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The effect of oral iodized oil on the breast milk iodine content

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The effect of oral iodized oil administration on the breast milk iodine concentrations was studied at different stages of lactation in two groups of women, one control and the other, experimental group. A single dose of iodized oil containing 400 mg iodine was given orally to each woman of experimental group during the third trimester of pregnancy. Casual urine samples before dosing and breast milk samples after parturition were collected. 37.5% of the subjects had low urinary iodine excretion (ie. less than 50 ug per gram creatinine). In the control group, milk iodine concentrations were low compared to Western data. Breast milk iodine concentrations were significantly higher in the experimental group, as compared to the control group up to six months postpartum. A greater part of iodine was found to be secreted in the colostrum and in most cases, milk iodine concentrations declined towards six months postpartum. The present study indicates that oral iodized oil containing 400 mg iodine given during the third trimester of pregnancy can effectively provide adequate iodine in the breast milk for the infants for the first six months.

INTRODUCTION

Human milk is the only source of food consumed by the babies during the first months of infancy. Therefore, it is essential that such food should contain all necessary nutrients in adequate amounts. This is particularly important for those elements which are not stored by the foetus in utero, eg. iodine. This applies for developing countries including Myanmar, where few can afford nutritionally adequate food supplements for very young infants. Published data on the breast milk iodine content of the lactating women and its variation during lactation and the effect of control measures of Iodine Deficiency Disorders (IDD) on the milk iodine content are scanty. There is no published report on the iodine content of human milk in Myanmar. Therefore, this study was conducted to measure the iodine content of breast

milk at different stages of lactation, and to determine the effect of oral iodized oil administered during the third trimester of pregnancy on the breast milk iodine concentration.

SUBJECTS AND METHODS

Twenty four apparently healthy non-goitrous pregnant women in the third trimester of pregnancy, aged between 20 to 30 years, belonging to moderate and low socio-economic classes were recruited for this study while attending the antenatal care clinic at Tower Lane Women Hospital, Yangon. After the collection of casual urine samples, each of the randomly selected 14 women was given two capsules of iodized walnut oil containing 200 mg elemental iodine per capsule. The remaining 10 served as control. After childbirth, breast milk samples were collected during the colostrum

(3 days postpartum) and transitional (14 days postpartum) periods and then at monthly intervals upto six months.

Urinary iodine excretion was measured according to Zak method modified by Benotti and Benotti [1]. Urinary creatinine was measured according to the alkaline picrate method of Bonsnes and Taussky [2]. Total iodine content of breast milk was determined by the method of Moxon and Dixon [3].

Student's 't' test was used for the statistical analysis.

RESULTS

Urinary iodine excretions

In the control group, urinary iodine excretions varied from 37.5 to 107.5 (Mean $\pm$ S.E. = 72.1 ± 7.34) μ g per gram creatinine. In the experimental group, urinary iodine excretions varied from 21.8 to 102.7 (Mean $\pm$ S.E. = 50 ± 5.04) μ g per gram creatinine. There was no significant difference in urinary iodine excretions between the control and experimental groups (Table 1).

Breast milk iodine concentrations

A total of 177 breast milk samples (69 from the control and 108 from the experimental subjects) were analysed. In the control group, milk iodine levels varied from 1.8 to 17.4 μ g per 100 mL. Mature milk iodine levels had a median value of 3.5 μ g per 100 mL. In the experimental group, milk iodine levels ranged from 3.6 to 1,300 μ g per 100 mL. Median value of mature milk iodine levels was 9.8 μ g per 100 mL. Very high values (1,300, 720 and 324 μ g per 100 mL) were found in the colostrum of three experimental subjects who delivered their babies only a few days after oral administration of iodized oil capsule. When mean milk iodine levels between the two groups

Table 1. Urinary iodine excretions (μ g/g creatinine)

Sr. No.	Control group	Experimental group
1	64.8	63.3
2	43.2	57.3
3	62.0	56.3
4	88.9	31.0
5	37.5	46.2
6	81.5	52.6
7	107.5	42.7
8	98.9	48.9
9	56.3	21.8
10	80.0	102.7
11		38.4
12		50.0
13		51.4
14		35.4
<hr/>		
n	10	14
Mean	72.1	50.0
S.D.	23.2	18.9
S.E.	7.34	5.04

were compared according to the stages of lactation, the levels of the experimental group were found to be significantly higher throughout the first six months. In both groups, with the exception of a few cases, milk iodine levels declined toward the end of the period of lactation (Table 2.).

The average amount of iodine consumed per day by the infant from the breast milk was computed from the average concentration of iodine found in 100 mL of milk at different stages of lactation and the average amount of milk consumed per day by the infant for that age (Table 3). The quantity of milk consumed by the infant is based on the breast milk intake of exclusively breast-fed Myanmar infants from birth to 5 months from the study by Khin Maung Naing and Tin Tin Oo [4] and for age six months, from the study by Khin Maung Naing *et al.* [5]. The

Table 2. Milk iodine levels (ug/100 ml)
(Mean $\pm$ S.D.)

Sr.No.	Time	I	II
1	Colostrum	7.24 $\pm$ 4.63 (8)	204.81 $\pm$ 367.37 (14) 47.47* $\pm$ 18.53 (11)
2	Transitional	7.23 $\pm$ 3.99 (10)	35.39 $\pm$ 20.05 (14)
3	One month	3.76 $\pm$ 0.87 (9)	19.99 $\pm$ 8.82 (14)
4	Three months	3.29 $\pm$ 0.59 (7)	9.87 $\pm$ 5.17 (14)
5	Six months	2.68 $\pm$ 0.69 (8)	6.74 $\pm$ 2.21 (11)

I = Control group

II = Experimental group

* 3 Subjects with high values were excluded
Parenthesis = No. of subjects

estimated average daily iodine consumption of the infant from the breast milk (Mean $\pm$ S.E.) was 19.5 ± 1.16 ug per day for the control group and 65.81 ± 8.3 ug per day for the experimental group.

DISCUSSION

Mean urinary iodine excretions between 50 and 75 ug per gram creatinine was graded as mild iodine deficiency. The low urinary iodine excretions of the pregnant women in this study indicate that they are mildly deficient in iodine probably reflecting the low iodine content in their food which are generally dependent on the geochemical environment in which they are grown.

Most of the iodine was found to be secreted in the colostrum, confirming

the statement of the importance of colostrum for the health and nutrition of the infant. Very high milk iodine levels found in the colostrum of the three experimental subjects provided the evidence that iodine from oral iodized oil capsule was readily absorbed from the gastrointestinal tract and quickly distributed to the mammary glands. Much higher levels (ie. mode = 150 ug/L, range 80 to 7,000 ug/L) had been reported in human milk samples from Japanese subjects who consumed dietary algae, known to be rich in iodine [7].

Individual variation of milk iodine levels was very wide among the experimental subjects and was probably due to the fact that breast milk iodine content was influenced by dietary iodine intake as well as by the contribution or subtraction from the body stores depending on the previous iodine status of the individual. The cost of maintenance apart from lactation which varied with the activity of the thyroid gland also influenced milk iodine concentration.

Mature milk iodine concentrations of the control group were low compared to the values from other countries [8] (Table 4), except Zaire where goitre was highly endemic. WHO report mentioned that the range of milk iodine concentrations observed under usual conditions was 5.5 to 6.5 ug/dL. Gushurst et al. [9] analysed the iodine content of mature milk samples from white middle-class volunteers living in North Carolina and reported the values ranging from 2.9 to 49 ug/dL, mean 17.8 ug/dL, median 14.6 ug/dL, S.D. 12.7 ug/dL. Mature milk iodine levels of the experimental group were quite comparable to their results.

Median iodine intakes of the infants reported by WHO/IAEA [8] were all, with the exception of Zaire, within the narrow range of 36-45 ug/d, and

Table 3. Estimated daily iodine consumption of infants (ug/d)

Month of lactation	Quantity of milk consumed daily (ml)	Estimated daily iodine intake (ug/d)	
		Control group	Experimental group
One	496*	18.64	99.15
Two	585*	16.15	79.85
Three	627*	20.63	61.88
Four	675*	24.37	59.67
Five	688*	19.61	50.10
Six	656**	17.58	44.21
Mean $\pm$ S.E.		19.5 $\pm$ 1.16	65.81 $\pm$ 8.3

*Khin Maung Naing & Tin Tin Oo (1991)

**Khin Maung Naing, Tin Tin Oo, Kywe Thein & Nwe Nwe Hlaing (1980)

Table 4. Amounts of iodine found in human milk (ug element per 100 ml milk)

Study area	No.	Milk iodine		
		Minimum	Median	Maximum
Myanmar**	7 (14)	2.6 (4.0)	3.2 (8.7)	4.4 (23.6)
Guatemala*	25	0.3	6.0	234.2
Hungary*	31	1.1	6.4	19.2
Nigeria*	7	1.3	6.2	12.4
Philippines*	11	1.5	5.7	13.1
Sweden*	16	1.7	5.6	17.2
Zaire*	9	0.3	0.5	153.7

**Present study

*WHO/IAEA collaborative study (1987)

- All samples from third month of lactation

- Unsupplemented group

- Parenthesis = Iodine supplemented group

was in remarkable agreement with the dietary allowance of 40 ug/d recommend by NAS [10]. When compared to this, the daily iodine intake of the infants was very low in the control group throughout the first six months. In the experimental group, the daily iodine intake was adequate for the infants upto six months. These findings emphasized the need to supplement iodine to the pregnant women living in iodine-deficient areas so that breast milk contains adequate iodine for young infants.

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Identification and anatomical characterisation of Yardan zeet

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An indigenous medicinal plant growing in Myeik, Tanintharyi division whose bitter fruit commonly known as Yardan zeet is very similar to the vernacular name of the Chinese drug "Yadanzi" the ripe bitter fruit of Brucea javanica (L.) Merr. Due to the similarity in the common names, the specific name of Yardan zeet plant obtained from Myeik, was identified taxonomically. Yardan zeet fruit available in Yangon market as antidiysenteric drug was also identified by comparing the macroscopic features and microscopic characters with that of the identified Yardan zeet from Myeik. Yardan zeet plant growing in Myeik was identified as Brucea javanica (L.) Merr., belonging to the family Simaroubaceae. The macroscopic features and microscopic characters of Myeik and market Yardan zeet fruits were identical. The findings from this study reveals that Yardan zeet fruits are of the same species as the Chinese drug "Yadanzi" the bitter fruit of Brucea javanica (L.) Merr., Simaroubaceae.

INTRODUCTION

An indigenous Myanmar medicinal plant whose bitter fruit locally known as "Yardan zeet" is very similar to the vernacular name of the Chinese drug "Yadanzi", a bitter fruit of Brucea javanica (L.) Merr. Yardan zeet is also a bitter fruit available in local drug store as an antidiysenteric drug.

The extremely bitter fruit "Yadanzi" have been used in traditional Chinese medicine for centuries, particularly for the treatment of malaria and dysentery. The antimalarial principal of the fruit was reported to be highly water soluble, heat stable and also possess antiplasmodial effect. The water soluble bitter substance was also reported for its amoebicidal activity [1].

Myint Oo [2] reported that 50 percent alcoholic extract of Brucea sumatrana Roxb. "Yardan zeet" possess amoebicidal activity on four strains of Entamoeba histolytica at 100 ug/ml concentration,

producing average mortality rates of 79.5 to 85.0 percent. The extract at dose level of 100 mg/kg was also tested against intestinal amoebiasis in guinea pigs and was found to be effective giving more than 35 percent residual infection rates.

Chang and But reported the *in vitro* anti-amoebic effect of the aqueous extract or ether extract of Yadanzi and also on the clinical findings of (Yadanzi) kernel, the crushed kernel and the powders of the defatted kernel respectively, for their therapeutic values in acute or chronic amoebic dysentery and in carriers when administered orally [1].

Mar Mar Nyein et al. [3] reported that 50 percent alcoholic extract of Brucea sumatrana Roxb. "Yardan zeet" when tested on 12 organisms of bacteria Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella flexneri,

Shigella shigae, Staphylococcus aureus aureus and Vibrio cholerae eltor shows a small zone of inhibition only on Proteus vulgaris, Salmonella paratyphi and Shigella boydii. Although a small zone of inhibition was observed on the above three organisms, it was suggested by the author that the extract does not possess any bactericidal or bacteriostatic activity, due to the high titre (5 mg/ml) concentration when tested for minimum inhibitory concentration.

It was also reported that "Yadanzi" (Brucea javanica (L.) Merr.) had no marked inhibitory or lethal action against Shigella dysenteriae, Salmonella typhi, Vibrio cholerae and other common pathogenic bacteria [1].

Pavanand et al. in 1986 [4] conducted the *in vitro* antimalarial activity with various crude extracts of B. javanica fruit, of which crude chloroform extract was found to be most active. He further confirmed the activity with the three compounds bruceine A, brucein B hydrate and brucein C isolated and purified from the chloroform extract against multidrug resistant Plasmodium falciparum. All the three compounds were reported to have the most potent antimalarial activity and the results were comparable to the new antimalarial drug mefloquine.

In 1987, O'Neil et al. [5] also reported the *in vitro* and *in vivo* antiplasmodial activities of the crude extracts of B. javanica fruit. Of the crude extracts, the methanolic, chloroformic, butanolic and aqueous were relatively highly active *in vitro* having IC₅₀ values of 0.5 ug/ml, whereas the petroleum ether extract showed low activity (IC₅₀ of 50 ug/ml). In the *in vivo* Plasmodium berghei infections, chloroformic and butanolic extracts were both shown to be highly active and highly toxic, whereas the aqueous extract was considerably

less active without any toxicity.

The *in vitro* and *in vivo* antimalarial activities of these aqueous extract lend some support to the highly water soluble antimalarial principle present in the fruit, as well as to the drinking of teas made from Brucea javanica (L.) Merr. fruits as antimalarials in traditional medicine [1,5].

Chang and But [1] reported on the clinical findings of a group of 27 malarial patients treated with capsules prepared from 5-15 kernels orally three times daily after meals for 5 days. After treatment 85.2% had no further malaria attacks and 25 had negative blood smears. Another group of 67 cases with tertian malaria were similarly treated. Of which 58 were also reported to be cured with no adverse reaction.

Two species of Brucea namely Brucea amarissima Desv. (synonym - Brucea sumatrana Roxb.) and Brucea mollis Wall. were mentioned in Hundley [6] to be distributed in Myanmar. The former is widely grown in Myeik and Tanintharyi, while the latter species in Maymyo, Pakokku and Tanintharyi. Among the synonyms of Brucea javanica (L.) Merr., are Brucea sumatrana Roxb. and Brucea amarissima Desv. [1,6,7]. The above two synonymous names give the information as to the distribution of Brucea javanica (L.) Merr. in Myanmar.

