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Potency assay of antivenom: Batch to batch variation of neutralising efficacy of antivenom Tun Pe, Aye Aye Myint & Kyi May Htwe	105
The association of enteropathogenic <i>Escherichia coli</i> (EPEC) and gastroenteritis Mar Mar Nyein, Aye Aye Than, Than Saw, Thuzar Myint, Tin Aye & Myint Lwin	108
Objective assessment of meconium content in amniotic fluid Than Than Tin, May Malar Swe, Aye Aye Min, San Shwe & Khin Win Thin	116
Seasonal variation of flea population and its relationship with small mammal hosts in sub-urban Yangon Pe Than Hutn, Win Htin, Moe Myint Kyu, Wai Si Thaung, Wah Wah Khet, Soe Soe Kyu & Hla Naing	120
The phytohemagglutinin (PHA) - induced peripheral lymphocyte blastogenic response in cancer patients Soe Soe, Than Than Htwe, Tint Swe, Sanda Win & Than Swe	125
Assessing the accuracy of respiratory rates at different counting periods and methods Khin Myat Tun, Phyu Phyu Aung, Thida Kyaw, Khin Saw Aye, Thein Shwe, Le Le Win & Soe Aung	130
Circulating and urinary cortisol, ACTH & HGH levels of normal Myanmar subjects Saw Naing, Tin Nu Swe, Mg Mg Thwin & Myo Khin	136
Comparative multiple-dose pharmacokinetics of chloroquine in <i>P. vivax</i> malaria patients and healthy volunteers Thaw Zin, Ye Thwe, May Aye Than, Khin Tint, Hla Yee, Thida Hmun & Nwe Nwe Yin	140
Potential use of Kyethingha-thee (<i>Momoridica charantia</i> L. fruit) in the treatment of maturity onset diabetes mellitus May Aye Than, Ye Thwe, Thaw Zin, Mu Mu Sein Myint, Hla Pe, Aung Naing & Maung Maung Wint	148
Male participation in birth spacing Than Than Tin, San Shwe, Thein Hlaing & Myint Myint Soe	155

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Potency assay of antivenom: Batch to batch variation of neutralising efficacy of antivenom

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Neutralisation of biological properties of Russell's viper venom (Thayawady) with 4 different batches of monospecific lyophilised antivenom manufactured by Myanmar Pharmaceutical Factory (batch DN 84560B exp. 2/90, DN 86608B exp. 4/92, DH 89652A exp. 9/94 and DD 90668B exp. 6/95) was assessed in rodents using WHO standard tests of neutralising activity. The amount of antivenom required to neutralise different biological properties of venom by different batches of antivenom varied from 2 to 50 times. Such variation of antivenom potency may explain failure of correction of some venom-induced effects seen in antivenom treated Russell's viper bite cases as well as variation in the composition of venom used as an immunogen. No quantitative differences in bands recognised by different antivenoms was observed in immunoblot. It is suggested that characterisation of widely pooled potent venoms to be used for antivenom production and potency assay of antivenom should be carried out routinely.

INTRODUCTION

It is desirable that antivenom should be effective in neutralising biological properties of venom and be raised with venoms of high biological activities. Moreover its potency be monitored using WHO standard tests of neutralising activity [1]. However, the potency of antivenom manufactured by Myanmar Pharmaceutical Factory has not been monitored except for antilethal potency. Variation in neutralisation of Russell's viper venoms by a monospecific antivenom has been reported [2]. In this communication, potency assay of four different batches of antivenom in neutralising biological properties of one Russell's viper venom (Thayawady) was carried out.

MATERIALS AND METHODS

Potency assay of three different batches of monospecific Russell's viper antivenom, DN 84560B exp. 2/90, DN 86608B exp. 4/92, DH

89652A exp. 9/94 were carried out in rodents with Russell's viper venom of Thayawady according to WHO standard tests of neutralising activity [1] in December 1989. A new batch of antivenom DD 90668B exp. 6/95 was tested in January 1995. All antivenoms were tested before the date of expiry. Lyophilised antivenom was reconstituted, aliquoted and stored frozen at -20°C until needed. Duplicate experiments were carried out with each antivenom. Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, mixed, incubated at 37°C for 30 min and then injected into the laboratory animals. The end point (ED_{50}) is taken as the amount of antivenom required to neutralise 50% of the biological activity of venom [2]. Biological properties of venom such as lethality, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing activity were also determined [1]. Immunoblotting of the venom with antivenoms was also carried out. Details of the methods have already been described

Table 1. ED₅₀ of 4 different batches of antivenom. ED₅₀ is defined as minimum amount of antivenom require to neutralise 50% of the biological activity of venom

ASV batch	5LD ₅₀ iv 23.4 ug /mouse # ul	10MCD 25.12 ug * ul	3MHD 126.5 ug /rat * ul	3MND 119.4 ug /rat * ul	5MDD 12 ug /mouse * ul	100MCPID 0.724 ug * ul
DN86608B	1.99 ±0.11	0.0625 ±0	25 ±0	25 ±0	6.25 ±0	6.25 ±0
DN84560B	3.62 ±0.18	0.0312 ±0	100 ±0	50 ±0	12.5 ±0	50 ±0
DN89652A	2.39 ±0.10	0.7812 ±0	25 ±0	25 ±0	50 ±0	100 ±0
DD90668B	6.25 ±0	1.562 ±0	50 ±0	12.5 ±0	3.125 ±0	12.5 ±0

LD₅₀ iv = lethality

MCD = Minimum coagulant dose

MHD = Minimum haemorrhagic dose

MND = Minimum necrotic dose

MDD = Minimum defibrinogenating dose

MCPID = Minimum capillary permeability increasing dose

= mean of duplicates ±1 SE

* = mean of triplicates ±1 SE

[2, 3]. Recently another attempt was made to see reproducibility of the potency tests.

RESULTS

Results of neutralisation of biological properties of the venom with four different batches of antivenom are shown in Table 1. There was variation in neutralisation of biological properties of venom by different batches of antivenom. It was found that batch DN86608B had potent anti-lethal, anti-haemorrhagic and anti-capillary permeability increasing activities whereas batch DD90668B had potent anti-necrotic and anti-defibrinogenating activities and weak anti-procoagulant and anti-lethality. The amount of antivenom required to neutralise potent and weak activities of different biological properties of the venom varied from 2 to 50 times. No quantitative differences in bands recognised by different antivenoms

was observed in immunoblot (Figure 1). An attempt made to see reproducibility of biological tests and potency

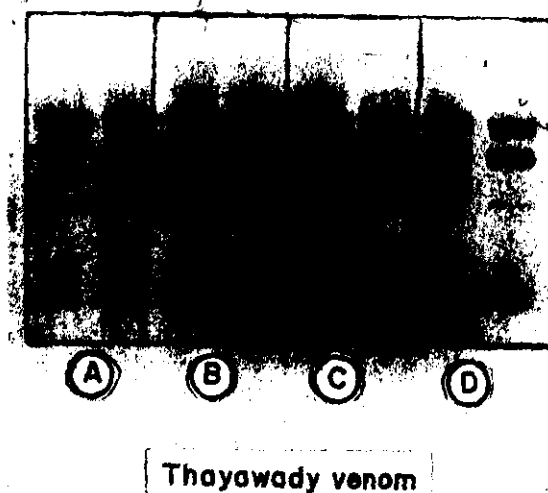


Fig. 1. A picture of immunoblot of Thayawady venom with 4 different batches of monospecific antivenom

A = DD90668B, B = DN89652A,
C = DN86608B and D = DN84560B

of expired antivenom stored frozen at -20°C revealed that tests are reproducible.

DISCUSSION

Although the source of pooled venom used for raising antivenom was not known, it was likely that antivenom was raised with pooled venom from snakes collected from lower Myanmar (Yangon, Bago and Ayeyawady Divisions) and it will have good neutralising activity of biological properties of the Thayawady (Bago Division) venom. However, variation in neutralising efficacy of antivenom was observed reflecting qualitative variation in immunogen used which was supported by immunoblot results. Antivenom if kept frozen at -20°C still keeps its potency even though it has reached its expiry date was observed and biological tests used for potency assays are reproducible.

Variation in potency of antivenom raised with venom of different geographical regions [4] and variation in neutralisation of biological properties of Russell's viper venoms had been reported [2]. Such variation in potency of antivenom may amount for failure of correction of some venom-induced effects seen in antivenom-treated Russell's viper bite cases (unpublished cases). Variation in venom composition with different milking and different batches [5] (unpublished observation) may also account for variation in antivenom potency. Poor efficacy of antivenom was due to use of weak venom immunogen. In order to raise a potent antivenom a widely pooled venom

with high biological activities should be used as an immunogen [6]. It is suggested that characterisation of venoms to be used for antivenom production and potency assay of antivenom should be carried out routinely.

REFERENCES

1. Theakston, R.D.G. & Reid, H.A. The development of simple standard assay procedures for characterisation of snake venoms. Bulletin of World Health Organization 1983; 61: 949-956.
2. Tun-Pe, Aye-Aye-Myint & Kyi-May-Htwe. Potency assay of antivenom: neutralisation of biological properties of three Russell's viper venoms by a monospecific antivenom. Myanmar Health Sciences Research Journal 1994; 6: 118-120.
3. Tun-Pe, Nu-Nu-Lwin, Aye-Aye-Myint, Kyi-May-Htwe & Khin-Aung-Cho. Biochemical and biological properties of the venom from Russell's viper (Daboia russelli siamensis) of varying ages. Toxicon 1995; 33: 817-821.
4. Kornalik, F. & Jaborska, E. Neutralisation of lethal, local and systemic effects of snake venoms by monovalent and polyvalent antivenoms. Proceedings of the 5th European symposium on animal, plant and microbial toxins, 1983; pp. 9-15.
5. Schaeffer, R.C. Jr, Chilton, S.M., Ren-Kiewicz, R.R. & Carlson, R.W. Variation in the composition of Echis venoms. Toxicon 1985; 23: 609.
6. Kornalik, F. & Jaborska, E. Correlation between the neutralising activity of monovalent antivenoms and the quality of venoms used for immunisation. Toxicon 1985; 23: 584.

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The association of enteropathogenic Escherichia coli (EPEC) and gastroenteritis

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A total of 619 cases of adults with gastroenteritis admitted to Yangon General Hospital, Workers' Hospital and Infectious Diseases Hospital were included in this study. It was observed that 33 per cent were suffering from gastroenteritis due to enteropathogenic Escherichia coli (EPEC). The most prominent EPEC serogroups encountered were O1, O26, O55, O86, O114, O125 and O128. The antibiotic sensitivity pattern showed that 87 per cent of enteropathogenic Escherichia coli (EPEC) were resistant to ampicillin, 58 per cent to chloramphenicol, 49 per cent to streptomycin, 55 per cent to benzyl penicillin, 44 per cent and 21 per cent respectively to tetracycline and septrin. They were all sensitive to nalidixic acid but 97 per cent to gentamicin, 96 per cent to sisomicin and 95 per cent to tobramycin respectively.

INTRODUCTION

The term acute infective gastroenteritis or infectious diarrhoea is applied to cases of gastroenteritis with frequent loose stools, with or without vomiting, in whom an infection of the gastrointestinal tract is presumed or known to be the cause. The principal microorganisms causing infective gastroenteritis are shigellae, salmonellae and enteropathogenic strains of Escherichia coli (EPEC). Extensive studies carried out on a world-wide basis have shown that specific bacterial pathogens could be isolated from 22 and 64 per cent of such cases. Thus about 50 per cent could not be diagnosed by conventional bacteriological techniques (Anathan et al., 1989; Regua et al., 1990) [1,2]. E. coli serotypes comprise the largest group of microorganisms inhabiting the intestinal tract. Infection with these strains sometimes occur without symptomatic illness even in the infant.

This study aims (1) to determine the

role of EPEC in adults with gastroenteritis; (2) to determine the distribution of serotypes in the adults and (3) to study the antibiotic sensitivity of these strains.

MATERIALS AND METHODS

Study cases

Random samples of 619 cases of adults with gastroenteritis admitted to Yangon General Hospital, Workers' Hospital and Infectious Diseases Hospital were included in this study. These comprised of 409 males and 210 females and their ages ranged from 18 to 65 years. The stool samples were transported in Cary Blair media or in clean containers.

Bacteriological examination of pathogens

It was done according to the method described by World Health Organization manual 1981 [3]. Routinely, MacConkey Agar (MA), Xylose Lysine Dextrose Agar (XLD) and Salmonella-

Shigella agar (SS) were used for the isolation of Enterobacteriaceae. Thioglycolate Citrate Bile Salt (TCBS) Agar was used for isolation of vibrios. For secondary culture, alkaline peptone water and Selenite F broth were used for isolation.

Identification of bacterial pathogens

Biochemical characterization was carried out by using Triple Sugar Iron Agar (TSI), Motility Sulphide Indole Agar (SIM), Lysine Iron Agar (LIA) or Lysine Indole Motility Agar (LIM). Urease tests, Gram staining, oxidase reaction and other biochemical reactions were done wherever necessary.

Serological testing

For *Shigella* and *Salmonella* species, serological tests were done with appropriate antisera available from Denka Seikan Co. Ltd., Japan. For *Escherichia coli*, the locally prepared pooled polyvalent antisera were used for screening the organisms and further confirmed by specific monovalent antisera. Further confirmation for specific somatic antigen 'O' and flageller antigen 'H' were also done by using homologous antisera.

Antibiotic sensitivity test

The available 21 types of antibiotic discs from BBL, USA and Denka Seikan Co., Ltd., Japan were used for antimicrobial sensitivity testing on Mueller Hinton agar and in broth media. A growth of approximately 10^8 organisms/ml adjusted by MacFarland turbidity tubes were spread on the media and sensitivity test was done using antibiotic discs.

Interpretation for zone size diameters

Interpretative zone standards were performed as described by Barry and Thernsberry, 1985 [4]. These were used to define the interpretive categories in our series.

RESULTS

It is shown in Table 1 that 33.0 per cent of adults were suffering from enteropathogenic *E. coli*. Out of these patients, 24.5 per cent passed blood and mucous, 1.5 per cent mucous only, 17.2 per cent watery diarrhoea and the remaining 56.9 per cent semisolid type of stool.

Table 2 represents the distribution of serogroups of enteropathogenic *Escherichia coli* among 204 cases. It is shown that the prevalence of O1 was (6.9) per cent, O26 (8.8) per cent, O55 (10.8) per cent, O86 (6.4) per cent, O114 (8.8) per cent, O125 (6.9) per cent, O126 (5.9) per cent, O128 (6.7) per cent and O142 (4.9) per cent. The other serogroups were found to be scattered.

Table 3 shows the resistance pattern to different antibiotics of Enteropathogenic *E. coli* (EPEC) and resistance to ampicillin was 87 per cent, chloramphenicol 58 per cent, streptomycin 49 per cent, benzyl penicillin 55 per cent, tetracycline 44 per cent and septrin 21 per cent respectively.

It was observed that 77.8 per cent O1 serogroups of *E. coli* were resistant to ampicillin, 22.2 per cent to chloramphenicol, 33.3 per cent to carbenicillin, 44.4 per cent to penicillin, 55.6 per cent to streptomycin, 55.6 per cent to tetracycline and 11.1 per cent to trimethoprim. They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, sisomycin and tobramycin (Table 4).

Of the *E. coli* serogroups O26, 88.9 per cent were resistant to ampicillin, 66.7 per cent to chloramphenicol, 44.4 per cent to carbenicillin, 33.3 per cent to colistin, 11.1 per cent to septrin, 11.1 per cent to sisomycin, 44.4 per cent to streptomycin, 44.4 per cent to tetracycline and 11.1 per cent to trimethoprim respectively. They were sensitive to amikacin,

Table 1. Types of stool in relation to enteropathogenic *Escherichia coli* serogroups (n = 619)

Sr. No.	<i>E. coli</i> serogroups	Cases isolated	Types of stool			
			Blood + mucous	Mucous	Watery	Semi-solid
1	01	14	8			6
2	06	5	3		1	1
3	08	7	3			4
4	025	10	1		2	7
5	026	18	3		6	9
6	027	8	1		1	6
7	028	5			1	4
8	044	1				1
9	055	22	3	1	2	16
10	078	2				2
11	086	13	3		2	8
12	0111	2				2
13	0112	2	2			
14	0114	18	4	1	3	10
15	0119	3			1	2
16	0124	1	1			
17	0125	14	2		4	8
18	0126	12	5		3	4
19	0127	2	2			
20	0128	14	3			11
21	0136	1				1
22	0142	10	2	1	2	5
23	0146	2			1	1
24	0148	4	1			3
25	0152	3	1		1	1
26	0157	2			1	1
27	0159	5	1		2	2
28	0164	4	1		2	1
Total		204	50	3	35	116
Per cent		33.0	24.5	1.5	17.2	56.9

furazolidone, gentamycin, nalidixic acid and tobramycin.

Of the *E. coli* serogroup 055, 72.7 per cent were resistant to ampicillin, 72.7 per cent to chloramphenicol, 27.3 per cent to carbenicillin, 36.4 per cent to colistin, 9.1 per cent to kanamycin, 27.3 per cent to penicillin, 18.2 per cent to septrin, 36.4 per cent to streptomycin, 36.4 per cent to tetracycline and 18.2 per cent to trimethoprim respectively. They were sensitive to amikacin, furazolidone, gentamycin, nalidixic acid,

sisomycin and tobramycin.

Of the *E. coli* 0114, 90 per cent were resistant to ampicillin, 50 per cent to chloramphenicol, 30 per cent to carbenicillin, 50 per cent to colistin, 30 per cent to penicillin, 40 per cent to streptomycin and 20 per cent to tetracycline respectively.

They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, septrin, sisomycin, tobramycin and trimethoprim.

