MYANMAR
HEALTH SCIENCES
RESEARCH JOURNAL

Volume 10, No. 1, April 1998

DEPARTMENT OF MEDICAL RESEARCH

No. 5, Ziwaka Road

Yangon 11191, Myanmar

CONTENTS

Prevalence of hepatitis B and C infections in hepatocellular carcinoma cases in Myanmar 1
Khin Pyone Kyi, Khin Maung Win, Myo Aye, Yi Yi Htwe, Khin May Oo, Than Aung & San San Oo

A case control study on the risk factors for diabetes mellitus 6
Khin Ye Myint, Myo Thet Htoon & Chit Soe

Susceptibility of *Culex quinquefasciatus* to Neem insecticide under laboratory conditions 11
Marcus Winn & Maung Maung

Maturation of foetal thyroid hormone synthesis and regulation 17
Soe Aung, Myo Khin, Kyaw Min, & Khin May Oo, Nilar Sein & Muya Than

Clinical significance of early intravenous antivenom in Russell's viper bite cases in Taungdwingyi District : A preliminary study 22
Tun Pe, Khin Aye Kyu, Aye Aye Myint, Nu Nu Aung, Sann Mya & Tin Oo

Russell's viper bites in Taungdwingyi Civil Hospital: Clinical features and response to antivenom 26
Sann Mya, Tun Pe, Aye Aye Myint, Nu Nu Aung, Khin Aye Kyu & Tin Oo

Salting and temperature effect on the occurrence of *Salmonella* spp. in hens' and ducks' eggs 31
Khin Nwe Oo, Hnit San Swe & Myat Thida

The association between faecal leukocytes and pathogens present in stool of children with diarrhoea and dysentery 35
Mar Mar Nyein, Khin Myat Tun, Moe Moe Win, Than Saw & Aye Than

Importance of plasma protein binding of antituberculosis drugs on the response to short-course chemotherapy 40
Thaw Zin, May Aye Than, Malar Myint, Khin Myo Myint, Thida Hmun, Nwe Nwe Yin & Htay Kyi

Glutaraldehyde gelification time - a highly acceptable bedside tool in the assessment of children tuberculosis 48
Ne Win, Khin Swe, Thein Thein Myint & Saw Mya Yi

Short Report: Clinical features and response to antivenom of Russell's viper bite cases of Kyaukse, Mandalay Division 51
Aung Myint, Tun Pe, Aye Aye Myint & Nu Nu Aung

Short Report : Clinical features and response to antivenom of Russell's viper bite cases of Nyaunglaybin, Bago Division 54
Min Than, Tun Pe, Aye Aye Myint & Nu Nu Aung

****

Editor-in-Chief
Dr. Than Swe

Editor
Dr. Soe Thein

Associate Editor
Dr. Myint Lwin
Dr. Tun Pe

Managing Editor
U Hla Myint

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

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Prevalence of hepatitis B and C infections in hepatocellular carcinoma cases in Myanmar

*Khin Pyone Kyi, **Khin Maung Win, *Myo Aye, *Yi Yi Htwe, *Khin May Oo, *Than Aung & *San San Oo

*Experimental Medicine Research Division
Department of Medical Research
**Yangon General Hospital

Hepatitis B and hepatitis C have been implicated with chronic liver disease in many parts of the world. This study was carried out on 40 patients with hepatocellular carcinoma, diagnosed clinically and confirmed by the positivity of alpha-fetoprotein by agar gel diffusion method. Sera were tested for HBsAg by AUSZYME ELISA test kits (Abbott Laboratories, U.S.A.) and for Anti-HCV by ORTHO HCV Antibody ELISA test system (Ortho Diagnostics, U.S.A.). Our findings confirmed the high incidence of hepatitis B infection (60%) and also of the association of hepatitis C infection (35%) among the hepatocellular carcinoma cases. The significance of the positivity of Anti-HCV by the ELISA tests was discussed.

INTRODUCTION

Myanmar has been listed among the top ten countries with the highest incidence of hepatocellular carcinoma (HCC) in the world [1]. Primary HCC is the second most common carcinoma in males and fourth most common in females in Myanmar [2]. Two different viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) have been implicated in the etiology of this carcinoma. Because of the similar epidemiology and disease spectrum caused by these two blood borne viruses, it is of interest and importance to study their roles in HCC in Myanmar.

Hepatitis B is highly endemic in Myanmar with a hepatitis B surface antigen (HBsAg) carrier rate of 9-12% and infection rate of 50%. Approximately, 90% of the infants and children who contracted the infection during early childhood and 10% of the acute hepatitis B cases may progress onto the carrier state, chronic hepatitis, cirrhosis and HCC [3,4,5]. HBsAg is the hallmark in the diagnosis of viral hepatitis type B.

Hepatitis C was previously designated as post-transfusion or parenterally transmitted non-A non-B hepatitis. Diagnostic test kits for hepatitis C became commercially available only in 1989. It was generally thought that hepatitis C virus (HCV) infection became chronic in 50% of the infected cases [6]. But the recent longitudinal study of post-transfusion hepatitis C patients by the National Institutes of Health in the United States showed that as many as 80% of such cases may ultimately progress to chronicity. Patients with HCV-related cirrhosis are at risk of developing HCC. A positive anti-HCV test indicates exposure to HCV and is a surrogate marker of ongoing HCV infection [7].

An increased level of alpha fetoprotein (AFP) above 400 ng/ml is an indicator of HCC and can be accomplished in suspected cases before the appearance of clinical features [8].

From 1993 to the middle of 1998, the number of HCC in-patient cases have increased fourfold according to the cancer statistics from the Cancer Ward, Radiotherapy Department, Yangon General Hospital [9]. The etiology of HCC needs to be studied for effective planning to reduce the incidence of HCC in Myanmar.

MATERIALS AND METHODS

A total of 40 serum samples were obtained from HCC patients who were diagnosed by their clinical features and the positivity for alpha fetoprotein (AFP) determined by agar gel diffusion method of Ouchterlony.

The serum samples were screened for HBsAg by using Auszyme ELISA test kits from the Abbott Laboratories, USA.

Ortho HCV Antibody ELISA test system from the Ortho Diagnostic System, USA was used to detect anti-HCV in the test samples.

RESULTS

Table 1 shows the detection of HBsAg and anti-HCV markers in HCC cases according to sex. In our study among the 40 HCC patients, the number of cases in males, predominates females by 7:1. Another finding in this study is that males predominate females in the positivity for both HBsAg and anti-HCV markers. HBsAg was positive in 60% (24 of 40) of all patients and anti-HCV was positive in 35% (14 of 40) of the study group.

Table 1. Detection of HBsAg & Anti-HCV in hepatocellular carcinoma patients according to sex.

Sex	No.	HBsAg positive HCC cases		AntiHCV positive HCC cases	
		No.	%	No.	%
Male	35	23	57.5	12	30
Female	5	1	2.5	2	5
Total	40	24	60.0	14	35

The prevalence of HBV and HCV markers in HCC patients according to age is shown in Table 2. In the 40 patients with their ages ranging from 20 years to 89 years, the peak incidence of HCC cases was seen in the 30-39 years age range (35%) where both HBsAg (27.5%) and anti-HCV (15%) were also at their highest distribution.

Table 2. Prevalence of HBsAg and Anti-HCV in hepatocellular carcinoma patients according to age.

Age Groups	HCC cases		HBsAg positive		AntiHCV positive	
	No.	%	No.	%	No.	%
20-29	4	10.0	3	7.5	3	7.5
30-39	14	35.0	11	27.5	6	15.0
40-49	7	17.5	6	15.0	1	2.5
50-59	4	10.0	2	5.0	1	2.5
60-69	6	15.0	1	2.5	3	7.5
70-79	4	10.0	1	2.5	-	-
80-89	1	2.5	-	-	-	-
All ages	40	100.0	24	60.0	14	35.0

The presence and absence of hepatitis markers in the study group are shown in Table 3. One or both markers, HBsAg and/or anti-HCV, were positive in 77.5% of patients. Both markers were positive in 17.5% of cases, and both markers were negative in 22.5% of the study group.

Table 3. Hepatitis B and C markers in hepatocellular carcinoma cases.

No. of HCC	Positive for either HBsAg and/or antiHCV		Positive for both HBsAg and Anti-HCV		Negative for both HBsAg or Anti-HCV		Positive for Anti-HCV only	
	No.	%	No.	%	No.	%	No.	%
40	31	77.5	7	17.5	9	22.5	7	17.5

From the data in Table 4, it could be seen that when divided into HCC patients positive and negative for HBsAg, it was positive for anti-

HCV in 29.17% in the former group and 43.75% in the latter group.

