

MYANMAR

Volume 11, No. 1-3, 1999

**HEALTH
SCIENCES**
RESEARCH JOURNAL

DEPARTMENT OF MEDICAL RESEARCH

(Lower Myanmar)

No. 5, Ziwaka Road

Yangon 11191, Myanmar



CONTENTS

The application of polymerase chain reaction technology in the diagnosis of tuberculous meningitis in Myanmar	1
<i>Htain Win, Ne Win, Khin Pyone Kyi, Khin May Oo & Myo Khin</i>	
Serum uric acid in preeclampsia.....	6
<i>Ye Tint Lwin, Lilian Po & Hnin Hline</i>	
Determination of glutaraldehyde gelification time in leprosy.....	11
<i>Ne Win, San San Aye, Khin Saw Aye & Charles Pang</i>	
Enteropathogenic <i>Escherichia coli</i> (EPEC) from acute diarrhoeic children with <i>in vitro</i> antibiotic susceptibility	14
<i>Mar Mar Nyein, Khin Myat Tun, Nyomie Kyaw, Thuzar Myint, Moh Moh Win, Wah Wah Aung & Than Nu Shwe</i>	
Cranial diabetes insipidus following a Russell's viper (<i>Daboia russelli siamensis</i>) bite in Myanmar.....	18
<i>Sann Mya, Tun Pe & Aye Aye Myint</i>	
Purification, characterization and properties of acid phosphomonoesterase from Russell's viper (<i>Vipera russelli siamensis</i>) venom	21
<i>Aye Kyaw, Hla Kathy Win, San Aye, Khin Khin Aye & Khin Pa Pa Kyaw</i>	
Plants possessing antibacterial activity.....	26
<i>Mar Mar Nyein, Nwe Yee Win, Win Myint, Aye Aye Thein, Mi Mi Htwe, Win Win Maw & Aye Than</i>	
Antivenom abuse: a review of antivenom policy in management of Russell's viper bite cases of six township hospitals.....	33
<i>Tun Pe, Aye Aye Myint & Nu Nu Aung</i>	
Use of PGL-1 ELISA test for serodiagnosis of <i>Mycobacterium leprae</i> infected individuals in a highly prevalent village in Myanmar.....	38
<i>Khin Nwe Oo, Nwe Nwe Yin, Kyaw Nyunt Sein, Kyaw Myint & Kyaw Kyaw</i>	

The establishment of Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) technique and its application for dengue virus serotype - specific diagnosis in Myanmar	41
---	----

Kyaw Zin Thant, Si Si Hla, Kay Thi Aye, Hlaing Myat Thu, Khin Mar Aye, Kyaw Moe, & Soe Thein

Short Report: Schizontaemia of <i>Plasmodium falciparum</i> in a non-fatal cerebral malaria patient.....	50
--	----

Khin Saw Aye, Soe Soe, Than Swe, Ko Ko Hla & Win Bo Kyaw

Short Report: Effect of freeze-drying on the formalinized Russell's viper venom toxoid	52
--	----

Win Aung, Khin Khin Hlaing & Aye Kyaw

Editor-in-Chief

Dr. Than Swe

Editor

Dr. Soe Thein

Associate Editor

Dr. Myint Lwin

Dr. Tun Pe

Managing Editor

Dr. Aye Kyaw

Editorial Board

Prof. Thein Thein Myint

Prof. Maung Maung Wint

Prof. Saw Naing

Prof. Myo Myint

Prof. Mala Maung

Prof. Win Maw

Prof. Htay Htay

Prof. Col. Marlar Than

Prof. Nyunt Wai

Prof. Tracy Sein

Publisher

U Aung Myint

Editorial Managers

Dr. Myint Oo

Dr. W. Tun Lin

Production Editors

Dr. Aye Kyaw

Dr. Tin Nu Swe

Production Manager

Daw Khin Sann Aung

Printed at the Publications Division, Department of Medical Research (Lower Myanmar)

The application of polymerase chain reaction technology in the diagnosis of tuberculous meningitis in Myanmar

*Htain Win, **Ne Win, ***Khin Pyone Kyi, ***Khin May Oo & ****Myo Khin

*Clinical Research Division

**Pathology Research Division

***Vaccine Production & Distribution Division

****Experimental Medicine Research Division

Department of Medical Research

Polymerase Chain Reaction (PCR) for enzymatic amplification of DNA is a very sensitive and useful diagnostic method for cases where early diagnosis and treatment is essential. In tuberculous meningitis cases, the concentration of antigen in the cerebrospinal fluid may be very low for detection by conventional methods and laboratory results can alter the prognosis of the patient. An *in vitro* method for PCR amplification of DNA, was developed for detection of *Mycobacterium tuberculosis* complex in clinical samples. In this test, a 245 bp sequence of the insertion IS 986 was amplified and detected by agarose gel electrophoresis in the presence of ethidium bromide. Twenty CSF samples were first decontaminated and treated with proteinase K detergent solution and extraction of DNA was performed. Part of each sample was spiked with 10^{-10} g/ml of purified *M. tuberculosis* DNA to detect the presence of inhibition. In this study 80% of the CSF samples were positive for tuberculosis by PCR method. The results were consistent with the clinical history of the patients, chest X-ray results and CSF findings.

INTRODUCTION

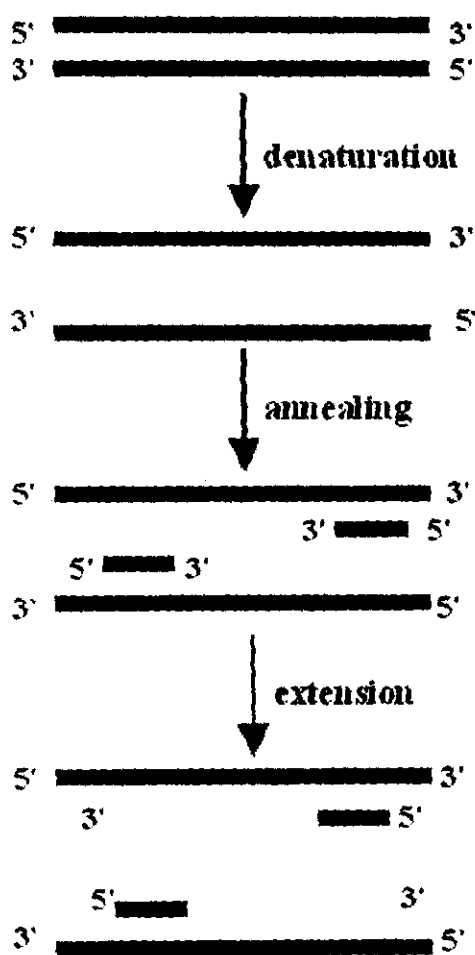
Tuberculosis is not a disease of the past and is still a disease of worldwide importance causing high morbidity and mortality despite the availability of effective chemotherapy. Tuberculous meningitis is the most dangerous form of extrapulmonary tuberculosis, occurring in 7-12% of tuberculous patients in developing countries [1]. Untreated tuberculous meningitis is fatal in a few weeks but recovery is the rule with modern treatment if it is started in time. When treatment is started at a later stage recovery rate drops to 60% or less and the survivors may be mentally deficient, epileptic, deaf, blind or may have some permanent sequelae [2]. Delay in diagnosis is directly related to poor outcome; there are

neurological sequelae in 20-25% of such patients.

Conventional laboratory diagnostic methods are inadequate for early diagnosis of tuberculous meningitis because there are too few organisms in the cerebrospinal fluid for consistent demonstration by direct smear, and also the ideal method of cultural identification takes 6-8 weeks [3].

A DNA amplification assay using the polymerase chain reaction (PCR) technique permits rapid detection of mycobacteria directly in clinical samples. PCR allows *in vitro* amplification of target DNA to a detectable level within a matter of hours [4,5]. PCR has been reported to amplify a single copy of DNA sequence by 10^{12} fold [6].

The Polymerase Chain Reaction



PCR amplification involves two oligonucleotide primers that flank the DNA segment to be amplified and repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences and extension of the annealed primers with DNA polymerase. These primers hybridize to opposite strands of the target sequence and are oriented, so DNA synthesis by the polymerase proceeds across the region between the primers, effectively doubling the amount of that DNA segment. Moreover, the extension products are also complementary to and capable of binding primers, each successive cycle essentially doubles the amount of DNA synthesized in the previous cycle. This results in the exponential accumulation of the specific target fragment.

MATERIALS AND METHODS

Equipment

Thermocycler, positive displacement pipettes, plugged tips, electrophoresis apparatus, UV transilluminator and polaroid camera.

Reagents (molecular biology grade)

Taq polymerase (Stratagene), primers INS1 and INS2 (Clontech Laboratories), nucleotides (United States Biochemicals), Tris HCl pH 8.3, 10 X digestion buffer, Proteinase K, Purified mycobacteria DNA, Potassium chloride, Magnesium chloride, gelatin, UNG and mineral oil.

Methods

For the establishment of the PCR test system in Myanmar, as a preliminary trial, 20 CSF specimens were collected from children admitted to the Yangon Children's Hospital with the provisional diagnosis of tuberculous meningitis before the investigations were completed. These patients gave a history of low grade fever, malaise and weight loss (non specific signs and symptoms of tuberculous infection). The following factors were recorded for each patient.

- (1) History of TB contact in the family
- (2) Chest X-ray
- (3) CSF results

DNA isolation from mycobacteria

The CSF samples were centrifuged at 12000xg in a microcentrifuge. The supernatant was discarded and the pellet was resuspended with 1 ml of 20 mM Tris-HCl (pH 8.3) and vortexed to loosen the pellet. It was spun at 12000g for 10 min. Again, the supernatant was discarded and the pellet was resuspended with 160 µl of 20 mM Tris-HCl (pH 8.3). Digestion buffer was prepared to a final concentration of 0.5% Tween 20 and 1 mg/ml proteinase K and the reaction mix was incubated at 60°C for 1 hour. The extract was boiled at 100°C

for 15 min to inactivate proteinase K. At this point each sample was divided into two aliquots, one of which was spiked with 10 μ l (10 μ g) of purified mycobacterial DNA. A control tube that contains only buffer (20 mM Tris-HCl, pH 8.3) was included in every assay. This tube was subjected to the same extraction procedure as the specimens, and split into two aliquot with one aliquot spiked with purified mycobacterial DNA as described above. The samples were stored at 4°C or used directly for PCR. Ten microlitre of the extract was used for amplification.

PCR

Two sets of primers were used for the PCR. The oligonucleotides INS 1 (5'-CGTGAGGGCATCGAGGTGGC-3') and INS 2 (5'-GCGTAGGCGTCGGTGACAAA-3') correspond to base pairs 631 to 650 and 856 to 875 respectively, of the IS 986 insertion element. The PCR was performed with the heat stable DNA polymerase from *Thermus aquaticus* (Taq polymerase; Perkin Elmer Cetus, Norwalk, Conn). The final composition of the PCR mix was 10 mM Tris HCl (pH 8.3) at 25°C, 50 mM KCl, 1.5 mM MgCl₂, 0.01% w/v gelatin, 0.2 mM each deoxynucleoside triphosphates (dATP, dCTP, dGTP) and 0.4 mM dUTP, 0.2 μ M (each) primers INS 1 and INS 2 (ie. 66 ng/50 μ l reaction vol), 1 U of Taq polymerase per 50 μ l reaction volume and 0.1 U of UNG per 50 μ l reaction volume for carry over prevention. The PCR mix was divided into aliquots in a final volume of 40 μ l per tube and stored at -20°C until use. When a test was performed, the reaction tubes were removed from the freezer, and 50 μ l of mineral oil (Sigma) was added to each vial before the 10 μ l sample was placed under the oil layer. For all pipetting, positive displacement pipette (Gilson, pipetteman) and plugged tips were used. The PCR was performed in a thermocycler (PTC-100), Programmable Thermal Controller, MJ Research). One cycle included 1.5 min of denaturation at 94°C, 2

min of annealing at 65°C, and 3 min of primer extension at 72°C. A total of 40 cycles were performed.

The presence of amplified DNA sequences was detected by agarose gel electrophoresis. Briefly aliquots of the amplified products were electrophoresed in 1.5% agarose gels stained with ethidium bromide. An amplified fragment of approximately 245 bp was considered a positive result.

RESULTS

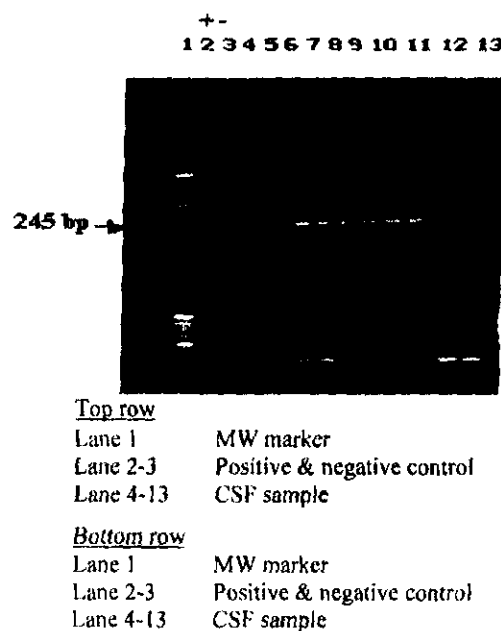


Fig. 1. Agarose gel electrophoresis of amplified CSF samples

Figure 1 shows the result of CSF specimens using ethidium bromide staining as a detection method. Each specimen was analysed in duplicate – 1 spiked and 1 unspiked. A positive result of a spiked and unspiked samples was interpreted as a positive result. A positive result of a spiked sample and a negative result of an unspiked sample was interpreted as a negative result. Specimens with *M. tuberculosis* would give positive signals for both samples. A positive result was seen as a band on the gel at the 245 bp region. Positive bands were seen in the reference control according to their molecular weight marker. Out of 20 CSF samples tested for the presence of

DNA for *Mycobacterium tuberculosis*, 16 samples were positive by PCR and 4 samples were negative (80% positive). All of the 20 patients were initially diagnosed as tuberculous meningitis on admission to the hospital. The case records of the patients and PCR results of the CSF samples were analysed to assess the relevance of PCR methodology in the diagnosis of TB meningitis cases.

DISCUSSION

Table 1 shows the results of PCR for TB DNA in the 20 CSF samples of meningitis cases. The results were also assessed with the clinical and laboratory findings of the patients.

Among the 20 cases, it could be seen that 10 cases were positive for all the three factors (criteria) collected for the meningitis

patients gave no history of TB contacts, the chest X-rays were normal and the CSF samples were either normal or suggestive of pyogenic meningitis.

The six remaining CSF samples gave controversial results for tuberculous infection regarding positivity for chest X-ray, history of TB contact and CSF findings. These six CSF samples were positive by PCR for TB DNA. PCR technique is invaluable in such cases. Positive controls and negative controls are included in each test run to validate the results.

PCR can specifically amplify discrete fragments of DNA in which target material is present in only picogram quantities. Thus researchers have been quick to apply the PCR to the diagnosis of tuberculosis [7]. False positives in the PCR can be caused by

Table 1. Relevance of PCR test with other findings in potential tuberculous meningitis patients

Group No.	TB contact in the family	Chest X-ray (primary complex)	CSF findings			PCR	No. of cases	Remarks
			Sugar	Protein	Cell count			
1	+	+	↓	↑	↑ (mainly L)	+	10	All three investigations points to TBM
2	-	-	↓	↑	↑ (mainly L)	+	2	Only CSF findings
3	+	+	Normal	Normal	↑ (mainly L)	+	2	CSF controversial
4	-	-	↓	↑	↑ (mainly N)	+	2	All 3 controversial
5	-	-	Normal	Normal	Normal	-	2	Not suggestive of TBM
6	-	-	Normal	Normal	↑ (mainly N)	-	1	? pyogenic M
7	-	-	↓	↑	↑ (mainly N)	-	1	? pyogenic M

TB = tuberculosis
TBM = tuberculous meningitis

N = neutrophils
L = lymphocytes

CSF = cerebrospinal fluid

patients which were favourable for the diagnosis of tuberculous meningitis – history of TB contact in the family, chest X-ray showing primary complex and CSF findings showing the features of TB infection – sugar content markedly reduced, protein content increased and cell count significantly increased with majority of lymphocytes. These 10 cases were straight forward cases of TB meningitis and were also positive by PCR.

Four CSF samples were negative for TB DNA by PCR. For all these 4 samples, the

cross contamination in the system. The sensitivity of the technique can itself be a drawback unless great care is taken in handling samples by taking special precautions as required. It was stated that 100% specificity is possible in PCR, which would be a definite advantage over the immunoassays. Previous studies have shown that PCR was more sensitive than the conventional bacteriological methods and antibody ELISA for diagnosis of tuberculous meningitis. In PCR the specificity is ensured by the choice of the amplified sequence [3].

In another study specimens from patients with a definite diagnosis of tuberculosis, 80% were positive by DNA amplification and only 67% by culture. In addition, 61 specimens from patients with strong evidence of tuberculosis were positive by DNA amplification only [8]. Similar observations were reported by Pao *et al* who examined 284 specimens with a PCR assay [9]. Inhibitors of the amplification reaction could also be detected in samples, mostly blood, sputum, gastric fluid and urines. They could result from residual traces of haemoglobin, sodium dodecyl sulfate or phenol which are known to be potent inhibitors of the Taq polymerase [8].

Another advantage of PCR is that it does not necessarily require viable cells. It has been reported that PCR tests were positive up to 16 weeks after anti-tuberculous therapy at which time bacterial culture was negative. This means that a diagnosis can be made even if a patient has already received chemotherapy under a tentative diagnosis of tuberculosis, and that when urgently required, therapy can be initiated before a definite diagnosis is reached [10].

CONCLUSION

The limitations to the routine use of PCR technology in clinical laboratories in developing countries are the high cost of the reagents and equipments and the expertise of the performer to prevent cross contamination of the specimens. The cost per test is approximately US\$ 50-60 exclusive of the expensive equipment. However, the PCR test is invaluable as a rapid test for diagnosis of tuberculous meningitis especially in controversial cases where early diagnosis is essential for effective treatment and prognosis of the patient.

ACKNOWLEDGEMENTS

The work of the authors was supported by Grant MYA 6018 from the International

Atomic Energy Agency (IAEA).

REFERENCES

1. Tandon, P.N. Tuberculous meningitis. In: Vinken, P.J., Bruyn, G.M. eds. *Handbook of clinical neurology*. Amsterdam: North Holland Publishing Co., 1978; 33: 195-262.
2. Simpson, J.A. Diseases of the nervous system. In: Macleod, J., Edwards, C. & Bouchier, I. eds. *Davidson's Principles and Practice of Medicine*. Fifteenth Edition. Churchill and Livingstone: Edinburgh, London, Melbourne and New York, 1987; 591-669.
3. Shankar, P., Manjunath, N., Mohan, K.K., Prasad, K., Behari, M. & Shrinivas, Ahuja, G.K. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. *Lancet* 1991; 337: 5-7.
4. Noordhock, G.T., Kolk, A.H.J., Bjune, Gunnar *et al*. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. *Journal of Clinical Microbiology* 1994; 147: 277-284.
5. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; 239: 487-491.
6. Li, H., Gyllenstein, U.B., Cui, X., Saiki, R.K., Erlich, H.A. & Arnheim, N. Amplification and analysis of DNA sequences in single human sperm and diploid cells. *Nature* 1988; 335: 414-417.
7. Cousino, D.V., Wilton, S.D., Francis, B.R. & Gow, B.L. Use of polymerase chain reaction for rapid diagnosis of tuberculosis. *Journal of Clinical Microbiology* 1992; 224: 255-258.
8. Noel, A.B., Aznar, C., Chureau, C., Nguyen, S., Pierre, C., Bartoli, M., Bonete, R., Pialoux, G., Gicquel, B. & Garrigue, G. Diagnosis of tuberculosis DNA amplification in clinical practice evaluation. *Lancet* 1991; 338: 364-366.
9. Pao, C.C., Yen, T.S., You, J.B. *et al*. Detection and identification of *Mycobacterium tuberculosis* by DNA amplification. *Journal of Clinical Microbiology* 1990; 28: 1877-1880.
10. Narita, M., Fogashi, T., Kobayashi, H. *et al*. Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. *Acta Paediatrica* 1992; 81: 141-144.

Serum uric acid in preeclampsia

Ye Tint Lwin, **Lilian Po & *Hnin Hline*

*Physiology Research Division
Department of Medical Research

**Physiology Department
Institute of Medicine (1), Yangon

***Physiology Department
Institute of Medicine (2), Yangon

Serum uric acid was measured in thirty, 20 to 40 years old, pregnant Myanmar women suffering from preeclampsia. Eighteen cases had mild preeclampsia and twelve cases suffered from severe preeclampsia. Hypertension and proteinuria subsided after puerperium in all subjects. The mean and standard deviation of serum uric acid level (8.83 ± 2.94 mg/dl) in severe preeclampsia was significantly higher ($p < 0.01$) than that (5.40 ± 1.24 mg/dl) of mild preeclampsia. There was significant positive correlation between serum uric acid level with either systolic blood pressure ($r = +0.75$, $p < 0.01$) or diastolic blood pressure ($r = +0.78$, $p < 0.01$). There was significant negative correlation between serum uric acid level and foetal birth weight ($r = -0.40$, $p < 0.05$). Thus the raised serum uric acid level could be useful as an additional criterion in assessing foetal prognosis and severity of preeclampsia.