Table 2. Percentage of enteropathogenic *Escherichia coli* by serogroups (n = 204)

Serogroup	Percentage	Serogroup	Percentage	Serogroup	Percentage
01	6.9	086	6.4	0136	0.5
06	2.5	0111	1.0	0142	4.9
08	3.4	0112	1.0	0146	1.0
025	4.9	0114	8.8	0148	2.0
026	8.8	0119	1.5	0152	1.5
027	3.9	0124	0.5	0157	1.0
028	2.5	0125	6.9	0159	2.5
044	0.5	0126	5.9	0164	2.0
055	10.8	0127	1.0		
078	1.0	0128	6.7	Total 204 cases	

Table 3. Resistance pattern to different antibiotics

Sr. No.	Antibiotics	No. of EPEC tested	Resistant cases (per cent)
1	Ampicillin Am2	77	87.01
2	Chloramphenicol C5	77	58.44
3	Carbenicillin CB100	77	28.57
4	Clindamycin CC2	77	98.70
5	Colistin CL10	68	47.05
6	Furazolidone Fx100	77	9.09
7	Penicillin, benzyl P10	44	55.28
8	Streptomycin S10	77	49.35
9	Septrin SXT	68	20.58
10	Tetracycline Te5	77	44.15
11	Trimethoprim IMP5	53	9.09

Table 4. Antibiotic resistance pattern of EPEC serogroups in percentage

Sr. No.	Antibiotics potency	<i>E. coli</i> serogroups							
		01	026	055	0114	0125	0126	0128	0142
		n = 9	9	11	10	14	7	9	8
1	Am2	77.8	88.9	72.7	90.0	100.0	85.7	77.8	100.0
2	An30	S	S	S	S	S	S	11.1	S
3	C5	22.2	66.7	72.7	50.0	71.4	42.9	55.6	75.0
4	CB100	33.3	44.4	27.3	30.0	28.6	14.3	11.1	37.5
5	CC2	100.0	100.0	90.9	100	100.0	100.0	100	100.0
6	CF30	NT	NT	NT	NT	7.2	NT	NT	NT
7	CL10	NT	33.3	36.4	50.0	35.7	42.9	66.7	75.0
8	FX100	S	S	S	S	14.3	S	33.3	12.5
9	GM10	S	S	S	S	S	S	11.1	12.5
10	K30	S	NT	9.1	S	7	S	22.2	12.5
11	P10	44.4	NT	27.3	30.0	100.0	NT	NT	NT

Table 4. Contd.

Sr. No.	Antibiotics potency	<u>E. coli</u> serogroups							
		01	026	055	0114	0125	0126	0128	0142
		n = 9	9	11	10	14	7	9	8
12	PB300	NT	11.1	NT	NT	NT	S	22.2	12.5
13	N30	S	NT	NT	S	7.1	S	NT	S
14	NA30	S	S	S	S	S	S	S	S
15	NE130	NT	NT	NT	NT	S	NT	NT	NT
16	SXT	NT	11.1	18.2	S	28.6	S	44.4	37.5
17	SIS10	S	11.1	S	S	S	S	11.1	12.5
18	S10	55.6	44.4	36.4	40.0	64.4	57.1	55.6	37.5
19	Te5	55.6	44.4	36.4	20.0	50.0	42.9	66.7	52.5
20	NN10	S	S	S	S	21.4	S	11.1	12.5
21	IMP5	11.1	11.1	18.2	S	21.4	NT	NT	NT

S = Sensitive

NT = Not tested

Of the E. coli serogroup 0125, 100 per cent were resistant to ampicillin, 71.4 per cent to chloramphenicol, 28.6 per cent to carbenicillin, 7.2 per cent to cephalothin, 35.7 per cent to colistin, 14.3 per cent to furozolidone, 7.1 per cent to kanamycin, 100 per cent to penicillin, 7.1 per cent to neomycin, 28.6 per cent to septrin, 64.4 per cent to streptomycin, 21.4 per cent to tobramycin and 21.4 per cent to trimethoprim respectively. They were sensitive to amikacin, gentamycin, nalidixic acid, netilmicin and sisomycin.

Of the E. coli serogroup 0126, 85.7 per cent were resistant to ampicillin, 42.9 per cent to chloramphenicol, 14.3 per cent to carbenicillin, 42.9 per cent to colistin, 42.9 per cent to tetracycline and 57.1 per cent to streptomycin respectively. They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, polymyxin B, neomycin, nalidixic acid, septrin, sisomycin and tobramycin.

Of the E. coli serogroup 0128, 77.8 per cent were resistant to ampicillin, 11.1 per cent of amikacin, 55.6 per cent to chloramphenicol, 11.1 per

cent to carbenicillin, 66.7 per cent to colistin, 33.3 per cent to furazolidone, 11.1 per cent to gentamycin, 22.2 per cent of kanamycin, 22.2 per cent to polymyxin B, 44.4 per cent to septrin, 11.1 per cent to sisomycin, 55.6 per cent to streptomycin and 11.1 per cent to tobramycin respectively. They were sensitive to nalidixic acid only.

Of the E. coli serogroup 0142, 100 per cent were resistant to ampicillin, 75 per cent to chloramphenicol, 37.5 per cent to carbenicillin, 75 per cent to colistin, 12.5 per cent to furazolidone, 12.5 per cent to gentamycin, 12.5 per cent to kanamycin, 12.5 per cent to polymyxin B, 37.5 per cent to septrin, 12.5 per cent to sisomycin, 37.5 per cent to streptomycin, 52.5 per cent to tetracycline and 12.5 per cent to tobramycin respectively. They were sensitive to amikacin, neomycin and nalidixic acid.

Table 5 shows that Flagella serotypes were scattered and restricted to some serogroups. Out of 172 cases tested 71 cases (41.2 per cent) were untypable and 13 cases showed no flagella. It is shown that H16 was

Table 5. Serotypes of flagella among different somatic serogroups

Sr. No.	'O' serogroups	Tested	Flagella 'H' type		
			H-	HUT	Others
1	01	14	3	4	H5(2), H15, H16, H21, H32, H48
2	06	5	0	0	H9(2), H16(3)
3	08	7	1	3	H9(2), H21
4	025	10	4	2	H5, H7, H9, H21
5	026	18	1	13	H5, H6, H11, H30
6	027	8	1	4	H11, H17, H21
7	055	22	0	11	H9, H15(2), H17(2), H19, H21, H34, H36, H45, H52
8	078	2	0	1	H16
9	086	13	2	1	H18(3), H19, H21, H30(2), H27, H51
10	0111	2			H31, H45
11	0119	3	0	2	H27
12	0124	1			H16
13	0125	14	0	9	H11, H17(2), H18(2)
14	0126	12	0	7	H10, H15, H18, H25(2)
15	0127	2			H15, H16
16	0128	14	0	6	H2(2), H12(2), H14, H21, H25, H26
17	0142	10	0	4	H6, H15(2), H38, H52(2)
18	0148	4	0	1	H28, H30(2)
19	0157	2	0	1	H6
20	0159	5	0	0	H4(5)
21	0164	4	1	2	H20
	Total	172	13	71	88
	Percent		0.77	41.2	54.2

*Figure in parenthesis denotes number of positive cases

more restricted to 06 serogroup and H4 to 0159 serogroup. It is also shown that 88 cases (51.2 per cent) belonged to different flagella serogroups.

DISCUSSION

In 1985, the role of enteropathogenic *E. coli* was evaluated in a group of children with endemic diarrhoea admitted to Dhaka hospital and it was reported that 23 per cent of them belonged to serogroups 020, 026 and 018 [5]. Anathan also reported 24.05 per cent of enteropathogenic *E. coli* were isolated from diarrhoea

cases [1]. In Sweden 143 strains of enteropathogenic *E. coli* of different serogroups were isolated from children with diarrhoea and most of them belonged to serogroups 055, 0119, 0126, 0127 and 0128 [6]. Siitonen reported that *E. coli* serotypes 01, 02, 06, 07, 018, 025 and Rough-type lipopolysaccharide accounted for 40 per cent of all isolates from 287 healthy adults [7].

Enteropathogenic *E. coli* strains which were isolated from 18 per cent of children with diarrhoea admitted to the hospital Municipal Salles Neto, Rio de Janeiro were reported to belong to serogroups 0111, 0125,

0126, 0127, 0142, 0128, 0119, 055 and 0114 [2]. In an epidemiological study of enteropathogenic *E. coli* by Olukoya et al. [8], they found that the most common serotype encountered was 055 followed by 026. Katouli et al. [9] also studied the prevalence of different types of diarrhoea-producing *E. coli* in 273 patients attending out-patient clinics in Banda-Abba during 1984 and reported that enteropathogenic *E. coli* belonging to 12 different serogroups of which 0128 and 0126 were the most common were found in 31 per cent of the patients. Sullivan also reported that classical enteropathogenic *E. coli* serotypes associated with diarrhoea were 018, 026, 0119 and 0126 in Gambia children [10]. In our study, out of 619 cases, 204 cases (32.4 per cent) yielded *E. coli* belonging to 28 different serogroups of which 055, 026, 0114, 0128, 0125 and 0126 were most common.

Antibiotics are primarily of value in dysentery. Many other treatments are available, most of which are intended to diminish stool volume, and shorten illness, but none has proven both safe and sufficiently effective to be used routinely in infants and young children [11]. However, no antibiotics or chemotherapeutic agent (including sulfaguanidine, other poorly absorbed sulfonamides, neomycin and halogenated oxyquinolines) has proven to be of value for the routine treatment of acute diarrhoea and their use is inappropriate and possibly dangerous. Olukoya et al., 1988 reported that all strains of enteropathogenic *E. coli* isolated were sensitive to colistin, nalidixic acid, nitrofurantoin, cefotaxime, amikacin and augmentin. Of 102 strains, 46 per cent were resistant to one or more of the following antimicrobial agents. Co-trimoxazole, tetracycline, ampicillin, streptomycin, sulfanomides and a combination of

ampicillin with sulbactam [8].

Takahashi [12] studied the antibiotic susceptibility of different pathogens and reported that *E. coli* demonstrated the highest level of susceptibility to ofloxacin and relatively high susceptibilities to oxolinic acid, ampicillin, kanamycin and chloramphenicol. In cases studied by Filali et al. [13] all 62 strains of *E. coli* serogroups 078, 01 and 02 were sensitive to colistin, flumequin and gentamycin but few strains were resistant to neomycin, nalidixic acid and trimethoprim and intermediate resistant to nitrofurane, sulfanomide, chloramphenicol, spectinomycin and ampicillin. Gonzalez and Blanco [14] reported that Verotoxic *E. coli* (VTEC) were resistant to antibiotics at higher rates than necrotizing *E. coli* (NTEC) and non VTEC and non NTEC also showed the highest multidrug resistant pattern. In our study, EPEC were also resistant to ampicillin and clindamycin at higher rates. They were also resistant to chloramphenicol, colistin, penicillin, streptomycin and tetracycline in more than 40 per cent of the cases tested. Most of the EPEC were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, sisomycin and tobramycin.

REFERENCES

1. Anathan, S., Subramanian, S., Thyagrajan, S.P. et al. Studies on the pathogenicity of classical enteropathogenic *Escherichia coli* strains isolated from acute diarrhoea among children 0-5 years of age. *Indian Journal of Pathology and Microbiology* 1989; 32: 198-206.
2. Regua, A.H., Bravo, V.L., Leal, M.C. & Lobo-Leite, M.E. Epidemiological survey of the enteropathogenic *Escherichia coli* isolated from children with diarrhoea. *Journal of Tropical Pediatrics* 1990; 36: 176-179.
3. World Health Organization. Manual of

- diagnostic laboratory procedures for acute infection. Simplified methods. Geneva, World Health Organization, 1981.
4. Barry, A.L. & Thornsberry, C. Susceptibility tests. Diffusion test procedures. In: Manual of Clinical Microbiology, 4th edition, Lennette, E.E. et al. (eds.), American Society for Microbiology, Washington D.C., 1985.
 5. Mayenuddin, M. & Rahman, K.M. Enteropathogenic Escherichia coli diarrhoea in hospitalized children in Bangladesh. Journal of Clinical Microbiology 1985; 22: 838-840.
 6. Katouli, M., Kuhn, I. & Mollby R. Biochemical phenotype of enteropathogenic Escherichia coli common to Iran and Sweden. Journal of Medical Microbiology 1991; 35: 270-277.
 7. Siitonen, A. Escherichia coli in fecal flora of healthy adults: serotypes and type K fimbriae, non-P mannose resistant adhesins and hemolytic activity. Journal of Infectious Diseases 1992; 166: 1058-1065.
 8. Olukoya, D.K., Daini, O., Alabi, S.A., Coker, A.O., Odugbemi, I. & Akiirimi, E.O. Antimicrobial resistance patterns and plasmids of enteropathogenic Escherichia coli isolated in Nigeria. European Journal of Epidemiology 1988; 4: 306-309.
 9. Katouli, M., Jaafari, A. & Ketabi, G.R. The role of diarrhoeagenic Escherichia coli in acute diarrhoeal diseases in Bandar-Abbas, Iran. Journal of Medical Microbiology 1988; 27: 71-74.
 10. Sullivan, P.B., Coles, M.A., Abera, G. & Ijungh, A. Enteropathogenic and enteroadherent-aggregative Escherichia coli in children with persistent diarrhoea and malnutrition. Annals of Tropical Pediatrics 1994; 14: 105-110.
 11. Diarrhoeal Diseases Control Programme. Drugs in the management of acute diarrhoea in infants and young children. WHO/CDD/CMT/86.1, 1986.
 12. Takahashi, I., Yoshida, I., Higashide, Y. & Sakano, I. Susceptibilities of Escherichia coli, Salmonella and Staphylococcus aureus isolated from animals to ofloxacin and commonly used antimicrobial agents. Japanese Journal of Antibiotics 1990; 43: 89-99.
 13. Filali, E., Bell, J.G., el-Houadft, H., Huggins, N.B. & Cook, J.K. Antibiotic resistance of Escherichia coli strains isolated from children with colisepticaemia in Morocco. Comparative Immunology, Microbiology and Infectious Diseases 1988; 11: 12-24.
 14. Gonzalel, E.A. & Blanco, J. Serotypes and antibiotic resistance of verotoxigenic (VTEC) and necrotizing (NTEC) Escherichia coli strains isolated from calves with diarrhoea. FEMS Microbiology Letter 1989; 51: 31-36.
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Objective assessment of meconium content in amniotic fluid

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In the first part, a sample set of meconium and amniotic fluid solutions with concentrations of 15%, 10%, 7.5%, 5%, 3% and 1.5% was prepared by mixing fresh meconium with clear amniotic fluid. After centrifugation, meconiumcrit values were measured similar to haematocrit measurement. A graph was constructed from meconium concentration and respective meconiumcrit values. A linear relationship was found. In the second part meconiumcrit readings were measured from meconium-stained amniotic fluid obtained from women in labour. Fetal and neonatal morbidity were increased significantly in cases where meconium level reached $\geq 10\%$. The cut-off level requiring tracheal intubation was found to be 15%. High meconiumcrit levels could alert the obstetrician to the possibility of neonatal morbidity and the need for intensive resuscitative measures.

INTRODUCTION

Amniotic fluid is meconium stained in 11-22% of all deliveries. The main clinical importance is the possibility of meconium aspiration syndrome, which complicates approximately 2% of these deliveries and may result in neonatal mortality as high as 40%.

Reed [1] and Brews [2] suggested that anoxia caused the sphincter to relax and meconium subsequently passed into the surrounding amniotic fluid. Saling [3] postulated that fetal hypoxia precipitated vasoconstriction in fetal gut, which caused hyperperistalsis, sphincter relaxation and passage of meconium. Walker [4] found that fetuses with meconium-stained fluid had lower umbilical vein oxygen saturation than normal fetuses at term.

Usually the amount of meconium in amniotic fluid is subjectively estimated by visual inspection and classified as thin (light), moderate or thick (heavy).

"Meconium crit" is a reproducible measure of objective assessment of meconium content of amniotic fluid [5].

This study was carried out to-

- (1) assess meconium content in meconium-stained amniotic fluid by meconium crit.
- (2) find out the relationship of meconium content with fetal condition at birth.
- (3) identify the cut-off point where tracheal visualization is required for suction.

PATIENTS AND METHODS

Part 1:

Clear amniotic fluid was obtained from patients who had no meconium-stained fluid during artificial rupture of membranes. It was stored at 22°C in opaque sterile containers.

Fresh meconium was obtained from term neonates.

Fifteen samples of amniotic fluid

from 15 women and 15 meconium samples from 15 infants were obtained.

3.0 g of meconium was placed into clear amniotic fluid to obtain 20.0 ml of stock solution with a concentration of 15.0 g meconium/100 ml amniotic fluid. This resulted in 15 independent stock solution samples. The stock solution was vortexed for 15 mins to ensure adequate mixing.

One milliliter of each stock sample was then diluted by adding 0.5, 1.0, 2.0, 4.0 or 9.0 ml of clear amniotic fluid. This produced a sample set with 6 concentrations.

15.0 g/100 ml	10.0 g/100 ml
7.5 g/100 ml	5.0 g/100 ml
3.0 g/100 ml	1.5 g/100 ml

One-tenth milliliter of each amniotic fluid/meconium mixture was passively drawn by capillary action into a standard haematocrit tube and centrifuged for 3 mins in the usual manner (12,700 g x 14,000 rpm) at room temperature.

The meconium crit was then measured directly as with a haematocrit. This was repeated 6 times for each concentration in every sample set. Meconium crit % and known meconium concentrations are shown in Table 1.

Table 1. Mean and standard deviation of meconium crit of known meconium concentration

Meconium concentration in g/100 ml	Meconium crit 10%	
	Mean	S.D.
15.0	17.67	1.11
10.0	13.93	0.98
7.5	8.13	1.47
5.0	6.40	0.85
3.0	3.77	0.50
1.5	1.69	0.24

Part II:

Amniotic fluid samples from 200

mothers with meconium-stained amniotic fluid in labour or during caesarean section were collected by sterile glass containers.

Then it was drawn up by capillary action into a standard haematocrit tube and centrifuged for 3 mins (12,700 g x 14,000 rpm) at room temperature. Measurement was done with a haematocrit.

RESULTS AND ANALYSIS

A linear relationship is shown between actual meconium concentration and meconium-crit value (Table 1).

Fig. 1 reveals a significant negative correlation between meconiumcrit values and corresponding Apgar scores.

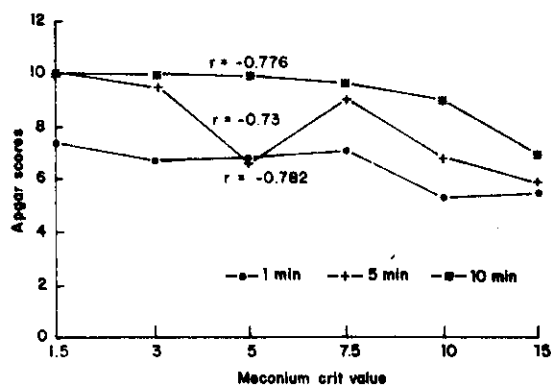


Fig. 1. Correlation between meconiumcrit values and Apgar scores

It is apparent that as meconiumcrit values increased, the requirement for suction and oxygen increased and at meconiumcrit 15% there was a 10% incidence of endotracheal intubation.

