrician.

With that history our second impression was that the child may be suffering from a rare connective tissue disorder and we were considering of taking liver biopsy. However, oral candidiasis of severe magnitude noted on the 2nd day of admission prompted us to exclude AIDS. Blood samples of the child was sent to National Health Laboratory for testing of HIV antibody and was found to be HIV antibody positive (positive by three screening tests and confirmed by western blot). We also checked patient's mother for HIV antibody but was negative. Thus this case could be labelled as first paediatric AIDS case in Myanmar [1] and the possible route of infection is through parenteral route.

According to reports from other countries, if congenitally infected, the symptoms of AIDS become evident between ages of 4 and 6 months. If perinatally infected, the mean age at diagnosis is 17 months. In children infected by transfusion, the interval between transfusion and diagnosis has been a mean of 24 months. Common clinical manifestations are: lymphaden-

pathy 90%, hepatomegaly 86%, splenomegaly 69%, failure to thrive 62%, thrush 48%, neurologic abnormalities 34% and diarrhoea 17%. In fact, abnormalities of all organ systems can be seen in AIDS, whether induced by opportunistic infection or by the HIV infection itself. Examples are: hepatitis, pancreatitis, renal disease (nephrotic syndrome), eye disease (perivasculitis of the retinal vessels) and blood abnormalities (thrombocytopenia, neutropenia) [2,3].

In Myanmar, according to the reports, no cases of HIV seropositives were detected among sexually promiscuous persons in 1985 and 1987. No tests for detection of HIV antibody was done in 1986. In 1988, serological surveillance was continued and one patient among the hospitalised patients tested was HIV seropositive (first adult AIDS case in Myanmar). HIV seroprevalence studies in 1990-91 in selected population revealed HIV seroprevalence rates of 16%, 71%, 0.3% and 0.5% in prostitutes, IVUs, blood donors and pregnant women respectively [4,5].

Paediatric HIV infection cases could have been resulted from HIV +ve pregnant women (through perinatal infection) as well as from the transfusion of HIV +ve blood. Such situation exists in Myanmar since 1990-91 (vide supra). Thus, although some of the manifestations (such as chronic

diarrhoea, prolonged fever, weight loss, oral candidiasis) can often be seen in malnourished and tuberculosis cases in paediatric practice, this case highlighted the need to keep in mind the possibility of AIDS in such cases.

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