Due to the similarity in the vernacular names and the medicinal value between Myanmar drug "Yardan zeet" and Chinese drug "Yadanzi" (Brucea javanica (L.) Merr.), it is of interest to identify the specific name of "Yardan zeet" and verify whether they are of the same species.

Therefore, this study was undertaken to identify the specific name of "Yardan zeet" plant from Myeik and also compare with Yangon market

sample, macroscopically and microscopically.

MATERIALS AND METHODS

The plant materials used for identification were obtained from Myeik, Tanintharyi division. Market Yardan zeet was purchased from the local drug store in Yangon. A voucher specimen has been deposited in the herbarium and catalogued in the crude drug museum of the Pharmacology Research Division, Department of Medical Research.

The plant was identified by references to description given by Nootboom [8], Hooker [9] and Brandis [10]. For anatomical studies permanent transverse sections of the fruits were made following the usual plant microtechnique by Johansen [11] and Gray [12].

RESULTS

Morphology

Shrubs with very large compound, alternately arranged, imparipinnate leaves; with 3-5 pairs of opposite ovate-oblong to ovate-lanceolate leaflets, with acuminate tip and flat spot-like glands along the serrate margins situated under the teeth on the under surface of the pubescent leaflets; petioles sparsely pubescent. Flowers very small, unisexual in axillary inflorescences, consisting of unbranched receme-like thyrses. Female flowers tetramerous, pubescent. Fruits dried ovoid drupes, 1-4 together of black to brownish black and reticulate surface, with pubescent pedicels were identified as Bucea javanica (L.) Merr., belonging to the family Simaroubaceae (Fig. 1 & 2).

The morphological characters of Yardan zeet plant from Myeik and characters of Bucea javanica (L.)

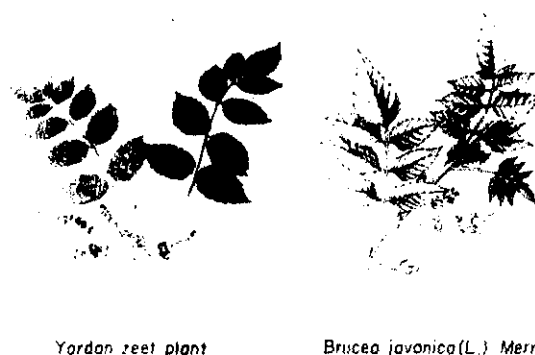


fig. 1. Twig in flower and fruit

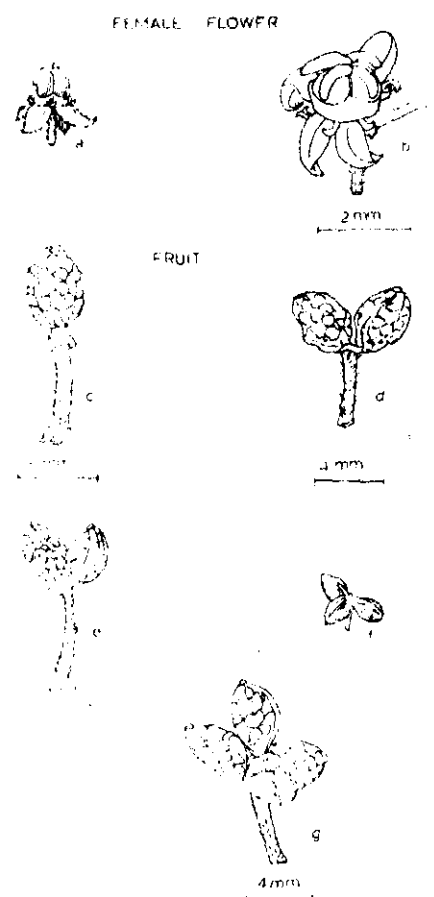


Fig. 2. Floral parts

Merr. cited from Nootboom [7], are presented (Table 1 & 2).

Table 1. Comparative morphological characters of Yardan zeet plant (Myeik) and Brucea javanica (L.) Merr.

Morphological characters	Yardan zeet plant (Myeik)	<u>Brucea javanica</u> (L.) Merr.
Habit	Shrub	Shrub
Leaves	Compound, alternate, 21.0-40.0 cm long imparipinnate	Compound, alternate, 20.0-50.0 cm long, imparipinnate
Petioles	8.0-12.0 cm, sparsely pubescent	5.0-10.0 cm, sparsely pubescent
Leaflets	Opposite, 3-5 pairs ovate-lanceolate, 3.0-9.0 cm x 1.5-5.0 cm, bluntly serrate with flat spot-like glands along the margin situated under the teeth on the under surface of the pubescent leaflets	Opposite, 3-6 pairs, ovate-oblong to ovate-lanceolate, 3.5-11.0 x 1.5-5.0 cm, bluntly serrate or creanate with flat spot-like glands along the margin situated under the teeth on the under surface of the pubescent leaflets
Petiolules		
(a) lateral	2-8 mm, pubescent	2-9 mm, pubescent
(b) terminal	4-22 mm, pubescent	3-40 mm, pubescent
Inflorescence	axillary, raceme-like thyse	axillary, raceme-like thyse

Table 2. Comparative floral parts of Yardan zeet plant (Myeik) and Brucea javanica (L.) Merr.

Floral parts	Yardan zeet plant (Myeik)	<u>Brucea javanica</u> (L.) Merr.
Female flowers (pistillate)	Small, unisexual	Small, unisexual
Pedicels	2-3 mm, pubescent	2.5 mm, pubescent
Sepals	4, connate at the base, ovate-elongate, small, 0.5-1 mm x 0.3-0.5 mm, pubescent	4, connate at the base, ovate-elongate small, 0.5-1 mm x 0.3-0.5 mm, pubescent
Petals	4, ovate-oblong, small, 1-2 mm x 0.5-1 mm, pubescent along the midvein on the outer surface	4, ovate-oblong, small 1-2 mm x 0.5-1 mm, sparsely pubescent to glabrous
Ovaries	4, free, ovoid, styles coherent at the base, stigma bent over the top of the ovary; disks thick with 4 lobes	4, free, ovoid, styles coherent at the base, stigma bent over the top of the ovary; disks thick with 4 lobes
Fruits	Dried ovoid drupes, 1-4 together	Dried ovoid drupes, 1-4 together

Table 3. Comparative macroscopical features of *Brucea javanica* (L.) Merr., Myeik and Yangon Market Yardan zeet fruits

Species	Fruit		Pedicel length
	Features	Size	
<i>Brucea javanica</i> (L.) Merr.	Ovoid, black, reticulate	4 - <u>5</u> - 7 mm	2 - 6 mm
Yardan zeet (Myeik)	Ovoid, black to brownish black, reticulate	4 - <u>5</u> - 7 mm	2 - <u>4</u> - 6 mm
Yardan zeet (Yangon market)	Ovoid, black to brownish black, reticulate	5 - <u>6</u> - 7 mm	2 - <u>4</u> - 6 mm

Table 4. Comparative microscopic characters of Myeik and Yangon market Yardan zeet fruits

Tissue	Yardan zeet fruit	
	Myeik	Yangon market
Epicarp	1-3 layers, cubical cells, 14 u - <u>26</u> u - 30 u	1-4 layers, irregular cells, 14 u - <u>28</u> u - 32 u
Mesocarp	15-20 layers, irregular thin-walled parenchymatous cells, 14 u - <u>20</u> u - 30 u, consisting of rosette aggregates of calcium oxalate	15-20 layers, irregular thin-parenchymatous cells, 15 u - <u>25</u> u - 30 u, consisting of rosette aggregates of calcium oxalate
Endocarp	Composed of lignified sclerenchyma cells; Outer region of oval, lignified sclerenchyma 14 - <u>24</u> u - 30 u. Inner region of rosette sclerenchyma cells, 10 u - <u>12</u> u - 16 u, with monoclinic prisms of calcium oxalate	Composed of lignified sclerenchyma cells; Outer region of oval, lignified sclerenchyma, 16 u - <u>30</u> u - 36 u. Inner region of rosette sclerenchyma cells, 10 u - <u>12</u> u - 16 u with monoclinic prisms of calcium oxalate
Testa (Seed coat)	Thin, with thin-walled polygonal parenchymatous cells, 14 u - <u>38</u> u - 78 u	Thin, with thin-walled polygonal parenchymatous cells, 16 u <u>40</u> u - 60 u
Cotyledons	Composed of thin-walled hexagonal parenchyma cells, 14 u - <u>44</u> u - 56 u, filled with starch grains	Composed of thin-walled hexagonal parenchyma cells, 12 u - <u>46</u> u - 68 u, filled with starch grains

Macroscopical features

Macroscopic features of the mature dried Yardan zeet fruits from Myeik and Yangon market samples are presented (Fig. 3 & Table 3).

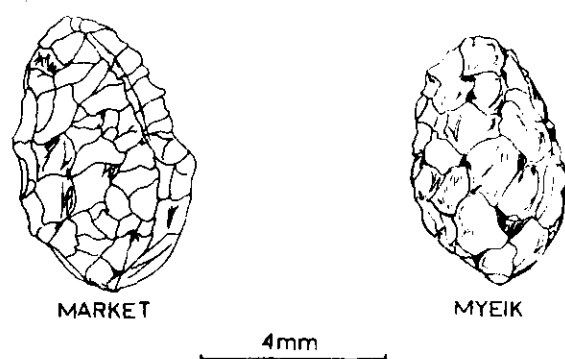


Fig. 3. Mature fruits

Microscopical characters

The comparative microscopical characters of the transverse sections of Yardan zeet fruit wall and seed from Myeik and Yangon market are presented (Table 4, Fig. 4 & 5).

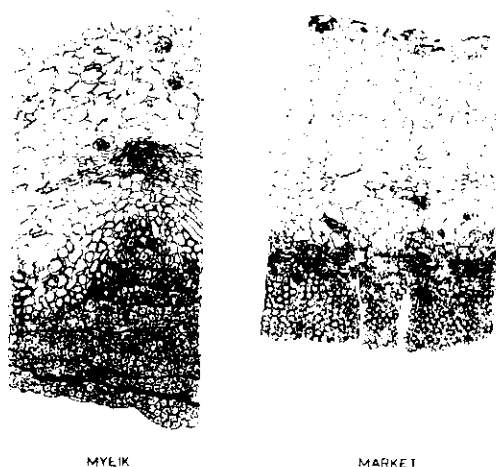


Fig 4. Transverse section of fruit wall

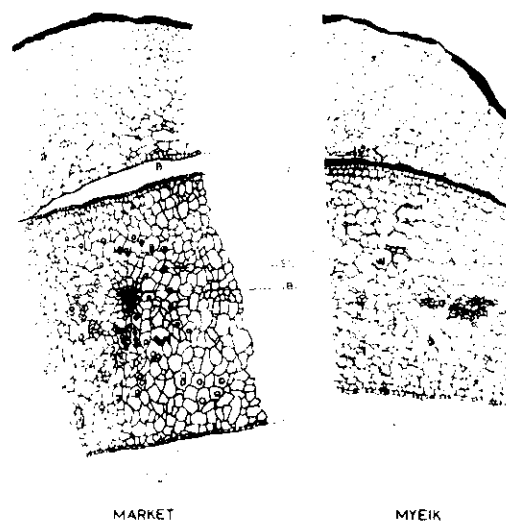


Fig. 5. Transverse section of seed

DISCUSSION

The morphological characters of the plant which bear the bitter fruit Yardan zeet from Myeik presented (Table 1) are in conformity with Brucea javanica (L.) Merr. by reference to description given by Nootboom [7]. From these taxonomic characters (Fig. 1 & 2), Yardan zeet plant grown in Myeik, Tanintharyi division was identified to be Brucea javanica (L.) Merr. belonging to the family Simaroubaceae.

On the basis of the similarities in the macroscopic features and microscopic characters (Fig. 3 Table 3 & Fig. 4, 5 & Table 4), Yangon market "Yardan zeet" was identified and confirmed to be Brucea javanica (L.) Merr.

The findings from this study reveals that "Yardan zeet" plant Brucea javanica (L.) Merr. from Myeik and the bitter fruit of Yangon market Yardan zeet are the same species as the Chinese drug "Yadanzi" the extremely bitter fruit of Brucea javanica (L.) Merr.

As reviewed in the literatures, the

Chinese drug "Yadanzi" was reported for the its antimalarial activity. Since Myanmar "Yardan zeet" and Chinese drug "Yadanzi" is exactly the same species, which is Brucea javanica (L.) Merr., it will be very interesting to investigate whether "Yardan zeet" Brucea javanica (L.) Merr. growing in Myanmar possesses similar antimalarial activity.

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Comparative study on detection of urease activity by CLO Test Kit and
Microtiter Biopsy Urease Test Kit for evaluation of
Helicobacter pylori

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A Microtiter Biopsy Urease Test (MBUT) for the detection of Helicobacter pylori was evaluated in the drug trial study conducted in the Endoscopy Department of New Yangon General Hospital. The MBUT was compared with commercially available urease test (CLO Test). Both CLO test and MBUT were performed on antral and fundal gastric tissues biopsied from 35 subjects who underwent upper gastrointestinal endoscopy. 29 (83%) cases were positive for Helicobacter pylori when tested by CLO test and MBUT. Thus the sensitivity and specificity of MBUT relative to CLO test was both 100% respectively. Tested by MBUT, the urease reaction was positive in 83% of cases within 2 hours and 90% within 3 hours. Tested by CLO test, it was positive in 60% of cases within 2 hours and 67% within 3 hours. The accuracy of MBUT was thus similar to that of CLO test for the detection of Helicobacter pylori. Moreover, MBUT is not expensive, can be prepared locally, packed in test kit form and can be supplied to gastroenteroscopy units for diagnostic and treatment purposes.

INTRODUCTION

Helicobacter pylori (HP) has been associated with active chronic gastritis. It is implicated as primary etiologic factor in the pathogenesis of gastric ulcer, duodenal ulcer disease and non-ulcer dyspepsia [1]. Recently, evidence has suggested that Helicobacter pylori infection may be associated with gastric cancer [2].

Because of Helicobacter pylori's etiologic role, various methods have been devised to detect its presence in human gastrointestinal tract.

The most commonly used methods are outlined as follows:

- (1) Rapid urease test [3]
- (2) Cultural method for isolation and identification of H. pylori
- (3) Histological examination of gastric biopsies
- (4) [^{13}C] labelled urea breath test
- (5) Serological assay

Each method has its distinct advantages, disadvantages and applicability in particular situations.

The parameters of sensitivity, specificity, reaction time for a positive result, simplicity, feasibility, invasiveness and cost of the test are the main factors to be considered for the recommendation for its use. Hence, rapid urease test, the most

simple, is one of the most suitable tests to be adopted in our study. After reviewing many references, we decide to choose the Microtiter Biopsy Urease Test (MBUT) as described by Hazell *et al.* [4].

