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Endemic goitre in Pindaya and Falam townships (1991-1992)

Maung Maung Cho, Khin Mg Mg, Hla Min, Myint Lwin, Aung Nyunt, Khin Mg Naing & Hla Pe

Nutrition Research Division,
Department of Medical Research

Baseline field studies including clinical and biochemical assessments on goitre had been conducted in Pindaya Township, Shan State and Falam Township, Chin State during November 1991 and March 1992. The subjects were school children of both sexes between the age of 5 to 14 years from both urban and rural areas. Clinical assessment was done on 1553 subjects from Pindaya Township and 1224 subjects from Falam Township. Moreover, 135 urine samples from Pindaya and 90 samples from Falam had been collected for laboratory analysis of iodine. 55.4% and 62.5% total goitre rates were observed in Pindaya and Falam respectively and the mean urinary iodine excretion indicated that the subjects were moderately deficient in iodine.

INTRODUCTION

Endemic goitre may have been present since time immemorial among the population of various parts of the world. Goitre in the hilly regions of Myanmar was first reported by Raymong (1940, unpublished). Again in 1954, Seagrave reported it in the Namkhan area where nearly half the population was affected by the disease [1]. The Interdepartmental Committee on Nutrition for National Defence (ICNND) survey reported that goitre was encountered during the clinical assessment of the population in varying intensities [2]. The Taron Scientific Expedition in 1962 showed that the prevalence of goitre was as high as 40 percent [3]. Report of the occurrence of endemic goitre also came from the flat plains and delta regions [4,5].

It is generally accepted that iodine deficiency is the dominant factor in the development of endemic goitre. In many countries it had been controlled through iodine prophylaxis, particularly consistant and regular use of iodized salt. The Government launched an iodized salt distribution programme in 1969 in Chin State resulting in a spectacular decline in goitre rate [6]. However, the programme was discontinued due to decontrol of salt trade in 1977 with the result that there was resurgence of goitre. National Committee for Control of Iodine Deficiency Disorders is trying to reactivate goitre control programme by iodized salt distribution starting from hilly regions. Nutrition Research Division, Department of Medical Research will undertake impact assessment of the control programme and the present paper is the report of baseline study in Pindaya and Falam townships where iodized salt distribution programme will be initiated in 1993.

MATERIALS AND METHODS

A cross sectional study on the prevalence of endemic goitre in Pindaya Township, Shan State and Falam Township, Chin State was carried out during November 1991 and March

1992 as a baseline study for the iodized salt distribution programme that will be launched in 1993.

Study area

Pindaya Township from Shan State and Falam Township from Chin State were chosen in our study as they had been randomly selected in the previous iodized oil injection programme of Department of Medical Research [7]. Thus we took these areas again for evaluation study.

Study population

A total of 1553 children from Pindaya Township and 1224 children from Falam Township between the age of 5 to 14 years (both sexes) were studied. They all are school going children. Altogether there were 105 schools (97 rural, 8 urban) in Pindaya Township and 177 schools (169 rural, 8 urban) in Falam Township [8]. 22 school (14 rural, 8 urban) from Pindaya and 14 schools (10 rural, 4 urban) from Falam Township were selected.

Because of financial, time and manpower limitations, schools located in inaccessible areas were excluded in both the study areas. 20% of the remaining schools were then selected randomly and included in the study.

Clinical assessment

All staff members of the survey team worked together at a survey site and standardization of clinical assessment was made. Classification of goitre size was done by the system recommended by the International Council for Control of Iodine Deficiency Disorder (ICCIDD), United Nation Children's Fund (UNICEF) and World Health Organization (WHO) [9] (Table 1).

Urinary iodine determination

Causal urine samples were collected from every 10th subject of clinical examination, and altogether 135 urine samples from Pindaya Township and 90 samples from Falam Township were analysed for urinary iodine excretion. The determination of urinary iodine was done according to the method described by Zak and modified by Benotti and Benotti [2]. Urinary iodine excretion was expressed in ugm iodine per 100 ml of urine.

Table 1. Classification of goitre size

Grade	Description
0	No goitre
1A	Thyroid lobes larger than ends of thumbs
1B	Thyroid enlarged, visible with head tilted back
2	Thyroid enlarged, visible with neck in normal position
3	Thyroid greatly enlarged, visible from about 10 metres

(ICCIDD, UNICEF, WHO, Classification System)

RESULTS

Total goitre rate of 55.4% in Pindaya Township, Shan State and 62.5% in Falam Township, Chin State were observed indicating high prevalence rate in survey areas (Table 2). Degrees of thyroid enlargement are shown in Table 3. It is shown that goitres observed in the study areas are small, not bigger than grade 2. The results of urinary iodine excretion are presented in Table 4. Eighty-one percent of boys and girls in Pindaya Township and 67% in Falam Township are in the range of iodine deficient level.

DISCUSSION

With the difficult geographical terrains and transportation difficulties in the study areas, and because of financial, time and manpower limitations as already stated, the sampling procedure

Table 2. Goitre rate in Pindaya and Falam Townships (1991 & 1992)

Area	Male			Female			Total		
	Total examined	Total goitre	Goitre %	Total examined	Total goitre	Goitre %	Total examined	Total goitre	Goitre %
Pindaya Township	722	361	50	831	499	61	1553	860	55.4
Falam Township	618	333	54	606	432	72	1224	765	62.5

Remark: Population at risk = Assumed that all individuals (inclusive of population with goitre) living in areas where total goitre rate 10%, are at risk.

Table 3. Degree of thyroid enlargement in school boys and girls (5 to 14 years)

Location	Pindaya Township		Falam Township	
	Boys	Girls	Boys	Girls
Group (0) (no goitre)	361	332	285	174
Group (1A) (very small)	158	182	142	152
Group (1B) (small)	192	308	186	272
Group (2) (medium)	11	9	5	8
Group (3) (large)	0	0	0	0

Table 4. Urinary iodine excretion of school boys and girls in Pindaya and Falam Townships

Pindaya Township			Falam Township		
No. of subjects	Range (ug/dl)	Percent	No. of subjects	Range (ug/dl)	Percent
45	0 - 2.0	33	15	0 - 2.0	17
52	2.0 - 3.5	39	21	2.0 - 3.5	23
12	3.5 - 5.0	9	24	3.5 - 5.0	27
26	> 5.0	19	30	> 5.0	33

Urinary iodine < 2.0 ug/dl = severe deficiency
" 2 - 3.5 ug/dl = moderate deficiency
" 3.5 - 5.0 ug/dl = mild deficiency
" > 5.0 ug/dl = not deficient

adopted for the study is the only practical method for this evaluation study.

The prevalence of endemic goitre in children of all the study areas constitute a public health problem since it well exceed 10% of the population surveyed. The results from urinary iodine excretion measurement also support the clinical findings. Information gathered from our study indicate that there still exists iodine deficiency in the area surveyed. It could be seen that goitre encountered in the study areas were small and not bigger than grade 2 which could be attribut-

able to the fact that only school boys and girls were studied. Previous iodized salt distribution study done by Department of Medical Research had shown effectiveness in reducing goitre rates in these study areas and the resurgence after discontinuation of the iodized salt distribution [6].

Present study has shown iodine deficiency disorder (IDD), which is known to impair the physical and mental developmental of children, is still a persisting important health problem in the hilly regions of Myanmar. According to the most recent survey in 1990 [10] about 18% of the country's population (22.66% in Shan State and 29.26% in Chin State), suffered from goitre, quite exceeding the 10% rate, the margin to take as the public health problem.

It is thus obvious that IDD is one of the important public health problems of Myanmar which need attention and priority. Authorities as well as international agencies should persue the committed efforts in the implementation of sustained iodized salt distribution programme that will be launched in 1993. Information with regards to availability, and consumption of iodized salt was collected during the survey in Pindaya and Falam Townships. It was found that iodized salt was not distributed yet during the survey period. We had also been informed that the current iodized salt distribution programme will be started only in 1993. Thus our study would serve as the baseline study for the future iodized salt distribution programme.

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Potency assay of liquid preparation of enzyme refined monospecific Russell's viper antivenoms

*Tun Pe, *Aye Aye Myint, **Than Win & ***Daw Nyein

*Immunology Research Division,
Department of Medical Research

**Kyauktaga Township Hospital

***Nyaunglaybin Township Hospital

Enzyme immunoassay technique (EIA) was used to assess the performance of liquid preparation of enzyme refined monospecific Russell's viper antivenoms manufactured by Myanmar Pharmaceutical Factory, stored at +4°C and room temperature at two township hospitals. The antivenoms stored at +4°C with a shelf life of less than ½ year, time expired precipitated antivenoms and batches of antivenom stored at room temperature with a shelf life of one year have lost venom neutralising activity and also venom antigen was detected in the urine. The poor performance of liquid antivenom probably results from batch to batch variation and incorrect storage of antivenom.

INTRODUCTION

According to the British Pharmacopoeia [1] pepsin treated antitoxins are more stable than the whole sera preparation and globulin precipitate and deterioration at temperature between 0° and 5°C is negligible. At 37°C the preparation may loose 10 to 20% of their activity in one year. The enzyme refined antivenom used for treating snake bite victims comes in liquid and lyophilised forms. Our rural hospitals are provided with liquid antivenoms, with or without cold storage facilities. Since potency of liquid antivenom depends on the stored temperature, we attempt to find out the effect of storage temperature on the potency of enzyme refined liquid antivenom commonly used in rural hospitals of Myanmar.

MATERIALS AND METHODS

Liquid preparation of enzyme refined monospecific Russell's viper antivenom (ASV) was manufactured by Myanmar Pharmaceutical Factory and the Central Medical Depot, Yangon distributes these to the rural hospitals. ASV from Nyaunglaybin was stored at +4°C referigerator and that of Kyauktaga hospital in the cupboard at room temperature. The study was conducted in the respective hospitals from March 1983 to August 1984.

Russell's viper bite victims were treated with either 40 or 80 mls of ASV. Serum samples were collected before and 2h following ASV therapy. Urine samples were collected upto 24h after ASV and its volume noted. Detection of venom antigen in the serum and urine was performed by enzyme immunoassay (EIA) technique [2].

In vivo potency assay of antivenom based on testing antilethal potency was carried out according to the method described by Theakston and Reid [3] on ASV stored at room

temperature with or without precipitate and lyophilised ASV stored at +4°C. In brief, $5LD_{50}$ iv of Russell's viper venom ($1LD_{50}$ iv = 2.5 ug/18g mouse) was mixed with doubling dilutions of ASV (starting from 200 ul to 12.5 ul), incubated at 37°C for 30 min and was injected intravenously into a group of mice (5 to 10 per dose). The number of dead animal following 24h after injection was noted and the end point is taken at 100% survival dose.