INTRODUCTION

At present, preeclampsia constitute one of the many threats to the mother's life during pregnancy, parturition and puerperium. It is also claimed to be one of the chief contributors to perinatal mortality. Although preeclampsia is a disease which has many pathophysiologic explanations, the importance of preventing eclamptic attack and foetal death is indisputable. Therefore, it is vital to assess the severity of the disease and to monitor closely the foetal prognosis.

Slemon and Bogert [1] first observed an association between the blood uric acid concentration and the presence of either preeclampsia or eclampsia. Since that time, a number of investigators [2,3,4,5,6] reported the etiology of hyperuricaemia of toxemia of pregnancy (preeclampsia) and

its relationship to the severity of toxæmic state. The degree of hyperuricaemia is well correlated with the severity of the clinical condition [2,3,4] and foetal prognosis [5,6]. However, other investigators failed to confirm the association between the severity of toxæmia and the degree of hyperuricaemia [7]. They found that patients with high serum uric acid of 10 mg/dl were not as ill as those with 6 mg/dl. Therefore the present study was conducted to find out whether the determination of serum uric acid level might be of help in assessing severity of maternal condition and foetal prognosis.

MATERIALS AND METHODS

Subjects

The subjects were from the Obstetrics and Gynaecological Unit of the Defense Services General Hospital and from the

maternity unit of the North Okkalapa General Hospital. They were selected according to the criteria laid down by Haynes [8]. Preeclampsia is characterized by any two of the triad of hypertension, proteinuria and edema or all three developing after 24th week of gestation. In this context, hypertension may be defined as a systolic blood pressure of 140 mmHg or above and a diastolic blood pressure of 90 mmHg or above. A significant proteinuria must be present in midstream urine specimen on two or more successive days during the third trimester. The edema must be present persistently in the face and hands.

The degree of preeclampsia is classified as mild if the blood pressure is between 140/90 mmHg and 160/100 mmHg after the 20th vweek of gestation with no proteinuria and as severe if the blood pressure is greater than 160/100 mmHg after the 20th week of gestation with edema and proteinuria [9].

The subjects taking diuretics and analgesics which could elevate serum uric acid were excluded from the study. Hypertension and proteinuria subsided after puerperium in all subjects.

Serum sample

The blood samples were taken from the antecubital vein on the patient's arrival to the hospital, usually in a sitting position. The blood samples were allowed to clot in a sterilized bottles and transported to the laboratory in the dark container and the serum were stored overnight at 4°C in a refrigerator.

Urine sample

Midstream specimen of urine samples were collected and tested for the presence of protein.

Methods

1. The serum uric acid level was measured by a simplified alkaline phosphotungs-tate assay method [10].

2. The blood pressure was measured with a mercury sphygmomanometer by the method described by World Health Organization [11].
3. The protein in urine was detected by sulphosalicylate test [12].

Statistical analysis

Mathematical evaluation of the results was performed by the Student's 't' test for the comparison of serum uric acid levels between mild and severe preeclampsia. Linear regression method was used for determining the relationship between serum uric acid level and blood pressure (either systolic or diastolic blood pressure) as well as for the relationship between serum uric acid level and foetal birth weight.

RESULTS

Some characteristics of the subjects are shown in Table 1. They were comparable in age, gravida, gestational period.

Table 1. Some characteristics of the subjects

	Mild preeclampsia	Severe preeclampsia
Age (years)	20-40	20-40
Gravida		
Primary gravida	13	11
Multigravida	5	1
Gestation (weeks) (mean±S.D)	37.67±1.81	37.00±2.76
Blood pressure (mmHg)		
Systolic pressure (mean±S.D)	146.57±7.67 140-160	176.25±21.23 160-240
Diastolic pressure (mean±S.D)	95.00±4.85 90-100	112.92±16.30 100-160
Edema	persistent	persistent
Proteinuria	nil	slight to solid

Table 2 shows the comparison of the mean serum uric acid levels between mild and severe preeclampsia in third trimester. The mean serum uric acid level of severe preeclampsia was significantly higher ($t>99.5$, $p<0.01$) than that of mild preeclampsia.

Table 2. Comparison between mean serum uric acid levels of mild and severe preeclampsia

	Mild preeclampsia	Severe preeclampsia	't'	'p'	Significance
No. of subjects	18	12			
Serum uric acid level (mg/dl)	5.40	8.83	>	<	Significant
(mean±S.D)	± 1.24	± 2.94	99.5	0.01	

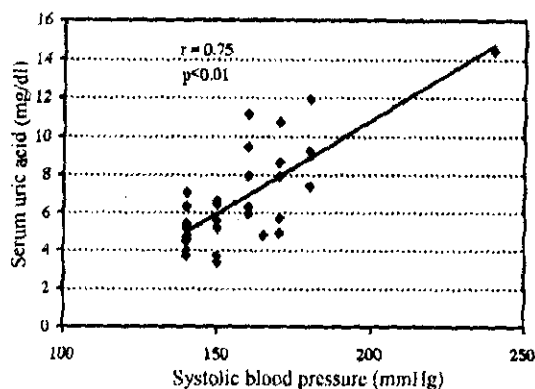


Fig. 1. Correlation between serum uric acid level and systolic blood pressure in preeclampsia

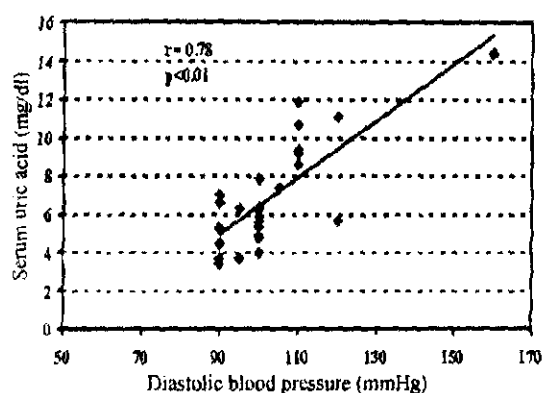


Fig.2. Correlation between serum uric acid level and diastolic blood pressure in preeclampsia

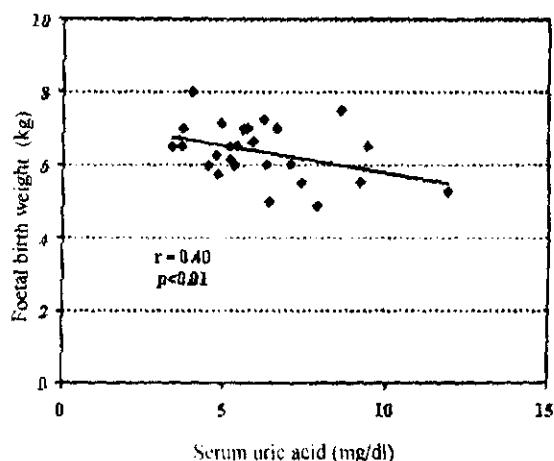


Fig.3. Correlation between serum uric acid level and foetal birth weight in preeclampsia

Figure 1 illustrates the correlation between serum uric acid level with systolic blood pressure in preeclamptic patients. A significant positive correlation was found ($r = 0.75$, $p < 0.01$).

Figure 2 illustrates the correlation between serum uric acid level with diastolic blood pressure in preeclamptic patients. There was also a significant positive correlation ($r = 0.78$, $p < 0.01$).

Figure 3 illustrates the significant negative correlation between serum uric acid level and foetal birth weight in preeclamptic patients who delivered at or after 38th week of gestation ($r = 0.4$, $p < 0.05$).

Table 3 shows clinical features, serum uric acid levels and foetal outcome of some severe preeclampsia cases who delivered prematurely due to maternal and foetal distress.

Table 3. Some severe preeclampsia cases who delivered prematurely due to maternal and foetal distress

Sr. No.	Blood pressure (mmHg)	Edema	Proteinuria	Serum uric acid level (mg/dl)	Period of gestation (weeks)	Foetal birth weight (kgs)
1	180/110	marked	+	11.1	36	2.15*
2	170/110	marked	++	10.7	34	1.43
3	170/100	marked	+	7.9	36	1.90
4	240/160	marked	+++	14.4	32	1.36*

* Still birth

DISCUSSION

Diet and drugs can modify the serum uric acid level. Since it is impossible to enforce specific dietary restriction, the subjects included in the study were those who took ordinary diet and those who took fish-eggs, bamboo-shoot, special high protein diet and drugs (analgesics and diuretics) within 2-3 days before serum sample collection were excluded from the study.

Many investigators had reported that the degree of hyperuricaemia correlated well with the severity of preeclampsia [2,3,4]. However, Pritchard and Stone [7] found no evidence to support the fact that patient with serum uric acid concentration greater than 10 mg/dl were more severely ill than those with concentration less than 6 mg/dl. Unfortunately the sizes of these groups were not reported and the characteristics of which the authors assessed the severity of this illness were not stated. In the present study, there was significant difference in mean serum uric acid level between mild and severe preeclampsia. Moreover, a significant positive correlation between serum uric acid level and blood pressure in preeclampsia was also noted.

Abnormal increase in serum uric acid level was associated with increased foetal morbidity and mortality [13]. Our finding supported this fact that those cases who delivered prematurely due to maternal and foetal distress were found to be associated with high maternal serum uric acid levels. Moreover, in those cases delivered at or after 38th week of gestation, there was a significant negative correlation between maternal serum uric acid level and foetal birth weight. This finding was in agreement with Anasura & Raman [14] and Widhole & Kuhlback [6].

The significant different level of mean serum uric acid between mild and severe preeclampsia and the significant positive correlation between serum uric acid level

and arterial blood pressure show that arising serum uric acid level could be used as an additional criterion to assess the severity of preeclampsia. A significant negative correlation between serum uric acid level and foetal birth weight also show that an abnormally raised serum uric acid level would predict a poor foetal prognosis.

ACKNOWLEDGEMENT

We wish to thank Prof. Myint Maung Maung and Prof. Daw Khin Nyunt for permission to conduct this research work at their departments and we are also grateful to all the subjects for their cooperation in our research.

REFERENCES

1. Slemons, J.M. & Bogert L.J. The serum uric acid content of maternal and foetal blood. *Journal of Biological Chemistry* 1917; 32: 63-65.
2. Stander, H.J. & Cadden, J.F. Blood chemistry in preeclampsia and eclampsia. *American Journal of Obstetric and Gynaecology* 1934; 28: 856-871.
3. Lancet M. and Fisher, J.L.. The value of blood uric acid levels in toxemia of pregnancy. *Journal of Obstetrics and Gynaecology of British Empire* 1956; 63: 110-119.
4. Pollak, V.E. & Nettles, J.B. The kidney in toxemia of pregnancy, a clinical and pathological study based on renal biopsies. *Medicine* 1960; 39: 469-526.
5. Dieckmann, W.J. The toxemia of pregnancy. 2nd ed., St. Louis, C.V. Mosby Company cited by Newcombe, D.S. Hyper-uricaemia of preeclampsia and eclampsia. *Obstetric and Gynaecology Survey* 1972; 27: 543-551.
6. Widholm, O. & Kuhlback, B. The prognosis of the foetus in relation to serum uric acid in toxemia of pregnancy. *Acta Obstetric and Gynaecology of Scandinavian* 1965; 43 (Suppl. 7): 137.
7. Pritchard, J.A. & Stone, S.R. Clinical and laboratory investigations on eclampsia. *American Journal of Obstetric and Gynaecology* 1967; 99: 754-765.

8. Haynes, D.M. Toxaemia of pregnancy. In: *Medical complication during pregnancy*. Haynes, D.M. (Ed.). McGraw Hill Book Company. The Blackiston Division, 1969; pp. 1-66.
9. Weir, R.J. Hypertension and eclampsia in pregnancy. In: *Practical Obstetrics Problems*. Donald, J. (Ed.). 5th Ed. Lloyd-Luke (Medical Books) Ltd, London, 1979; pp. 284-320.
10. Carrol, J.J., Coburn, H., Douglass, R. & Babson, A.L. A simplified alkaline phospho-tungstate assay for uric acid in serum. *Clinical Chemistry* 1971; 17: 158-160.
11. A World Health Organization study group. Arterial hypertension. *Technical Report Series* 1978; No. 628.
12. Varley, H., Gowenlock, A.H. & Bell, M. *Practical Clinical Biochemistry*, 5th ed. William Heineman Medical Books Ltd. London, 1980; 1: 100.
13. Redman, C.W.G., Beilin, L.J., Bonnar, J. & Wilkinson, H. Plasma urate measurement in predicting foetal death in hypertensive pregnancy. *Lancet* 1976; i: 1270-1373.
14. Anasuya, A. & Raman, L. Significance of hyperuricaemia in preeclampsia. *Indian Journal of Medical Research* 1974; 70: 767-770.

Determination of glutaraldehyde gelification time in leprosy

**Ne Win, *San San Aye, *Khin Saw Aye & **Charles Pangi*

**Pathology Research Division
Department of Medical Research*

***Central Special Skin Clinic
Yangon General Hospital*

As a preliminary study, glutaraldehyde gelification time (GGT) was determined in 151 cases of leprosy (129 old cases and 22 new cases). It is significantly shortened in lepromatous leprosy (LL) and multibacillary (MB) cases in comparison to that of normal controls. No significant change is observed in tuberculoid leprosy (TT) and paucibacillary (PB) cases. GGT is not shortened in old cases under appropriate treatment. Since the sample size is small, a conclusive recommendation can not be given yet. But it is primarily observed that GGT test might have a potential to be applied in the establishment of diagnosis of leprosy either LL or TT and MB or PB at least at the peripheral health centres where facilities are much limited.

INTRODUCTION

Glutaraldehyde causes gelification of blood and the time required to form a gel is known as glutaraldehyde gelification time (GGT). It varies with globulin and fibrinogen level in blood. It is shortened not only by increased gamma-globulin levels but also by increased amount of fibrinogen, singly or both [1]. In pulmonary tuberculosis it is significantly shortened [2]. For its great advantages like ease, rapidity, negligible cost, acceptable specificity and sensitivity, GGT has been proposed to be used as a reliable screening test for pulmonary tuberculosis [2,3,4]. GGT was also proposed to be used as a reliable indicator for the assessment of response to anti-tuberculous treatment in children [5].

With regards to causal agents, course of the disease, changes of protein profile and immunopathogenesis, leprosy is close to tuberculosis. So there is a high possibility that some significant changes in GGT may also occur in leprosy. With such hypothesis a cross-sectional study was conducted

focusing on changes of GGT in various types of leprosy.

MATERIALS AND METHODS

Study design

A one-year cross-sectional study effective July 1994.

Patients/subjects

Total 151 cases of leprosy attending to Central Special Skin Clinic, YGH. Among them 22 cases were new (before initiation of treatment) and 129 cases were old (under multidrug therapy-MDT).

Clinical data

A senior consultant dermatopathologist, in-charge of the Central Special Skin Clinic, YGH, examined the cases and classified according to Ridley and Jopling [6]. After microscopic examination, cases were classified into multibacillary (MB) and paucibacillary (PB) leprosy according to bacteria density or count.

Materials and reagents

- One and half millilitre (1.5 ml) of venous blood in anticoagulant EDTA.
- 2.5% glutaraldehyde solution in normal saline
- Sterile test tubes or vials
- Clock or timer

Method (GGT test)

To one ml of 2.5% glutaraldehyde solution in normal saline in a test tube or a vial 1 ml of whole blood in EDTA was added and gently mixed. The formation of a gel was then observed every minute for at least 20 minutes. Two to five blood donors from National Blood Bank were tested as normal controls on every day of test done on patients. There were altogether 47 cases as normal controls in this study. To prevent from personal variation of the result, GGT was performed by only one researcher throughout the study.

RESULTS AND FINDINGS

The age of the youngest patient was 9 years and the oldest was 69 years. GGT values in normal control blood donors ranged from 10 minutes to more than 20 minutes.

Table 1 shows GGT results in 22 new cases. Three of four lepromatous cases have GGT <10 min (75%). Among 18 cases of tuberculoïd leprosy, four cases have <10 min of GGT (28%).

Table 1. Distribution of GGT in different types of leprosy (new cases)

Types/cases	GGT (<5min)	GGT (6-9 min)	GGT (>10min)
LL/0	-	-	-
BL/4	1	2	1
BB/1	-	1	-
BT/9	1	1	7
TT/8	1	1	6

LL = lepromatous

BL = borderline lepromatous

BB = indeterminate

BT = borderline tuberculoïd

TT = tuberculoïd leprosy

Table 1a. Distribution of GGT among new leprosy cases classified by bacilli count

Groups	GGT (<5 min)	GGT (5-9 min)	GGT (>10min)	Total
MB	2	3	1	6
PB	1	2	13	16

MB = multibacillary

PB = paucibacillary

Table 2. Distribution of GGT among old leprosy cases

Treatment Duration (yrs)	GGT <5min	GGT 5-9 min	GGT >10 min	Total
<1/2	3	2	41	46
1/2-1	1	6	24	31
>1-2	3	5	21	29
2-5	0	3	10	13
>5	0	2	8	10
Total	07	18	104	129

In relation to bacteria count, 5 out of 6 MB leprosy cases have GGT <10 min (83.3%). Only 3 cases among 16 cases of PB leprosy have <10 min of GGT (18.7%) (Table 1a).

Table 2 shows GGT results in 129 old cases of lepromatous leprosy under different duration of treatment, 15 cases have GGT <10 min (12.6%). Out of 37 cases of tuberculoïd cases under treatment, 10 cases have GGT <10 min (27.3%).

Statistically, GGT in overall cases of leprosy is observed as follows: sensitivity 90.0%, specificity and positive predictive value 100%.

DISCUSSION

GGT is found markedly changed in leprosy. It is shortened in significant portion of new cases. More than 75% of new cases of LL and more than 83% of new cases of MB leprosy are associated with shortened GGT (<10 min) compared to normal controls (>10min). Majority of patients (>85%) under MDT and other forms of appropriate

antileprosy treatment have no shortened GGT. In some of these cases it is observed that GGT is even prolonged compared to normal controls. Although the sample size is small the results obtained from this study are encouraging.

The distinction between MBL and PBL is important from management point of view and emergence of drug resistant mutant. The most important advance in management and control of leprosy is introduction of multidrug therapy (MDT) by WHO since 1982 [7]. But the distinction is not without difficulty especially in peripheral health posts where microscopy and other facilities are lacking.

The finding from this preliminary study indicates GGT test has a high potential to be used as a simple, rapid, reliable and cheap test in the identification of MBL leprosy especially in the peripheral health centres. The study is in progress. Specificity, sensitivity and cut-off values of GGT in different types of leprosy would be established if larger samples could be used in the future studies.

REFERENCES

1. Richard, F.M. & Knowles, J.R. Glutaraldehyde as a protein cross-linking reagent. *Journal of Molecular Biology* 1968; 37(3): 231-235.
2. Larssons, P., Upadhyay, M.P. & Shrestha, KB. The glutaraldehyde test as a rapid screening method for tuberculosis. A preliminary report. *Annals of Tropical Medicine and Parasitology* 1990; 84(2): 111-121.
3. Ei Ei Khin. Changes of glutaraldehyde gelification time in children with tuberculosis. *Thesis, Institute of Medicine 1, Yangon, 1993.*
4. Tin Htay. A study of glutaraldehyde gelification time as a screening test for pulmonary tuberculosis. M.Med.Sc. (Pathology). *Thesis, Institute of Medicine 1, Yangon, 1993.*
5. Khin Swe. Changes of glutaraldehyde gelification time with antituberculous treatment in children. M.Med.Sc. (Pediatrics). *Thesis, Institute of Medicine 1, Yangon, 1995.*
6. Ridely, D.S. & Jopling, W.H.O. A classification of leprosy according to immunity. A five group system. *International Journal of Leprosy* 1962; 34: 255-259.
7. WHO. Chemotherapy of leprosy for control programmes. World Health Organization. *Technical Report Series* 1982, No. 675: 25-29

**Enteropathogenic *Escherichia coli* (EPEC) from acute diarrhoeic children with
in vitro antibiotic susceptibility**

*Mar Mar Nyein, **Khin Myat Tun, Nyomie Kyaw, *Thuzar Myint, *Moh Moh Win

*Wah Wah Aung & ***Than Nu Shwe

*Bacteriology Research Division

**Clinical Research Division

Department of Medical Research

***Yangon Children's Hospital

A descriptive hospital-based study was conducted on 335 children, aged less than five years, with diarrhoea attending Children's Hospital during 1996 using convenience-sampling method. Enteropathogenic *Escherichia coli* (EPEC) was isolated from 61 cases (18.21%) of which 17.77 percent was from age group of less than six months, 13.24% from >6-12 months, and 37.50% from >12-24 months. The antibiotics resistance pattern using agar disk diffusion test shows that 90.32% of the cases of EPEC was resistant to aminobenzyl penicillin, 88.53% to streptomycin, 88.50% to ampicillin, 86.89% to tetracycline, 80.33% to carbenicillin and septrin (trimethoprim/sulfamethoxazole) and 62.30% to chloramphenicol. The sensitivity pattern of isolated EPEC to antibiotics were 98.36% to amikacin, 88.53% to gentamicin, 85.25% to sisomicin, 83.61% to nalidixic acid, 81.97% to norfloxacin, 78.69% furazolidone, 67.21% to minocycline and 59.02% to cephalothin.