The aim of this study was to evaluate MBUT and to compare it with CLO test (Commercially available rapid urease test by Delta West, Bentley, Australia).

MATERIALS AND METHODS

Patients

The total number of subjects studied was 35 (23 males and 12 females; mean age, 38 years; range, 23-58 years).

Detailed history and medical examination were recorded in a standardized protocol.

Informed consent was obtained from all cases taking part in the study.

Endoscopy was performed with overnight fasting as a routine procedure in Endoscopy Room in New Yangon General Hospital. At each endoscopy, two antral and two fundal biopsies were taken simultaneously from each case for comparative study of CLO test and MBUT.

Rapid urease detection

CLO test: was used as standard to compare with locally prepared MBUT throughout the study. CLO test was performed according to the instruction manual provided by the manufacturer (Delta West Limited, Australia).

MBUT: Reagent containing urea (2%), phenol red (0.05%) and sodium azide (0.02%) in 100 ml of 0.01 M sodium phosphate buffer pH 6.5.

Procedure: By a dropper bottle, approximately about 50 ul of the reagent was added to each well of the microtiter tray. Tissue from a pinch biopsy specimen of the gastric mucosa

was placed into the well and sealed with clear adhesive tape. The elapsed time for a predetermined end point colour (dark pink) to be reached was recorded for all urease positive biopsies. The tissue was classed negative if no significant colour change developed overnight when compared with an uninoculated control. Half hourly readings to detect colour change were done up to 6 hours and final reading was done after overnight incubation.

RESULTS

Of the 35 cases, 29 (82.8%) were positive and 6 (17.1%) were negative by both CLO and MBUT. Both tests performed simultaneously gave similar results showing a complete concordance (Table 1).

Table 1. The results of MBUT compared with CLO test for rapid detection of urease in *Helicobacter pylori* infected cases

	MBUT	
	(+)	(-)
CLO (+)	29	0
(-)	0	6
Total no. of cases	29	6 = 35

However, there was difference in the reaction time observed between these two tests. MBUT was positive in 83% of cases within two hours and 90% within three hours compared with 60 and 67% of cases for CLO test at the same time, whereas about 90% of cases were positive in 6 hours by both tests. The time course for positive results for both tests was shown in Figure 1.

Thus MBUT performed in our study had the same sensitivity and specificity when evaluated against CLO test. In this study, histology was positive in 24 (69%), thus it showed

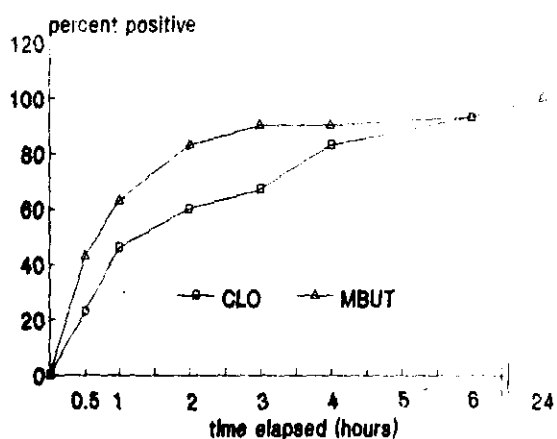


Fig. 1. Time elapsed before a positive result for the MBUT and CLO test

that MBUT was more sensitive than histology.

Of the 35 cases studied, endoscopy revealed duodenal ulcer in 8 (23%), erosive gastritis in 6 (17%), chronic gastritis in 6 (17%), superficial gastritis in 10 (29%) and 5 (14%) with normal endoscopic findings. Rapid urease tests gave positive results in all cases associated with ulcers, whereas, endoscopic findings in cases without ulcers were not helpful in predicting the presence of *H. pylori*. In this study, normal endoscopic findings were observed in 5 cases, of which, 2 (40%) cases gave positive results in both urease tests and histology and 4 (80%) cases were positive in urease tests (Table 2).

DISCUSSION

Rapid urease test is a sensitive and highly specific indicator of *H. pylori* infection. It is used as one of the routine tests by the gastroenterologists. It is an invaluable aid in the primary patient assessment and follow-up cases especially when the antibacterial therapy benefits cases with active chronic gastritis associated with *H. pylori* [4].

Table 2. Predictive value of endoscopic findings in relation to rapid urease test

No. of cases	Endoscopic findings	Rapid urease tests			
		CLO		MBUT	
		+	-	+	-
8	Duodenal ulcers	8	0	8	0
6	Erosive gastritis	6	0	6	0
6	Chronic gastritis	5	1	5	1
10	Superficial gastritis	6	4	6	4
5	Normal endoscopy	4	1	4	1
35		29	6	29	6

Various kinds of urease tests had been formulated. Urea broth used in the routine bacteriology was introduced first. Later, development of commercially available CLO test followed. It is amounted gel pellet containing urea, phenol red indicator and bacteriostatic agent. Addition of bacteriostatic agent obviates the need of the aseptic techniques in collection and biopsies. However, CLO test is expensive with limited shelf life.

Reagents needed for preparation of rapid urease test kits are available in most microbiology laboratories. MBUT was prepared locally and standardized as specified. The study carried out to evaluate its sensitivity and specificity with CLO test shows that it was as good as CLO test even though the study number was small. Similar results were also reported by others [5].

MBUT contains not only the reagents mentioned in CLO test kit, but also sodium phosphate which has a buffering property to protect the solution from the changes of pH as well as to use the test in semiquantitative studies [5].

Each component of the reagents from MBUT can be prepared, premixed

and stored in 4°C for certain period of time. Those urease test formulae without the addition of buffering chemicals has to be premixed just before use to prevent the shift in pH.

MBUT is in liquid form. This is the reason why positive test reaction takes shorter time to develop when compared to CLO test.

Above all, MBUT is simple, not expensive, locally available, and test results manageable by the staff from the Endoscopy Room and most of the positive results can be obtained in 3 hours before the patients leave the hospital.

In this study, MBUT is more sensitive than histology as reported by other authors also [5].

From above results, normal endoscopic findings in the patients do not predict the exclusion of *Helicobacter pylori* infection. Thus, it is essential to perform the required investigations for patients presenting with gastrointestinal complaints [3,5].

Thus we propose that MBUT is the most appropriate and feasible test to be established for use in Myanmar.

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**Assessment of the erythrocyte protoporphyrin test
for screening of iron deficiency anaemia**

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A total of 84 apparently healthy children between ages of 1-12 years were screened for iron deficiency with the erythrocyte protoporphyrin (EP) test. Serum iron, total iron binding capacity in serum, percent transferrin saturation, haematocrit and haemoglobin values were also determined together with the screening test. Comparing the relationship between EP and percent transferrin saturation, using a percent transferrin saturation value $< 10\%$ as the criterion of iron deficiency, the optimal cut off limit for the EP test appears to be 80 ug/dl of rbc's. At this level, this screening test showed a sensitivity of 74% and specificity of 95% and the positive and negative predictive values were 82% and 93% respectively. Thus EP assay which is less expensive and easy to perform can be used as a screening test for the detection of iron deficiency.

INTRODUCTION

Iron deficiency has been detected by the use of battery of tests like serum iron, total iron binding capacity in serum, percent transferrin saturation and serum ferritin measurements in our country. However, the use of erythrocyte protoporphyrin assay has never been tried for the measurement of iron status in Myanmar. An elevation of erythrocyte protoporphyrin is commonly found in association with iron deficiency or lead poisoning [1,2]. The EP assay has only recently gained wider use for the measurement of iron nutritional status, where in even minor insufficiencies are detectable as increased protoporphyrin concentration [4]. Furthermore, the EP assay is intended to detect a disease while it is asymptomatic or in its earliest stages in order to provide a better outcome through early intervention [4]. The objective of this study was to evaluate the usefulness of the EP assay as a

screening test, which is inexpensive, technically simple and effective for the diagnostic use of iron deficiency.

MATERIALS AND METHODS

Subjects

1-12 year old children from Intakaw township, Bago division were recruited for the study. A total number of 84 subjects comprising 44 males and 40 females and all children were free of acute illness at the time of testing. During a three months period analyses for EP, serum iron, total iron binding capacity in serum and percent transferrin saturation were performed together with the routine determination of haemoglobin and haematocrit (PCV).

Blood collection

5 ml of venous blood was drawn from each subject and 2.5 ml was placed in EDTA anticoagulated tube for whole blood and another 2.5 ml

was collected in a plain tube as clotted blood simultaneously. Serum was separated carefully from clotted blood in order to avoid haemolysis. Whole blood and serum were stored at 4°C and -20°C respectively for later assay. The time of storage in these studies usually did not exceed 1 to 2 weeks and all the samples were taken from the subjects in the morning.

Methods

Haematocrit (PCV) was measured by centrifugation of 0.1 ml of whole blood in the CC VI Red Cell Counter. Serum iron and total iron binding capacity in serum were measured according to the method as described by the International Committee for Standardization in Haematology using bathophenanthroline as a colour reagent and magnesium carbonate as adsorbent [5]. To determine EP concentration 2 ml of whole blood was extracted by the acetone: ethylacetate, formic acid: diethyl ether extraction method of Samuel R. Heller *et al.*, 1971. Results were expressed as ug/dl of rbc.

Diagnostic criteria for iron deficiency

Our diagnostic criteria for anaemia were based on the haemoglobin values by age groups [6] and these values were as follows:

Age 1-10 years -- < 11.0 g/dl

Age 10-12 years -- < 12.0 g/dl

Percent transferrin saturation $\leq 10\%$ was selected to reflect iron deficiency [7]. This value corresponded to 2 SD below the mean for the entire study population after excluding the subjects with evidence of iron deficiency. Our limit of $\leq 10\%$ transferrin saturation was slightly lower than the commonly reported one 12 to 14% [6] for children under 12 years.

RESULTS

Of the 84 subjects studied the overall rate of anaemia was 26.19% while the rate of iron deficiency based on a percent transferrin saturation $\leq 10\%$ was 22.6%. The anaemia and iron deficiency rate for these children is shown in Table 1.

Table 1. Subjects with anaemia and low level of percent transferrin saturation ($\leq 10\%$) by age

Age	No. of cases	% with anaemia	% with low transferrin saturation
1-10 yrs	73	20(27.39%)	18(24.65%)
10-12 yrs	11	2(18.18%)	1(9.09%)
Total	84	22(26.19%)	19(22.60%)

We found that anaemia and iron deficiency was more common in the children between the ages of 1-10 years. 27.39% (20/73) of the 1-10 years old children tested were found to be anaemic and out of which 24.65% (18/73) had iron deficiency when the cut off criteria was percent transferrin saturation $\leq 10\%$. For 10-12 years age group only 18.18% (2/11) had anaemia and 9.09% had iron deficiency using similar criterion.

The Pearson's correlation coefficient of EP and percent transferrin saturation for the entire study group was ($r = -0.43$, $p < 0.001$) indicating a moderate inverse relationship between the two tests.

In this study we also observed that the upper normal limit of 95% confidence +2 SD for EP is approximately 80 ug/dl rbc. This is the optimal cut off value when screened for iron deficiency with the EP test (Table 2 & Fig. 1).

Table 2. EP at different cut off levels in detection of subjects with low levels of percent transferrin saturation ($\leq 10\%$)

EP screening cut off (ug/dl rbc)	No. of subjects whose EP exceeded cut off limit	Sensitivity	Specificity
70	21	74%	86%
80*	17	74%	95%
90	15	68%	98%
100	13	63%	98%

EP = Erythrocyte Protoporphyrin

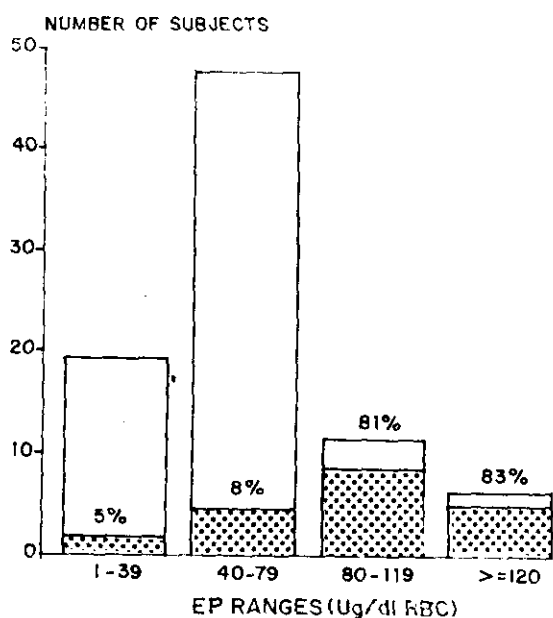


Fig. 1. Frequency distribution of subjects according to EP ranges. Shaded area represents distribution of subjects who also had Low Levels of percent transferrin saturation ($< 10\%$). Percentage of subjects with Low Levels of percent transferrin saturation at each EP range is expressed in each bar

DISCUSSION

In this study, based on analyzing the relationship of EP and low percent transferrin saturation, we found the optimal cut off value for screening test of iron deficiency is > 80 ug/dl of rbc. Using this cut off point, it has the advantage of detecting most of the subjects (74%) with low level of percent transferrin saturation without markedly increasing the number of false positive cases. Measurement of serum ferritin which is highly specific for iron deficiency was not included in the test, because the assay kit using radioimmunoassay was not available at the time of testing. Among the biochemical test that can be used to assess iron status, the EP assay is effective for being used as a screening test in routine laboratory because of its stable index, ease of performance and inexpensive. On the other hand, the serum iron, total iron binding capacity in serum, transferrin saturation and serum ferritin measurements are more difficult to perform because of the longer time required to complete the assay and the reagents used are expensive. Moreover, the measurement of EP has recently been further simplified by the haematofluorometer method [8], in which the analysis requires only a small amount of capillary blood and the result can be obtained within a few seconds. Our observations show that many non anaemic individuals have biochemical evidence of iron deficiency similar to that found in individuals who have frank anaemia are in agreement with the suggestions of Ray Yip *et al.* [4] that measurement of EP test offers the advantage of detecting iron deficiency prior to the manifestation of anaemia. The EP screening test will not only detect iron deficiency but

also detect lead poisoning [9], which will also result in an elevated EP. In the latter case, measurement of the blood lead levels are the main aids in detecting the presence of a lead burden. In children who are found to have an EP ≥ 80 ug/dl of rbc's, a course of oral iron therapy which is followed by a rise in haemoglobin level after one month can confirm the diagnosis of iron deficiency [10], without performing to other biochemical tests such as the serum ferritin or percent transferrin assays.