DISCUSSION

The male predominance of 7:1 in HCC cases found in this study was higher than the previous study in Myanmar which was recorded to be 4:1 for male-female occurrence [3]. HCC incidence has been known to be higher in males than in females in most populations of the world. The male excess is recorded to be greatest generally between three and four to one, in places and populations in which HCC incidence is highest. It has been expressed that the sex ratio does not relate to gender differences in HBV carrier rates or to aflatoxin exposure and that sex hormones probably mediated differences in responses to the etiologic agents [10].

Globally, HBV has been recorded to be the probable etiological agent responsible for 75% to 90% of HCC cases [11]. Hepatitis C virus has also been studied to be involved in the development of HCC [12]. In this study HBsAg was positive in 60% of cases confirming the close association of HBV with HCC. A previous study in Myanmar showed a 70% positivity for HBsAg in HCC cases [2]. This study also showed that HCV played a significant role in the etiology of HCC as it was present in 35% of HCC cases. In this study, males predominate females in the positivity for both HBsAg and anti-HCV.

This study showed that the peak incidence of HCC cases was seen in 30-39 years age range (35%) where both HBsAg (27.5%) and anti-HCV (15%) were also at their highest distribution. This was contrary to a previous study by Muir *et. al* who reported in 1987 that the HCC incidence increased with advancing age in both sexes. The incidence appeared to be linear with increasing age from early adulthood, with a steeper slope in males in most populations [13]. However, the pattern of HCC incidence in Myanmar was similar to the one in China as reported by Yeh in 1989 in which the HCC incidence appeared to rise with increasing age and then declined [14].

The high incidence of HBsAg and anti-HCV either alone (42.5% and 17.5%) or in combined forms (17.5%) in HCC cases in Myanmar showed that HCV infection has now made an additional contribution to the severe forms of liver disease either as super infection or co-infection with HBV (Table 3). A co-infection with both HCV and HBV was also reported in 26.7% of HCC cases by Liaw and Chien from Taiwan [15]. A study in Japan also established that anti-HCV correlated with clinical severity of hepatitis B chronic liver diseases and that anti-HCV was not detected in asymptomatic HBsAg carriers [16]. In this study 22.5% of cases were negative for both HBV and HCV markers which was similar to a study in China which reported 23.1% of such chronic liver diseases which was not due to either HBV or HCV.

Table 4 shows that there were 24 HBsAg positive HCC patients (60%) and 16 (40%) HBsAg negative HCC patients. HBsAg positivity was therefore 1.5 times greater than HBsAg negativity in HCC patients. Of the 24 HBsAg positive HCC cases, 7 patients were positive for anti-HCV (29.17%). These were higher than results obtained in other hepatitis B endemic countries; 13% in Singapore, 16% in Japan, 17% in Taiwan and 16.3% in Korea[18].

Table. 4. Prevalence of Anti-HCV in HCC cases positive or negative for HBsAg

Total No. of HCC cases	No. tested	Anti-HCV positive	
		No.	%
HBsAg (+) cases	24	7	29.17
HBsAg (-) cases	16	7	43.75
Total	40	14	35.0

Of the 16 HBsAg negative HCC cases 7 (43.7%) were positive for anti-HCV in this study. In Singapore 71% of HBsAg negative HCC cases were anti-HCV positive, in Japan the figure was 76% and in Taiwan it was 63% [12].

Some authors have also speculated upon the possibility of interaction between HBV and HCV that might alter the natural course of each separate viral disease. A possible synergistic effect of past or current HBV and HCV infection on HCC risk has also been suggested.

The prevalence of HBV and HCV infection in HCC cases varied in different countries as evident in Table 5. The incidence of HBsAg positive in HCC cases was highest in Taiwan (83.5%) where as anti-HCV prevalence in HCC patients was highest in Japan (76.2%). The prevalence of HBsAg ranged from 12% - 83.5% where as for anti-HCV it was 0-76.2% in HCC cases in different countries [19].

Table 5. Prevalence of hepatitis B and C markers in HCC cases in different countries.

No.	Country	No. of HCC cases tested	HBsAg positive (%)	AntiHCV positive (%)
1	Japan	880	21.8	76.2
2	China	52	67.3	38.5
3	Korea	75	29.3	57.3
4	Taiwan	103	83.5	32.0
5	Philippine	11	45.4	18.0
6	India	33	15.2	42.0
7	Indonesia	70	58.6	34.3
8	Thailand	20	12.0	-

CONCLUSION

The results of this study indicate that both HBV and HCV are strongly associated with HCC. These data emphasize the importance of effective planning strategies for the prevention and control of viral hepatitis in Myanmar.

REFERENCES

1. Bosch,F.X & Munoz. Hepatocellular carcinoma in the world. International Agency on Research and cancer. 15 Courts Albert Thomas, France, 1989.
2. Khin-Maung-Tin. Overview of viral hepatitis in Myanmar. Proceedings of the National Workshop on Viral Hepatitis; 1990 July 31 - August 3: Yangon, Myanmar: Department of Medical Research , 1990; 23-32.
3. Khin-Maung-Tin. Studies in hepatitis B virus in Myanmar: Prevalence, distribution and transmission. WHO Regional Publications South-East Asia Series. Research Abstracts. 1987; 1: No.16: 8.
4. Khin-Maung-Tin. Efficacy of hepatitis vaccine in high risk infants of hepatitis B virus carriers. WHO Regional Publication South-East Asia Series. Research Abstracts. 1987; 1: No.16: 8.
5. Khin-Pyone-Kyi, Than-Swe, Khin-Mya-Lwin, Khin-Yi-Oo & Soe-Lwin. Prevalence of hepatitis B infection in health care personnel. Paper presented at the 35th Burma Medical Conference 1988.
6. Kou, C & Alter, H.J. An assay for circulating antibodies to a major etiologic virus of human Non-A Non-B hepatitis. Science, 1989; 244: 362-364.
7. Copolla, R., Rizetto, M., Bradley, D.W., Crivelli, O. (ed) Hepatitis C. In Viral Hepatitis Handbook. Sorin Biomedica Diagnostics, S.P.A. Italy, 1996; 57-84.
8. Kew, M.C. Detection and treatment of small hepatocellular carcinoma. In: Hollinger, F.B., S.M., Margolis, H., editors. Viral Hepatitis and Liver Diseases. Proceedings of the 1990 Inter-national Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Aspects; 1990 April 4-8. Houston, Texas, U.S.A. Williams and Wilkins. 1991; 535-540.
9. Soe-Aung. Cancer Statistics, Cancer Ward, Radiotherapy Department, Yangon General Hospital, 1998.
10. Beasley, R.P. & Hwang, Lu-Yu. Overview on the epidemiology of hepatocellular carcinoma. In: Hollinger, F.B., Lemon, S.M., Margolis, H., editors. Viral Hepatitis and Liver Diseases. Proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Aspects; 1990 April 4-8. Houston, Texas, U.S.A. Williams and Wilkins. 1991; 535-540.
11. Beasley, R.P. Hepatitis B virus as the cause of viral hepatitis and hepatocellular carcinoma. In: Proceedings of the Second International Symposium on Viral Hepatitis and Hepatocellular carcinoma, Taipei, 1988 Dec. 7-9. Excerpta Medica Current Clinical Practice Series 57.

