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Role of intramuscular anti-snake venom administration as a first-aid measure in the field

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Effectiveness of intramuscular (i.m) anti-snake venom (ASV) administration immediately after bite as a first-aid measure in the field followed by standard hospital treatment in the management of Russell's viper bite patients was studied in 12 victims and was compared with that of standard hospital management alone in 82 victims. There was a marked reduction in the number of patients with systemic envenomation ie. disseminated intravascular coagulation (DIC), clinical proteinuria, oliguric acute renal failure (ARF), systemic bleeding, hypotension and fatality rate of Russell's viper bite victims who received initial i.m ASV prior to the hospitalization compared with those who did not. It is recommended that i.m ASV could be administered to the Russell's viper bite patients at the site of incident as a first-aid method in places where no facility for giving intravenous ASV therapy prior to hospitalization.

INTRODUCTION

Vast amount of research and clinical trial on Russell's viper (Daboia russelli siamensis) bite envenomation have been conducted by many researchers and authorities concerned from Ministry of Health in order to reduce morbidity and mortality of snake bite in Myanmar. In spite of the availability and wide-spread use of potent anti-snake venom (ASV) produced by Myanmar Pharmaceutical Factory (MPF), snake bite fatality rate still remains considerably high in our country. One of the reasons is delayed in getting antivenom probably due to late arrival to hospital since almost all the snake bite cases occur in paddy fields which are far away from hospital and where an immediate administration of intravenous (i.v) antisera is impossible. Therefore, an alternative measure to i.v ASV therapy is the intramuscular (i.m) injection of ASV, to be given

immediately after bite at the site of incident prior to hospitalization as a first-aid measure in situation where i.v administration is impossible or transportation to hospital is likely to be delayed. Preliminary studies on i.m administration of ASV on experimentally envenomed mice and rats revealed that immediate administration of antisera by i.m route could reduce the lethality [1,2]. A study on clinical trial of i.m antisera administration as a first-aid measure in the field in Thayawady Township, Bago Division, Myanmar during 1991-1994 showed that there was a definite reduction in the number of patients with systemic envenomation, complications and fatality in 34 Russell's viper bite victims who had received i.m ASV prior to hospitalization [3]. Therefore this promising study was extended as a research-cum-action program in other areas of Myanmar where snake bite morbidity and mortality rates are high.

EXPERIMENTAL DESIGN AND METHODOLOGY

The study was carried out in Meiktila Township, Mandalay Division for 2 successive years, 1994 and 1995 with the active participation of the Basic Health Staff (BHS) in the field and Medical Officers in Meiktila District Hospital. The BHS from all villages served as a mobile task force prior to the snake bite season. They were trained for giving i.m ASV and provided with 3 pairs of lyophilised antisera, disposable syringes with needles and methylated spirit swabs. They were stationed at the respective village tract and were instructed to give a total of 10 ml ASV (5 ml to each buttock) intramuscularly to a victim within 2 hours after Russell's viper bite at the site of incident in the field. Those who received i.m ASV at the field and were transferred immediately to the district hospital for further standard hospital management were regarded as "test cases". Those victims who did not receive i.m ASV were regarded as "control cases". Patients of both groups were given the same hospital management and were kept in the hospital for a minimum of 5 days. Blood and urine samples were taken at the time of admission, and then daily for 5 days to detect development of disseminated intravascular coagulation (DIC) and clinical proteinuria by 20 minute whole blood clotting test [4] and boiling test [5] respectively. The complications following Russell's viper envenomation such as oliguric acute renal failure (ARF), systemic bleeding, hypotension and final outcome were noted. Appropriate statistical tests such as Student's unpaired 't' test, Chi-square test and Fisher's exact test were used for analysis of data.

RESULTS

A total of 94 Russell's viper bite patients confirmed by snake seen

and/or dead snake brought were available for the study. Of which 12 received 10 ml of i.m ASV at the field (test) and 82 did not (control). Comparison of bio-physical data of the two groups is shown in Table 1. The initial serum venom antigen levels of the subjects could not be determined in our study. As shown

Table 1. Bio-physical data of control and test cases

	Control n=82	Test n=12	Signifi- cance
Age (years)	32±1.63 (12-66)	29±4.42 (13-59)	NS*
Sex (M:F)	54:28	8:4	NS**
Snake length (mm)	378±34 (200-680)	345±58 (150-1200)	NS*
Distance from site of inci- dent to hospi- tal (miles)	8.3±1.04 (3-25)	10.7±2.15 (10-22)	NS*
Interval between bite and admission (hours)	2.3±0.24 (0.5-7.5)	2.9±0.35 (2.0-4.75)	NS*

- Each value represents the mean ± SEM.
- Values in the parenthesis indicate the ranges.
- Data between the two groups were compared using Student's unpaired 't' test* and Chi-square test**.
- NS means not significant at the 5% probability level.

in Table 2, the number of patients who developed systemic envenomation (ie. DIC) was significantly less in the tests and the other parameters such as clinical proteinuria, oliguric ARF, systemic bleeding, hypotension, change from coagulable to in-coagulable state in hospital and death were also found to be markedly reduced in the tests compared to the controls.

DISCUSSION

Although the majority of the juvenile or young snake bites result in blank bite and the snakes responsible for

Table 2. Clinical data between control and test cases

	Control n=82	Test n=12	Signifi- cance
DIC (incoagulable blood) (no.)	46 (56.1%)	1 (8.3%)	p<0.01
Clinical proteinuria (no.)	35 (42.7%)	3 (25%)	NS*
Oliguric ARF (no.)	23 (28.1%)	1 (8.3%)	NS*
Systemic bleeding (no.)	8 (9.1%)	Nil	NS**
Hypotension (no.)	7 (8.5%)	Nil	NS**
Change from coagulable to incoagulable state in hospital (no.)	1 (1.2%)	Nil	NS**
Death (no.)	7 (8.5%)	Nil	NS**

- Data between the two groups were compared using Chi-square test* and Fisher's exact test**.

- NS means not significant at the 5% probability level.

the bite in this study were juvenile snakes, their venom have a greater lethal, defibrinogenating and edema-inducing activities than adult viper consequently leading to more severe envenomation [6]. Since initial venom antigen levels of the victims could not be determined, the true proportion of systemic envenomed cases could not be ascertained. Moreover, the lengths of the snakes of the control and the test were not significantly different in the study. There were also no significant differences in the other clinical criteria between two groups indicating the validity of experimental design (Table 1).

It is well known that administration of specific antisera in adequate dose intravenously and immediately after the bite is an ideal and effective therapy for Russell's viper bite victims. However, i.v antisera could be given by trained medical personnel only at the hospital and at the later

hours (ie. about 2-4 hours after bite). Therefore systemic envenomation and mortality resulted from Russell's viper bite remain high in our country. In our study, 10 ml i.m administration of antisera within 2 hours after the bite as a first-aid measure in the field to the Russell's viper bite victims could significantly prevent the onset of systemic envenomation. Besides, less number of patients developed clinical proteinuria and oliguric ARF. There were no cases of systemic bleeding, hypotension and death in those who received i.m ASV prior to hospitalization. Although most of the findings were not statistically significant, marked clinical significance was observed in the tests compared to the controls.

From our present and the past studies [3] it can be concluded that in the management of Russell's viper bite victims, administration of i.m ASV immediately after the bite may be of value as a first-aid measure in the field where i.v route of ASV is impossible or transport to hospital is likely to be delayed for more than 2 hours. Hence, these findings could be used in formulating national guidelines in the management of Russell's viper bite patients in Myanmar.

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**Acceptability of condoms among family planning clinic attenders in Waybagi,
North Okkalapa**

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A hundred currently married women of 20-49 years were interviewed to assess the knowledge, attitude and practice of male condoms among family planning clinic attenders of Waybagi in North Okkalapa Township and to identify their acceptance and perception towards male condoms. The majority of wives were 30 to 34 years old and primary school level whereas the majority of husbands were between 35 to 39 years of age, educated up to high school level. Most wives (75%) were dependents whereas majority of husbands (38%) were semi-skilled workers. Although 75% have heard of condoms only 36% had actually seen one. Most knew that condoms were meant for contraception (86.7%). Sixty-eight per cent knew condoms could prevent transmission of sexually transmissible diseases; 8% thought condoms were meant for extra-marital sex; 77.3% knew that condoms were meant for single use only. Over half thought condom is important for family planning and prevention of STD, less than half thought it was good for sexual relationship, that every adult male should use it, that it was good for male use, and that it would not create any problem between husband and wife. As regards statements with negative view, more than half disagreed with statements that condoms could disturb sexual relationship and that it could not be used for monogamous marriage. Overall, the respondents seemed to regard condoms positively rather than negatively. Only 5% had ever used condoms out of which only one liked it whereas one disliked it due to condom rupture. None were currently using condoms. It indicated that proper counselling and appropriate training is essential to promote condom acceptability.

INTRODUCTION

The National Health Plan (NHP) of Myanmar has accepted birth spacing as a national priority [1]. Closely related to birth spacing is the prevention of STD.

In a baseline study of one rural area of Myanmar, the use of barrier method was found to be virtually non-existent [2]. Furthermore, in the same rural study, knowledge of condoms was 20.3% among married

women while only 2.1% of couples were using them [2].

The MYA/94/PO4 project on "Contraceptive Acceptability" is underway at the present moment, one of the study centres being North Okkalapa primary health center.

This study is therefore undertaken with the objective of assessing the acceptability and utilization of condoms when offered after proper counselling.

Objectives

General:

To evaluate the acceptability and utilization of condoms among family planning clinic attenders.

Specific:

- (1) To assess the baseline knowledge, attitude and practice of condoms among family planning clinic attenders of Waybagi (North Okkalapa Township).
- (2) To identify their acceptance and perception towards condom.

METHODOLOGY

A cross-sectional descriptive study was undertaken among family planning clinic attenders of Waybagi in North Okkalapa Township. One hundred currently married women who attended the family planning clinic during the study period were included and those women who refused to answer the questionnaire were excluded from the study. Face-to-face interview with standardized questionnaire was used for data collection after pretest. Knowledge about condoms, attitudes towards and practice on condom were asked by trained technicians. Informed consent was obtained before interview.

RESULTS

Table 1 reveals demographic characteristics of respondents and their husbands. The majority of wives were 30 to 34 years old whereas the majority of husbands were between 35 to 39 years of age. Primary school leavers formed the modal group among wives whereas most of the husbands were educated up to high school level. Most wives (75%) were dependents.

In Table 2 responses to questions on knowledge are shown. Although 75% have heard of condoms only 36% had actually seen one. Among those who

Table 1. Demographic features of respondents

Factors	Frequency (%)	
	Wife	Husband
Age		
20 to 24	10	5
25 to 29	25	17
30 to 34	32	23
35 to 39	22	29
40 to 44	9	11
45 to 49	2	12
50 to 54	-	2
65 to 69	-	1
Total (n=100)	100	100
Education level		
Read & write	8	5
Primary	36	24
Middle	24	21
High	27	41
College	5	9
Total (n=100)	100	100
Occupation		
Professional	6	13
Skilled	7	12
Semi-skilled	9	38
Odd jobs	3	27
Dependent	75	1
Total (n=100)	100	100

have knowledge of condom 86.7% knew the reasons for condom use as a form of contraception. Over half (68%) knew condoms could prevent transmission of sexually transmissible diseases. Only a few 8% thought condoms were meant for extra-marital sex, although 100% thought condoms were used only for sexual purpose.

Most of the 98.7% responded that condoms were meant for males only. Some 77.3% knew that condoms were meant for single use only. Only 33% knew how to apply although 64% knew that condoms could be applied only after erection.

Some 72% responded that proper disposal of condoms was required, and 34.7% realized that condoms

Table 2. Knowledge on condoms

Question	Yes (%)	No (%)	Don't know (%)
Have you heard of condoms?	75.0	24.0	1.0
Do you know the reason for using condoms?			
If so, is it for birth spacing?	86.7	13.3	
is it for preventing STDs?	68.0	32.0	
is it for extra-marital sex?	8.0	92.0	
Condom is used only for sexual relationship, is it true?	100		
Condoms are used by men only. Is it true?	98.7		1.3
Condom can be used only once. Is it true?	77.3		22.7
Do you know how to use condoms?	33.3	66.7	
Condoms are applied only after erection. Is it true?	64.0	36.0	
Condoms must be disposed properly. Is it true?	72.6	27.4	
Do you know condoms can rupture?	34.7	65.3	
Do you know what to do when condoms rupture?	66.0	12.0	
If it ruptures, do you know you have to use another one?	100		
If there is accidental rupture, do you visit the family planning clinic?	92.0	4.0	4.0

Table 3. Frequency of responses to attitudinal statements

Statements	Responses (%)		
	Yes	No	Don't know
1. I think condom is important for family planning (+)	71	29	
2. I think condom is very useful for preventing STD/HIV infection (+)	60	40	
3. I think it is good for sexual relationship (+)	26	74	
4. I think every adult married male should use it (+)	34	66	
5. It can disturb sexual relationship (-)	29	70	1
6. It is good for male (+)	34	66	
7. I don't think it can be used for single marriage (-)	17	61	
8. Using condom won't be a problem between husband and wife (+)	32	68	

(+) = Positive attitude

(-) = Negative attitude

Table 4. Frequency of responses to questions on condom usage (practice)

Question	Yes (%)	No (%)	Don't know (%)
Have you ever used condoms?	5.0	95.0	
If yes, are you now using it?		100.0	
Do you like condoms?	20.0	20.0	60.0
Reason for not liking condom rupture	100.0		
Are you using other methods?	88.0	10.7	1.3
If so, do you use COC?	37.2	62.8	
Injectable?	60.5	39.5	
IUCD?	7.0	93.0	

could rupture and among them, 84% admitted that they knew what to do when condom ruptured, such as using a new one or visiting the family planning clinic.

Table 3 tabulates the responses to attitudinal statements regarding condom use. Over half thought condom is important for family planning and prevention of STD, less than half thought it was good for sexual relationship, that every adult male should use it, that it was good for male use, and that it would not create any problem between husband and wife. As regards statements with negative view more than half disagreed with statements that condoms could disturb sexual relationship and that it could not be used for single marriage. Overall, the respondents seemed to regard condoms positively rather than negatively.

Table 4 shows responses to questions on male condom usage. Only 5% had even used condoms. Only one liked it but one disliked it due to rupture. None were currently using condoms. Some 88% were using other types of contraception, 37.2% on oral contraception, 60.5% on injectables and 7% on IUCD.

DISCUSSION

Condoms are supposed to be blessed with the qualities of safety i.e. avoiding the minor and major side-effects of hormones and IUCDs. In addition, it provides a barrier against sexually transmitted diseases especially the dreaded HIV infection. However, from this small study following deficiencies have been brought into light.

- (1) Knowledge about condoms is not as widespread as it should be (<90%).
- (2) Practice is among 5% of respondents.
- (3) Rupture is the sole shortcoming of condom use.

Theoretical safety ends in practical

failure. It could be due to improper application which can be corrected with proper counselling or it could be due to poor quality of condoms which can be remedied by proper distribution of good quality ones.