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Birth weight estimation of newborns utilizing chest and arm circumference

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A study of 547 births in Thaketa township showed a strong correlation between birth weight with chest circumference ($r = 0.71$) and mid arm circumference ($r = 0.70$). Multiple regression analysis of birth weight on chest and mid arm circumference was: Birth weight = $-1870.41 + (\text{mid arm circumference} \times 192.03) + (\text{chest circumference} \times 91.97)$. The value of F statistic (371.97) was significant ($p < 0.001$) indicating the usefulness of chest and mid arm circumference in predicting birth weight.

INTRODUCTION

The weight of a baby taken at birth is a reliable and sensitive indicator for predicting the immediate or later outcome of a newborn child. The average birth weight of newborn babies of a certain village, village tract or township reflects the maternal and child health and socioeconomic status of that area. It also indicates the effectiveness of the maternal and child health services provided in that area. In developing countries a high percentage of babies are delivered by untrained and trained birth attendants even in periurban areas [1] and are not weighed because weighing scales are not available. To alleviate this problem, this study attempts to explore the possibility of utilizing certain simple anthropometric measurements to estimate the weight of newborns after delivery by the TBAs either by ten household health workers or community health workers in villages or in periurban areas where the services of a midwife or an auxiliary midwife are not available.

MATERIALS AND METHODS

This study is part of a larger study which is "A Prospective Study of Growth and Development in Yangon Children, Thaketa Township" where a cohort of 547 children are being followed up from birth till the age of 3 years with regards to their growth and development.

In Thaketa township 12 wards (clusters) out of a total of 19 wards (clusters) were chosen randomly and 547 consecutive births that were delivered between 12th October 1992 and 12th December 1992 were included in the study.

Anthropometric measurements and developmental scales were measured by the project staff within 24 hours for children who were delivered in the local maternity home. Children who were delivered at home were examined within 48 hours after delivery.

The sample size for growth and development study was a cohort of 200 children which was calculated as

follows:

$N = 3,000 \times \log \text{ to the base } 10 \text{ of } -$
 $700 \text{ the no. of cells}$

A sample of 547 was taken as there would be dropouts due to deaths and lost to follow up by the end of three years, in which there would be at least a minimum of 200 children left in the cohort.

There were two teams in the field each led by a medical officer. Each team had an infantometer, a tape measure, weighing scales and a sphygmomanometer. Both of the teams were trained at the Yangon Children's Hospital.

To minimize the interpersonal error in the anthropometric measurements, a quality control session was held during a pilot study in Hlaing township where each persons in the teams measured length, chest circumference, head circumference, mid arm circumference and weight of a sample of 20 infants. The values of the standard deviations of the difference between duplicate measurements was less than 0.19 cm which encompasses well within the value of 0.18 cm found for Mr. R.H. Whitehouse (Whitehouse, Tanner and Healy, 1974).

Data analysis was carried out using Epi info version 5 which is a statistical software programme.

Standard statistical methods of linear regression, correlation and multiple regression were applied to analyze the data.

RESULTS

Chest circumference had the highest correlation with birth weight ($r = 0.71$) with 95% confidence limits $0.66 < r < 0.75$ followed by mid arm circumference ($r = 0.70$) with 95%

confidence limits $0.66 < r < 0.74$.

A mid arm circumference of 8.66 cm corresponded to a birth weight of 2501 gms (sensitivity 79.4% and specificity 91.96%) calculated from the regression line equation of:

$$\text{Birth weight} = -388.6113 + (333.7189 \times \text{mid arm circumference})$$

The 95% confidence limit for birth weight using a mid arm circumference of 8.7 cm was (2514.75 + 248.239 gms).

Similarly chest circumference of 28.5 cm corresponded with birth weight of 2499.9 gms (sensitivity of 81.48% and specificity of 89.17%) calculated from the regression line equation of:

$$\text{Birth weight} = -1903.5912 + (154.5095 \times \text{chest circumference})$$

The 95% confidence limit for birth weight using a chest circumference of 28.5 cm was (2499.9 + 372.223 gms).

Multiple regression analysis of birth weight on chest and mid arm circumference was:

$$\text{Birth weight} = -1870.41 + (\text{MAC} \times 192.03) + (\text{chest circum.} \times 91.97)$$

The value of F statistic was significant ($p < 0.001$) indicating the usefulness of chest and mid arm circumference in predicting birth weight.

The 95% confidence limit for birth weight with a mid arm circumference of 8.7 cm and a chest circumference of 28.5 cm was (2421.4 + 804.3 gms).

DISCUSSION

Birth weight is a reliable and sensitive indicator for predicting the immediate or later outcome of a newborn child. In Myanmar even in periurban areas

Table 1. Birth weight in relation to Midarm circumference and Chest circumference

Birth MAC Birth chest	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
26	1384.9	1480.9	1576.9	1672.9	1769.0	1865.0	1961.0	2057.0	2249.0	2249.0	2345.0	2441.1	2537.1	2633.1	2729.1	2825.1	2921.1	3017.2	3113.2	3209.2	3305.2	3401.2
26.5	1430.9	1526.9	1622.9	1718.9	1814.9	1911.0	2007.0	2103.0	2199.0	2295.0	2391.0	2487.0	2583.1	2679.1	2775.1	2871.1	2967.1	3063.1	3159.2	3255.2	3351.2	3447.2
27	1476.9	1572.9	1668.9	1764.9	1860.9	1956.9	2053.0	2149.0	2245.0	2341.0	2437.0	2533.0	2629.0	2725.1	2821.1	2917.1	3013.1	3109.1	3205.1	3301.2	3397.2	3493.2
27.5	1522.9	1618.9	1714.9	1810.9	1906.9	2002.9	2098.9	2195.0	2291.0	2387.0	2483.0	2579.0	2675.0	2771.0	2867.1	2963.1	3059.1	3155.1	3251.1	3347.1	3443.2	3539.2
28	1568.8	1664.9	1760.9	1856.9	1952.9	2048.9	2144.9	2240.9	2337.0	2433.0	2529.0	2625.0	2721.0	2817.0	2913.0	3009.1	3105.1	3201.1	3297.1	3393.1	3489.1	3585.2
28.5	1614.8	1710.8	1806.9	1902.9	1998.9	2094.9	2190.9	2286.9	2382.9	2479.0	2575.0	2671.0	2767.0	2863.0	2959.0	3055.0	3151.1	3247.1	3343.1	3439.1	3535.1	3631.1
29	1660.8	1756.8	1852.8	1948.9	2044.9	2140.9	2236.9	2332.9	2428.9	2524.9	2621.0	2717.0	2813.0	2909.0	3005.0	3101.0	3197.0	3293.1	3389.1	3485.1	3581.1	3677.1
29.5	1706.8	1802.8	1898.8	1994.8	2090.9	2186.9	2282.9	2378.9	2474.9	2570.9	2666.9	2763.0	2859.0	2955.0	3051.0	3147.0	3243.0	3339.0	3435.1	3531.1	3627.1	3723.1
30	1752.8	1848.8	1944.8	2040.8	2136.8	2232.9	2328.9	2424.9	2520.9	2616.9	2712.9	2808.9	2905.0	3001.0	3097.0	3193.0	3289.0	3385.0	3481.0	3577.1	3673.1	3769.1
30.5	1798.8	1894.8	1990.8	2086.8	2182.8	2278.8	2374.9	2470.9	2566.9	2662.9	2758.9	2854.9	2950.9	3047.0	3143.0	3239.0	3335.0	3431.0	3527.0	3623.0	3719.1	3815.1
31	1844.7	1940.8	2036.8	2132.8	2228.8	2324.8	2420.8	2516.9	2612.9	2708.9	2804.9	2900.9	2996.9	3092.9	3189.0	3285.0	3381.0	3477.0	3573.0	3669.0	3765.0	3861.1
31.5	1890.7	1986.7	2082.8	2178.8	2274.8	2370.8	2466.8	2562.8	2658.9	2754.9	2850.9	2946.9	3042.9	3138.9	3234.9	3331.0	3427.0	3523.0	3619.0	3715.0	3811.0	3907.0
32	1936.7	2032.7	2128.7	2224.8	2320.8	2416.8	2512.8	2608.8	2704.8	2800.9	2896.9	2992.9	3088.9	3184.9	3280.9	3376.9	3473.0	3569.0	3665.0	3761.0	3857.0	3953.0
32.5	1982.7	2078.7	2174.7	2270.7	2366.8	2462.8	2558.8	2654.8	2750.8	2846.8	2942.9	3038.9	3134.9	3230.9	3326.9	3422.9	3518.9	3615.0	3711.0	3807.0	3903.0	3999.0
33	2028.7	2124.7	2220.7	2316.7	2412.7	2508.8	2604.8	2700.8	2796.8	2892.8	2988.8	3084.9	3180.9	3276.9	3372.9	3468.9	3564.9	3660.9	3757.0	3853.0	3949.0	4045.0
33.5	2074.7	2170.7	2266.7	2362.7	2458.7	2554.7	2650.8	2746.8	2842.8	2938.8	3034.8	3130.8	3226.9	3322.9	3418.9	3514.9	3610.9	3706.9	3802.9	3899.0	3995.0	4091.0
34	2120.7	2216.7	2312.7	2408.7	2504.7	2600.7	2696.7	2792.8	2888.8	2984.8	3080.8	3176.8	3272.8	3368.9	3464.9	3560.9	3656.9	3752.9	3848.9	3944.9	4041.0	4137.0
34.5	2166.6	2262.7	2358.7	2454.7	2550.7	2646.7	2742.7	2838.7	2934.8	3030.8	3126.8	3222.8	3318.8	3414.8	3510.9	3606.9	3702.9	3798.9	3894.9	3990.9	4086.9	4183.0
35	2212.6	2308.6	2404.7	2500.7	2596.7	2692.7	2788.7	2884.7	2980.7	3076.8	3172.8	3268.8	3364.8	3460.8	3556.8	3652.9	3748.9	3844.9	3940.9	4036.9	4132.9	4228.9
35.5	2258.6	2354.6	2450.6	2546.7	2642.7	2738.7	2834.7	2930.7	3026.7	3122.7	3218.8	3314.8	3410.8	3506.8	3602.8	3698.8	3794.9	3890.9	3986.9	4082.9	4178.9	4274.9
36	2304.6	2400.6	2496.6	2592.6	2688.7	2784.7	2880.7	2976.7	3072.7	3168.7	3264.7	3360.8	3456.8	3552.8	3648.8	3744.8	3840.8	3936.9	4032.9	4128.9	4224.9	4320.9
36.5	2350.6	2446.6	2542.6	2638.6	2734.6	2830.7	2926.7	3022.7	3118.7	3214.7	3310.7	3406.7	3502.8	3598.8	3694.8	3790.8	3886.8	3982.8	4078.9	4174.9	4270.9	4366.9
37	2396.6	2492.6	2588.6	2684.6	2780.6	2876.6	2972.7	3068.7	3164.7	3260.7	3356.7	3452.7	3548.7	3644.8	3740.8	3836.8	3932.8	4028.8	4124.8	4220.9	4316.9	4412.9
37.5	2442.6	2538.6	2634.6	2730.6	2826.6	2922.6	3018.6	3114.7	3210.7	3306.7	3402.7	3498.7	3594.7	3690.7	3786.8	3882.8	3978.8	4074.8	4170.8	4266.8	4362.9	4458.9
38	2488.5	2584.6	2680.6	2776.6	2872.6	2968.6	3064.6	3160.6	3256.7	3352.7	3448.7	3544.7	3640.7	3736.7	3832.7	3928.8	4024.8	4120.8	4216.8	4312.8	4408.8	4504.9
38.5	2534.5	2630.5	2726.6	2822.6	2918.6	3014.6	3110.6	3206.6	3302.6	3398.7	3494.7	3590.7	3686.7	3782.7	3878.7	3974.7	4070.8	4166.8	4262.8	4358.8	4454.8	4550.8
39	2580.5	2676.5	2772.5	2868.6	2964.6	3060.6	3156.6	3252.6	3348.6	3444.6	3540.7	3636.7	3732.7	3828.7	3924.7	4020.7	4116.7	4212.8	4308.8	4404.8	4500.8	4596.8
39.5	2626.5	2722.5	2818.5	2914.5	3010.6	3106.6	3202.6	3298.6	3394.6	3490.6	3586.6	3682.7	3778.7	3874.7	3970.7	4066.7	4162.7	4258.7	4354.8	4450.8	4546.8	4642.8
40	2672.5	2768.5	2864.5	2960.5	3056.5	3152.6	3248.6	3344.6	3440.6	3536.6	3632.6	3728.6	3824.7	3920.7	4016.7	4112.7	4208.7	4304.7	4400.7	4496.8	4592.8	4688.8

such as Thaketa township 12.9% of deliveries [1] are conducted either by relatives, TBAs or trained TBAs where recording the weight of every child may not be feasible.

The present study shows chest and mid arm circumference had the best correlation with birth weight. A mid arm circumference of ≤ 8.7 cm and a chest circumference of < 28.5 cm were used as cut off values for low birth and the 95% confidence limit for birth weight the above two was (2421.4 + 804.3) gms which is comparable to the study by S.K. Bhagara [2] in which their cut off point for low birth weight was ≤ 8.7 cm for mid arm circumference and ≤ 30 cm for chest circumference and the 95% confidence limit for birth weight with the above two was (2539.2 $\pm$ 621.3 gm).

This clearly shows that simple measurements such as mid arm circumference and chest circumference can estimate birth weight when recording the birth weight is not feasible in instances where the deliveries take place where there are no midwives or auxiliary midwives. These measurements are easy to learn and can conveniently be introduced into the existing systems of health care for use by ten household health workers and community health workers. A chart (Table 1) and birth weight table (Table 2) has been derived from the data obtained in which the chest and mid arm circumference are measured, a new born's weight can be estimated. This chart can easily be incorporated into the existing manuals of ten household health workers for use in the community.