12. Khin, L. W., Teo, C.J & Guan, R. Seroprevalence of hepatitis B and C viral markers in patients with primary hepatocellularcarcinoma in Singapore. *Singapore Medical Journal* 1996; 37: 492-496.
13. Muir, C., Waterhouse, J., Mack, T., Powell, J & Whelan, S. Cancer in five continents. Vol V. Lyon: International Agency for Research on Cancer, 1987.
14. Yeh, F.S., Yu, M.C., Luo, S., Tong, M.J & Henderson, B.E. Hepatitis B virus, aflatoxin and hepatocellular carcinoma in Southern Guangxi. *China. Cancer Research* 1989; 49: 2506-2509.
15. Liaw, Y.F., Chien, R., Sheen, I & Shyan. Hepatitis C in patients with chronic liver diseases in an endemic area for hepatitis B virus infection. *Gastroenterologica Japonica* 1991; 26 suppl 3: 167-169.
16. Yoshigawa, M., Tsujii, T., Fukui, H. Hepatitis C virus infection in patients with chronic liver diseases. *Gastroenterologica Japonica* 1991; 26 suppl 3: 202-205.
17. Tao, Q.M., Wang, Y., Wang, H., Chen, W.R., Sun, Y & Meng, O. Seroepidemiology of hepatitis C virus and hepatitis B virus infections in Northern China. *Gastroenterologica Japonica* 1991; 26 suppl 3: 156-158.
18. Park, Y.M., Kim, I.S., Lee, C.D & Kim, B.S.Seroprevalence of antibody against hepatitis C virus (anti-HCV) in various groups of indi-viduals in Korea. *Gastroenterologica Japonica* 1991; 26 suppl 3: 159-163.
19. The Japanese Society of Gastroenterology. Seroepidemiology of hepatitis C virus. *Gastroenterologica Japonica* 1991; 26 suppl 3: 152-221.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

A case control study on the risk factors for diabetes mellitus

*Khin Ye Myint, ** Myo Thet Htoon & ***Chit Soe

*Institute of Medicine (Mandalay)
**Department of Health
***Yangon General Hospital

The development of prevention programs for non insulin dependent diabetes mellitus is greatly needed. Before such programs are implemented, proper community assessment is essential and this includes a knowledge of relevant risk factors for non insulin dependent diabetes. To determine such risk factors, a study was done on 118 cases of diabetes mellitus from Diabetic Clinic and Wards 1 & 2, Yangon General Hospital. A questionnaire of the patient's family history and drug history, smoking and alcohol history among others were noted. Statistical analysis was done by Odds ratio and 95% confidence interval of Odds ratio. In this study significant risk factors for diabetes were physical inactivity, family history of diabetes, especially parenteral, history of taking diuretics and dietary excesses like high carbohydrate and high fat diet. We recommend that exercise be an adjunct to proper diet and weight control for the prevention of non insulin dependent diabetes mellitus.

INTRODUCTION

Diabetes affects large numbers of people in all social conditions throughout the world. The personal and public health problem of diabetics, already of vast proportions continues to grow despite advances in every field of diabetic research and patient care. Epidemiological clinical and laboratory investigations have revealed evidence of several possible mechanisms in diabetes. In many communities non insulin depended diabetes mellitus appears to be linked with sedentary life style, overnutrition and obesity, correction of which may reduce the risk of both diabetes and its complications. The development of prevention programs for non insulin dependent diabetes mellitus is greatly needed. Before such programs are implemented, proper community assessment is essential, and this includes a knowledge of relevent risk factors for non insulin depended diabetes mellitus. Local environmental factors thought to be diabetogenic should be investigated and identified. The risk factors for diabetes in Myanmar have not yet been identified.

The aim of this study was to identify the risk factors for diabetes mellitus in YGH.

MATERIALS AND METHODS

Study design - A case control retrospective study with matching

Study area - Yangon General Hospital

Study population

Cases - From out-patients of diabetics attending Diabetic Clinic and in-patients of Wards 1 & 2, Yangon General Hospital

Control - From in-patients of non diabetics admitted to YGH for other reasons other than cardiovascular disease, hypertension, and ischaemic heart disease.

There were two controls for each diabetic and controls were matched for sex and age ±5 years. A questionnaire of variables noted were the patient's age, sex, race, residence, family history of diabetes, migration to urban areas, physical activities, smoking and alcohol intake, history of drug ingestion, body weight and height, body mass index, dietary history of stress e.g., infections and surgery.

Criterion for diagnosis of diabetes was according to the W.H.O criterion of fasting blood sugar of 140 mg% (7.8 mmol/dl). Those who gave history of diabetes but did not meet this criterion were considered diabetic if they were taking insulin or oral hypoglycemic agents.

Statistical analysis was done by Odds ratio and 95% confidence interval of Odds ratio.

RESULTS

1. Effects of physical activities on the development of diabetes (Table 1): Those with no exercise are 9,9 times more likely to develop diabetes.Physical inactivity is a significant risk factor.

Table 1. Effects of physical activity on the development of diabetes.

Physical Activity	Cases	Control	Odds ratio	95% CI of OR
No exercise	25	16	9.90	3.41 - 28.73
Sedentary life	36	92	2.48	0.97 - 6.37
House work/ no fixed work	43	77	3.54	1.38 - 9.05
Daily exercise	8	13	3.90	1.14 - 13.37
Physical labor	6	38	1	1

2. Effects of family history of diabetes on development of diabetes (Table 2):

Those with both parents or one parent with diabetes are 35 times more likely to develop diabetes. Siblings are 11.37 times more likely to develop diabetes than those with no family history. Family history is a significant risk factor.

Table 2. Effects of family history of diabetes on development of diabetes.

Family history	Cases	Control	Odds Ratio	95%CI of OR
Both parents/ one parent	14	1	35.38	4.59-272.09
Sibling	9	2	11.37	2.41-53.64
Grand-parent	2	1	5.05	0.45-56.38
Other family members	4	2	5.05	0.91-28.05
No family history	89	230	1	1

3. Effects of diet on the development of diabetes (Table 3):

Those with high carbohydrate diet, high fat diet and protein deficient diet were 16.75, 11.63 and 3.1 times more likely to develop diabetes and thus, diet is a significant risk factor.

Table 3. Effect of diet on the development of diabetes

Type of diet	Cases	Control	Odds Radio	95% CI of OR
High carbo-hydrates	27	5	16.75	6.21-45.19
High fat	15	4	11.63	3.73-36.23
Rice, fish & vegetable	7	15	1.44	0.56-3.68
Protein def.	1	1	3.10	0.19-50.23
Eats every thing	68	211	1	1

4. History of taking medication and development of diabetes (Table 4):

In those with history of taking thiazides, Odd's radio was 7.55 and those with history of taking betablockers, Odd s ratio was 5.81 and taking these drugs is a significant risk factor.

Table 4. History of taking medications and the development of diabetes

Type of medication		Odds Radio	95% CI of OR
Phenytoin	1	-	
Thiazides	4	7.55	2.40-23.77
Steroids	6	0.77	0.15-3.88
Oral Contraceptives	7	1.66	0.51-5.36
Beta blockers	2	5.81	1.11-30.49
No history of medication	216	1	1

5. Risk factors for diabetes mellitus in YGH (Table 5):

It is seen that physical inactivity, family history of diabetes, high carbohydrate and high fat diet and the use of thiazides are significant risk factors.

Table 5. Risk factors for diabetes mellitus in YGH

Variables	Odds ratio	Risk
1. B.M.I. and obesity	4.98	+
2. Urban life	3.80	+
3. Physical inactivity	9.90	S
4. Family history Parental and siblling	35.38	S
5. Diet		
- High carbohyrate	16.75	S
- High fat	11.63	S
6. Stress		
- Infections	2.29	+
- Surgery/Trauma	1.83	+
7. Medications		
- Thiazides	7.55	S
- Beta blockers	5.81	+

S = Significant risk
+ = Risk present

DISCUSSION

The majority of the cases in this study were in the 45-54 and 55-65-year age group. Helmrich has shown that age was positively associated with development of non-insulin dependent diabetes [1].

In our study obesity and increased body mass index of 26 and above is a risk factor. Knowler has described that incidence of diabetes in Pima Indians was strongly related to preceding obesity as estimated by body mass index [2].

In this study the effects of urban life increase the risk of diabetes. Knowler reported that in Pima Indians, disruption of their traditional agricultural life style and introduction of new food by whites and changing food consumption with resultant obesity have contributed to their present high incidence of diabetes [2].

Physical inactivity is a significant risk factor in this study. Helmrich reported that increased physical activity is effective in preventing non-insulin dependent diabetes mellitus and the protective benefit is especially pronounced in persons at the highest risk for the disease. i.e. with high body mass index, history of hypertension or a parenteral history of diabetes [1]. Physical activity increases sensitivity to insulin and regular endurance exercise induces weight loss and improves glucose tolerance. Horton has reported that a critical factor in preventing non-insulin dependent diabetes mellitus is prevention and treatment of obesity through dietary restriction and increase energy expenditure [3].

Alcohol drinking was not significant in our study. Stampfed has shown that there is no support for the hypothesis that moderate alcohol intake increases the risk of non-insulin dependent diabetes mellitus in females [4]. However Holbrook has reported that moderate to heavy alcohol intake appears to be associated with risk of non-insulin dependent diabetes mellitus in men but not in women [5].

Smoking was significant in our study. Facchmi has reported that chronic cigarette smokers are insulin resistant, hyperinsulinemic, and dyslipaedemic compared to a matched group of non-smokers [6].