RESULTS

Results of venom antigen detected in serum and urine of patients treated with liquid ASV stored at room temperature with or without precipitate and at +4°C are shown in Table 1. Serum venom antigen level at 2h after ASV in antigen detectable cases ranged from 10-67 ng/ml and that of urine was 1.05-67.5 ug/24h.

Results of in vivo antilethal potency

Table 1. Neutralisation of venom antigen of patients treated with antivenoms stored at different temperature.

Preparation/storage of ASV	Antivenom batch/expiry	Shelf life months	ASV dose ml	initial venom level (ng/ml) range	Venom antigen detected in 2h after ASV serum	urine
Liquid ASV, room temperature with precipitate	C80330,3/83	3(exp.)	40	70-78	*2/2	*2/2
Liquid ASV, +4°C	F80362,7/83	4	40	35-100	4/7	6/7
	C81406,4/84	10	40	47-72	0/4	3/4
	E81413,6/84	12	40	22-70	0/4	0/4
Liquid ASV, room temperature	D81409,5/84	12	40	30-95	4/6	6/6
	H81433,8/84	12	40	28-98	1/5	2/5
	F82482,5/85	12-18	40	25-50	0/5	0/5
	"	"	80	25-82	0/6	0/6
	G82490,8/85	8-12	40	14-77	2/3	0/3
	"	"	80	43-88	2/4	0/4

ASV = antivenom, * = number of antigen positive cases detected at 2h after ASV.

Table 2. In vivo potency assay of antivenoms. $5LD_{50}$ iv (12.5 ug venom) was mixed with variable volumes of antivenom, incubated for 30 min at 37°C before injecting intravenously into mice.

Preparation/storage of antivenom	Antivenom batch/expiry	Precipitate	Antivenom volume (ul) 200	100	50	25	12.5
Liquid ASV room temperature	E79279,6/82	+	*2/5	*3/5	*5/5	*5/5	
	C80311,3/83	+	0/6	2/6	4/6	6/6	
	D81409,5/89	-	0/5	1/5	5/5	5/5	
Lyophilised ASV, +4°C	DC84547A,3/89	-	0/10	0/10	0/10	0/10	2/10
	DC84547B,3/89	-	0/10	0/10	0/10	1/10	5/10
	DC84547D,3/89	-	0/10	0/10	0/10	0/10	NT

NT = not tested, * = number of dead mice 24h following intravenous injection of venom-antivenom mixture

of ASV are shown in Table 2. Lyophilised preparation has far better antilethal potency than liquid ASV and precipitated ASV has little neutralising activity.

DISCUSSION

It is recommended that lyophilised form of ASV should be used in preference to the liquid preparation especially in the tropical countries [4]. Since lyophilised ASV is not generally available in Myanmar, the commonly used liquid ASV of rural hospitals, stored at different environmental temperature needs assessment of its potency before we can assess the efficacy of a particular dose of ASV used. We observed that ASV stored at +4°C from our rural hospitals with a shelf life of less than one year and time expired precipitated ASV stored at room temperature have lost venom neutralising property indicated by presence of venom antigen in the serum and urine after ASV. In vivo study of liquid ASV supports our observations (Table 2). The possibility of losing efficacy of ASV stored at +4°C in rural hospitals is the result of faulty storage of ASV since enzyme refined liquid ASV if stored at recommended temperature of +4°C will keep its potency upto the end of its shelf life stated by the manufacturer [5].

Variation in venom neutralising potency of liquid and lyophilised ASV observed in our studies [6] suggested that the present method of potency assay of ASV using three pigeons test by Myanmar Pharmaceutical Factory is not satisfactory and will need to use more refined method of potency assay.

It has been reported [7] that enzyme refined liquid ASV on storage at 37°C became cloudy after 6 months of storage and precipitated out by the end of a year. Opacity signifies loss of potency and is not recommended for injecting into human. The loss of potency of batches of 1984 expiry ASV stored at room temperature was probably result of storage at room temperature or due to batch to batch variation. A similar loss of potency of liquid ASV stored at room temperature was observed in our previous study [2]. The better performance of batch 5/85 expiry although stored at room temperature probably results of getting fresh stock of ASV from the Central Depot and used up before long while kept at room temperature. The other reason may be due to batch to batch variation in potency of ASV.

Therefore standardisation of ASV is needed in future so that potency of ASV can be compared and a working dose of ASV can be recommended for treatment of snake bite cases. If feasible, proper cold storage facilities should be provided in rural hospitals or production of lyophilised ASV should be encouraged. The other suggestion is that quick turnover of fresh stock from the Central Depot should be practised instead of stocking liquid ASV at room temperature especially in midland where the room temperature is always above 25-27°C. Practice of administration of liquid ASV which turns cloudy or has precipitate in it should be stopped completely.

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Psychiatric sequelae of female sterilization

*Mya Mya, **Hla Pe & ***Tin Aung

*Department of Obstetrics and Gynaecology, Institute of Medicine (1)

**Yangon General Hospital

***Yangon Psychiatric Hospital

Prior to sterilization 272 cases were subjected to assessment of their personality. Six months after the operation 190 respondents were interviewed regarding the post-sterilization sequelae at the Central Women's Hospital. Housewives were the commonest occupational group, and the socio-economic problem being the most frequent reason besides the basic medical and psychological reasons for sterilization. Belief in the rumours of post-sterilization complications was found to be insignificant contrary to our suspicion. Over half of the sterilized cases were found to suffer post-sterilization sequelae either in isolation or in multiple of symptoms, the most outstanding symptoms being that of nuchal neuromuscular tension. Unstable personality types were more prone to have post-sterilization sequelae significantly than the stable types.

INTRODUCTION

More often than by chance we have encountered cases who had undergone sterilization complaining of psychiatric symptoms, and although some were having pre-sterilization symptoms quite a number of cases were perfectly well before the operation and yet they did suffer these symptoms sometimes after the operation. Therefore the objectives of the study were to determine the psychiatric sequelae of female sterilization and to find out the effect of previous personality of the individuals on the post-sterilization sequelae.

MATERIALS AND METHODS

Tow hundred and seventy two cases of varying age, educational level and socio-economic standard who have been registered for female sterilization at the Central Women's Hospital constituted the basis of this study. These cases had tubal ligation in Unit I of Central Women's Hospital, Yangon during the period of five years from 1979 to 1984. The research instruments used were: Personality inventory (Myanmar adaptation of the Eysenck's Personality Inventory); neuroticism questionnaire (Myanmar adaptation of Middlesex Hospital Health Questionnaire); and clinical interview method.

The inventory and questionnaire were administered to the subjects prior to the operation. They were requested to come for follow up after a period of six months for clinical interview regarding the consequences of female sterilization. Complete confidentiality was assured to the subjects. Out of 272 cases only 190 came for the follow-up sessions. They were thoroughly interviewed and examined psychiatrically to get the comprehensive picture of clinical symptoms, if any.

RESULTS

Personal characteristics

The commonest age groups were 30-34 years at 49.5%, followed by 35-39 years at 30.5%. The average age was 34.1 years. Regarding the parity, 56 cases (29.5%) were found to have 5 living children. The average number of children was 5.2 per person. Housewives were the most common group to apply for sterilization at 63.2% of the total with the sellers group taking up the second place at 22.1% and the rest of the occupation group taking up only few percentages between 3.7% to 1.6% of the total.

Reasons for sterilization

Cardiovascular disease appeared at the top of the list of 23.7%, followed by socio-economic reason (21.6%). Anxiety and obstetric and gynaecological diseases were third and fourth places (16.8% and 16.3%). General debility, respiratory disorders, other physical illnesses and neurological diseases trailed behind at 7.4%, 5.3%, 5.3% and 3.7% respectively.

Personality pattern and neurotic trait

Personality dimension of the total is shown in Table 1. The majority (93.2%) of the cases were introvert in basic personality, consisting of stable type (72.1%) and unstable type (21.1%). Ambivert and Extrovert personality comprised only 6.85% of the total. In all the different personality groups, the stable type is 77.37% with 22.63% as unstable. The percentages of cases scored high on common neurotic traits were anxiety (22.1%), obsessionality (18.9%), phobia (17.4%), somatic (15.3%), depression (11.1%) and hysteria (3.7%).

Post-sterilization sequalae

Pre-operative belief in the rumours of post-sterilization complications was assessed. Believers in the rumours of post-sterilization complications were low in number (4.2%) whereas non-believers were high (81.1%). Those who were not sure whether to believe it or not comprised 14.7%.

Table 1. Personality Dimension of the study subjects

Personality type	Stable		Unstable	
	Number of cases	%	Number of cases	%
Introvert	137	72.1	40	21.1
Ambivert	6	3.1	3	1.6
Extrovert	4	2.1	0	0
Total	147	77.3	43	22.7

Post-sterilization sequelae with any one symptoms were among 52.1% of the cases, 40% were without any disturbances and 7.9% reported even better in healths after the operation. Of the 99 affected cases, 78 (78.8%) showed appearance of symptoms within 6 months of post-sterilization period, whereas 19 cases (19.2%) within 6 months to one year and only 2 cases (2%) within 1 to 2 years. Symptoms presented as post-sterilization sequelae are shown in Table 2. Neuro-muscular tension was the most common symptoms (61.6%), followed by a whole nuttitude of other symptoms in varying degree.

Personality vs post-sterilization sequalae

The relationship between basic personality and post-sterilization sequelae is shown in Table 3. There was a definite relationship between personality stability status and presence or absence of post-sterilization sequelae. Persons of introvert with instable personality had about three times risk of acquiring the psychiatric

symptoms after sterilization than introvert with stable personality.

Table 2. Symptoms presented in percentage of the overall total study subjects

Symptom	Number	%
Neuro-muscular tension	61	61.6
Giddiness	22	22.2
Headache	19	19.2
Aches and pain	17	17.2
Tiredness	16	16.2
Palpitation	11	11.1
Bizarre symptoms	10	10.1
Insomnia	9	9.1
Irritability	7	7.1
General weakness	6	6.1
Weight loss	6	6.1
Poor vision	6	6.1
Depression	4	4.0
Poor memory	4	4.0
Breathlessness	3	3.0
Weight gain	3	3.0
Pain over the scar	3	3.0
Menstrual irregularity	3	3.0
Guilt feeling (regret)	3	3.0
Anxiety	2	2.0
Sensitive to cold	1	1.0
Epigastric pain	1	1.0

Table 3. Relationship between personality and post-sterilization sequelae

	Introvert		Total*	
	Unstable	Stable	Unstable	Stable
Present	28	64	31	68
Absent	11**	75***	11	80****

Odds ratio = 3.0 Odds ratio = 3.3
X^2=7.09; $p < 0.01$ X^2= 9.09; $p < 0.01$

*Includes other type of personality such as ambivert and extrovert. The number of cases, either stable or unstable, that had with or without the sequelae, ranged from 0 to 3.