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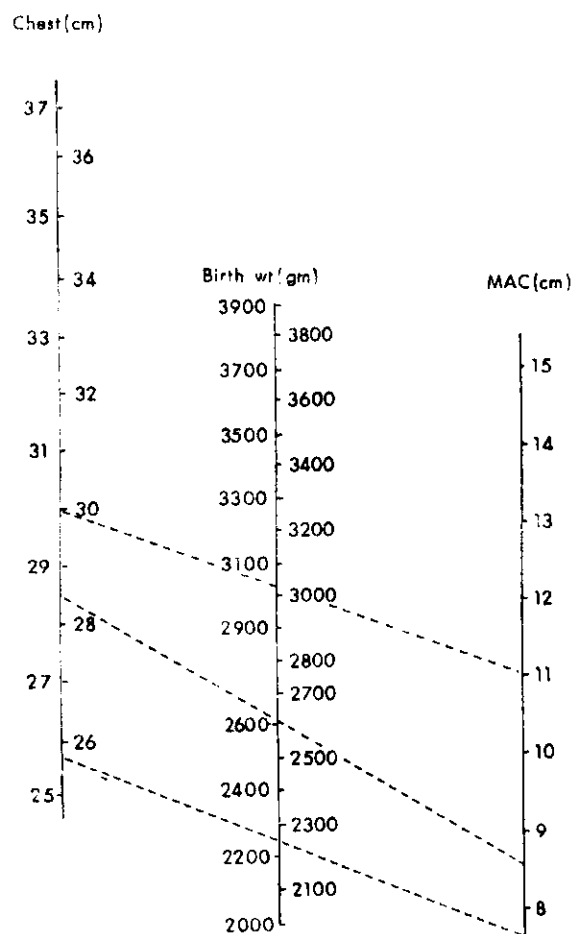


Table 2. Thaketa birth weight table

assignments often in difficult conditions. Dr. U Than Win, Township Medical Officer of Thaketa Township and to all the mothers and children of Thaketa Township who willingly took part in the studies.

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Potency assay of antivenom: neutralisation of biological properties of three Russell's viper venoms by a monospecific antivenom

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Potency assay of a monovalent Russell's viper antivenom manufactured by Myanmar Pharmaceutical Factory (MPF) batch DN 866088 exp. 4/92 was assessed with Russell's viper venoms of Tharyawady, Kungyankone and Daedaye in rodents using WHO standard tests of neutralising activity. There is variation in the amount of antivenom requires to neutralise individual biological property of three venoms and it is noteworthy that 4 times greater amount of antivenom is needed to neutralise haemorrhagic activity of Tharyawady and defibrinogenating activity of Kungyankone than the other two venoms. This variable performance of antivenom may be responsible for failure of correction of some venom induced effects seen in Russell's viper bite cases. It is suggested that a wider range of standard assays should be included in characterising the potency of antivenom.

INTRODUCTION

Russell's viper bite is endemic in 6 rice growing divisions of Myanmar. The potency of antivenoms (manufactured by Myanmar Pharmaceutical Factory) used for treating Russell's viper bite cases in these areas has not been assessed. The current practice of potency assay of antivenom is mainly based on testing antilethality potency. However, WHO has recommended for testing of other biological properties of venom in assessing potency of antivenom [1]. This paper describes the potency assay of antivenom and determination of its efficacy in neutralising biological properties of Russell's viper venoms of three different localities of Myanmar.

MATERIALS AND METHODS

Neutralisation of biological properties of three Russell's viper venoms such as lethality (5LD₅₀ iv), coagulant

(10 MCD), haemorrhagic (3 MHD), necrotic (3 MND), defibrinogenating (5 MDD) and capillary permeability increasing activities (100 MCPID) of six rice growing divisions of Myanmar namely Daedaye (Ayeyarwady division), Tharyawady (Bago division) and Kungyankone (Yangon division) (Table 1) with a monovalent Russell's viper antivenom DN 866088 exp. 4/92 was tested according to WHO standard tests of neutralising activity [2]. Briefly a fixed amount of venom is mixed with variable dilutions of antivenom, incubated at 37°C for 30 min. and injected into the laboratory animals. The end point is taken as the minimum amount of antivenom requires to completely neutralise the biological effect of venom.

RESULTS

Results of neutralisation of biological properties of three venoms by a single batch of monovalent antivenom are shown in Table 2. There is

Table 1. The amount of venom used in testing neutralising activity

Source of venom	5LD ₅₀ i.v ug/mouse	10 MCD ug	3 MHD ug/rat	3 MND ug/rat	5 MDD ug/mouse	100 MCPID ug
Daedaye	14.3	0.135	84.5	83.6	8.9	0.892
Tharyarwady	23.4	2.512	126.5	119.4	12.0	0.724
Kungyankone	30.2	3.125	79.0	70.3	25.5	0.115

Table 2. The amount of antivenom required to completely neutralise the biological effects of venom

Source of venom	5LD ₅₀ i.v ul	10 MCD ul	3 MHD ul	3 MND ul	5 MDD ul	100 MCPID ul
Daedaye	2.5	0.125	0.625	1.25	0.625	2.5
Tharyarwady	2.5	0.0625	2.5	2.5	0.625	2.5
Kungyankone	5.0	0.0625	0.625	2.5	2.5	1.25

LD₅₀ = lethality
MCD = minimum coagulant dose
MHD = minimum haemorrhagic dose
MND = minimum necrotic dose
MDD = minimum defibrinogenating dose
MCPID = minimum capillary permeability increasing dose

variation in the amount of antivenom requires to neutralise individual property of three venoms. Four times greater amount of antivenom is needed to neutralise haemorrhagic activity of Tharyarwady and defibrinogenating activity of Kungyankone than that of the other two venoms.

DISCUSSION

The antivenoms manufactured by MPF are recommended for use in treating Russell's viper bite cases throughout Myanmar however their neutralising efficacies against venoms from different localities have not been tested. It is observed that the antivenom DN 86608B failed to equally neutralise all the biological activities of 3 Russell's viper venoms reflecting that there is geographical variation of biological property of venom [3-4]. Moreover 4 times greater amount of

antivenom is needed to neutralise haemorrhagic activity of Tharyarwady and defibrinogenating activity of Kungyankone than the other two venoms supports the observation that antivenoms failed to correct some venom induced effects in Russell's viper bite cases in spite of repeated antivenom therapy. It is suggested that a wide range of neutralisation tests should be included in potency assay in addition to current practice of testing antilethality potency only and widely pooled venoms should be used for raising antivenom for common use.

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Immunophenotyping of acute leukaemia

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Immunophenotyping of peripheral blood mononuclear cells from 36 patients (Male 23, female 13; age range 3-80 yrs) newly diagnosed as acute leukaemia were done during May 1991 to November 1992. All cases were diagnosed as acute leukaemias based on clinical features and peripheral blood and bone marrow findings under light microscopy. Using the indirect immunofluorescent method immunophenotypes of the leukaemic blasts were identified on peripheral blood mononuclear cells using monoclonal antibodies (CD13 as myeloid marker; CD3, CD10 and CD19 as lymphoid markers). Out of 36 patients, 19 were myeloid and 14 were lymphoid leukaemias and 3 were non-reactive with monoclonals used. The study showed a significant positive correlation between morphological diagnosis and immunophenotyping. It had established the diagnosis in 3 cases where morphology alone was unable to do it. Discrepancy between morphological diagnosis and immunophenotyping was observed in 4 cases. The present study showed that it will be of great diagnostic as well as therapeutic value if an appropriate and wider panel of monoclonal antibodies can be used.

INTRODUCTION

The acute leukaemias are described pathologically as blast cell leukaemias or malignancies of immature haematopoietic cells. In acute leukaemia, one of the reasons for the poor prognosis is delay in commencement of combination chemotherapy. For prompt commencement of intensive chemotherapy, accurate diagnosis is necessary [1].

In recent years immunophenotyping has proven valuable for the accurate diagnosis and classification of leukaemias. Although the diagnosis of acute myeloid leukaemia is still based on morphological and cytochemical criteria, the diagnosis of acute lymphoid leukaemia depends greatly on immunophenotyping of leukaemic

cells [2,3].

In Myanmar, the diagnosis and subclassification of acute leukaemias is based on clinical features supported by morphological examination of blood film and/or bone marrow aspirate under light microscopy. This has the disadvantage of inability to differentiate a number of acute leukaemia cases into lymphoblastic or non-lymphoblastic in general. Furthermore precise subclassification of ALL, AML and others are still lacking and there has been only a few studies on acute leukaemias in Myanmar particularly regarding the immunological diagnosis and classification of it.

The present study has been undertaken with the following objectives:-

- (1) to introduce an immunological method in DMR for the diagnosis and subclassification of acute leukaemias,
- (2) to define phenotypes of different kinds of acute leukaemias and to correlate those with morphologic subtypes.
- (3) to assess how this method could contribute to the more precise subclassification of acute leukaemias and hence to get improvements in the diagnosis and treatment of the disease.

MATERIALS AND METHODS

Patients

Thirty six newly diagnosed acute leukaemia patients (male 23, female 13; age ranges from 3-80 yrs) who were admitted to Yangon General Hospital, New Yangon General Hospital, Yangon Children's Hospital and North Okkalapa General Hospital were included in this study. The blast cells of these patients were classified morphologically according to F.A.B. classification after bone marrow examination [4,5,6] and immunologically by the use of Monoclonal antibodies. The 2 procedures were done on the day of blood sampling independently.

Materials

Monoclonal antibodies (Mo Abs) used are

1. DAKO-T3 (CD3) (Monoclonal mouse antibodies to human) DAKOPATTS a/s Denmark, for Pan-T-Cell surface antigen
2. DAKO-CALLA (CD10) (Monoclonal mouse antibodies to human) for common acute lymphoblastic leukaemia antigen.
3. DAKO-CD19 (Monoclonal mouse antibodies to human) for Pan-B-Cell surface antigen.

4. Fluorescence - conjugated F(ab')₂ fragment of rabbit immunoglobulin to mouse immunoglobulin (Rabbit antimouse FITC) DAKO-PATTS a/s Denmark.

Rabbit antimouse IgG - FITC labelled used as secondary antiserum for fluorescence labelling. This antibody will react with all classes of mouse immunoglobulin (Ig) due to Fab specificity.

5. My7 lyophilized MoAb reagent (Coulter immunology - Hialeach Florida) for myeloid antigen.

Preparation of PBMC (Peripheral blood mononuclear cells)

Peripheral blood samples were taken in heparinized tubes (151U/ml). PBMC were separated by Ficoll-Hypaque density gradient centrifugation [7] and washed 3 times in RPMI.

Detection of cell surface antigen by Indirect Immunofluorescence (I.I.F.)

[12]
50 ul PBMC (2×10^7 /ml) was incubated with the different MoAbs used in the panel for 30 min. at 4°C. After that, the cells were washed thrice with RPMI medium (+5% sodium azide + FCS). A second antibody labelled with fluorescence (GAM-FITC) was added and incubated for a further 30 min. at 4°C. Then, the cells were washed three times to remove the excess fluorescence. The cell suspension was then placed on a cover slip and examined under fluorescence microscope (Olympus BH, U.V. microscope with a FITC, 485 nm excitation primary filter).

RESULTS

Out of total 36 leukaemias, 23 were males (13 adults, 10 children) and 13 were females (7 adults, 6 children) (Table 1).

Morphologically, 18 cases were AML,

Table 1. Age and sex distribution of acute leukaemias (by FAB morphological classification)

Type	No. of cases	Adult		Children	
		Male	Female	Male	Female
A.N.L.L.	18	8	5	3	2
A.L.L.	16	3	2	7	4
A.U.L.	2	2			
Total	36	13	7	10	6

Table 2. Distribution of subtypes (FAB morphological classification)

Type	Subtype	No. of cases
A.L.L.	L-1	6
	L-2	10
A.M.L. (A.N.L.L.)	M-1	3
	M-2	9
	M-3	3
	M-4	3
A.U.L.	-	2
Total		36

Table 3. Distribution of immunophenotypes

Immunophenotypes	Marker	Reactive cases
A.L.L.	CD10	10
	CD3	3
	CD19	1
A.N.L.L.	My7 (CD13)	19
Total		33
Non reactive cases		3
Grand total		36

16 ALL and 2 AUL. Immunophenotypically, 19 cases have myeloid phenotypes, 14 cases of lymphoid phenotypes and 3 cases of non reac-

Table 4. Age and sex distribution of immunophenotypes

Type	No. of cases	Adult		Children	
		Male	Female	Male	Female
Lymphoid	14	4	0	6	4
Myeloid	19	8	5	4	2
Non-reactive	3	1	2	0	0
Total	36	13	7	10	6

Table 5. Correlation between morphological types and membrane phenotypes

Morpho-logical types	Morpho-logical subtypes	No. of cases	Membrane phenotypes				
			CD3	CD10	CD19	My7	NR
A.L.L.	L-1	6	-	6	-	-	-
	L-2	10	3	4	1	1	1
A.M.L. (ANLL)	M-1	3	-	-	-	2	1
	M-2	9	-	-	-	9	-
	M-3	3	-	-	-	3	-
	M-4	3	-	-	-	2	-
A.U.L.	-	2	-	-	-	2	1
Total		36	3	10	1	19	3

tive with all monoclonal antibodies used (Table 2).

Regarding the correlation between morphology and immunophenotypes (Table 5), it was found that:

- All cases (6 cases of ALL-L1 were CD10⁺
- 10 cases of ALL-L2 reacted as follows:
 - CD10⁺ = 4 cases
 - CD3⁺ = 3 cases
 - CD19⁺ = 1 case
 - My7⁺ = 1 case
 - N.R⁺ = 1 case
- Out of 18 cases of ANLL,
 - My7⁺ = 16 cases
 - N.R. = 2 cases (one from

- AML-M1, other from AML-M4)
(d) Both cases of AUL turned out to be My7⁺ Myeloid leukaemias.

Out of 16 cases of ALL, 10 ALL-L2 cases showed very marked variable membrane phenotypic characteristic (Table 5). Three were CD3⁺, 9 were CD10⁺, 1 CD19⁺, 1 My7⁺ and 1 NR. This finding proves heterogenicity within ALL. This heterogenicity within ALL can only be recognized by membrane marker studies which allows a clear separation between B-lineage and T-lineage ALL [8].

DISCUSSION

In this study a strongly positive correlation was found between morphological diagnosis and membrane phenotypes in ANLL cases. 16 out of 18 cases (88.8%) diagnosed as ANLL were positive with myeloid marker (M7). In the remaining 2 cases, the blast cells do not react with any of the MoAbs used. This finding does not exclude these 2 cases being myeloid since we were able to use only one antibody i.e M7(CD13) to identify the myeloid precursors. We might be able to define the membrane phenotype as myeloid in these cases if and additional myeloid marker eg. My9, gpA, gpIIb/IIIa etc; could be used in our panel of MoAbs.

None of the morphologically diagnosed ANLL were found to be lymphoid lineage on phenotyping. This suggest that the morphological diagnosis alone in experienced hands is acceptable for routine diagnosis of ANLL.

One non-reactive case of ALL could be a case of null-ALL. One case of morphologically diagnosed L-2 ALL turned out to be myeloid. This finding suggest that precaution should be taken in diagnosing L-2 since this could be mistaken for AML.

As mentioned above, discrepancy between morphology and immunophenotypes was observed only in 4 out of 36 cases (2 cases of AML showed NR, one ALL-L2 showed NR, one ALL-L2 became My7⁺). One possible factor leading to this discrepancy may be the limited use of MoAbs.