Family history was a very significant risk in our study, especially parental. Helmrich reported that a parental history of diabetes was a significant predictor of non-insulin dependent diabetes mellitus [1]. Rilery reported that non-diabetic relatives of probands with insulin dependent diabetes mellitus who are in the first two decades of life, are members of multiplex pedigrees and have increased titres of islet cell antibodies are the most likely to contract insulin dependent diabetes mellitus themselves [7]. Pyke has reported that family studies have shown the existence of strong genetic components in insulin dependent diabetes mellitus as well as in non-insulin dependent deabetes mellitus [8].

In our study a high fat diet as well as high carbohydrate diet was a significant risk factor and protein deficiency is a risk. Results from cross sectional studies such as in native Americans show that diet is an important risk. In malnutrition related diabetes history of nutritional and protein deficiency is present. Khardoir has reported that experimental induction of protein malnutrition in rhesus monkey produces metabolic features that resemble protein deficient pancreatic diabetes [9].

Migration to urban areas was not significant in our study. Kawate reported that diabetes prevalence was 1.8 times as high in Japanese migrants from Hiroshima to Hawaii as in Japanese living in Hiroshima [10].

Severe infection and surgery are risks in our study. But emotional stress was not a risk. Several states of physical stress are associated with glucose intolearance. Epidemiological studies have evidence for a role of viral infections in insulin dependent diabetes mellitus [11].

Taking thiazides and beta-blockers are significant risks in our study. Pollarc has reported that hydrochlothiazide used for treatment of hypertension has adverse effects on glucose and lipid metabolism [12]. Bengtsson reported that treatment with thiazides and beta-blockers caused impaired glucose tolerance and precipitates overt diabetes [13].

CONCLUSION

This study has identified some of the risk factors for diabetes mellitus in YGH. A parenteral family history of diabetes, physical inactivity dietary excess like a high carbohydrate and high fat diet and medication with thiazides are significant risk factors. We recommend that exercise be an adjunct to proper diet and weight control for the prevention of non-insulin dependent diabetes mellitus.

REFERENCES

1. Helmrich, S.P., Ragland, D.R., Leury, R.W., & Patterbasger, R.S., Physical activity and reduced occurence of non-insulin dependent diabetes mellitus. *New England Journal of Medicine* 1991; 325-147-52.
2. Knowler, W.C., Pettitt, D.J., Savage, P.J. & Bernett, P.H. Diabetes incidence in Pima Indians: Contributions of obesity and parental diabetes. *American Journal of Epidemiology* 1981; 113(2): 144-156.
3. Horton, E.S. Exercise and decreased risk of NIDDM. Editorial: *New England Journal of Medicine* 1991; 325: 196-197.
4. Stampfed, M.J., Colditz, G.A., Willett, W.C. & Menson, J.E. A prospective study of moderated alcohol drinking and role of diabetes in women. *American Journal of Epidemiology* 1988; 128: 549-558.
5. Holbrook, T.L., Barrett, C.E. & Wingard, D.L. Prospective population-based study of alcohol use and NIDDM. *American Journal of Epidemiology* 1990; 132: 902-909.
6. Facchmi, F.S., Hollenbech, C.B., Jeppeser, J. ZDA Chen, YD. & Reaven, G.M. Insulin resistance and cigerette smoking. *Lancet* 1992; 339: 1128-1130.
7. Rilery, W.J., Maclorn, N.K., Krischen, J.et al. A prospective study of the development of diabetes in

relatives of patients with insulin-dependent diabetes. *New England Journal of Medicine* 1990 ; 323: 1167-1172.

8. Pyke, D.A. Diabetes; the genetic connections. *Diabetologica* 1979; 17: 33-343.
9. Khardori, R. et al, Insulin secretion and carbohydrate metabolism in experimental protein malnutrition. *Journal of Endocrinological Investigation* 1980; 3: 273.
10. Kawate, R., Yamakido, M., Nishimoto, Y. et al. Diabetes mellius and its vascular complications in Japenese migrant on the Island of Hawaii. *Diabetes Care* 1979; 2: 161-170.
11. Cudwarth, A.G.,& Gorscreb, A.N. Autoimmunity and viruses in type 1 (insulin-dependent) diabetes. In: Eleenberg, M. & Rifkin, H. Ed: Diabetes mellitus, memory and practice. 3rd ed., New York, Medical Examination Publishing Co: 1983; 505-517.
12. Pollarc, T., Litnell, H. & Bevre, C. A comparison of the effects of hydrochlorothizide and captoril on glucose and lipid metabolism in patients with hypertension. *New England Journal of Medicine* 1989; 321: 868-873.
13. Bengtsson, C., Blohme, G., Lapidus, L., Lindquist, O. et al. Do antihypertensive drug precipitate diabetes? *British Medical Journal 1984; 289: 4495-*4497.
14. Borch-Johnsen, K. et al. Relationship between breast feeding and incidence rates of insulin dependent diabetes mellitus; A hypothesis. *Lancet* 1984; 2: 1083-1086.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Susceptibility of *Culex quinquefasciatus* to Neem insecticide under laboratory conditions

*Marcus Winn & **Maung Maung
Department of Health

The effect of the azadirachtin, an extract of indigenous neem, was tested on larvae of *Culex quinquefasciatus*. The test results showed that *Cx quinquefasciatus*, the vector of lymphatic filariasis is susceptible to azadirachtin, neem extract. The tests were conducted with water from various sources i.e. concrete drain water, earthen drain water, septic tank water and tap water. The most effective water using neem against *Cx. quinquefasciatus* was found to be concrete drain water and tap water followed by septic tank water and earthern drain water. The Lc_{50} values for *Cx quinquefasciatus* in various water ranged from 0.022% to 0.028%. The potential of the future use of this natural compound in the control of filaria was discussed.

INTRODUCTION

There are many varieties of neem trees all over the world. It grows in most tropical countries of Asia and Africa. In Myanmar, seven varieties of neem are well established. They are three species of Melia and one species each of *Azadizachta indica, Cedrela toona, Polyalthia longifolia and Micromelum hirsutum. Melia volkensi* is found to grow only in Kenya.

The species found in Myanmar are as follows [1]:

Scientific name

1. *Azandirachta indica J.(A. Juss)*
2. *Melia azedarch L. (Linn)*
3. *M. dubia H. (Hiern)*
4. *M. excelsa J. (Jack)*
5. *Cedrela toona R. (Roxb)*
6. *Polyalthia longifolia B. (Benth)*

Myanmar name

1. Tamarkha,Thinbaw-tamarkha, Kalartamar
2. Pantamar, Tamarkha
3. Pantamar, Tawtamarkha
4. Tamargalay
5. Tawtamar
6. Thinbawtamar, Thinbawte or Lantamar
7. Tamarcho (Pyintawthein)

Neem has been known to have some medicinal effects for over two thousand years. It has been written in sanskrit in medical pharmacopoeia and is translated into Myanmar. In Ayurveda Pharmacopoeia, the neem seed extract is used to treat infections of the skin. Various uses of neem extract are described in it [2].

The active chemicals that act as insect growth inhibitor, antifeedant, repellency and chemosterilant properties are due to the limonoid contents of these seeds. There are nine limonoids found in neem extract. The most important of them are azadirachtin, selanin, meliantriol and nimbidin [3].

Azadirachtin inhibits the growth of the insect and interferes with the power of taste and also acts as a repellent. All the limonoids have the same molecular structure except meliantriol and nimbidin. The first three limonoids act on the brain not to produce hormones necessary for the growth and development resulting in the incompletion of moulting process and the insect eventually dies. These deep-seated hormonal effects are the main powerful growth inhibiting properties of the neem extract [4].

The azadirachtin is the compound extracted from the neem kernels. It is believed to be an

insect growth regulator. Here it is tested as an insecticide and it is successful against larvae of *Cx quinquefasciatus* in various breeding habitats. By using this extract it will prevent the toxic hazards induced by organophosphorous compound, which were commonly used in the control of filariasis in Myanmar.

In Myanmar the azadirachtin has been used by the Myanmar Agriculture Service against some important agriculture pests of chickpea, peanut, cotton, cabbage and cauliflower [5].

The effect on *Ae. aegypti* larvae was studied by Mwangi and Rembold. They found that the neem extract can be used as an invaluable agent for growth inhibition in Aedes control since "it is simple, reliable and quick" [6] by using 0.005% to get 100% mortality of *Cx quinquefasciatus*. It also kills Gambusia and tad pole at 0.04% concentration [7].

MATERIAL AND METHODS

Neem extract contains 1% azadirachtin active ingredient. This was available as an emulsifiable concentrate of 35% neem extract. It was provided in 500ml bottle by the Agriculture Department by neem insecticide factory in Paleik, Mandalay Division. Tests were carried out in the laboratory of Vector Borne Diseases Control, Yangon for seven concentrations starting from 0.005%, 0.01%, 0.04%, 0.08%, 0.16% and 0.32% against active and vigorous late third and early fourth instar larvae of *Cx. quinquefasciatus*. These tests were carried out according to the W.H.O instructions on larval susceptibility test method [8].