In the 1995 Progress in Human Reproduction Research report, it has been reported that most men reported knowing the condom (in some countries the awareness was nearly 100%) [1]. It was concluded that lack of awareness of a male method was probably not the chief obstacle to more men using such methods. Condom use was low even in countries that are relatively more affected by the AIDS pandemic. In the Contraceptive study in Taikkyi rural area [2] there was very poor knowledge of condoms. In the MYA 94/PO4 project on contraceptive acceptability in urban, semi-urban and rural areas condom use was very low [3]. Basing on the above inferences, the following recommendations are made -

- (1) training on proper counselling on condom use
- (2) procurement and proper distribution of condoms of optimum standard
- (3) provision of emergency contraception in case of rupture.

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Effectiveness of health education methods displayed at the selected health centres, Yangon Division

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A clinic-based cross-sectional survey was conducted at outpatient departments of Hlegu and Kungyangone Township Hospitals to explore the existing situation of effectiveness of health education (HE) methods displayed at health centres. Two types of study population were involved: clinic attendants and health care providers and face-to-face interviewing method was performed by using separate questionnaires. Clinic attenders who were 13 years old and above and who have been visiting more than once were considered to be involved. During the study period of 2 weeks each at respective health centre, 99 clinic attenders (74.7% patients and 25.3% their companions) were observed. Only 32.3% of total attenders were aware of and noticed HE posters displayed at health centres and read them. Only 26.3% said that they had seen HE activities done at health centres during their presence. Regarding the HE activities performed at health centres, 19.2% were satisfied with the works of health centres and staff, whereas, about 71% had no idea due to their unawareness. Apart from HE talk and poster display, other HE activities were rarely observed. Due to availability during the study period, 12 health care providers could respond to our questionnaire. Their mean service year at the study place was 2.2 years. All respondents were aware of the HE activities performing at their health centres. All of them were found to be satisfied with their own performance, and only 3 (25%) satisfied with their health centre's job.

INTRODUCTION

Individuals and families are most of the important decisions that affect their health, and health-related knowledge and skill are needed to promote their healthy behaviours. Thus, disseminating health education (HE) to reach the grass-root level is essential, so that, it can provide benefits to all deminsions of society, ie. the individual, the family, the community and the nation. Health centres are one of the major sources for disseminating HE by using different health education methods, such as posters, pamphlets, billboards, health talks,

discussion, demonstration, in health centres to obtain the awareness of existing health problems and promotion of health care, and getting people with knowledge, facts, ideas and attitudes they need to make informed decisions about their health.

If these disseminated messages are inclined to be believed and have the positive effects to change their behaviours if necessary, good communication had been taken place and then health education methods are said to be effective [1,2,3].

Objectives

The general objective is to explore the existing situation of effectiveness of health education methods displayed at health centres. The specific objectives are to explore the clinic attendants' awareness of and attitudes toward HE methods displayed at health centres and to identify the utilization pattern of HE methods displayed at health centres by clinic attendants, and to find out the health care provider's awareness of and attitudes toward HE methods displayed at health centres.

MATERIALS AND METHODS

A clinic-based cross-sectional study design was carried out at Hlegu and Kungyangone Townships respectively. These townships were selected purposely due to feasibility and accessibility of transportation.

Clinic attendants (ie. patients and their companions) who visited outpatient departments (OPD) during the study period were included by using convenience sampling. In addition, all health care providers who were dealing with HE were requested to involve in the study.

Clinic attendants who have been visiting more than once during the study period of 2 weeks at each study site were included in the study. Those who had met the following criteria were excluded from the study.

- The subsequent visit(s) of the same attendant during the study period was not considered for the interview.
- Those who visited for the first time on the interviewing day were also excluded, because such a person could have no chance for acquainting with the health centre.
- If either a patient or a companion was a child (ie. 12 years or less), he or she was excluded from inter-

viewing. For a child patient, the respective adult companion was interviewed.

All the eligible respondents were interviewed at the time of exist from the clinic. They were interviewed by the trained interviewers with a set of pretested questionnaire involving awareness of the attitudes toward HE methods displayed at the health centre. Health care providers were interviewed with another set of questionnaire on the last day of the study period. The questionnaire includes only awareness of and attitudes toward HE methods displayed at their health centres. Pretesting was done at Letyetsan rural health centre, Thanlyin Township and at Kyimyindine urban health centre.

RESULTS

1. Clinic attendants' aspect

During the study period of 2 weeks each at the respective health centre, a total of 99 clinic attendants at OPDs (51 from Hlegu and 48 from Kungyangone) were included in the study. Of which 74.7% were patients and 25.3% were their companions. The mean age was 40.11 years with the range of 13 to 76 years and most of them (63.6%) were found to be female. The highest percentage (39.4%) had passed middle school level, followed by lower school level and those who can read and write (18.2% each).

1.1. Awareness of HE methods

Out of the total attendants, only 32.3% were aware of and noticed HE posters displayed at health centres and read them. Of which, 78.1% were patients and 21.9% were their companions. For the HE activities, only 26.3% stated that they had seen the activities done at the health centres. Among them, health talk was found to be the most common exposed HE method at health centres

(69.2%), followed by poster display (30.8%) and video show (26.9%), which were identified by all clinic attendants. None of the companions said they had seen demonstrations and received pamphlets at the health centres (Table 1).

Table 1. Percent distribution of exposed HE methods among who saw HE activities done at health centres*

HE methods	Patient n=21	Companion n=5	Total n=26
Health talk	76.2	40	69.2
Poster display	28.6	40	30.8
Showing video	23.8	40	26.9
Demonstration	9.5	0	7.6
Distributing pamphlets	9.5	0	7.6
ORI corner	4.8	20	7.6

*Responses are more than one for some attendants

1.2. Health messages obtained

The majority (61.6%) of health messages disseminated were found to be delivered by the health staff and 29.3% were from health centres. The messages received from other places rather than health centres and from non health staff were found to be 25.3% and 23.2% respectively. Most of the messages reached directly to the patients and not to the companions from various sources (Table 2).

1.3. Utilization of health messages

It was observed that 75% of those who were aware of and read HE posters utilised the health messages obtained from the health centres. About 53% had stated that they disseminated the health messages to their families, 31% disseminated to others and 28% followed the instructions of health messages. The disseminated health messages were more utilized by patients (59.4% vs 15.6%) (Table 3).

1.4. Preferred HE methods

HE messages depicted with pictures

Table 2. Source of health messages obtained by type of clinic attendants*

Sources	Patients(%) n=74	Companions(%) n=25	Total(%) n=99
Health staff	64.9	52.0	61.6
Health centre	27.03	36.0	29.3
Other places	25.7	24.0	25.3
Non health staff	27.02	12.0	23.2
GP	5.4	8.0	6.1
Others	16.2	16.0	16.2

* Responses are more than one for some attendants

Table 3. Percent distribution of utilization of health messages obtained through HE methods at health centres among clinic attendants who were aware of and read HE posters*

Utilization type	Patients n=25	Companions n=7	Total n=32
Disseminate to family	48	71.4	53.1
Disseminate to others	32	28.6	31.3
Follow the messages	32	14.3	28.1
Memorize the facts	28	14.3	25.0
None	24	28.6	25.0

* Responses are more than one for some attendants

and cartoons were observed to be the most preferred methods of the attendants (37.4% and 32.3% respectively). About 16% of them liked posters with written descriptions. None of the companions mentioned about movie, video, TV, radio and individual talk as their preferences.

1.5. Attitudes toward HE activities performed at health centres

Regarding HE activities done at the health centres, 19.2% said they satis-

fied with the jobs of health centres and the staff, 5% said the staff had done for the sake of patients' health, and 2% expressed their attitudes as it depends on the type of staff's dealing. The majority (ie. 71%) had no idea due to their unawareness. The patients had more positive attitudes than their companions.

1.6. Suggestions for improving HE activities

In order to improve HE activities done at the health centres, 23.2% suggested to do more HE talks alone while 8.1% suggested to perform more HE talks together with the other HE methods. Some 9.1% suggested that staff should visit to every house and disseminate health education. Other suggestions like, to show movie, to organize and supervise by community authorities, to place the posters and billboards at road-side, to distribute pamphlets to everyone were given by another 23.2%. Most of the suggestions were given by patients than by companions (Table 4).

II. Providers' aspect

Due to availability during the study period, 12 health care providers were involved in the study: 2 township medical officers (TMO), 1 medical officer (MO), 1 dentist, 1 health assistant (HA), 2 blue staff, 3 nurses and 2 student nurses. Their mean service year at the study place was 2.2 years (range 6 months to 10 years).

2.1. Awareness and responsibilities of HE activities

All health staff who were dealing with health education, were fully aware of and taken responsibilities for HE activities. All, except a dentist, said they had responsibilities for these activities.

2.2. Performance of HE activities

Nearly all staff had performed more than one activity. Health talk and

Table 4. Suggestions for improving HE activities, given by type of clinic attendants

Suggestions	Patients (%)	Companions (%)	Total (%)
Should do more HE talks	21.6	28	23.2
Staff should visit every house	9.5	8	9.1
Should do more HE talks along with other HE methods	8.1	8	8.1
HE should be disseminated through TV/video	6.8	8	7.1
Staff should talk to to every person at the clinic	6.8	-	5
Community should follow health messages & impart to others	4.1	-	3.03
People should be encouraged to read when visiting clinic	1.4	-	1.01
Others	24.3	20	23.2
Don't know	17.6	28	20.2
Total	74	25	99

demonstration were found to be the activities they performed most (27.3% each).

2.3. Receiving instructions and supervision for HE activities

Among 11 staff who had HE responsibilities, 7 persons (63.3%) knew that there was an instruction for HE activities, whereas 18.2% said there was no instruction and another 18.2% did not know about it. Except MO and HA, all of them were found to be instructed and supervised for HE activities by his or her immediate superior. Instructions received by the health staff were found to be relevant with the nature of their work.

2.4. Attitudes toward HE activities

All staff were found to be satisfied with their own performance of HE activities. When attitudes toward HE activities performed at their health centres were asked, 25% satisfied with the activities and half of them said the emphasis on expansion of HE activities will be needed for those who were not understood about such activities. About 17% said the activities could be managed for the existing patients' magnitude with the existing staff.

2.5. Need for improvement of HE activities

Health care providers felt that giving HE alone is not enough, some kinds of provision should be considered for community and the staff to encourage them to be involved in HE activities, such as, sufficient amount of required medicine, milk powder for the patients and supported HE materials, basic needs of household for the staff. Some of the identified statements for improving of involvement of both community and health care providers, were found to be manageable solutions which can handle at their health centre level, namely, to demonstrate all HE activities of possible, to explain with pictorial to illiterates, to share knowledge among staff, to organize the staff to be involved in such activities.

DISCUSSION

The findings revealed that the percentage of clinic attendants who had awareness of HE posters displayed and HE activities conducted at the health centres were very low. As we have expected, patients were found to be more exposed to these HE methods than their companions by nature: they could express their preferred HE methods and the use of health message, they had positive attitudes toward HE methods done, and could give more suggestions.

But HE methods can only be effective, if the entire community within the jurisdiction of health centre understand the health messages given and willingly accept and change their behaviour. Thus companions of the patients, who are also the members of community, should have the same attitudes and be interested in HE activities. To achieve this goal, health staff should encourage them to read HE posters displayed at health centres and make opportunities to acquaint with HE activities during their visit to the health centres. Clinical attendants gave impression that other HE methods, apart from health talk and poster display, were not very effective.

On the other hand, all health staff who were dealing with HE, were fully aware of and taken responsibilities for HE activities. Nearly all staff had performed more than one activity. Every staff was found to be instructed and supervised for HE activities by his or her immediate superior. Instructions received by the health staff were found to be relevant with the nature of their work. Although all staff were found to be satisfied with their own performance, only 25% satisfied with their health centre's activities.

On the whole, both clinic attendants and health care providers agreed that, not all HE methods displayed at health centres were seemed to reach all community levels. The findings also revealed that, it is not always true about health centres which are considered as the best places for communicating health messages. Health care providers should also consider about the suitable time and places for delivering the health messages needed by the community. This study is an attempt to obtain the information for the health managers in evaluating and implementing the health education materials and methods to be effectively displayed at health centres.

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An epidemiological study of ocular trauma in rural community in Myanmar

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The epidemiological study of ocular trauma in rural setting of Chauk, Meiktila and Bago area was carried out by the ophthalmologists and a team of IC & PBL named as "Model Village Eye Health Survey". The prevalence was 248 cases (1.57 case per 1000). The rate of 0.5 case per 1000 in under 14 years increased to 2.4 per 1000 in 45 to 59 age group. The older age groups were exposed to trauma almost 5 times more than the younger group. The prevalence of ocular trauma was about the same in both sexes 1.8 and 1.4 per 1000 in male and female respectively. 213 persons (85.9%) sustained the injuries in agricultural environment and only 35 (14.2%) were injured by other sources. The number of blunt injury was 93 (37.5%) and injuries by agricultural equipments contributed to 87 cases (35.1%). It was followed by 57 foreign body cases (23%) and others about 4.4%. Cornea was the commonest anatomical site sustaining 64% of injury followed by 12.4% in conjunctiva, 10.8% in the eye ball and 2.8% in the lens. This study illuminates the fact that the strategies to prevent blindness through ocular trauma are to render extensive eye health education down to the communities at the village level and to recruit, train and equip primary eye care workers at least one for each village to deliver primary eye care services and referral throughout the country.

INTRODUCTION

The epidemiological study of ocular trauma has been carried out in rural setting of Meiktila, Chauk and Bago area. Most of the injuries are sustained in agricultural environment. Being a point prevalence study, the exact nature of injury, the initial visual acuity and physical findings could not be presented. The defective vision, corneal opacity, presence of cataract, phthisis, staphyloma and old uveitis found on examination during the randomised morbidity survey are used as indicators of ocular trauma and thus ~~as for~~ of minor injuries might be missed and the actual prevalence under recorded.

Majority of the ocular trauma usually occurs in one eye leading to uniocular

blindness and only occasionally both eyes are involved causing bilateral blindness. Chemical injury and road traffic accidents are such instances.

The tragic outcome of blindness in a large number of cases are due to the lack of primary eye care and timely treatment. Moreover the "harmful eye practice" is an aggravating factor contributing to blindness.

MATERIALS AND METHODS

The 'Model Village Eye Health Survey' programme was carried out to assess the morbidity, to study the impact of the programme and to know the incidence of eye diseases. Moreover this programme aimed at practical training of the TC & PBL, BHS and

and volunteer eye care workers. The survey was undertaken by ophthalmologists and a team of TC & PBL ophthalmic assistants including Health Assistants, Field Workers and Dressers.

The villages were chosen at random representing one per cent of the total number of villages in the township. The personal data of the villagers, visual acuity assessment using pinhole were recorded by the team. History taking and eye examination were carried out by ophthalmologists. The type, cause, site, complication, treatment received and time lapsed before seeking treatment of the ocular trauma were recorded.

RESULTS AND DISCUSSION

Prevalence of eye trauma

A total of 248 persons out of 157,249 examined population have history, signs and sequelae of trauma. Thus the rate of ocular trauma is 0.16% and the distribution in different geographic area is 0.27% in Chauk, 0.12% in Bago and 0.22% in Meiktila. The blindness rate due to trauma is 2% compared to blindness due to cataract 59%, glaucoma 20%, trachoma 7%. The finding of slightly higher prevalence could be due to the nature of agriculture work. Most of the lower Myanmar area represented by Bago usually plant paddy employing mechanical means whereas cultivation of beans, pulses and to a lesser extent paddy is carried out by using traditional methods and appliances seems to favour more eye injuries in Chauk and Meiktila (Figure 1).