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) organisms adhering to intestinal epithelial cells are important pathogens of acute diarrhoeal diseases in young children. EPEC induces host cells protein phosphorylation and increases intracellular calcium level that may initiate cytoskeletal rearrangement. The outbreaks and sporadic cases of bloody diarrhoea and colitis are due to *E. coli* 0157: H7 [1]. In some outbreaks, EPEC serotypes were incriminated such as 0142:H6 [2]; a nursery and community outbreak by 086:B7 [3]; 0114 [4] and 0126 [5]. Although EPEC enteritis now appears to be of minor importance in temperate areas with good standards of hygiene, the

studies have confirmed that EPEC is still a common cause of enteritis in tropical countries [6,7] and among communities with poor standards of hygiene [8]. The antibiotics resistance of bacterial organisms is also increasing nowadays. The aim of this study is to find out the distribution of EPEC among children with acute diarrhoea and to find out the antibiotics susceptibility pattern of those strains.

MATERIALS AND METHODS

Case selection

A descriptive hospital-based study on gastroenteritis cases attending Yangon Children's Hospital during March to

October 1996 was carried out using convenience-sampling method. A total of 335 cases (195 males and 140 females) diagnosed as acute diarrhoea, were selected and their stools were collected in Cary Blair's transport media and proceeded for bacteriological examination. The definition of diarrhoea was an increase in the frequency of stools (>3 times per day) and/or increase in the liquidity of stools.

Isolation of bacterial pathogens

It was done by standard methods [9, 10].

Antibiotic susceptibility testing

It was done as described by Kirby Bauer method [11] with discs from BBL (USA), Hi (India) and Showa (Japan).

RESULTS

Age and sex distribution of Enteropathogenic E. coli (EPEC) among acute diarrhoea cases

Distribution of Enteropathogenic *E. coli* (EPEC) among acute diarrhoeal cases is shown in Table 1. From the age group of less than six months, EPEC was isolated from 17.77% of the tested cases. Similarly, EPEC was isolated from 13.24% from the age group of >6-12 months and 37.50% from the age group of >12-24 months. EPEC was not isolated from the age groups of >24-36, >36-48 and >48-60 months in this study. EPEC was isolated from 31/195 males (15.90%) and 20/140 females (14.29%) samples. Totally it was isolated from 61/335 tested cases (18.21%).

Serogroups of EPEC

The serogroups encountered in this study are shown in Table 2. The most common serogroup is O125K70 (19.67%) which is followed by O114K90 (13.11%), O126 K71 (9.84%) and O119 K69 (8.20%).

Table 1. Age and sex distribution of Enteropathogenic *Escherichia coli* (EPEC)

Age group months	Sex		Total EPEC/case
	male EPEC/case	female EPEC/case	
<6	9/50 (18.00)	7/40 (17.50)	16/90 (17.77)
>6-12	5/82 (6.09)	13/54 (24.07)	18/136 (13.24)
>12-24	17/46 (36.96)	10/26 (38.46)	27/72 (37.50)
>24-36	0/7	0/16	0/23
>36-48	0/4	0/2	0/6
>48-60	0/6	0/2	0/8
Total	31/195 (15.90)	20/140 (14.29)	61/335 (18.21)

Figures in parentheses denote percentages

Table 2. Distribution of Enteropathogenic *Escherichia coli* (EPEC) (n=61)

Sr. No.	Serogroups	Number	
		of isolates	Percentage
1	O1 K51	1	1.64
2	O6 K15	2	3.28
3	O8 K40	2	3.28
4	O25 K+	4	6.55
5	O26 K70	4	6.55
6	O27 K+	1	1.64
7	O55 K59	4	6.55
8	O78 K80	1	1.64
9	O86 K62	1	1.64
10	O112 K66	2	3.28
11	O114 K90	8	13.11
12	O119 K69	5	8.20
13	O124 K72	1	1.64
14	O125 K70	12	19.67
15	O126 K71	6	9.84
16	O127 K63	1	1.64
17	O128 K67	1	1.64
18	O142 K+	1	1.64
19	O148 K+	1	1.64
20	O157 K+	1	1.64
21	O159 K+	2	3.28

Antibiotic susceptibility pattern of EPEC

Antibiotics susceptibility pattern of isolated EPEC from acute diarrhoeal cases is shown in Table 3. In this study all the antibiotics except netilmicin and penicillin were tested on 61 isolates of EPEC. These two antibiotics were tested on 10 and 31 cases respectively due to the availability of discs.

It was clearly seen that 60 isolates (98.36%) were sensitive to amikacin, 36 (59.02%) to cephalothin, 48 (78.69%) to furazolidone, 54 (88.53%) to gentamicin, 50 (81.97%) to

protracted diarrhoea. It is non enterotoxigenic and non-invasive but biopsy showed adhesion and damage to the intestinal brush border. A similar report

Table 3. Antibiotic susceptibility of EPEC isolated from diarrhoeal cases (n=61)

Sr. No.	Antibiotics	R	I	MS	S
1	Amikacin, AN30, BBL	1	0	0	60 (98.36)
2	Ampicillin, AM10, BL	54 (88.53)	0	1	6 (9.84)
3	Carbenicillin, CB100, BBL	49 (80.33)	0	0	12 (19.67)
4	Cephalothin, CF30, BBL	6 (9.84)	1	18 (29.51)	36 (59.02)
5	Chloramphenicol, C30, BBL	38 (62.30)	0	0	23 (37.71)
6	Furazolidone, Fx50, Hi	9 (14.75)	4	0	48 (78.69)
7	Gentamicin, GM10, BBL	7 (11.48)	0	0	54 (88.53)
8	Kanamycin, K30, BBL	8 (13.12)	2	1	50 (81.97)
9	Minocycline, MnO30, Showa	10 (16.39)	9	1	41 (67.21)
10	Nalidixic acid, NA30, BBL	10 (16.39)	0	0	51 (83.61)
11	Netilmicin, NET30*, BBL	0	0	0	10 (100)
12	Norfloracin, NOR10, BBL	10 (16.39)0	0	1	50 (81.97)
13	Penicillin, P10**, Showa	28 (93.33)	1	0	2 (6.67)
14	Streptomycin, S10, BBL	54 (88.53)	1	0	6 (9.84)
15	Sisomycin, SIS10, BBL	9 (14.75)	0	0	52 (85.25)
16	Seprtin, SXT, BBL	49 (80.34)	0	1	11 (18.03)
17	Tetracycline, T30, BBL	53 (86.89)	1	0	7 (11.48)

R = Resistant

I = Intermediate

* tested on 10 cases

MS = Moderately sensitive

S = Sensitive

**tested on 31 cases

kanamycin, 50 (81.97%) to norfloxacin, and 52 (85.25%) to sisomycin.

DISCUSSION

Various studies have confirmed that EPEC is still a common cause of enteritis [12,13]. Oral challenge experiments in infants and mechanism remain have confirmed the ability of EPEC strains to cause diarrhoea, but their pathogenic mechanism remains obscure. Post-mortem studies and intubation techniques have shown that colonization of the duodenum and ileum occurs, although these regions of the gut have usually lesser *E. coli* in healthy individuals. Most EPEC strains do not produce heat labile toxin (LT) or heat stable toxin (ST) and are non invasive, although a few strains of *E. coli* 0114 and 0128 have been shown to be enterotoxigenic. Furthermore, some strains of *E. coli* 026 and 0126 produce cytotoxin, which can be detected by using vero cells. In 1980, a strain of 0125 was isolated from the duodenal aspirate of an infant with

describing a strain of *E. coli* 0111 isolated from the small bowel of two infants with chronic diarrhoea support the pathogenicity of EPEC strains [14]. Persistent diarrhoea and malnutrition [15] and antibiotics resistance of EPEC [16] was also demonstrated.

In this the study common serogroups in acute diarrhoea are 026K60, 055K59, 0114K90, 0119K69, 0125K70, 0126K71 and 0127K63. Thus, the distribution of these *E. coli* serotypes in children with acute diarrhoea might lead to prolong diarrhoea which retards the growth of the children.

The resistance pattern to commonly used antibiotics such as ampicillin, chloramphenicol and tetracycline is also an important finding because it might carry the resistant gene as other organisms, which will effect in treatment. During the last decade quinolones such as norfloxacin, ciprofloxacin and ofloxacin have emerged as drugs of choice for treatment of various enteric bacterial infections. However, usage of quinolone in treatment of bacterial enteritis

is furthermore limited for rapid development of resistance and is not used in cases of children. In this study most of the isolate organisms are sensitive to new antibiotics such as amikacin, minocycline and nalidixic acid. The susceptibility of antibiotics needs to be monitored to assess the resistance pattern throughout the year.

ACKNOWLEDGEMENTS

To Director-General Professor Dr. U Paing Soe, Deputy Director-General Dr. U Soe Thein and Director Dr. U Tun Pe for their keen interest and various supports in research activities. To the Medical Superintendent and Wards in Charge and staff of Yangon Children's Hospital their kind co-operation.

REFERENCES

- Ong, J., Zhe, L., Robins-Browne, R., Gapes, M. & O'Loughlin, E.V. Prevalence of verocytotoxic *Escherichia coli* serotype O157:H7 in children with diarrhoea attending a Sydney hospital. *Journal of Paediatrics Child Health* 1993; 29(3): 185-187.
- Kennedy, D.H., Walker, G.H., Fallow, R.J., Boyde, J.F., Gross, R.J. & Rowe, B. An outbreak of infantile gastroenteritis due to *E. coli* O142. *Journal of Clinical Pathology* 1973; 26: 731-737.
- Pal, S.C., Rao, C.K., Keresclidze, T., Krishnaswami, A.K., Murty, D.K., Pandit, C.G. & Shrivastan, J.B. An extensive community outbreak of enteropathogenic *Escherichia coli* O86:B7 gastroenteritis. *Bulletin of World Health Organization* 1969; 41: 851-858.
- Jacobs, S.L., Holzel, A., Wolman, B., Keen, J.H., Miller, V., Taylor, J. & Gross, R.J. Outbreak of infantile gastroenteritis caused by *Escherichia coli* O114. *Archives of Diseases of Childhood* 1970; 45: 656-663.
- Boris, M., Thaomason, B.M., Hines, V.D. *et al.* A community epidemic of enteropathogenic *Escherichia coli* O126:B16:NM gastroenteritis associated with asymptomatic respiratory infection. *Pediatrics* 1964; 33: 18-29.
- Hoque, S.S., Faruque, A.S., Mahalanabis, D. & Hasnat, A.S.O. Infectious agents causing acute watery diarrhoea in infants and young children in Bangladesh and their public health implications. *Journal of Tropical Pediatrics* 1994; 40(6): 351-354.
- Mayatepek, E., Seebass, E., Hingst, V., Kroeger, A. & Sonntag, H.G. Prevalence of enteropathogenic and enterotoxigenic *Escherichia coli* in children with and without diarrhoea in Esteli, Nicaragua. *Journal of Diarrhoeal Disease Research* 1993; 11(3): 169-171.
- Levine, M.M., Ferreccio, C., Prado, V., Cayazzo, M. *et al.* Epidemiologic studies of *Escherichia coli* diarrhoeal infections in a low socioeconomic level peri-urban community in Santiago, Chile. *American Journal of Epidemiology* 1993; 138(10): 849-869.
- World Health Organization. Manual of diagnostics laboratory procedure for acute enteric infections. Simplified method. Geneva: World Health Organization 1962.
- Ewing, W.H. *Edwards and Ewing's Identification of Enterobacteriaceae*. Fourth edition, 1986, Elsevier Science Publishing Company Incorporation, New York.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. & Turck, M. Antibiotic susceptibility testing by a standardized simple disc method. *American Journal of Clinical Pathology* 1966; 45: 493-496.
- Rademaker, C.M., Fluit, A.C., Jansze, M., Jansen, W.H., Glerum, J.H. & Verhoef, J. Frequency of enterovirulent *Escherichia coli* in diarrhoeal disease in The Netherlands. *European Journal of Clinical Microbiology and Infectious Disease* 1993; 12(2): 93-97.
- Hart, C.A., Batt, R.M. & Saunders, J.R. Diarrhoea caused by *Escherichia coli*. *Annals of Tropical Paediatrics* 1993; 13(2): 121-131.
- Scotland, S.M., Willshaw, G.A., Smith, H.R., Said, B., Stokes, N. & Rowe, B. Virulence properties of *Escherichia coli* strains belonging to serogroups O26, O55, O111 and O128 isolated in the United Kingdom in 1991 from patients with diarrhoea. *Epidemiology of Infection* 1993; 111(3): 429-438.
- Sullivan, P.B., Coles, M.A., Aberra, G. & Jungh, A.S.O. Enteropathogenic and enteroadherent-aggregative *Escherichia coli* in children with persistent diarrhoea and malnutrition. *Annals of Tropical Paediatrics* 1994; 14(2): 105-110.
- Senerwa, D., Mutanda, L.N., Gathoma, J.M. & Olsvik, O. Antimicrobial resistance of enteropathogenic *Escherichia coli* strains from a nosocomial. *APMIS* 1991; 99: 728-734.

**Cranial diabetes insipidus following a Russell's viper
(*Daboia russelli siamensis*) bite in Myanmar**

**Sann Mya, **Tun Pe & **Aye Aye Myint*

**Department of Medicine
Institute of Medicine (1), Yangon
**Venom Research Laboratory
Department of Medical Research*

Development of a mild cranial diabetes insipidus following a Russell's viper (*Daboia russelli siamensis*) bite was described. The victim had systemic envenoming with systemic bleeding manifestations; haemoptysis, haematemesis and melena. He developed polyuria 4 days after the bite. Loss of body hairs, loss of libido, lack of concentration, hoarseness of voice, intolerance to cold and loss of weight were observed 4 weeks after the bite. Diagnosis of cranial diabetes insipidus was confirmed by fluid deprivation test. The patient recovered from the illness 28 weeks after the bite.

INTRODUCTION

Snake bite is a national health problem. Russell's viper bite constitutes about 90% of the total snakebite cases. Anterior pituitary insufficiency has been reported following Bothrops bites (*Bothrop jararacussu*) bite in southern Brazil [1], Indian Russell's viper (*V. russelii russelii*) bite in southern India [2-3], Russell's viper (*D. r. siamensis*) bites in Myanmar [4]. However, there was no report of posterior pituitary dysfunction following Russell's viper bite. Development of mild cranial diabetes insipidus in a systemic envenomed Russell's viper bite patient was described.

Case report

A 29 year old male from Shwebo Township was bitten on dorsum of right foot on 22 April 1992 while in the field. He was brought to his friend's house by a cart after walking for a distance of 400 ft. Three hours after the bite, he took 10-12 indigenous pills. Seven hours later, his eyes were swollen, vomited blood, coughing up

blood stained sputum and passed black tarry stools. He was admitted to Shwebo hospital 20hr after the bite. At admission, he had puffy face, blurred vision, pain in epigastrium, and local pain and swelling. His blood pressure measured 100/60 mmHg and admission blood was incoagulable. Forty millilitres of monospecific antivenom, antitetanus toxoid, antitetanus serum 5000iu, 2 amps intravenous frusemide injections, dextrose saline infusion and intravenous hydrocortisone 100mg, 4 hourly were given. Blood was clotted when tested 1h after the antivenom. Intermittent fever was treated with chloroquine, primaquine and pyrexin.

From 4th day onwards, he started passing urine ranging from 6 to 6½ litres per 24h upto the time of discharge from the hospital. Two pkts of oral rehydration salt (ORS) was taken per day. On the 7th day, his blood pressure fell to 70/50 mmHg and parental feeding + ORS was reintroduced. On 9th day his blood pressure rose to 80/60 mmHg and chloramphenicol 250mg 6 hourly and

prednisolone 5mg twice a day were given until discharged from the hospital on day 11.

Four weeks after the bite he noticed loosening of body hair, loss of libido, lack of concentration, hoarseness of voice, intolerance to cold, feeling tired easily and loss of weight. Six weeks after the bite he was admitted to the North Okkalapa Hospital. At admission, he was pale anaemic with sparse secondary sexual hairs and blood pressure of 110/70 mmHg and pulse rate 80/min. Clinically no abnormality was detected. No history of head injury and habitual taking of indigenous medicine but history of malarial attacks was given.

INVESTIGATIONS

The volume of urine for 24h was 3L (average) (5-6L/24h) with a specific gravity of 1.005 (n=4 days). Urine Bence Jones protein was not detected. Random blood sugar level was 75 mg%. Serum electrolytes were Na⁺ 130 meq/L, K⁺ 4.2 meq/L, chloride-100 meq/L, bicarbonate-24.2 meq/L. Serum calcium level was 9.3 mg%, blood urea 25 mg% and serum creatinine 1.6 mg%. X-ray chest and skull showed no abnormality.

Hormonal levels determined at 12-19 wks after the bite were: ACTH 17 u.iu/ml (1-37u.iu/ml), cortisol 1 nmol/L (138-690 nmol/L), serum T4- 31.7 nmol (55-156 nmol), T3- 0.84 nmol (0.8-2.5 nmol), TSH- 1.537 mU/L, prolactin 3 ng/ml (0.1-6ng/ml), LH 0.78 mIU/ml (2-12mIU/ml), FSH 1.73 mIU/ml (1-12mIU/ml).

Fluid deprivation test plus vasopressin challenge carried out on the patient at 16 and 18 weeks after the bite showed that urine failed to concentrate after 8h of fluid deprivation (urine osmolality remains the same). However a double increase in urine osmolality was observed after s.c injection

of 10 units of aq. vasopressin. Water deprivation test carried out at 28 weeks after the bite showed kidney restored its concentrating power.

DISCUSSION

The study highlighted that both anterior and posterior pituitary dysfunction could develop following Russell's viper (*D. r. siamensis*) bite. Earlier studies had documented development of anterior pituitary dysfunction following *Bothrops jararacussu* [1], *Vipera russelii russelii* [2-3] and *D. russelii siamensis* bites [4].

A fluid deprivation test confirms the diagnosis of posterior pituitary dysfunction. Nephrogenic and other causes of diabetes insipidus have been ruled out. It was observed that cranial diabetes insipidus and anterior pituitary dysfunction could develop 4 days and 4 weeks after the bite respectively.

Local vascular occlusion by fibrin compounded by venom haemorrhagin damaging pituitary capillary endothelium could be major cause of the pituitary lesion in Russell's viper bite cases who developed disseminated intravascular coagulation following systemic envenoming [4]. The symptoms, 24 h urine volume and clinical recovery observed in our case are compatible with a mild form of cranial diabetes insipidus associated with anterior pituitary dysfunction. The primary lesion of the neurophysis itself or of the stalk below the median eminence could be responsible for it [5].

ACKNOWLEDGEMENTS

We would like to acknowledge the staff of the Nuclear Medicine Division, Department of Medical Research for performing hormonal assays.

REFERENCES

1. Wolff, H. Insuficiencia hipofisaria anterior por picada de ofido. *Arq bras Endocrinol Metab* 1958; 7: 25-47.
2. Eapen, C.K., Chandy, N., Kochuvarkey, K.L. *et al.* Unusual complication of snake bite: hypopituitarism after viper bite. In: Ohsaka, A., Hayashi, K., Sawai, Y. (eds.), *Animal, plant and microbial toxins*, Vol. 2, New York: Plenum, 1976; pp. 467-73.
3. Eapen, C.K., Chandy, N. & Joseph, J.K. A study of 1000 cases of snake envenomation. In: XI International Congress of Tropical Medicine and Malaria, Calgary, 1984.
4. Tun-Pe, Warrell, D.A., Tin-Nu-Swe, Phillips, R.E., Moore, R.A. & Myint-Lwin. Acute and chronic pituitary failure resembling Sheehan's syndrome following bites by Russell's viper in Burma. *Lancet* 1987; ii: 763-767.
5. Hall, R. Pituitary and hypothalamic disorders in Oxford Textbook of Medicine, eds. Weatherall, D.J., Ledingham, J.G.G. & Warrell, D.A. Oxford University Press, 1987.

Purification, characterization and properties of acid phosphomonoesterase from Russell's viper (*Vipera russelli siamensis*) venom

Aye Kyaw, Hla Kathy Win, San Aye, Khin Khin Aye & Khin Pa Pa Kyaw

Biochemistry Research Division
Department of Medical Research

The acid phosphomonoesterase has been purified to electrophoretic homogeneity from the venom of *Vipera russelli siamensis* (Russell's viper). The molecular weight of the purified enzyme was estimated to be 23,000 by SDS-polyacrylamide gel electrophoresis. The enzyme exhibited an apparent K_m value of 2.6×10^{-3} M. The acid phosphomonoesterase was activated by Mg^{++} and inhibited by EDTA, fluoride and Zn^{++} . Ca^{++} showed slight activating effect but cysteine did not influence the enzyme activity. The enzyme showed edema forming and myonecrotic activities but not hemorrhagic activity.

INTRODUCTION

Snake venoms belonging to Elapidae, Hydrophiidae, Viperidae and Chrotalidae show both acid and alkaline phosphomonoesterase (PME) activities having optimal pH at 5.0 and 9.5 respectively [1]. Hassan *et al.* also reported the presence of acid and alkaline PME in the venoms of four Egyptian snakes, namely two viperids (*Cerastes* and *Cerastes vipers*) and two elapids (*Naja* and *Naja nigricollis*) [2]. Besides, snake venom PME has been reported to show toxic action [3]. It has been suggested that venom (antigen) preparation consisting of important venom components only might be more appropriate for the production of highly effective antivenoms [4]. With this view in mind, purified caseinolytic activity and phospholipase A from locally available Russell's viper venom and their biological activities have been studied [5, 6]. In this paper, we report the purification and characterization of another enzyme, acid PME (EC 3.1.3.2) from the venom of *Vipera russelli siamensis* found in Myanmar (Burma).