Morbidity increased with increase in meconiumcrit values with 4.1% and 5.0% mortality at MCV 10% and 15% respectively (Table 3).

DISCUSSION

The appearance of meconium during any stage of labour has long been considered a clinical sign of fetal

Table 2. Requirement for resuscitation according to meconium crit value

Meconium crit value	Not required	Required		
		suction	suction + O ₂	Endotracheal
(n ₁ = 53) 1.5%	51	2(3.7%)		
(n ₂ = 51) 3%	49	2(3.9%)		
(n ₃ = 30) 5%	27	2(6.6%)	1(3.3%)	
(n ₄ = 22) 7.5%	17	3(13.6%)	2(9.1%)	
(n ₅ = 24) 10%	18	3(12.5%)	3(12.5%)	
(n ₆ = 20) 15%	12	1(5.0%)	5(25.0%)	2(10%)

Table 3. Neonatal morbidity and mortality according to meconium crit value

Meconium crit values	Total number of neonates	Morbidity	Mortality
1.5%	53	3(5.6%)	
3%	51	7(13.7%)	
5%	30	9(30.0%)	
7.5%	22	10(45.4%)	
10%	24	11(45.8%)	1(4.1%)
15%	20	15(75.9%)	1(5.0%)

distress. Lately, the appearance of meconium as a sign of fetal distress has been questioned. According to Wood, Pinkerton and Leslie [5], there is no difference in fetal outcome when cases in which meconium appeared during delivery are compared to cases in which the amniotic fluid remains clear. However, in the CWH study [6], the presence of meconium was found to be significantly associated with poor fetal outcome (still - birth, early neonatal death and Apgar score ≤ 6).

Meconium passage per se does not indicate fetal distress. The occurrence of fetal asphyxia when meconium is present enhances the potential for meconium aspiration and a poor neonatal outcome. The presence of meconium should serve to alert the physician to a high risk fetal condition. Special care must be taken to

avoid conditions which would cause fetal asphyxia, such as those caused by uterine hyperstimulation and maternal hypotension, either positional or secondary to induction anaesthesia and/or other medication. Every attempt should be made to avoid difficult and/or traumatic delivery with the inherent increase in fetal compromise and asphyxia. Although unanswered questions remain regarding the significance of meconium passage, a more rational approach is now possible for the management of labour with meconium in the amniotic fluid.

CONCLUSION

- Meconium crit has a direct relationship with meconium concentration.
- With increasing meconium crit, Apgar scores at 1 min, 5 min and 10 min decreased.
- Need for resuscitation increases with increasing meconium crit values.
- Meconium crit 15% is the cutoff point where tracheal visualization is required for suction.

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REFERENCES

1. Reed, C.B. Physiology of meconium passage. Surgery, Gynaecology and Obstetrics, 26: 550. Cited by Gregory, C. (1980). Journal of Obstetrics and Gynaecology of British Commonwealth 1948; 55: 604-9.
2. Brews, A. Fetal asphyxia: Eden and Hollands Manual of Obstetrics, 9th ed., London J. and A. Churchill, 1948, 611. Cited by Gregory C. (1980). Journal of Obstetrics and Gynaecology of British Commonwealth 1948; 55: 604-9.
3. Saling, E.W. Fetal scalp pH sampling. Archives of Gynaecology 1962; 198: 82.
4. Walker, J. Meconium staining and lower umbilical vein oxygen saturation. Journal of Obstetrics and Gynaecology of British Commonwealth 1954; 61: 162.
5. Wood, C. & Pinkerton, J. Foetal distress. Journal of Obstetrics of Gynaecology of British Commonwealth 1961; 68: 427.
6. Than-Ihan-lin. fetal outcome and meconium stained liquor. Myanmar Medical Journal 1993; 39: 51-56.

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Seasonal variation of flea population and its relationship with small mammal hosts in sub-urban Yangon

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Monthly flea and small mammal surveys covering three seasons (cool dry, hot dry and monsoon) were carried out at three townships in Yangon. From more than 400 small mammals captured, two species of fleas totalling about 700 were collected and identified as Xenopsylla astia and Xenopsylla cheopis. General flea index was found to be highest in May (dry hot) with 6.7 and lowest in November (cool dry) with 0.09. Total ratio of collected X. astia and X. cheopis was found to be about 2:1. However, host-vector specificity was pronounced between these two species especially on B. bengalensis. The great majority of X. astia were infested on B. bengalensis ($p < 0.05$) and the majority of X. cheopis were infested on other aboveground species namely Rattus exulans, R. rattus and Suncus murinus mainly in dry hot seasons. Several factors which might govern the host-vector specificity are outlined. Due to the host-vector specificity, the limitations and the use of various flea indices are discussed and suggestions are made for a proper surveillance system. The prospect of the timely flea control program as a preventive plague control measure is also discussed.

INTRODUCTION

Plague continues to pose a threat to human health in certain regions of the world and recent bubonic and pneumonic plague outbreaks in India during 1994 illustrate how quickly plague can spread through human populations.

At present in Myanmar, the disease is endemic over a vast area of the country except in Ayeyawady Division, Taninthayi Division and Chin State. There has not been any pneumonic plague outbreak cases reported since 1975. Velimirovic (1972) summarised the history and geographic distribution of plague in Myanmar [1]. The presence of Yersinia pestis among small mammals in Yangon as well as human cases of plague has been reported in 1977 [2].

As in many other vector-borne diseases, the occurrence of plague is usually associated with the seasonal abundance of flea population. The ecological conditions in Yangon permit the development and maintenance of large populations of several species of commensal small mammals. Investigations of the rat and rat-flea vectors of plague in Yangon have been made at irregular intervals since 1920. The classic plague vector, X. cheopis, and X. astia occur in large numbers on the small mammals in Yangon.

Surveillance of vector populations has been regarded as one of the important preventive measures and knowledge of local flea species and their hosts is essential for estimating risks of human plague infection. Therefore, a one year study was carried out in

three townships in Yangon which cover most of the land entrance routes to the city.

Objectives

To provide the necessary information on fleas for plague surveillance and control.

To assess and update the seasonal variation of flea population and its relationship with small mammal host.

To be able to design specific vector control measures that are appropriate for local situations.

MATERIALS AND METHODS

The survey was conducted in three townships in Yangon, namely Insein, Hsing and Mayangon. Animals were captured in locally-made wooden live traps baited with dried fish. These collections covered every month from January to December 1995. Each monthly trapping lasted for three nights and about 60 households were usually involved. Three traps per house were distributed in a designated neighbourhood in the afternoon and rechecked the next morning. Traps were substituted as necessary to maintain 180 traps per night of the study area.

Captured animals were brought alive to the laboratory while still in the traps. There they were placed in plastic bags containing chloroform. The animals were brushed over a white enamel pan to remove fleas. These fleas and any found in the plastic bags were placed in numbered vials of 70% alcohol and later identified by direct microscopic examination. Then, individual animals were sexed, measured, weighed and the reproductive conditions noted.

RESULTS

Five species of small mammals were captured from the residential neigh-

bourhood of the above-mentioned three townships in Yangon. The distribution of four frequently captured species: Bandicoota bengalensis, Rattus exulans, Rattus rattus, Suncus murinus and one least frequently captured species, Mus musculus are depicted in Figure 1.

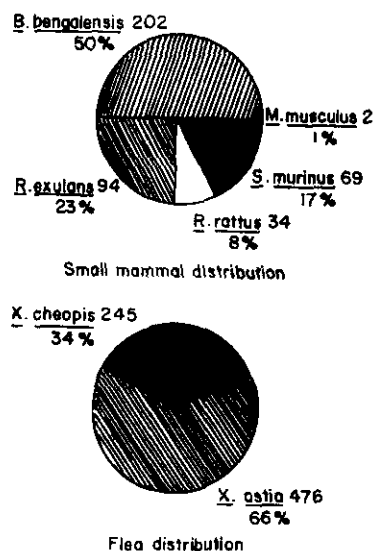


Fig. 1. Species distribution of total captured small mammals and fleas from study area

Two flea species namely Xenopsylla astia and X. cheopis were identified and the ratio of X. astia to X. cheopis for the whole study was found to be 1.94:1.

The monthly general indices for both X. astia and X. cheopis were determined and the results are shown in Figure 2. The general index was found to be highest in May (hot dry season) with 6.7 and declined during the rainy season and reached the minimum towards the beginning of the cool dry season. When general indices of the two flea species were determined separately, sharp increase and decrease were observed in X. astia while gradual increase and decrease were found in X. cheopis. However, high infestation rate was evident in dry hot season for both species especially for X. astia in

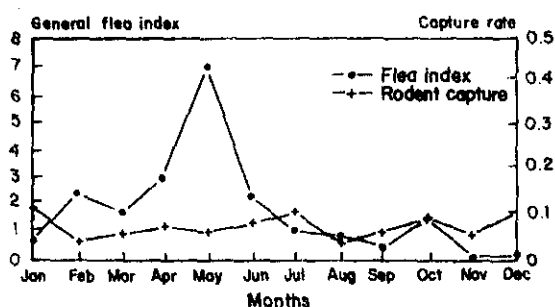


Fig. 2. Seasonal variation of flea density Insein, Mayangon and Hlaing

May (Figure 3).

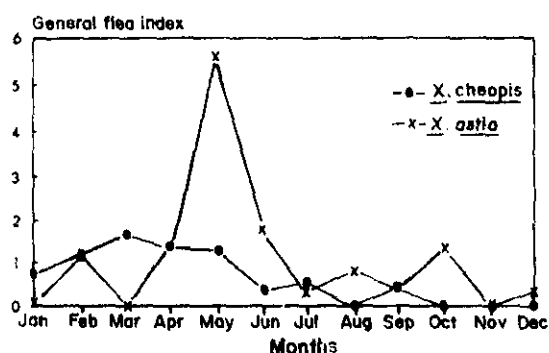


Fig. 3. Seasonal variation of flea density for *X. cheopis* and *X. astia*

The proportion of flea-infected animals among the most commonly captured species was not significantly different ranging between 40% and 52% (Chi-square: $p > 0.05$) (Table 1).

The majority of *X. astia* were found on *B. bengalensis* and the majority

of *X. cheopis* were found on *R. exulans*, *R. rattus* and *S. murinus*. *X. astia* indices were high throughout the survey on *B. bengalensis* compared to *X. cheopis* indices (t-test: $p < 0.05$). Although statistically significant results were not obtained, *X. cheopis* indices were all time higher than *X. astia* on *R. exulans* and *R. rattus*. The same applied to *S. murinus* except in cool dry season. The comparisons are depicted in Figures 4, 5 and 6.

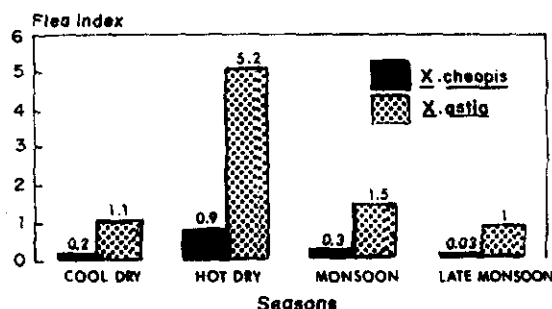


Fig. 4. Level of *X. cheopis* and *X. astia* infestation on *B. bengalensis*

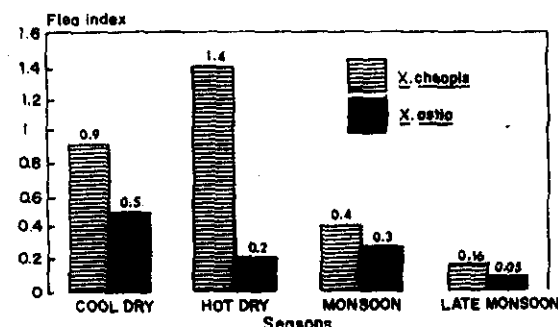


Fig. 5. Level of *X. cheopis* and *X. astia* infestation on *R. exulans* & *R. rattus*

Table 1. Total number of small mammal species and the status of flea infestation

Species	Cool dry	Hot dry	Monsoon	Late monsoon	All seasons	Infested animals No.	%
<i>B. benglaensis</i>	38	41	50	73	202	106	52.48
<i>R. exulans</i>	24	28	29	13	94	38	40.43
<i>R. rattus</i>	9	9	11	5	34	17	50.00
<i>M. musculus</i>	1	1	0	0	2	0	0.00
<i>S. murinus</i>	22	18	16	13	69	28	40.58
Total	94	97	106	104	401	189	47.13

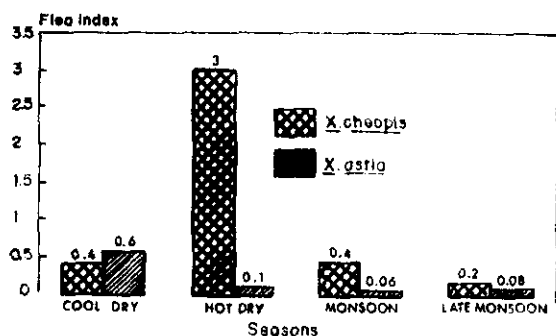


Fig. 6. level of *X. cheopis* and *X. astia* infestation on *S. murinus*

DISCUSSION

In previous studies in Myanmar, rat flea surveys in the Port area of Yangon showed that *X. astia* constituted about 95% and *X. cheopis* 5% of the total flea fauna on the small mammals [3]. Harrison also reported only *X. astia* and *X. cheopis* from small mammals in Yangon [4]. Walton & Tun reported that *X. astia* and *X. cheopis* occurred in large number and an occasional cat flea *Ctenocephalides felis* (less than 1% of total) can be found [5].

The same authors reported that the ratio of the number of *X. astia* to *X. cheopis* recovered was approximately 4:1.

In previous studies, *X. cheopis* were vastly outnumbered by *X. astia*. In the present study, the ratio of the number of *X. astia* to *X. cheopis* recovered was found to be approximately 2:1. The occurrence of *X. cheopis* seemed to be increasing. This point should not be ignored and needs verification. At least seasonal check up should be followed up in the future in the neighbouring townships.

Fluctuation in abundance of both flea species in the study area appeared to be closely tied to climate, and heavy rainfall caused a decline in the number of these fleas recovered. The present finding generally

agreed with the findings of Walton and Tun [5] with the exception of lower *X. astia* indices in several months throughout the year. However, hot dry season is the most favourable for both species and occurrence is in large numbers. During this season, there are risks of epizootic plague and sporadic occurrence of human cases in Yangon and climatically similar areas. Therefore, constant surveillance of flea density especially at the beginning of the dry hot season is necessary. Depending on the available results e.g. high cheopis index for particular area with previous history of rat fall or human cases, preventive flea control measures can be carried out. Since many flea population have been found to be resistant to DDT, Actellic 2% dust (Pyrimiphos methyl) is the proposed insecticide for flea control. Patch dusting in and around houses and burrows is recommended.

In relation to host specificity between the two flea species, *X. astia* was most abundant on the underground species (*B. bengalensis*) and *X. cheopis* on the three aboveground species (*R. exulans*, *R. rattus* and *S. murinus*). In this respect, it is considered that environmental condition i.e. microclimate may play a major role in host specificity. Although *X. astia* occurred in greater number, it is considered to have a narrow range of temperature, being adversely affected by cool or cold climate. *X. cheopis* appears to have the great tolerance for dry conditions. In all seasons, inside the burrows, moisture might be greater than in an aboveground environment. Since fleas are nest dwelling animals, host associated factors such as construction and location of nests or burrows tend to modify the effect of an ambient climate, creating microclimatic condition favourable or unfavourable to a species. The present study indicated that host specificity is likely due to

the effect of underground and above ground environment. Thus, it may be appropriate to calculate the flea indices separately for underground and aboveground species rather than solely based on general index when using as a predictor value.

The most commonly used indices are as follows:

General flea index =

$$\frac{\text{Total no. of fleas collected}}{\text{Total no. of host species collected}}$$

General cheopis index =

$$\frac{\text{Total no. of cheopis collected}}{\text{Total no. of host species collected}}$$

Specific flea index =

$$\frac{\text{No. of flea sp. A collected from host Y}}{\text{No. of host sp Y examined}}$$

Total flea index =

$$\frac{\text{Total no. of fleas collected}}{\text{Host species Y examined}}$$

S F I $X. c > 1$: Regarded as indicative of dangerous situation (Pollitzer, 1954)

For comparison between survey sites: standardized as much as possible.

As few as 20 rats is sufficient to establish a reliable SFI for X. cheopis (Schwan, 1984).

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REFERENCES

1. Velimirovic, B. Plague in Southeast Asia. Transactions of the Royal Society of Tropical Medicine and Hygiene 1972; 66: 479.
2. Brooks, J.E. et al. Plague in small mammals and humans in Rangoon, Burma. Southeast Asian Journal of Tropical Medicine & Public Health 1977; 8: 335-344.
3. Jolly, G.G. et al. Rat flea survey of Rangoon, Part I: The Port Area. Indian Journal of Medical Research 1931; 28: 1231.
4. Harrison, J.L. Rat vectors of plague. Nature 1946; 157: 483.
5. Walton, D.W. & Iun, M.M. Fleas of small mammals from Rangoon, Burma. Southeast Asian Journal of Tropical Medicine and Public Health 1978; 9: 369-377.
6. Schwan, T.G. Sequential sampling to determine the minimum number of host examinations required to provide a reliable flea (Siphonaptera) index. Journal of Medical Entomology 1984; 21: 670-674.

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The phytohemagglutinin (PHA) - induced peripheral lymphocyte blastogenic response in cancer patients

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The *in vitro* blastogenic response of peripheral blood lymphocytes was determined in 50 patients with various types of malignancies by quantitation of phytohemagglutinin PHA-induced tritiated thymidine uptake. PHA-induced blastogenic response was impaired in patients with malignancy more so in patients with squamous cell carcinoma in whom there was an inverse correlation with the stage of the tumor and the reduction. No correlation was seen in patients with adenocarcinoma, lymphoma or sarcoma. These data indicate that PHA-induced blastogenic response is a potentially useful assay for the detection of immunocompromized persons especially in patients suffering from squamous cell carcinoma.