Distribution by age group

Although no age group is exempted from ocular trauma, it was found that less trauma occurred up to the age of 14. The trauma usually resulted from playing with catapult, sticks, pens, pencils and stones. A few cases of chemical injuries by lime were recorded as the children played

with plastic packages containing lime which bursted and sprayed the eye while playing. The prevalence rises 3 to 4 folds from an average 0.05 to 0.12 in 15-29 age group. The highest prevalence of 0.73% was found to occur in 45-59 age group in Chauk and it could be said that majority of the injury are found in 30 year and above age group. This may again be due to more exposure to risk factors by advancing age and/or the examiners are finding cumulative trauma signs (Figure 2).

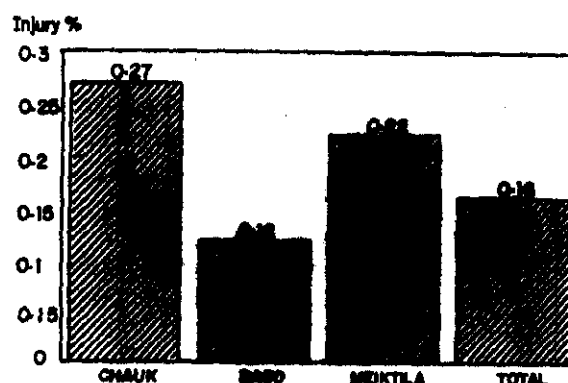


Fig. 1. Prevalence of ocular injury

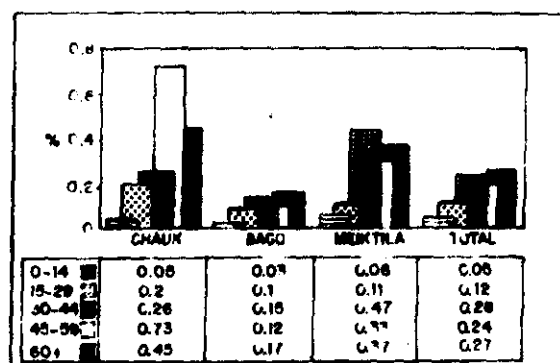


Fig. 2. Ocular injury by age group

Distribution by sex

It was found that 137 males out of 75,608 (0.18%) and 111 females out of 81,648 (0.14%) suffered from ocular trauma respectively. Males were injured more than females as they have to lead an outdoor life and worked and travelled much more than females. At the same time

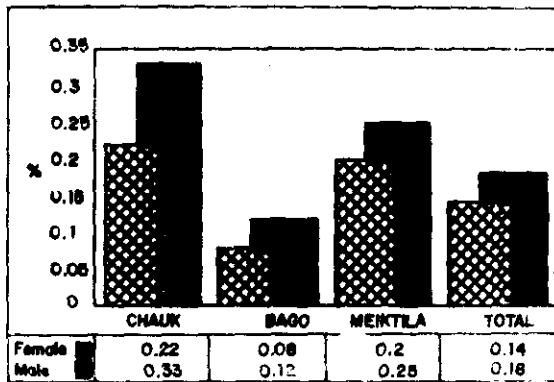


Fig. 3. Ocular injury by sex

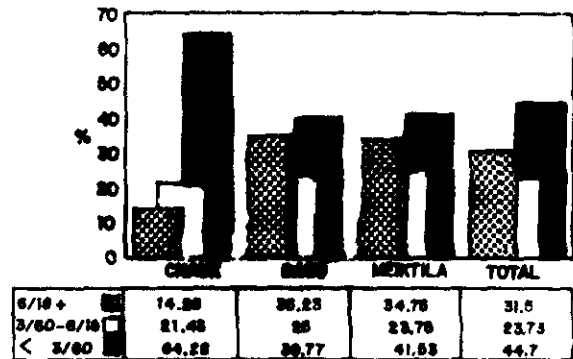


Fig. 6. Final visual acuity

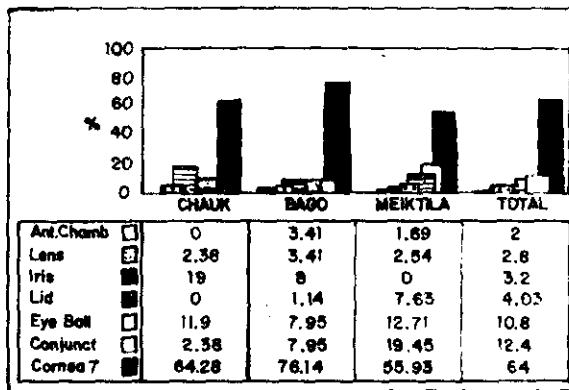


Fig. 4. Ocular injury by site

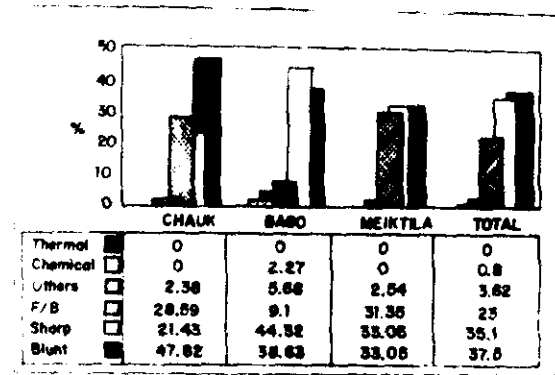


Fig. 7. Nature of injury

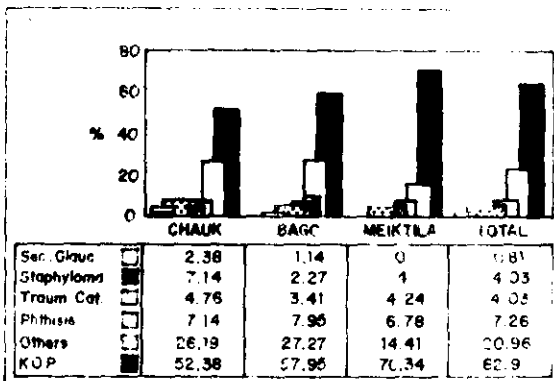


Fig. 5. Ocular injury by sequelae

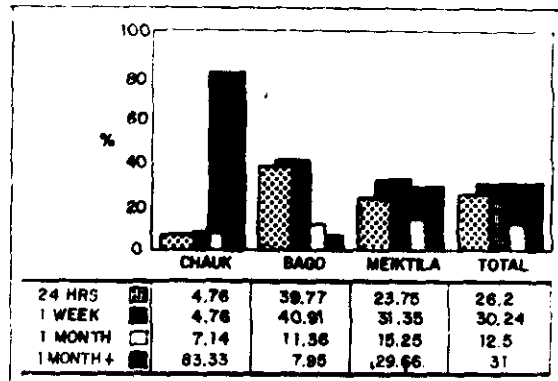


Fig. 8. Time lapsed before coming to hospital

females who now-a-days are working shoulder to shoulder with their opposite sex suffered a little bit less than their counterparts (Figure 3).

Site of injury

The commonest site of injury is cornea 64.0% followed by 12.4% in conjunctiva, 10.8% in the eye-ball,

4.03% in the lid, 3.2% in the iris and 2.8% in the lens. The cornea being the most exposed part of the eye sustains more eye injuries despite protective mechanism of the eye such as blinking and protective anatomical structures such as bony orbit, eye-brow, eye-lashes and eye-lids. The survey pointed out the fact that

only 6% of the injury (iris 3.2% & lens 2.8%) penetrates the eyes and the blindness due to trauma is cornea in origin, corneal ulcer, corneal opacity, phthisis and staphyloma. This situation also calls for better management of corneal injuries by primary health care workers and primary eye care workers who are men on the spot at grass-root level. More should be trained and equipped with appropriate eye ointments and drops for first aid treatment. Health education should be disseminated up to the village level imparting knowledge about how best to take care of common blinding eye conditions (Figure 4).

Outcome of eye injury

About 62.9% of the eye injury ended with corneal opacity and 7.26% with phthisis. There also were cases with staphyloma, traumatic cataract and secondary glaucoma etc. The secondary preventive measures thus become necessary to treat corneal opacities by keratoplasty, an eye bank to supply corneal tissue (Figure 5).

Resultant visual acuity

Nearly half of the injured eyes (44.7%) found are blind with a visual acuity of less than 3/60. Severe visual impairment with a visual acuity of 6/60 or less are found in 23.8% and only 31.5% have a mild to moderate visual impairment.

The visual outcome depends on a lot of factors and to mention a few, eye health knowledge, severity, primary eye care, personal and environmental hygiene, accessibility of proper eye care at affordable cost and availability of required medicine. The high blindness rate in this study could be due to one or a combination of above-mentioned factors (Figure 6).

Nature of injury

Blunt and sharp injuries are at the top of the list contributing to 37.5%

and 35.1% respectively and foreign body with 23.0% trails at the third place. Only 2 out of 248 cases of injury are due to chemical injury. Both the chemical injuries are caused by accidental acid splashing while handling batteries which are commonly used now-a-days as alternative light source in the villages (Figure 7).

Time lapsed before seeking medical treatment

Only 65 patients (26.2%) sought medical treatment within 24 hours whereas the remaining 183 patients (73.8%) took one week or more to do so. The delay in seeking medical treatment might be due to lack of eye health education, lack of fund to go to the eye centre or other health facilities are located very far from the village. The delay in going to the eye centre enhance the patient to become a prey to harmful eye practice, which again leads to blindness. Time lapse may on the other hand depend on the degree of severity of the trauma. Those with severe trauma seeking medical attention more quickly, but the severity again may influence the visual outcome. The patients with minor quickly, but the severity again may influence the visual outcome. The patients with minor trauma though late in seeking medical treatment may escape blindness and severe loss of vision (Figure 8).

CONCLUSION

Ocular trauma and ocular blindness mostly occurred early in life between the productive years of 30-50 age group unlike cataract the majority of which are found later in life. As the blind years from trauma could be longer than that of cataract, it is important that ways and means should be created to improve the environmental factors and health care facilities. The strengthening of primary eye care services by training more primary

eye care workers and equipping them should be regarded as an important strategy in addition to disseminating eye health education down to the grass-root level and improving eye care facilities. The successful management of ocular trauma and corneal ulcer largely depends on easy availability of appropriate anti-microbial, anti-fungal, anti-mycotic drugs etc and reliable laboratory services to perform identification of causal organism and culture and sensitivity.

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**Effectiveness of sodium bisulfite on the stability of Russell's viper
(*Daboia russelli siamensis*) venom toxoid under various storage conditions**

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Crude Russell's viper venom toxoid was prepared by slow and step-wise formalinization to a final concentration of 0.8% formalin. Then 35% sodium bisulfite was added to half volume of the toxoid. Two types of toxoid were stored at different storage temperatures. At two monthly interval, the immunogenicity of these toxoid were tested in mice. The antibody levels in immunized mice were measured by ELISA. The toxoid with sodium bisulfite was more immunogenic than the toxoid without it. Therefore the reversion of formaldehyde linkage during storage was less in toxoid which contained sodium bisulfite.

INTRODUCTION

Russell's viper envenomation is a health problem in Myanmar. It had been treated with specific monovalent antivenom produced by Myanmar Pharmaceutical Factory (MPF), Myanmar. However, there is still a high mortality due to delayed arrival to hospital [1,2]. It was reported that lethal cases of Habu (*Trimeresurus flavoviridis*) snake bite occurred not from inadequate or delayed treatment with antivenom, but from large amount of venom being injected into the victims [3]. The amount of venom was so large that intoxication developed before the victims received proper medical treatment. In such situations, prophylactic active immunization with specific venom toxoid seems to be useful [4]. Many investigators had tried various methods to produce a potent and safe toxoid of Russell's viper venom. Habu (*Trimeresurus flavoviridis*) venom toxoid had been reported to be effectively used in man [5]. Crude Russell's viper venom toxoid had been prepared by slow and step-wise formalinization. The immunogenicity of such toxoid was

reported in experimental animals [6] and in human volunteers [7]. Formalinized cobra venom toxoid kept at 37°C for 20 days showed reversion to toxicity [8]. Free formaldehyde reappeared in Russell's viper venom toxoid after one month of storage at -20°C and after 3 months of storage at 4°C and at room temperature (18°C) [9].

Artemenko and Nenashev [10] reported that there was no reversion of toxicity in toxoid kept under the action of formalin-binding agent (ie. sodium bisulfite). In this study, we tried to observe the action of sodium bisulfite on formalin linkage to have a potent and safe toxoid.

MATERIALS AND METHODS

Toxoidation of venom

Toxoidation of venom was done according to a modified method described by Aung-Khin et al. [11]. To one gram percent of Russell's viper venom solution, dissolved in M/30 phosphate buffered saline (pH 7.0), formaldehyde was initially added to get 0.2% and

increasing concentration by 0.2% at two days interval to obtain a final concentration of 0.8% at day 10. During the process of toxoidation, the solution was kept at 20-22°C and pH was adjusted to 7.0 with 0.1N NaOH. The solution was dialyzed overnight using seamless cellulose tubing against 200 ml of M/30 phosphate buffered saline to take out the excess formaldehyde used in toxoidation of venom. Then 35% sodium bisulfite solution was added to half volume of toxoid at the rate of 0.1 ml of 35% sodium bisulfite per 5 ml of toxoid (0.8% formalinized). The control toxoid (without sodium bisulfite) and sample toxoid (with sodium bisulfite) were stored at two different storage temperatures (ie. 4°C and room temperature, 28°C-30°C).

Immunogenicity test of toxoid

Immunogenicity test was done initially before storage condition (ie. at the time of completion of 0.8% formalinized toxoid). Aluminium phosphate (M/8.45) was added to the toxoid solution as an adjuvant in the ratio of 1:1 by volume. The adsorbed toxoid was then diluted to 1:9 with M/30 phosphate buffered saline. Then 0.1 ml of diluted adsorbed toxoid (containing 0.05 mg protein) was given subcutaneously to a group of mice (n=5) and three booster injections of the same dosage were given at weekly interval commencing three weeks after the first injection [11]. Blood was taken from the heart one week after the third booster injection and the antibody level against toxoid was estimated by indirect enzyme immunoassay technique as described by Tun-Pe et al. [12]. The same immunization schedule [11] was followed for testing of immunogenicity of the toxoid stored for 2 and 4 months (Table 1 & 2).

RESULTS

The immunogenicity of stored toxoid

was determined by its ability to produce antibody in mice after challenging with toxoid as described. The antibody level was expressed in optical density measured at 405 nm wavelength.

At zero month storage, toxoid with sodium bisulfite produced 0.344 absorbance of antibody and toxoid without sodium bisulfite produced 0.472 absorbance of antibody. They were considered as initial antibody level (ie. 100%) (Table 1 & 2).

After two months storage at 4°C, toxoid with sodium bisulfite produced 0.144 absorbance of antibody (ie. 42% of initial antibody level) and toxoid without sodium bisulfite produced 0.154 absorbance of antibody (ie. 33% of initial antibody level) (Table 1).

After 4 months of storage at 4°C, toxoid with sodium bisulfite and toxoid without sodium bisulfite produced 0.186 absorbance of antibody (54% of initial antibody level) and 0.114 absorbance of antibody (24% of initial antibody level) respectively (Table 1).