Cx. quinquefasciatus larvae were collected from the field and brought in clean plastic to the laboratory. They were kept for one hour in order to acclimatize them to the testing conditions. Then the larvae were transferred into clean water in an enameled tray measuring (46 cms × 35cms × 4cms). The larvae from the field were used in all these test replicates. This provides stronger and more vigorous larvae of the field than the laboratory reared specimens.

Stock solutions were prepared as follows:-

Two stock solutions were prepared by diluting 35% neem extract (a.i 1% azadirachtin) with ethyl alcohol. The first stock solution was prepared as 20% neem extract. It was prepared by diluting 40ml of 35% neem extract with 30ml of ethyl alcohol. A second stock solution prepared was 2.5% of neem extract. It was prepared by diluting 5ml of 35% neem extract with 65ml of ethyl alcohol. For example a concentration of 0.005% neem extract was prepared by adding 0.5ml of 2.5% neem extract (stock solution) in 249.5ml of water containing 25 larvae. In this way various concentrations were prepared by adding the appropriate stock solution to 249ml or (250 ml stack solution) of water containing 25 larvae accordingly. In each of the control cups containing no insecticide, 1ml of ethyl alcohol/249ml of water added for comparison.

Moribund and dead larvae were counted as dead after 24 hours exposure. The mortality rate was recorded and plotted on the probability graph paper. Percentage mortality of Lc_{50} and Lc_{90} were processed statistically. The tests were done in ten replicates. During the test the temperature and humidity were recorded. The temperature ranges from 27° C to 30° C and the humidity ranges from 60% to 80%.

RESULTS

Cx. quinquefasciatus was tested against azadirachtin of neem extract. The test results show that highest Lc_{50} value is found in earthen drain water (0.028%) followed by septic tank water (0.024%), tap water (0.022%) and concrete drain water (0.022%). The highest Lc_{90} value is found in earthen drain water

(0.13%) and septic tank water (0.13%) followed by tap water (0.12%) and concrete drain water (0.11%) (Table 1,2.3,4 and Figure 1,2,3,4).

Although the Lc values of different polluted water are within statistically insignificant

Using the statistical analysis adopted by S.Swaroop's Chisquare test, it is found that the test results are highly significant (P=0.001) [9]. This shows that *Cx. quinquefasciatus* is a heterogeneous population and is highly resistant to chlorinated hydrocarbons.

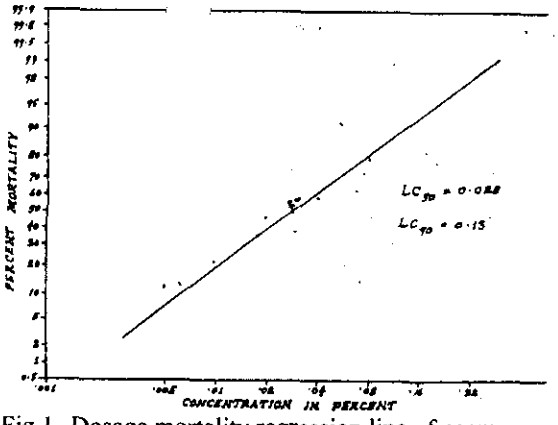


Fig.1. Dosage mortality regression line of neem insecticide against larvae of *Culex quinquefasciatus* in water from earthern drain.

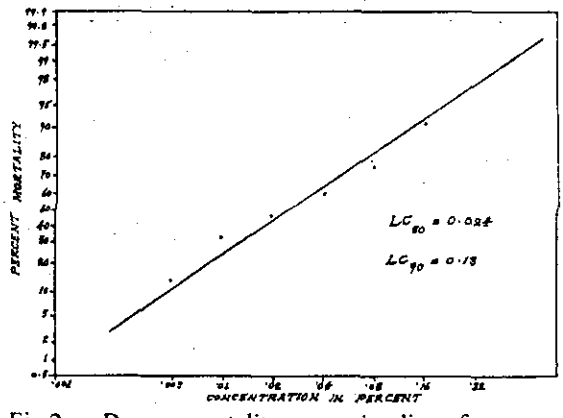


Fig.2. Dosage mortality regression line of neem insecticide against larvae of *Culex quinquefasciatus* in water from septic tank

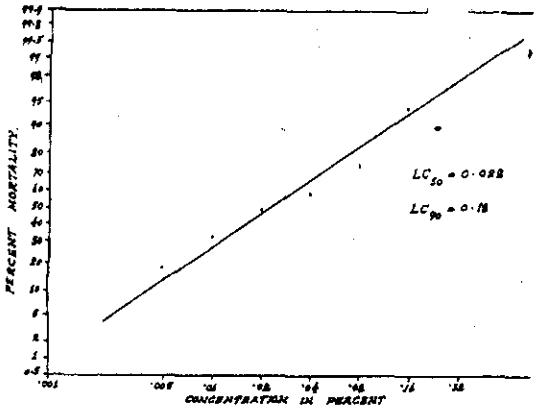


Fig.3. Dosage mortality regression line of neem insecticide against larvae of *Culex quinquefasciatus* in water in tap water drain.

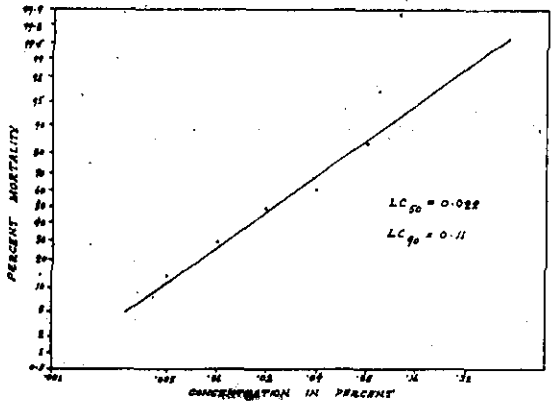


Fig.4. Dosage mortality regression line of neem insecticide against larvae of *Culex quinquefasciatus* in water in the breeding water from concrete drain.

range, they show that the mosquitoes population is heterogeneous. The regression lines are sloping and the range of concentration to get a kill of 50% to 90% mortality is nearly 16 times higher than of that of the initial concentration.

This type of trend of Lc_{50} and Lc_{90} values was found in the test of *Cx. quinquefasciatus* larvae in polluted drain and septic tanks as reported by L.S. Self and M.M.Tun [10,11] although their values were very much lower as expressed in PPM but the trend is exactly the same. All their

Table 1. Calculation of lethal concentration (Lc_{50} and Lc_{90}) from dosage mortality regression line for *Culex quinquefasciatus* larvae tested in water from earthen drain using χ^2 methods

Concen-tration	Dead/ Tested	Observed mortality rate % (O)	Expected mortality rate % (E)	O-E	$(O-E)^2$	χ^2
0.005	30/250	12	7.5	4.5	20.25	0.0291
0.01	53/250	21.2	19	2.2	4.84	0.0029
0.02	115/250	46	39.5	6.5	42.25	0.0176
0.04	145/250	58	62	-4	16	0.0067
0.08	198/250	79.2	81	-1.8	3.24	0.0021
0.16	250/250	100	93	7	49	0.0752
0.32	250/250	100	98	2	4	0.0204
					Total	0.154

Lc_{50} = 0.028% 95% Upper Confidence Limit of Lc_{50} = 0.031
95% Lower Confidence Limit of Lc_{50} = 0.025
Lc_{90} = 0.13% 95% Upper Confidence Limit of Lc_{90} = 0.143
95% Lower Confidence Limit of Lc_{90} = 0.118

Table 2. Calculation of lethal concentration (Lc_{50} and Lc_{90}) from dosage mortality regression line for *Cx. quinquefasciatus* larvae tested in water from septic tank using χ^2 methods

Concen-tration	Dead/ Tested	Observed mortality rate % (O)	Expected mortality rate % (E)	O-E	$(O-E)^2$	χ^2
0.005	32/250	12.8	11	1.8	3.24	0.0033
0.01	83/250	33.2	24	9.2	84.64	0.0464
0.02	116/250	46.4	44	2.4	5.76	0.0023
0.04	148/250	59.2	64.5	-5.3	28.1	0.0123
0.08	185/250	74	82	-8	64	0.0434
0.16	228/250	91.2	92.5	-1.3	1.69	0.0024
0.32	250/250	100	97.2	2.8	7.84	0.0288
					Total	0.1389

Lc_{50} = 0.024% 95% Upper Confidence Limit of Lc_{50} = 0.027
95% Lower Confidence Limit of Lc_{50} = 0.0201
Lc_{90} = 0.13% 95% Upper Confidence Limit of Lc_{90} = 0.144
95% Lower Confidence Limit of Lc_{90} = 0.117

trials were being carried out using the organophosphorous compound, which are highly potent but toxic hazards to man and domestic animal are unavoidable.