MATERIALS AND METHODS

Materials

The lyophilized venom of Russell's viper was obtained from Myanmar Pharmaceutical Factory, Yangon (Rangoon). Albino mice were supplied by the Laboratory Animal Services Division, Department of Medical Research. CM-Sephadex C-50 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Molecular weight markers for SDS-polyacrylamide gel electrophoresis (PAGE), *p*-nitrophenylphosphate (phosphatase substrate) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Purification of acid PME

All steps were carried out at 4°C whenever necessary.

CM-Sephadex C-50 column chromatography: A sample of 150 mg of Russell's viper venom (RVV) was dissolved in 1.0 ml of 0.05 M Tris-HCl buffer (pH 7.0) and applied to a CM-Sephadex C-50 column (2.5 × 30 cm) which was preequilibrated

with the same buffer. Elution was carried out with the same buffer under a linear gradient of 0.0 – 0.2 M NaCl (200 – 200 ml). The flow rate was adjusted to 18 ml per hour and 3 ml fractions were collected. Fractions exhibiting high acid PME activities (i.e. tube 12 – 20 for PME1 and tube 30 – 45 for PME2) were separately pooled, concentrated and subjected to SDS-PAGE. The resulting PME1 was also subjected to pH profile, kinetic profile and biological activity measurements.

Acid PME activity was measured by the method of Sulkowski *et al.* (1963) using *p*-nitrophenylphosphate as substrate [7]. One enzyme unit (EU) is defined as the amount of enzyme which liberates 1 micromole of *p*-nitrophenol per minute. Protein was determined by the method of Miller (1959) [8]. SDS-PAGE was carried out according to Weber and Osborn (1969) [9]. Edema forming activity was assayed according to Vishwanath *et al.* (1987) [10].

Myonecrotic activity was measured according to Kasturi and Gowda (1989) [11] and hemorrhagic activity by the method of Kondo *et al.* (1960) [12].

RESULTS AND DISCUSSION

Crude RVV when applied to CM-Sephadex C-50 chromatography was resolved into three protein peaks 1, 2 and 3 (fig.1). When each tube was monitored for acid PME activity, two acid PME peaks were obtained and they were termed PME1 and PME2. PME1 was apparently separated from protein peak 1 whereas PME2 coincided with protein peak 2. PME1 showed a more distinct purification (about 3 fold) over the crude venom when compared to PME2 for which a purification of about 2 fold over crude venom was obtained (Table 1). It is possible that PME2, which was just stuck by secondary forces to the protein peak 2 and eluted out together with it, may represent part of PME1, the major acid PME peak.

Fractions constituting the major PME1 peak (i.e. tubes 8-12), the acid PME2 peak (i.e. tubes 30-45) and the protein peak 3 (i.e. tubes 46-70) were pooled and assayed for PME activity and protein content. The remaining fractions were also pooled and analyzed for PME and protein content to check protein recovery of the entire chromatographic procedure (Table 1). The

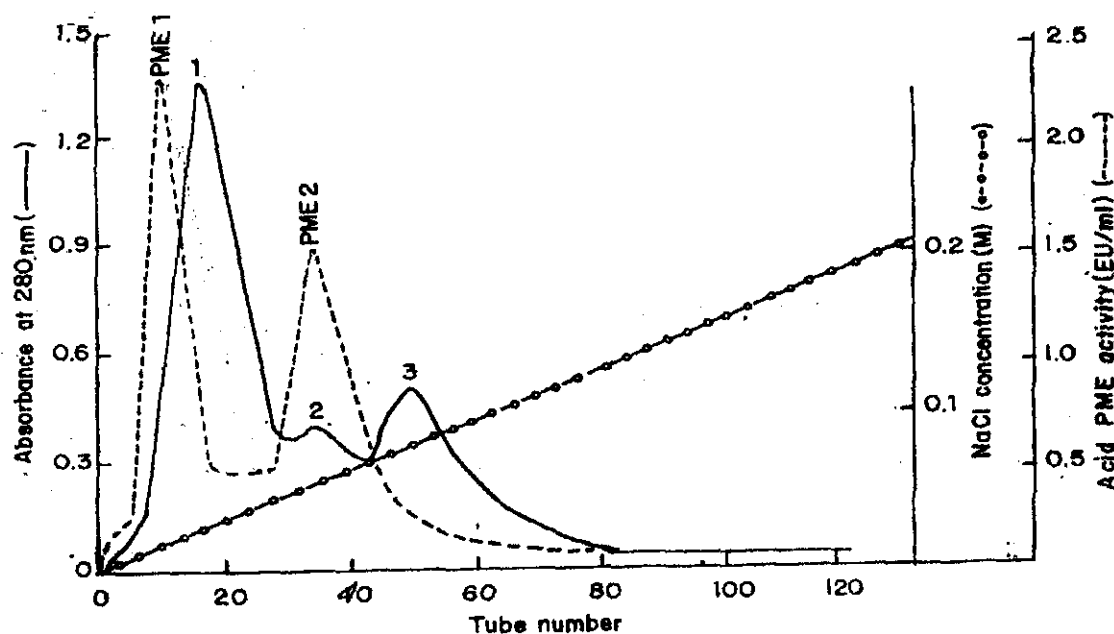


Fig. 1. CM-Sephadex C-50 ion exchange chromatography of Russell's viper venom

protein recovery was found to be 75.6%.

Table 1. Distribution of protein and acid PME activities in CM-Sephadex C-50 chromatographic fractions

Fraction	Total protein (mg)	Acid PME	
		Total EU*	EU*/mg protein
Crude RVV	104.0	160.5	1.54(1.0)**
Peak PME1	14.9	62.8	4.41(2.9)**
Peak PME2	17.9	54.7	3.05(1.9)**
Protein Peak 3	13.2	0.6	0.05
Remaining fractions	32.6	5.5	0.17
Recovery %	75.6		

*EU (Enzyme Unit) is defined as the amount of enzyme which liberates 1 umole of *p*-nitrophenol per minute

** Purification factor

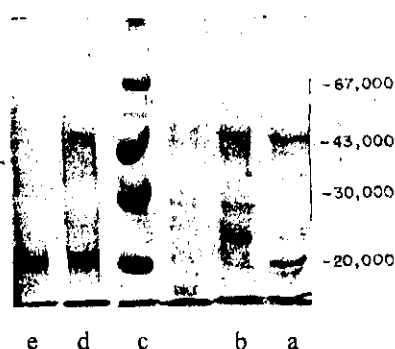


Fig. 2. SDS- PAGE of crude venom (lane a & b), molecular weight marker proteins (lane c), PME2 (lane d) and purified acid phosphomonoesterase (PME1) (lane e) of the CM-Sephadex C-50 elutes

The homogeneity of PME1 and PME2 were tested by SDS-PAGE. PME1 showed a single band (lane e in Fig.2) and its molecular weight was calculated to be about 23,000 whereas PME2 (lane d in Fig. 2) showed an extra band at about 57,000 molecular weight probably indicating that PME2 was contaminated by other proteins. It may be of interest to note that PME was apparently isolated by a single step purification on Sephadex C-50 ion exchange rechange resin probably indicating that the cat-ion is most effective in separating acid PME from other proteins of RVV which are mostly basic in nature under the conditions used in the present study.

The molecular weight of about 23,000 for acid PME as evidenced in this study is consistent with that of acid phosphatase from beef spleen according to Barman (1969) [13]. The purified acid PME (i.e. PME1) was found to have an optimum pH at 5.0 (Fig. 3) and was apparently free from alkaline PME since no optimum pH at alkaline medium was obtained.

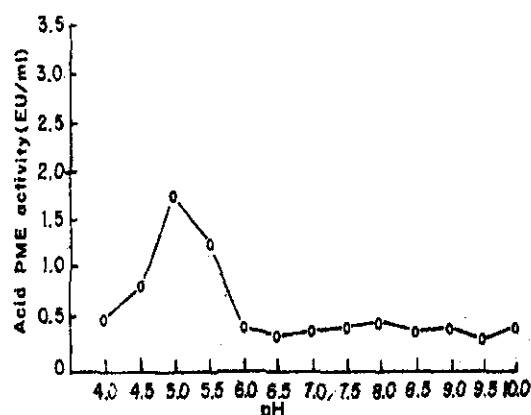


Fig. 3. pH profile of purified acid phosphomonoesterase (PME1). The following buffers were used; acetate buffer (pH4.0- 5.5), phosphate buffer (pH 6.0-8.0), glycine-NaOH buffer (pH 8.5-11.0)

It may be of interest to note that the major trace elements reported to be present in the venom of *Vipera russelli siamensis* are Ca^{++} , Mg^{++} and Zn^{++} and moreover, it was also reported that Ca^{++} and Mg^{++} present in the venom were completely removed by treatment with EDTA whereas Zn^{++} remained at 10% of the original content [14]. Thus, the effect of Ca^{++} , Mg^{++} , Zn^{++} and EDTA on the purified acid PME was studied (Table 2).

Table 2. Effect of ions, EDTA and cysteine on purified acid PME

Addition	Relative activity* (%)
None (Control)	100
Mg^{++} (0.3 mM)**	123
Ca^{++} (0.3 mM)	107
F (0.3 mM)	71
Zn^{++} (0.3 mM)	66
EDTA (0.3 mM)	59
Cysteine (0.3 mM)	98

* Relative to control. Activities were first expressed as EU per ml of enzyme solution before relative activities were calculated

** Final concentration in incubation mixture

In addition, the response of this acid PME to fluoride (the common non-specific phosphatase inhibitor) and cysteine (a thiol compound) was tested. It was found that the acid PME was activated by Mg^{++} and inhibited by EDTA, fluoride and Zn^{++} . Ca^{++} showed slight activating effect. However, cysteine did not have any influence on acid PME probably indicating that the enzyme is thiol independent.

Purified acid PME exhibited edema forming and myonecrotic activity. However, it did not show hemorrhagic activity. The edema ratios were $124.2 \pm 4.7\%$ (mean $\pm$ SE) and $137.9 \pm 2.4\%$ when 7.5 μ g each of crude venom and the purified acid PME were injected into the foot pad of mice ($n = 3$). The myonecrotic activity of the purified acid PME was observed when 5 μ g was injected into thigh muscle of mice. Macroscopically, the thigh muscle became swollen and reddish brown in color. Microscopically, there was infiltration of inflammatory cells and disappearance of nucleus in muscle cells.

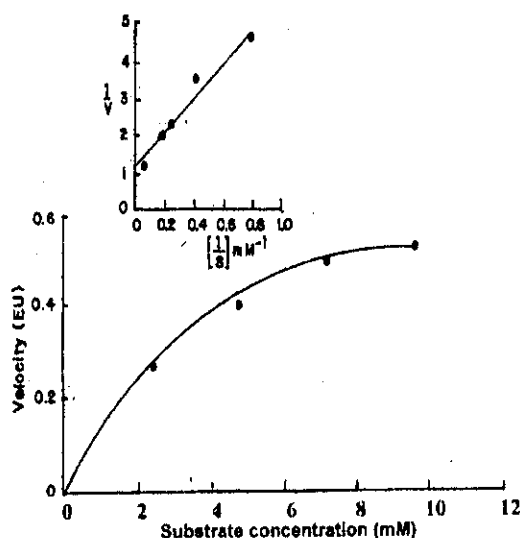


Fig. 4. The effect of substrate concentration on the rate of hydrolysis of *p*-nitrophenyl-phosphate by the purified acid PME (PME1) of Russell's viper venom

The effect of substrate concentrations on the hydrolysis of *p*-nitrophenyl phosphate by the purified enzyme is shown in Fig. 4. The

apparent K_m for the hydrolysis was found to be 2.6×10^{-3} M.

REFERENCES

1. Inwanaga, S. & Suzuki, T. Enzymes in snake venom. In: *Handbook of Experimental Pharmacology, Snake Venoms* (Edited by Lee, C.Y.), 1979; Vol. 52, pp. 61-158, Springer, Berlin.
2. Hassan, F., El-Hawary, M.F. & Ghazawy, A. Acid and alkaline phosphomonoesterases in Egyptian snake venoms. *Z. Ernährungswiss* 1981; 20: 44-54.
3. Zeller, E.A. Snake venom action. Are enzymes involved in it? *Experientia* 1977; 33: 143-284.
4. Mebs, D. & Doll, B. Snake venom enzymes: Inhibition by commercial antivenoms. In *10th World Congress on Animal, Plant and Microbial Toxins I* (Programme and Abstracts) (Edited by Gopalakrishnakone, P. & Tan, C.K.), 1991; pp. 88, National University of Singapore, Singapore.
5. Aye Kyaw, Thant Zin & Aung Khin. Partial purification and some properties of caseinolytic activity in Russell's viper venom. *Snake* 1982; 14: 18-22.
6. Aye Kyaw, San Aye, Tin Win, Hla Pe & Mya Maung. Purification, characterization and biological activities of phospholipase A from Russell's viper (*Vipera russelli*) venom. *International Journal of Biochemistry* 1994; 26: 79-83.
7. Sulkowski, E., Bjork, W. & Laskowski, M. Sr. A specific and non specific alkaline monophosphatase in the venom of *Bothrops atrox* and their occurrence in the purified venom phosphodiesterase. *Journal of Biological Chemistry* 1963; 238: 2477-2486.
8. Miller, G.L. Protein determination for large number of samples. *Analytical Biochemistry* 1959; 31: 964.
9. Weber, K. & Osborn, M. The reliability of molecular weight determination by SDS-PAGE. *Journal of Biological Chemistry* 1967; 244: 4406-4412.
10. Vishwanath, B.S., Kini, R.M. & Gowda, T.V. Characterization of three edema inducing phospholipase A₂ from Habu (*Trimeresurus flavoviridis*) venom and their interaction with the alkaloid aristolochic acid. *Toxicon* 1987; 25: 501-505.

11. Kasturi, S. & Gowda, T.V. Purification and characterization of a major phosphor-lipases A₂ from Russell's viper (*Vipera russelli*) venom. *Toxicon* 1989; 27: 229-237.
12. Kondo, H., Kondo, S., Ikezawa, H., Murata, R. & Ohsaka, A. Studies on the quantitative method for determination of hemorrhagic activity of Habu snake venom. *Japanese Journal of Medical Science and Biology* 1960; 13: 43-51.
13. Barman, T.E. enzyme Handbook, 1969, Vol. 2, pp. 521-524, Springer-Verlag, Berlin.
14. Frederick, C. & Tu, A.T. Role of metals in snake venom for hemorrhagic, esterase and proteolytic activities. *Biochemistry and Pharmacology* 1971; 20: 1549-1556.

Plants possessing antibacterial activity

*Mar Mar Nyein, **Nwe Yee Win, ***Win Myint, ***Aye Aye Thein, *Mi Mi Htwe,

***Win Win Maw & ***Aye Than

*Bacteriology Research Division, DMR

**Advanced Science and Technology, Yangon

***Pharmacology Research Division, DMR

Forty one plants were tested for antibacterial activity by using 18 species of bacteria and found to be active on some bacteria by 28 plants. The tested bacteria include: five species of *Escherichia coli*; four species of *Shigella*; three species of *Vibrios*; and one each of *Klebsiella aerogenes*, *Plesiomonas shigelloides*, *Proteus morgani*, *Pseudomonas pyocyanea*, *Salmonella typhi* and *Staphylococcus aureus*. Plants having antibacterial activity include *Ageratum conyzoides* (hkwe-thai-pan), *Azadirachta indica* (tamar); *Cassia fistula* (Ngu); *Coleus aromaticus* (ziyarywethtu); *Cuminum cyminum* (ziyarzai); *Cyperus scariosus* (Nwamyetyin); *Embelia robusta* (Eikmwethee); *Embilica officinalis* Zibyu); *Eugenia caryophyllata* (Layhnin); *Eugenia jambolana* (Thabye); *Eupatorium odoratum* (Bizat); *Euphorbia milli* (Shahzaungtinga-neah); *Euphorbia splendens* (Shahzaungtinga-neah-ni and wah); *Garcinia mangostana* (Min-good); *Girardinia zeylonica* (Petya); *Leucaena glauca* (Bawsakaine); *Neptunia oleracea* (Yehtikayone); *Nerium oleandes* (Nwethagi); *Nyctanthes arborescens* (Seikphalu); *Phyllanthus urinaria* (taungzibyu); *Pinus kesiya* (Htinyu); *Piper betle* (Kun); *Piper nigrum* (Ngayokekaung); *Plumeria rubra* (Tayokesaka-ni); *Rhoeo discolor* (Mikwingamone); *Terminalia chebula* (Panga); and *Vinca rosea* (Thinbawmany).

INTRODUCTION

Since early days, plants have been used in management of some diseases. Many drugs were produced from different kinds of plants. They owe various pharmacological activities: hypotensive, laxative, analgesic, diuretic, antidiabetic, antimicrobial action etc. Antimicrobial activity is that a substance which inhibits the growth and activity of pathogenic microbes or kills them. This antibacterial activities are not only present in moulds, actinomycetes and bacteria; but also in some. The antibiotic principle obtained from plants have been reported [1]. The objective of this study is to carry out the antibacterial activities of some plants by using the bacterial strains isolated from our clinical specimens.

MATERIALS AND METHODS

Bacterial strains used: It is shown in Table 1.

Plants used: It is recorded as shown in Table 2.

Plant extraction: Ethanolic, 50-percent ethanolic, watery extracts and essential oil extracts were prepared as described in the previous experiments [2-4]. Petroleum ether extract was also included in this study.

Determination of antibacterial activity: It was done by using impregnated discs [2] using Mueller Hinton broth and Mueller Hinton agar or Nutrient agar.

Table 1. Bacterial strains tested for antibacterial activity

Sr. No.	Bacterial strains	Code No.
1	<i>Escherichia coli</i> ETEC	DMR-H-1-3
2	<i>Escherichia coli</i> EPEC O128 K67	DMR-N-10-83
3	<i>Escherichia coli</i> EAEC O128 K67	DMR-N-3-83
4	<i>Escherichia coli</i> VTEC	Sumon -10-1
5	<i>Escherichia coli</i> ATCC	ATCC 25922
6	<i>Klebsiella aerogenes</i>	BIK418
7	<i>Plesiomonas shigelloides</i>	DMR-WT-8
8	<i>Proteus morganii</i>	HAY-1
9	<i>Pseudomonas pyocyanea</i>	DMR- ID-74
10	<i>Salmonella typhi</i>	BIK-ID-3
11	<i>Shigella boydii</i>	DMR-N-136-7
12	<i>Shigella dysenteriae</i>	DMR-ID-25
13	<i>Shigella flexneri</i>	DMR-NOGH-11-2
14	<i>Shigella sonnei</i>	DMR-NO-398-2
15	<i>Staphylococcus aureus</i>	DMR-ID-15
16	<i>Vibrio cholerae</i> Inaba	DMR-ID-26
17	<i>Vibrio cholerae</i> O139	DMR-A-535
18	<i>Vibrio cholerae</i> O1 Ogawa	DMR-A-532

RESULTS

Out of 41 plants tested 28 plants possessed antibacterial activity. In this study the petroleum ether extracts of the tested plants did not show any antibacterial activity. Plants having antibacterial activity of the tested extracts are shown in Table 3. The most effective activity was obtained in essential oil followed by 50 percent ethanolic extracts, watery extracts and the last in 95 percent ethanolic extracts. Plants having antibacterial activity include *Ageratum conyzoides* (Hkwe-thai-pan), *Azadirachta indica* (Tamar); *Cassia fistula* (Ngu); *Coleus aromaticus* (Ziyarywethtu); *Cuminum cyminum* (Ziyarzai); *Cyperus scariosus* (Nwamyetyin); *Embelia robusta* (Eikmwethee); *Emblica officinalis* (Zibyu); *Eugenia caryophyllata* (Layhnin); *Eugenia jambolana* (Thabye); *Eupatorium odoratum* (Bizat); *Euphorbia milli* (Shahzaungtinga-neah); *Euphorbia splendens* (Shahzaungtinga-neah-ni and wah); *Garcinia mangostana* (Min-good); *Girardinia zeylanica* (Petya); *Leucaena glauca* (Bawsakaine); *Neptunia oleracea* (Yehtikayone); *Nerium indicum* (Nwethagi); *Nyctanthes arbor-tritis* (Seikphalu); *Phyllanthus urinaria* (Taung-zibyu); *Pinus kesiya* (Htinyu); *Piper betle* (Kun); *Piper nigrum* (Ngayokekaung); *Plumeria rubra* (Tayokesaka-ni); *Rhoeo*

discolor (Mikwingamone); *Terminalia chebula* (Panga); and *Vinca rosea* (Thinbawmanyo).