**Include 2 cases

***Include 11 cases = who felt better after

****Include 2 cases sterilization

DISCUSSION

The commonest age group of 30-39 years found in our study with the median age of 34.1 years is about five years older than the age group of other studies [1-3]. Cut-off point given by our sterilization board in Yangon is 30 years. The number of living children varied from 2-14 with the average number of 5.8 is much higher than in the study of Smith [3] where 2 is the average number. This could probably be due to the socio-cultural background where their ideal family size is 2. Our study is quite comparable to that of the Singaporean study of Cheng et al. [4] where the average family size was 4.4, although age distribution was slightly younger in his study.

The official indications for granting the sterilization are either medical or psychological. However, 41 cases reported socio-economic problems alone as the reason for sterilization, and 59 more cases had some amount of socio-economic problem in addition to their basic reasons, suggesting that in reality, socio-economic problem was the most prominent factor for sterilization in our study. This is also quite true in other study carried by Paniagui et al. [2], who found that with few exceptions, the sterilization were requested to limit the size of the family for social and economic reason than medical ones.

In our community we heard rumours circulating that sterilization could result in ill health and this kind of hearsay was also common in the Singaporean study [4]. They suspected that it might result in a high incidence of psychological sequelae, and they felt that the seriousness of the sequelae appears to depend on cultural beliefs and religious convictions.

There has been some similar reports on such rumours in the studies of literature by Mathis [5] and Barglow [6]. In our study we found that only 4.2% of the cases were found to be believers of the rumours. This resolved our suspicion that believers would be much higher in number. It could probably be due to better health education programme and, or better knowledge in the efficacy of sterilization procedure in our community.

Over half of the cases (52.1%) had post-sterilization sequelae, the symptom being either singly or combination of multiple symptoms. Fifteen cases (7.9%) of the cases without the sequelae felt even better in their general health condition. This is most likely to be due to their relief from having further pregnancies and their adjustability to socio-economic and occupational situation.

Among the cases with neuromuscular tension, highly significant number of women complained of nuchal tension. This symptom was not even mentioned in other studies. So it seemed that this symptom is singularly peculiar to our Myanmar women.

We found that post-sterilization sequelae occurred significantly in unstable introvert personality than in stable introvert personality which is in agreement with the findings of other study [7].

Although 52.1% of the cases suffered post-sterilization sequelae it is encouraging to find that more than two-thirds of the cases (67.7%) have only mild symptoms who did not require much attention, and one-third of the cases (32.3%) had moderate degree of symptoms, out of which only few needed therapy.

Regret to sterilization of 3% in our study is quite comparable to that study by Sim et al. [8] which gave a value of 3.5%. But it is much higher in other studies [1,4,9] which gave the figures of 11%, 6.3% and 8% respectively. In the study by Barnes and Zuspan [10], it was much higher (25%) because they were done during caesarean section without ample time to psychologically prepare them of the oncoming change.

In view of the above findings we feel that the anticipation of having post-sterilization sequelae should not be of great concern for those needing such as operation. At the same time, one should consider the possible multifactorial predisposing factors such as basic personality, cultural beliefs and religious convictions, age at operation, number of living children, the timing of operative procedure and the medical and psychological disorder prior to the sterilization.

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The relationship between congo red binding capacity and the virulence of Shigella strains

*Phyu Phyu Win, **Htay Htay Kyi, *Thuzar Myint & *Tin Aye

*Bacteriology Research Division,
Department of Medical Research

**Bacteriology Division, Pathology Department,
Yangon General Hospital

Study of pathogenic mechanisms of bacteria has been facilitated by the discovery of easily identifiable markers that are used to differentiate between virulent and avirulent organisms of a given bacterial species. One of these markers is a congo red binding phenotype. In this study, 55 isolates of shigellae were tested for their congo red binding capacity and the results were compared with those of HEp-2 tissue culture assay. Shigella isolates (85.45 per cent) which invade into HEp-2 cells can also absorb congo red dye from the agar medium as well as from the solution. Non-invasive or avirulent Shigella (14.55 per cent) cannot bind congo red resulting in white colonies formation. The present study shows that congo red binding capacity of virulent Shigella indicating that this assay can be used as a quick marker and inexpensive alternative to other invasive assays to determine the virulence of these bacteria.

INTRODUCTION

An essential factor in the ability of Shigella to provoke bacillary dysentry is invasion and proliferation in the cells of human colonic epithelium [1]. Commonly used methods for determining the invasive capacity of Shigella spp. are rather time consuming, expensive and need experienced hands and specialized facilities. So, study of the pathogenic mechanism of bacteria has been facilitated by the discovery of easily identifiable markers that are used to differentiate virulent from avirulent strains of a given bacteria. One of these markers is a congo red binding phenotype. Virulent isolates of certain gram-negative bacteria such as, Yersinia pestis, Shigella flexneri, Aeromonas salmonicida, Escherichia coli, Vibrio cholerae, and Neisseria meningitis, bind the dye congo red from solid agar media producing red colonies. Isolates which fail to bind the dye produce white colonies [2].

The biochemical basis for congo red binding and the virulence phenotype is, however, not clear. The importance of being able to establish an association between pigmentation and virulence is apparent.

In Myanmar, bacillary dysentry caused by shigellae is one of the major health problem as in other parts of the world. The death rate among the patients with shigellosis is high especially in extreme ages. To control the disease effectively, prompt treatment with effective drugs are necessary. In addition, rapid and inexpensive methods to identify the virulent organisms are essential. This study has set out to determine the reliability of using pigmentation as a marker to distinguish virulent strains from avirulent ones and thus establishing a sensitive in vitro model

for studying invasion by Shigella strains.

MATERIALS AND METHODS

Bacterial strains

The study consists of 22 Shigella dysenteriae strains, 22 Shigella flexneri strains, 7 Shigella sonnei and 4 Shigella boydii strains obtained from dysentry patients involved in the W.H.O. study of shigellosis in Yangon, Myanmar (1989-90). These strains were biochemically and serologically characterized as the genus Shigella. Nonpathogenic E. coli strain and virulent Shigella dysenteriae type 1 strain (encoding 140 mdal plasmid, provided by Japan) were used as negative and positive controls respectively.

Virulence assays

Virulent properties of Shigella strains were measured by HEp-2 cell invasion assay described by Hale and Formal (1986). Nonconfluent monolayers of HEp-2 cells (Flow Laboratories, USA) were used for the experiment. Bacterial strains were prepared into suspension containing 10^7 bacteria per ml. Then the monolayers were challenged with bacteria and incubated at 37'Celcius for 2.5 h. After washing 3 times with sterile PBS (pH 7.2) to remove the majority of extracellular bacteria, the cells were covered with MEM supplemented with 15 per cent FCS, glutamine 1 mM, Kanamycin 20 ug per ml and incubated for another 2.5 h at 37'Celcius.

Then the monolayer cells were washed and fixed in 100 per cent ethanol for 10 minutes and stained for 30 minutes with 10 per cent Giemsa stain. Then the slides were cleared by passing through acetone, acetone-xylene (50:50) and xylene and mounted. After that 200 cells were examined for the presence of intracellular bacteria.

Congo red binding experiment

To examine the ability to bind congo red, cells were grown at 37'Celcius on Congo-red-TSA medium, (prepared by adding 0.01 per cent congo red dye (Wako Pure Chemical Industries Limited, Japan) into TSA (Trypticase Soy Agar, Difco, Detriot, USA) medium. On the next day, growth of colonies appeared on the medium was examined to differentiate between pigmented and non-pigmented colonies.

Congo red binding to cells was done according to the method described by Panagiotis et al. [4] to study congo red binding from the solution.

Cells were grown to late log phase (A_{650}, 1.5) in TSB (Trypticase Soy Broth, Difco, Detriot, USA), centrifuged, washed once with Phosphate Buffered Saline (PBS, pH 7.2) and suspended in one-fifth the original volume of TSB. To 1 ml of bacterial solution containing approximately 10^9 cells, 50 ug of congo red dye was added. After a 20 minutes incubation at 37'Celcius, cells were palleted by centrifugation in an Eppendorf centrifuge tube and binding of the dye was determined by assaying the residual dye in the supernatant spectrophotomaterically at 480 nm for congo red dye.

The amount of the residual dye was then compared with a standard curve of the congo red in TSB (Fig. 1), and the amoung of congo red bound by the bacteria was determined by substraction.

RESULTS

The invasive potential of different strains of Shigella on HEp-2 cells, tested by Hanle and Formal method is summarized in Table 1. Among 55 Shigella isolates, all Shigella

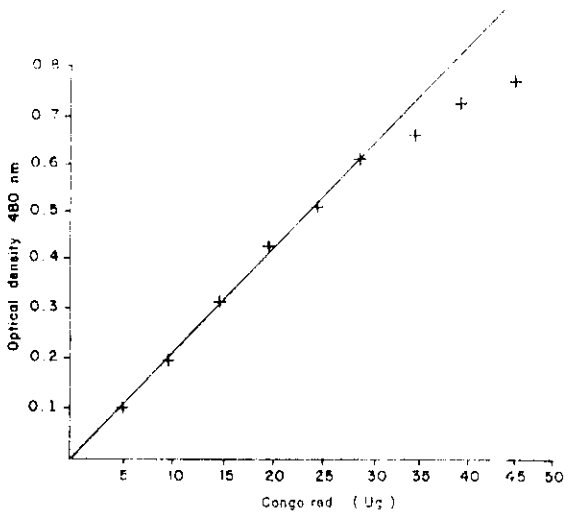

Fig. 1. Standard curve of congo red dye in Trypticase Soy Broth (TSB)

with a red centre and a lighter peripheral zone. In contrast, the strain which could not absorb the dye from agar medium formed relatively larger white colonies.

This congo red binding property was observed in all (100%) Shigella dysenteriae strains, 21 (95.45%) out of 22 Shigella flexneri strains, 4 (57.1%) out of 7 Shigella sonnei strains. The rest 4 strains of Shigella boydii revealed that they have no congo red binding capacity (Table 1). The nonpathogenic E. coli produced white colonies after the same incubation period at 37'Celcius on congo-red-TSA medium. The positive control strain (Shigella dysenteriae type 1) formed pigmented colonies on congo-red-TSA medium and it can bind the dye of 42 Ug/10^9 cells from the solution.