INTRODUCTION

Impairment of cellular immunity in patients with cancer had been well documented by the decreased ability of such patients to develop delayed type hypersensitivity reaction [1,2]. Many studies had revealed that *in vitro* lymphocyte reactivity to PHA quantitated *in vivo* cell mediated immune competence [3,4,5]. Evidences accumulated that abnormalities of *in vitro* blastogenic response in patients with lymphoreticular malignancies correlated with the clinical stage of the disease [3,6,7]. However, similar studies in patients with non-lymphoid tumors had yielded conflicting results. Some demonstrated impaired PHA response and others reported no reduction in the lymphocyte blastogenic reactions [8,9].

The purpose of the study is to find out whether there is an impaired blastogenic response in cancer patients and if possible to correlate the depressed reactivity with tumor histology and stage of the disease.

MATERIALS AND METHODS

Fifty cancer patients attending the

Cancer Ward, Yangon General Hospital, before receiving chemotherapy or radiation were studied (Table 1). Five milliliter of heparinized blood (10 IU heparin/ml of blood) was collected for mitogenic response. Tritiated thymidine uptake in three days *in vitro* culture at the optimal condition for maximum stimulation of lymphocyte was performed. PHA from Sigma Co. was used. The detailed procedure was described elsewhere [10]. Control values were obtained from previous study on healthy blood donors (Table 2). For statistical analysis the blastogenic response of each cancer patient was determined and compared with the appropriate age and sex group of normal control. Correlation between lymphocyte reactivity and tumor histology and stage of the disease were evaluated by Chi² test.

RESULTS

The lymphocyte blastogenic response of control

Using the arbitrary classification of lymphocyte blastogenic response defined in relation to the mean reactivity ± 1 SD for each age and

Table 1. Mitogen response of 50 cancer patients

Sr. No.	Sex	Age	Type of cancer	PHA response	Hb	TWBC
1	F	44	Ca cervix	15.65	8.00	6.2
2	F	67	"	5.17	12.12	7.2
3	F	60	"	26.35	19.57	10.2
4	F	45	"	9.93	13.64	8.7
5	F	35	"	22.25	11.19	7.8
6	F	64	"	51.11	10.72	11.8
7	F	33	"	16.21	8.44	4.2
8	F	*43	"	19.40	13.05	5.2
9	F	*52	"	14.64	12.20	12.4
10	F	56	Ca breast	15.68	12.23	6.8
11	F	*44	"	23.57	12.12	5.8
12	F	50	"	32.31	12.24	4.0
13	F	29	"	29.01	13.05	9.7
14	F	63	"	17.58	10.61	10.2
15	F	53	"	22.07	11.77	9.4
16	F	*49	Ca Lung	19.51	11.30	8.9
17	F	55	"	13.02	8.58	6.8
18	F	66	"	1.44	15.15	12.8
19	F	59	"	58.82	12.94	11.6
20	M	63	"	31.23	12.59	10.2
21	M	57	"	42.16	9.32	6.4
22	M	*44	"	17.91	15.38	10.4
23	M	80	"	6.76	13.01	4.2
24	M	58	"	7.56	8.23	10.9
25	M	*66	"	15.69	12.70	9.2
26	F	56	Ca endometrium	2.31	14.50	9.8
27	M	60	Ca rectum	50.53	13.75	9.2
28	M	30	Pinealoma	7.20	15.38	9.8
29	F	27	Malignant lymphoma	14.65	8.76	8.7
30	M	52	"	11.11	10.83	6.9
31	M	38	Hodgkin's	36.66	12.70	9.7
32	M	20	All	3.44	4.66	48.0
33	F	49	Ca ovary	17.05	12.35	10.8
34	F	*63	Ca oesophagus	20.13	13.40	8.7
35	M	68	"	14.67	11.42	5.4
36	F	52	Ca buccal mucosa	21.62	9.79	4.1
37	F	*67	"	50.12	10.72	12.7
38	M	*31	"	1.84	15.70	11.7
39	M	*61	"	15.43	10.61	12.8
40	M	*37	Ca PNS	9.44	12.70	5.8
41	M	*71	Ca tongue	23.00	10.80	5.2
42	M	56	Ca pyriform	25.82	13.98	8.7
43	M	57	Ca larynx	21.84	15.61	9.6
44	M	48	Metastatic gland	29.32	13.05	7.2
45	M	57	"	2.35	10.95	12.6
46	M	68	Ca nose	26.39	12.12	4.0
47	M	55	Ca jaw	22.11	13.17	5.6
48	M	24	Ca paranasal sinus	36.99	9.90	6.2

to be continued

Sr. No.	Sex	Age	Type of cancer	PHA response	Hb	TWBC
49	M	46	Meduloblastoma	19.47	12.59	4.2
50	M	45	Liposarcoma	21.13	7.57	8.7

Hb = Hemoglobin concentration (gm/dl)

TWBC = Total white cell count ($\times 10^9$ /dl)

PHA = Phytochemagglutinin response (cpm)

* = Regional lymph node involvement or distant metastasis

Table 2. Age and sex correlated PHA-induced lymphocyte blastogenic response of 50 healthy blood donors

Age group (years)	Sex	No. of subjects	Mean reactivity (cpm) $\pm$ 1SD	Range of normal reactivity(cpm)
15-35	Male	13	30.14 $\pm$ 13.17	16.97-43.31
	Female	14	12.41 $\pm$ 5.61	6.80-18.02
35-55	Male	12	38.23 $\pm$ 17.23	21.00-55.46
	Female	11	15.28 $\pm$ 8.27	7.01-23.55

Table 3. Age and sex correlated PHA-induced lymphocyte blastogenic response of 50 cancer patients compared with 50 controls

Patient group	No. of patients	Percent (low*)	Percent (high**)	Percent (normal***)
Control	50	11	14	75
Cancer	50	38	12	49
Squamous (localized)	25	32	16	52
Squamous (metastasized)	10	56	11	33
Adenocarcinoma (localized)	19	21	32	47
Adenocarcinoma (metastasized)	4	50	0	50
Lymphoma & Leukemia	4	50	0	50
Sarcoma	2	50	0	50

* <1SD of mean of control

** $\pm$ 1SD of mean of control

*** >1SD of mean of control

sex group 75% of healthy blood donors were scored as normal reactivity, 11% had low reactivity and 14% had high reactivity (Table 3).

The lymphocyte blastogenic response of cancer patients

The frequency distributions of the lymphocyte reactivities of cancer patients were compared with those

of the controls of the same age and sex group. As a group cancer patients had significantly ($P=0.002$) low lymphocyte reactivity. Low reactivity in 38% (Table 3). When lymphocyte reactivity was correlated with tumor histology and stage of the disease, significantly more patients with squamous cell carcinoma ($P=0.04$) had reduced lymphocyte reactivity.

Reduced reactivity was noted in 32%, normal reactivity in 52% and high reactivity in 16% of squamous cell carcinoma patients. Patients with adenocarcinoma had no significant diminution in lymphocyte reactivity ($P=0.12$), lymphoma and leukemia ($P=0.05$) and sarcoma ($P=0.19$). Dividing the cancer patients into two groups namely one with localized tumor and the other with regional lymph node or distant metastasis, there were significant differences between two groups in patients with squamous cell carcinoma ($P=0.002$). However, there was no significant difference between the localized and metastasized tumors in adenocarcinoma patients ($P=0.05$).

DISCUSSION

The results of the present study indicate that a greater incidence of low reactivity was found in patients with malignancy and the association was more significant in squamous cell carcinoma. In patients with squamous cell carcinoma the reduction in lymphocyte reactivity correlated with the stage of the disease in an inverse relation. No significant diminution in lymphocyte reactivity was seen in adenocarcinoma cases whether they were localized or had spread to regional or distant sites. William JC et al. [8] has also reported reduced lymphocyte reactivity in squamous cell carcinoma and no correlation with adenocarcinoma cases. However, they could not correlate the stage of the tumor with the lymphocyte reactivity. Garrioch DB et al. [11] demonstrated distinct deficiency of lymphocyte reactivity in patients with nonlymphoid tumor and suggested that the degree of deficit may be related to the extent of neoplasia. Nicole et al. [12] had shown that the responsiveness of lymphocytes from cancer patients to PHA was significantly lower than that of normal control and an inverse

correlation had been found between the blastogenic response and the extent of the disease.

The significant reduction of lymphocyte reactivity in lymphoma and lympholeukaemia was documented by many investigators [13,14,15]. The lack of association between lymphoma and lymphoid leukemia and impaired lymphocyte reactivity in our study may be due to a small number of patients studied.

From our data it is concluded that the in vitro PHA-induced peripheral lymphocyte mitogenic response at optimal stimulation could discriminate between normal control and cancer patients especially in patients with squamous cell carcinoma. The reduction was more significant in patients with regional or distant metastasis than the localized tumor.

Thus the assay may be potentially useful at least in patients with squamous cell carcinoma for the prediction of prognosis or detection of hidden metastasis.

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REFERENCES

1. Eliber, F.R. & Morton, D.L. Impaired immunologic reactivity and recurrence following cancer surgery. Cancer 1970; 25: 362-367.
2. Hughes, L.E. & Mackay, W.D. Suppression of tuberculin response in malignant disease. British Medical Journal 1965; 2: 1346-1348.
3. Hersh, E.M. & Oppenheim J.J. Impaired in vitro lymphocyte transformation in Hodgkin's disease. New England Journal of Medicine 1965; 273: 1006-1012.

4. Huber, H., Pastner, D., Dittrich, P. & Braunsteiner, H. In vitro reactivity of human lymphocytes in uremia: a comparison with the impairment of delayed hypersensitivity. Clinical and Experimental Immunology 1969; 5: 75-82.
 5. Sharma, O.P., James, D.G. & Fox, R.A. A correlation of in vitro delayed-type hypersensitivity with in vivo lymphocyte transformation in sarcoidosis. Chest 1971; 60: 35-37.
 6. Rubin, A.D., Havemann, K. & Dameshek, W. Studies in chronic lymphocytic leukaemia: further studies of the proliferative abnormalities of the blood lymphocyte. Blood 1969; 33: 313-328.
 7. Surtherland, R.M., Inch, W.R. & McCredie, J.A. Phytohemagglutinin (PHA)-induced transformation of lymphocytes from patients with cancer. Cancer 1971; 27: 574-577.
 8. William, J.C., Sample, W.F. & Chretien, P.B. Lymphocyte reactivity in cancer patients: correlation with tumor histology and clinical stage. Cancer 1973; 31: 65-71.
 9. Golub, S.H., O'Connell, I.X. & Morton D.L. Correlation of in-vivo and in-vitro assay of immunocompetence in cancer patients. Cancer Research 1974; 34: 1833-1837.
 10. Soe-Soe, Tint-Swe, Than-Than-Htwe, Khin-Ei-Han & Than-Swe. The peripheral lymphocyte mitogenic responses of apparently healthy Myanmar blood donors. Myanmar Health Sciences Research Journal 1995; 7(1): 14-17.
 11. Garrioch, D.B., Good, R.A. & Gatti, R.A. Lymphocyte response to PHA in patients with non-lymphoid tumors. Lancet 1970; i: 618.
 12. Nicole, S.F., Buda, J., Theim, T. & Rsemtsma, K. Impaired responsiveness of lymphocytes and serum-inhibitory factors in patients with cancer. Cancer Research 1973; 33: 2373-2377.
 13. Astaldi, G., Costa, G. & Airo, R. Phytochemagglutinin in leukemia. Lancet 1965; i: 1394.
 14. Brown, R.S., Haynes, H.A., Foley, H.T., Godwin, H.A., Berard, C.W. & Carbone, P.P. Hodgkin's disease: Immunologic, clinical and histologic features of 50 untreated patients. Annals of Internal Medicine 1967; 67: 291-302.
 15. Lamb, D., Pilney, F., Kelley, J. & Good, R.A. A comparative study of the incidence of energy in patients with carcinoma, leukemia, Hodgkin's disease and other lymphomas. Journal of Immunology 1962; 89: 555-558.
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Assessing the accuracy of respiratory rates at different counting periods and methods

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The respiratory rates of 198 children (aged 2-59 months) suffering from acute respiratory infections (ARI) were monitored by observation, palpation and auscultation (Gold standard). Two 30-second and one 60-second counts were obtained. The difference among respiratory rate counts determined simultaneously by observation, palpation and auscultation in relation to their mean count was analyzed for 60-second counting period, 30 plus 30-second period and 30-second doubled. The variability among the different counting methods and counting periods was not significantly different whether the children were feeding, sleeping, resting and awake or awake but not resting. The data from the study suggest that respiratory rate counting either by observation or palpation are similar in accuracy and one minute's counting either at a stretch or 30-second doubled or two blocks of 30-second intervals are also similarly accurate.

INTRODUCTION

The WHO protocol for the management of acute respiratory infections (ARI) in children allows pneumonia to be diagnosed on the basis of a few objective clinical signs, without using a stethoscope or radiology. Elevated respiratory rate has been shown to be an important indicator of underlying pulmonary diseases such as pneumonia, asthma, bronchiolitis and pulmonary oedema. However, the movement of respiratory rate is often a difficult manoeuvre for health workers especially in infants. Several factors such as methods of counting (auscultation, palpation, observation), duration of counting methods, state of the child (awake, sleeping, feeding and calm or agitated) will affect the respiratory rate and its variability. The usual method recommended for measuring respiratory rate is to count number of breaths taken during a specific period of time, usually one minute. Since accurate measurement of the respiratory rate is an essential step in assessment of a

child with acute respiratory infection, a simple device to measure the rate is required.

The purpose of the study was to determine the accuracy of respiratory rate counting by observation and palpation by comparing with those obtained by auscultation, to determine the effects of different counting methods, state of the child and age on the accuracy of counting and on the frequency of counting failures.

MATERIALS AND METHODS

The study was a hospital-based study, conducted on 2-59-month-old children, suffering from ARI and admitted to medical wards of Yangon Children's Hospital during the winter-months of 1992. 198 children were included in the study, out of which 95 were 12-59 months of age and 103 were 2-11 month-old infants. Those children who had any chronic illness, an illness which would likely involve dehydration and who were unconsolably agitated were not included in the

study.

Before the actual study took place, three medical officers and three assessors were trained for a week in counting the respiratory rate on children with ARI and high fever at the Out-Patient Department of Yangon Children's Hospital, to minimize the interobserver and intraobserver variation. Three medical officers were trained for counting the respiration rate by auscultation method and three assessors by observation and palpation techniques.

A medical history focussing on the child's current illness and any previous respiratory conditions was obtained before doing physical examination. In each child the respiratory rate was assessed by a medical officer and two assessors when the child was either awake and resting or awake and not resting or sleeping or feeding. respiratory rate was not measured in infants and children who were unsettled or crying. As a gold standard a medical officer counted the respiratory rate for full 60 seconds by stethoscope, after waiting patiently for the child to be accustomed to the application of stethoscope on its chest.

A trained assessor counted the respiratory rate by palpating the abdomen very lightly for full 60 seconds, waited for a period of one minute and repeated the above procedure for 30 seconds and after waiting again for one minute, another 30-second count was made. The count was taken only when the child was accustomed to the touching of its abdomen. Another trained assessor counted the respiratory rate by observing the chest wall movement according to the procedure described above. For each state respiratory rate was assessed simultaneously by one medical officer and two trained assessors. During each counting interval frequency of counting failures according to counting periods, counting methods

and state of the child were also recorded.

Variance of the difference among respiratory rate measurements by different counting methods, different counting periods, different child age and state were compared employing the ANOVA test using the Epi Info software. The data for the counting failures are reported as the percentage of failures out of the total observations.

RESULTS

The numbers, ranges and (mean $\pm$ SD) of the respiratory rates were determined simultaneously by auscultation, palpation and observation for full 60 seconds are presented in Table 1. From the analysis of variance no significant difference was found among the mean respiratory rates of all 3 methods.

Respiratory rate counts determined by observation and palpation during the 60-second, 30-second and repeat 30-second of the same counting intervals (within 5 minutes) were analysed when the child remained in the same state during the counting period. The differences among the means ($\pm$ SD) were analysed for the different counting periods. There was no significant difference in variability among the different counting periods as shown in Table 1.

The influence of age (2-11 months and 12-59 months) and child state (resting and awake, awake but not resting, feeding and sleeping) on the mean respiratory rates of different counting methods is shown in Table 2 & 3. From the analysis of variance it was found that there was no significant difference in any condition (awake and not resting condition was not analyzed as the sample size was too small).