DISCUSSION

Antimicrobial activity from plants had been tested by various investigators in many parts of the world. [4-13]. Antibacterial activity of *Piper betle* [14]; *Euphorbia milli* [15]; *Phyllanthus niruri* and *Terminalia chebula* [16]; *Emblica officinalis* and *Syzygium aromaticum* [17] and *Ageratum conyzoides*, *Coleus aromaticus*, *Cuminum cyminum*, *Nyctanthes arbor-tritis*, *Piper nigrum* and *Vinca rosea* [18]; and *Cassia fistula*, *Eugenia caryophyllata* [19] had been shown. These results suggest that plants which are widely used in remedies of traditional medicine are also proved to be of valuable antibacterial agents.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to Director-General Professor U Paing Soe and Deputy Director Dr. U Soe Thein for their keen interests and supports for conducting the research. To Directors, Dr. U Tun Pe and Dr U Myint Oo for their

Table 2. Plants tested for antibacterial activity in this study

Plants	Myanmar name	Family
1. <i>Aegle marmelos</i> (L.) Correa	Okshit	Rutaceae
2. <i>Ageratum conyzoides</i> Linn.	Hkwe-thai-pan	Compositae
3. <i>Amaranthus spinosus</i> Linn	Hinnunwe-subauk	Amaranthaceae
4. <i>Azadirachta indica</i> A. Juss	Tamar	Meliaceae
5. <i>Brassica napus</i> Linn	Monhnyin	Cruciferae
6. <i>Cassia fistula</i> Linn.	Ngu	Caesalpiniaceae
7. <i>Cassia siamea</i> Lam.	Mezali	Caesalpiniaceae
8. <i>Clerodendrum siphonanthus</i> R.Br.	Ngayanpadu	Verbenaceae
9. <i>Coleus aromaticus</i> Benth.	Ziyarywethtu	Labiatae
10. <i>Crataeva nurvala</i> Ham.	Kadet	Capparidaceae
11. <i>Cuminum cyminum</i> Linn.	Ziyarza	Umbelliferae
12. <i>Cyperus scariosus</i> Br.	Nwa Myet Yin	Cyperaceae
13. <i>Embelia robusta</i> Roxb.	Eikmwethee	Myrsinaceae
14. <i>Emblia officinalis</i> Gaertn.	Zibyu	Euphorbiaceae
15. <i>Eugenia caryophyllata</i> Thunb.	layhnin	Myrtaceae
16. <i>Eugenia jambolan</i> Lam.	Thabye	Myrtaceae
17. <i>Eupatorium odoratum</i> Linn.	Bizat	Compositae
18. <i>Euphorbia milli</i> Desmoul ex. Boiss	Shahzaungtinga-neah	Euphorbiaceae
19. <i>Euphorbia splendens</i> Boj. ex. Hook	Shahzaungtinga-neah-ni	Euphorbiaceae
20. <i>Euphorbia splendens</i> Boj. ex. Hook	Shahzaungtinga-neah-wah	Euphorbiaceae
21. <i>Foeniculum vulgare</i> Mill.	Samon-saba	Umbelliferae
22. <i>Garcinia mangostana</i> Linn.	Min-good	Guttiferae
23. <i>Girardinia zeylanica</i> Dcne.	Petya	Urticaceae
24. <i>Hydrocotyle asiatica</i> Linn.	Myinkhwa	Hydrocotylaceae
25. <i>Ipomoea aquatica</i> Forsk.	Kazunni	Convolvulaceae
26. <i>Ipomoea reniformis</i> Chios.	Myin-khwa-tu	Convolvulaceae
27. <i>Leucaena glauca</i> Benth.	Bawsakaine	Mimosaceae
28. <i>Momordica charantia</i> Linn.	Kyet-hin-ga	Cucurbitaceae
29. <i>Neptunia oleracea</i> Lour.	Yehtikayone	Mimosaceae
30. <i>Nerium oleander</i> L.	Nwe-thagi	Apocynaceae
31. <i>Nyctanthes arbor-tristis</i> Linn.	Seik-phalu	Oleaceae
32. <i>Phyllanthus urinaria</i> Linn.	Taung zibyu	Euphorbiaceae
33. <i>Pinus kesiya</i> Royle ex Gordon	Htin-yu	Pinaceae
34. <i>Piper betle</i> Linn.	Kun	Piperaceae
35. <i>Piper nigrum</i> Linn	Nganyokekaung	Piperaceae
36. <i>Plumeria rubra</i> Linn.	Tayoke-sa-ka-ni	Aocynaceae
37. <i>Rhoeo discolor</i> Hance	Mi-kwin-gamone	Commelinaceae
38. <i>Ricinus communis</i> Linn.	Kyet su	Euphorbiaceae
39. <i>Terminalia chebula</i> Retz.	Panga	Combretaceae
40. <i>Tinospora cordifolia</i> Miers	Sindonmanwe	Menispermaceae
41. <i>Vinca rosea</i> Linn.	Thinbawmany	Apocynaceae

Table 3. Plants showing antibacterial activity

Plants	Parts	Extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
<i>Aegle marmelos</i> (L.) Corr. (Ok-shit)	rind	95ETOH	-	-	-	-	-	-	-	-	-	-	-	-	12	-	N	-	-	12	
Rutaceae		50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ageratum conyzoides</i> Linn. (Hikwa-thai-pan)	whole plant	50ETOH	-	-	-	-	-	-	-	-	-	-	12	16	-	12	-	17	-	-	
Compositae		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Amaranthus spinosus</i> Linn. (Hinnunwesubauk)	whole plant	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	
Amaranthaceae		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-
<i>Azadirachta indica</i> A. Juss (Tamar)	leaves	70ETOH	-	N	N	N	-	N	-	-	-	-	N	-	-	N	-	-	-	N	
		watery	-	N	N	N	-	N	-	-	-	-	N	-	-	N	10	-	-	N	
	bark	70ETOH	-	N	N	N	-	N	13	12	10	-	N	13	-	N	15	-	14	N	
		watery	-	N	N	N	-	N	13	12	10	-	N	13	-	N	15	-	14	N	
	xylem	70ETOH	-	N	N	N	-	N	-	-	-	-	N	-	-	N	-	-	-	N	
		watery	-	N	N	N	-	-	-	-	-	-	N	-	-	N	-	-	13	-	
Meliaceae	seeds	70ETOH	-	N	N	N	-	N	-	-	-	-	N	-	-	N	-	-	-	N	
		watery	-	N	N	N	-	-	-	-	-	-	N	-	-	-	-	-	-	-	
<i>Brassica napus</i> Linn. (Monnyin)	seeds	ess. oil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	
Cruciferae																					
<i>Cassia fistula</i> Linn. (Ngu)	bark	50ETOH	-	9	-	-	-	-	-	-	-	12	-	15	12	-	12	12	N	N	
Caesalpinaceae		watery	-	-	-	-	-	-	-	-	-	-	-	N	15	-	-	-	12	N	N
<i>Cassia siamea</i> Lam. (Mezali)		50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	
Caesalpinaceae		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Clerodendron siphonanthus</i> R.Br. (Ngayanpadu)	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	
Verbenaceae		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
<i>Coleus aromaticus</i> Benth. (Ziyarywelhtu)	leaves	50ETOH	-	-	-	-	-	-	-	-	-	20	-	14	N	16	14	21	N	N	
Labiatae		watery	-	-	-	-	-	-	-	-	-	-	-	14	18	18	-	18	-	12	20
		ess. oil	28	30	26	-	26	-	-	30	-	30	28	35	N	30	30	30	N	N	
<i>Crataeva nurvala</i> Ham. (Kadet)	bark	95ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Capparidaceae		50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Cuminum cyminum</i> Linn. (Ziyarzal)	seeds	50ETOH	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	18	14	-	
Umbelliferae		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	14	14	
<i>Cyperus scariosus</i> Br. (Nwa Myet Yin)	roots	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N	
Cyperaceae		95ETOH	-	-	-	-	-	-	-	-	-	-	-	18	-	14	-	10	14	12	15
		50ETOH	-	-	-	-	-	-	-	-	-	-	-	16	-	15	-	N	13	-	15
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	
<i>Embelia robusta</i> Roxb. (Eikmwethae)	fruits	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	-	
Myrsinaceae		95ETOH	-	-	-	-	-	-	-	-	-	-	-	13	-	11	-	N	12	11	13
		50ETOH	-	-	-	-	-	-	-	-	-	-	-	16	-	12	-	N	-	-	12
		watery	16	-	-	-	-	-	-	-	-	-	20	12	15	-	N	-	-	16	
<i>Embilca officinalis</i> Gaertn. (Zilyu)	fruits	95ETOH	10	20	-	-	-	12	-	-	13	15	14	11	11	-	16	-	-	-	
		50ETOH	12	12	13	15	13	13	13	13	14	12	14	13	10	13	17	-	N	N	
		watery	13	15	-	15	-	-	15	-	-	15	13	10	15	-	17	15	10	-	
	bark	95ETOH	-	-	-	-	-	N	-	-	-	-	-	12	N	-	17	15	10	-	
		50ETOH	-	-	-	-	-	N	-	-	-	-	-	12	N	-	19	N	-	-	
Euphorbiaceae		watery	-	10	-	10	10	-	11	10	-	11	16	13	-	-	22	N	12	-	
		95ETOH	13	12	15	13	-	N	-	-	-	18	13	10	N	15	14	N	13	-	
		50ETOH	-	-	-	-	-	12	-	-	-	-	11	22	-	-	18	N	14	-	
		watery	20	15	15	-	-	12	10	10	-	14	14	13	12	20	15	N	15	-	

Table 3. Plants showing antibacterial activity (contd.)

Plants	Parts	Extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Eugenia caryophyllata</i> Thunb. (Layhnin) Myrtaceae	flower	ETOH	15	15	N	15	-	N	-	10	15	10	14	N	N	10	18	N	20	10
		50ETOH	10	-	-	-	-	13	15	-	10	16	17	16	16	-	16	10	N	N
		watery	-	-	-	15	-	-	10	10	-	16	10	10	13	-	20	10	15	-
		ess. oil (Local)	20	20	20	19	18	20	20	20	23	25	N	21	22	19	19	25	-	-
		ess. oil (BDH)	16	15	16	17	12	11	12	16	15	20	N	20	20	14	15	32	N	N
<i>Eugenia jambolan</i> Lam. (Thabye) Myrtaceae	leaves	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		95ETOH	-	-	-	-	-	-	-	17	-	15	13	18	-	15	24	16	16	-
		50ETOH	-	-	-	-	-	-	12	-	17	-	18	14	19	-	25	26	20	27
		watery	-	-	-	-	-	-	-	14	20	19	15	17	11	15	18	20	24	-
<i>Eupatorium odoratum</i> Linn. (Bizat) Compositae	leaves	95ETOH	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-
		50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Euphorbia milii</i> Desmoul ex. Boiss (Shahzaungtinga-neah) Euphorbiaceae	leaves	watery	-	-	-	-	-	-	10	-	-	-	-	-	-	-	13	-	-	-
		95ETOH	-	-	-	-	-	-	10	13	-	-	12	-	17	20	14	N	N	-
		79ETOH	-	-	-	-	-	-	15	12	-	-	14	-	18	21	15	N	N	-
		50ETOH	-	-	-	-	-	-	20	15	-	-	17	14	20	23	20	N	N	12
<i>Euphorbia splendens</i> Boj. ex Hook (Shahzaungtinga-neah-ni) var. 1 Euphorbiaceae	leaves	50ETOH	-	-	-	-	-	N	-	-	-	-	16	13	22	-	17	19	17	19
		watery	-	-	-	-	-	-	-	-	-	12	13	12	20	-	20	-	13	-
<i>Euphorbia splendens</i> Boj. ex Hook (Shahzaungtinga-neah-wah) var. 2 Euphorbiaceae	leaves	50ETOH	-	-	-	-	-	N	-	-	-	-	12	12	15	-	N	16	12	16
		watery	-	-	-	-	-	-	10	-	15	15	13	11	-	-	N	10	N	N
<i>Foeniculum vulgare</i> Mill. (Samonsaba) Umbelliferae	seed	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	13	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	N	N
<i>Garcinia mangostana</i> Linn. (Min-good) Guttiferae	rind	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N
		95ETOH	-	-	-	-	-	-	-	-	-	14	-	14	-	13	15	-	15	-
		50ETOH	-	-	-	-	-	-	-	-	-	11	-	-	-	15	14	12	12	-
		watery	-	-	-	-	-	-	-	11	-	13	12	12	-	12	13	12	13	-
<i>Girardinia zeylonica</i> Don. (Petya) Urticaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		watery	-	-	-	-	-	-	15	15	-	-	-	-	-	-	16	-	-	-
<i>Hydrocotyl asiatica</i> Linn. (Myinkhwa) Hydrocotylaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Ipomoea aquatica</i> Forsk. (Kazunni) Convolvulaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Ipomoea reniformis</i> Chiosy (Myin-khwa-tu) Convolvulaceae	whole plant	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Leucaena glauca</i> Benth. (Bawsakaine) Mimosaceae	leaves	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N
		95ETOH	-	-	-	-	-	-	-	-	-	10	13	19	-	-	18	13	20	-
		50ETOH	-	-	-	-	-	-	-	-	-	15	14	16	-	26	17	17	22	-
		watery	-	-	10	-	-	-	23	-	15	-	15	15	16	-	24	11	18	22
<i>Momordica charantia</i> Linn. (Kyot-hin-ga) Cucurbitaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
<i>Neptunia oleracea</i> Lour. (Yahlikayone) Mimosaceae	whole plant	PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N
		95ETOH	-	-	-	-	-	-	-	-	-	-	-	14	-	N	15	-	15	-
		50ETOH	-	-	-	-	-	-	-	-	-	14	-	14	-	N	13	12	14	-
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	13	-	-
<i>Nerium oleander</i> Linn. (Nwe-thagi) Apocynaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-
		watery	-	-	-	-	-	-	-	-	-	-	-	14	-	12	-	18	16	-

Table 3. Plants showing antibacterial activity (contd.)

Plants	Parts	Extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Nyctanthes arborescens</i> Linn. (Seik-phalu) Oleaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	16	17	12	-	13	18	21	10	-
		watery	-	-	-	-	-	-	-	-	-	-	20	12	14	-	17	-	18	14
<i>Phyllanthus urinaria</i> Linn. (Taung zibyu) Euphorbiaceae		ETOH	-	15	-	-	-	N	-	-	-	-	10	N	-	-	10	N	10	N
		50ETOH	-	10	-	-	-	-	-	-	-	-	20	-	-	-	17	10	N	N
		watery	-	-	-	-	-	-	12	-	-	10	22	-	-	-	22	20	-	-
<i>Pinus kesiya</i> Royce ex Gordon (Hin-yu) Pinaceae	xylem	ess. oil	12	12	12	11	-	-	-	10	-	10	-	12	11	-	12	12	N	N
		ETOH	20	20	18	13	20	18	19	20	15	25	20	20	19	21	19	22	20	20
<i>Piper batte</i> Linn. (Kun) Piperaceae	leaves	50ETOH	16	16	18	16	16	15	17	16	15	19	23	17	18	15	15	20	22	20
		watery	13	14	13	13	15	15	17	13	15	16	24	14	15	20	25	18	20	20
		ess. oil	20	18	16	13	20	18	18	18	18	25	23	25	18	20	16	20	20	20
<i>Piper nigrum</i> Linn. (Nganyokakaung) Piperaceae	seeds	50ETOH	-	10	-	-	-	-	-	-	-	14	14	-	-	12	14	15	-	-
		watery	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	12	12
<i>Plumeria rubra</i> (Tayoke-sa-ka-ni) Apocynaceae	flower	50ETOH	-	-	-	-	-	-	-	-	-	-	14	-	16	-	-	17	-	-
		watery	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-
<i>Rhoeo discolor</i> Hance (Mi-kwin-gamone) Commelinaceae	leaves	crystals	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N	N
		PE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	N
		ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		50ETOH	-	-	-	-	-	-	-	-	-	-	14	-	12	-	N	12	-	-
		watery	9	12	-	10	-	-	-	-	-	-	-	-	-	-	N	-	-	-
<i>Ricinus communis</i> Linn. (Kyat su) Euphorbiaceae	seeds	ess. oil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
	leaves	ETOH	18	20	15	13	16	N	11	15	-	20	15	20	N	15	18	N	20	-
		50ETOH	-	-	-	-	-	N	-	-	-	-	17	20	N	-	17	N	-	-
		watery	20	20	23	13	13	-	-	17	-	22	16	20	20	20	18	N	20	-
<i>Terminalia chebula</i> Retz. (Panga) Combretaceae	bark	ETOH	-	-	-	-	-	N	-	-	-	-	-	19	N	-	17	N	-	-
		50ETOH	-	-	-	-	-	N	-	-	-	-	-	15	N	-	17	N	-	-
		watery	14	13	-	-	-	-	-	-	-	18	16	15	N	15	13	N	20	-
	fruit	ETOH	10	20	N	-	-	N	-	-	-	-	20	N	N	-	26	N	-	10
		50ETOH	12	12	14	-	-	12	-	15	12	12	15	15	15	15	19	17	20	N
		watery	13	-	15	15	-	-	16	15	-	15	20	15	13	-	23	20	N	N
<i>Tinospora cordifolia</i> Miers (Sindonmanwe) Menispermaceae	creeper	50ETOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Vinca rosea</i> Linn. (Thinbawmanyu) Apocynaceae	leaves	50ETOH	-	-	-	-	-	-	-	-	-	18	16	13	-	12	13	17	-	-
		watery	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-

figures = zone size diameter in millimeters

N = Not tested

1= ETEC

4= VTEC

7= *Plesiomonas shigelloides*10= *Salmonella typhi*13= *Shigella flexneri*16= *Vibrio cholerae* O1 Inaba

2= EPEC

5= ATCC

8= *Proteus morganii*11= *Shigella boydii*14= *Shigella sonnei*17= *Vibrio cholerae* O139

3= EAEC

6= *Klebsiella aeruginosa*9= *Pseudomonas pyocyanea*12= *Shigella dysenteriae*15= *Staphylococcus aureus*18= *Vibrio cholerae* O1 Ogawa

suggestions and criticisms for the research.

Last, but not the least to the staff of Bacteriology and Pharmacology Research Divisions for their technical assistance.

REFERENCES

1. Eilert, U., Wolters, B. and Nahrstedt A. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. *Planta Medica*, 1981; 42: 55-61.

2. Mar Mar Nyein, Chit Maung, Mya Bwin and S. J. Tha. *In vitro* testing of various indigenous plant extracts on human pathogenic bacteria. *Myanmar Health Sciences Research Journal*, 1991; 3: 89-99.
3. Mar Mar Nyein, Chit Maung and Mya Bwin. Investigation of antibacterial activity of three traditional medicine formulations. *Myanmar Health Sciences Research Journal*, 1996; 8(1):20-24.
4. Mar Mar Nyein, Win Myint, Mu Mu Sein Myint, Mya Bwin and Tin Aye. Antibacterial properties of essential oils from six medicinal plants. *Myanmar Health Sciences Research Journal*, 1996, 8(2): 62-65.
5. Rios, J. L., Recio, M. C. and Villar, A. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *Journal Ethnopharmacology*, 1987; 21(2): 139-152.
6. le-Grand, A., Wondergem, P. A., Verpoorte, R. and Poussel, J. L. Anti-infectious phytotherapies of the tree-savannah of Senegal (West Africa) II. Antimicrobial activity of 33 species. *Journal Ethnopharmacology*, 1988; 22(1): 25-31.
7. Forestieri, A. M., Pizzimenti, F. C., Monforte, M. T. and Bisignano, G. Antibacterial activity of some African medicinal plants. *Pharmacology Research Community*, 1988, 20, Suppl. 5: 33-36.
8. Phadke, S. A. and Kulkarni, S. D. Screening of *in vitro* antibacterial activity of *Terminalia chebula*, *Eclapta alba*, and *Ocimum sanctum*. *Indian Journal Medical Sciences*, 1989, 43(5): 113-117.
9. Chen, C. P., Lin, C. C. and Namba, T. Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*. *Journal Ethnopharmacology*, 1989; 27(3): 285-295.
10. Caceres, A., Fletes, L., Aguilar, L., Ramirez, O., Taracena, A. M. and Samayoa, B. Plants used in Guatemala for the treatment of gastrointestinal disorders. 3. Confirmation of activity against enterobacteria of 16 plants. *Journal Ethnopharmacology*, 1993, 38(1): 31-38.
11. Perez, C. and Anesini, C. Antibacterial activity of alimentary plants against *Staphylococcus aureus* growth. *American Journal Chinese Medicine*, 1994, 22(2): 169-174.
12. Grosvenor, P. W., Gothard, P. K., McWilliam, N. C., Supriono, A. and Gray, D. O. Medicinal plants from Riau province, Sumatra, Indonesia. Part 1: Uses. *Journal Ethnopharmacology*, 1995, 45(2): 75-95.
13. Rabe, T. and van-Staden, J. Antibacterial activity of South African plants used for medicinal purposes. *Journal Ethnopharmacology*, 1997, 56(1): 8187.
14. Win Myint and Mar Mar Nyein. Antibacterial activity of *Piper betle* Linn. leaf. *DMR Bulletin*; 1993; 1: 10.
15. Nwe Yee Win. Bactericidal activity and chemical constituents of Shahzaung-tinga-neah. M.Sc. (Chemistry) Thesis; 1996; Yangon University
16. Thandar Oo. Studies on the *in vitro* bacterial activity of some indigenous plant extracts. M. Sc. (Botany) Thesis; 1998. Yangon University
17. Khin Min Min Phy. The *in vitro* antimicrobial activity of some indigenous plant extracts. M. Sc. (Botany) Thesis; 1998; Yangon University
18. Mi Mi Htwe. Evaluation of the effect of some indigenous plant extracts on bacterial activities. M. Sc (Zoology) Thesis; 1998. Yangon University
19. Mu Mu Thin. Antibacterial properties of some Myanmar medicinal herbs. M. Sc (Zoology) Thesis; 1998; Yangon University.