In this series, immunophenotyping has confirmed the morphological diagnosis in 30 out of a total of 36 cases of acute leukaemias. This has established the diagnosis in 2 cases of AUL into My7⁺ myeloid leukaemia and 1 case of FAB-L2 into CD10⁺, totally 3 cases, where morphology alone fail to differentiate between ALL and ANLL.

Immunophenotyping in this study has made possible to the accurate diagnosis and subclassification of 14 cases of ALL into 10 CALLA, 3 T-A11 and 1 BALL.

CONCLUSION

This work has supported the fact that immunophenotyping is undoubtedly contributing to the accurate diagnosis and subclassification of acute leukaemias. However, the study is by no means complete of a number because of limitations such as limited panel of MoAbs used, eg. GpA for AML-M6, gpIIa/IIIb for AML-M7, My9(CD33) for AML M-M1, M5, CD45 for leucocyte common antigen, CD21 for malignant B-cells with receptors for E.B. virus, CD25 for T cells with I.L.2. receptors etc; can not be used for detection of lineage heterogenicity, lineage promiscuity and rare aberrant phenotypes of acute leukaemias [10,11].

In FAB classification cytochemistry is essential for fulfillment of FAB criteria especially in M1, M2, M6 and L2 cases. Currently, in almost all of the laboratories in Myanmar,

the facilities for cytochemical staining to be applied in diagnosis of acute leukaemia is very much limited or even absent.

Fluorescence microscope is the only elaborate equipment needed in immunophenotyping by indirect immunofluorescence technique. The availability of it is also very limited at present. Persons skilled or expert in fluorescence microscopy are also very few. Arrangements should be made to introduce immunoenzyme methods such as immunoperoxidase (P.A.P) or immuno-alkalinephosphatase (APAAP) techniques where a single light microscope will serve the purpose.

This work introduced a reproducible method of immunophenotyping using MoAbs by I.I.F. technique and the finding leads to draw conclusions that immunophenotyping definitely plays as an essential diagnostic tool for accurate diagnosis and subclassification of acute leukaemias. It will be of greater value if the more specific and wider panel of MoAbs could be used and if the cost and sophistication in using I.I.F. technique and a fluorescence microscope could be overcome by simple immunoenzyme method like immunoperoxidase or immuno-alkalinephosphatase methods [9].

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**Clinical trial to determine the antidiarrhoeal potential of Traditional Medicine
Formulations TMFs-16, TMF-35a and TMF-43**

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Clinical trial to determine the therapeutic efficacy of three Traditional Medicine Formulations, claimed to have antidiarrhoeal action, were studied on 150 acute diarrhoeal patients admitted to the Infectious Diseases Hospital, Yangon. TMF-16 was found to possess a good antidiarrhoeal action with the antidiarrhoeal index (ADI) of 28.71%, which is approximately equal to that of the standard drug, loparamide which had the ADI of 27.94%. TMF-35a also possess a mild to moderate antidiarrhoeal action (ADI = 21.5%), but TMF-43 showed little or no antidiarrhoeal action (ADI = 9.64%). The clinical significance of the study is that both TMF-16 and loparamide were found to reduce the stool output as well as the amount of fluid replacement required. TMF-16 is well tolerated, available locally and cheaply, and thus, may prove beneficial in the symptomatic relief of non-specific acute diarrhoea.

INTRODUCTION

Acute nonspecific diarrhoea is one of the commonest symptom encountered in medical practice and in tropical countries like Myanmar, diarrhoea constitute a major health problem [1,2]. But despite its common occurrence, majority of the patients experienced limited disability often leading to self medication with symptomatic antidiarrhoeal agents whereas a minority suffered considerable distress, which necessitates physicians' advice, hospitalization and subsequently affecting both life style and productivity [3]. In Myanmar, a large segment of the country folk in rural areas were found to rely on traditional drugs and herbal remedies and traditional medicine formulations have

been used for symptomatic relief of acute non-specific diarrhoea by many traditional medicine practitioners [4]. Since there is yet no systematic and scientific appraisal of the therapeutic claims has been made according to the principle and technique of western modern medicine, a clinical trial was conducted on patients admitted to the Infectious Diseases Hospital for acute diarrhoea with the object to:

- (1) To find out whether the 3 TMFs, TMF-16, TMF-35a and TMF-43 have antidiarrhoeal effect on acute diarrhoeal patients and
- (2) If so, to compare the antidiarrhoeal efficacy with that of the standard antidiarrhoeal drug, loparamide.

TMF-16 and TMF-35a have been proven to be the most promising by

both in vivo and in vitro test models [5], and easy availability and low cost of these drugs justified their clinical evaluation.

PATIENTS AND METHODS

A total of 160 acute diarrhoeal patients of both sexes, aged 16-60 years, who were admitted to the Infectious Diseases Hospital were selected on the basis of a satisfactory history and medical examination. The patients included in the study were considered healthy except for having diarrhoea, have not been taking medication prior to hospitalization, and fulfill the criteria for acute diarrhoea according to the WHO/CDD, 1984 [6] (ie. acute onset of watery motions of more than 3 times within the past 24 hours). Appropriate laboratory investigations and rectal swab for culture of V. cholerae was done on admission.

The trial was a double blind, parallel group design where each patient entered into the study was allocated by random numbers to receive either of the 5 packets containing one of the 3 TMFs or loparamide or placebo, after an initial rehydration therapy of 3 hours (ie. the usual time taken for patient to become clinically stable). Body weight was taken on admission, before starting rehydration therapy and on discharge, IV and oral rehydration therapy was continued throughout the study period as necessary, but drug medication was restricted up to 24 hours after test drug administration. After this, oral tetracycline 250 mg QID was started as routine. Four-hourly monitoring of vital signs, intake and output as well as careful observation for potential adverse effect was done up to 48 hours after which soft food and liquid was allowed, the amount of food intake calculated by differential

weight of dishes.

Statistical analysis

Statistical comparison of each group with the control group was done by unpaired student's 't' test with the p value of 0.05 as the minimal level of significance. Comparative efficacy between the test groups was also done by one-way analysis of variance (ANOVA).

RESULTS

Our of a total of 160 patients who met the requirements for enrollment in the study, 150 patients completed the study without significant discomfort or adverse effect requiring cessation of therapy. The baseline clinical characteristics of patients shown in Table (1) showed that the treatment groups were comparable. All patients gave history of acute onset of watery motion of more than 3 times within 24 hours prior to admission and more than 70% of the patients gave history of vomiting before admission. All patients were severely dehydrated and 10 were in a state of shock on admission but all patients recovered after 3 hours initial rehydration therapy. Sixteen percent of the patients studied were positive for cholera. Table (2) showed the comparison of patients' response in the 5 groups tested. The 4 groups receiving loparamide and the 4 TMF's showed some degree of antidiarrhoeal action regarding duration of diarrhoea (which is taken from the time of initial treatment to the last watery stool passed, provided the next one is semisolid or solid), the frequency of diarrhoea (which is the number of unformed stool passed during the study period), purging volume (which is the volume of stool passed) as well as the amount of fluid replacement required, when compared with

Table 1. Baseline (clinical) data of patients of different groups studied

No.	Data	Individual drug studied (test drug)					TOTAL n=150
		LOPERAMIDE n=29	TMF-16 n=32	TMF-35a n=31	TMF-43 n=30	PLACEBO n=28	
1.	Age (average in years)	32.0	34.5	31.2	33.1	28.8	31.9
2.	Body weight (average in kg)	45.1	46.9	47.9	45.0	46.8	46.3
	DOD(hr)	18.9	16.4	20.1	16.9	18.0	17.1
3.	Average no. of stools passed before admission	11.1	10.4	9.5	11.7	13.2	11.2
	FOD (no.)	11.1	10.4	9.5	11.7	13.2	11.2
	No. of patients	19	21	23	21	24	108
4.	Vomiting before admission	6.5	4.9	4.4	4.0	3.9	4.7
	Average no. of vomiting	6.5	4.9	4.4	4.0	3.9	4.7
	G III	26	29	29	29	27	140
5.	State of hydration at the time of admission						
	Shock	3	3	2	1	1	10
	C positive	0	5	6	4	9	24
6.	Stool culture for cholera						
	C negative	29	27	25	26	19	126

Table 2. Comparison of patients' response to antidiarrhoeal treatment (mean value)

Group	Diarrhoea		Purging volume (litres)					Rehydration (litres)	
	Duration (hours)	Frequency (times)	(0-8)hr	(0-24)hr	(24-48)hr	After 48 hr	Total	IV	ORS
TMF-35a	30.35	12.32	0.855	1.212	0.340	0.024	1.551	4.774	1.284
Placebo	34.57	19.57	0.787	1.688	0.766	0.151	2.455	4.675	2.252
Loperamide	28.21	14.31	0.654	1.135	0.404	0.016	1.539	3.517	1.167
TMF-16	27.06	13.19	0.478	1.051	0.277	0.022	1.328	4.101	1.327
TMF-43	28.33	16.03	0.775	1.411	0.321	0.008	1.733	5.108	1.728

the group receiving placebo. No significant difference was seen between groups regarding the duration and frequency of diarrhoea as well as fluid replacement given, but TMF-16 was found to significantly reduce the total volume of stool output ($t=2.1340$, p 0.02) as compared with the control group, the maximum action seen between 24-48 hours ($t=2.1454$, p 0.02). TMF-35a and loperamide were also found to reduce the total stool output (p 0.05), but

no significant difference was noted with TMF-43.

Fig. 1 showed the changes in the mean volumes of diarrhoeal stools produced by different groups after oral administration of either of the 3 TMF's or loperamide or placebo. Loperamide and the 3 TMF's were found to reduce the stool output as compared to placebo and the maximal effect was seen at 24-48 hours after drug administration.

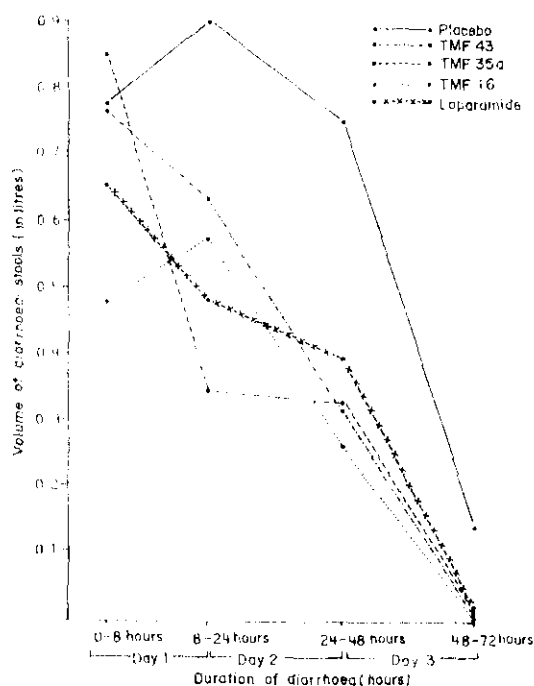


Fig. 1. Changes in mean volumes of diarrhoeal stools produced by different groups after oral administration of either of the 3 TMF's or loperamide or placebo

Table 3 showed the relative antidiarrhoeal efficacy of the 3 TMF's as compared with placebo by calculating the antidiarrhoeal index using the formula:-

$$\text{Antidiarrhoeal Index (ADI)} = \frac{4 D_{\text{dur}} \times D_{\text{freq}} \times P_{\text{vol}} \times F_{\text{rep}}}{\text{Placebo}}$$

TMF-16 was found to possess a good antidiarrhoeal action with the antidiarrhoeal index (ADI) of 28.71%, which is approximately equal to that of the standard drug, loperamide which had the ADI of 27.94%. TMF-35a also possess a mild to moderate antidiarrhoeal action (ADI = 21.5%), but TMF-43 showed little or no antidiarrhoeal action (ADI = 9.64%). Table 4 showed ingredient of TMF-16, (APU-NYEIN THWESE).

All 4 drugs tested were well tolerated. Adverse effect were minimal (eg. nausea, vomiting and abdominal discomfort), inconsistent, some what equally distributed between treatments and for the most part, indistinguishable from the symptom commonly associated with diarrhoea syndrome.

DISCUSSION

In most people, acute diarrhoea is a common, self limiting, non-fatal symptom which rarely last for more than 2-5 days [6]. Aetiology of acute diarrhoea is many and although it has been claimed that appropriate specific therapy can shorten the illness and may even be life saving [7], the aetiological agent, even when aggressively sought, is rarely found and by the time the organism is identified, the patient is usually well [8]. Thus, replacement of fluid and electrolytes loss remains the main stay of treatment and antimicrobials are not indicated for the routine treatment of diarrhoea [9].

In mild to moderate diarrhoea, people rarely seek physician's advice, but to overcome the morbidity and loss of working time during diarrhoea, it is not uncommon to find patients taking symptomatic treatment with over-the-counter antidiarrhoeal agents and, in Myanmar, indigenous antidiarrhoeal agents are of no exception. TMF-16, TMF-35a and TMF-43 has been approved as well as produced locally by the Department of Traditional Medicine and since they has been widely used for the symptomatic relief of diarrhoea, there is a need to know the efficacy as well as adverse effects in diarrhoeal patients. Since their antidiarrhoeal action in *in vitro* and *in vivo* animal models was said to resemble that of opiates [5], loperamide was used as a positive

Table 3. Relative efficacy of antidiarrhoeal agents

	D _{dur}	D _{freq}	P _{vol}	P _{rep}	ADI
Loperamide	18.40	26.88	37.31	32.38	27.94
TMF-16	21.72	32.60	45.91	21.64	28.71
TMF-35a	12.21	37.05	36.82	12.55	21.50
TMF-43	18.05	18.09	29.41	1.31	9.64

$$\text{Antidiarrhoeal Index (ADI)} = 4 \times D_{\text{dur}} \times D_{\text{freq}} \times P_{\text{vol}} \times F_{\text{rep}}$$

D_{dur} = Reduction in duration of diarrhoea as % of control

D_{freq} = Reduction in frequency of diarrhoea as % of control

P_{vol} = Reduction in purging volume as % of control

F_{rep} = Reduction in fluid replacement required as % of control

Table 4. Ingredients of TMF-16 (Apu-nye-in thwese)

Description of item			Part used	Amount in %
Scientific name	Family	Burmese name		
1. Glycyrrhiza glabra L.	Papilionaceae	Nwegyo	Stem	14.1
2. Mesua ferrea L.	Guttiferae	Gangaw wutsan	Stamen	14.1
3. Pterocarpus santalinus L.	Papilionaceae	Na-tha-ni	Wood	14.1
4. Barley sugar		Thagya ge	Sucrose	14.1
5. Santalum album L.	Santalaceae	Na-thabyu	Wood	12.7
6. Unidentified	Unidentified	Dandaku-ni	Bark	12.7
7. Cuminum cyminum L.	Umbelliferae	Ziya	Fruit	1.4
8. Elettaria cardamomum Maton var. miniscula	Zingiberaceae	Phalagyi*	Seed	1.4
9. Jatropha multifida L.	Euphorbiaceae	Semakhan*	Stem	1.4
10. Myristica fragrans Houtt	Myristicaceae	Zadeikpho thi	Fruit	1.4
11. Saussurea sp.	Compositae	Pannu	Root	1.4
12. Syzygium aromaticum L. Merr. & Perry	Myrtaceae	Lehnyin*	Flower	1.4
13. Terminalia chebula Retz.	Combretaceae	Kattaratthincha	Leaf gall	1.4
14. Unidentified	Unidentified	Panma	Bark	1.4
15. Unidentified	Unidentified	Sepazundok	Rhizome	1.4
16. Unidentified	Unidentified	Yoton	Stem	1.4
17. Unidentified	Unidentified	Chaunggya	Stem	1.4
18. Unidentified	Unidentified	Dandagubyu	Bark	1.4
19. Babulus babulis	Bovine	Kywe thwe	Blood	1.4

*San-Khin (1970), otherwise Hundley and Chit-Ko-Ko (1961)

control in the present study.