The distribution and frequency of failures in counting by age are asses-

Table 1. Respiratory rates (means $\pm$ SD) according to different methods and different counting period (n = 198)

Methods	Counting period	No. of counts	Maximum	Minimum	Means ($\pm$ SD)
Auscultation	60 sec	10334	102	23	52.19(15.9)
Observation	60 sec	10220	110	21	51.62(15.3)
Palpation	60 sec	10052	98	21	50.77(14.3)
Observation	60 sec	10220	110	21	51.62(15.3)
	30 + 30 sec	10116	104	21	51.09(15.1)
	30 x 2 sec	10101	104	22	51.02(14.9)
Palpation	60 sec	10052	98	21	50.77(14.3)
	30 + 30 sec	10116	95	24	51.17(15.3)
	30 x 2 sec	10115	94	24	51.09(15.3)

Table 2. Distribution of respiratory rates (means $\pm$ SD) in 2-11 month-old infants at different states

State	Counting method	No. of counts	Maximum	Minimum	Means ($\pm$ SD)
RA (n = 48)	Aus (60)	2607	98	24	55.47(17.0)
	Obs (60)	2555	90	25	54.36(17.2)
	Pal (60)	2542	110	22	54.09(26.4)
F (n = 16)	Aus (60)	960	102	32	60.00(18.0)
	Obs (60)	1026	96	25	64.13(10.2)
	Pal (60)	938	98	28	58.63(17.3)
S (n = 36)	Aus (60)	1993	78	30	55.36(14.2)
	Obs (60)	1946	98	30	54.06(14.3)
	Pal (60)	1895	72	32	52.64(12.1)
ANR (n = 3)	Aus (60)	165	80	28	55.00(26.1)
	Obs (60)	134	88	31	44.67(19.4)
	Pal (60)	175	66	30	58.33(28.6)

RA = Rest & awake; F = feeding; S = Sleeping; ANR = Awake but not rest
 Aus = Auscultation; Obs = Observation; Pal = Palpation

sed according to counting period and counting method for child age between 2-11 months and between 12-59 months (Table 4). Failures were significantly higher in children above one year than between 2-11 months. When the period of counting was taken as a criterion (60 seconds vs 30 second doubled vs the sum of the

two 30-second), it was found that there was significant increase in the overall proportion of failures in 60-second count at a stretch and the sum of two 30-second counts than 30-second doubled. It is possible that counting for a longer period of time might result in more failed attempts because of movements of

Table 3. Distribution of respiratory rates (means $\pm$ SD) in 12-59 month-old children at different states

State	Counting method	No. of counts	Maximum	Minimum	Means ($\pm$ SD)
RA (n = 71)	Aus (60)	3369	92	22	48.14(13.1)
	Obs (60)	3320	98	21	47.43(12.7)
	Pal (60)	3332	81	25	47.60(11.5)
F (n = 6)	Aus (60)	367	73	40	61.17(13.0)
	Obs (60)	371	80	45	61.83(11.7)
	Pal (60)	361	71	47	60.17(9.0)
S (n = 13)	Aus (60)	476	64	23	36.62(13.1)
	Obs (60)	500	63	22	38.46(14.1)
	Pal (60)	515	66	21	39.62(14.6)
ANR (n = 5)	Aus (60)	299	87	45	59.80(17.3)
	Obs (60)	280	66	45	56.00(5.7)
	Pal (60)	236	62	42	47.20(12.0)

RA = Rest & awake; F = Feeding; S = Sleeping; ANR = Awake but not rest
Aus = Auscultation; Obs = Observation; Pal = Palpation

Table 4. Frequency of counting failures according to counting periods and counting methods, child state and age

	2-11 months			12-59 months		
	AUS (%)	OBS (%)	PAL (%)	AUS (%)	OBS (%)	PAL (%)
Counting period	(n = 103)			(n = 95)		
60 sec	15 (14.6)	7 (6.8)	24 (23.3)	22 (23.2)	8 (8.4)	27 (28.4)
30 + 30 sec	12 (11.7)	8 (7.8)	19 (18.4)	21 (22.1)	7 (7.4)	24 (25.3)
30 x 2 sec	5 (4.9)	2 (1.9)	10 (9.7)	7 (7.4)	4 (4.2)	14 (14.7)
Child state						
RA	(n = 48)			(n = 71)		
	11 (22.9)	5 (10.4)	16 (33.3)	16 (22.5)	5 (7.0)	20 (22.8)
F	(n = 16)			(n = 6)		
	2 (12.5)	1 (6.3)	4 (25.0)	2 (33.3)	1 (16.7)	2 (33.3)
S	(n = 36)			(n = 13)		
	0 (0)	0 (0)	2 (5.6)	0 (0)	0 (0)	0 (0)
ANR	(n = 3)			(n = 5)		
	2 (66.7)	1 (33.3)	2 (66.7)	4 (80.0)	2 (40.0)	5 (100)

RA = Rest & awake; F = Feeding; S = Sleeping; ANR = Awake but not rest

the child, or other distractions. If the counting method was taken as a criterion it was seen that failure rate was lowest in observation.

Frequency of counting failure rates was found to have an increasing trend from sleeping, feeding, resting and awake to awake but not resting.

DISCUSSION

The aim of the study was to determine the more accurate time period and method for counting the respiratory rate by the basic health staff. Respiratory rate counting for full 60 seconds by medical officer, using the stethoscope is taken as a Gold standard, being short of having continuous electronic monitoring. Work in Colorado suggests that counting respirations with a stethoscope is similar to counting respiration with an electronic monitor [1]. Although some suggested that touching a child with a stethoscope or hand may cause an increased level of agitation, we waited till the child was accustomed to the touching and regular respiration resumed in those who were agitated.

Based on the results from our study it is obvious that in children 2 months of age and older, the respiratory rate can be counted over a 30-second period instead of a full minute. A 30-second count must be doubled to give the respiratory rate for one minute. This agreed with the results from five studies in Nigeria, Switzerland, Egypt and USA - San Francisco and Denver [2]. The shorter counting period requires less patience and concentration and gives less time in which the child can become upset and the count interrupted as suggested by the findings of lower rate of counting failures in 30-second doubled counting. A failed count means that the basic health staff must start again, which wastes time and discourages the use of this technique. Although many suggested [3,4] that touching a child may give an agitation that increases the respiratory rate, the findings from the present study suggest that respiratory rate counting by palpation is as accurate as observation.

Another important factor in determining the optimum method of counting the respiratory rate in children is

the number of attempts that must be made before an acceptable count can be obtained. It was found that although the accuracy between the palpation and observation methods were similar, counting failure was much higher in palpatory method. Finding of lesser counting failure rates in children between 2-11 months old suggests that children of older age group were more aware of they being examined and thus more difficult to stay in resting condition.

Considering the child's state, sleeping and feeding were the best conditions to count the respiratory rate while awake and resting was the better condition than awake but not resting, which resulted in the highest failure rates.

In conclusion, the data from this study indicated that one-minute counting either at a stretch or in two blocks of 30-second counting or doubling the 30-second counting either by observation or palpation are similar in accuracy, but observation by 30-second count doubled is preferable method in children more than 2 months of age as the counting failure rate is lowest. The respiratory rate taken when the child is in a state of sleeping, feeding or resting and awake is more suitable rather than when the child is awake but not testing.

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REFERENCES

1. Morley, C.J., Thornton, A.J., Fowler, M.A. et al. Respiratory rate and severity of illness in babies under 6 months old. Archives of Diseases in Childhood 1990; 65: 834-837.
2. Cain, N. How to assess for fast breathing. ARI News 1992; 22: 4.
3. Charian, T., John, T.J., Simoes, E.A.F. Steinhoff, M.C. & John, M. Evaluation of simple clinical signs for the diagnosis of acute lower respiratory tract infections. Lancet 1988; ii: 125-128.
4. Berman, S., Simoes, E.A.F. & Lanata, C. Respiratory rate and pneumonia in infancy. Archives of Diseases of Childhood 1991; 66: 81-84.

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Circulating and urinary cortisol, ACTH & HGH levels of normal Myanmar subjects

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Cortisol, adrenocorticotrophic hormone (ACTH), human growth hormone (HGH), overnight urinary cortisol and HGH levels were measured in 31 apparently normal healthy volunteers (21 males and 10 females) at the Thayawady Township Hospital. Measurement of circulating hormonal levels was performed by using commercial radioimmunoassay kits. All volunteers were admitted to the hospital for a period of 48 hours and cortisol, ACTH, HGH were measured four times in 24 hours (9:00 hr, 12 noon, 18 hr and 24 hr) consecutively for 48 hours. Overnight urinary cortisol and HGH levels were also measured after a protein load was given on the second night. Serum ACTH level was 15.82 uU/L (range 0.02 to 150.0 uU/L) and the average 24 hour serum HGH level was 7.55 mU/L (range 0.01 to 37.0 mU/L). Serum cortisol was found to be in the range of 21.0 nmol/L to 700.0 nmol/L with a 24 hr average of 227.95 nmol/L. Overnight urinary cortisol and HGH ranged from 0.089 to 27.7 ug and 4.0 to 230 uU respectively.

INTRODUCTION

Normal hormonal levels of cortisol, ACTH, HGH and urinary cortisol, ACTH, HGH levels in normal individuals have never been established in Myanmar and hence the normal standard of Western countries had to be used as reference in clinical practice. As a result of differences in diet, life style, climatic and other biological factors, there is bound to be a variation between these populations. 24 hours urinary total neutral 17 Oxosteroid (ketosteroid) in normal adult Burmese males was found to be lower than the European standard [1]. Since estimation of these hormonal levels was not studied in normal Myanmar subjects, the differences could not be demonstrated. This study was carried out with the objective to obtain baseline data of cortisol, ACTH, HGH and overnight urinary cortisol and HGH levels in order to establish a standard of reference for further routine investigations.

MATERIALS AND METHODS

Subjects

This study was undertaken at Thayawady Township Hospital in 1990-91. Thirty one apparently healthy individuals (21 male and 10 female) were kept in the Thayawady Township Hospital for 48 hours. The age range of male and female were 18 to 68 years and 16 to 39 years respectively. These volunteers have different social strata and occupations (housewives, students, farmers, manual labourers and office workers). Subjects with previous snakebite history, pregnancy, past and/or recent history of any endocrine diseases or of any illnesses, alcoholism, history of steroid medication or of receiving any form of hormonal therapy including contraceptives (oral and parenteral) and high potency vitamins were excluded from the study. Thorough history taking and clinical examination were performed by the project medical

officers.

Collection of samples

Four ml of venous blood was drawn from the study subjects at three to six hours intervals (at 9:00 hr, 12 noon, 18:00 hr and 24:00 hr) consecutively for 48 hours. The samples were centrifuged at 2500 rpm for 15 minutes and the supernatant serum was stored at (-20°C) until transport to the Department of Medical Research.

Overnight urine specimens (9 pm to 9 am) were collected in urine collectors containing sodium azide. Fifty ml aliquots of urine were stored at (-20°C) until analysed. A protein load (2 eggs) was given on the second night prior to the collection of the second urine specimen.

Laboratory methods

Measurement of circulating and urinary hormonal levels was performed by using commercial radioimmunoassay kits. Cortisol and ACTH levels were determined by using commercially available RIA kits (Double Antibody Cortisol and ACTH kits) from Diagnostics Products Co-operation (DPC), Los Angeles, CA, USA. The lower detection limit for cortisol and ACTH were 0.35 ug/dl and 8.5 pg/ml respectively and both intra-assay and inter assay precisions are less than 10%. The serum and urinary HGH were detected by using commercially available HGH, Netria IRMA kits (Double Antibody HGH kits) from St. Bartholomew's Hospital, London, UK. The lower detection limit for HGH was 0.25 mU/L.

RESULTS

Serum cortisol level

Average serum cortisol level was observed to be higher at 6 am, 9 am and 12 noon in both male and female volunteers and was lowest at midnight (Fig. 1). The values for males were

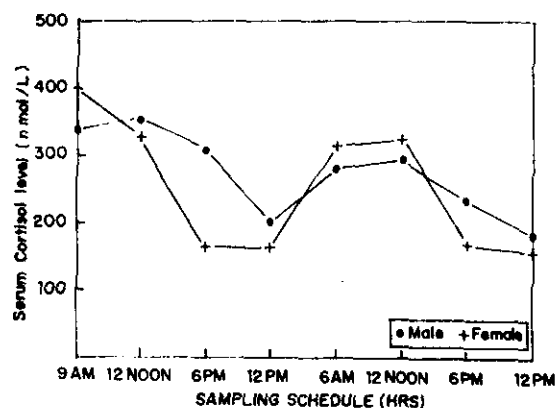


Fig. 1. Average serum cortisol levels in normal individuals (male = 21, female = 10)

higher than that of females with the exception of two values at 6 am and 12 noon of the second day. As a whole, it showed the usual circadian rhythm as observed in other studies [2, 3].

Serum adrenocorticotrophic hormone (ACTH) level

Figure 2 shows the average serum ACTH levels in normal individuals.

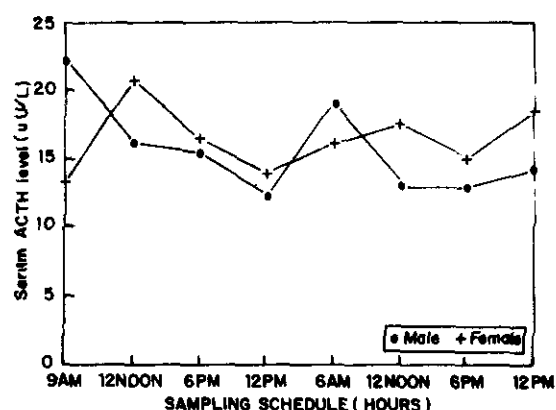


Fig. 2. Average serum ACTH levels in normal individuals (male = 21, female = 10)

It peaks at 9 am in male (12 noon for female) and reaches its lowest level at 12 midnight (for both sexes). The peaks were unchanged on the second day, but the levels were found to be rising at 24 hr. On

both days, a gradual fall of ACTH as the day progressed was seen.

Serum human growth hormone (HGH) level

The average serum HGH level in normal individuals is shown in Fig. 3. In both gender, the peak value was noted at 9 am and lowest at 6 pm (in male) and at 12 noon in females.

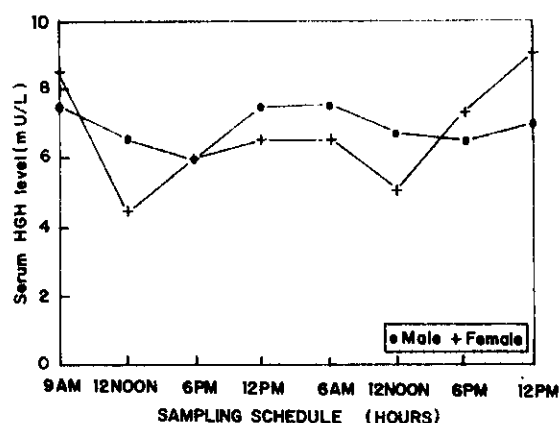


Fig. 3. Average serum HGH levels in normal individuals (male = 21, female = 10)

There is a rise of overnight urinary cortisol and HGH levels on the second day (after protein load) in both sexes.

DISCUSSION

The normal and average values of cortisol, ACTH and HGH are shown in Table 1. The reference kit value of cortisol for morning was 138-690 nmol/L and the value for evening is approximately half of the morning. Although some of the subjects had low serum cortisol levels, the average and highest values conformed to the reference values. As expected from other studies evening values were approximately half of morning values.

The range of serum ACTH was found to be nondetectable to 40 uU/ml (excluding one subject with 150 uU/ml, the cause of high value could

Table 1. Normal values of blood ACTH, HGH and cortisol in normal Myanmar individuals

Type of hormone	Average 24 hour values	Range
Blood cortisol (nmol/L)	277.33	21.0-700.0
	Male 281.61	27.0-660.0
	Female 268.34	21.0-700.0
Blood ACTH (uU/L)	15.82	0.0-165.0
	Male 15.49	0.0-165.0
	Female 16.32	0.0- 95.0
Plasma HGH (mU/L)	7.55	0.0- 39.0
	Male 4.58	0.0- 39.0
	Female 16.32	0.0- 27.0

not be ascertained), which conforms to the kit value (range is nondetectable to 20 uU/ml). The average 24 hr value was 15.82 uU/ml.

The range of serum HGH for both gender was found to be 0-39.0 mU/L and the expected kit value was 0-10.0 mU/L and 0-20.0 mU/L for males and females respectively.

Overnight urinary cortisol and HGH

The urinary cortisol excretion was increased in both males and females after a protein load on the second night. There is usually an increased cortisol excretion in response to eating and exercise. The values for overnight urinary HGH for both gender were 36.7 uU and 83.7 uU for first and second night respectively.

Both male and female showed a good response to protein load producing a higher value on the second night. A protein meal or IV infusion of amino acid (eg. arginine) causes HGH release [4, 5].

Average values and ranges of serum cortisol, ACTH and HGH in Myanmar subjects were found to be in conformity with the expected kit values and those reported by others [2, 3]. All the subjects had circadian or diurnal rhythm in relation to serum

ACTH and cortisol. The serum HGH was found to have its own rhythm, peak HGH level were seen 1-4 hrs after the onset of sleep (during stages 3 & 4) and nocturnal sleep bursts accounting for nearly 70% of daily secretion [5]. The subjects have a normal endocrine response by showing an increase in excretion of overnight urinary cortisol and HGH after protein load.

There was considerable intra and inter individual variation which may be due to short plasma half life, instability in human plasma, structural homology and pulsatile (episodic) secretion of these hormones and various forms of stresses imposed on these volunteers.

REFERENCES

1. May-May-Yi & Sein-Gwan. Urinary 17-oxosteroid (ketosteroids) excretion in normal adult Burmese Males. Union of Burma Journal of Life Sciences 1968; 1(3): 343-345.
2. Giles, A.M. & Ross, B.D. Normal or reference values for biochemical data. In: Oxford Text Book of Medicine. Weatherall D.J. et al. (eds.), Oxford University Press, Oxford, New York, Toronto, 1983; 2: 273-278.
3. Tietz Norpert, W. Reference ranges and laboratory values of clinical importance. In: Cecil Text Book of Medicine. Wyngarden Smith (ed.), Igaku-Shoin/Saunders, International edition, 16th Edition, 1982; 2: 2320-2349.
4. Tyrrell, J.B. & Forsham, P.H. Basic and Clinical Endocrinology. Greenspan, F.S. & Forsham, P.H. (eds.), 2nd edition, Medical Publication/Los Altos-California, Appleton Century-Crofts/East-Norwalk, Connecticut, 1986; pp. 272-309.
5. Findling, J.W. & Tyrrell, J.B. Basic and Clinical and Clinical Endocrinology. Greenspan, F.S. & Forsham, P.H. (eds.), 2nd edition, Lange Medical Publication/Los Altos-California, Appleton Century-Crofts/East-Norwalk, Connecticut, 1986; pp. 50-51.

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Comparative multiple-dose pharmacokinetics of chloroquine in *P. vivax* malaria patients and healthy volunteers

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Recent evidence of the emergence of resistance of *P. vivax* to chloroquine in Myanmar has increased the importance and urgency of understanding the cause of resistance as well as the need for devising the strategies to limit its spread. A comparative multiple-dose pharmacokinetic study was conducted on 5 clinically healthy volunteers and 10 malaria patients with *P. vivax*, admitted to the No. 2 Military Hospital, Yangon, with the object to study whether there is any pharmacokinetic-dynamic relationship underlying the response of patients to standard chloroquine (1500 mg given over 3 days) therapy. Serum chloroquine concentrations reached well above the MIC level in all subjects with the patients' serum concentration (both peak and trough) and the $AUC_{0-\infty}$ being significantly (2-3 times) higher than normal volunteers ($p < 0.02$). Both the clearance and the volume of distribution were also significantly lower in the malaria patients as compared to the healthy volunteers ($p < 0.05$). The elimination half-life ($t_{1/2e}$) was shorter in malaria patients but the difference was not statistically significant. No significant difference was seen with other pharmacokinetic parameters, between normal volunteers and patients and between patients who do and do not recrudescence. The study supports the emergence of chloroquine-resistant *P. vivax* in Myanmar and also excludes the possibility of apparent resistance due to pharmacokinetic causes, especially reduced bioavailability.