Table 1. Congo red binding and invasive characteristics of Shigella strains

No.	Species	No. of strains tested	Invasion into HEp-2	Congo red binding	
				From media	From solution (Ug/10^9 cells)
1.	Sh. dysenteriae	22	22(100%)	22(100%)	22(30-45)
2.	Sh. flexneri	22	21(95.45%)	21(95.45%)	22(10-47)
3.	Sh. sonnei	7	4(57.1%)	4(57.1%)	7(10-34)
4.	Sh. boydii	4	0(0%)	0(0%)	4(8-12)
	Total	55	47(85.45%)	47(85.45%)	55(8-47)

dysenteriae showed invasiveness (100%) whereas one Shigella flexneri, (4.55 per cent), 3 Shigella sonnei (42.9%) and all Shigella boydii (100%) tested were found to be non-invasive. The mean number of cell infected varied between 5 and 36 per cent and the mean number of intracellular bacteria per infected cell ranged from 4 to 15.

The results of the congo red binding experiment showed that after overnight incubation at 37'Celcius on congo-red-TSA agar, pigmented strains formed colonies which were small in size

Strains of Shigella dysenteriae bound dye from the solution in the range between 30-45 Ug/10^9 cells (Table 1). Shigella flexneri strains bound the dye in the range between 10 and 47 Ug/10^9 cells. The rate of binding capacity of Shigella sonnei and Shigella boydii strains showed between 10-34 and 8-12 Ug/10^9 cells respectively. It was also noted that the isolates which showed the congo red binding capacity in the solution

between 8 and 12 ug could not absorb the dye from the agar.

DISCUSSION

The present study confirms the two parameters for the virulence of Shigella bacilli: (i) The ability to invade the tissue culture cells and (ii) the ability to bind congo red dye. The results from the congo red binding test run parallel with those of the HEp-2 tissue culture test. In other words, Shigella strains (87.3 per cent) which were virulent in tissue culture assay can absorb congo red dye from agar medium as well as from the solution. Those that were non invasive (12.7 per cent) or avirulent can not bind congo red properly.

The difference between virulent and avirulent strains is also seen in other invasive organisms like Yersinia pestis and Yersinia enterocolitica [5]. Results of earlier studies with Yersinia pestis indicated that isolates which absorbed hemin from agar media also absorbed congo red and can be differentiated from avirulent isolates, which bound neither hemin nor congo red [6].

More recently, results from studies on Aeromonas salmonicida have been reported that congo red, hemin, photoporphyrin IX, ALL Bind to the same site of an 49,000 m protein, which is the principal component of the cell surface protein assay designated "A" layer. Aeromonas salmonicida which possess the "A" layer bind the dye or hemin are virulent, whereas Aeromonas isolates which are unable to bind hemin are avirulent [7].

Most studies of invasion mechanisms have used Shigella flexneri whereas studies with Shigella dysenteriae are very few. However, in recent years Shigella dysenteriae has been the causative agents of most of the outbreaks of shigellosis in developing countries. This study has established a sensitive in-vitro model for studying invasion by these bacteria.

Several assay systems employed for distinguishing the different steps in pathogenesis of Shigella, include Sereny test and tissue culture assay which were rather time-consuming, expensive and need experienced persons and special facilities. From this study, the property that virulent Shigella strains bind congo red from solid media has been exploited as a rapid test for distinguishing the virulent from the avirulent strains. The virulent strains formed red colonies on plates containing congo red. This indicates that congo red agar may be used as a quick and inexpensive alternative to the tissue culture assay to determine the virulence of these bacteria.

It was reported earlier that the ability of Shigella strains to bind congo red can be used as a reliable phenotypic marker of invasive character. The correlation among invasion and congo red binding capacity suggests that they are the same genetic determinants. Although the invasiveness of Shigella has been considered as phenotype expressed through cooperative actions of complex genes in the large plasmid, genetic analysis of virulence determinant has yet not been properly undertaken. The reasons may be due to the large size of the plasmid, the difficulty in isolating it into pure form, and the lack of an easily scorable marker to differentiate between invasive and non-invasive bacteria. However, it is still necessary to identify the DNA sequence associated with virulence in shigellae.

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Anticholinesterase activities of some anthelmintic agents and some medicinal plants

Tin Win, Hla Pe & Khin Khin Su

Biochemistry Research Division,
Department of Medical Research

It is known that anthelmintic drugs are potent cholinesterase inhibitors. A biochemical system for measurement of anticholinesterase activity of chemical agents and some reputed medicinal plants was established. Some of Myanmar medicinal plants were found to be cholinesterase inhibitors. Lettokegyi, an antidysenteric medicinal plant which was found to contain high anticholinesterase activity, can therefore be considered as a potential anthelmintic drug.

INTRODUCTION

Cholinesterase has been identified histochemically [1,2] and measured biochemically [3,4] in several parasitic nematodes including Ascarides. Evidence indicates that most of the conventional chemotherapeutic agents used for the treatment of worm infestations might act on cholinergic receptors [5,6,7] of the parasites. It was shown that piperazine was a cholinesterase inhibitor [3] and caused paralysis of Ascaris by direct blocking of neuromuscular junction [5]. It was suggested that the mechanism of action of santonin, another potent cholinesterase inhibitor, is due to a disturbance on the cholinergic system of the Ascaris [3]. Further evidence that anthelmintic drugs might act on cholinergic receptors is supported by the fact that many organophosphorus compounds which have a potent anticholinesterase action have been shown to have anthelmintic activity [4,5]. Present paper reports anticholinesterase activities of the chemotherapeutic agents and reputed medicinal plants as measured by dye-linked method.

MATERIALS AND METHODS

Biological materials

Pig parasites - Ascaris suum were collected from the intestine of pigs at Sinmalaik slaughterhouse.

Drugs and chemicals

Combantrin (Pyrantel pamoate) was obtained from PFIZER INC., New York, USA.

Mintezol (Thiabendazole) was purchased from Merck & Co., Inc., N.J., USA.

Ketrex (Levamisole HCl) was purchased from ICI (Imperial Chemical Industries Ltd.), Pharmaceutical Division, England. Santonin, Acetylcholine and Acetylcholinesterase were purchased from Sigma Chemical Co., USA.

Lettoke-gyi bark was purchased from Ba-ya-sei (Indigenous medicine) shops.

Preparation of Ascaris AChE enzyme

Ascaris enzymes were prepared according to Mansour [6]. The worms were cleaned with several washes of

normal saline. The intestine was removed and washed with distilled water. 1 g of the washed intestine was homogenized in 3 ml of buffered-sucrose (0.25 M sucrose in 0.005 M Tris-HCl buffer pH 7.5 containing 1% Tween 20). The homogenates were centrifuged in a high speed refrigerated centrifuge at 18,000 rpm for 30 min. The supernatant was removed, the residue was resuspended in the buffered-sucrose and the suspension was used for AChE enzyme assay. The suspension may be stored at -20°C.

Extraction of plants and chemical agents

Finely powdered air-dried plant or bark was extracted in 20 volumes (w/v) of 50% aqueous ethanol containing 100 mg% calcium chloride with stirring overnight. The suspension was placed in boiling water-bath for 10 min and was clarified by filtration or centrifugation. The extraction was repeated two more times and the extracts were pooled for the measurement. Chemical agents and drug preparations were extracted for 2 hours followed by 10 min heating in boiling water-bath and centrifugation.

Anticholinesterase activity

This activity was measured by phenol red dye-linked enzymic method modified from published methods [8,9]. The clarified extract (0.1 ml diluted to 0.95 ml with distilled water, which equals to 5 mg if 50 g of the dried plant powder is used for extraction) was incubated for 30 minutes with a known excess of acetylcholinesterase (1 millimole/0.1 ml) in buffer solution (PBS pH 7.5). At the end of this "inhibition" period substrate acetylcholine 0.2 ml (final concentration 0.6 millimole per incubation mixture of 2.5 ml) with 1.25 ml phenol red dye-buffer solution in PBS (pH 7.5) was added and incubated for further 60 minutes at 37°C. After the incubation, Optical Density (O.D.) which is a function of acetic acid produced by the hydrolysis of acetylcholine was read at 550 nm in a "Shimadzu" spectrophotometer. Cholinesterase inhibitor activity was expressed as units of AChE inhibited by per gram of the dry substance per hour at pH 7.5 and 37°C. In the control experiment, various concentrations of the drug or plant extracts added to the reaction mixture after the 60 minutes incubation instead of adding in the "inhibitory" period, showed no interference or alteration of O.D. of the reaction mixture. This indicates that colour or buffering action of the extracts did not interfere with the assay method.

(1) Cholinesterase + inhibitor ⟶ inhibited cholinesterase + free cholinesterase

(2) Acetylcholine $\xrightarrow{\text{free ChE}}$ acetic acid + choline + unhydrolysed acetylcholine

The less cholinesterase is inhibited in reaction (1), the more acetic acid will be formed in reaction (2) during the specified time of hydrolysis.

The acetic acid produced was read from the standard curve of acetic acid which ranged from 0 to 3.75 micromoles per tube, with gradations of 0.25 micromoles, giving different colours by phenol red dye.

The inhibition activity is calculated according to the formula:

$$U/g = \frac{A_{-h} - A_{+h}}{\text{inhibitor mg per tube}} \times 1000$$

where, U/g = units of AChE inhibited by per gram of dry substance per hour

A_{-h} = micromoles of acetic acid liberated in 1 hr at 37°C (without inhibitor)

A_{+h} = micromoles of acetic acid liberated in 1 hr at 37°C (with inhibitor)

RESULTS

Table 1 shows the anticholinesterase activities of various chemotherapeutic agents and some medicinal plants. It can be seen that pyrantel pamoate (Combantrin) was found to be the most potent inhibitor of cholinesterase, twice as active as santonin or ten times as active as quinidine sulphate or fifty times piperazine. Levamisole hydrochloride (Ketrex) and thiabendazole (Mintezol) are equally as active as quinidine sulphate. Acetylcholinesterase inhibition activity was found in some of the medicinal plants examined. The bark of Lettoke-gyi (Hollarrhena antidysenterica) was found to contain a high concentration of the inhibitor. Tea leaves and lauk-thay-ywet (Desmodium triquetrum) had some activity. Table 2 shows the inhibition action of various agents and plants on acetylcholinesterases of different sources, namely, eel, Ascaris intestine and human serum.