The study is also considered ethical as there has been no disruption in the routine treatment for diarrhoea (It has been recognised by the WHO, 1990, that 90% of patients required nothing more than prompt and adequate replacement of water and electrolytes lost in the diarrhoeal stools and vomiting). Since the study was conducted during the diarrhoea season where cholera outbreak is very common, oral tetracycline was given to prevent excretion of V. cholerae in diarrhoeal stools.

The present study showed that the 2 TMF's, TMF-16 and TMF-35a, were effective in the treatment of diarrhoea by decreasing the duration and frequency of diarrhoea as well as decreasing the amount (volume) of stool passed and this has ultimately led to a quicker recovery with less amount of fluid replacement required. Calculation of antidiarrhoeal index showed TMF-16 to be the most potent, having the antidiarrhoeal efficacy approximately equal to that of loparamide. But all drugs, although given in maximal allowable daily dose for at least 2 days, did not produce any significant adverse effect of discomfort which required treatment or cessation of therapy.

TMF-16 and TMF-35a are available locally and cheaply, and thus, may prove beneficial for the symptomatic relief of mild to moderate nonspecific acute diarrhoea. But since it has been suggested that their antidiarrhoeal action resembles that of opiates, it may not be advisable to use such agents in patients experiencing fever or blood in stool, where opiates and ant motility agents are contra indicated [10].

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Study of KAP on HIV infection/AIDS among medical doctors in Yangon

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HIV infection/AIDS related knowledge, attitudes and precautionary practices were studied among medical doctors in various health institutions, which included general hospitals, specialist hospitals, township health centers and GP clinics in Yangon, between June and August of 1991. The main objective of the study is to provide necessary information that may promote the appropriate use of health education services for the prevention of HIV infection/AIDS in health workers, particularly those at high risk. A total of 425 questionnaires were delivered and 378 (99.9%) returned. The survey results indicated that (99%) of the respondents had ever heard of AIDS and only (74%) knew the difference between AIDS and HIV infection. Knowledge concerning aetiology, transmission, diagnosis, control and prevention of the disease were revealed to provide the necessary information for effective management. Positive and negative attitudes towards HIV infected person were assessed and practices towards prevention of HIV infection/AIDS in health care settings were discussed in detail.

INTRODUCTION

The World Health Organization (WHO) estimates that between 5 and 10 million persons are currently infected with HIV and the cumulative number of cases of AIDS globally may exceed one million by 1991 [1]. In Myanmar, the magnitude and spread of HIV infection has been increasing since 1988 [2]. As there is no vaccine nor effective treatment, strategies for the control of AIDS is mainly on prevention of HIV infection. Thus, public education is the main weapon in the fight to limit the spread of HIV infection in this situation. Since AIDS has a great socio-medical impact on community, education programmes need to be developed so that the community will understand about the disease and accept the HIV infected patients and support well. Not only health workers are

important as a category to play in the education of the community but also they are dealing with infected patients in the health care settings. Taking into consideration that, it is an attempt to assess the level of knowledge, the attitudes and precautionary practices of medical doctors on HIV infection/AIDS to assist effective control and management of the disease in Myanmar.

MATERIALS AND METHODS

Medical doctors of 11 categories, viz: physicians, surgeons, obstetricians and gynecologists, paediatricians, anaesthetists, radiologists, pathologists, assistant surgeons, township medical officers, dental surgeons and general practitioners of various health care settings, viz: 9 general and specialist hospitals, 5 township health centres

and 18 GP clinics in Yangon Civil Development Committee area were included in this study. Self-administered questionnaires including 60 different questions on knowledge, attitudes and practices were delivered to 425 medical doctors. This study was conducted during three months period from June to August, 1991. Data analysis was carried out by computer assistance in Epidemiology Research Division.

RESULTS

Of the 425 medical doctors, 378 returned the questionnaires i.e. the response rate was (88.9%). Regarding sex distribution of the respondents, 39% were male and 61% were female with the age range of 25 to 66 years. 48% of assistant surgeons formed the major portion, followed by surgeon (11.6%), physician (7.6%), and other category including house-surgeon, specialists, dental surgeon (10.3%) and 1-3% of medical doctors in special fields i.e. pathologist, paediatricians and public health.

Knowledge of AIDS/HIV infection

Disease aetiology: The majority of subjects (88.7%) have ever heard of AIDS. As regards the difference between AIDS and HIV infection, 74.3 knew the difference, 21.9% thought that the two terms were the same, 2.4% didn't know the difference and 1.3% gave no answer at all. Among the respondents who had heard of AIDS, 59.7% provided correct answers like AIDS is caused by a kind of virus, is a life-threatening disease and is a preventable disease. Some (2.4%) had wrong conception of describing the diseases as a type of cancer, disease caused by illegal drug use or a type of bacterial infection (Table 1).

Transmission: Regarding transmission

Table 1. Cause of AIDS

Causes	No.	%
1. Most correct answers including - caused by a kind of virus - a life threatening disease - preventable disease	225	59.7
2. It is cause by a kind of virus only	37	9.8
3. Correct answer including caused by a kind of virus and a life threatening disease	85	22.6
4. A life threatening disease which can be preventable	5	1.3
5. Incorrect answer describing a type of cancer, illegal drug use and caused by bacteria	9	2.4
6. It is a preventable disease only	6	1.6
7. It is a life threatening disease only	10	2.6
Total	337	100.0
*No answer	1	

of the disease, 96% of doctors were aware of disease transmission through infected needles. But there were still a number of doctors that gave incorrect answers like getting infection through utensils (16.1%), infection from toilet seats (12.7%) and a afraid of working together with HIV infected person (7%), mosquito carrying the infection and getting infection after hand shaking with the infected person (1.1%) (Figure 1).

Diagnosis: Concerning diagnosis of AIDS, 98.8% of respondents answered that AIDS can be diagnosed and 94.4% knew the place for the testing. Only 2% did not know the exact place for testing. Some (91.8%) answered that early HIV testing should be done for health personnel who are at high risk and only 5.8% did not think it was a need. Most (94.2%) of doctors had an idea of

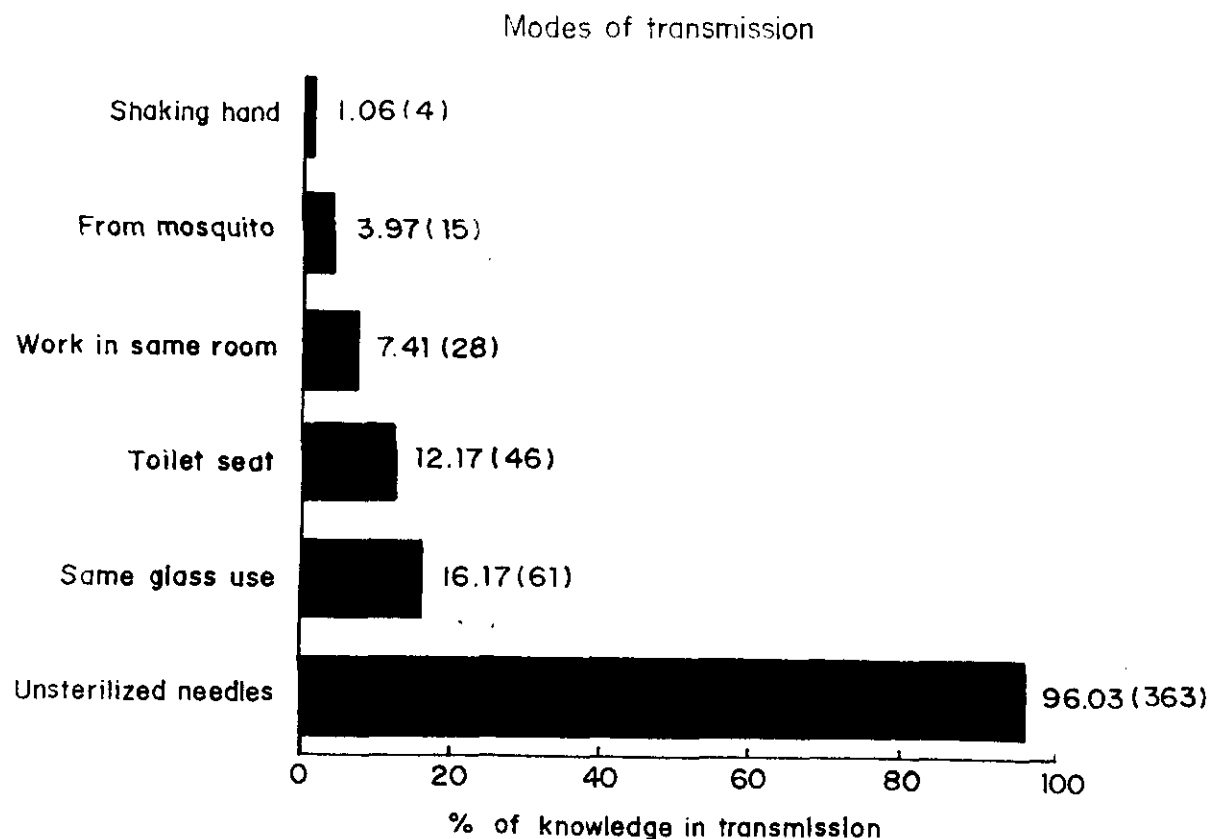


Fig. 1. Beliefs of medical doctors about transmission of AIDS/HIV infection

performing blood test before surgery, marriage or donation of blood.

Prevention: Regarding prevention of AIDS/HIV infection, 97% revealed that the infection can be prevented. Knowledge towards prevention of AIDS/HIV infection by the doctors included: safe blood transfusion, safe sex, proper sterilization techniques and training to promote adequate sterilization, but a few favoured isolation of suspected cases. For prevention aspect, 97% knew what condom was and only 86.8% had conception of condom use as an effective measure for HIV infection. Majority (95.5%) of health care workers thought that they should take precaution against HIV infection by universal precaution in addition to on the job training.

Counselling: Eighty two per cent of the doctors have heard of counselling and some (5.8%) did not know what counselling was. Only a few (12.2%) of the doctors really had a comprehensive meaning of counselling and there were some misconception thinking it is just like a disease surveillance programme.

Attitudes

Regarding attitudinal questionnaires, 95.5% answered that AIDS is an important problem in the country. Most (97.1%) doctors were afraid of getting this infection and 91.2% were afraid if someone in the family get the disease. There were only 32.8% who would like to take care of AIDS patients in their wards or rooms (Figure 2).

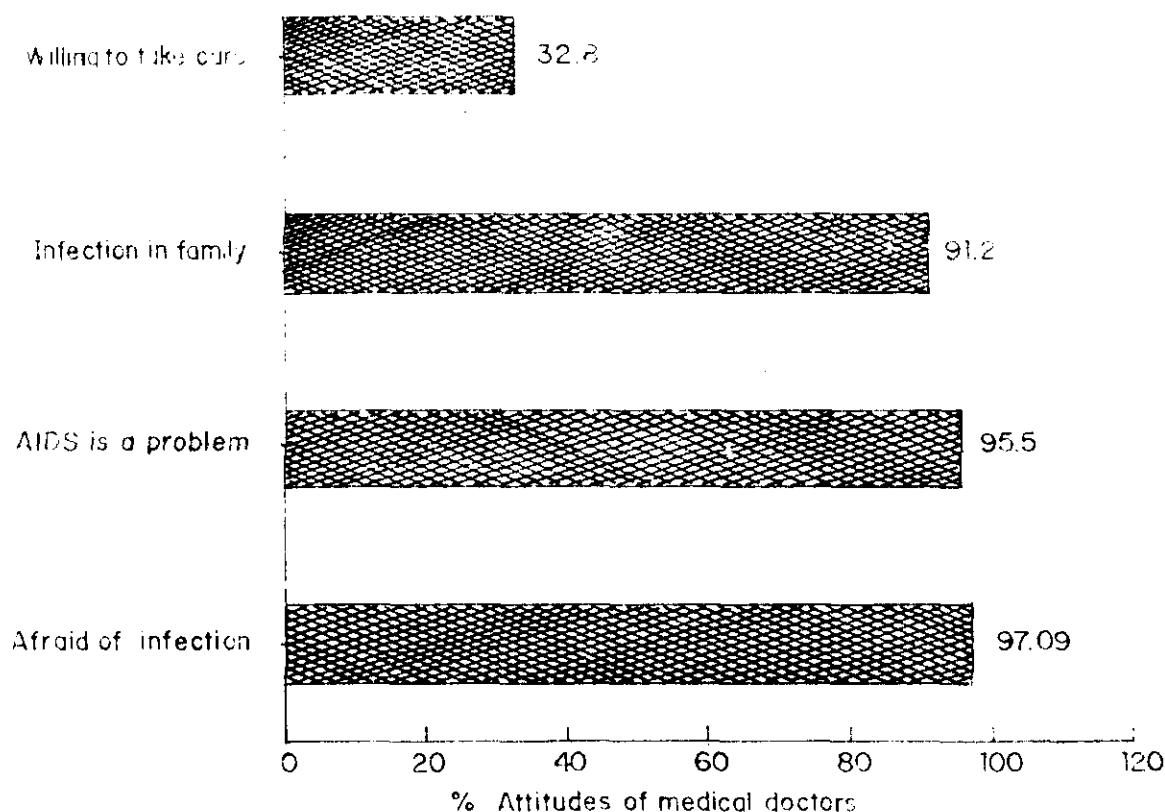


Fig. 2. Attitudes towards HIV infection

Practices: Ninety five percent of the respondents were aware of taking strict precaution to fight against HIV infection but 82.3% were able to protect themselves from HIV infection. Majority of doctors thought that a motivated working group would be needed in each hospital. A group of respondents (73.8%) had an idea to have preventive practices by giving appropriate information about AIDS to patients in hospitals. The other measures they mostly like to do were described in Figure 3.