INTRODUCTION

Despite the relentless advance of drug resistance of *P. falciparum* malaria, chloroquine still remains the treatment of choice for sensitive *P. vivax* strains in Myanmar and many developing countries [1,2]. This is because chloroquine is inexpensive, widely available, well tolerated and cures malaria with relatively few doses. But the indication that *P. vivax* may develop resistance to chloroquine has been reported by various authors since 1989 [3-5] and the finding of inability of chloroquine to suppress vivax malaria in spite of adequate serum concentrations of chloroquine, has also been reported in Myanmar [6,7].

Chloroquine-resistance in *P. falciparum* has contributed to a world-wide resurgence in malaria and to increasing difficulties with prophylaxis and treatment. In Myanmar, an increasing incidence has been reported since 1989 in the Northern Shan State, where *P. vivax* is a major cause of morbidity, and the possibility that the resurgence was due to the emergence of chloroquine resistant strains has been mentioned by Myint-Oo et al. [8]. In tropical countries like Myanmar, the upsurge of drug-resistance constitute a major health problem and the principal objective underlying the present study is to investigate whether there is any pharmacokinetic-dynamic relationship underlying the response of patients

to standard chloroquine therapy in *P. vivax* infected malaria patients, so as to provide a rational basis for optimising dosage regimen and for prevention of further development of resistant strains due to suboptimal drug serum levels.

SUBJECTS AND METHODS

A total of 10 patients, aged 18-50 years admitted to the No. 2 Military Hospital for signs and symptoms of malaria and confirmed by the presence of asexual *P. vivax* malarial parasites of more than 1000 parasites/mm³ in blood smear, were recruited into the study. A standard history, clinical examination and appropriate laboratory investigations were done to exclude the presence of any organic disease or dysfunction and having taken any antimalarials within the past 2 weeks which was confirmed by spot detection of antimalarials in the urine. Non-infected healthy volunteers of comparable age and body weight were also selected. The research was approved by the Ethics Committee on Medical Research and each subject gave an informed written consent.

Eligible patients were admitted to the hospital for a minimum of 7 days and were followed weekly up to 8 weeks. They were given 3 days' treatment with standard regimen of Chloroquine Phosphate (CHLOROFOS; Myanmar Pharmaceutical Factory), 600 mg, 600 mg, 300 mg followed by 45 mg primaquine immediately after the chloroquine course, followed by 15 mg twice daily for 14 days and was continued weekly for 8 weeks to eradicate the exoerythrocytic cycle of *P. vivax*. A control 0-hour blood and urine sample was taken immediately before the first dose of chloroquine and blood collection was continued at 1, 3, 6, 24, 27, 48, 51, 72, 96 and 168 hours. The serum was separated immediately and stored at -20°C until the time of assay.

Vital signs were recorded every 4

hours until resolution of fever; and thick- or thin-film parasite counts were made every 12 hours until clearance. Blood film examinations were continued daily until day 7 and then weekly until week 8. Routine biochemical and haematological tests were done at admission and discharge from the hospital.

Analytical methods

The chloroquine concentration in the serum was analysed within 2 weeks by the double extraction fluorometric technique of Ensor [9], using a Computerised Fluorescent Spectrophotometer, Model 850, Hitachi, Japan.

Serum chloroquine concentration-time curves were fitted to a two compartment open model with first order absorption, and the standard pharmacokinetic parameters calculated using a non-linear curve fitting software programme (TOPFIT). The terminal elimination rate constant (β) was derived from the terminal series of chloroquine serum concentrations by log-linear regression. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated according to the Trapezoidal Rule up to the time of the last sample and extrapolating to infinity by integrating (C_t/K_{el}); where C_t is the last drug concentration value. The volume of distribution (V_d/f) was calculated as C_1/β and clearance (Cl/f) as dose/ AUC .

Statistical analysis of group data was performed by Student's 't' test and the correlation between different parameters and clinical and laboratory findings was done by linear regression analysis.

RESULTS

A total of 5 clinically healthy volunteers and 10 patients infected with *P. vivax* malaria took part in the study and their baseline clinical and laboratory data shown in Table 1 were in line with the criteria for

Table 1. Comparative demographic, clinical and laboratory data of healthy volunteers and *P. vivax* malaria patients

	<i>P. vivax</i> malaria patients	Healthy volunteers
<u>Demographic data</u>		
Age (years)	20.0±2.4	29.2±2.7
Height (cm)	158.2±1.4	163.8±1.3
Weight (kg)	46.5±1.6	51.6±1.8
<u>Clinic data</u>		
Temperature (°C)	38.9±0.01	36.7±0.01
Heart rate (beats/min)	92.3±2.5	89.4±1.7
Blood pressure (mmHg)	100/70	100/70
<u>Laboratory data</u>		
Haemoglobin (g/dl)	10.2±0.5	12.6±0.4
WBC	5840.2±402.5	4208.1±275.5
Total protein (g/dl)	6.6±0.2	6.7±0.2
albumin (g/dl)	3.4±0.1	3.8±0.1
globulin (g/dl)	3.2±0.1	2.9±0.1
Urea (mg/dl)	9.54±0.81	8.72±0.6
Bilirubin	0.71±0.13	
SGOT	43.0	
SGPT	46.0	

selection of subjects and comparability, since there is no significant differences in age and body weight, admission parasitaemia, haematology or biochemistry.

Table 2 summarizes the history and clinical response of the patients to standard chloroquine therapy. All patients had signs and symptoms of acute attack of malaria and almost all gave history of previous attacks. The mean initial parasite count was 7960.2 ± 1144.0 parasites/mm³ and all patients showed a rapid initial response to the standard treatment with the mean fever clearance time (FCT) of 18.1 ± 2.6 hours and the mean parasite clearance time (PCT) of 36.2 ± 4.8 hours. Eight out of ten patients remained free of symptoms throughout the period of study but parasites reappeared in two patients, one (Pv-6) on day 33 and another (Pv-9) on day 43. The parasites were cleared again after a second course of chloroquine (1500 mg) followed by primaquine but reappeared after day 24 and day 17 respectively in both patients out of

Table 2. Clinical history and response of the patients to treatment

	Patients treated with standard therapy		
	Total patients (n = 10)	Sensitive (n = 8)	Recrudescence/Relapse (n = 2)
1. Previous history of attack of malaria	all	all	all
(a) 1-2	7	7	0
(b) >10	3	1	2
2. Previous anti-malarial therapy		CQ/Qu S-P MQ	CQ/S-P Qu + tetracycline MQ, HF Artemisinin
3. Mean parasite count	7960.2	7840.0	8440.0
4. Mean parasite clearance time (PCT) [hours]	36.2±4.8	31.3±3.6	56.0±10.0
5. Mean fever clearance time (FCT) [hours]	18.1±2.6	18.3±2.9	17.0±7.0

CQ = Chloroquine

S-P = Sulphadoxine-pyremethamine

HF = Halofantrine

Qu = Quinine

MQ = Mefloquine

Table 3. Comparison of pharmacokinetic parameters between healthy volunteers and *P. vivax* malaria patients treated with standard chloroquine therapy

Pharmacokinetic parameters		Healthy volunteers	<i>P. vivax</i> malaria patients		
			Total	Sensitive	Recrudescence/Relapse
K_{ab}	[hr ⁻¹]	0.78	0.81	0.82	0.76
$T_{1/2ab}$	[hr]	0.96	1.02	1.04	0.92
K_{el}	[hr ⁻¹]	0.0096	0.0180	0.0176	0.0194
$T_{1/2el}$	[hr]	84.91	44.47	46.04	38.21
α	[hr ⁻¹]	0.96	1.02	1.04	0.92
$T_{1/2\alpha}$	[hr]	9.79	17.01	16.72	18.14
β	[hr ⁻¹]	0.0028	0.0030	0.0031	0.0030
$T_{1/2\beta}$	[hr]	268.41	231.84	228.88	232.72
$AUC_{0-\alpha}$	[ug/mL.hr]	13.23	29.11	28.54	31.36
Cl/f	[L/hr]	134.10	77.30	84.50	48.47
Vd/f	[L/Kg]	470.46	246.06	267.66	184.30
$A(Co)$	[ug/mL]	2.95	2.54	2.47	4.87
B	[ug/mL]	0.04	0.16	0.17	0.11
C_{peak}	[ug/mL]	0.14	0.36	0.32	0.51
C_{trough}	[ug/mL]	0.04	0.17	0.17	0.17

which Pv-6 had mixed vivax and falciparum infection. They were retreated with artemether-mefloquine followed by primaquine and no further recrudescence or relapse was detected after further blood film examinations up to 8 weeks post-treatment. Both these patients had had previous repeated attacks of more than 10 times and had been treated with various antimalarial drug regimens, including drug combinations like quinine + tetracycline. Both also showed a slower response to treatment with the mean PCT of 56.0 ± 10.0 hours. Except for 2 patients and 4 volunteers who complained of having dizziness and 1 patient having headache, no serious adverse reactions were reported.

Figure 1 shows the comparative serum concentration-time profile of healthy volunteers and malaria patients. Serum chloroquine concentrations were found to reach well above the MIC level within the first hour and persisted well above this level throughout the period of study in all subjects with the patients' chloroquine

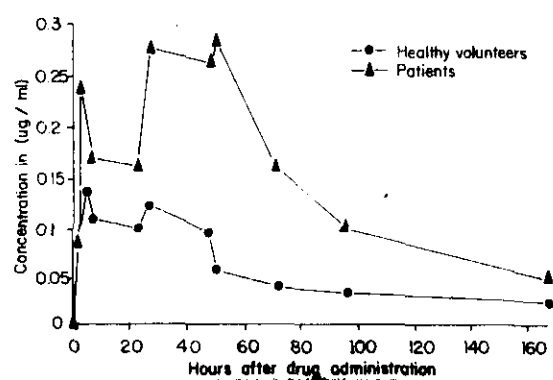


Fig. 1. Comparative chloroquine multiple-dose kinetics in malaria patients and healthy volunteers

concentration being 2-3 times higher than healthy volunteers.

Table 3 shows the comparative pharmacokinetic parameters of the healthy volunteers and patients. The mean serum concentration (both peak and trough) and the $AUC_{0-\alpha}$ was significantly higher than normal volunteers ($p < 0.02$). Both the clearance and the volume of distribution were also significantly lower in the malaria patients as compared to the healthy

volunteers ($p < 0.05$). The elimination half-life was shorter in malaria patients but the difference was not statistically significant. No significant difference was seen with other pharmacokinetic parameters, between normal volunteers and patients, and between patients who do and who do not recrudescenced.

DISCUSSION

Soon after the first report of chloroquine resistant *P. vivax* in 2 Australian soldiers returning from Papua New Guinea [3], there have been an increasing reports chloroquine resistant *P. vivax* in other countries such as Indonesia [4], Papua New Guinea [5], Brazil [10], Guatemala [11] and India [12]. In Myanmar, also a malaria endemic area, recent reports of resistance of *P. vivax* to chloroquine [6, 7] has increased the importance and urgency of understanding the development of drug resistance and the need for devising strategies to limit its spread.

Antimalarial treatment may fail for many reasons. Patient's non-compliance with treatment, vomiting or malabsorption related to patho-physiological changes associated with disease severity, or the pharmacokinetic properties of the drug being unusual in a particular individual, and as a consequence blood concentration of the drug may be subtherapeutic. Alternatively, the malaria parasite causing infection may be resistant to the drug given. In many cases it is a combination of these factors. When malaria parasites are highly drug sensitive, the range of blood concentrations that cures the infection is wide, so relatively low blood levels are still effective. As resistance increases, these low levels are no longer effective and the factors which either predispose to lower drug bioavailability mentioned above, or assist in the pharmacodynamic response (such as age-related

immunity), will then increasingly predispose to treatment failure. The present study, however, did not indicate any likely pharmacokinetic basis underlying the development of drug resistance, especially reduced bioavailability. All patients were found to have serum chloroquine levels greater than those deemed effective for the suppression of acute attack of sensitive vivax malaria (i.e. 15 ug/L plasma or 30 ug/L serum [2, 13]), up to 7 days of treatment suggesting decreased susceptibility of the erythrocytic parasites to chloroquine.

The finding of a significantly higher serum chloroquine concentration and the $AUC_{0-\infty}$ seen in our study was similar to the study reported in Thailand [14] but the latter did not mention about the pharmacokinetics in chloroquine-resistant *P. vivax* patients. The derived pharmacokinetic parameters from the present study were also comparable with the previous single dose study in healthy Myanmar volunteers [15].

Chloroquine has a very large volume of distribution (a V_d of more than 1000 litres) which indicate extensive tissue distribution and binding with the resultant slow elimination, and this contributes to the cumulative effect with subsequent dosing [16]. Similarly, as with many highly basic drugs, chloroquine binds exclusively to α_1 acid glycoprotein (AGG), an acute phase reactant protein which increases in the blood when the body is subjected to acute stressful conditions including acute malaria [17], and increased binding may probably contribute to decreased distribution and clearance with the resultant 2-3 times higher concentration in malaria patients than in healthy volunteers. Since serum samples are used, the preferential accumulation of chloroquine within the parasitized erythrocytes of up to 10-25 times may also contribute to the higher concentration seen in malaria patients [18].

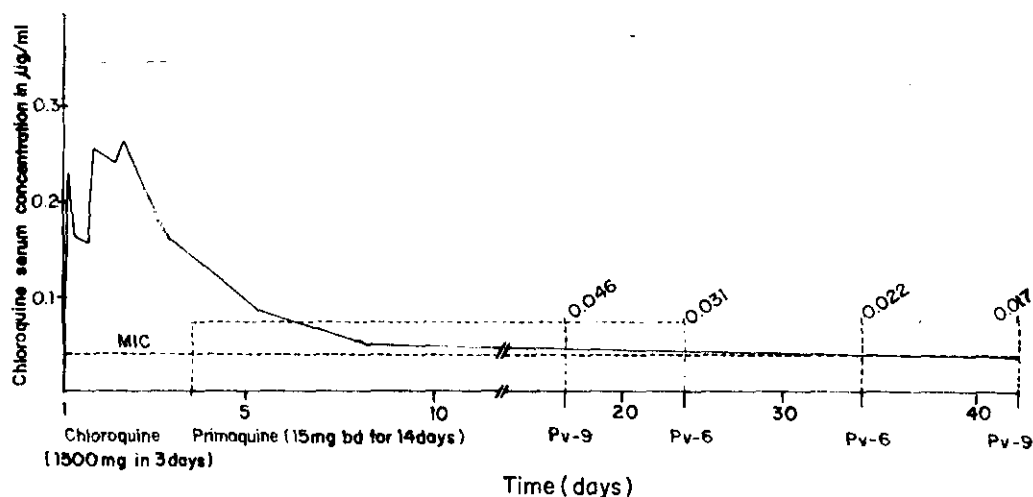


Fig. 2. Extrapolation of the terminal elimination phase of chloroquine using a two-compartment open model

On extrapolation of the terminal elimination phase, using $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for a two-compartment open model (Fig. 2), it can be seen that chloroquine concentrations become subtherapeutic after week 5. This finding supports the general assumption that recrudescences in vivax malaria occur within 4-5 weeks of treatment, while relapses/reinfection usually occur later [19]. Reinfection seems unlikely as there has been no previous reports of urban transmission in Yangon but relapse is characteristic of vivax malaria which results from the persisting exoerythrocytic forms in the liver (hypnozoites) and have been reported to have variable sensitivity to the hypnozoitocidal action of primaquine [20]. *P. vivax* strains from New Guinea had been found to require a higher dose of primaquine (22.5 mg of base/day) for radical cure [21]. It has also been reported that relapses were found to occur in 6-17% of patients even after standard treatment with chloroquine followed by primaquine [22]. Thus, the finding of reappearance of parasites after 5 weeks in the first course of treatment seems more likely to be a relapse but one still cannot exclude the low-

grade chloroquine resistance which could be masked by the asexual blood stage activity of primaquine (daily for 14 days) and could appear to be a relapse [23]. Since primaquine has a very short half-life (< 6 hours) and is barely detectable in blood after 24 hours, recrudescence can become apparent any time after 24 hours of termination of primaquine [24]. Therefore, strain typing is probably needed to clarify between the two. On the other hand, reappearance of parasites on day 24 and 17 seen after the second course of chloroquine, despite having adequate serum levels (0.031-0.046 ug/ml), proves the existence of chloroquine resistant *P. vivax* malaria in Myanmar.

Although no significant pharmacokinetic difference was seen between patients who do and who do not recrudescenced, more studies are needed as the number of failure cases in the present study can also be assumed as too small for meaningful analysis. Chloroquine resistant *P. vivax* malaria, if become widespread, would undoubtedly have a significant implication for the prophylaxis and treatment in Myanmar and would

also add an extreme burden to the multi-drug resistant problem of *P. falciparum*. The present study indicated that pharmacokinetic studies can be helpful in clinical practice and that predictors of treatment failure as with mefloquine [25], such as previous repeated drug administration, prolong PCT, severe anaemia and severity of parasitaemia need also be explored for chloroquine.