When the toxoid were stored at room temperature (28-30°C), the toxoid with sodium bisulfite produced, after 2 months of storage, 0.080 absorbance of antibody (23% of initial antibody level) and after 4 months storage, 0.074 absorbance of antibody (22% of initial antibody level). On the other hand, toxoid without sodium bisulfite produced, after 2 months of storage, 0.088 absorbance of antibody (19% of initial antibody level) and after 4 months of storage, 0.062 absorbance of antibody (13% of initial antibody level) (Table 2).

DISCUSSION

The initial antibody level produced by toxoid with sodium bisulfite and toxoid without sodium biosulfite were 0.344 and 0.472 absorbance of antibody

Table 1. Antibody levels produced by two different toxoids stored at 4°C

Duration of storage (month)	Antibody level (expressed in OD ₄₀₅)			
	Toxoid with sod. bisulfite		Toxoid without sod. bisulfite	
	n=5	Mean ($\pm$ SD)	n=5	Mean ($\pm$ SD)
0	0.25		0.52	
	0.38		0.50	
	0.44	0.344 ($\pm$ 0.013)	0.48	0.472 ($\pm$ 0.034)
	0.35	(100%)*	0.31	(100%)*
	0.30		0.55	
2	0.08		0.11	
	0.12		0.19	
	0.21	0.144 ($\pm$ 0.048)	0.17	0.154 ($\pm$ 0.023)
	0.15	(42%)*	0.15	(33%)*
	0.16		0.15	
4	0.18		0.14	
	0.22		0.12	
	0.17	0.186 ($\pm$ 0.024)	0.17	0.114 ($\pm$ 0.046)
	0.16	(54%)*	0.09	(24%)*
	0.20		0.05	

* Percentage of initial antibody level

Table 2. Antibody levels produced by two different toxoids stored at room temperature (28-30°C)

Duration of storage (month)	Antibody level (expressed in OD ₄₀₅)			
	Toxoid with sod. bisulfite		Toxoid without sod. bisulfite	
	n=5	Mean ($\pm$ SD)	n=5	Mean ($\pm$ SD)
0	0.25		0.52	
	0.38		0.50	
	0.44	0.344 ($\pm$ 0.013)	0.48	0.472 ($\pm$ 0.034)
	0.35	(100%)*	0.31	(100%)*
	0.30		0.55	
2	0.10		0.07	
	0.05		0.07	
	0.04	0.080 ($\pm$ 0.033)	0.10	0.088 ($\pm$ 0.012)
	0.09	(23%)*	0.11	(19%)*
	0.12		0.09	
4	0.07		0.10	
	0.06		0.06	
	0.08	0.074 ($\pm$ 0.011)	0.06	0.062 ($\pm$ 0.023)
	0.09	(22%)*	0.04	(13%)*
	0.07		0.05	

* Percentage of initial antibody level

respectively. They were regarded as the initial antibody level (100%) at the beginning of storage time (ie. zero month storage).

After two months of storage time, at 4°C, the toxoid with sodium bisulfite produced more antibody (42% of initial antibody level) than the toxoid without sodium bisulfite (33% of initial antibody level). As the duration of storage became longer (at 4 months of storage time, at 4°C), the toxoid without sodium bisulfite produced less antibody level (only 24% of initial antibody level) as compared with 54% of initial antibody level produced by toxoid with sodium bisulfite.

Similarly, the toxoid with sodium bisulfite also produced more antibody level than the toxoid without sodium bisulfite, when it was stored at room temperature (Table 2).

Regarding storage temperature, the toxoid with sodium bisulfite stored at 4°C produced more antibody level (42% & 54% of initial antibody level) than the same toxoid stored at room temperature (23% & 22% of initial antibody level).

Therefore, it was found that toxoid with sodium bisulfite preparation was more immunogenic than the toxoid alone and more stable when stored at 4°C.

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Comparison of lactose breath hydrogen test and milk breath hydrogen test in the diagnosis of lactose malabsorption

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In both clinical and field situations, breath hydrogen analysis has been widely used in evaluating lactose malabsorption. It is as accurate as other tolerance tests and has an advantage of being noninvasive. The use of milk (360 ml) as a test meal for breath hydrogen test was evaluated in 16 adult subjects (8M:8F), age ranging from 20 to 50 years. Lactose (17.6 g) breath hydrogen test was used as standard test. Of 16 subjects tested, 5 subjects were found to be lactose absorbers and 11 subjects were lactose malabsorbers by lactose breath hydrogen test. In milk breath hydrogen test, the same 5 subjects were found to be lactose absorbers and 8 out of 11 subjects (lactose malabsorbers) were detected as lactose malabsorbers. The present study demonstrates that breath hydrogen test using milk (360 ml) as a test meal has a sensitivity of 73% and a specificity of 100% and could be used as an alternative test in the diagnosis of lactose malabsorption.

INTRODUCTION

The principle that the colonic fermentation of incompletely absorbed carbohydrates results in increased excretion of breath hydrogen has led to the development of noninvasive procedures for diagnosing carbohydrate malabsorption. Through the pioneering work done by Calloway and associates in the past decade the breath hydrogen method has been widely used clinically in the study of lactose intolerance [1]. Originally the dose of substrate has been 50 g of lactose which is equivalent to 1000 ml of milk. Moreover that dose can induce severe abdominal pain and diarrhoea in some lactose intolerant persons. Since the breath hydrogen testing has been demonstrated as sufficiently sensitive to detect the malabsorption of as little as 2 g of incompletely absorbed carbohydrate [2], the appli-

cation of oral lactose test meal doses in the range that one would consume in an ordinary meal i.e. 10 g to 15 g has been advocated [3] and recently the application of normal food sources of lactose (milk) instead of pure lactose aqueous solution becomes increasingly popular. However, a reduced rate of hydrogen production, has been demonstrated clearly by comparing lactose in whole milk with aqueous lactose in preschool children and in older children [4,5]. Therefore, the present investigation is aimed to compare lactose breath hydrogen test and milk breath hydrogen test in the diagnosis of lactose malabsorption in Myanmar adults.

MATERIALS AND METHODS

Subjects

The study involved 16 adults of both

sexes (8 males and 8 females) between 20 to 50 years of age from the Department of Medical Research. All were apparently healthy, had not taken any antibiotics and no gastrointestinal diseases during previous 2 weeks. The informed verbal consent was taken from each subject. Smoking was prohibited during the test.

Breath collection and analysis

Lactose breath hydrogen test was carried out as follows. After overnight fast (asking the subjects to take the last meal at 10:00 p.m and the test was conducted at 8:00 a.m on the next morning), each subject was given lactose 17.6 g dissolved in 360 ml of water. Breath samples were taken immediately before giving lactose and every 15 minutes for 1 hour and every 30 minutes for another 3 hours. The subject was asked to breathe once into a collecting bag. To ensure that we obtain end expiratory breath samples, we used T valve breath sampling plastic bags which allow expired air from the dead space to be discarded. The samples were immediately transferred to 30 ml airtight plastic syringes and measured hydrogen and oxygen within 24 hours. The subjects were monitored for evidence of symptoms of lactose intolerance for 24 hours.

Milk breath hydrogen test was carried out as the same procedures as above but commercial cow's milk powder 46.8 g (containing 17.6 g lactose) dissolved in 360 ml of water was used as a test meal instead of aqueous lactose solution. It was done on all the subjects two days after the lactose breath hydrogen test.

Analysis of hydrogen in the breath samples and normalization of hydrogen concentrations were carried out as methods described by Khin Maung U et al. [6]. Quintron microlyzer was used for measuring hydrogen in breath samples. A portable oxygen analyzer (Oxywarm 100 I) was used to measure oxygen.

Symptom score

The subjects were asked to record the presence or absence of the 3 symptoms: flatulence, meteorism and/or diarrhoea experienced during the test period.

Carbohydrate dose

For lactose (aqueous) solution, 17.6 g of lactose was dissolved in 360 ml of water. For milk (test meal), commercial whole cow's milk (Nespray full cream milk) 46.8 g was dissolved in 360 ml of water. Water was permitted *ad libitum* throughout the study.

Working definitions

Lactose intolerance - was defined as any symptoms (flatulence, meteorism and/or diarrhoea) which appear after the ingestion of a test dose of lactose in water.

Lactose malabsorption - A peak hydrogen concentration >20 ppm above baseline values in the 90, 120, 150, 180 or 240 min sample after a lactose test meal.

Statistical analysis

Data were expressed as mean $\pm$ SD. Sensitivity and specificity of milk breath hydrogen test were calculated using formula of validity of a screening test [7].

RESULTS

Table 1 shows that out of 16 subjects tested, 5 subjects were found to be lactose absorbers by both tests. Out of 11 lactose malabsorbers detected by lactose breath hydrogen test, only 8 persons were shown to be lactose malabsorbers by milk breath hydrogen test which has a sensitivity of 72.73% and a specificity of 100%.

Figure 1 shows that breath hydrogen concentrations were found to be lower in milk breath hydrogen test. Breath hydrogen concentration was found to rise from 0.5 hours and

Table 1. Sensitivity and specificity of milk breath hydrogen test

		LBH	
		positive	negative
MBH	positive	8	0
	negative	3	5

Note: LBH = Lactose Breath Hydrogen test

MBH = Milk Breath Hydrogen test

Positive = lactose malabsorber

Negative = Lactose absorber

Sensitivity = 72.73%

Specificity = 100%

Positive predictive value = 100%

Negative predictive value = 62.5%

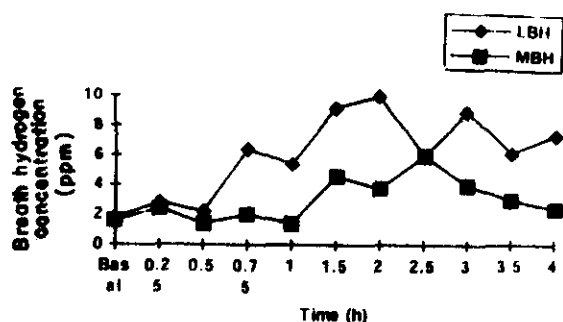


Fig. 1. Comparison of mean breath hydrogen concentrations determined by lactose breath hydrogen test and milk breath hydrogen test at various time intervals in lactose absorbers

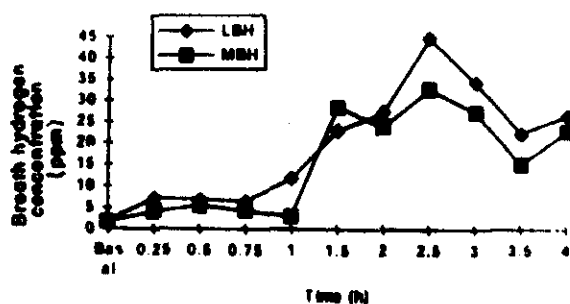


Fig. 2. Comparison of mean breath hydrogen concentrations determined by lactose breath hydrogen test and milk breath hydrogen test at various time intervals in lactose malabsorbers

reach the peak at 2.5 to 3 hours and

and decline at 3.5 hours after lactose loading in lactose absorbers.

Figure 2 shows that the two curves are more or less similar in pattern and breath hydrogen concentrations were found not much different from each other. Breath hydrogen concentration was found to rise from 1.0 hour and reach the peak at 2.5 hours and decline at 3.0 hours after lactose loading in lactose malabsorbers.

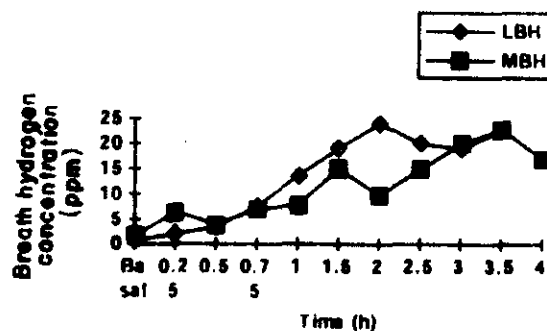


Fig. 3. Comparison of mean hydrogen concentrations determined by lactose breath hydrogen test and milk breath hydrogen test at various time intervals in lactose intolerance

Figure 3 also shows that the two curves show more or less similar pattern but the time required to reach peak breath hydrogen concentration was different. Breath hydrogen concentration was found to rise from 0.5 hours and fall down at 3.5 hours after lactose loading in lactose intolerance.

Table 2 shows that there was some delay in time interval to reach peak breath hydrogen concentration by milk breath hydrogen test than lactose breath hydrogen test.

Table 3 shows that peak breath hydrogen concentration values measured by lactose breath hydrogen test were found to be higher than those values by milk breath hydrogen test in lactose absorbers, malabsorbers and lactose intolerant persons.

Table 2. Comparison of time required to reach peak breath hydrogen concentration after lactose loading between lactose breath hydrogen test and milk breath hydrogen test

	Lactose breath hydrogen test (min)	Milk breath hydrogen test (min)
Lactose absorber	156.00±32.86	156.00±44.49
Lactose malabsorber	157.50±34.95	168.75±50.55
Lactose intolerance	120.00±30.00	200.00±17.32

Table 3. Comparison of peak breath hydrogen concentration above basal level between lactose breath hydrogen test and milk breath hydrogen test

	Lactose breath hydrogen test (ppm)	Milk breath hydrogen test (ppm)
Lactose absorber	11.63±6.33	8.12±6.11
Lactose malabsorber	50.52±37.53	41.82±36.02
Lactose intolerance	25.40±5.39	23.80±3.53

DISCUSSION

The breath hydrogen test is the most valid indirect method of detecting lactose malabsorption being 100% specific and 100% sensitive [8]. Therefore lactose breath hydrogen test was used as a standard test in this study. All subjects in this study were hydrogen producers proved by breath hydrogen test with lactulose meal.

In the present study, 5 out of 16 subjects tested (31.3%) were found to be lactose absorbers by both tests. Of 11 subjects identified as lactose malabsorbers by lactose breath hydrogen test, 8 were found to be

lactose malabsorbers as determined by milk breath hydrogen test. Therefore, milk breath hydrogen test has a sensitivity of 73% and a specificity of 100%.

As shown in figure 2 and 3 the mean breath hydrogen concentration at various time intervals after lactose in aqueous solution and that after milk meal changed more or less in similar pattern in lactose malabsorbers and lactose intolerant persons. Mean breath hydrogen concentrations above basal level appeared to be greater with lactose breath hydrogen test than those with milk breath hydrogen test but the difference seemed not to be statistically significant. Since there was the wide range of hydrogen production by its nature among any individual, the results could not possibly avoid high SD values. These findings were in agreement with Calloway et al. [1] and Solomon et al. [9] who showed that little difference in hydrogen excretion seen with lactose malabsorption from aqueous solution or from milk in adults. There was also time delay to reach the peak hydrogen concentration in lactose malabsorbers and lactose intolerant subjects tested with milk (milk breath hydrogen test) on comparison with lactose aqueous solution (lactose breath hydrogen test). These findings might be due to retardation of gastric emptying by fat and protein contents of milk leading to decreased rate of delivery of unabsorbed lactose to the colon. The slower delivery of the substrates increases the time of contact between lactose and available mucosal lactase resulting in real increase in hydrolysis and absorption. Another reason might be due to the presence of other milk components that may interfere with the normal bacteria fermentation of lactose.