DISCUSSION

In India and almost all over the world where neem extract has been tested the results seem to be effective against larvae of *Cx. quinquefasciatus* and *Ae. Aegypti*. Previous laboratory studies regarding the effect of neem products on *Cx. quinquefasciatus, Anopheles stephensi* and *Aedes aegypti.* found that there were conspicious disruption of growth, prolongation of larval period and mortality rate of 90%. It was also found that exposure of fourth instar larvae of *Cx. quninquefasciatus* to water soaked neem cake powder resulted in a 50% mortality and 50% of deformed larvae at a concentration of 0.5% and 100% mortality at high concentration [12].

Table 3. Calculation of lethal concentration (Lc_{50} and Lc_{90}) from dosage mortality regression line for *Cx. quinquefasciatus* larvae tested in tap water using χ^2 methods

Concentration	Dead/ Tested	Observed mortality rate % (O)	Expected mortality rate % (E)	O-E	$(O-E)^2$	χ^2
0.005	46/250	18.4	13.5	4.9	24.01	0.0205
0.01	82/250	32.8	28	4.8	23.04	0.0114
0.02	119/250	47.6	48	-0.4	0.16	0.0
0.04	147/250	58.8	68	-9.2	84.64	0.0388
0.08	184/250	75.6	84	-10.4	108.16	0.0804
0.16	236/250	94/4	93.5	0.9	0.81	0.0013
0.32	250/250	100	97.5	2.5	6.25	0.0256
					Total	0.178

Lc_{50} = 0.022% 95% Upper Confidence Limit of Lc_{50} = 0.0246
95% Lower Confidence Limit of Lc_{50} = 0.0196
Lc_{90} = 0.12% 95% Upper Confidence Limit of Lc_{90} = 0.133
95% Lower Confidence Limit of Lc_{90} = 0.108

Table 4. Calculation of lethal concentration (Lc_{50} and Lc_{90}) from dosage mortality regression line for *Cx. quiquefasciatus* larvae tested in water from concrete drain using χ^2 methods

Concentration %	Dead/Tested	Observed mortality rate % (O)	Expected mortality rate % (E)	O - E	$(O - E)^2$	χ^2
0.005	34/250	13.6	12	1.6	2.56	0.0024
0.01	72/250	28.8	26	2.8	7.84	0.0040
0.02	117/250	46.8	47	-0.2	0.04	0.0
0.04	150/250	60.0	68	-8	64	0.0294
0.08	209/250	83.6	85	-1.4	1.96	0.0015
0.16	250/250	100	94.5	5.5	30.25	0.0582
0.32	250/250	100	98.3	1.7	2.89	0.0173
					Total	0.1128

Lc_{50} = 0.022% 95% Upper Confidence Limit of Lc_{50} = 0.0245
95% Lower Confidence Limit of Lc_{50} = 0.0197
Lc_{90} = 0.11% 95% Upper Confidence Limit of Lc_{90} = 0.121
95% Lower Confidence Limit of Lc_{90} = 0.099

In Myanmar, the test conducted also shows good impact on the larvae but a high concentration has to be utilized compared to other countries such as India. This might be due to the fact that these two species are highly resistant to DDT and other chlorinated hydrocarbons. They are under the influence of these insecticides all over the country since 1951 either in the malaria eradication programme or in the control of agriculture pests by the Department of Agriculture and Forestry.

Further studies on the effect of azadirachtin on the larval growth by inhibiting the production of hormones as well as insecticide properties should be carried out. This can be utilized in the control of the vector of malaria and Dengue Haemorrhagic Fever.

This neem extract is not at all hazardous but of high medicinal value for many diseases as described in Ayurveda sanskrit medical pharmacologica. The extract of neem insecticide can be prepared very easily all over the country where neem trees are in abundance. The kernels of neem seeds soaking in water to form a concentrated solution of neem extract are ready to be used in the control of *Cx. quinquefasciatus* larvae [13]. The longer this extract in contact with the larvae the greater is the prolonged growth inhibiting effect as well

as the contac insecticidal effect. In this way the insecticide can be extracted cheaply. It is by far the most economical method of control for developing countries. Neem kernels can be obtained without any cost and is within the reach of the common people who are interested in controlling the mosquitoes.

ACKNOWLEDGEMENT

The authors are grateful to Dr. U Soe Aung, Deputy Director (Malaria) for his permission to conduct the study of susceptibility test. We wish to thank Dr. U Tun Tun Aung, Deputy Project Manager V.B.D.C for his suggestion and advice. We wish to thank Dr. U Thein Htay, Deputy General Manager of Myanmar Agriculture Service, Yangon, for his kind permission for access to the library. Our heartfelt thanks are extended to the staff of the filariasis section for lending their invaluable assistance in field collection where their services are very much appreciated.

REFERENCES

1. Hla-Tin-Oo. Burmese-German Plant Protection and Rodent Control Project. Neem tree research, terminal report, 1987; 1-3.
2. Pranacharya, K.V.J & Verma, G.S. Miracles of neem tree. Resayan Pharmacy, 3, New Delhi, 110 - 002. 1984; 1-3.
3. Rambold, H., Forster, H., Czoppelt, C.H, et.al. The azadirachtin, a group of insect growth regulators from the neem tree.Proceedings of the second internationalneemconference,Rauischnolzhausen,Germany, 1983; 153-158.
4. Ruscoe, L.N.E. Growth disruption effects of an insect antifeedent. Nature-London. 1977: 236.
5. Myanmar Agriculture Service. Monthly report (July) 1992.
6. Mwangi, R. W. & Rambold, H. Growth regulating activity of Melia volkensi extract against the larvae of Aedes aegypti. Proceedings of the third international neem conference, Nairobi, Kenya, 1986; 679.
7. Jotmani, M.G. & Srivastana, K.P. A review of neem research in relation to insect. proceedings of the second international neem conference, Rauischnolzhausen, Germany, 1983; 43-56.
8. World Health Organization. Technical Report Series No. 443. Insecticide Resistance and Vector Control, 1970; 73-76.
9. Swaroop, S. Statistical methods in malaria eradication. W.H.O. Monograph Series. No. 51, 1963; 117-129.
10. Self, L. S. & M. M. Tun. Evaluation of organophosphorus sprays and organophosphorus oil spray against Culex pipiens fatigans in polluted drains and septic tanks. WHO/VBC/68.81, 1968; 1-7.
11. Self, L.S. & M.M.Tun. Summary of field trial in 1964-1969 in Rangoon, Burma, of organophosphorus larvicides and oil against Culex pipiens fatigans lervae in polluted water. *W.H.O. Bulletin* 43/1970; 841-850.
12. Curtis, C. F. Appropriate Technology in Vector Control. In: Biological control methods suitable for community use. Boca Raton, Florida, C. RE Press, 1990; 151-155.
13. Vietmeyer, N. D. Neem. A Tree for Solving Global Problems. Report of an Ad Hoc Panel of the Board on Science and Technology for International Development National Research Council, 1970; 31-51.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Maturation of foetal thyroid hormone synthesis and regulation

*Soe Aung, **Myo Khin, ***Kyaw Min, & ****Khin May Oo,
**Nilar Sein & ** Muya Than

*Clinical Research Division
**Nuclear Medicine Research Division
***Computer Research Division
****Virology Research Division
Department of Medical Research

A total of 343 pairs of maternal serum and foetal cord serum samples were assayed for thyroxine (T4), triiodothyronine (T3) and thyrotropin (TSH) levels. Foetal serum total T4 levels showed a positive correlation not only with gestational age(r=0.14) but also with maternal serum T4 levels (r=0.42). Although these correlations were not very strong, they were statistically significant. Foetuses were found to be T3 deficient for all gestational age groups in the study. Despite these deficient levels, there was still a significant positive correlation between foetal serum T3 levels and the maternal serum T3 levels (r=0.24). Foetal serum TSH levels were about three times higher than the corresponding maternal levels for all gestational age groups. High TSH levels in the face of deficient T3 levels for all gestational age groups in the study indicated that the function of the foetal hypothalamic-pituitary feedback control system of the thyroid gland has been well established even before the foetal maturity.