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REFERENCES

1. Mya-Oo. The current situation of malaria and antimalarial programmes. Burma Medical Journal 1980; 26: 179-190.
2. World Health Organization. Chemotherapy of malaria, 2nd ed., Bruce-Chwatt, L. (ed.), Geneva, 1986; pp. 68.
3. Rieckmann, K.H., Davis, D.R. & Hutton, D.C. *Plasmodium vivax* resistant to chloroquine? Lancet 1989; ii: 1183-1184.
4. Schwartz, I.K., Lackritz, E.M. & Patchen, M.S. Chloroquine-resistant *Plasmodium vivax* from Indonesia. New England Journal of Medicine 1991; 3324: 927.
5. Schuurkamp, G.J., Spicer, P.K., Kerreu, R.K., Bulongol, P.K. & Rieckmann, K.H. Chloroquine-resistant *Plasmodium vivax* in Papua New Guinea. Transactions of the Royal Society of Tropical Medicine and Hygiene 1992; 86: 121-122.
6. Myat-Phone-Kyaw, Myint-Oo, Myint-Lwin, Thaw-Zin, Kyin-Hla-Aye & Nwe-Nwe-Yin. Emergence of chloroquine-resistant *Plasmodium vivax* in Myanmar (Burma). Transactions of the Royal Society of Tropical Medicine and Hygiene 1993; 87: 687.
7. Marlar-Ihan, Myat-Phone-Kyaw, Aye-Yu-Soe, Khaing-Khaing-Gyi, Ma-Sabai & Myint-Oo. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. Transactions of the Royal Society of Tropical Medicine and Hygiene 1995; 89: 307-308.
8. Myint-Oo, Than-Swe, Tin-Shwe, Ye-Htut, Nyunt-Win, Aung-Win, Kyin-Hla-Aye & Thi-Thi-Aye. The changing incidence of *P. vivax* infection in subjects with malaria. Myanmar Health Sciences Research Journal 1992; 4: 61-63.
9. Ensor, E.M. Estimation of chloroquine in blood serum. Transactions of the Royal Society of Tropical Medicine and Hygiene 1966; 66: 75-78.
10. Canessa, A., Mazzarello, G., Cruciani, M. & Bassetti, D. Chloroquine-resistant *Plasmodium vivax* in Brazil. Transactions of the Royal Society of Tropical Medicine and Hygiene 1992; 86: 570-571.
11. Gascon, J., Gomez-Arce, J.E., Menendez, C., Valls, M.E. & Corachan, M. Poor response to primaquine in two cases of *Plasmodium vivax* malaria from Guatemala. Tropical and Geographical Medicine 1994; 46: 32-33.
12. Goyal, S., Gupta, R. & Bhandari, B. Chloroquine resistance in malaria. Indian Pediatrics 1994; 31: 1550-1552.
13. Bergqvist, Y. & Domeig-Nyberg, B. Distribution of chloroquine and its metabolite desethylchloroquine in human blood cells and its implication for the quantitative determination of these compounds in serum and plasma. Journal of Chromatography 1983; 272: 137-148.
14. Na-Bangheang, K., Limpabul, L., Thanavibul, A., Tan-Ariya, P. & Karbwang, J. The pharmacokinetics of chloroquine in healthy Thai subjects and patients with *Plasmodium vivax* malaria. British Journal of Clinical Pharmacology 1994; 38: 278-281.
15. Thaw-Zin, Aung-Naing, S.J. Tha, Khin-Mg-U & Khin-Kyi-Kyi. Effect of acute diarrhoea on the bioavailability of chloroquine in Myanmar subjects. Myanmar Health Sciences Research Journal 1992; 4: 25-34.
16. Frisk-Holmberg, M., Bergqvist, Y., Termond, E. & Domeij-Nyberg, B. Single-dose kinetics of chloroquine and its major metabolite, desethylchloroquine in healthy subjects. European Journal of

- Clinical Pharmacology 1984; 26: 521-530.
17. Pfafsky, K.M. Disease induced changes in plasma protein binding of basic drugs. Clinical Pharmacokinetics 1980; 4: 246-262.
 18. Ferrari, V. & Cutler, D.J. Kinetics and thermodynamics of chloroquine and dihydrochloroquine transport across the human erythrocyte membrane. Biochemical Pharmacology 1991; 41: 23-30.
 19. WHO Practical chemotherapy of malaria. World Health Organization. Technical Report Series, No. 805, 1990; WHO, Geneva.
 20. Webster, L.I. Drugs used in the chemotherapy of protozoal infections: malaria. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th ed., Macmillan Publishing Co., Inc., NY, 1990; pp. 978-998.
 21. Cooper, W.C., Myatt, A.V., Hernandez, Z.I., JEFFERY, G.M. & Coatney, G.R. Studies in human malaria XXXI. Comparison of primaquine, isopentaquine Sn-3883 and pamaquine as curative agent against Chesson strain vivax malaria. American Journal of Tropical Medicine and Hygiene 1953; 2: 949-957.
 22. Murphy, G.S., Basri, H. & Purnomo, E. Vivax malaria resistant to treatment and prophylaxis with chloroquine. Lancet 1993; 341: 96-100.
 23. Pukrittayakamee, S., Vanijanonta, S., Chantira, A., Clemens, R. & White, N.J. Blood stage antimalarial efficacy of primaquine in P. vivax malaria. Journal of Infectious Diseases 1994; 169: 932-935.
 24. Fletcher, K.A., Prince-Evans, D.A., Gilles, H.M., Bunnag, D. & Harinasuta, T. Studies on pharmacokinetics of primaquine. Bulletin of World Health Organization 1981; 59: 407-412.
 25. ter Kuile, F.O., Luxenberger, C., Nosten, F., Kyaw Lay Thwia, Chongsuphajaisiddhi, I. & White, N.J. Predictors of mefloquine treatment failure: a prospective study of 1590 patients with uncomplicated falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene 1995; 89: 660-664.
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Potential use of Kyethingha-thee (Momordica charantia L. fruit) in the treatment of maturity onset diabetes mellitus

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The hypoglycemic efficacy of Kyethingha-thee dried powder capsule was conducted on five uncomplicated type II non insulin dependent diabetes mellitus patients, who were admitted to the No. 2 Military Hospital, Yangon. Preliminary study revealed that it has hypoglycaemic effect with minimum effective dose of 3 grams for each patient and the time of maximum effect was 4 to 6 hours respectively. Kyethingha-thee was found to be 79.94% as effective as tolbutamide and 154.53% as effective as TMF 32. So far no adverse side effects were observed in any of these patients.

INTRODUCTION

Diabetes mellitus is a chronic debilitating and costly disease with severe complications including blindness, heart and renal diseases. It already represents a significant burden on the public health services of member states especially in developing countries [1] like Myanmar. In Myanmar, diabetes mellitus is one of the common health problems. Majority of patients have been using traditional methods using various plants even though they have never been subjected to scientifically controlled trial in human patients.

Among the traditionally reputed antidiabetic plants, Momordica charantia L. has been shown to be very effective by in vivo studies in animal models [2-4]. Studies on diabetic patients [5,6] have also demonstrated that Momordica charantia L. could

significantly lower the mean blood sugar level. An in vivo study in animal model [7] has shown Momordica charantia L. to be the most promising antidiabetic drug without showing any adverse effects. Kyethingha-thee (Momordica charantia L. fruit) is included in the daily normal Myanmar foods and it is cheap and easily available in Myanmar markets. Kyethingha-thee is bitter in taste and its juice has been shown to contain an active principle responsible for hypoglycemic effect. Diabetic patients generally require prolong therapy (sometimes life long) and thus bitterness, although tolerable by most Myanmar people, may still cause a decrease in compliance by these patients. Therefore powdered form, presented as tablet or capsule, also needs to be considered. This study was a preliminary trial, conducted with an attempt to find out

the dose-response relationship of Kyethingha-thee, so as to establish its therapeutic status in diabetes mellitus patients.

MATERIALS AND METHODS

Plant materials

Fresh green fruits of Kyethingha-thee (*Momordica charantia* L.) were carefully washed with tap water to remove dust and any foreign material and sliced into pieces and dried under shade. The dried pieces were powdered with an electric grinder and capsulated 300 mg in each by machine.

Patient selection

Five type II, non insulin dependent diabetes patients (females, aged 51.2 $\pm$ 1.38 yr) from diabetic clinic of No. 2 Military Hospital were selected according to the National Diabetic Data Criteria [8] for diabetes, which are:

- (1) Fasting blood glucose level of more than 140 mg%
- (2) Random blood glucose level of more than 200 mg%. The patients were clinically free from other diseases or complications of diabetes and had not been taking any medication for the past 2 weeks. Pregnant and lactating mothers were also excluded from the study.

Experimental design

The trial was a cross-over design. The hypoglycemic efficacy of different doses of Kyethingha-thee (2 g and 3 g) was evaluated on the same patient with washout period of 24 hours between each dose of the same drug.

Eligible patients from the No. 2 Military Hospital were selected according to the criteria. Only those patients who gave voluntary informed consents were allowed to participate in the study and they had the right to withdrawl at any stage without

prejudice. All subjects were abstained from smoking and strenous activity throughout the study period. The trial was conducted under strict diet control of approximately 1800 Kcals per day [9].

Trial procedure

All previous antidiabetic medications of these selected patients were completely stopped for 2 days prior to the study. Then oral glucose tolerance tests were carried out. Their blood sugar levels were determined at fasting and at $\frac{1}{2}$ hour, 1 hour, 2 hour and 4 hour intervals after food. The blood sugar levels were determined immediately by O-toluidine method.

Each of the patients was given Kyethingha-thee capsule (2 g) daily for 3 days. They were asked not to take any other medication. Daily urine sugar was tested during the trial by using Benedict's reagent. After 3 days of drug treatment, the oral glucose tolerance tests were again performed. After 24 hours washout period, the procedure was repeated with 3 g Kyethingha-thee capsule.

Clinical assessment

Thorough clinical examination was done on admission and then daily. Recording of vital signs including pulse, blood pressure, body temperature and respiration as well as fluid intake and output monitoring and recording of drug toxicity were done throughout the trial.

Data analysis

The blood glucose concentration time curve for each patient was drawn. Mean post treatment blood glucose levels at all the time intervals were plotted against pretreatment values. The areas under blood glucose concentration curve (AUC) for different dose of drug was calculated. The mean blood glucose reduction AUC% (percentage) of the five patients studied was then plotted against

logarithm of the dosage of Kyethingha-thee and the mean blood glucose reduction AUC percentage of six patients studied with tolbutamide and TMF 32 [10] were compared. In addition, point by point comparison of mean blood glucose levels at various time intervals was done by Student's paired 't' test.

RESULTS

A total of five diabetes patients age between 40 to 60 years took part in this study. The diabetic patients had a fasting blood glucose level of 168.2 ± 9.11 mg% and 2 hours post prandial blood glucose level of 292.0 ± 26.44 mg%.

The glucose tolerance curves of each patient of pretreatment and post treatment values of different doses of Kyethingha-thee capsule are shown in Fig. 1-5. All of them were found to show reduced blood glucose levels at 3 g Kyethingha-thee capsule. Fig. 6 shows that the mean pretreatment and post treatment blood glucose levels of the patients treated with Kyethingha-thee (2 g) at 1,2,4 hour time intervals after glucose intake were from 260.4 ± 31.34 to 188 ± 21.2 ,

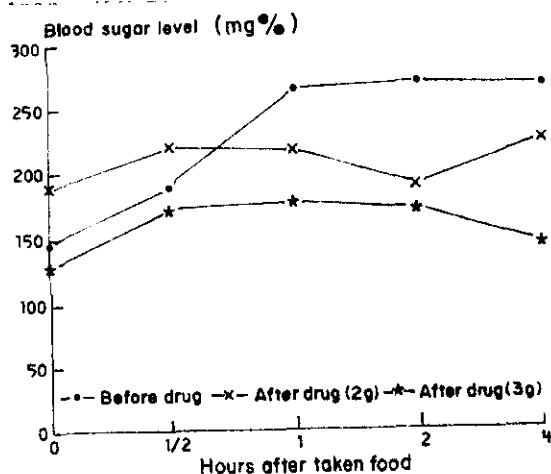


Fig. 1. Glucose tolerance curves of patient A with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee (2 g & 3 g) daily

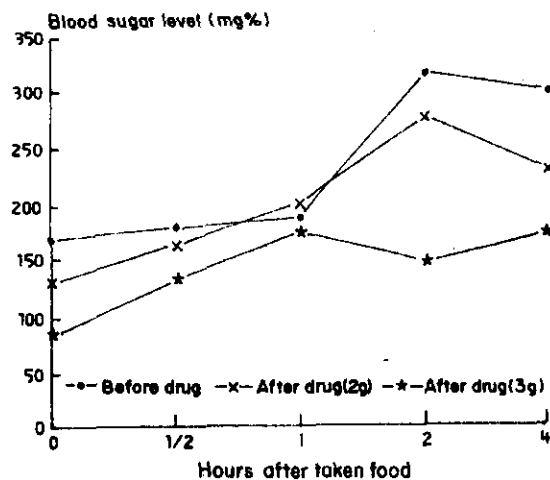


Fig. 2. Glucose tolerance curves of patient B with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee (2 g & 3 g) daily

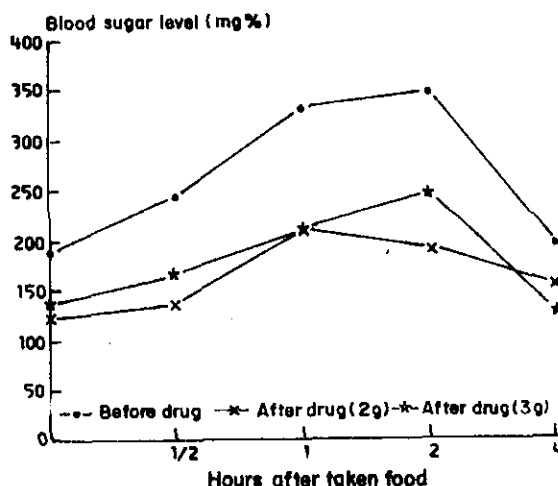


Fig. 3. Glucose tolerance curves of patient C with maturity onset diabetes before and after 3-days treatment with Kyethingha-thee (2 g & 3 g) daily

292.0 ± 26.44 to 197.4 ± 34.67 , 229.0 ± 23.18 to 174.2 ± 23.39 mg% respectively. All the values were found to be significantly ($p < 0.05$, $p < 0.002$, $p < 0.001$) different from each other.

Figure 7 shows that the mean post treatment of Kyethingha-thee (3 g) blood glucose levels at all the time intervals were significantly lower than their respective pretreatment

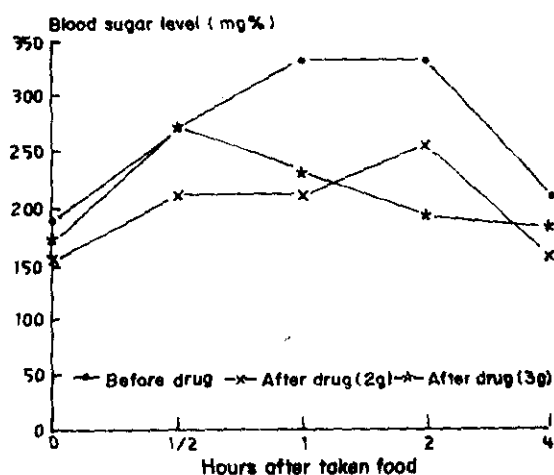


Fig. 4. Glucose tolerance curves of patient 8 with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee (2 g & 3 g) daily

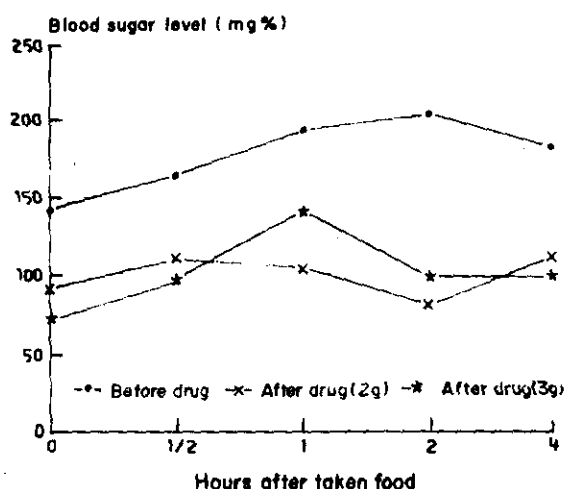


Fig. 5. Glucose tolerance curves of patient E with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee (2 g & 3 g) daily

values. After 3 days of treatment with 3 g Kyethingha-thee, fasting blood glucose level decreased from 168.2 ± 10.18 to 119 ± 18.05 mg% and the blood glucose levels at $\frac{1}{2}$, 1, 2, 4 hours time intervals after OGTT were 157.6 ± 21.65 vs 208.8 ± 20.36 , 186.4 ± 16.11 vs 260.4 ± 31.43 , 169.96 ± 34.47 vs 292.0 ± 26.44 and 144.36 ± 15.93 vs 229.0 ± 23.18 mg% respectively. All

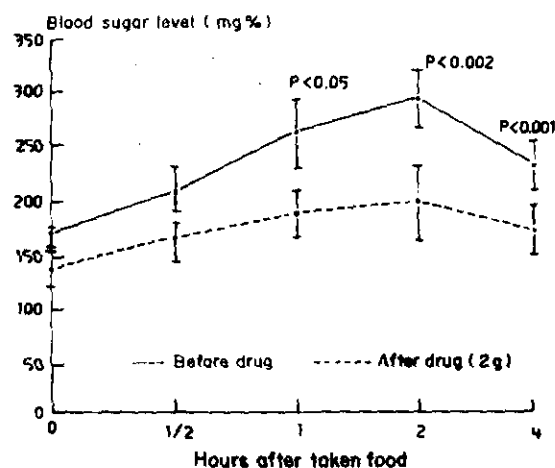


Fig. 6. Glucose tolerance curves of patients with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee 3 g daily

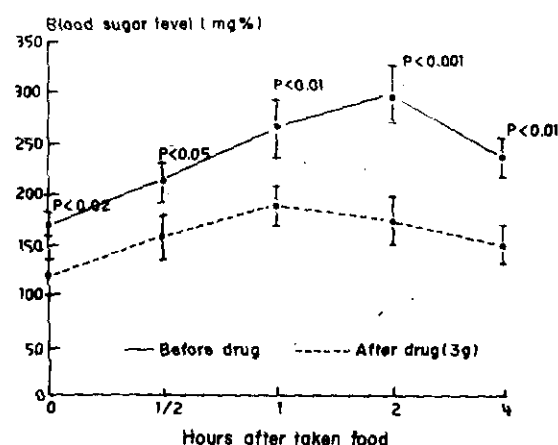


Fig. 7. Glucose tolerance curves of patients with maturity onset diabetes before and after 3-day treatment with Kyethingha-thee 3 g daily

these values were found to be significantly different from each other ($p < 0.02$, $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.01$).

None of the patients showed any visible signs of toxicity nor other side effect.

Table 1 shows the comparison of hypoglycemic potency given as reduction area under the OGTT curves between Kyethingha-thee, tolbutamide and TMF 32 (data from previous study [10]). It shows production in plasma glucose AUC% of 23.93% in

Table 1. Relative hypoglycemic potency of Kyethingha-thee capsules as compared with tolbutamide and TMF-32 (Plasma glucose AUC in g% hr)

Patient	OGTT	Tolbutamide		TMF-32		Patient	OGTT	Kyethingha-thee	
		0.5g	1g	2g	3g			2g	3g
A	1.535	1.755	1.385	1.250	1.165	A	0.997	0.830	0.644
B	2.070	1.785	1.525	1.425	1.150	B	1.047	0.912	0.620
C	2.985	2.840	2.610	2.430	2.490	C	1.132	0.695	0.768
D	0.855	0.875	0.905	0.690	0.715	D	1.333	0.832	0.796
E	2.980	2.490	1.860	2.260	2.450	E	0.736	0.380	0.409
F	3.365	2.560	2.205	3.060	2.620				
Total	13.790	12.305	10.490	11.115	10.590	Total	5.045	3.649	3.237
Mean	2.298	2.051	1.748	1.853	1.765	Mean	1.009	0.730	0.647
S.D. $\pm$	0.979	0.723	0.609	0.878	0.845	S.D. $\pm$	0.163	0.210	0.153
Sugar reducing (%)		10.75	23.93	19.36	23.19	Sugar reducing (%)		27.75	35.84

tolbutamide (1 g) which is significant blood glucose lowering dose, 23.19% in TMF 32 (3 g) and 35.84% in Kyethingha-thee (3 g) respectively.