Although Ladas et al. [10] showed that mean breath hydrogen concentration had positive correlation with severity of lactose intolerance, the

present study showed that the mean peak hydrogen concentration of lactose intolerant persons (with diarrhoea) was less than that of lactose malabsorbers. This might be probably due to increased colonic activity in diarrhoea which could reduce the time for bacteria fermentation of lactose and absorption of hydrogen produced.

Conclusion

The present study demonstrates that commercial milk powder dissolved in 360 ml of water (containing 17.6 g lactose) could be used as an alternative test meal in the diagnosis of lactose malabsorption.

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The usefulness of *Moringa oleifera* (dan-da-lun) seed for the sedimentation and decontamination of turbid water from ponds and rivers

Part I: Laboratory investigations

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The seeds of *Moringa oleifera* were tested as the clearing and sedimentation agents in turbid water from various sources (1) DMR pond (2) Yangoon River (3) Bago River (4) Hline River (5) Hlinethaya Pond (6) artesian well from Laydaunkkan and (7) Thanlyin River. The turbidity reduction of 80.00 to 99.5 percent paralleled by a primary bacterial reduction of 90.00 to 99.99 per cent were obtained within the first one to two hours of treatment, the bacteria being concentrated in the coagulated sediment. It was observed that 100 mg (one ywe) of the seed powder served as a clearing agent in one litre of turbid water. However, the difference of clearing activity was not significant with different amounts of powder, when 50 mg, 100 mg, 150 mg and 200 mg respectively were used with constant water quantity (one litre) within 24 hours. Moreover, the effect of the seeds could not alter the original pH of the tested water samples. The powder of *M. oleifera* leaves, bark and roots had no power to clear the turbidity of tested water samples.

INTRODUCTION

Surface waters from rivers, ponds, water holes or shallow wells are still the main water source for rural areas in tropical countries. These types of water usually become muddy and turbid during the rainy season and it may be turbid throughout the year. For these reasons it is evident that there is a need for people from those areas to have simple, quick and inexpensive method for water purification, which is effective, simple, quick, low in costs and suited for human use. *Moringa oleifera* Lam is a native tree of India. It was introduced to the Northern Sudan by the British as an ornamental tree. It has been noted that in some parts of Africa, *Moringa oleifera* seeds are effective in water coagulation and is

traditionally used as turbidity reduction and removable of faecal indicator bacteria as well as selected enteric bacterial pathogens. A turbidity reduction of 80.0-99.5 per cent paralleled by a primary bacterial reduction of 1-4 log units (90.00-99.99 per cent) was obtained within the first 1 to 2 hours of treatment. The bacteria were observed to be concentrated in the coagulated sediment [1]. The coagulating activity of *Moringa* seeds has been ascribed to polypeptides acting as cationic polymers [2]. Therefore, this study was conducted to determine the decontamination effect of *Moringa oleifera* seeds on water from various sources.

MATERIALS AND METHODS

Sedimentation effect of Moringa

oleifera seeds on turbid water from various water sources

The seeds of *Moringa oleifera* were tested as the sedimentation agents on turbid water from various sources (a) Ngamoeyeik River (b) Yangon River (c) Bago River (d) Hline River (e) Hlinethaya Pond and (f) an artesian well from Laydaunkkan. The experiments were repeated at least three times. Various amount of water and different contents of *Moringa oleifera* seeds were tested in different experiments.

The turbidity of water was measured by a Spectrophotometer, Hitachi 100-50. Total bacterial count was done according to the method described by Wright [3].

Bacteriological examination

Bacterial contamination and total coliform count of water was assessed according to the method of World Health Organization [4,5].

RESULTS

Clearing of water by Moringa oleifera seeds

Figure 1 represents the sedimentation of water by *Moringa oleifera* seeds in the laboratory. Water samples were collected from (a) an artesian well from Laydaunkkan (b) Yangon River (c) Hlinethaya Pond and (d) Ngamoeyeik Creek. The reduction of turbidity was observed in samples of water with various turbidity according to different sources. The maximum turbidity reduction was obtained within four hours after treatment with *Moringa oleifera* seed powder. The tap water quality was obtained after treating different types of turbid water with the seed powder.

One litre of turbid water from Yangon River treated with different amount of powder viz. 50 mg, 100 mg, 150 mg and 200 mg respectively and it was found that there was no difference in the turbidity reduction (Fig.

2a). The optical density of water before treatment was 1.04. The optical density was measured after 30 minutes of treatment and then followed hourly up to 9 hours and then measured again in 20 hours. The optical density decreased to 0.13 after 30 minutes treatment. The pH had not changed throughout the experiment. It was also observed that the untreated water remained turbid up to 20 hours. The turbidity reduction markedly decreased after one hour of treatment. As little as 50 mg of *Moringa oleifera* seed powder was found to have the activity to clear one litre of turbid water.

The optical density of Bago River water decreased from 1.00 to 0.06 after sedimentation with *Moringa oleifera* (Fig. 2b). Different amount of water (one litre, two litres, five litres and ten litres) were treated with different amount of seed powder (100 mg, 200 mg, 500 mg and one gram respectively). Samples were tested in one, two, three, six and 20 hours after treatment. The pH also did not alter by the effect of seed powder. *Moringa oleifera* seed powder when used with proportionate amount of water also showed clearing activity. However, the untreated water did not reached tap water quality up to 20 hours. The similar results were obtained with waters from Yangon River and Hline River (Figure 2c and 2d).

Total bacterial counts after treated with *Moringa oleifera* seed powder are shown in Figure 3. Water samples were tested hours up to six hours and then at 20 hours and 48 hours after treatment. It was observed that total bacterial count decreased hourly, but increased again after 48 hours. Although the seed had the activity to clear the water and allowed to sediment the bacteria within six hours of treatment, it is not advisable to keep the treated water for more than 20 hours because the bacteria growth increased again

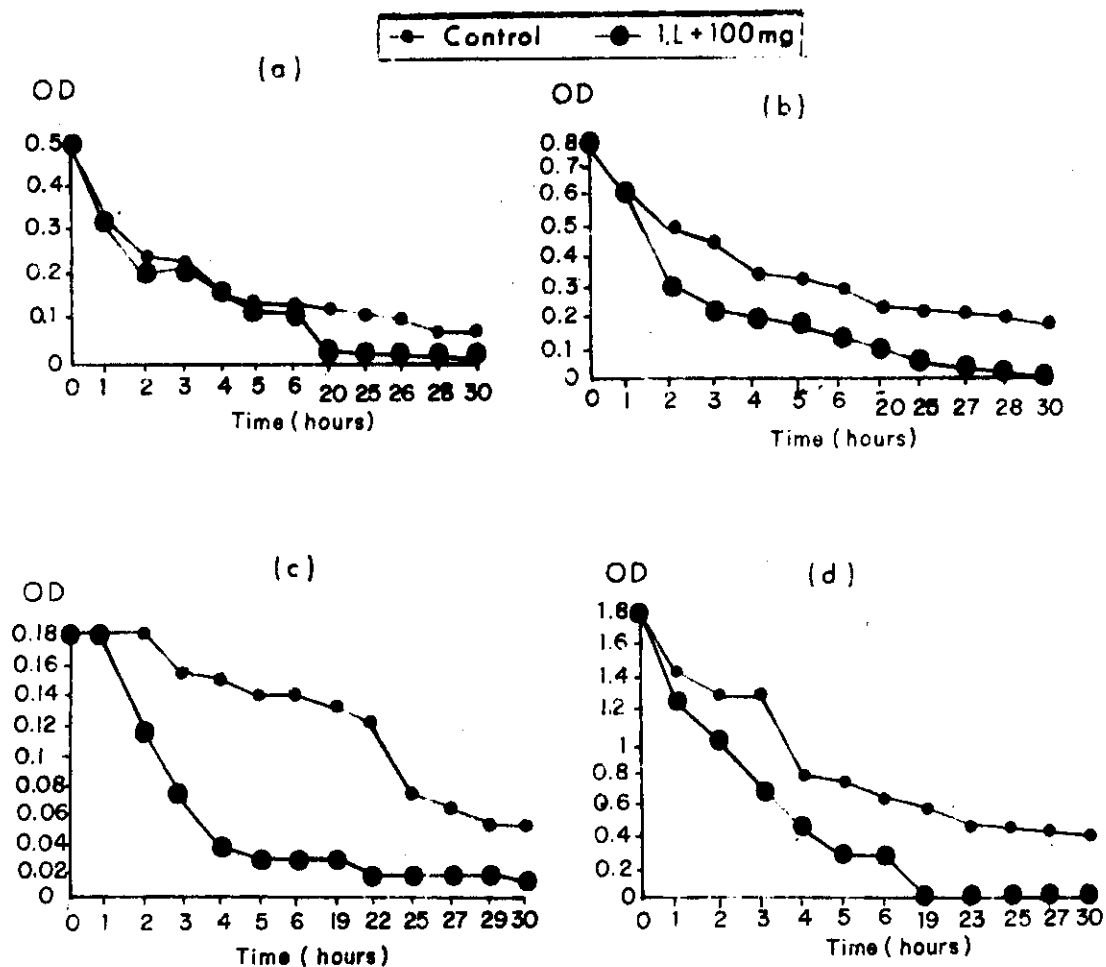


Fig. 1. Sedimentation effect of *Moringa oleifera* seeds on turbid water from various sources
 (a) Artesian well from Laydaungkan
 (b) Yangon River
 (c) Hlinethaya Pond
 (d) Ngamoeyeik Creek

after 20 hours. The initial bacterial count before treatment with *Moringa oleifera* was 6.6×10^4 and it decreased to 2.8×10^4 after one hour treatment. After six hours of treatment, it decreased to 3×10^3 and to 3.3×10^2 after 20 hours of treatment. However if the treated water was kept up to 48 hours, the total bacterial count increased to 2×10^3 organisms per milliliter of water.

Similar findings were recorded when waters from (a) Yangon River (b) Hlinethaya Pond (c) Ngamoeyeik Creek and (d) an artesian well from Laydaungkan were tested. The reduc-

tion of total coliforms and faecal coliforms was observed in all the water tested which originally had high density of bacterial count (uncountable). The total bacterial from its original count (uncountable/100 ml) decreased to (35 organism/100 ml) after 4 hrs treatment in water samples from Yangon river. Moreover, after 2,3,4 hours and overnight treatment, the total coliform decreased to 70,50,35 and 60 organisms per 100 ml respectively and increased to uncountable organisms/100 ml after keeping for 36 hrs. The faecal coliforms counts were also decreased.

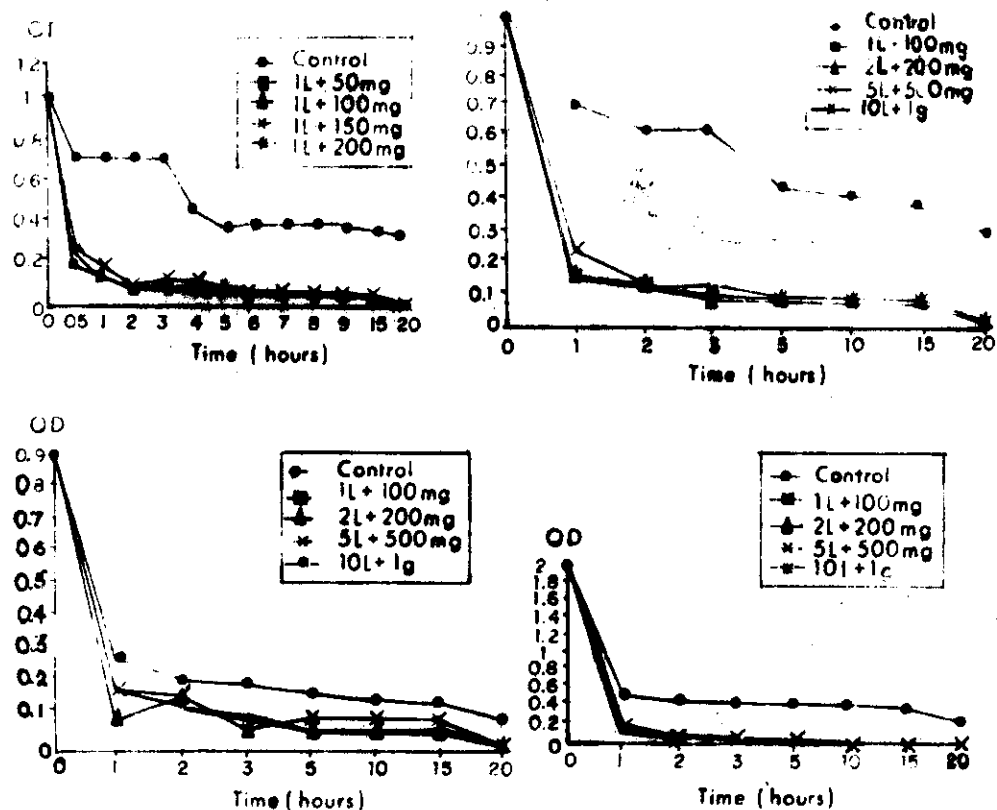


Fig. 2. Effect of different concentrations of *Moringa oleifera* seed powder on the sedimentation of turbid water from various sources
(a) Yangon River (c) Mline River
(b) Bago River (d) Yangon River

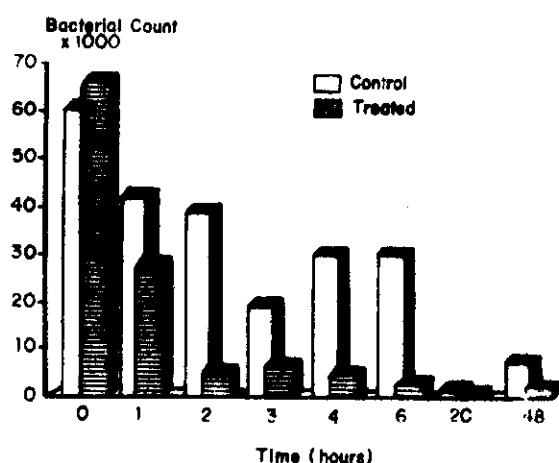


Fig. 3. Total bacterial counts after treating the water samples with *Moringa oleifera* seed powder

Similar findings were recorded with the remaining water samples from various sources. Although the bark powder of *Moringa oleifera* had no activity to clear turbid water, it showed the activity to decrease the total coliforms and faecal coliforms. The dried powdered forms of bark, leaves and roots were tested for the sedimentation effect on turbid water from Laydaungkan. In contrast to the effect of *Moringa oleifera* seed powder, all the tested agents had no activity to clear the turbidity of the tested water samples. The turbidity reduction was measured up to six hours and continuously measured again from 19 to 30 hours in all three agents. In all the tested cases

there was no reduction of turbidity, only making water more dirty. When the effect of bark and leaves of Moringa oleifera was tested on the bacterial content of water samples, reduction of bacteria was observed in day one but the organisms were increased in day three. In case of root powder, there was a reduction of faecal bacteria in day one and day two.

DISCUSSION

Surface water from rivers, ponds, water holes or shallow wells is still the main water source for rural areas in tropical countries. This type water of usually becomes muddy during the rainy season or it may be turbid throughout the year. Therefore it is essential for people from those areas to have a method for water purification, which is effective, simple, quick, low in costs and suited for human use. Natural local coagulants, which are able to settle the turbidity of water, would be a matter of first choice [6].