**Antivenom abuse: a review of antivenom policy in management of Russell's
viper bite cases of six township hospitals**

Tun Pe, Aye Aye Myint & Nu Nu Aung

Venom Research Laboratory
Department of Medical Research

Antivenom policy in management of 312 Russell's viper (*Daboia russelii siamensis*) bite cases of six township hospitals (Danubyu, Yegyi, Nyaunglaybin, Myinmu, Kyaukse and Taungdwingyi) was reviewed. The victims (7-29%) from Danubyu, Yegyi and Taungdwingyi received one to 4 ampoules of monospecific liquid antivenom at the villages. Antivenom was given at the discretion of the medical officer in charge of the hospitals. Majority of the cases were treated with liquid antivenom. 28.2% of the victims suffered from no envenoming, 29.4% local and 42.3% systemic envenoming, of which 41.7% developed complications like systemic bleeding, hypotension and renal failure. 50% of non-envenomed cases were given 1-4 ampoules of antivenom. 72.8% of local envenomed cases received 3-4 ampoules, which could have been managed with 1-2 ampoules of antivenom. 72.7% of systemic cases were treated with 4 ampoules of antivenom and 29% of systemic cases with complications received 5-30 ampoules of the antivenom. Pyrogen reactions developed in 50.4% of the cases. Normal clot restoration fails to occur in 51.5% of the systemic cases treated with 4 ampoules of antivenom in 6hr (8-48h). The study highlighted that in order to save expensive antivenom, the guidelines for antivenom therapy practiced in township hospitals should be reviewed and continuing medical education should be given to medical officers looking after snakebite cases. Pyrogen free potent antivenom should be produced by antivenom manufacturer that will help in saving substantial amount of antivenom in treating systemic cases with or without complications.

INTRODUCTION

Snakebite is an occupational hazard to our farmers. With the introduction of multiple cropping the chance of contact between men and snakes becomes more and the incidence of snakebite is expected to be on the rise as well as demand for increase production of antivenom. Monospecific antivenom manufactured by Myanmar Pharmaceutical Factory is used for treating Russell's viper bite cases throughout the country. It is specific for Russell's viper (*Daboia russelii siamensis*) of Myanmar and found to be more effective than Thai Red Cross and Indian Serum Institute antivenom [1]. Four

ampoules of monospecific antivenom (1ml neutralises 2 mg of venom) are recommended for treating Russell's viper bite cases with systemic envenoming [2]. If the clotting state of the patient remains incoagulable 6h after the initial dose, another 4 ampoules of antivenom are recommended. In cases of unknown bite, antivenom is given at the discretion of the medical officer in charge. It has been found that 1-4 ampoules of antivenom were given to non-envenomed cases in our earlier study[3]. Since production of expensive antivenom could not meet the increasing demand of antivenom, it is high time to review the antivenom policy of township

MATERIALS AND METHODS

Antivenom policy of 312 Russell's viper bite cases treated at the six snakebite endemic township hospitals (Danubyu, Yegyi, Nyaunglaybin, Myinmu, Kyaukse and Taungdwingyi) was reviewed retrospectively. Twenty minute whole blood clotting test was performed according to Warrell *et al.* [4] on admission. Clotting test was carried out at 6hr intervals in patients presented with incoagulable blood (systemic envenomed cases) until normal clot restoration occurred. Clinical details of the cases were recorded in standard proforma. Antivenom was given at the discretion of the medical officer in charge of the hospital. According to the guidelines given in National Snakebite Seminar booklet, 4 ampoules of antivenom should be given to patient presented with non-clotting blood on admission and 8 ampoules to systemic cases with complications [2]. Two milliliters of blood were needed for clotting test. Serum samples from clotting tests and sample taken at 1, 2, 3 and 4hr after antivenom were collected onto filter paper strip, air dried, sealed in a polythene bag and were dispatched to Venom Research Laboratory, Department of Medical Research, Yangon for determination of venom antigen levels by enzyme immunoassay technique [5].

A total of 312 Russell's viper bite cases (excluding 8 Cobras and 2 green pit vipers) treated in 6 township hospitals were studied [3, 6-10]. Patients received 1-4 ampoules of antivenom in villages of Danuphyu (7%), Yaekyi (3%) and Taungdwingyi (22%) before admitted to the respective hospitals. It was observed that the dose of antivenom given to clotted cases in Taungdwingyi was based on the size of the snake; 2 ampoules for young snake and 4 ampoules for adult snakebites. However, in general the medical officer in charge of the hospital decided the antivenom dosage. Majority of the cases (98%) were treated with liquid antivenom.

Prehospital antivenom therapy

Two and 3 ampoules of antivenom were given to 2/30 (6.6%) of snake bite cases in Danubyu, 1-4 ampoules in Yegyi 29.4% (10/34) and 22.4% (30/134) in Taungdwingyi.

Degree of envenoming (Table).

Non-envenoming: 28.2% (88/312) of the cases had no envenoming and antivenom was not given to 50% (44/88) of them. However 50% (44/88) received antivenom: 1 ampoule (6.8%) (n=3) and 2-4 ampoules in 93.2% (41/44).

Table. Degree of envenoming of 312 Russell's viper bite vs antivenom dosage

Degree of envenoming	None		Local		Systemic envenoming	
					Systemic	Systemic + complications
Total 312	88	28.2%	92	29.4%	132 (42.3%)	
No ASV	44	50%			77 (58.3%)	55 (41.7%)
ASV	44	50%				
1 amp	3	6.8%	9	27%		
2 amps	23	52.3%	16			
3 amps	3	6.8%	11			
4 amps	15	34.1%	55	73%	56 (72.7%)	39(71%)(21*)
4.5 amps			1			
5-12 amps					21(27.3%) (2*)	
5-30 amps						16** (29%)(9*)

* Fatality

** One patient was given 30 amp. of antivenom

Local envenoming: 29.4% (92/312) of the cases suffered from local envenoming 27% (25/92) of them received 1-2 ampoules of antivenom and 73% (67/92) received 3-4½ ampoules of antivenom.

Systemic envenoming: 42.3% (132/312) of the cases developed systemic envenoming manifested by development of incoagulable blood. 77/132 (58.3%) are systemic cases; four ampoules of antivenom were given to 72.7% of the cases and 5-12 amp. of antivenom to 27.3% (21/77) of which 9.5% (2/21) died.

41.7% (55/132) of the systemic envenomed cases developed systemic bleeding complications. 5-30 amp. were given to 29.1% (16/55) of the cases. Thirty ampoules of antivenom were given to a patient presented with shock in Yaekyi. 53.8% (21/39) cases treated with 4 ampoules and 56.3% (9/16) cases treated with 5-30 ampoules are fatal.

Pyrogen reactions and clot restoration time

Pyrogen reactions following administration of liquid antivenom such as chills and rigors occurred in 50.4% (70/139) (range 21-82%). 51.5% (53/103) of the systemic envenomed cases treated with 4 ampoules of antivenom failed to have normal clot restoration in 6h (8-48h).

Venom levels and venom antigen clearance time

The average venom level of local envenomed cases is 24 ng/ml (10-45 ng/ml) (n=82) and that of systemic cases is 66.1 ng/ml (30-100 ng/ml) (n=106). In local envenomed cases 2 ampoules of antivenom took 1.19h (0.5-2h) to clear a venom level of 10-40 ng/ml (n=23).

Systemic envenomed cases treated with 4 ampoules of antivenom cleared a venom level of 30-75 ng/ml (n=48) in 2.81h (1-6h) and a venom level of 80-100 ng/ml (n=27) in 5.29h (2-12h).

DISCUSSION

Monospecific antivenom manufactured by Myanmar Pharmaceutical Factory is used for treating Russell's viper bite cases throughout the country. It is specific for Myanmar Russell's viper (*Daboia russelii siamensis*) and is more potent than Thai Red Cross and Serum Institute of India [1]. Antivenom policy and guideline for management of Russell's viper bite was clearly defined in the National Seminar on prevention and management of Russell's viper bite, September 1989 [2]. However, some medical officers looking after snakebite patients were probably not well informed of the guidelines, which are as follows:

Indications for antivenom are: (a) clinical; local swelling and snake identified, local swelling (grade 2 & above), spontaneous systemic bleeding, hypotension and (b) laboratory; non-clotting blood, serum FDP more than 80ug/ml, urine proteinuria plus 3 & above. Presence of any one of the clinical or laboratory criteria indicates antivenom therapy.

For patients with severe local swelling or incoagulable blood alone (in absence of heavy proteinuria or systemic bleeding) 4 ampoules of antivenom given intravenously in 10 minutes is indicated. If blood is still incoagulable after 6h, another dose of 4 ampoules should be repeated. For patients with incoagulable blood, heavy proteinuria and or systemic bleeding on admission, a bolus dose of 8 ampoules of antivenom given intravenously in 20 minutes is recommended.

Snake bite cases admitted to 3 hospitals received 1-4 ampoules of pre hospital antivenom therapy, which helps in early neutralisation of venom released, from the site of bite. Pre hospital antivenom therapy combined with a local compression immobilisation first aid technique was found to be effective in delaying spread of

Russell's viper venom [11]. It is difficult for local health worker to access degree of envenoming. One to 2 ampoules of antivenom are indicated for local envenomed cases [11] and 4 ampoules for patients who meet the criteria for antivenom indications [2]. It is important that antivenom should be stored in villages in an environment where it will keep its potency. Recently, a simple method of storage of antivenom in the field was described [12].

According to the findings, 28.2% (88/312) of Russell's viper bite cases were not envenomed and 50% of them were given 118 ampoules of antivenom. 29.4% of local envenomed cases were treated with 1-4.5 ampoules of antivenom, of which 73% were given 3-4.5 ampoules (257.2 ampoules), which could have been managed with 1-2 ampoules of antivenom [11]. In this context, a total of 241.5 ampoules of antivenom could have been saved in treating 312 Russell's viper bite cases if the guidelines were followed. Unknown bites whose blood is clotted on admission with minimal local swelling should be kept under observation for 24h where clotting test should be performed at 2h intervals. If the patient develops incoagulable blood during monitoring, 4 ampoules of antivenom should be given.

Twenty nine percent of systemic envenomed cases with systemic bleeding and complications, treated with 5-10 ampoules of antivenom carry a mortality rate of 56.5% (9/16). Often antivenom was given in small divided doses. A bolus dose of 8 ampoules is preferred since it clears the circulating venom rapidly. One patient from Yegyi was given 30 ampoules of antivenom to combat hypotension. It is envisaged that administration of antivenom more than 8 ampoules carries no beneficial effects and expensive antivenom is wasted. Moreover it sensitises the patients with equine gamma globulin and carries risk of developing hypersensitivity.

Delay in clot restoration time (51.5%) indicates poor efficacy of antivenom in neutralising venom procoagulant. Pyrogen reactions (50.4%) gave unpleasant experiences to snake bite patients. Production of potent pyrogen free antivenom and continuing medical education of the medical officers who are looking after snakebite cases will minimise wastage of expensive antivenom and solve the issue of increase demand for production of antivenom. It could save a substantial amount of governmental spending on health budget. Wearing of protective shoes among farmers will reduce incidence of snakebite and requirement of antivenom.

ACKNOWLEDGEMENTS

We would like to acknowledge Drs. U Kyaw Than (Danubyu), U Aung Myint (Kyaukse), U Tint Lwin (Yegyi), U Myint Soe (Myinmu), U Min Than (Nyaunglaybin), Daw Khin Aye Kyu (Taungdwingyi) and Professor Daw Sann Mya (Medical Institute I) for allowing us to use their clinical data generated from our joint collaborative studies.

REFERENCES

1. Tun Pe, Aye Aye Myint, Kyi May Htwe & Nu Nu Aung. Potency assay of antivenom: neutralisation of Russell's viper (*Daboia russelii siamensis*) and cobra (*Naja kaouthia*) venoms by Myanmar, Thai Red Cross and Indian SII antivenoms. *Myanmar Health Research Congress* 1997, Abstract, p. 36.
2. National Seminar on Prevention and Management of Russell's Viper bite. Department of Medical Research, Ministry of Health, Myanmar, August 1990.
3. Sann Mya, Tun Pe, Aye Aye Myint, Nu Nu Aung, Khin Aye Kyu & Tin Oo. Russell's viper bites in Taungdwingyi: clinical features and response to antivenom. *Myanmar Health Sciences Research Journal*.
4. Warrell, D.A., Davidson, N.McD., Greenwood, B.M., Omerod, L.D., Pope, H.M., Watkins, B.J. & Prentice, C.R.M. Poisoning by bites of the saw-scaled or carpet viper (*Echis carinatus*) in

- Nigeria. *Quarterly Journal of Medicine* 1977; 46: 33-62.
5. Tun Pe, Ba Aye, Aye Aye Myint, Tin Nu Swe & Warrell, D.A. Bites by Russell's viper (*Daboia russelii siamensis*) in Myanmar: effect of the snake's length and recent feeding on venom antigenemia and severity of envenoming. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991; 85: 804-808.
 6. Kyaw Than, Tun Pe, Aye Aye Myint & Nu Nu Aung. Clinical features and response to antivenom of Russell's viper bite cases of Danubyu, Ayarwady Division. *Myanmar Health Sciences Research Journal* 1997; 9: 93-96.
 7. Aung Myint, Tun Pe, Aye Aye Myint & Nu Nu Aung. Clinical features and response to antivenom of Russell's viper bite cases of Kyauksae, Mandalay Division. *Myanmar Health Sciences Research Journal* 1998; 19(1): 51-53.
 8. Min Than, Tun Pe, Aye Aye Myint & Nu Nu Aung. Clinical features and response to antivenom of Russell's viper bite cases of Nyaunglaybin, Bago Division. *Myanmar Health Sciences Research Journal* 1998; 19(1): 54-56.
 9. Tint Lwin, Tun Pe, Aye Aye Myint & Nu Nu Aung. Clinical features and response to antivenom of Russell's viper bite cases of Yaekyi, Ayarwady Division. *Myanmar Health Sciences Research Journal* 1998; 10(2): 102-104.
 10. Myint Soe, Tun Pe, Aye Aye Myint & Nu Nu Aung. Clinical features and response to antivenom of Russell's viper bite cases of Myinmu, Sagaing Division. *Myanmar Health Sciences Research Journal* 1997; 9(3): 159-162.
 11. Tun Pe, Khin Aye Kyu, Aye Aye Myint, Nu Nu Aung, Sann Mya & Tin Oo. Clinical significance of early intravenous antivenom in Russell's viper bite cases in Taungdwingyi district: a preliminary study. *Myanmar Health Sciences Research Journal* 1998; 10(1): 22-25.
 12. Aye Aye Myint, Tun Pe, Kyi May Htwe & Khin Aye Kyu. Potency assay of the liquid Russell's viper (*Daboia russelii siamensis*) antivenom stored at different environment. *Myanmar Health Sciences Research Journal* 1998; 10(3): 131-134.

Use of PGL-1 ELISA test for serodiagnosis of *Mycobacterium leprae* infected individuals in a highly prevalent village in Myanmar

Khin Nwe Oo, *Nwe Nwe Yin, **Kyaw Nyunt Sein, **Kyaw Myint, *Kyaw Kyaw*

*Immunology Research Division
Department of Medical Research
**Department of Health
***Central Special Skin Center

Usefulness of PGL-1 antigen for serodiagnosis of leprosy was tested in this study. The study populations were the residents of Kyan-Bo-Kone village, Nyaunglaybin Township, Bago Division. Sera from all of these residents were tested by enzyme linked immunosorbent assay at a dilution of 1:300 for immunoglobulin M(IgM) antibodies to NT-P-BSA. All results were expressed as optical density with a control pool of sera derived from 10 multibacillary patients. Geographically matched normal control sera were obtained from 60 clinically normal residents of a nearby village. A total of 268 sera were tested with ELISA on first year (2000) and 230 on second year (2001). The seropositives and seronegatives on first year were 62 (23.13%) and 206 (76.87%) second year were 38 (16.52%) and 192 (83.48%). On second year, negative to positive seroconversion was 14, positive to negative was 35 and persistent seropositives were 19 subjects. The highly positives were 6 and 8 subjects on first and second year respectively. This study reports on the usefulness of an IgM PGL-1 ELISA for serodiagnosis of leprosy in the first and second year of a prospective longitudinal community survey in a highly prevalent village in Myanmar.

INTRODUCTION

Leprosy has been endemic in Myanmar since the ancient days. Among the communicable diseases, it causes the greatest socio-economic problem due to disfigurement and disabilities. With the advice of World Health Organization (WHO), the present Multi Drug Therapy (MDT) regimen with fixed duration was initiated in 6 hyperendemic divisions, ie. Ayeyarwady, Bago, Magway, Mandalay, Sagaing and Yangon in a phase by vertical staff since 1988. But at the end of 1990, it was recognized that the present manpower of leprosy control programme would not be sufficient to cover all the case within the short period. Hence in addition to previously integrated activities, MDT

activities was also integrated into Basic Health Services by the mid 1991 [1].

Leprosy still remains to be a public health problem of the country and it ranks No. 8 in National Health Plan II (1993-1996). At the end of 1996 there were only 18,969 cases and prevalence rate was 4.2/10,000 in the whole country. New cases detection in the whole country was 10.6/100,000 populations at the end of 1996. In 1999 the prevalence rate of Bago Division was 9.2/10,000 and new case detection rate was 102.3/100,000 populations. The 10-year period prevalence rate of Kyan-bo-kone village was 98%. The village is in Nyaunglaybin Township, Bago Division. MDT coverage was 100% at that time. Therefore it was chosen as a study area.

Early diagnosis of asymptomatic cases of leprosy would greatly improve the prospects for successful leprosy control [2]. It has long been hoped that this could be achieved with a specific serological test and studies of selected patients using an IgM PGL-1 ELISA have been encouraging [3]. Thus, IgM antibodies to PGL-1 were detected by ELISA in 90% and 40-60% of recently diagnosed multibacillary (MB) and paucibacillary (PB) cases respectively [4]. The current study report on the usefulness of this assay for serodagnosis in the first and second year of a prospective longitudinal community survey of leprosy in a highly prevalent village in Myanmar.

MATERIALS AND METHODS

The study populations were the residents of Kyan-Bo-Kone village, Nyaunglaybin Township, Bago Division. Ethical approval was obtained from the Ethical Committee of Department of Medical Research. Since 1991, all previous leprosy cases and new leprosy patients received WHO MDT [1]. Sera from all of these residents were tested by enzyme linked immunosorbent assay at a dilution of 1:300 for immunoglobulin M (IgM) antibodies to NT-P-BSA (kindly provided by Dr. Fujiwara, Tokyo, Japan). All results are expressed as optical density (OD) with a control pool of sera derived from 10 multibacillary patients. Geographically matched normal control sera were obtained from 60 clinically normal residents of a nearby village.

ELISA technique

ELISA microtitre plates (Fastec microplate U, Fujirebio, Inc.) with 96 U shaped wells, were coated with 50 μ l of 1 μ g/ml concentration of soluble antigen and placed at 4°C overnight. The antigen was diluted with carbonate bicarbonate buffer to get the final concentration. Next day the solution was sucked out and the plates were then washed 3 times with phosphate buffer (PBS)

containing 0.05% Tween 20 (PBST). One hundred microlitre of blocking agent was added to each well and incubated at 37°C for 1 hour. The plate was washed with PBST for 3 times again. Fifty microlitre of test or control sera, diluted in 1:300 with dilution buffer was added to each well and incubated at 37°C for 1 hour. The plate was washed with PBST for 3 times again. Fifty microlitre of goat antihuman IgM conjugated to horse-radish peroxidase, diluted in 1:2000 with dilution buffer was added to each well. It was incubated at 37°C for 1 hour. The plate was then washed with PBST for 3 times again. One hundred microlitre of substrate solution (ortho phenylene diamine) was added to each well and incubated at 37°C for 15 min. Then the reaction was stopped by adding stopping solution. Optical density (OD) was measured by ELISA reader with wavelength of 492 nm. Mean OD was calculated from duplicate results.

RESULTS

The total subjects sera tested with ELISA were 268 nos. on first year and 230 on second year. The dropouts were 38 subjects in second year. The seropositives and seronegatives in first year were 62 (23.13%) and 206 (76.87%) and second year were 38 (16.52%) and 192 (83.48%). On second year, negative to positive seroconversion was 14, positive to negative seroconversion was 35 and still positives were 19 subjects. The highly positives were 6 and 8 subjects on first and second year respectively.

DISCUSSION

In this study NT-P-BSA antigen which is trisaccharide determinant of PGL-1 was used as an antigen. The serological activities of the specific PGL-1 from *Mycobacterium leprae*, its dissected parts, and related glycolipids from other mycobacteria were examined by enzyme linked immunosorbent assay against

hyperimmune anti- *M. leprae* rabbit antiserum and sera from patients with leprosy and other mycobacterial diseases. High anti-PGL-1 IgM antibodies were found in 23 of 24 (96%) of lepromatous leprosy patients on short term chemotherapy and in 8 of 13 (62%) of tuberculoid leprosy patients. Sera from patients with tuberculosis or atypical mycobacterial infections were devoid of anti-PGL-1 activity. The structurally related PGLs from *M. kansasii* and *M. bovis* and the trisaccharide determinant of the *M. leprae* product showed no significant activity. Thus, the trisaccharide determinant of PGL-1 is specific in its structure and serological activity [4].