INTRODUCTION

Thyroid hormones are essential to the growth and development of human foetus. They are particularly important in stimulating neural growth and pulmonary development. Synthesis of thyroid binding globulin (TBG) had been detected as early as the 29th day of human development [1]. Foetal thyroxine and TSH had also been identified in a 78-day-old foetus [2]. Foetal hypothalamic-pituitary control system had been reported to function autonomously as early as 12 weeks of gestation. TSH in cord blood increased abruptly between 18 and 22 weeks to levels characteristic of term infants and T4 in cord blood increased progressively between 22 weeks of gestation and term [3,4]. On the other hand foetuses and newborn infants had been shown to be T3 deficient [6-9]. In a recent study, foetal serum TSH, TBG and total and free T4 and T3 concentrations were found to increase significantly with the length of gestation [7]. The present study was carried out with the objective of determining the maturity status of foetal thyroid-pituitary-hypothalamus axis in Myanmar infants between 28 and 40 weeks of gestation.

MATERIALS AND METHODS

A total of 343 pairs of maternal venous and foetal cord serum samples were taken at the time of uncomplicated deliveries at the Central Women's Hospital, Yangon, Myanmar. An informed consent was taken from each mother before recruitment. All mothers were clinically euthyroid at the time of delivery and none of them had been taking any medication that could interfere with the tests throughout their pregnancies. Their ages ranged from 16 to 48 years with a mean of 26.6 years. Maternal blood was collected from the antecubital vein

immediately after delivery. Foetal samples were mixed venous and arterial cord blood samples collected from the cord stumps immediately after severance. The maturity of the babies ranged from 28 to 40 weeks of gestation.

After collection, the samples were immediately sent to the Department of Medical Research on ice packs (2° - 8° C). the sera were then separated by centrifuging at 2000 rpm for 10 minutes and then stored at -20° C until measurements were made. The thyroxine (T4), triiodothyronine (T3) and thyrotropin (TSH) levels in serum samples were determined by radio immunoassay using Amerlex-M RIA kits (Radiochemical Centre, AMERSHAM, England). All analyses were performed in duplicates within two months after collection of the samples. Due to the limited availability of the assay kits, both maternal and foetal T4 levels were measured in 329 samples; T3 levels were measured in 320 maternal and 323 foetal samples; TSH levels were determined only in 71 maternal and 72 foetal samples. All radioactive measurements were carried out using a Pakard Auto-Gamma Scintillation Spectrometer, Model 5230. The coefficients of variation of all the tests were less than 10%.

Data analysis was performed with SPSS (Statistical Package for Social Scientists Ver 5.0 ; SPSS Corporation, Chicago, IL, U.S.A) on an IBM PC computer. Data are expressed as mean ± SEM. The differences between maternal and cord thyroid hormonal levels were analyzed by means of Student's 't' tests for unpaired samples (two tailed). Differences were considered significant if 'p' value is less than 0.05. Pearson's correlation coefficient was determined for correlations between maternal and cord hormonal levels and also between gestational age and cord hormonal levels.

RESULTS

Comparison between maternal and cord hormone levels

Table 1 summarises the results of inter-comparisons between maternal and foetal (cord) T4, T3 and TSH levels for various gestational age groups. The mean cord serum T4 and T3 levels were found to be significantly lower than the corresponding maternal levels for all gestational age groups. While mean cord serum T3 levels for all groups were strikingly lower than the corresponding mean maternal levels, the differences in mean maternal and cord serum T4 levels were less marked. On the other hand, the mean cord serum TSH levels were found to be significantly higher than the maternal levels irrespective of gestational age. Mean cord serum TSH levels were two- to three-folds higher than the corresponding maternal serum levels.

A significant positive correlation was found between maternal and cord serum T4 levels ($r=0.42$; $n=326$). This correlation was stronger for premature infants ($r=0.74$; $n=32$) than for mature infants ($r=0.37$; $n=294$). There was also a significant positive correlation between maternal and cord serum T3 levels ($r=0.24$; $n=315$). However, no significant correlation was found between maternal and cord TSH levels ($r=0.22$; $n=70$).

Correlation between gestational age and hormone levels

Figure 1a through 1c show correlations of maternal and cord hormonal levels with the gestational age. The shaded area in each figure represents normal non pregnant adults levels in Myanmar as used by the Nuclear Medicine Department of the Yangon General Hospital (55-156 nmol/l for total T4, 0.8-2.5 nmol/l for total T3 and 0-10 uU/l for TSH) (Personal

communication with the Nuclear Medicine Department of The Yangon General Hospital).

Figure 1a shows correlation of maternal and cord T4 levels with gestational age. Maternal T4 showed a significant negative correlation (r=0.12; n=329) and cord T4 showed a significant positive correlation (r=0.14; n=329).

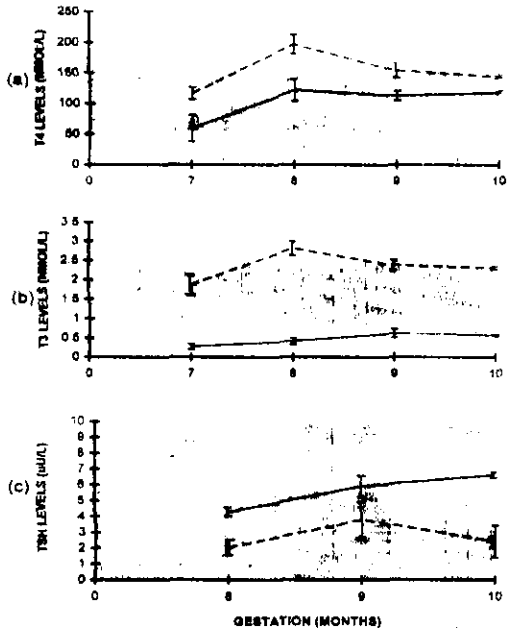


Fig.1. Total serum T4 (a),T3 (b) and TSH (c) concentrations (mean±SEM) in paired maternal and cord blood specimens plotted as a function of length of gestation. The interrupted lines represent maternal values and the continuous lines represent cord values. Shaded areas represent normal nonpregnant adult levels

Figure 1b shows maternal and cord serum T3 levels for different gestational age groups. Cord serum T3 levels never reached the adult normal non pregnant range for all gestational age groups in the study. No significant correlations were found between the gestational age and serum T3 levels in both maternal and foetal cases.

Figure 1c shows maternal and cord serum TSH levels for different gestational age groups. Cord serum TSH levels were two-to-three fold higher than the corresponding maternal levels for all gestational age groups. These high cord TSH levels did not show any significant correlation with gestational age of the foetus.

DISCUSSION

In our study, cord serum T4 levels were found to be significantly lower than the corresponding maternal levels for all gestational age groups (Table 1).

Although the majority of cord serum T4 levels were lower than the maternal levels most of them were well within normal limits for non pregnant normal adult female (Figure 1a). Even one 28 week old foetus had the level of a normal non pregnant adult female (103 nmol/l). This finding is in agreement with the findings of other workers [3,7].

Table 1. Comparison between maternal and foetal hormonal levels for various gestational age groups

Hormone	Gestation (Months)	No. of subjects	Maternal (Mean±SEM)	Foetal (Cord) Mean±SEM)	Students t test (p value)
T4 nmol/l	7	3	117.000 ± 10.000	59.333 ± 21.881	<0.05
	8	6	197.333 ± 15.601	122.667 ± 18.299	<0.01
	9	23	155.043 ± 12.291	113.043 ± 7.734	<0.0001
	10	294	144.575 ± 1.998	118.439 ± 1.975	<0.0001
T3 nmol/l	7	3	1.867 ± 0.267	0.273 ± 0.073	<0.05
	8	6	2.817 ± 0.182	0.418 ± 0.089	<0.0001
	9	23	2.387 ± 0.136	0.626 ± 0.117	<0.0001
	10	283	2.312 ± 0.041	0.570 ± 0.019	<0.0001
TSH *(uU/ml)*	8	4	2.055 ± 0.278	4.275 ± 0.478	<0.05
	9	9	3.819 ± 0.669	5.921 ± 1.111	<0.05
	10	57	2.438 ± 0.170	6.641 ± 1.022	<0.001

Although Thorpe-Beeston et al. did not find any significa it relation between foetal and maternal hor nonal levels [7], a significant positive corr·lation was found in our study between mat rnal and cord serum T4 levels. This correlat on was stronger for premature infants than for mature infants. This indicates that there might be more maternal contribution to foetal T4 in premature infants than in mature infants. Cord T4 levels also showed a significant positive correlation with gestational age of the foetuses (Fugure 1a). this finding is in agreement with the findings of other investigators [3,7].