This tree Moringa oleifera Lam is a native of India [7] and was introduced to the Northern Sudan by the British as ornamental tree [8]. The flocculation properties of the seeds of Moringa oleifera and Moringa stenopetala, a wild species from Kenya were reported in 1979 [9]. Additional experiments on the acute toxicity in mice with Moringa oleifera seeds revealed no specific effect on animals, although a high dose (5 g/kg) was applied [10]. To estimate a possible health risk for humans, it was reported that there were no toxic effects and no histopathologic changes in 28 organs of rats after 6 weeks application of powdered seeds from Moringa oleifera and Moringa stenopetala [11]. The plants also possessed antibacterial activity [12,13].

It was clearly demonstrated that only

the seeds of Moringa oleifera possess the clearing substance whereas the bark, stem and roots did not neither in the wet form nor in the dried state. The optical densities of waters from Yangon river, Bago river and Hline river dropped to zero from 1.98, 0.98 and 0.84 respectively after overnight treatment with the dried mature seed powder. It was also noted that in general turbidity reduced to 10-fold times within one hour and to 20-folds within 5 hours of treatment. The total bacterial count also decreased up to 10-15-fold within 4 hours. From various experiments conducting in the laboratory it was advisable to treat water within 4 hours and the cleared water could be decanted and use for general purposes. However, there was a secondary growth of bacteria in the supernatant water after 24 hours treatment and this finding agrees with that of Madsen et al. [1].

From the present study it was observed that 100 mg of the seed powder which is equivalent to one ywe in Myanmar weight could clarify one litre of turbid water. For one gallon of water approximately one seed will be enough to be used as a clearing agent. Moreover the advantages of Moringa oleifera seeds were (i) no change in pH value of water up to 3 days of storage at 25°C, (ii) growing wild in most parts of Myanmar, (iii) very easy change into powder form by using kitchen utensils, and (iv) no toxicity and getting ready acceptance of many people as they are well-known edible seeds. The procedure for treatment of water is also very simple.

From these experiments it was noted that though the seeds had a sedimentation effect on turbid water, the powdered dried leaves, flowers and bark had no effect. However, the bark powder showed some activity to inhibit the total coliforms and faecal coliforms.

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In-vitro nitric oxide (NO) production of murine cell line and human monocyte-derived macrophages after cytokine stimulation and activation

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Nitrite (NO₂) and nitrate (NO₃) production were determined using J774-murine cell line and human monocyte-derived macrophages after activation of these cells with Interferon gamma (IFN-γ), Lipopolysaccharides (LPS) and Tumor Necrotic Factor α (TNF-α) in different concentration and in different combination. Both simple colorimetric assay and chemiluminescence assay were used for comparison. Simple colorimetric assay could detect nitrite produced by J774 cells only. However, with chemiluminescence assay nitrite production by both J774 cells and human monocyte-derived macrophages could be measured. This study was aimed to investigate a possible potent killing mechanism of monocytic cells in infection with intracellular microorganisms, particularly mycobacterium.

INTRODUCTION

Nitric oxide (NO) is a short-lived gaseous radical with potent multifunctional activities; like neurotransmission, immunosuppression, vasodilation, blocking of cellular adhesion and in host defense mechanism. It is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS), with the formation of citrulline and NO. NO has a very short half-life and is unstable. It converts into either nitrite (NO₂) or nitrate (NO₃) which are the more stable end products. Thus, measurement of these end products serve as crude, yet reliable quantitative indicators of NO production [1]. Experiments have proved that murine macrophages can be stimulated for NO release by several agents: including cytokines, microbial products and tumor cells [2,3]. Rodent macrophages possess inducible nitric oxide synthase (iNOS) [4], but it is controversial whether this enzyme exists in human mononuclear phagocytes or not [5-9]. Moreover, NO has been implicated in the killing activity of macrophages against many intracellular pathogens including

Toxoplasma gondii, Chlamydia psittaci, Leishmania donovani, L. major and Schistosoma mansoni [10]. We are interested in the mycobactericidal activity of NO and as a preliminary study, we are trying to detect NO production from murine and human macrophages after activating them with various cytokines. Tuberculosis due to intracellular mycobacterium infection is an important health problem in Myanmar, ranking second position, next to malaria in National Health Plan [11]. Therefore, it will be useful if we can measure NO production from murine and human macrophages that are infected with mycobacterium for a better understanding of its pathogenesis and immunity against the infection.

Aim and objective

To detect NO production by murine and human macrophages after cytokine stimulation with various agents.

MATERIALS AND METHODS

Culture of cells

- (i) J774 murine cell line

J774 was grown in Eagles medium (GIBCO) supplemented with 10% foetal calf serum (FCS) in 25 ml plastic tissue culture flasks at a concentration of $2-10 \times 10^5/\text{ml}$ at 37°C in a 5% CO_2 incubator. Cell viability was determined by trypan blue exclusion.

(ii) Human monocyte-derived macrophages

These were obtained from healthy blood donors. Peripheral blood was collected in heparinized tubes and processed within 60 minutes of drawing. Separation of monolayer was done by Ficoll-Hypaque separation. Cells were resuspended in RPMI-1640 + glutamine + 10% heat inactivated AB + human serum. Cell suspension of $2.5 \times 10^6/\text{ml}$ was used.

Cytokine stimulation and activation

Both murine and human monocyte-derived macrophages were cultured in 24-wells tissue culture plates for 7 days prior to the addition of following agents of $\text{IFN-}\gamma$ (50U/ml), LPS (3 $\mu\text{g}/\text{ml}$), $\text{TNF-}\alpha$ (50U/ml), $\text{IFN-}\gamma$ + LPS and $\text{IFN-}\gamma$ + $\text{TNF-}\alpha$ (all from the Institute of Biological Standard). Cell culture without any prior activation was included as control.

Detection of nitrite production

Nitrite production was measured in culture supernatants after 48 hr of incubation.

(i) Colorimetric assay

50 μl of supernatant solution was mixed with 100 μl of freshly prepared Greiss reagent (i.e. 1% sulphanilamide in D/W and 0.1% N-1 naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid in D/W) (all from Sigma), for 5 minutes at room temperature in day light and read with ELISA reader (Titertek MultiskanR MCC) at 540 nm. Greiss reagent + D/W was used as blank. A standard graph was plotted with NaNO_2 (sodium nitrite) at different concentrations in $\mu\text{g}/\text{ml}$.

(ii) Chemiluminescence assay

In this assay chemiluminescence is measured by Sievers NOA 270B Nitric Oxide Analyser (Boulder, Colorado, USA); an inert gas purge vessel for biological fluids [12,13]. This method is based on the gas phase reaction between NO and ozone. Emission from electronically excited nitrogen dioxide is in the red and near infrared region of the spectrum and is detected by a thermoelectrically cooled, red-sensitive photomultiplier tube. Sodium iodide (Sigma) and strong glacial acetic acid (Sigma) were added in the purge vessel to allow complete liberation of nitrite from the added sample. By reducing dissolved nitrite to NO in a nitrogen purge vessel NO gas was liberated.

RESULTS

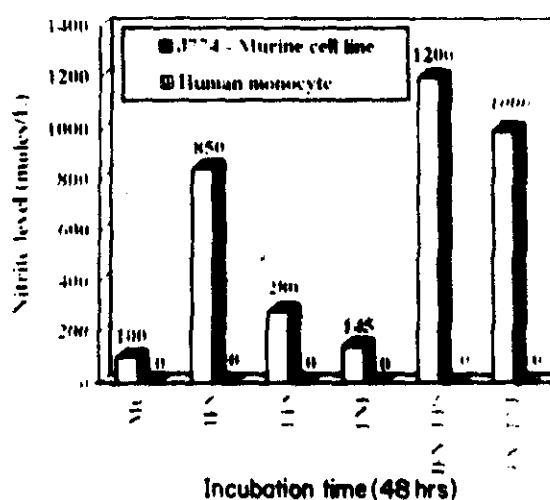


Fig. 1. Nitrite production from treated J774 cells and human monocyte with colorimetric assay

Fig. 1 shows nitrite production in treated J774 cells after 48 hrs of incubation. Only the murine J774 cells had detectable nitrite production following activation with $\text{IFN-}\gamma$, $\text{TNF-}\alpha$ and LPS alone and in combination. It was noted that stimulation with $\text{TNF-}\alpha$ alone had minimum

nitrite production and a marked production was observed in IFN- γ + TNF- α and IFN- γ + LPS combination. However, nitrite production was undetectable in human-macrophages with this method.

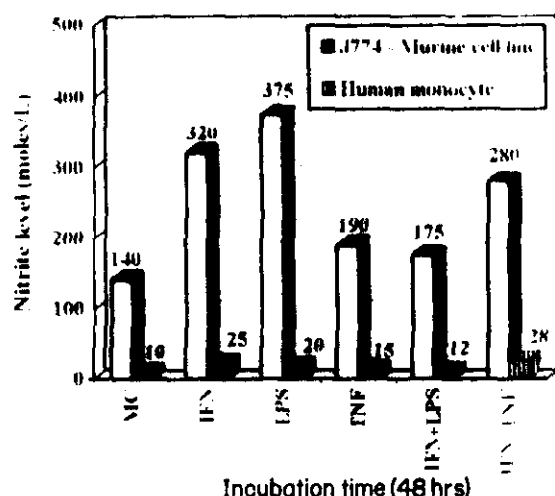


Fig. 2. Nitrite production from treated J774 cells and human monocyte with chemiluminescence assay

Fig. 2 shows detection of nitrite from both J774 and human macrophages with chemiluminescence assay. With this method, a higher level of nitrite was produced by J774 cells following treatment which was most prominent with IFN- γ + LPS in combination. In contrast, human macrophages produced much lower levels of nitrite compared to J774, but they were still easily detectable and well within the sensitivity range of the assay.

DISCUSSION

From the results, it was observed that both J774 and human macrophages had nitrite production after cytokine activation. However, it was undetectable for the human macrophages with colorimetric assay. Thus, measurement of nitrite by chemiluminescence assay was much more sensitive than the former assay, being able to detect nitrite up to 10 moles/L.

However, this is only one set of result obtained from a single experiment and therefore requires repetition. For future studies, it is recommended that all nitrite assays be done by chemiluminescence. Failure to detect nitrite production from human macrophages may be due to lack of sensitivity of the first assay.

The results obtained in this study however would suggest that there is production of NO from human macrophages which is detectable only with sensitive assay method and such pathway of RNI (Reactive Nitrogen Intermediates) might be an effective and potent pathway of antimycobactericidal effect.

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Snake bite in Loikaw with special reference to Malayan pit viper
(Calloselasma rhodostoma) bite

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A retrospective study of 38 snakebite cases admitted to Loikaw Divisional Hospital during 1995-96 was carried out. Twenty four were bitten by green pit viper (GPV) (Trimeresurus erythrurus), 13 Russell's viper (Daboia russelli siamensis) and one attributed to Malayan pit viper (Calloselasma rhodostoma). The median age of the victim is 21 year (5-66 yr) and 66% of them are less than 30 years old. It took 11.20 hours (0.30 to 97 h) to get to the hospital. Lower limbs (12/13) were predominantly bitten in Russell's viper bite and both upper and lower limbs were bitten in Tr. erythrurus bite. Day bite is 4 times more common than after dark bite. Clinical features of snakebites of both species are similar to those reported ones except spontaneous bleeding: bleeding gums 38.5% (5/13) and epistaxis 23% (3/13) are common in Russell's viper bites. One C. rhodostoma bite confirmed by immunodiagnosis, presented with incoagulable blood developed a deep ulcer which took over 100 days to heal leaving a big scar.

INTRODUCTION

Snakebite occurs throughout 14 states and divisions of Myanmar. Russell's viper (RV) bite occurs in 9 out of 14 states and divisions and is endemic in 6 rice-growing divisions (annual bite of >150 cases) (1990-92, 114 hospital-based study). National survey of venomous snake of Myanmar and observation of recent emerging RV bite cases in Loikaw (there was no report of RV bite between 1990-94) by the co-author (TP) lead to discovery of Malayan pit viper (MPV) bite in Loikaw divisional hospital in 1996 and is reported in this text. Although there are scanty bites in states, it will be of interest to study clinical features of RV bite cases from these places because of geographical variation of Russell's viper venom [1-3]. Although Loikaw, Kayah State is close to Thai border, there were no reports of MPV bite on Myanmar land whereas there are many bites in Thailand [4].

MATERIAL AND METHODS

A retrospective study of clinical details and outcome of snakebite cases admitted to the Loikaw Divisional Hospital during 1995-96 was carried out. Confirmation of MPV bite was made by detection of anti MPV antibody in the patient's serum by enzyme immunoassay technique [5]. Serum was also screened for presence of anti Russell's viper and anti Trimeresurus erythrurus antibodies since the patient had incoagulable blood on admission.

RESULTS

There were 38 snakebite cases, 24 green pit viper (Tr. erythrurus), 13 Russell's viper (D. r. siamensis) and one Malayan pit viper (C. rhodostoma) bite. The median age of the victim is 21 years (5-66 yr). Sixty-six per cent of them are less than 30 years old. Male to female ratio is 2:1.

Day (6am-6pm) bite is 4 times more common than after dark (6pm-6am) bite.

Russell's viper bite predominantly occurs in lower limb (12/13) whereas both upper and lower limbs are equally bitten in *Tr. erythrurus* bite. The mean intervals between the bite and the time of admission is 11.20 h (0.30-97 h); for Russell's viper is 19.30 h (0.30-97 h) and that of *Tr. erythrurus* bite is 6.25 h (0.30-31.30 h).

Clinical features of 13 Russell's viper bites are: systemic envenoming (13) (100%), local swelling and pain (13) (100%), abdominal pain (4) (30.7%), vomiting (3) (23%), spontaneous bleeding: bleeding gums (5) (38.5%), haemoptysis (3) (23%), epistaxis (3) (23%), haemetemesis (1) (7.7%) and haematuria (1) (7.7%), shock (4) (30%), anuria (3) (23%) and conjunctival oedema and haemorrhage (1) (7.7%). Two died of renal failure.

Green pit viper bites presented with local pain and swelling (24/24) of grade I to II which peaks at day 2-3 and subsided gradually thereafter. All cases have clottable blood (5-8 min) at admission.

Malayan pit viper bite

A 28-year-old farmer from Loikaw was bitten on the dorsum of left foot at the root of fourth and fifth toes, in a rice field by an unknown snake at 14:30 hr on 10th June 1996. He suffered from severe local pain which intensified on walking and eventually had to stop and asked for help to get home. Locally there were two fang marks measuring 1 cm apart. Local swelling rapidly spread to the dorsum of the foot, ankle and to the lower half of the foot distal to the tourniquet reaching maximum on third day. A blister appeared on the third day, became infected and was incised four days later leaving a deep ulcer measuring

4 in x 3 in exposing underlying structures like tendon and metatarsal bones. An admission clotting test was 15 min and subsequent tests were not performed. There were no systemic symptoms such as haemoptysis, petechial haemorrhages and bleeding gums following the bite. Four ampoules of RV antivenom were given. The victim did not see the snake. Daily wound dressing was needed which took over 100 days to heal leaving a final scar which measured 3 in x 1 in. Anti MPV antibody was detected by ELISA in the patient's serum collected at 9 months after the bite. Anti RV and anti *Tr. erythrurus* antibodies were not detected in the serum.