A positive test result cannot display a high predictive value for overt disease since infection is far more common than is evidenced by clinically manifested cases. Thus, the test should be useful for screening populations or groups of individuals at risk and would allow to detect the small proportion that are seropositive for clinical examination [5]. Only a minority of persistent seropositive persons developed leprosy. These observations suggest that subclinical infection with *M. leprae* is common in endemic countries and that PGL-1 seropositivity is a marker of subclinical infection, with poor specificity for overt disease [6]. Therefore we need to carry out follow-up studies to get persistent seropositive subjects for prophylactic chemotherapy.

This study reports on the usefulness of an IgM PGL-1 ELISA for serodiagnosis of leprosy in the first and second year of a prospective community in a highly

prevalent village in Myanmar. The IgM PGL-1 had limited value as a screening method for detection of new cases. Many normal persons had elevated IgM PGL-1 antibodies, presumably a consequence of early subclinical infection.

ACKNOWLEDGEMENT

This study was supported by a grant from Technical Core Group of Leprosy Research Grant. We thank Dr. Fujiwara, Tokyo for gifting NT-P-BSA antigen and Dr. Namisato and Prof. Kashiwabara, Tokyo for giving some supplies to carry out this research work.

REFERENCES

1. Tin Maung Aye. Leprosy Control Programme in Kalay Zone, Sagaing Division, Union of Myanmar. *Japanese Journal of Leprosy* 1997; 66: 186-189.
2. Fine PEM. Leprosy: the epidemiology of a slow bacterium. *Leprosy Review* 1982; 4: 161-188.
3. Melson, R. Editorial: Serodiagnosis of leprosy: the past, the present and some prospects for the future. *International Journal of Leprosy* 1983; 51: 235-252.
4. Cho, S.N., Yanagihara, D.L., Hunter, S.W. et al. Serological specificity of PGL-1 from *M. leprae* and use in serodiagnosis of leprosy. *Infection Immunology* 1983, 41(3): 1077-1083.
5. Gonzalez-Abreu, E., Pon, J.A., Hrnapez, P. et al. Serological reactivity to a synthetic analog of PGL-1 and the detection in an area of low endemicity. *Leprosy Review* 1996; 62: 4-12.
6. Baumgart, K.W., Britton, D.L., Mullins, R.J. et al. Subclinical infection with *M. leprae*: a problem for leprosy control strategies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1993; 87: 412-418

The establishment of Reverse transcriptase - Polymerase chain reaction (RT-PCR) technique and its application for dengue virus serotype - specific diagnosis in Myanmar

**Kyaw Zin Thant, **Si Si Hla, *Kay Thi Aye,
*Hlaing Myat Thu, *Khin Mar Aye, *Kyaw Moe, & *Soe Thein*

**Virology Research Division
Department of Medical Research (Lower Myanmar)
**Biotechnology Department
Yangon Technological University*

Since dengue virus belongs to RNA viruses, it is essential to undergo reverse transcriptase (RT) step prior to the polymerase chain reaction (PCR) procedure. We have successfully established the RT-PCR technique for the detection of RNA virus genome in our own laboratory setting for the first time in Myanmar. This study has been conducted to apply the advanced PCR technology for rapid diagnosis of dengue virus infection and serotyping in Myanmar. Serum samples were obtained from the clinically diagnosed dengue patients admitted to Yangon Children's Hospital in 1998. All samples were subjected to classical haemagglutination-inhibition (HI) assay to get the laboratory confirmation. RNA extraction from the positive samples was done by using QIAamp viral RNA purification kit. The resultant RNA was amplified by RT-PCR technique using the (4) sets of dengue serotype-specific primers. Some of the positive serum samples were undergone the Indirect fluorescent antibody technique (IFAT) which could also identify the causal dengue serotypes. The results will be discussed to highlight the advantages of RT-PCR technique to get the rapid serotype-specific diagnosis in several hours. This study indicated that the Department of Medical Research (Lower Myanmar) is capable to utilize the PCR technology, an access to the era of Molecular biology and Biotechnology, and also imparting its expertise to other scientific communities in Myanmar as being a leading research institution.

INTRODUCTION

Dengue viruses are single-stranded, positive-polarity, enveloped, RNA viruses, belonging to the genus *Flavivirus* in family *Flaviviridae* [1]. Dengue virus infection may lead to Dengue fever (DF), Dengue haemorrhagic fever (DHF) and can sometimes lead to sudden and often fatal hypovolemic shock, Dengue shock syndrome (DSS) [2,3,4]. It is caused by four serotypes of Dengue virus (DEN 1, DEN 2, DEN 3, DEN 4). Each of four Dengue serotypes can be genetically [5,6,7] and antigenically [8] subdivided into subtypes that may vary in virulence [9].

Dengue virus infection continues to present a serious health problem in many tropical areas of the world, especially in Southeast Asia [10]. Dengue fever is the most important mosquito-borne viral disease of man, causing in tropical latitude millions of cases annually [2]. The global distribution of Dengue includes the Americas, Asia and the Pacific Islands [11]. The rise of epidemics outside the Southeast Asian endemic part of the world results from the introduction of new variant and serotypes, mainly by means of air travel [12].

The basic method of a routine serological diagnosis of dengue virus infection is anti-dengue virus antibody detection in blood

samples (enzyme immunoassay, haemagglutination-inhibition (HI) or complement fixation). The increase of antibody titre in convalescent serum could be false positively produced as the result of cross-reaction among flaviviruses. Serologic identification usually take one or more week before the diagnosis could be confirmed, because it needs paired samples (acute and convalescence).

The dengue virus isolation is more definitive than the other techniques. The virus isolations either by mosquito cell culture or mosquito inoculation are most successful during acute infection, 3-5 days after onset of the illness. However, it requires a relatively high level of technical skills, it is time consuming and high cost.

Recently, Polymerase Chain Reaction (PCR) technique has been introduced for the detection, identification and typing of dengue virus isolates. PCR is a sequential *in vitro* enzymatic amplification of specific template DNA segment that lies between two regions of known sequence, using heat-resistant DNA polymerase. Since the PCR was invented in 1985 [13], many DNA viruses had been detected by this advanced technique. RNA viruses had also been detected by the variant of PCR, namely Reverse transcriptase-polymerase chain reaction (RT-PCR). Nowadays, PCR technology plays an important role in the detection and characterization of the genomes of many living organisms and in the manipulation of the recombinant genes. For serotyping, PCR technique would be more rapid and more specific than the conventional serological techniques [12,14,15,16].

This research study aims at the application of PCR technology and evaluation of its role in the diagnosis of dengue virus infection in Myanmar with the following objectives.

- (1) to apply PCR technology for dengue virus detection and serotyping in

Myanmar.

- (2) to identify the advantages of PCR technology in comparison with the existing routine HI test and Indirect fluorescent antibody technique (IFAT).
- (3) to explore the feasibility and logistics support for the application of PCR technology at the central level laboratory.

MATERIALS AND METHODS

Blood Collection

Blood samples of about 2 ml in volume (both from acute phase, S_1 , and convalescent phase, S_2 , of each patient) were collected from a total of (50) patients admitted to Yangon Children's Hospital (YCH) being diagnosed clinically as dengue virus infection according to the WHO criteria [4].

Blood collection was commenced from August, 1998 until the desired number of specimen was collected. Venepuncture was done on the day of admission (for S_1) and 14 days after admission (for S_2). Serum separation was done prior to the storage at 80°C . The samples were kept in cold storage until the further experimental steps were proceeded.

Haemagglutination-Inhibition test

Classical Haemagglutination-Inhibition (HI) test was performed on all serum samples according to the method of Clarke and Casals [17].

Virus isolation and serotyping of dengue virus by Indirect Fluorescent Antibody Technique (IFAT)

Selected dengue positive serum samples tested by HI test were inoculated into *Aedes albopictus* clone C6/36 cell lines. The infected culture fluid was harvested 7 days after incubation. Serotyping was done by Indirect fluorescent antibody technique

Table 1. Nucleotide sequences of dengue virus primers

Code	Sequence	Position	Reference(s)
D1S	GGACTGCGTATGGAGTTTTG	2229-2248	19
D1C	ATGGGTTGTGGCCTAATCAT	2718-2699	19
D2S	GTTCTCTGCAAACACTCCA	1203-1222	20,21
D2C	GTGTTATTTGATTCCTTG	1432-1413	20,21
D3S	GTGCTTACACAGCCCTATTT	2253-2272	22
D3C	TCCATTCTCCCAAGCGCCT	2572-2553	22
D4S	CCATTATGGCTGTGTTGTTT	3973-3992	23,24
D4C	CTTCATCCTGCTTCACTTCT	4370-4351	23,24

*S. sense primer; C. complementary primer; D1,-2,-3,-4; dengue virus types 1,2,3 and 4

(IFAT) according to the method of Kuberski and Rosen [18].

RNA extraction by QIAamp Viral RNA purification kit

Dengue RNA extraction from selected serum samples already tested as dengue positive by HI test, were performed by the QIAamp Viral RNA purification kit according to the manufacturer's instructions [QIAGEN Inc., USA].

Optimization of Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) technique using dengue seed viruses

Dengue seed viruses: The following dengue seed viruses (three local strains and one reference strain) were used to optimize successfully the RT-PCR reaction. These seed viruses are dengue virus infected C6/36 culture fluid which were inoculated to C6/36 mosquito cells culture and were harvested one week after incubation at 28°C and stored at -80°C until use.

- (1) Dengue serotype 1 - D1-008 (local strain)
- (2) Dengue serotype 2 - D2-009 (local strain)
- (3) Dengue serotype 3 - H-87 (reference strain)
- (4) Dengue serotype 4 - D4-007 (local strain)

Primers

Four primer pairs which were selected on the basis of the published sequence data of four dengue virus serotypes were either ordered from the commercially available

source or were synthesized with an Applied Bio-systems DNA synthesizer (model 392) at the Institute of Tropical Medicine, Nagasaki University, Japan. The nucleotide sequence and reference information of the primers are listed in Table 1.

RT-PCR procedure

RT-PCR procedure was done according to the method of Morita et al [14]. Briefly, five microlitres of infected culture fluid was incubated with an equal volume of detergent mix [1% Nonidet P-40 in phosphate - buffered saline (-)] in a 500 µl volume microcentrifuge tube for one minute at room temperature. This was followed by the addition of 90 µl of RT-PCR mix containing 100-200 pmoles of each primer, 0.2 mM deoxynucleoside triphosphate, 10 mM Tris (pH 8.9), 1.5 mM MgCl₂, 80 mM KCl, 0.5 mg of bovine serum albumin per ml, 0.1% sodium cholate, 0.1% Triton X-100, 10 U of Avian myeloblastosis virus reverse transcriptase (Amersham Life Science Inc., U.S.A), 4 U of Tth DNA polymerase, a thermostable DNA polymerase, (Toyobo Co., Osaka, Japan) and 10 U of RNase inhibitor (Boehringer Mannheim, Germany). In case of negative control, five microlitres of double distilled H₂O (dd H₂O) was used in stead of infected culture fluid. The reaction mixture was mixed thoroughly by vortexing followed by quick spin for few seconds. Then the reaction mixture was covered by 2 drops of mineral oil (Aldrich Chemical Co., U.S.A.) and the reaction tube was placed in a thermal cycler (Quick

Thermo Personal; Nippon Genetics, Tokyo, Japan). The first step of reverse transcriptase reaction was carried out for 10 minutes at 47°C for dengue-2 and at 50°C for dengue-1, -3 and -4, respectively. This step was followed by 35-40 cycles of PCR amplification, involving 1 minute denaturation at 94°C, 1.5 minutes annealing at 45°C and 2 minutes extension at 72°C. Annealing temperature for dengue-2 virus was 47°C in stead of 45°C for dengue -1, -3 and -4. After the amplification, 10µl of PCR product was subjected to agarose gel electrophoresis in a composite gel consisting of 2.8% NuSieve GTG Agarose (FMC Bio Products, U.S.A.) and 0.2% Agarose NA (Pharmacia, Sweden) using a running buffer 40 mM Tris acetate and 1 mM EDTA, pH 8.0. The seperated DNA fragments were visualized by ethidium bromide staining under a UV transilluminator, and their sizes were estimated by comparing with the pHY DNA molecular weight marker (Takara Biochemicals, Japan). Amplified DNA fragment from dengue viral genome by serotype-specific primer pairs were 490 base pairs (bp) for dengue -1, 230 bp for dengue- 2, 320 bp for dengue-3 and 398 bp for dengue-4, respectively. Photodocumen-

tation was made by Polaroid Direct Screen Instant Camera DS 34 for the permanent record.

RESULTS

Haemagglutination-Inhibition (HI) Assay

Fifty paired serum samples collected from the patients admitted to YCH in the year 1998, being diagnosed clinically as dengue virus infection were undergone HI assay as described in the Materials and Methods section.

The results shown that 27 serum samples (54 %) had primary dengue infection and 23 serum samples (46%) had secondary dengue infection.

Indirect Fluorescent Antibody Technique (IFAT)

Among the laboratory confirmed dengue serum samples tested by HI assay, (nine) samples have been undergone Indirect Fluorescent Antibody Technique (IFAT) as described in the Materials and Methods section. The results of IFAT method are described in the following table (Table 2).

Table 2. Results of the Indirect Fluorescent Antibody Technique (IFAT) done on dengue serum samples collected in 1998

Sr. No	Sample No.	Pt's Name initial	Age (Yrs)	Sex	Clinical diagnosis	HI Antibody titre	Dengue serotype
1	31804	HMH	14 6/12	M	DHF-GI	<1:10	D3
2	31838	SSW	1	F	DHF-GI	1:40	D2
3	31987	HMMA	6 6/12	F	DHF-GII	<1:10	D2
4	32036	KTDA	6	F	DHF-GI	1:40	D2
5	32202	WWTN	4	F	DSS	1:80	D3
6	32274	TD	3	F	DSS	1:20	D2
7	323089	OMM	12	M	DHF-GIII	1:20	D2
8	32338	NHZW	14	M	DHF-GII	<1:10	D3
9	32514	KZWK	7	F	DHF-GII	<1:10	D1

DHF = Dengue haemorrhagic fever G I, II, III = Grade I, II, III
 D1 = Dengue serotype 1 D2= Dengue serotype 2 DSS = Dengue shock syndrome
 D4 = Dengue serotype 4 D3 =Dengue serotype 3

Table 3. Results of the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) technique using dengue virus infected tissue culture fluid (TCF) or dengue viral RNA extracted by QIA amp RNA Purification kit as templates.

Sr. No	Sample No.	Pt's Name initial	Age (Yrs)	Sex	Clinical diagnosis	HI Antibody titre	Template	Dengue serotype
1	24510	TZM	10	F	DHF-GI	<1:10	TCF	D2
2	24511	TNNC	10	M	DHF-GII	1:20	TCF	D2
3	31594	TTS	8	F	DSS	1:2560	RNA	D3
4	31742	STY	7	F	DHF-GI	<1:10	RNA	D2
5	31804	HMH	14 6/12	M	DHF-GI	<1:10	TCF	D3
6	31838	SSW	1	F	DHF-GI	1:40	TCF	D2
7	31912	KMK	11	M	DHF-GI	1:20	RNA	D3
8	31987	HMMA	6 6/12	F	DHF-GII	<1:10	TCF	D2
9	32124	TA	6	F	DHF-GII	<1:10	TCF	D2
10	32202	WWTN	4	F	DSS	1:80	TCF	D3
11	32309	ONN	12	M	DHF-GIII	1:20	TCF	D2
12	32338	NHZW	14	M	DHF-GII	<1:10	TCF	D3
13	15791	TSHH	28	M	DF	1:10240	RNA	D3

DF = Dengue Fever,

DSS = Dengue shock syndrome,

RNA = Dengue viral RNA

D1 = Dengue serotype 1,

D3 = Dengue serotype 3,

DHF = Dengue haemorrhagic fever,

TCF = Dengue virus infected tissue culture fluid,

D2 = Dengue serotype 2,

D4 = Dengue serotype 4

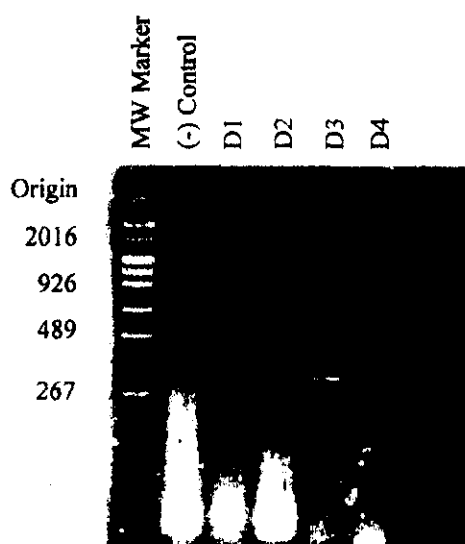


Fig. 1. Identification of dengue virus serotype by dengue virus-specific primer pairs. Infected fluid of each dengue seed virus was subjected to the RT-PCR procedure as described in Materials and Methods. MW= Molecular weight marker, (-) control=negative control, D1 – dengue virus type 1, D2 – dengue virus type 2, D3 – dengue virus type 3, D4 – dengue virus type 4 seed viruses. Dengue virus types 1,2,3 and 4 show unique length of amplified DNA fragment: (490, 230, 320 and 398 base pairs for D1, D2, D3 and D4, respectively), which was calculated from the nucleotide sequences.

Optimization and Performance of the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) technique

The optimization of the RT-PCR technique using both local and reference dengue virus strains and four pairs of dengue serotype-specific primers was successfully done. The results are shown in Fig.1.

After the optimization, RT-PCR technique has been applied on two kinds of templates, including dengue virus infected tissue culture fluid (TCF) and dengue viral RNA extracted by QIAamp RNA purification kit.

Positive bands were found in altogether (13) samples, including (nine) TCF samples and (four) samples extracted by QIAamp kit. The results are shown in Table 3 and in Fig. 2.

DISCUSSION

In this study, several laboratory techniques have been employed to get the confirmed laboratory diagnosis of dengue virus infection in Myanmar, namely HI test, IFAT test, dengue viral RNA extraction by QIAamp viral RNA purification kit and RT-PCR technique. It is the first ever reported

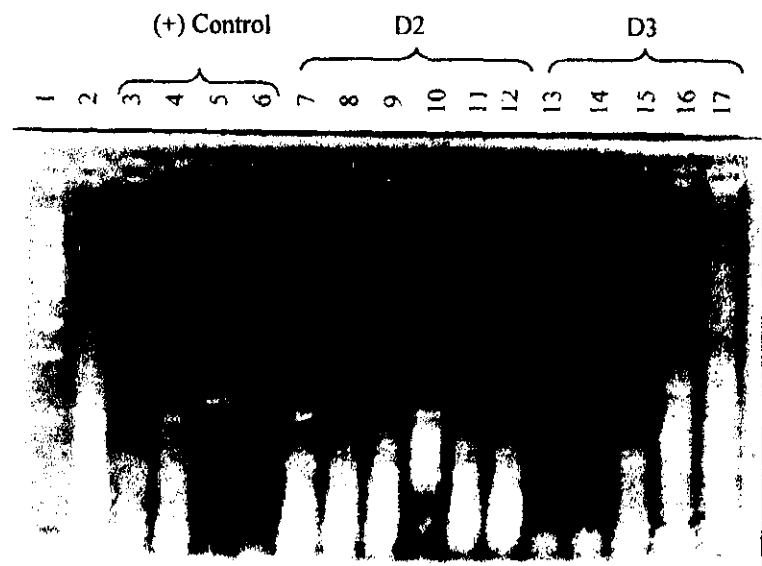


Fig. 2. Photograph of an agarose gel stained with ethidium bromide, demonstrating the serotype specificity of the four primer pairs among selected dengue virus strains from Myanmar. Lanes; 1, MW Marker; 2, (-) control; 3, D1 (+) control; 4, D2 (+) control; 5, D3 (+) control; 6, D4 (+) control; 7, 24510; 8, 24511; 9, 31838; 10, 31987; 11, 32124; 12, 32309; 13, 32202; 14, 32338; 15, 31804; 16, 31912; 17, 15791.

application of QIAamp viral RNA purification kit and RT-PCR technique in Virology Research Division of the Department of Medical Research (Lower Myanmar). The most striking feature of this study is the successful optimization and establishment of RT-PCR technique and application of this technique for serotype-specific diagnosis of dengue virus infection in Myanmar for the first time.

It has been clearly evident that DHF is endemic in Myanmar with a 3-4 year cyclical epidemic pattern. According to the Vector Borne Diseases Control Project (VBDC) report, two major DHF epidemics have occurred in Myanmar in the past 10 years, particularly in 1994 and in 1998. The number of cases were 11647 and 12668, the number of deaths were 444 and 192, and the case fatality rate (CFR) was 3.81 and 1.51, in the years 1994 and 1998, respectively [25].

According to the Yangon Children's Hospital Registry, the total number of DHF cases admitted to YCH in 1994 and in 1998 were 4376 and 4596, respectively (Unpublished data).

In the year 1998 (from January 1998 to December 1998), acute phase serum samples were collected from 2845 clinically diagnosed dengue patients admitted to the medical wards of YCH. Among these, convalescent serum samples were collected from 1061 patients. Paired serum samples were titrated for dengue haemagglutination-inhibition titres by the method of Clarke and Casals [17]. Out of 709 pairs tested, 476 (67.1%) were serologically diagnosed as dengue infection among which 129 (27.1%) had primary dengue infection and 347 (72.9%) had secondary dengue infection. Several acute phase serum samples from serologically diagnosed dengue cases were passaged in the C6/36 mosquito cell line for further isolation [26].