Figure 8 shows the comparison of log of dose response curves of tolbutamide, TMF 32 and Kyethingha-thee with blood glucose reduction AUC%. All of these drugs showed a good

correlation between logarithm of dose and blood glucose reduction AUC% ($r = 1$ in each case). Kyethingha-thee was found to be 79.94% as effective as tolbutamide and 154.53% as effective as TMF 32.

DISCUSSION

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by elevated plasma glucose concentration resulting from insufficient insulin or insulin resistance or both [11]. It is also a chronic, often debilitating disease with severe complications including ketoacidosis, blindness, neuropathy, heart and kidney diseases and affects many millions of people throughout the world [1].

Effective treatment strategies must include control of blood pressure, regulation of plasma lipids, reduction in body weight, and modification of life-style (cigarette smoking, physical activity, together with the maintenance of blood glucose levels as near as normal possible without major iatrogenic disturbances. Oral hypoglycemic agents occupy an important

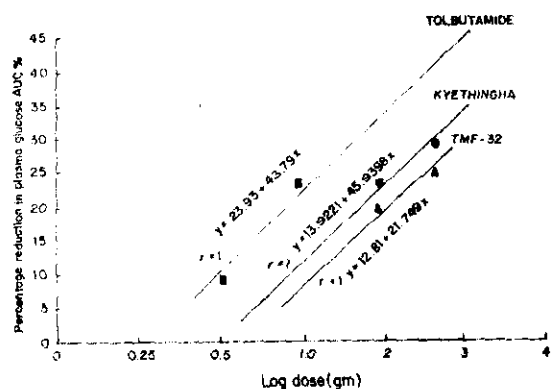


Fig. 8. Relative hypoglycemic potency of Kyethingha-thee capsule compared with standard drugs tolbutamide and TMF 32 by reduction in plasma glucose AUC% (Kyethingha-thee is 79.98% as effective as Tolbutamide and 154.57% as effective as TMF 32)

role in the control of blood glucose levels especially in maturity onset type II non insulin dependent diabetes mellitus [12].

There are two main aims in treatment of diabetes with oral hypoglycemic agents. The short-term aim is to control symptoms by restoring to near normal levels. The long-term aim is to prevent micro- and macro-vascular complications by maintaining fasting blood glucose at or below 115 mg% and glycosylated haemoglobin (HbA₁) concentration not higher than 9.0% [12].

The aims of our study is to find out whether Kyethingha-thee dried powder capsule is capable of effectively lowering the plasma glucose levels without major iatrogenic disturbances as a short-term aim.

The findings showed Kyethingha-thee to be a potent hypoglycemic agent having the potency of 79.95% of tolbutamide and 154.54% of TMF 32. The onset of hypoglycemic action occurred at approximately 2 hours after drug administration and the maximal hypoglycemic action occurred between 4 to 6 hours.

In present study, the effective minimum dose of Kyethingha-thee was 3 g. So far, no adverse side effects were observed in any of these patients.

According to chemical investigation, the active principle will possibly be vegetable insulin (V-insulin) or some similar substance which has been extracted [13] and a clinical study showed a consistent hypoglycemic effect in patients with diabetes mellitus [14]. The hypoglycemic effect of the active compound(s) of *M. charantia* which is non-sapogenin, is probably mediated either by improving the insulin-secretory capacity of β cells or by improving the action of insulin [15].

Diabetic patients require prolong

therapy (sometimes life long), and therefore availability, low cost and low toxicity, as well as good compliance play important roles in achieving successful anti-diabetic therapy.

In conclusion, Kyethingha-thee can be a potent drug being easily available as low cost in Myanmar markets. The continuous use of Kyethingha-thee capsule may well control the diabetes [6]. Of course, clinical trial on larger scale would be needed to further establish its antidiabetic efficacy, duration of action, various effects on intermediary metabolism in human beings and to search for active principle to reduce the effective dose and increase compliances. Chronic toxicity studies in animals must be carried out to declare its total safety for prolong use in human beings.

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REFERENCES

1. Prevention of diabetes mellitus, WHO Technical Report Series 844, WHO, Geneva, 1993; 3-4.
2. Welihinda, J., Arvidson, G.E., Hellman, B. & Karlsson, E. The insulin releasing activity of the tropical plant *Momordica charantia*. *Acta Bio. Med. Ges.*, 1982; 41(12): 1229-40.
3. Singh, N., Tyagi, S.D. & Agarwal, S.C. The effects of long term feeding of acetone extract of *Momordica charantia* (whole fruit powder) on alloxan diabetic albino rats. *Indian Journal of Physiology and Pharmacology* 1989; 33(2): 17-100.
4. Day, C., Cartwright, I., Provost, J. & Bailey, C.J. Hypoglycaemic effect of *Momordica charantia* extracts. *Planta Medica* 1990; 56: 426-429.

5. Wilihinda, J., Karunanayake, E.H., Sheriff, M.H. & Jayasinghe, K.S. Effects of Momordica charantia on the glucose tolerance in maturity onset diabetes. Journal of Ethnopharmacology 1986; 17(3): 277-82.
6. Akhtar, M.S. Trial of Momordica charantia Linn. (Karela) powder in patients with maturity onset diabetes. Journal of Pakistan Medical Association 1982; 32: 106-7.
7. Aye-Than, Win-Myint, Tin-Myint, Mu-Mu-Sein-Myint, Win-Myint & Mya-Bwin. Blood sugar lowering effect of Momordica charantia L. Myanmar Health Sciences Research Journal 1993; 5(2): 72-78.
8. National diabetes data group. Classification and diagnosis of diabetes mellitus and other categories of glucose tolerance. Diabetes 1979; 28: 1039-57.
9. Tin-Shwe & Win-Myint. See-cho-yaw-gar. Ngwe-ba-day-thar Press, Yangon, 1992; 111-117.
10. Thaw-Zin, Nwe-Nwe-Win, Win-Myint et al. Clinical trial of anti-diabetic traditional medicine formulation TMF 32. Myanmar Health Sciences Research Journal 1994; 6(1): 11-18.
11. Singer, M.D., Christopher, M., Coley, M.D., Jeffrey, H., Samet, M.D. & Nadhan, D.M. Test of glycemia in diabetes mellitus. Annals of Internal Medicine 1989; 2: 125-137.
12. Marchetti, P. & Navalsei, R. Pharmacokinetic, pharmacodynamic relationships of oral hypoglycemic agents. Clinical Pharmacokinetic 1989; 16: 100-128.
13. Khanna, P., Nag, I.N. & Jain, S.C. Extraction of insulin from plant cultures in vitro. 3rd International Congress of Plant Tissue and Cell Culture, held at Leicester, England, July 1974.
14. Baldwa, V.S., Bhandari, C.M., Pangaria, A. & Goyal, R.K. Clinical trial in patients with diabetes mellitus of an insulin-like compound obtained from plant source. Upsala Journal of Medical Science 1977; 82: 39.
15. Ali, L., Khan, A.A., Lgbal, M. et al. Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of Momordica charantia. Planta Medica 1993; 59: 408-412.

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Male participation in birth spacing

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A cross-sectional, descriptive, community-based survey using pre-tested pre-formed questionnaire was carried out on 100 randomly selected husbands of 1000 currently married women of age 15-49 years residing in rural area of Taikkyi Township where 24.2% of the married women were current users of contraceptives. Major portion (89%) had heard of contraception and 85% knew one or more method(s) of spacing birth. Only 1% knew what safe period meant although 75.3% claimed to have knowledge of the method. The majority desired three children per family. As regards attitudinal responses, 80% thought contraception should be the wife's responsibility, 72% thought it should be theirs, 23% thought contraception should begin at the start of married life, 71% preferred male method, 51% agreed that contraception should not be practiced, 48% disliked methods requiring medical examination and 78% thought contraception should be used during lactation. As for effect on health, 70% considered it good for mother, 55% did not think so and 74% considered it good for the child. Contraception was negatively reviewed by some as to be against religion (22%), against culture (18%) and against nature (17%).

INTRODUCTION

The Ministry of Health of the Union of Myanmar has put priority on MCH services since the country's independence in 1948. With the setting up of Primary Health Care (including MCH) network, human resources have expanded. Birth spacing program needs expansion and implementation on country-wide scale. On reviewing the existing data on contraception in Myanmar, there were isolated studies on knowledge and practice in peri-urban area of Yangon [1], rural area in Hlegu [2,3] and urban area of Mandalay [4]. However the role of male in birth spacing remains to be identified. This study is therefore carried out as part of WHO funded study on contraception in rural Yangon, to assess the level of KAP on contraception in currently married men of rural area in Yangon Division.

MATERIALS AND METHODS

One thousand currently married women of 15-49 years were chosen by systematic random sampling from 40 clusters of 36 villages belonging to 2 rural health centres of Taikkyi Township. A total of 40 clusters were obtained by sampling of probability proportional to household size and 10% of spouses were then concurrently randomly selected. At the time of study, the contraceptive prevalence among the current users of the married women was 24.2%.

Pre-formed and pre-tested questionnaire was then administered to the male spouses by trained technician on knowledge, attitude and practice of contraception.

After data checking and cleaning, entry and analysis were carried out by the use of Dbase III and Stata software.

Table 1. Background variable of husbands (n = 100)

Age group (in years)		Religion		Level of schooling		Income in kyats per month	
	%		%		%		%
15-19	2	Buddhists	97	Monastery	12	<1000	2
20-24	9	Christians	3	Primary	51	1000-<2000	33
25-29	25			Secondary	28	2000-<3000	29
30-34	20			High school	7	3000-<4000	26
35-39	23			College	2	4000-<5000	7
40-44	15					>5000	3
45-49	5						
>50	1						

RESULTS

The majority of male respondents were 25 to 35 years old. Most of them were Buddhists (97%) and farmers (60%). All had attended school, mostly (51%) up to primary level. Most of them had Ks. 1000 to Ks. 2000 monthly income. Some 89% had heard of contraception and 85% knew how to space births. Mostly, 94.4% mentioned pills, 92.1% injectables, 36.0% IUCD, 48.3% condoms, 14.6% diaphragm, 4% spermicide, 14.6% withdrawal, 4.5% abstinence, 75.3% safe periods. Regarding safe period, only 1% knew correctly about the days to avoid. Regarding traditional methods, 13.5% knew about uterine massage, 29.2% about uterine repositioning, 3% about others like alcohol and herbs. Most of them (93.3%) had heard of female sterilization and 92.1% of male sterilization (Table 1 & 2).

As regards practice of contraception, none of the male respondents were using contraception, although 3% admitted to ever use. The method used was safe period. Most of the respondents said the optimum number of children desired was three. Regarding attitude on contraception, 80% thought it was mainly the wife's responsibility, 72% thought the husband's responsibility, 23% thought it

Table 2. Knowledge on birth spacing methods

Knowledge		%
Birth spacing	Heard of	89
	Not heard of	11
	Total	100
Methods known	Pills	94.4
	Injectable	92.1
	IUCD	36.0
	Condom	48.3
	Diaphragm	14.6
	Spermicide	3.4
	Withdrawal	14.6
	Abstinence	4.5
	Safe period	75.3
	Female sterilization	93.3
	Male sterilization	92.1
	Uterine massage	13.5
	Uterine retroversion	29.2

should begin with the start of marriage, 71% preferred male method dependent on medical examination, 78% had the opinion that contraception should be used during lactation, 70% thought it was good for maternal health, 55% thought it could sometimes affect maternal health, 74% thought it was good for child health, 22% regarded it was against religion, 18% against culture and 17% against nature (Table 3).

Table 3. Attitudes towards contraception

Attitudinal questions	Yes	No	Don't know
1. Contraception is mainly the wife's responsibility	80.0	8.0	12.0
2. Contraception is mainly husband's responsibility	72.0	14.0	14.0
3. Contraception should be practised at the start of marriage	23.0	70.0	7.0
4. Male method is preferable	71.0	21.0	8.0
5. Contraception should not be practised	51.0	49.0	-
6. Contraceptive method dependent on medical examination should be used	48.0	51.0	1.0
7. Contraception should be used during lactation	78.0	15.0	7.0
8. Birth spacing is good for maternal health	70.0	18.0	12.0
9. Birth spacing can sometimes affect maternal health	55.0	29.0	16.0
10. Birth spacing can improve child health	74.0	14.0	12.0
11. Contraception is against religion	22.0	69.0	9.0
12. Contraception is against culture	18.0	74.0	8.0
13. Contraception is against nature	17.0	75.0	8.0

DISCUSSION

From this study, it is apparent that although the male spouses expressed their responsible role in birth spacing, in practice, use of male method was virtually non-existent. Knowledge about condom was affirmative in 48.3%, withdrawal in 14.6% and abstinence in 5.6% but there were neither current nor ever user of those male-controlled methods. As regards attitude, more males were in favour

of contraception. The gap between belief and practice as regards male method may stem from non-availability of condoms. Furthermore, wives have the opinion that they should take responsibility for contraception as most of them were dependent on their husbands as bread-winners and would not like to trouble the latter [5].

Therefore in planning counselling on family planning in rural area, couple counselling should be considered as males appear to be open-minded and approachable as this study has shown. Male method needs promotion as the large gap in knowledge and practice is apparent from this study.

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REFERENCES

1. Kyaw-Tint. A KAP study on child spacing behaviour in peri-urban areas. Abstract of papers, Burma Medical Association, 2nd Medical Specialities Conference 1983.
2. Hla-Pe. Birth spacing practices among rural population of Kyo Kone and Sarbudaung rural health centre in Hlegu township. Myanmar Medical Journal 1992; 37: 1-11.
3. Mya-Mya & Si-Lay-Khaing. Contraceptive prevalence survey in rural Hlegu. Myanmar Health Sciences Research Journal 1990; 4: 81-85.
4. Mya-Mya, Than-Win, Mi-Mi-Khaing & Si-Lay-Khaing. Preliminary report on the contraceptive prevalence survey in Mandalay (phase I urban). Abstract of papers, Myanmar Research Congress 1989.
5. Report on studies on contraception in rural Yangon, Myanmar. Project funded by WHO/HRP-LID grant, May 1995.

MYANMAR
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SCIENCES
RESEARCH JOURNAL

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Ministry of Health, Yangon, Myanmar

INDEX TO VOLUME 8 (1996)

Author Index

A

Aung-Naing - 41,148
Aye-Aye-Min - 116
Aye-Aye-Myint - 51,59,105
Aye-Aye-Than - 108
Aye-Than - 1,36,74,83

C

Chit-Maung - 14,20
Chit-Soe - 25

H

Hla-Min - 1,98
Hla-Naing - 120
Hla-Pe - 148
Hla-Yee - 140
Htain-Win - 86
Htain-Win - 1,66

K

Khin-Aye-Tha - 56
Khin-Hlaing - 54
Khin-Khin-Aye - 66
Khin-Mar-Oo - 86
Khin-Maung-U - 7
Khin-Myat-Tun - 130
Khin-Myo-Chit - 47
Khin-Nwe-Oo - 71,83,102
Khin-Sann-Aung - 71
Khin-Saw-Aye - 130
Khin-Tint - 140
Khin-Win-Thin - 116
Khin-Ye-Myint - 25
Ko-Ko-Hla - 1,54,66,98
Kyi-Kyi-Ngwe - 1
Kyi-May-Htwe - 51,59,105
Kyin-Hla-Aye - 102
Kyin-Htwe - 1,66

L

Le-Le-Win - 130

M

Marlar-Aung - 83,102
Mar-Mar-Nyein - 7,20,62,108
Maung-Maung-Thwin - see
Mg-Mg-Thwin
Maung-Maung-Wint - 148
see also Mg-Mg-Wint
May-Aye-Than - 41,140,148
May-Malar-Swe - 116
Mg-Mg-Thwin - 136
Mg-Mg-Wint - 41
see also Maung-Maung-Wint

Mi-Mi-Cho - 71
Mie-Mie-Khine - 83
Moe-Myint-Kyu - 120
Moe-Sunn - 92
Mu-Mu-Sein-Myint - 36,62,74,148
Mya-Bwin - 20,62
Myat-Thida - 71
Myint-Lwin - 1,108
Myint-Myint-Soe - 155
Myint-Oo - 83
Myo-Khin - 7,29,56,86,136

N

Nilar-Win - 1
Nu-Nu-Aung - 51
Nwe-Nwe-Yin - 140
Nyunt-Wai - 47,92

O

Ohnmar - 47

P

Pe-Than-Htun - 120
Phyu-Phyu-Aung - 130
Phyu-Phyu-Win - 7

S

San-Hla-Mu - 98
San-Shwe - 116,155
Sanda-Win - 125
Saw-Naing - 136
Saw-Phone-Naing - 98
Soe-Aung - 1,29,130
Soe-Soe - 125
Soe-Soe-Kyu - 120
Soe-Thein - 98
Su-Su-Hlaing - 74

T

Tan-Myint-Maung - 1
Tha, S.J. - 14
Than-Htay - 1
Than-Lwin - 78
Than-Saw - 83,108
Than-Swe - 125
Than-Than-Htwe - 125
Than-Than-Kyaing - 25
Than-Than-Tin - 116,155
Thaw-Zin - 41,140,148
Thein-Hlaing - 155
Thein-Shwe - 130
Thein-Toe - 7
Thet-Lwin - 29

Thet-Thet-Mar - 102
Thida-Hmun - 140
Thida-Kyaw - 130
Thuzar-Myint - 108
Ti-Ti - 78
Tin-Aye - 7,62,108
Tin-Myint - 36,74
Tin-Nu-Swe - 78,98,136
Tin-Oo - 29,86
Tin-Shwe - 1,54,66,98
Tin-Tin-Lay - 41

Tint-Swe - 125
Tun-Pe - 51,59,105

W

Wah-Wah-Khet - 120
Wai-Si-Thaung - 120
Win-Htin - 120
Win-Myint - 36,62,74

Y

Ye-Thwe - 140,148
Yi-Mon-Myint - 14

Venoms - 59