DISCUSSION

Commercial eel AChE (EC 3.1.1.7; Type III, SIGMA) resembles Ascaris enzymes with respect to substrate Km value 5 mM and response to inhibitors [10]. Therefore, the commercial eel AChE enzyme could be used in place of Ascaris enzymes for screening anticholinesterase agents. Phenol red dye-linked enzymic method was used for its simplicity since it measures the pH change due to acetic acid formed from acetylcholine hydrolysis. Trace amount of calcium (100 mg per 100 ml) was incorporated in the aqueous ethanol since santonin-

Table 1. Anticholinesterase activities of various anthelmintic agents and some medicinal plants

Agent/Plant	Anticholinesterase activity U/g	Effects
Pyrantel pamoate (Combantrin)	15,200 (1085)*	- Neuromuscular paralysis
Santonin	8,000 (570)	- Neuromuscular paralysis
Quinidine sulphate	1,400 (100)	- Not known
Levamisole HCl (Ketrex)	1,520 (108)	- It kills adult worms
Thiabendazole (Mintezol)	1,200 (86)	- It kills adult worms
Piperazine citrate	333 (23)	- Neuromuscular paralysis
Lettoke-gyi (Holarrhena antidysenterica)	1,040 (74)	- Not known
Lauk-thay-ywet (Desmodium triquetrum)	640 (45)	- Not known
Tea leaves	680 (48)	- Not known
Ya-dan-se (Brucia javanica)	260 (18)	- Not known
Thin-baw-se	0	- Not known
Daw-na-pan	0	- Not known
Potato leaves	0	- Not known

* The results are the means of three tests done

U/g = Units of AChE inhibited by per gram of the dry substance per hour at pH 7.5 and 37°C

( ) = Relative anticholinesterase potency, quinidine sulphate as 100 per cent

Table 2. Inhibition action of various agents and plants on acetylcholinesterase of different sources

Agent/Plant	Anticholinesterase activity U/g		
	Eel*	Ascaris intestine*	Human serum*
Pyrantel pamoate	15,200*	16,000*	12,800*
Santonin	8,000	9,000	5,000
Levamisole HCl	1,500	1,200	1,100
Lettoke-gyi	1,040	1,600	1,520
Piperazine citrate	333	n.d.	n.d.
Tea leaves	680	260	165
Lauk-thay-ywet	640	200	240

* The results are the means of three tests done
U/g = Units of AChE inhibited by per gram of the dry substance per hour at pH 7.5 and 37°C
Eel = Cholinesterase, acetyl; Type III, SIGMA
Ascaris intestine = Tween 20 homogenate
Human serum = Pooled human serum
n.d. = Not done

like alkaloids are more soluble in the presence of calcium ion.

Several chemotherapeutic agents that are effective against helminths effect specific mechanisms that regulate motility. Standon [7] demonstrated that Ascaris incubated with piperazine is paralysed. When the drug was introduced as anthelmintic it was realised that worms expelled from the patients were paralysed. It has been suggested [3] that the mechanism of action of santonin is due to paralytic action on the anterior part of the worm, where the central nervous control is located. Thus santonin deprives the worm of the coordinating impulses which comes from the central nervous ring. The end result of the action is that the parasite is no longer able to maintain its normal position in the host and can be expelled by purgative. Other evidence that anthelmintic drugs act on cholinergic receptors in the nematodes comes from the studies of organophosphorus compounds that are potent anticholinesterases and have anthelmintic effects [4,5].

CONCLUSION

Our findings indicated that most of the commercially available anthelmintic drugs are potent anticholinesterase agents (Table 1). Thiabendazole, which is reportedly a succinic dehydrogenase (SDH) inhibitor [11], also showed anticholinesterase action. Natrual occurrence of anticholinesterase agents has been reported [12]. We found that Lettoke-gyi bark (Holarrhena antidysenterica), a reputed anti-dysenteric drug, was found to contain a high concentration of anticholinesterase agent. It can, therefore, be considered as a potential anthelmintic drug.

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Plasma level of quinidine in Myanmar patients with falciparum malaria

*Cho Cho Myint, **Tin Shwe & ***Pe Than Myint

*Department of Pharmacology,
Institute of Medicine (I)

**Clinical Research Unit (Cerebral and Complicated Malaria),
Department of Medical Research

***Institute of Medicine (I)

Plasma level of quinidine on 14 adult male falciparum malaria patients were studied. The patients consisted of 7 with low level of parasites in blood and 7 with high level of parasites (>5% RBC parasitised). All the patients received infusion of quinidine 15 mg/kg body weight diluted in 500 cc normal saline as initial loading dose, followed by infusion 7.5 mg/kg body weight 8 hourly for another two doses and than followed by oral quinidine 7.5 mg/kg 3 times a day for 7 days. Plasma for quinidine estimation was collected at the following hours: 0,1,2,4,6,9,12,36,48 hours and on days 3, 4 and 7 of the study period. In patients with low level of parasites, the maximum plasma quinidine level reached the peak 6.8 ug/ml on the 4th day of treatment. After the first dose of treatment, it reached 4.3 ug/ml at 2nd hour. Among patients with high level of parasites, the maximum plasma quinidine concentration was 6.6 ug/ml and this concentration was obtained at 9th hour after the first dose. The mean plasma concentration of the 2 groups was not statistically different.

INTRODUCTION

Quinidine is the dextroisomer of quinine. There is a wealth of information on the Pharmacokinetics and toxicity of quinidine in healthy subjects and in patients with cardiovascular disease [1]. But there is relatively little information on its disposition in malaria patients. So far, the blood level of quinidine in Myanmar patients has not been reported. Hence, this study is necessary.

PATIENTS AND METHODS

A total of 14 adult male falciparum malaria patients who attended Tharyarwady Civil Hospital during 1988 malaria season were studied. The patients consisted of 7 with low level of parasites in blood and 7 with high level of parasites (> 5% of RBC parasitised). Patients with quinine in urine as tested by Mayer Tunrent reagent were not included in the study.

All the patients received infusion of quinidine 15 mg/kg body weight diluted in 500 ml normal saline as initial loading dose, followed by infusion of 75 mg/kg body weight 8 hourly for another 2 doses and then followed by oral quinidine 7.5 mg/kg three times a day for seven days. All patients were admitted to the hospital for seven days and followed up on days 14, 21 and 28. While in the hospital body temperature was recorded every 6 hours and blood films were cleared from the blood. Plasma for quinidine estimation was collected at the following hours. 0,1,2,4,6,9,12,36,48 and on day 3,4

and 7 of the study. Plasma was separated immediately after collection and stored at -20°C until assayed by the benzene extraction fluorescence method using Turner Spectrofluorometer [2]. The mean plasma quinidine concentrations achieved by the two groups of subjects were plotted against time, and the data were analysed statistically using Student's 't' test.

RESULTS

The ages of the patients were between 21 and 51 years. Their body weight was between 40-52 kg. All patients responded well to the treatment and were free from signs and symptoms of drug toxicity. The mean fever clearance time and parasite clearance time of all patients were 44.9 hours and 54.4 hours respectively.

In patients with low level of parasites the maximum plasma quinidine level reached the peak of 6.8 ug/ml on the 4th day of treatment. After the first dose of treatment, it reached 4.3 ug/ml at 2nd hour.

Among patients with high level of parasites, the maximum plasma quinidine concentration was 6.6 ug/ml and this concentration was obtained at 9th hour after the first dose.

In this latter group, there was bimodal distribution of graph at 9th hour and 3rd day. Apart from the 2nd, 36th, 48th hour and 4th day samples, the patients with high level of parasites showed higher levels of quinidine in the plasma throughout the study (Fig. 1). Overall the mean plasma concentration of the 2 groups was not statistically different.

DISCUSSION

In this study, the maximum peak plasma concentration was 6.8 ug/ml among patients with low level of parasites at 4th day of drug administration and 6.6 ug/ml was reached among patients with high level of parasites at 9th hour.

The latter group had higher blood level of quinidine concentration almost throughout the seven days of treatment. But this is much lower than the mean peak plasma concentration

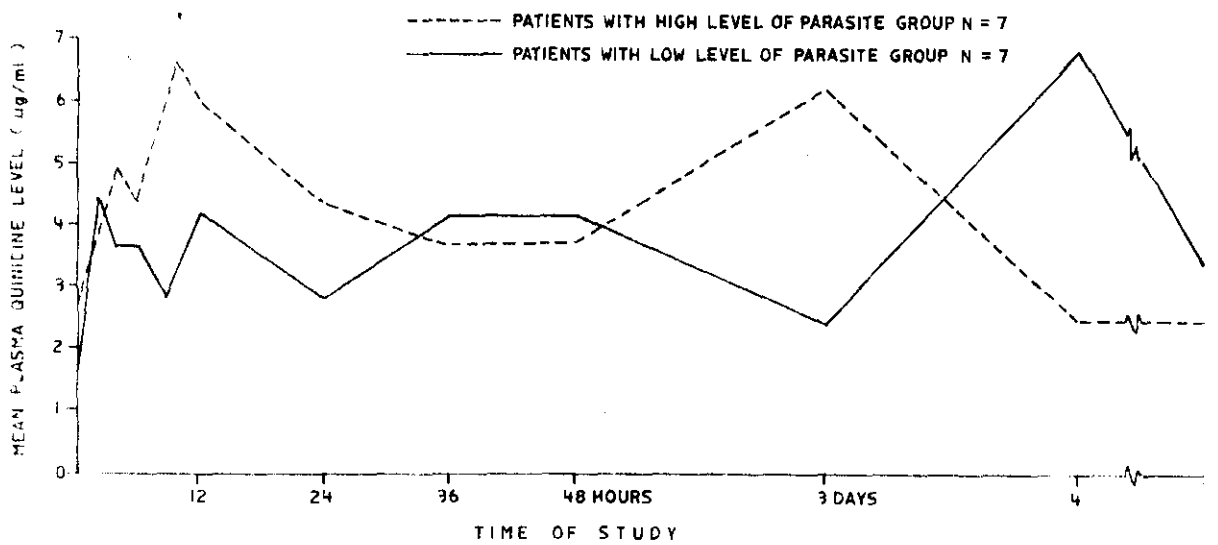

Fig. 1. Mean plasma quinidine level in patients with falciparum malaria

among comparable patients treated with quinine at the same dose and at the same hospital, where the peak concentration of quinine was 17.5 ug/ml for patients with low level of parasites and 17.1 ug/ml for patients with high level of parasites [3].