In this study, altogether (50) paired serum samples were tested by HI assay among which 27 (54%) had primary dengue infection and 23 (46%) had secondary dengue infection.

Out of these samples, nine samples were undergone IFAT test. The results revealed that one sample (11%) of dengue serotype-1, five samples (56%) of dengue serotype-2

and three samples (33%) of dengue serotype-3. Dengue serotype-4 was not detected by IFAT test.

We have made several attempts on optimization and establishment of RT-PCR technique in the Virology Research Division, Department of Medical Research (Lower Myanmar), and have encountered a number of obstacles including template problems, primer concentration, annealing temperature for RT and PCR reactions, and ratio of the components of RT-PCR mixtures, etc. Over thirty RT-PCR reactions have been done and different types of templates, different sets of primers, different annealing temperatures and different ratios of the components of RT-PCR mixtures have been exercised. There was no contamination among the positive controls, negative controls and samples tested except the appearance of non-specific bands in some reactions.

The pre-PCR step (preparation of RT-PCR mixture) was done at one specific area of the laboratory room whereas PCR step (thermal cycling) and post-PCR step (agarose gel electrophoresis) were done at the different laboratory room. Photodocumentation by Polaroid instant camera was done at the PCR laboratory situated in another building.

Altogether (13) samples were found to be positive by RT-PCR technique out of which seven samples (54%) were dengue serotype-2 and six samples (46%) were dengue serotype-3, respectively. Several negative results have yet to be determined whether true negative samples or dengue viral antigen level below the detectable limit (data not shown).

In one of the studies conducted by our group, the Immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA) was applied on seventy serum samples from clinically diagnosed dengue patients admitted to the North

Okkalapa General Hospital, Yangon, in 1994 [27]. Confirmed virological diagnosis was made by IgM-capture ELISA using all four types of dengue antigens raised by cell culture method. Sixty-four ELISA unit dengue-2 antigen could detect 95.15% of sixty-two total positive sera, whereas 16 unit dengue-1 antigen, 3 unit dengue-3 antigen and 4 unit dengue-4 antigen could detect 77.4%, 75.8% and 51.6% of total positive sera, respectively. It might be due to the fact that high titred dengue antigen could detect the higher number of serum samples than the low titred antigens do. On the other hand, it might possibly reflect the distribution pattern or the prevalence of dengue serotypes in Yangon community in 1994 [27].

Dengue samples of 1998 outbreak were tested in this study by conventional HI assay as well as by IFAT test and latest molecular biology technique known as RT-PCR technique. The results showed that dengue serotype-2 is the leading serotype responsible for this outbreak and serotype-3 stood next to serotype-2. The prevalence of dengue serotype-3 infection seems to have increasing trend. This data may not be fully representative for the circulating dengue serotypes in our local community because of the limited number of samples tested by IFAT test and RT-PCR technique. Nevertheless, it could be used as an indicator to assess the real image as a "tip of the iceberg" phenomenon.

The clinicians and the public health administrators should be fully aware of this laboratory finding that dengue serotypes 2 and 3 are co-circulating in our community as leading figures which could trigger the new episode of dengue outbreak in the year 2001 or 2002 according to the antibody-dependent enhancement of infection theory by Professor Halstead [28, 29].

The gold standard of dengue viral diagnostic methods, virus isolation, takes about one week to get the confirmed diagnosis while

HI test and ELISA techniques usually take two days, and IFAT method takes about a week to get the results. Employing the RT-PCR technique for dengue viral diagnosis could obtain the quick results in six hours which could facilitate simultaneous identification and serotyping. This technique has advantages in its rapidity, sensitivity and serotype-specificity, but the major disadvantages include its high cost and the need of technical expertise. However, it is high time to establish and utilize this technology for speedy diagnosis of various infectious diseases in Myanmar, at least at the central level laboratories.

This study indicates that the Department of Medical Research (Lower Myanmar) is capable to utilize the PCR technology, an access to the era of Molecular Biology and Biotechnology, and also imparting its expertise to other scientific communities in Myanmar as being a leading research institution.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Dr. U Soe Aung, Deputy Director-General (Disease Control and Public Health), Department of Health, for the kind supply of some necessary reagents for RT-PCR technique. Thanks are also due to Professor Akira Igarashi, Former Dean and Professor of the Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan, for the generous supply of dengue serotype-specific primers, and also to Dr. Daw Myint Aye Mu, Head of the Nuclear Medicine Research Division, for her kind allowance to use the Photodocumentation facilities at the PCR laboratory.

REFERENCES

1. Rice, C.M., Lenches, E.M., Eddy, S.R., *et al.* Nucleotide sequence of Yellow Fever virus: implications for flavivirus gene expression and evolution. *Science* 1985; 229: 726-733.
2. Halstead, S.B. Pathogenesis of dengue: challenges to molecular biology. *Science* 1988; 239: 476-481.
3. Allen, D. Dengue haemorrhagic fever: Clinical features and management. In: *Pathology Today: Dengue Haemorrhagic Fever*. Sng. JEH. (ed) The Department of Pathology, Singapore General Hospital, Singapore. 1992; 23-26.
4. World Health Organization. Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control. 2nd Edition. *World Health Organization*, Geneva, 1997.
5. Blok, J., Samuel, S., Gibbs, A.J. *et al.* Variation of the nucleotide and encoded amino acid sequences of the envelope gene from eight dengue 2 viruses. *Archives of Virology* 1989; 105: 39-53.
6. Chu, M.C., O'Rourke, E.J. and Trent, D.W. Genetic relatedness among structural protein genes of dengue 1 virus strains. *Journal of General Virology* 1989; 70: 1701-1702.
7. Trent, D.W., Manske, C.L., Chu, M.C. *et al.* Genetic variation and microevolution of dengue 2 virus in Southeast Asia. *Virology* 1989; 172: 523-535.
8. Monath, T.P. Pathobiology of the flaviviruses. In: *The togaviridae and flaviviridae*. Schlesinger, S. and Schlesinger, M.J. (eds), Plenum Press, New York. 1986; 375-440.
9. Rosen, L. The Emperor's new clothes revisited, or reflections on the patho-genesis of dengue hemorrhagic fever. *American Journal of Tropical Medicine and Hygiene*. 1977; 26: 337-343.
10. Halstead, S.B. Immunological parameters of togavirus disease syndromes. In: *The Togaviruses*. R.W. Schlesinger (Ed). Academic Press, New York. 1980; 107-173.
11. Chungue, E., Deubel, V., Cassar, O. *et al.* Molecular epidemiology of dengue 3 viruses and genetic relatedness among dengue 3 strains isolated from patient with mild or severe form of dengue fever in French Polynesia. *Journal of General Virology* 1993; 74: 1765-1770.
12. Deubel, V., Laille, N., Hugnot, J.P. *et al.* Identification of dengue sequences by genomic amplification: rapid diagnosis of dengue virus serotypes in peripheral blood. *Journal of Virological Methods* 1990; 30: 41-54.
13. Mullis, K.B., and Faloona, F.A. Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. *Methods in Enzymology* 1987; 155: 335-350.

14. Morita, K., Tanaka, M., and Igarashi, A. Rapid identification of dengue virus serotypes by using Polymerase Chain Reaction. *Journal of Clinical Micro-biology* 1991; 29: 2107-2110.
15. Chungue, .E, Roche , C., Fevre, M.F. *et al.* Ultra-rapid, simple, sensitive and economical Silica Method for extraction of Dengue virus RNA from clinical specimens and mosquitoes by RT-PCR. *Journal of Medical Virology.* 1993; 40: 142-145.
16. Puri, B., Henchal, E.A., Burans, J. *et al.* A rapid method for detection and identi-fication of flaviviruses by polymerase chain reaction and nucleic acid hybridization. *Archives of Virology* 1994; 134: 29-37.
17. Clarke, D.H. and Casals, J. Techniques for haemagglutination and haemaggluti-nation-inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene* 1958; 7: 561-573.
18. Kuberski, T., and Rosen, L. A. simple method for the detection of dengue antigen in mosquitoes by immuno-fluorescence. *American Journal of Tropical Medicine and Hygiene* 1977; 26: 533-537.
19. Mason, P.W., McAda, P. C., Mason, T.L *et al.* Sequence of the Dengue-1 virus genome in the region encoding the three structural proteins and the major nonstructural protein NS1. *Virology* 1987; 161: 262-267.
20. Deubel, V., Kinney, R.M., Trent, D.W. Nucleotide sequence and deduced amino acid sequence of the structural proteins of Dengue type 2 virus; Jamaica genotype. *Virology* 1986; 155: 365-377.
21. Deubel, V., Kinney, R.M., and Trent, D.W. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of Dengue type 2 virus, Jamaica genotype: comparative analysis of the full-length genome. *Virology* 1988; 165: 234-244.
22. Osatomi, K., and Sumiyoshi, H. Complete nucleotide sequence of Dengue type 3 virus genome RNA. *Virology* 1990; 176: 643-647.
23. Mackow, E., Makino, Y., Zhao, B. *et al.* The nucleotide sequence of dengue type 4 virus: analysis of genes coding for nonstructural proteins. *Virology* 1987; 159: 217-228.
24. Zhao, B., Mackow, E., Buckler-White, A. *et al.* Cloning full-length dengue type 4 viral RNA sequence: analysis of genes coding for structural proteins. *Virology* 1986; 155: 77-88.
25. Khin-Mon-Mon, Saw-Lwin, Soe-Aung *et al.* Epidemiology of dengue haemorr-hagic fever in Myanmar: 1991-1998. *Dengue Bulletin* 1998; 22: 49-52.
26. *Department of Medical Research. Annual Report.* 1998; 87-88.
27. Kyaw-Zin-Thant, Khin-Mar-Aye, Soe-Thein *et al.* Application of IgM-capture ELISA on Myanmar dengue patients' serum samples collected in 1994. *Tropical Medicine* 1996; 38(1); 1-6.
28. Halstead, S. B., Chow, J. S., and Marchette, N. J. Immunological enhan-cement of dengue virus replication. *Nature* 1973; 243: 24-26.
29. Halstead, S. B. The pathogenesis of dengue: molecular epidemiology in infectious disease. *American Journal of Epidemiology* 1981; 114:632-648.

SHORT REPORT

Schizontaemia of *Plasmodium falciparum* in a non-fatal cerebral malaria patient

*Khin Saw Aye, *Soe Soe, *Than Swe, **Ko Ko Hla & **Win Bo Kyaw

*Department of Medical Research

**North Okkalapa General Hospital

Severe malaria especially cerebral malaria is seen only in *P. falciparum* infection. The severity of the disease in *P. falciparum* generally rests on the peripheral parasitaemia [1], and high parasite load is usually associated with severe forms. However, there are cases where there is low parasite count or even no parasite with severe malaria [2]. This phenomenon is explained by the sequestration of the maturing rings, trophozoites and schizonts in the deep-seated capillaries of the internal organs mainly the cerebral vessels. The sequestration process, the peculiarity of the *P. falciparum* species is thought to be responsible for the severe disease [3] and the absence of mature stages in the peripheral blood. Other three human malarial species namely *P. vivax*, *P. malariae* and *P. ovale* have their asexual erythrocytic schizogony in the peripheral circulation and all stages can be visualized in the peripheral blood [4]. Thus, presence of later stages of parasites in *P. falciparum* malaria is a rare event and if present carries a poor prognosis with fatal consequences.

A 21 year-old Myanmar female was admitted to the North Okkalapa General Hospital for fever, chills, cough with yellow sputum for 7 days duration. On admission, the temperature was 104°F. Physical examination showed a fully conscious young lady without abnormal signs except for few crepitations in both lung fields. She gave history of visit to a malaria endemic area 3 months ago. Because of lung signs

she was given a course of amoxicillin (SmithKline Beecham) and blood film for malaria parasite was sent to the hospital emergency laboratory, operated by a trained technician. The results came back with presence of ring and schizont stages of *P. vivax* (+++++) (more than 10 parasites per one thick film field [1]). Thus she was given chloroquine tablet (Myanmar Pharmaceutical Factory) 25 mg/kg in three divided doses as recommended by the National Malaria Campaign, Myanmar. However on the next day, her consciousness deteriorated and scored 6 in the modified Glasgow Coma Scale (MGCS). According to the WHO criteria (1986). GCS ≤ 7 is regarded as cerebral malaria. Her MGCS on admission was 10. To exclude hypoglycemic attack or ketoacidosis and mixed infection with *P. falciparum* and *P. vivax*, random blood sugar and immuno-chromatographic test (ICT Diagnostics, Sydney, Australia) for the qualitative detection of *P. falciparum* antigen were performed immediately. Cerebro-spinal fluid examination was not carried out due to the absence of neck stiffness. Blood sugar level was within normal value (90 mg/dl) and ICT test for *P. falciparum* was positive. Therefore, she was diagnosed as cerebral malaria and treated with intramuscular injection of Artemeter 160 mg loading dose with 80 mg 12 hourly for 2 days and Mefloquine 15 mg/kg single dose through intra-nasal tube. Intramuscular crystalline penicillin and gentamycin were given in place of amoxicillin. She improved pro-

gressively and was fully conscious on day 6. She was discharged 9 days after admission. Her follow up blood for malaria parasite before discharge was negative.

When the pretreatment blood films, both thick and thin films stained with Giemsa's were examined by one of the authors, the schizonts were small in size and occupied two-thirds of the red cells. In segmental stage the number of merozoites were few only about 12-16 in numbers. The host RBCs was not enlarged as in *P. vivax* species. The ring forms were small, and occupied one-fifth of the diameter of the red blood cells. Ring forms with two chromatin granules and marginal forms were also present. The mature gametocytes were banana-shaped. The parasitemia was 45 schizonts, 96 ring trophozoites and 1 gametocyte per 300WBCs.

Thus a case of non-fatal *P. falciparum* cerebral malaria with schizontaemia was confirmed.

Silamut and White 1993 reported that fatal cases of *P. falciparum* malaria had more mature parasites in the blood than those from survivors and concluded that

predominance of mature parasites in the peripheral blood reflects a greater sequestered biomass and is diagnostic of a more severe form of *P. falciparum* malaria and thus simple microscopical assessment of parasite maturity on an admission blood slide provides important prognostic information. In their series, there was only one survivor who had schizontaemia compared with 22/50 fatal cases. This may be the reason why our patient survived, as she received prompt and adequate treatment.

REFERENCES

1. WHO. Severe and complicated malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1993; 80: 436-443.
2. Field, J.W. Blood examination and prognosis in acute falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1949; 43: 33-48.
3. White, N.J. & Ho, M. Pathophysiology of malaria. *Advances in Parasitology* 1992; 31: 84-175.
4. Field, J.W. & Shute, P.G. The Microscopic Diagnosis of Human Malaria. II. A morphological study of erythrocytic parasites. Kuala Lumpur: Studies from the Institute for Medical Research, Federation of Malaya 1956; 24: 23-28.

SHORT REPORT

Effect of freeze-drying on the formalinized Russell's viper venom toxoid

Win Aung, Khin Khin Hlaing & Aye Kyaw

Biochemistry Research Division
Department of Medical Research (Lower Myanmar)

A previously developed Russell's viper (*Daboia russelii siamensis*) venom (RVV) toxoid as a liquid form in Department of Medical Research was prepared from crude RVV by slow and step-wise formalinization [1]. However, it showed reversion in formaldehyde linkage and toxicity on storage at 37°C and room temperature (RT) from three months onwards [2,3]. It is necessary to develop a toxoid which is safe and immunogenic on storage even at RT. It is a well known fact that freeze-dried biological have the advantage over liquid products in that they can be stored at ordinary room temperatures for a long time without deterioration in this proportions [4]. In this study the conventional liquid toxoid was frozen at -80°C for 48 hours followed by lyophilization using a freeze-dryer model no. 5, Labconco. The obtained lyophilized form of the toxoid was reconstituted with same volume of distilled water just before the test. Then, pH, protein contents, amount of free formalin released, activities of various enzymes present in RVV such as proteinase, phospholipase (PLA), phosphodiesterase (PDE) and arginine esterase (AE) in both liquid and lyophilized toxoid was determined by standard assay procedures. The comparison of the findings between these two toxoids was shown in the following table.

The data in the table displayed that pHs of both toxoids showed no apparent difference whereas other parameters were found to be significantly reduced from initial activities after lyophilization.

Table Comparison of the characteristics of liquid and lyophilized forms of toxoid just after lyophilization

	Liquid toxoid	Lyophilized toxoid	Percentage of reduction
pH	7.0	7.0	Nil
Protein (mg/ml)	8.27	5.38	35%
Proteinase (EU/mg protein)	4.05	2.95	27%
PLA (EU/mg protein)	6.69	1.38	73%
PDE (EU/mg protein)	0.025	0.014	44%
AE (EU/mg protein)	7.09	5.67	20%
Free formalin released (Ug/ml)	95.1	31.7	67%

In conclusion, lyophilization of the formalinized RVV toxoid induced a significant reduction in its contents. This undesirable effects should not be ignored in preparation of lyophilized form of RVV toxoid which was found to be more stable and potent even stored at RT compared to the conventional liquid toxoid [5].

REFERENCES

1. Aung Khin, M., Khin Ohn Lwin & Thant Zin. Immunogenicity of the toxoid of Russell's viper venom. *The Snake* 1980; 12: 45-53.

2. Aye Kyaw, Khin Aye Thar, Hla Pe, Khin Pa Pa Kyaw & San Kun. Reversion of formaldehyde linkage in Russell's viper venom toxoid on storage. *The Snake* 1992; 24: 147-150.
3. Khin Pa Pa Kyaw, Khin Maung Maung, Aye Aye Myint, Aye Kyaw & Hla Pe. Effect of storage temperature on the stability of Russell's viper venom toxoid. *Abstract of Papers, Myanmar Health Research Congress 1994*, 12-19 December, DMR, Yangon, pp. 59.
4. Ko Ko Gyi, Myint Lwin, Khin Su Su, Han Sein & Stanley Maung Sein. Freeze-drying of anti-snake venom sera. *Union of Burma Journal of Life Science* 1969; 2(3): 349-351.
5. Win Aung, Khin Khin Hlaing, Khin Pa Pa Kyaw, Mya Mya Win & Aye Kyaw. Stability of Russell's viper venom toxoid (lyophilized) on storage. *Abstract of papers, Medical Research Congress, Yangon, 1998*, 19-23 December, p. 95.

RISO KS 500
10.2.2004/500 ***

MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

INDEX TO VOLUME 11 (1999)

Author Index

A

Aye-Aye-Myint - 18, 33
Aye-Aye-Thein - 26
Aye-Kyaw - 21, 52
Aye-Thin - 26

H

Hla-Kathy-Win - 21
Hlaing-Myat-Thu - 41
Hnin-Hline - 6
Htain-Win - 1

K

Kay-Thi-Aye - 41
Khin-Khin-Aye - 21
Khin-Khin-Hlaing - 52
Khin-Mar-Aye - 41
Khin-May-Oo - 1
Khin-Myat-Tun - 14
Khin-New-Oo - 38
Khin-Pa-Pa-Kyaw - 21
Khin-Pyone-Kyi - 1
Khin-Saw-Aye - 11, 50
Ko-Ko-Hla - 50
Kyaw-Kyaw - 38
Kyaw-Moe - 41
Kyaw-Myint - 38
Kyaw-Nyunt-Sein - 38
Kyaw-Zin-Thant - 41

M

Mar-Mar-Nyein - 14, 26
Mi-Mi-Htwe - 26
Moh-Moh-Win - 14
Myo-Khin - 1

N

Ne-Win - 1, 11
Nu-Nu-Aung - 33
Nwe-Nwe-Yin - 38
Nwe-Yee-Win - 26
Nyomie-Kyaw - 14

P

Pangi, Charles - 11
Po, Lilian - 6

S

San-Aye - 21
San-San-Aye - 11
Sann-Mya - 18
Si-Si-Hla - 41
Soe-Soe - 50
Soe-Thein - 41

T

Than-Nu-Shwe - 14
Than-Swe - 50
Thuzar-Myint - 14
Tun-Pe - 33, 18

W

Wah-Wah-Aung - 14
Win-Aung - 52
Win-Bo-Kyaw - 50
Win-Myint - 26
Win-Win-Maw - 26

Y

Ye-Tint-Lwin - 6

Subject Index

A

Antibiotics - 14
Antivenins - 33

D

Dengue Virus - 41
Diabetes Insipidus - 18

E

Enzyme-Linked Immunosorbent Assay - 38
Escherichia coli - 14

F

Freeze-Drying - 52

G

Glutaral - 11
Glutaraldehyde see Glutaral

L

Leprosy - 11

M

Malaria, Cerebral - 50
Mycobacterium leprae - 38

P

Plant Extract - 26
Plants, Medicinal - 26
Plasmodium falciparum - 50
Polymerase Chain Reaction - 1
Pre-Eclampsia - 6

R

Reverse Transcriptase - Polymerase Chain Reaction - 41
Russell's Viper - 18, 21, 33, 52

S

Serologic Tests - 38
Serodiagnosis see Serologic Tests
Snake Venoms - 18, 33, 21

T

Toxoids - 52
Tuberculosis, Meningeal - 1

U

Uric Acid - 6

V

Viper Venoms - 18, 21, 33, 52,