Source of information

The important issue of health promotion strategy is the way of how to reach the public with AIDS information. Out of 378 doctors, 94.4% have heard of AIDS since more than

2 years ago and very few noticed within a year or less than 6 months. In relation to source of information, about 40% have received information mainly from magazines, 17.9% by watching radio or TV, and 9.8% by a combination of radio or TV programme together with reading papers and pamphlets. Some (75.4%) thought that they did not get enough information about AIDS.

DISCUSSION

The findings in this survey revealed that most of medical doctors had been well informed of about AIDS but not HIV infection. A number of misconceptions existed about transmission by mosquito bite and working together with infected person. The

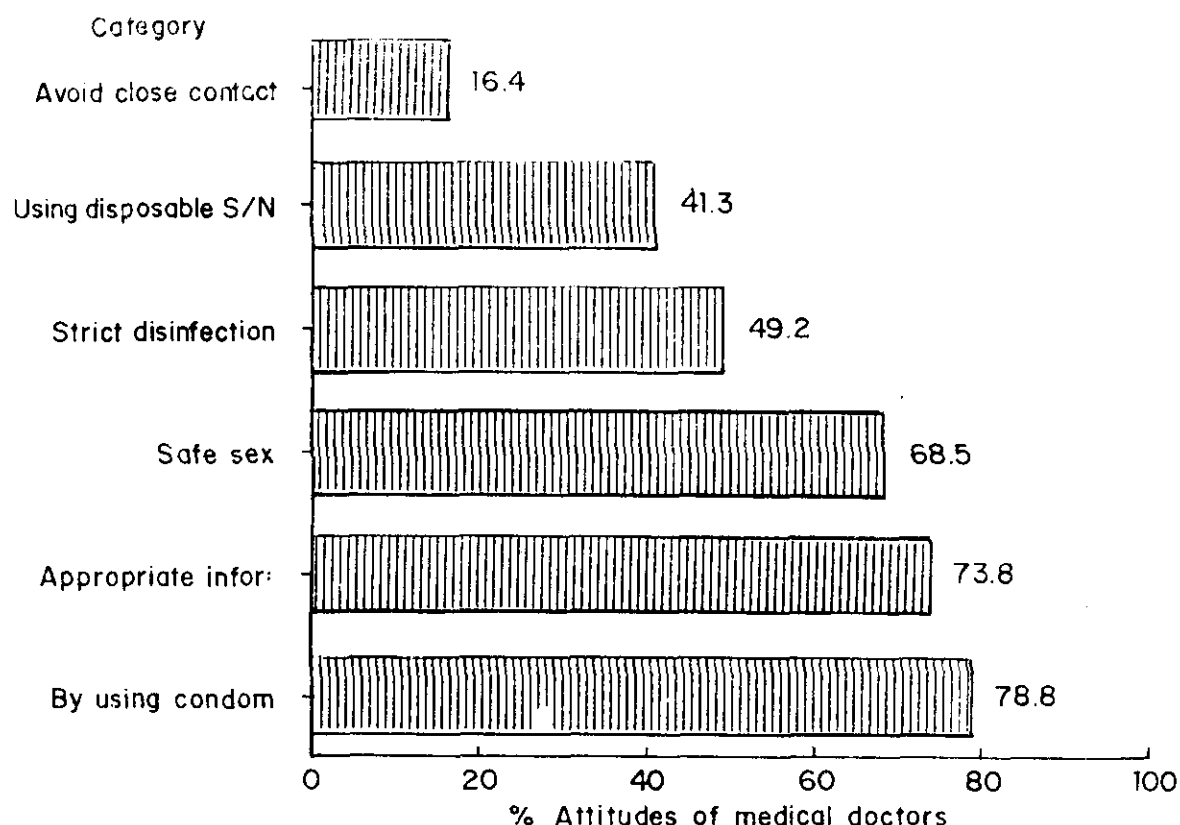


Fig. 3. Prevention of AIDS by strict precaution
S/N = syringes & needles

other thing to be considered is about "counselling". Most of the respondents confused with counselling as a special organization giving services towards HIV infected or AIDS patients and some thought of an organization like a surveillance programme. Thus it highlighted us to disseminate more information on the subject of counselling to the professional groups.

Most doctors were reluctant to take care of the infected patients because they might be infected in this environment. The doctors liked to have well equipped facilities for AIDS/HIV infection prevention in their daily practices. It is generally true that the more the health personnel has knowledge concerning AIDS, the more likely he or she is willing to

implement the universal precautions. In a study in Uganda they described that promoting of any health education strategy should be direct and include more visual and dramatic means [13]. Thus, in our health care setting, an effective and informative health education programme along with proper counselling on HIV infection/AIDS should be disseminated for each target group as necessary.

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Analysis of arsenic content in "Khun-hnit-par-shaung"

Myanmar indigenous medicine

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"Khun-hnit-par-shaung" (ခုန်နီပါရှောင့်) indigenous Myanmar medicine has been used widely for the treatment of tingling and numbness of extremities, and for a wide variety of the joint disease. It is a mixture of sein-nee-myin-thwa (ဆိုင်နီမိုင်ထွာ) (As_2S_3), kyauk-say-dan (ကျွန်ဆိုင်ဌာန်) (As_2S_3), kant (ကန်) (sulphur), za-wet-tha (ဆာဇေတ်ထာ) (ammonium chloride), hin-yaing-ni (ဟိန်ယိုင်နီ) (mercury II sulphate) let-char (လတ်ချာ) (borax), dotehtar (ဒုတ်ထာ) (copper II sulphate) in equal proportion. Arsenic content of sein-nee-myin-thwa, kyauk-say-dan and the formulated "khun-hnit-par-shaung" medicine was analysed by nuclear method. 66.89% of sein-nee-myin-thwa and 36.37% of kyauk-say-dan were found to be arsenic. The arsenic content of formulated "khun-hnit-par-shaung" medicine depends on the heating time during the preparation. The content of arsenic in the mixture become higher according to the exposure time of heat.

INTRODUCTION

"Khun-hnit-par-shaung" indigenous Myanmar medicine has been used widely for external application for the treatment of ailments such as neuritis, tingling and numbness due to prolonged healing of sores. Some Myanmar physicians claim that the medicine can cure eczema.

"Khun-hnit-par-shaung" medicine formulated by seven kinds of indigenous raw materials. Two indigenous raw materials were "sein-nee-myin-thwa" and "kyauk-say-dan", which are arsenic compounds, probably the arsenic disulphide and arsenic trisulphide respectively.

Arsenic compounds as poisonous substances are most often used if the poison plots of antiquity [1]. The alchemists were acquainted with the oxide, As_4O_6 . Certain brightly coloured arsenic compounds were used by the Greeks and Romans as

pigments. Inorganic arsenic compounds such as arsenic trioxide, arsenic pentoxide and sodium and potassium arsenite and arsenate are found in insecticides, rodenticides, fungicides, wood preservatives, herbicides and compounds used in glass manufacturing. Organic arsenic is widely distributed in the environment. Arsine gas is produced in the smelting and refining of metals, is galvanizing and etching, is lead plating, and in making silicon microchips [2].

The physical properties of the element had caused it to be classified with the metals but its similarity in chemical behaviour to nitrogen and phosphorous had caused it to be classified as non-metallic.

Arsenic has an appearance something like that of a metal, but lacks strength and can be easily broken up into small particles. It is a dark gray, crystalline solid. It sublimes, readily giving a yellow, poisonous

vapour which possesses the odor of garlic. The molecules of the vapour correspond to the formulas As_4 or As_2 , depending upon the temperature. It occurs in at least three allotropic forms, changing quickly, even at ordinary temperature into gray arsenic.

Chemically, arsenic resembles phosphorus in many ways. The main difference between the properties of the two elements is a slightly greater tendency of arsenic to act as a metal [1].

Arsenic compounds have been used as therapeutic agents. Arsenic sulfide (probably realgar) has been used for the treatment of ulcerative abscesses. Similar arsenic preparations for skin disorders, tuberculosis, asthma and leprosy [3]. Arsenic trioxide is given internally because of its beneficial effect on metabolism, for its values as a general tonic and for the use in improving the appetite. Inorganic forms of arsenic were used formerly in the treatment of protozoal diseases such as syphilis, malaria and trypanosomiasis, but organic arsenical compounds are now employed [4].

Arsenic compounds have been used for spirochetal infections, blood dyscrasias, dermatitides. Human Toxicity: most forms of are highly toxic. Acute symptoms following ingestion relate to irradiation of the G.I. tract, nausea, vomiting, diarrhoea which can progress to shock and death. Chronic poisoning can result in exfoliation and pigmentating of skin, herpes, polyneuritis, altered hematopoiesis, degeneration of liver and kidneys [5].

The reported lethal dose for arsenic ranges from 130 to 300 mg [2].

This research is to determine the content of arsenic in "sein-nee-myin-thwa" "kyauk-say-dan" and formulated "khun-hnit-par-shaung" medicine which depends on the heating time

during the preparation.

MATERIALS AND METHODS

In our experiment seven kinds of substances are used for "khun-hnit-par-shaung". The are sein-nee-myin-thwa, kyauk-say-dan, kant, za-wet-tha, hin-yaing-ni, let-char and dotehtar.

The formulation procedure mentioned below was that recommended by U Hla Myint, former Medical Superintendent Civil Hospital (Yangon), Department of Indigenous Medicine, Ministry of Health.

Each of these substances in 4 gm amount was taken and mixed together thoroughly on a glass plate. Then the mixture was placed in the middle portion of an iron pan and the mixture was then covered with a porcelain cup. Sand was placed around the cup. The sand was soaked with a small amount of water. About 2 cm³ of water was placed in the shallow place at the top of the cup. Then the pan was heated on a charcoal stove. In the course of heating, some water at the top of the cup got evaporated. To compensate the vaporation effect some water was again put into the shallow place at the top of the cup (This kept the temperature more or less constant at the top of the cup).

The sand was also always kept moist. A thermometer was placed in the soaked sand and the temperature was kept monitored. The heating intervals employed were two, four and six hours. At the end of heating, the iron pan was removed from the charcoal stove and allowed to cool. The porcelain cup and sand were removed, the substance collected at the inner top surface of the cup was taken and powdered. Then, the medicine thus obtained was fabricated into targets of known weights.

The determination of "khun-hnit-par-shaung" Myanmar indigenous medicine was done by thermal neutron activation analysis technique. Subsequent activity monitoring will be carried out by gamma scintillation counting technique.

Neutron activation analysis is a very common and useful nuclear instrumental technique. It is a method of quantitative elemental analysis [6,7].

Activation analysis consists of two major stages:

- (a) the production of a radioactive nuclide from the desired element through some nuclear reaction and
- (b) the measurement the amount of the product radioactive nuclide, of which in turn is related to the amount of the desired element present in the sample [8].

Natural arsenic is monoisotopic and undergoes thermal neutron reaction.

^{75}As (n,r) ^{76}As with the product nuclide having half-life of 26.4 hours. The reaction cross section is only about 4.5 barns.

The nuclide ^{76}As was the activation and detection.

The sample target was fabricated and irradiated with thermal neutrons from Am(Be) source for four days to attain saturation activity. The target was sealed in a thin polythene bag. Then it was fastened onto a plastic strip (specially made for this purpose so as to ensure reproducible irradiation geometry). The plastic strips were fixed in position so that the target was exactly placed in front of Am(Be) neutron source and at a distance of 3.5 cm from the source (The source and target were placed in water tank). After the end of irradiation, the target was removed and its activity monitored.

Am(Be) neutron source is a reactor

neutron and has a very long half-life of 450 years.

The activated sample target can be detected by Bicron gamma scintillation counter.

RESULTS

It was found that arsenic contents of "sein-nee-myin-thwa" and "kyauk-say-dan" were 66.89% and 36.37% respectively. The arsenic content of formulated "khun-hnit-par-shaung" medicine depends on the heating time during the preparation. The content of arsenic were 15.68% of formulated "khun-hnit-par-shaung" medicine after 2 hrs heating time, 18.20% of formulated "khun-hnit-par-shaung" medicine after 4 hrs heating time and 20.16% of formulated "khun-hnit-par-shaung" medicine after 6 hrs heating time.

Table Effects of heating intervals on the arsenic content of the formulated "Khun-hnit-par-shaung" medicine

Sr. No.	Heating interval (hr)	Arsenic content (% of wt)			
		Experiment		Average $\pm$	
		I	II	III	
1	2	15.45	15.30	16.30	15.68 $\pm$ 0.54
2	4	17.62	18.25	18.19	18.72 $\pm$ 0.55
3	6	20.04	19.38	21.05	20.16 $\pm$ 0.84

DISCUSSION

It can be observed that increase in the heating time resulted in the increase of the arsenic content in "khun-hnit-par-shaung" medicine. This may be due to the nature of some ingredients which might change with heating. The compound $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ on heating to 110°C can lose four molecules of water of crystallization. Sulphur, which on heating, can

combine with oxygen of the air to form sulphur dioxide. Ammonium chloride, which on heating, can undergo thermal decomposition and also sublimation. "Sein-nee-myin-thwa" and "kyauk-say-dan" are arsenic compounds, according to literature it can react on heating in air to form the volatile As_2O_3 and SO_2 compounds [9], which are untrapped during the heating. These untrapped compound is named as "khun-hnit-par-shaung".

The greater the heating time the greater will be the chemical and physical changes and greater will be the changes of weight. Subsequently, one can find from Table, by changing heating intervals, arsenic content can be changed from about 16% to about 20%. This observation stress the importance of heating interval in the formulation of "khun-hnit-par-shaung" medicine.

This research has demonstrated that the content of arsenic in Myanmar indigenous medicine "khun-hnit-par-shaung" can be studied by Thermal Neutron Activation Analysis employing low neutron flux source like $\text{Am}(\text{Be})$ source. This knowledge is important because arsenic content cannot be determined easily by other instrumental techniques such as atomic absorption spectrometry. The study by chemical analysis on this medicine will be quite tedious and may not be very pragmatic.

The study of the variation of arsenic content in "khun-hnit-par-shaung" medicine formulated under heating interval contributed knowledge which will be vary important to both indigenous medical practitioners and to the patients. Arsenic is absorbed through the skin, lungs and gastrointestinal tract. Inorganic compounds are absorbed more readily than organic. Arsenic is distributed from blood to liver, kidney, lungs and spleen within 24 hrs of ingestion and

to skin, hair and bone within 2 weeks [2]. So one should be careful in preparing and using "khun-hnit-par-shaung" medicine as the arsenic content becomes higher on longer exposure to heat.

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