White et al. [4] have shown that against malarial parasites, minimum inhibitory concentration was consistently lower for quinidine than for quinine. This explains the adequate clinical efficacy of quinidine with low blood level. Hence quinidine is to be considered as an effective antimalarial drug for Plasmodium falciparum infection and may be more potent than quinine. In this study, allthe patients responded well to treatment and were free from toxicity of the drug.

White et al. [4] and Phillips et al. [5] have also shown that quinidine was effective for patients with Plasmodium falciparum infection, even when the infection was severe. Although electrocardiographic changes are common with the drug [6] there were no dysarrhythmias encountered in the present study. Hence, quinidine may be effectively used in patients with falciparum malaria when quinine is not available.

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The effect of intramuscular loading dose of quinine on Myanmar patients (children and adults) with falciparum malaria

*Thein Thein Myint, **Cho Cho Myint, ***Tin Shwe, ****Pe Than Myint, ***Min Thwe, *****Khin Shein, *****Aung Myint & ***Kyin Htwe

*Department of Child Health, Institute of Medicine 1, Yangon

**Pharmacology Department, Institute of Medicine 1, Yangon

***Clinical Research Unit (Cerebral and Complicated Malaria) Department of Medical Research

****Institute of Medicine 1, Yangon

*****Myanmar Pharmaceutical Factory, Yangon

The effect of three doses of intramuscular quinine followed by oral quinine on ten adults and ten children with falciparum malaria (half of each group were highly parasitised) were studied. There were no complications associated with this method of therapy. The level of serum quinine in all the adults reached above the minimal inhibitory concentration (MIC) from the 2nd hour of the drug administration. So this method of administration should be recommended for severely ill patients before referral to hospitals. Among the children, eight responded well to the therapy and the serum quinine level rose above MIC level from the second hour as in adults. There were two patients who failed to respond to the treatment. One had persistantly high level of quinine and was misdiagnosed as a case of cerebral malaria instead of quinine toxicity. He responded well when quinine was omitted and replaced with mefloquine. Another child had persistantly low level of quinine. He developed cerebral signs and symptoms and also responded well to mefloquine. Thus it is suggested that the level of serum quinine should be monitored in children if possible, or toxicity to quinine should be watched.

INTRODUCTION

Quinine is the drug of choice for the treatment of severe and complicated falciparum malaria. Conventional method of four hour intravenous infusion is safe and reliable [1], however, this method of administration is not always possible in developing countries. In rural areas, where there are no skilled medical personnel to give intravenous injections, an alternative reliable route of administration of quinine to raise the drug level quickly is by intramuscular injection [2,3]. This study is to determine the effect of intramuscular quinine in both adults and children with falciparum malaria.

PATIENTS AND METHODS

A total of 20 patients with falciparum malaria who attended Tharyarwady Civil Hospital during 1988 malaria season were studied. Patients with quinine in urine as tested by Mayer Tanret reagent test, were excluded

from the study. The study group consisted of 10 adults and 10 children (under 12 years of age). Half of each group of patients had high level of parasites in blood (>5% of RBCs parasitised).

Quinine dihydrochloride which was specially prepared from Myanmar Pharmaceutical Factory was used for the intramuscular injection. All patients were treated with intramuscular quinine 20 mg/kg followed by 10 mg/kg 8 hourly for a total of 3 doses and they were followed by 10 mg/kg 8 hourly of oral quinine for a total of 7 days.

RESULTS

All the adults responded well to the treatment. After the loading dose of quinine, the level of serum quinine in 9 out of 10 patients were above 6.9 mg/l at the end of first hour, which were well above the minimal inhibitory concentration (MIC) level of quinine for Myanmar patients (5.7 mg/l for 1987, Myint Lwin personal communication). The serum quinine level of the remaining patient was 6.5 mg/l at the first hour but it rose to 9.8 mg/l at the second hour Fig. (1). The mean parasite clearance time (± SD) and the mean fever clearance time (± SD) were 65.2±19.8 hours and 45.0±20.8 hours respectively (Table 1).

Among children, only 8 out of 10 patients responded well to the therapy. The mean serum quinine level (7.8 mg/l) which was well above the MIC level, was reached at the second hour. The other two children responded to intramuscular quinine therapy differently. In one child, the level of serum quinine fell to 2.9 mg/l and 2.6 mg/l at 24 and 36 hours, respectively. He developed cerebral signs and symptoms and his clinical condition deteriorated at 36

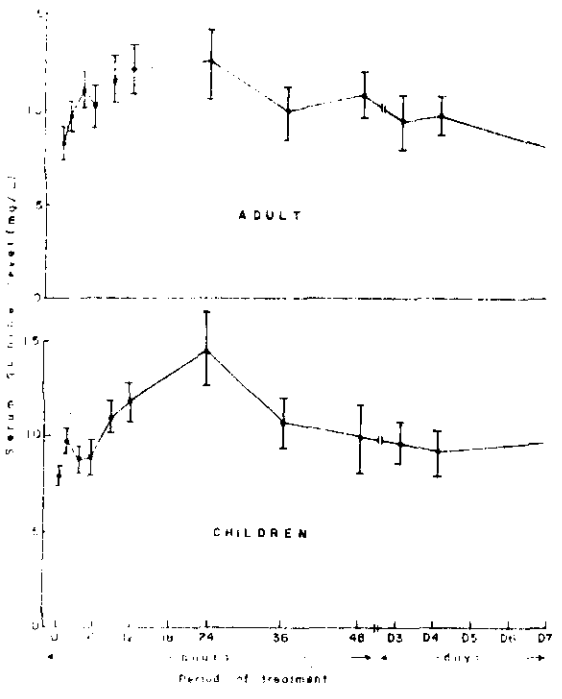

Fig. 1. Serum quinine level (SE) in children and adults treated with intramuscular quinine N = 10 each

hours and we had to change the drug to mefloquine and he responded well to it. In the other child, serum quinine level was raised above 18.2 mg/l at 4,6,9,12 and 24 hours (Fig. 2). He also developed signs and symptoms similar to cerebral malaria at 24 hours and the therapy was changed to mefloquine with good response. In the children, the mean parasite clearance time (± SD) and the mean fever clearance time (± SD) were 60.4±13.0 hours and 53.1± 15.6 hours respectively (Table 1).

There was no associated complications such as tissue necrosis, abscess formation or severe induration at the site of injections in all patients.

DISCUSSION

In this study, intramuscular loading dose of quinine was found to be safe and effective in all the adults. The

Table 1. Mean parasite clearance time (± SD) and mean fever clearance time (± SD) of the different groups of the patients (both adult and children)

Sr.No.	Patient group	No. of patients studied	Mean parasite clearance time ± SD (in hours)	Mean fever clearance time ± SD (in hours)
1	Adults			
(a)	- Uncomplicated malaria	5	51.5±16.8	43.2±10.4
(b)	- Highly parasitised malaria	5	78.8±22.7	46.8±20.7
	Both groups of adults	10	65.2±19.8	45.0±20.6
2	Children			
(a)	- Uncomplicated malaria	5	63.6±13.7	46.6± 8.6
(b)	- Highly parasitised malaria	5	57.2±12.2	59.6±22.6
	Both groups of children	10	60.4±13.0	53.1±15.6

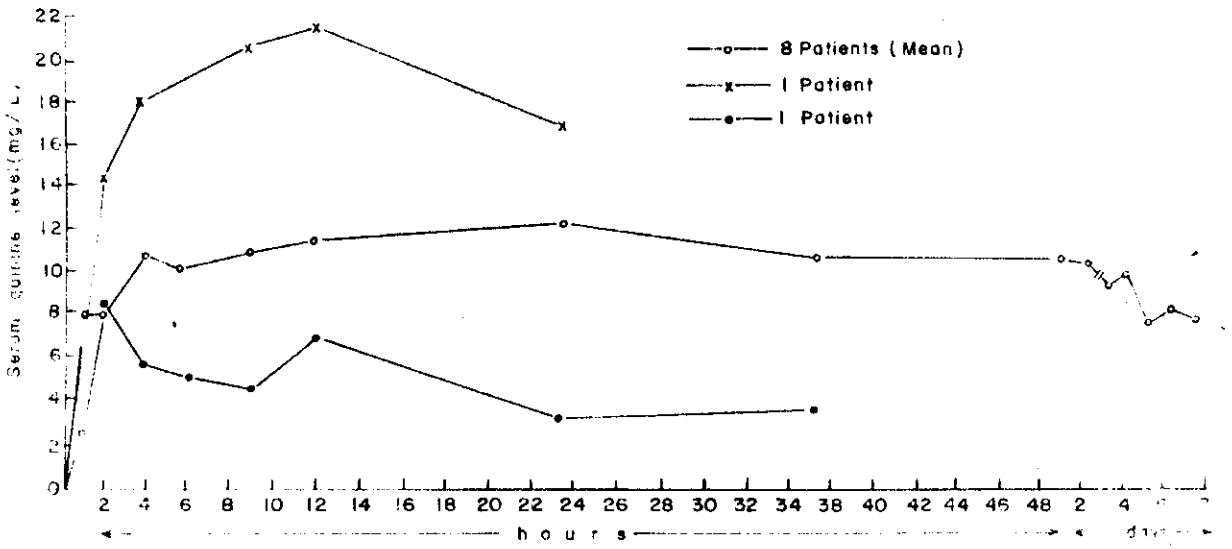

Fig. 2. Serum quinine level in children treated with intramuscular quinine

level of serum quinine reached above MIC level within the first 2 hours of injections. This was an advantage over both the oral and infusion quinine therapy. In the former, it took 4 to 6 hours and in latter it took 4 hours to reach the peak level [1]. This method is less expensive and no special nursing supervision is necessary and no dangerous peak level has occurred. Serum quinine level after intramuscular injection of quinine closely resembled to that of quinine infusion followed by oral therapy [2,3].

There was also lack of local pain and tissue necrosis probably due to

the addition of 1.5% benzyl alcohol to the standard preparation. In this study, although the sample size was small, parasite clearance time and fever clearance time were comparable to those treated by quinine infusion [1].

In many rural clinics, intravenous fluid administration sets are not easily available and infusion cannot always be supervised. Slow intravenous injection (diluted with 20 cc of normal saline taking 20 minutes for the administration of the drug) is also out of question for semi-trained personnels [4]. Rectal administration of quinine was found to be too irritant to get the therapeutic effect [5].

Hence for adults who are severely ill and needed urgent quinine administration, this method is recommended before they are referred to the hospitals. Chongsuphajaisiddhi [3] and Wattanagoon et al. [6] had also reported the effectiveness of intramuscular quinine on 40 patients. In this study, one child had a very high serum quinine level. He was clinically mis-diagnosed as a case of cerebral malaria and quinine therapy was omitted and treated with mefloquine. His clinical condition improved after omitting quinine. Later only, it was proved to be due to quinine toxicity. Another child had persistantly low level of serum quinine. The level at 36 hours was only 2.6 mg/l. He developed cerebral signs and symptoms while on quinine therapy. He was diagnosed as quinine resistant case and was saved with mefloquine (Fig. 1).