DISCUSSION

Study of snakebite cases of Loikaw, Kayah State pointed out that the working force of farmers are young people. 13/38 (34%) total bites in 2 yr were due to Russell's viper bites. Clinical features and the site of bite of Russell's viper cases are similar to those reported earlier [6,7]. However, high incidence of systemic envenoming (100%) and spontaneous haemorrhage such as bleeding gums (38.5%) and epistaxis (23%) occurred in Loikaw was probably due to late admission (5/13 took 19-97 h to get to the hospital) or geographical variation of venom properties [1-3].

Although most victims failed to bring the green snakes responsible for the bites, *Tr. erythrurus* is the commonest species of green pit viper (GPV) found throughout Myanmar. 24/38 (63%) of the total bites in 2 yr were due to GPV bites. Its bite produces local swelling and pain with incoagulable blood. In our cases, the victims presented with local swelling and pain with clottable blood on admission. Slight prolongation of clotting time 6-8 min was observed. Since serial monitoring of clotting test was not

carried out, time of onset of incoagulable blood was not known. The change from clotted to incoagulable blood could be detected as early as 1.15-2.4 h after the bite or delay for 9 h following *Tr. erythrurus* bite (unpublished observation) or 9-47 h in *T. albolabris* bite [8]. This lag phase probably represents the period before plasma fibrinogen has fallen to a critical level, which depends on the amount of venom injected into the victim.

Malayan pit viper bite has not been reported in Myanmar before. It has been suggested that may possibly inhabited in Myanmar [4]. In Thailand this species represents the commonest venomous snakes responsible for bites. It is an occupational disease of rubber plantation and other plantation workers. Thirty-eight per cent of the dead snakes brought by the patients in Thailand which had bitten them were MPV [9]. Thirty-eight per cent of snakebites in Indonesia are due to MPV bite with 3.5% fatality rate [10]. Sixty-three per cent of identified snake bite in north Malaya were attributed to this species [11]. About half of the 250 proven MPV bites studied at Sungi Patani Hospital in Malaya by Reid and colleagues [12] developed slight or no envenoming. Its bite produces incoagulable blood, pain, swelling and superficial necrosis [12] leading to scarring and deformity, osteomyelitis, gangrene and malignant change [4]. Blood became incoagulable in all systemic envenomed cases which could be detected as early as 30 min after the bite. In untreated cases this coagulopathy may have persisted for 1-26 days [13]. In mild envenomed cases there are no systemic symptoms and spontaneous haemorrhages [12]. Systemic haemorrhage was seen in only 15% of the cases. Spontaneous systemic bleeding was seen in 24% of those with systemic envenoming [4]. No systemic haemorrhage was detected in our case

suggesting that it was a mild envenomed one. Local necrosis developed in 11% of the patients which healed in 13-86 (mean 47) days. In most severe cases there was 300 cm² of skin loss which healed in 322 days. In our case, it took over 100 days. Local necrosis was always preceded by blistering but not all patients with blistering developed necrosis. Mortality from MPV bites, attributed to haemorrhage or secondary infection of necrotic wounds (including tetanus), is relatively low.

Although the inflicting snake was not identified in our case, clinical features of it fit MPV bite and the diagnosis was confirmed by detection of specific anti MPV venom antibody in the patient's serum. If immunodiagnosis could not be done, MPV bite will have to be differentiated from other bites which produce incoagulable blood and a big scar on healing. Cobra (*Naja kaouthia*) bite results in neurotoxic envenoming with local scarring and is not associated with development of incoagulable blood. Moreover *Tr. erythrurus* bite produces incoagulable blood with local swelling only. Mild envenomed Russell's viper bite cases may present with incoagulable blood with local swelling. However, severe envenomed cases will have local blister, necrosis, spontaneous haemorrhages and renal failure. Monospecific Russell's viper antivenom was given because the patient presented with incoagulable blood.

However there were two snakebite cases (one from Man-sae-nan, Kayah State and another from Kyauk-me, Shan State by the author) labelled as Russell's viper bite, which had clinical features and outcome that fit MPV bite when they were interviewed retrospectively (personal communication). Moreover, farmers and natives from Phasaung and Man-sae-nan of Kayah State have seen MPV around when they were shown of the picture of the snake. Wood

cutters and log transporters of Kayah State working near Myanmar-Thai border are familiar seeing the snake around and among teak logs. It is probable that the snakes could be transported from place to place by these trucks. A higher incidence of MPV bite is expected if further follow-up of the cases in states is carried out.

It is highlighted that a patient presented with incoagulable blood with or without systemic symptoms and develops a local wound which took months to heal leaving a scar should be suspicious of MPV bite.

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Clinical features and response to antivenom of Russell's viper bite cases of

Danubyu, Ayeyawady Division

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A total of 31 (excluding one cobra bite) Russell's viper bite cases were available for study during 1995-96. The median age of the victim is 24 yr (11-68 yr) and the interval between bite and admission is 4.45 h (2.30-12.00 h). Eighty per cent of the bites occurred in lower limb and 65% were bitten while at work in the field. Length of the snakes varies from 23-110 cm (median 26 cm). Thirteen per cent of the bites are local, 29% no envenoming and 58% systemic of which 67% developed spontaneous systemic bleeding. Nineteen per cent died of renal failure and shock. Venom levels of local and systemic are 10-40 ng/ml and 20-199 ng/ml respectively. Clot restoration time in 59% of the cases takes more than 6 h indicating variable efficacy of antivenom in neutralising procoagulant activity of local venom. Albuminuria was detected in 56%. Local blackening (45%) is probably the result of ischaemia and local venom effect.

INTRODUCTION

Russell's viper (*Daboia russelli siamensis*) bite is endemic in 6 rice-growing divisions of Myanmar. Recent reports on variation of biological properties of Russell's viper venom [1-6] and clinical features of Russell's viper bite cases from different localities [7-8] suggested that there is variation in clinical features of Russell's viper bite cases from different localities. This study concerns on Russell's viper bite cases of Danubyu, Ayeyawady Division.

MATERIALS AND METHODS

All snakebite cases admitted to Danubyu Hospital, Ayeyawady Division during 1995-1996 were studied. Clinical details of the patients were recorded in standard proforma. Twenty minute clotting test [9] was used to determine degree of envenoming. A total of 40 ml of monospecific antivenom was given to patients at

the discretion of the medical officer. Clotting test was carried out at 2 h intervals on cases presented with clottable blood and 6 hly after antivenom on systemic cases until normal clot restoration occurred. Two milliliters of blood is needed for clotting test. Sera were collected onto filter paper strips, air dried, stored in a sealed polythene bag and later transported to the Department of Medical Research once a month. Venom antigen level of the samples were determined by enzyme immunoassay technique [10] at the Immunology Research Division.

RESULTS

A total of 31 Russell's viper bites (excluding one cobra bite) were studied. Clinical details of 31 Russell's viper bite and 22 envenomed cases are shown in Table 1 and 2 respectively.

Table 1. Clinical details of 31 Russell's viper bite cases

Total (31) (18 systemic+4 local+9 non envenoming)		%
Sex; male : female	22:9	71:29
Time bite; Day (6am-6pm) : night (6pm-6am)	24:7	77:23
Age (yr) (median)	24 (11-68)	
Activity when bitten; working : walking	20:11	65:35
Site of bite; lower limb : upper limb	25:6	80:20
First aid; Tourniquet	24	77
Immobilisation	3	10
Suction	3	10
Incision	3	10
Herbal	2	6.5
Tattoo	8	26
Cheroot burning	1	3
Distance travelled (miles) (median)	4 (0.5-21)	
Bite and admission (h)	4.45 (2.30-12.00)	
Antivenom pretreatment at village	2/32	6
Bite and antivenom therapy (h)	4 (0.20-12.30)	
Dead snake brought	7	23
Length of the dead snake (cm) (median)	26 (23-110)	

Table 2. Clinical details of 22 envenomed cases

Symptoms: (22)	%		Signs: Local & systemic (22)	%	
Local pain at site	21	95	Local swelling	21	95
Epigastric pain	15	68	Bleeding at site of bite	4	18
Loin pain	16	73	Local blackening	10	45
Vomiting	12	55	Local bruising	5	23
Dyspnea	2	9	Local necrosis	2	9
Drowsiness	2	9	Lymph node enlargement	8	36
Dysphagia	2	9	Systemic (18)		
			Spontaneous systemic bleeding	12	67
			Haemoptysis	8	44
			Gingival	5	28
			Haematuria	7	39
			Haematemesis	4	22
			Malena	1	6
			Subconjunctival	2	11
			Epistaxis	3	17
			Hypotension (<80 mmHg)	5	28
			Oliguria (<400 ml/24 h)	9	50
			Conjunctival oedema	2	11
			Periorbital oedema	2	11
			Renal failure	7	39
			Fatality	6	19

Response to antivenom

Of 31 bites, 58% (18/31) are systemic, 13% (4/31) local and 29% (9/31) no envenoming. Antivenom was given on clinical judgement of the cases. Clot restoration time after antivenom therapy is 59% (10/17) in 6 h, 29% (5/17) in 12 h, 6% (1/17) in 20 h and 6% (1/17) in 36 h.

Venom antigen levels

Venom antigen was not detected in 29% (9/31) Russell's viper bite cases and no antivenom was given to them. Antigen level of 10-40 ng/ml were detected in 13% (4/31) local cases, in two even after administration of one ampoule of antivenom at the village. Venom level of systemic cases ranges from 20-100 ng/ml and 4-8 ampoules of antivenom were given to them.

Initial wound treatment and local wound complications

Local wound complications such as blackening 45% (10/22), bruising 23% (5/22), bleeding 18% (4/22), necrosis 9% (2/22) and regional lymph node enlargement 36% (8/22) were observed.

Thirty-six per cent (8/22) of the bites with initial wound treatment (suction, burning, incision either singly or in combination) developed local complications and 18% (4/22) wound treated cases did not. However, 41% (9/22) of the cases with no initial wound treatment developed local complications.

Clinical features of systemic cases

Of 18 systemic cases, 12 (67%) developed spontaneous systemic bleeding. Epigastric pain 68%, vomiting 55%, haemoptysis 44%, haematuria 39%, gingival bleeding 28%, haematemesis 22%, epistaxis 17% and hypotension 28% are observed. Nineteen per cent (6/31) died of shock [5] and renal failure [1].

DISCUSSION

Clinical feature of Russell's viper

bite cases of Danubyu, differs from Yegyi (both are from Ayeyawady Division) and Myinmu, Sagaing Division. In the present study, no antivenom was given to non envenomed cases which shows clinical indications for antivenom therapy could be followed by the township medical officer and a substantial amount of antivenom was saved.

Since 41% of the untreated and 36% of the treated wounds developed local complications, it is suggested that these are result of direct venom-induced effects compounded by initial wound treatment.

The incidence of development of spontaneous systemic bleeding (67%), haemoptysis (44%), gingival bleeding (28%), epistaxis (17%), hypotension (28%) is 1 to 11 times more common in Danubyu when compared with cases from Yegyi whereas malena (12%) and albuminuria (77%) are more common in the latter (unpublished observation). Variation in biological properties of venom from different localities of Ayeyawady may account for it [2]. Low incidence of systemic envenoming (17%) in the Russell's viper bite cases from Myinmu, Sagaing Division compared to 58% in Danubyu is the result of early administration of high dose of antivenom routinely combined with immobilisation of the limb in the former which helps in preventing local envenoming state turns into systemic. The incidence of local blackening (45%) is high compared to reports from other localities (4%) in Yegyi and 14% in Myinmu (unpublished observation) which is the result of ischaemia and local venom effect. High mortality rate (19%) compared to Yegyi, Taungdwingyi and Thayawady which are about 9% is attributed to high proportion of cases (67%) developed spontaneous systemic bleeding, shock and albuminuria.

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A clinical profile of diabetic polyneuropathy in Mandalay General Hospital

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A cross-sectional study of risk factors, associated complications and nerve conduction studies on diabetic polyneuropathy was conducted on 68 diabetics with and without clinical evidence of polyneuropathy in Mandalay General Hospital over a 9-month study period. Diabetic polyneuropathy was seen in older age groups. The duration of diabetes is a significant risk factor for diabetic polyneuropathy. There was no association with fasting blood sugar levels, smoking, hypertension and hypercholesterolaemia in our study. Diabetic polyneuropathy has increased association with retinopathy, though ischaemic heart disease and nephropathy have no association in our study. On performing nerve conduction studies, diabetic polyneuropathy patients have significantly lower nerve conduction velocity than those without polyneuropathy. Subclinical polyneuropathy was detected in 15% of diabetic cases, without clinical evidence of polyneuropathy. It is concluded that early detection of diabetic subclinical polyneuropathy is possible with electrophysiologic studies. Early detection of subclinical polyneuropathy and early detection and alleviation of risk factors and intensive therapy to control blood glucose may prevent irreversible nerve damage and associated complications.

INTRODUCTION

Diabetes mellitus is a global health problem. Globally there are at least 30 million diabetics [1]. According to data from the Department of Planning and Statistics, Ministry of Health, morbidity for all age groups in Myanmar diabetics (1989, 1990, 1991) significantly reveals the increasing trend (almost double within 3 years). The overall mortality is 5.19% and is therefore of considerable epidemiological significance [2,3,4]. In a study conducted in Yangon General Hospital, neuropathy was present in 33.5% of non insulin dependent diabetes mellitus (NIDDM) and 14.7% of insulin dependent diabetes mellitus (IDDM) [5].

The clinical manifestations of neuropathy are protean. A peripheral symmetric sensory-motor neuropathy is the most common form of diabetic neuropathy, while other forms include cranial and peripheral neuropathies

and autonomic neuropathy. Diabetic somatic neuropathy is a common but often forgotten complication of diabetes mellitus. Although there is indeed no treatment known for the reversal of diabetic somatic neuropathy, significant improvement in symptoms and quality of life is possible [1].

Abnormal nerve conduction in diabetes mellitus is more likely to be due to a generalized metabolic disturbance rather than ischaemia, hence it is preventable. Prevention of nerve damage by maintenance of optimal diabetic control and early detection of neuropathy are of major importance. Established neuropathy may be irreversible and risk of serious consequences such as soft tissue and joint destruction ending in non-traumatic amputation may occur.

In the DCCT, intensive therapy in those with impaired nerve conduction velocity decreased the occurrence of

clinical polyneuropathy by 60% [6]. Early detection of diabetic neuropathy by nerve conduction studies and alteration of risk factors may play crucial role in prevention of polyneuropathy and enable the patient to have a normal life.

Aims and objectives

1. To compare the nerve conduction velocity among diabetics with and without polyneuropathy.
2. To detect any subclinical diabetic polyneuropathy cases by means of nerve conduction velocity.
3. To detect any correlation between diabetic polyneuropathy and associated complications and risk factors.

PATIENTS AND METHODS

This is a cross-sectional analytic study for a period of 9 months starting from September 1994.

Thirty-four cases of diabetes with peripheral neuropathy and 34 cases of diabetes without peripheral neuropathy were selected by convenience sampling method from diabetics of diabetic clinic and in-patients of Medical Unit (II), Mandalay General Hospital.