Hence only the first dose of intramuscular quinine may be used safely in children. It is advisable to monitor the level of quinine in children at the later period. If monitoring of the drug level is not possible, signs and symptoms of drug toxicity should be watched carefully. If in doubt, the dose of quinine should be reduced to one half or one third from the third day after the clinical condition has improved to avoid cumulative toxicity of quinine as suggested by WHO [7] (Fig. 2).

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Vitamin A status of the newborn in relation to birth weight, gestational age and maternal vitamin A levels

Myo Khin, Khin Maung Naing, Khin May Oo & Thane Toe

Department of Medical Research

Vitamin A concentrations of 53 newborns delivered at Central Women's Hospital, Yangon, and their mothers were studied. The mean cord vitamin A concentration (17.19 ± 7.5 ug/dL) was significantly less than mean maternal vitamin A concentration (63.58 ± 37.5 ug/dL) ($p < 0.0001$). The mean cord vitamin A concentration of newborn to mothers with vitamin A concentration less than 20 ug/dL was significantly less than those of newborns born to mothers with maternal vitamin A concentrations more than 20 ug/dL (12.82 ± 1.8 vs 17.74 ± 7.3 ug/dL) ($p < 0.01$). Variation in birth weight was associated with differences in maternal vitamin A concentrations; lower maternal vitamin A was seen in low birth weight babies as compared to normal birth weight babies (44.13 ± 37.7 ug/dL vs 69.28 ± 35.8 ug/dL). No significant correlation between cord vitamin A levels and gestational age was found. The present study demonstrates that the vitamin A status of the newborn is influenced by maternal vitamin A status.

INTRODUCTION

Vitamin A deficiency is a major problem among young children in many areas of the world resulting in blindness and significant mortality. Children are usually affected, but vitamin A deficiency symptoms can occur at any age. Pregnant mothers are no exception and it was shown that up to 25% of third trimester pregnant mother may have vitamin A serum concentration below a level considered minimally acceptable, i.e., 20 ug/dL [1,2]. Raksha and Rajalakshmi (1984) suggested that maternal vitamin A status is one of the correlates of prematurity based on their findings of lower levels of serum vitamin A in mothers of premature infants than those of full-term infants [3]. Their findings supports the previous findings where difference in neonatal vitamin A status as judged by cord serum vitamin between full-term and premature infants [4,5]. Baker et al. (1977) have also reported differences in maternal serum vitamin A levels in full-term infants with normal and low birth weights [6].

Although ICNND survey in 1963 reported that vitamin A presented no problem in Myanmar [7], recent reports suggest that vitamin A deficiency problem exists in some areas of Myanmar [8]. In a recent study where liver stores of vitamin A concentration were determined it was observed that 18.2% of neonates have low vitamin A concentrations below 20 ug/g [9]. There is lack of information concerning vitamin A nutriture of newborns and pregnant mothers in Myanmar.

The present study was undertaken to determine maternal and newborn vitamin A status and to assess newborn vitamin A levels in relation to birth weight, gestational age and maternal vitamin A levels.

SUBJECTS AND METHODS

The study was carried out at Central Women's Hospital, Yangon, Myanmar, during the month of February, 1988. The study group consisted of 53 consenting mothers and their newborns. Information regarding maternal age, parity, gestational age, history of taking vitamin A containing drugs, were recorded. Mixed venous-arterial cord blood was obtained immediately after delivery and maternal venous blood was collected within 4 hours after delivery. The samples were stored protected from light and were transported to laboratory for analysis of vitamin A concentrations.

The mother was questioned about the last menstrual date for assessment of gestational age. Gestational age was taken as menstrual age minus 2 weeks to allow for ovulation. Fundal height examination, ultrasonography and radiological examinations were carried out in doubtful cases. Vitamin A concentrations in cord and venous blood was determined by standard colorimetry method using trifluroacetic acid [10].

Statistical analysis was performed by means of SPSS/PC+ (Statistical Package of Social Sciences) Version 3 (Chicago, Illinois, USA), on an IBM-PC computer. Data was checked for accuracy and completeness before data entry. Descriptive statistics (mean, standard deviation) was calculated for each major outcome variable (cord vitamin A concentration, maternal vitamin A concentration) for each group (sex, birth weight and maturity of the newborn, maternal vitamin A adequate and inadequate groups). The mean values (t-test) were compared separately for each group to determine the relationships between cord vitamin A concentrations and birth weight, gestational age and maternal vitamin A concentrations.

RESULTS

The age of the mothers ranged from 16 to 45 years with a mean age (SD) of 27.8 ± 5.7 years. Their parity ranged from 1-8. The mean parity was 2.7 ± 1.9. The gestational age ranges from 30 to 40 weeks with a mean (SD) value of 38.9 ± 2.3 weeks. Although a positive trend was observed between maternal age and serum vitamin A concentrations, a significant correlation cannot be demonstrated. No correlation was also found between age of the mother and cord vitamin A concentrations.

The mean cord vitamin A concentration (17.19 ± 7.52 ug/dL) was found to be significantly lower than that of mean maternal vitamin A concentration (63.58 ± 37.47 ug/dL) ($p < 0.0001$) at an approximate ratio of 1:4. Cord and maternal vitamin A concentrations were analyzed in relation to birth weight of the infant (Table 1). Variation in birth weight

Table 1. Maternal and cord vitamin A concentrations in relation to birth weight of new born

	Newborn birth weight		
	Low birth weight (<2.5 Kgs)	Normal birth weight (>2.5 Kgs)	p value (Student's t-test)
Maternal vitamin A concentrations (ug/dL)	44.13±37.7 (n=12)	69.28±35.9 (n=41)	0.0396
Cord vitamin A concentrations (ug/dL)	15.26± 7.9 (n=9)	17.47± 6.9 (n=41)	0.318 (n.s.)

was associated with differences in cord vitamin A concentrations as

well as maternal vitamin A concentrations. Maternal vitamin A levels of infants with low birth weight (birth weight less than 2.5 kg (LBW) were significantly lower than that of vitamin A levels of mothers of normal birth weight (NBW) babies. Lower cord vitamin A concentrations were found in LBW babies compared to NBW babies, but the mean difference was not statistically significant.

The cord vitamin A concentrations were further analyzed in relation to maternal vitamin A concentrations. The mean cord vitamin A concentrations of newborns born to mothers with maternal vitamin A concentrations less than 20 ug/dL (Maternal vitamin A inadequate group (MVAIG) was significantly lower than of those born to mothers with maternal vitamin A concentration more than 20 ug/dL (Maternal Vitamin A Adequate Group (MVAAG). The mean birth weight of the newborns, born to mothers belonging to MVAIG was also lower than those born to mothers belonging to MVAAG, but statistical significant level was not achieved (Table 2). No significant difference of mean cord vitamin A concentration regarding sex and gestational age of the newborns was also found (Table 3).

Table 2. Newborn birth weight and cord vitamin A concentrations in relation to maternal vitamin A status

	Maternal vitamin A concentrations		
	Inadequate group (n=6)	Adequate group (n=47)	p value (Student's t-test)
Newborn birth weight (kg)	2.55±0.44	2.92±0.56	0.126 (n.s.)
Cord vitamin A concentration (ug/dL)	12.82±1.9	17.74±7.8	0.001

Table 3. Cord vitamin A concentrations in relation to newborn sex and gestational age

Relationship with		Cord vitamin A concentration (ug/dL)
Sex of newborn	Male (22)	16.67± 4.81*
	Female (31)	17.97±10.49*
Gestational age	<37 wks (9)	18.35± 8.89*
	37 wks (44)	16.95± 7.3*

*Student's 't' test (Not significant)

DISCUSSION

The maternal serum vitamin A concentrations in the present study are within the range as reported by others [2]. However, the mean value of maternal serum vitamin A concentration is slightly higher than that of others. As the present study had been conducted at a tertiary hospital, 80% of the mothers were taking regular vitamin supplements prior to delivery.

The mean values for serum vitamin A concentrations had been shown to fall in early pregnancy, followed by an increase in later stages, significantly at the third trimester [11]. In the present study, the mean gestational weeks is 38.94 ± 2.3 weeks, suggesting that the maternal serum vitamin A values represent the maximum serum vitamin A concentrations. Even then, 11.3% of the subjects had vitamin A levels lower than adequate levels. It has been reported that ten percent of United States pregnant Navajo women with docu-

mented sub-optimal nutritional status also had plasma vitamin A values under 20 ug/dL [12]. Kusin et al. (1981) also reported that in a poor rural community of East Java, Indonesia, where malnutrition was documented, 23% of the pregnant women compared with 13% of non-pregnant controls had serum vitamin A levels below 10 ug/dL [13]. Maternal undernutrition is presumed to be associated with socio-economic status as a higher prevalence of low serum vitamin A is often found among pregnant women from low socioeconomic groups [3]. In addition, Baker et al. (1975) reported that 33% of US low-income pregnant women not taking vitamin supplements had plasma vitamin A levels below 20 ug/dL, compared to 17% for those taking supplements containing vitamin A [1].

In the present study, cord vitamin A concentrations is found to be significantly lower than maternal vitamin A concentration. This is in agreement with findings reported by various authors [1,2]. The cord vitamin A concentration is also found to be independent of sex of the fetus, in accord with the study reported by Vobecky [15]. The effect of birth weight on cord vitamin A concentration in the LBW group is demonstrated in the present study but the difference is not statistically significant. Baker et al. (1977), studying full term pregnancy had also reported no significant difference between cord vitamin A concentration of low birth weight infants compared to controls of normal birth weight in full term pregnancy. The mean cord vitamin A level was not significantly lower in the infants born prematurely (less than 37 weeks of pregnancy) than infants of term gestation [6]. However 98% (8/9) of preterm infants in our study had cord vitamin A concentration of inadequate level as compared to 80% (35/44) of term infants. Various workers had reported lowest cord vitamin A concentrations in 30-33 weeks old preterm infants [4,5]. However, significant correlation between cord vitamin A levels and gestational age were not observed [14,15]. Also in the present study, gestational age did not have any effect on cord vitamin A levels.

The difference in mean birth weight of newborns born to MVAIG and MVAAG is not significant. The poor maternal vitamin A status together with poor nourishment may play a role in determining the birth weight of the infants.

In conclusion, the present study shows that vitamin A status of the new born is influenced by factors such as birth weight and maternal vitamin A status. It also suggests poor vitamin A status of mothers to be one of the features associated with intrauterine growth retardation. The present study also indicates the importance to maintain vitamin A status of mothers during pregnancy and in areas characterized by high prevalence of vitamin A deficiency, supplementation with physiologically safe doses of vitamin A during pregnancy is desirable.

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Geographical variation of biological properties of Russell's viper (Doboia russelii siamensis) venom: Yangon division

*Aye Aye Myint, *Tun Pe, *Kyi May Htwe, **Khin Aung Cho & **Theingie

*Immunology Research Division,
Department of Medical Research

**Veterinary Division,
Myanmar Pharmaceutical Factory

Biological properties of Russell's viper (Daboia russelii siamensis) venoms collected from 5 localities (Htantabin, Kungyankone, Hmawbi, Hlegu and Indaing) of Yangon division were studied according to WHO recommended methods. Htantabin venom is the most potent among others. Both adult and young viper venoms of Htantabin possess potent lethality, capillary permeability increasing activity; necrotic and defibrinogenating activities in the former and haemorrhagic in the latter. In contrast, adult venoms of Kungyankone has potent haemorrhagic and coagulant activities and the young has potent necortic and defibrinogenating activities. Such variation in venom properties may be responsible for variation in clinical pictures of Russell's viper bite cases seen within the same division and variable performance of antivenom.

INTRODUCTION

Geographical variation of composition of venoms [1-3] including Russell's viper [4-5] has been reported. Variation in the clinical pictures following Russell's viper bite from different localities and failure of antivenom to correct certain properties of venom like procoagulant or haemorrhagic activities may be attributed to such variation. Intradivisional variation of venom composition of Ayarwady division [6] leads us to study geographical variation of venom from different rice growing divisions of Myanmar where Russell's viper bite constitutes a major occupational health hazard.

MATERIALS AND METHODS

Venoms collected from 5 localities of Yangon division (Htantabin, Kungyankone, Indaing, Hlegu and Hmawbi) (Figure 1) were lyophilised and pooled according to their length into 2 groups (<90 cm and >90 cm) (Table 1). SDS-PAGE electrophoresis and biological properties of venom were carried out according to the recommended methods [7]. Details of the methods have been already described [5,6].

RESULTS

Results of biological properties of venoms were shown in the table. Among adult vipers (> 90 cm) Htantabin venom possesses potent lethality, capillary permeability increasing activity (CPI), necrotic and defibrinogenating activities. Kungyankone venom has strong procoagulant activity. Young viper venom of Htantabin is equally as potent as that of adult in lethality and capillary permeability increasing activity and

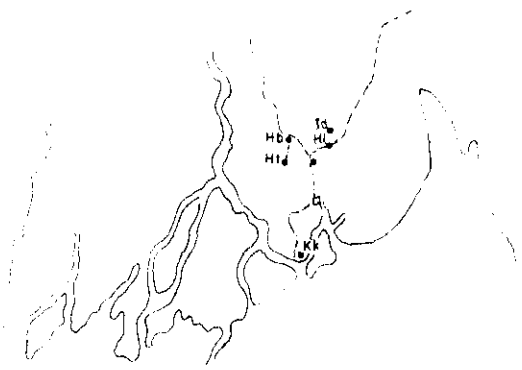

Fig. 1. A map showing 5 localities of Yangon division where venoms were available for study. Id = Indaing, Hl = Hlegu, Hb = Hmawbi, Ht = Htantabin and Kk Kungyankone.

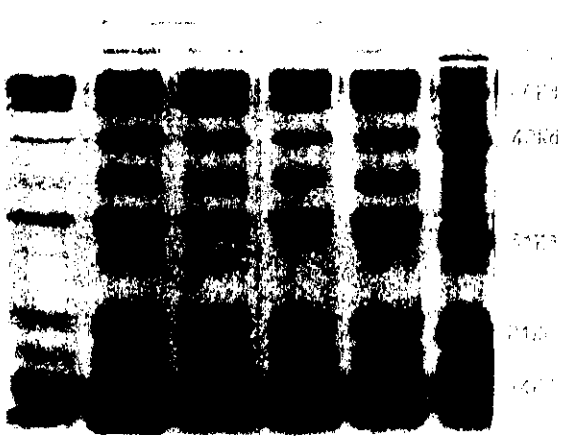

Fig. 2. SDS-PAGE electrophoresis of adult Russell's viper venoms of Yangon Division. Code are as above

Table 1. Biological properties of young and adult venoms of 5 localities of Yangon division

Source of venom	Length cm	No. snake	LD_{50}app. ug/mouse	MCD ug	MHD ug/rat	MND ug/rat	MDD ug/mouse	MCPID
Htantabin	61-90	19	4.63±2.63	1.38	30.9	33.1	4.2	0.014
	91-120	15	3.26±2.78	0.98	35.1	21.6	1.8	0.008
Kungyankone	61-90	27	8.74±3.94	0.63	32.4	20.4	1.8	0.033
	91-110	25	6.05±0.98	0.31	26.3	23.4	5.1	0.115
Hmawbi	61-90	7	5.76±3.23	0.12	56.9	52.5	6.0	0.063
	91-110	8	5.18±2.23	0.34	52.5	40.7	6.0	0.063
Hlegu	61-90	6	5.47±1.94	0.93	37.2	23.4	4.2	0.050
	91-110	6	9.31±2.65	0.48	32.7	50.1	6.3	0.030
Indaing	61-90	20	6.38±0.98	0.54	49.0	67.6	6.3	0.032
	91-120	17	4.97±3.70	0.47	44.9	56.9	5.1	0.025

possess strong haemorrhagic activity. However, young viper venom of Kungyankone has potent necrotic and defibrinogenating activities.

In SDS-PAGE electrophoresis of venoms, qualitative and quantitative differences in protein bands were observed among venoms (Figure 2 & 3).

DISCUSSION

This is the second of the division-wise study of geographical variation of Russell's viper venom of Myanmar. Htantabin venom is the most potent among venoms of Yangon division followed by Kungyankone. Its biological properties are comparable to

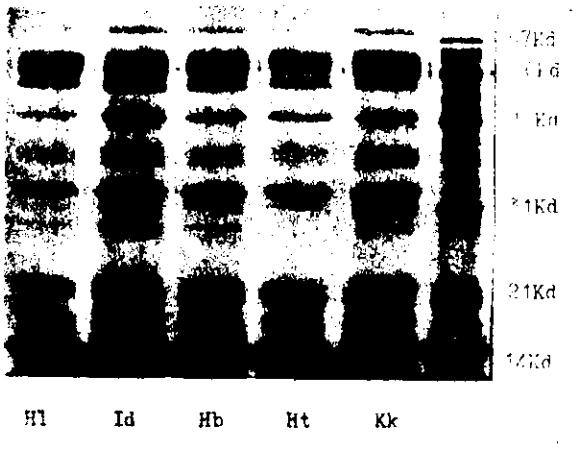

Fig. 3. SDS-PAGE electrophoresis of young adult Russell's viper venoms of Yangon Division. Code are as above

that of Daedaye venom which is the most potent venom of Ayarwady division [6] except the latter has strong procoagulant activity. Batch to batch variation of biologicical properties of venoms of Htantabin and Kungyankone is also observed [5]. It is expected that variation in the clinical pictures following Russell's viper bite of snakes of different lengths and localities within the same division will occur. Such variation in venom composition may lead to variable performance of antivenom. It is suggested that potent venoms should be pooled and used for immunising horse for antivenom production.

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SHORT REPORT

Total protein and albumin in cerebrospinal fluid of patients with cerebral malaria

*Khin Maung Maung & **Tin Shwe

*Biochemistry Research Division &

**Clinical Research Unit, Cerebral and Complicated Malaria, Department of Medical Research

Of the various mechanism for the cause of cerebral malaria the hypothesis of Maegrith and Fletcher [1] had attracted some interest among the clinicians. They considered that toxic factors perhaps a kinin, released by Plasmodium falciparum produced increased permeability of the blood brain barrier allowing protein and water to leak into the cerebrospinal fluid (CSF) resulting in cerebral oedema, cerebral vascular stasis and coma. The following study was conducted with the view to find out the possible causes of cerebral malaria.

Thirty five cerebral malaria patients admitted to Clinical Research Unit (Cerebral & Complicated Malaria), Department of Medical Research, during 1987-88 malaria season were studied. Twenty six patients admitted to the same hospital for non-inflammatory conditions such as hernia and hydrocele served as controls. Patients were studied within 4 hours following admission. Lumbar puncture was performed and CSF opening pressure was measured. CSF was examined under light microscope immediately. Aliquots were stored at -80°C for total protein and albumin estimation. CSF total protein was measured by Lowry's method [2] and CSF albumin by method of Doumas, Watson & Beggs [3].

CSF opening pressure was normal (70-180 mm H_2O) in all patients. CSF was clear and contained 1-3 lymphocytes only. The mean±SD of CSF protein of patients and control were 81.3 ± 57.6 mg/dl and 55.3 ± 47.0 mg/dl respectively. There was no significant difference ($p < 0.1$) between two groups. However CSF total protein of 4 fatal cases were 203.8, 148.0, 275.5 and 49.5 mg/dl. The mean±SD of CSF albumin and control were 49.3 ± 35.6 mg/dl and 31.6 ± 36.0 mg/dl respectively. There was no significant difference ($p < 0.1$) between the two groups. CSF albumin concentration of 4 fatal cases were 133.3, 96.6, 133.3 and 33.3 mg/dl. The Glasgow Coma scale of the patients had significant correlation with the concentration of CSF albumin ($r = -0.79$, $p < 0.001$) and total protein ($r = -0.81$, $p < 0.001$). Normal CSF opening pressure in cerebral malaria patients suggested that there is no obstruction in regular CSF flow which was observed by Warrell and his group [4]. No significant increase in CSF total protein and albumin among the patients supports the hypothesis that blood brain barrier is essentially intact in these patients which was observed by Warrell et al. [4]. Increased CSF total protein and albumin in 3 out of 4 expired patients suggested that

blood brain barrier may be impaired at terminal stages of illness. Serum CSF index is commonly used to demonstrate the integrity of blood brain barrier. If serum total protein and albumin of these patients had been determined at the same time as CSF, we would be able to draw a firm conclusion that there was no impairment of blood brain barrier at the early hours of cerebral malaria. Thus we wish to conclude that blood CSF barrier is likely to be intact in most patients with cerebral malaria and cerebral oedema may not be the cause of coma.

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