Alcoholics, patients who had exposure to neurotoxic agents, those with vitamin deficiencies, patients with systemic diseases and patients with other metabolic diseases such as hypothyroidism and thyrotoxicosis were excluded in this study.

According to proforma, thorough history taking, physical examination, investigations, some sociodemographic variables (age, sex) types of diabetes mellitus, associated risk factors such as smoking, duration of diabetes mellitus, hypertension, hypercholesterolaemia, blood sugar control and associated complications such as retinopathy, ischaemic heart disease and nephropathy were recorded. The

selected patients were sent to Physiology Department for nerve conduction study.

The sensory and motor nerve conduction of median and ulnar nerve were calculated by using the following formula.

$$V = D/T$$

V = The velocity of nerve impulse (m/sec)

D = The conduction distance (mm)

T = Conduction time

Data analysis

At the end of the study data were analyzed by χ^2 test.

RESULTS

Age and sex distribution of diabetic cases are shown in Table 1. The youngest patient was 13 years and the oldest was 78 years. The commonest age group is 41 to 60-year group. Majority of the patients (80.88%) were female patients. Out of 34 diabetic patients without polyneuropathy 8 patients were male and 26 were female. Out of 34 patients with polyneuropathy 5 were male and 29 were female. Male:female ratio was 1:4.2.

Table 2 shows types of diabetes. Out of 68 cases with and without polyneuropathy, 8 were IDDM and 60 were NIDDM. Out of 8 IDDM cases, 62.67% had polyneuropathy and out of 60 NIDDM cases, 48.33% had polyneuropathy.

Table 3 shows mean duration of diabetes in this study. Mean $\pm$ SD duration of diabetes mellitus in patients with polyneuropathy was 6.97 ± 6.05 years and mean $\pm$ SD duration of diabetes mellitus in patients without polyneuropathy was 3.032 ± 3.81 years. P value was <0.01 .

Table 4 shows mean $\pm$ SD fasting blood sugar level of diabetic cases. Mean fasting blood sugar level (FBS) of diabetes mellitus with neuropathy was 12.09 ± 5.12 mmol/L and in

Table 1. Age and sex distribution of diabetic cases

Age group	With neuropathy		Without neuropathy		Total
	Male	Female	Male	Female	
0-20 years	-	-	-	1	1
21-40 "	-	4	1	8	13
41-60 "	4	19	5	11	39
61-80 "	1	6	2	6	15
Total	5	29	8	26	68

Male : Female = 1:4.2

Table 2. Types of diabetic cases

Diabetic cases	IDDM		NIDDM		Total
	No. of cases	Percentage	No. of cases	Percentage	
With neuropathy	5	14.7	29	85.3	34
Without neuropathy	3	8.8	31	91.2	34
Total	8	11.8	60	88.2	68

Table 3. Duration of diabetes mellitus

	Mean $\pm$ Duration of diabetes mellitus (in yrs)
With neuropathy	6.97 $\pm$ 6.05
Without neuropathy	3.032 $\pm$ 3.81

P = <0.01

Table 4. Fasting blood sugar level in diabetic cases

	Mean $\pm$ SD FBS (mmol/L)
With neuropathy	12.09 $\pm$ 5.12
Without neuropathy	11.19 $\pm$ 4.07

Not significant

Table 5. Nerve conduction velocity (mean $\pm$ SD) in diabetic cases

	Median (m/sec)		Ulnar (m/sec)	
	Motor	Sensory	Motor	Sensory
With neuropathy	49.04 $\pm$ 9.16	37.68 $\pm$ 14.5	49 $\pm$ 6.74	26.51 $\pm$ 14.91
Without neuropathy	54.94 $\pm$ 8.34	45.35 $\pm$ 14.75	65.62 $\pm$ 9	36.24 $\pm$ 11.99
P value	<0.05	<0.05	<0.01	<0.05

those without neuropathy was 11.19 $\pm$ 4.07 mmol/L. In this study FBS has no significant difference between diabetic neuropathy and diabetic without neuropathy cases.

Table 5 shows mean $\pm$ SD nerve

conduction velocity of diabetes in this study. Mean motor conduction velocity of median nerve of diabetic neuropathy patients was 49.04 $\pm$ 9.16 m/sec. This was significantly less than the mean value of diabetic

Table 6. Associated complications in diabetic cases

	Hypertension				IHD				Retinopathy				Nephropathy			
	+	%	-	%	+	%	-	%	+	%	-	%	+	%	-	%
With neuropathy	22	64.7	12	35.3	16	47.1	18	52.9	21	61.8	13	38.2	3	8.8	31	91.2
Without neuropathy	14	41.2	20	58.8	14	41.2	20	58.8	6	17.6	28	82.4	4	11.8	30	88.2

without polyneuropathy which was 54.94 ± 8.34 m/sec. Mean sensory conduction velocity of median nerve of diabetic polyneuropathy patients was 37.68 ± 14.5 m/sec. This was lesser than diabetic without polyneuropathy cases which was 45.35 ± 14.75 m/sec ($P < 0.05$). Mean motor conduction velocity of ulnar nerve of diabetic polyneuropathy patients was 49 ± 6.74 m/sec. This was significantly less than the mean value of diabetic without polyneuropathy which was 56.62 ± 9 m/sec ($P < 0.01$). Mean sensory conduction velocity of ulnar nerve of diabetic polyneuropathy patients was 26.51 ± 14.91 m/sec. This was significantly less than the mean value of diabetic without polyneuropathy which was 36.24 ± 11.99 m/sec ($P < 0.05$). Five out of 34 cases of diabetics clinically without neuropathy (i.e. 15%) had no action potential on nerve conduction study.

Table 6 shows associated complications of diabetic polyneuropathy. Out of 34 cases of diabetic polyneuropathy, hypertension, IHD, retinopathy and nephropathy were detected in 22 (64.7%), 16 (47.1%), 21 (61.8%) and 3 (8.8%) respectively. Out of 34 cases of diabetes mellitus without polyneuropathy 14 (41.2%) of each had hypertension and ischaemic heart disease, 6 patients (17.6%) had retinopathy and 4 patients (11.8%) had frank nephropathy.

Among the diabetic complications, retinopathy has strong association with diabetic polyneuropathy ($P < 0.001$). The association with other complications and risk factors such as nephro-

pathy ($\chi^2 = 0.156$), ischaemic heart disease ($\chi^2 = 0.24$), hypertension ($\chi^2 = 3.78$, $P = < 0.1$), hypercholesterolaemia ($\chi^2 = 2.12$, $P = < 0.5$) and smoking ($\chi^2 = 0.12$) were statistically not significant in this study.

DISCUSSION

In this study, majority of patients (80%) with polyneuropathy were between 40 to 80 years of age. Male and female ratio is 1:4. This can be comparable with Wetterhall et al. stated that the incidence of diabetes increased among women ($P = 0.003$) particularly among those >65 years old ($P = 0.02$) [7].

Pfeifer et al. pointed that age, hypertension, duration of diabetes, blood glucose control and possible smoking and cholesterol are cross-sectional risk factors for the development of diabetic polyneuropathy [8]. Kyaw Min's study showed a significant negative linear correlation between the duration of diabetes mellitus and nerve conduction velocity [9]. Chopra et al. reported that a longer duration diabetes showed greater abnormalities in the sural nerves and slower nerve conduction [10]. In this study mean duration of diabetes was significantly longer in patients with polyneuropathy than those without polyneuropathy ($P = < 0.01$) and supported the above studies, indicating increasing risk of developing diabetic neuropathy with time.

In one of the animal studies, Jakobsen concluded that early structural axon abnormality results from diabetic

state [11]. This relationship between diabetic metabolic control and nerve damage was supported by other human studies. In Pivart's study, patients with excellent glucose control for 25 years showed 10% of diabetic somatic neuropathy and those with poor glucose control the prevalence of diabetic neuropathy increased nearly 70% by 25 years [8]. Gregersen found mean motor nerve conduction velocity (MNCV) lower in the patients in whom metabolic state was poorly controlled than well controlled [12]. In contrast, in this study fasting blood sugar level (FBS) between diabetics with and without neuropathy showed no significant difference. It may be that FBS may not represent long standing diabetic control.

According to animal studies, nerve conduction velocity was significantly reduced in the diabetic animals [11, 13]. Noel studied 59 diabetic patients, sensory nerve potentials were recorded at various sites along the course of the median nerve. He pointed out the presence and severity of clinical neuropathy in the upper limb could be related to decreased maximal sensory nerve conduction [14]. Kyaw Min reported that nerve conduction velocity was significantly delayed in diabetes [9]. The DCCT (Diabetes Control and Complication Trial) research group showed that electrophysiologic studies demonstrate subclinical abnormalities, including slow nerve conduction after 5 to 10 years of diabetes [15]. Lamontagne and Buchthal found abnormalities of sensory potentials in 24 patients with diabetes, 12 of whom had no sensory symptom or sign of neuropathy and 5 of whom had no other clinical or electrophysiological evidence of neuropathy in the upper extremities. The sensory potentials were the most sensitive indicators of subclinical involvement of nerves in diabetes [16]. In this study, median and ulnar nerve conduction velocity was significantly delayed in diabetics with polyneuropathy compared to

those without polyneuropathy. Fifteen per cent clinically normal diabetic patients have no action potential on nerve conduction study. It may be that these patients may suffer from subclinical diabetic polyneuropathy.

Bell observed 78 patients of diabetes. Out of these 25 cases 32% showed microalbuminuria and 51% had neuropathy and showed a significant association between microalbuminuria and diabetic neuropathy. This concluded that microalbuminuria is independently associated with diabetic polyneuropathy [17]. In this study, among the diabetic complications retinopathy had strong association with diabetic polyneuropathy ($P < 0.001$) but other diabetic complications such as ischaemic heart disease and nephropathy had no significant association with polyneuropathy. Lack of association with nephropathy may be due to our limited facilities to detect microalbuminuria cases.

In this study smoking, hypertension and hypercholesterolaemia had no significant association with diabetic neuropathy. It may be due to cultural differences.

CONCLUSION

It can be concluded that nerve conduction studies have value in confirming polyneuropathy. Thus early detection of subclinical cases are possible by means of nerve conduction study which shows delayed or absent action potential even in patients who do not have clinical evidence of polyneuropathy. Prevention of nerve damage by early detection of neuropathy and intensive therapy is of major importance, since established neuropathy may be irreversible.

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SHORT REPORT

Survival of Salmonella typhi in natural waters of Yangon

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Salmonellae are the pathogenic micro-organisms most frequently found in polluted water. Salmonellae were isolated from river water of Yangon by simple methods such as cotton wool filtration and multiple tube method [1]. Prolonged survival of salmonellae in water and soil has also been described. The only source of typhoid bacilli is the infected person who excretes the bacilli from the intestine or urinary tract. The main route of epidemic spread is by food and water. Thus the survival of S. typhi in natural waters of Yangon was studied.

The water samples collected were: (1) municipal water from Kamayut Township, (2) shallow well water from Hline and Kamayut townships, (3) tube-well water from Hline and Kamayut townships, (4) pond water from Bahan, Hline and Shwepyitha townships and (5) river water from Hline, Kyimyindine and Yangon rivers.

The first three sources of water are used for drinking as well as for domestic purposes and the latter two are used for bathing and washing. One litre of water from each of the above sources were collected in sterile containers employing the standard method of collection [2]. Eight samples of each type of water were tested and therefore a total of 40 water samples were tested in this study. The water samples were immediately transported to the laboratory and processed within one hour after arrival. S. typhi strain was used as contaminant in the study. It was maintained on trypticase soy

agar medium at room temperature and subcultured at weekly intervals. Physicochemical analyses of these natural water samples were made for appearance, colour, smell and pH.

Eighteen-hour peptone-water cultures of S. typhi kept overnight at 37°C was used as inoculum. One hundred milliliter of water sample from each source was inoculated with the above S. typhi to a final concentration of 10^4 - 10^6 colony forming units per ml. The contaminated sample was then incubated at room temperature (24°C) and viable counts of S. typhi were determined by drop plate method of Miles and Misra [3] using XLD (xylose lysine desoxycholate) agar at one hour intervals for 4 hours on the 1st day and once daily until the viable counts were negative. The bacilli from XLD agar was identified as S. typhi strain using salmonella-shigella agar, short biochemical tests with triple sugar iron agar, lysine iron agar, urease agar and sulphide indole motility agar. It was then confirmed by serotyping with group antisera 'D group' and V antisera. This was done at every 2 weeks interval.

The municipal, shallow well, tube well and pond water samples were all clear. The river water samples were turbid. There was no smell. The average pH of these natural water samples were as follows: municipal water - pH 7.2, tube well water - pH 7.0, shallow well water - pH 6.4, river water - pH 7.1 and pond water - pH 8.2. Salmonella grows at a pH range of 4 to 8 [4].

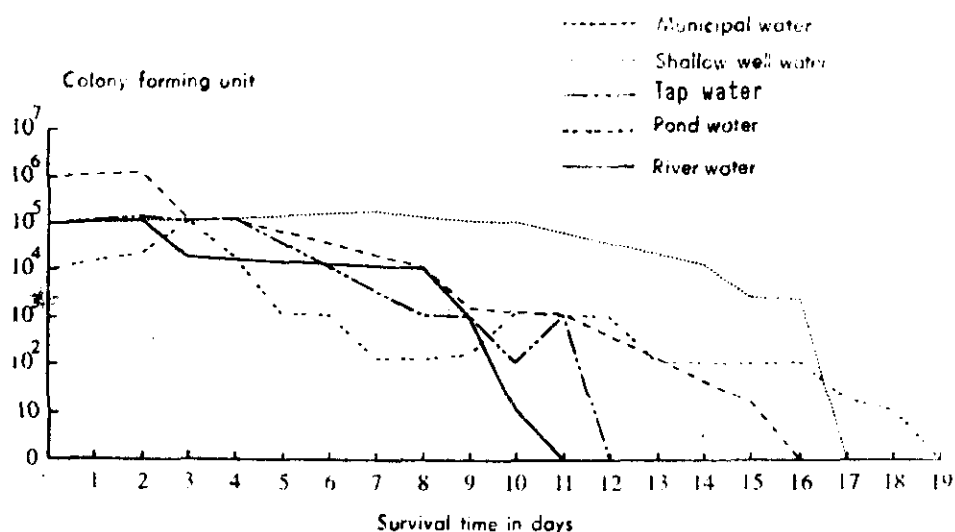


Fig. 1. Survival time of *S. typhi* in natural waters of Yangon

On the first day the growth curve of *S. typhi* in all natural waters were similar in all 0, 1, 2, 3 and 4 hour growth curve. There was no difference. The generation time of salmonellae is 20 mins/cycle.

S. typhi survived up to 16-19 days in municipal, shallow-well and pond water samples in this study. However it survived for only 11-12 days in river water and tube-well water samples. This may be due to the fact that *S. typhi* has to compete with other bacteria that are also present in river water since it is the most heavily contaminated water among all the natural waters of Yangon. Tube-well water is the least contaminated source of water. However there are less nutrients in it for survival of *S. typhi* and thus it survived for only 12 days (Figure 1).

This study indicates that such waters if contaminated with *S. typhi*, can transmit infection and contribute a health hazard to the public. In typhoid outbreaks, the fact that *S. typhi* can survive for about 2 weeks

in natural waters should be kept in mind in carrying out control measures.

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