Our finding of low cord T3 levels throughout the entire period of gestation is in agreement with other workers [6,9]. Cord T3 levels never reached the range of a normal non pregnant adult woman (Figure 1b). Despite their low levels, they still showed a significant positive correlation with corresponding maternal T3 levels. Although T3 is knows to cross the placenta more readily than T4 [10], the correlation between maternal and cord hormonal levels was not so strong in case of T3 as in case of T4. The reason for this paradox could not be explained in the present study.

No significant correlation was found between cord serum T3 levels and gestational age. This is in disagreement with the findings of a study where foetal serum TSH, TBG, and total and free T4 and T3 concentration were found to increase significantly with the length of gestation [7].

Our finding of significantly higher cord TSH levels as compared with maternal levels is in agreement with the findings of other workers [4,7-9,11]. Since TSH is not transferred across the placenta [10], all cord TSH must come from foetal pituitary gland. There is also no significant correlation between maternal and cord TSH levels in our study. The fact that cord TSH levels were very high even for premature gestational age groups indicated that maturity of foetal TSH secretion took place rather early in pregnancy. This finding is also in agreement with that of other workers [3,7], but in contrast to those studies we did not find any significant correlation between cord TSH levels and gestational age of the foetuses.

In conclusion, our findings indicated that foetuses were T3 deficient for all gestational age groups, and that the maturity of the foetal hypothalamic-pituitary feedback control system of the thyroid gland took place relatively early in pregnancy.

ACKNOWLEDGEMENT

We would like to express or gratitude to the Medical Superintendent of the Central Women's Hospital, Yangon, for his kind permission to conduct this study and Dr. Daw Win Mar, Professsor and Head of the Nuclear Medicine Department of the Yangon General Hospital for providing us with normal standard values for Myanmar women. This study was partly supported by the United Nations Children's Fund.

REFERENCES

1. Gitlin, D. & Biasucci, A. Ontogenesis of immunoreactive growth hormone, Follicle-stimutating hormone, thyroid-stimulating hormone, luteinizing hormone, chorionic prolactin and chorionic gonadotropin in the human conceptus. *Journal of Clinical Endocrinology* 1969; 29(b): 926-935.
2. Greenberg, A.H., Czernichow, P., Rega, R.C., Tyson, J. & Blizzard, R.M. Observations on the maturation of thyroid function in early foetal life. *Journal of Clinical Investigation* 1970; 49: 1790-1803.
3. Fisher, D.A., Hobel, C.J., Garza, R. & Pierce, C.A. Thyroid function in the preterm foetus. *Pediatrics* 1970; 46: 208-216.
4. Fisher, D.A., Odell, W.D., Hobel, C.J. & Garza, R. Thyroid function in the term foetus. *Pediatrics* 1969; 44: 526-535.

5. Lemarchand - Beraud, T.H., Genazzani, A.R., Bagnoli, F. & Casoli, M. Thyroid function in the premature and the full term newborn. *Acta Endocrinologica* 1972; 70: 445-453.
6. Erenverg, A., Phelps, D.L., Lam, R. & Fisher, D.A. Total and free thyroid hormone concentrations in the neonatal period. *Pediatrics* 1974; 53: 211-216.
7. Thorpe-Beeston, J.G., Kypros, H., Nicolaides, Felton, C.V., Butler, J. & McGregor, A.M. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. *New England Journal of Medicine* 1991; 324: 532-536.
8. Abuid, J., Klein, A.H., Foley, Jr T.P., & Larsen, P.R. Total and free triiodothyronine and thyroxine in early infancy. *Journal Clinical Endocrinology and Metabolism* 1974; 39(2): 263-268.
9. Lieblich, J.M. & utiger, R.D. Triiodothyronine in cord serum. *Journal of Pediatrics* 1973; 82(2): 290-292.
10. Scott, M.D. maternal-foetal thyroid disorders. In: Cloherty, J.P., Stark, A.R. Editors. *Manual of Neonatal Care.* Boston: Little, Brown and Company, 1980.
11. Delange, F., Camus, M., Winkler, M. & Dodion, J. Serum thyrotropin determination on days of life as screening procedure for congenital hypothyroidism. *Archives of Disease in Childhood* 1977; 52: 89-96.

CENTRAL BIOMEDICAL LIBRARY
5, ZIWAKA ROAD,
YANGON.
DEPARTMENT OF MEDICAL RESEARCH

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Clinical significance of early intravenous antivenom in Russell's viper bite cases in Taungdwingyi District : A preliminary study

*Tun Pe, **Khin Aye Kyu. *Aye Aye Myint, *Nu Nu Aung,
***Sann Mya & **Tin Oo

*Immunology Research Division
Department of Medical Research
**Taungdwingyi Civil Hospital
***Institute of Medicine II

A retrospective study of 26 Russell's viper (*Daboia russelii siamensis*) bite cases who received 1-2 ampoules of antivenom at the villages in Taungdwingyi Township was carried out. Sixteen pad-applied cases were included in the study. Pad-treated cases who received early antivenom irrespective of the time after the bite developed no systemic complications (n=0/4) compared to those without pad (n=7/8) or cases who received 4 ampoules of antivenom within 4 hours after the bite at the hospital (n=5/10). Those who received antivenom 4 hours after the bite at the villages irrespective of pad carried the same risk of developing systemic complications compared to those who received 4 ampoules of antivenom at the hospital. Early administration of 1-2 ampoules of antivenom is indicated for treatment of local envenomed cases and 4 ampoules of antivenom should be given to early systemic cases following bites of big snakes. Application of compression immobilisation first-aid technique with an early intravenous antivenom at the villages is to be recommended in Russell's viper bite cases. Antivenom could have been saved in 24% of snake bite cases if guidelines for antivenom therapy were properly followed.

INTRODUCTION

Most snake bites occurred to farmers while they were harvesting in the fields where immediate access to the medical treatment was limited. Early administration of antivenom (ASV) to victim plays an important role in predicting the outcome of the patient. Because of poor transportation most victims received treatment at the hospital no sooner than 4 hours after the bite when systemic envenoming has already occurred. In order to delay spread of venom, various first-aid measures have been used with little success [1]. Recently compression immobilisation technique was found to be effective in delaying spread of venom in prospective Russell's viper bite cases [2]. Early neutralisation of injected venom with ASV by intravenous or intramuscular route [3] is another alternative. It has been practiced in Taungdwingyi Township that 1-2 ampoules of ASV was given intravenously to the victims irrespective of the clotting status by the local health workers at the villages and were transported to the hospital for further management. However, the efficacy of early prehospital ASV therapy in preventing development of systemic complications has not been studied.

MATERIALS AND METHODS

Russell's viper bite cases admitted to the Taungdwingyi Civil Hospital from June to December 1994 were analyzed retrospectively. Cases who received 1-2 ampoules of prehospital ASV at the villages and those who did not were studied and their clinical details

were analyzed. The routine practice at the hospital is a total dose of 4 ampoules ASV was given to the patients on admission irrespective of their clotting status. Most pretreated snake bite cases received remaining 2-3 ampoules ASV on admission. Cases who did not receive ASV pretreatment were given 4 ampoules of ASV on arrival. Time of receiving first and second dose of ASV were recorded. Clinical details of the cases were recorded in standard proforma. Admission venom antigen levels of all patients were determined by enzyme immunoassay technique [4].

RESULTS

Altogether 171 snakebite cases were available for study. After exclusion of 30 non envenomed cases, 15% (26/141) received ASV at villages and 85% (115/141) at the hospital.

In prehospital ASV treated group 23% (6/26) had no envenoming, 31% (8/26) had local and 46% (12/26) had systemic envenoming whereas in hospital ASV treated group 26% (30/115) had no envenoming, 37% (42/115) had local and 37% (43/115) had systemic envenoming. Clinical details of the systemic cases are presented in Table 1.

Average age of the victim is 24 years (13-45yr). The site of bites includes around malleolus (14/26), dorsum of foot (9/26) and toes (3/26). Five were bitten while walking and 21 while working.

In general prehospital ASV cases received ASV at the villages on average 1.44 hours (0.25-6.50 h) after the bite. Ninety-two percent (24/26) of the prehospital ASV victims received first dose of 1-2 ampoules of ASV (mean 11.6ml) (except in 2 cases, 5 ml and 5 ampoules) intravenously within 1.19 hours (025-310h) after the bite (except in two late arrivals, 6.20 and 6.50 hours after the bites received 2 and 4 ampoules of ASV respectively). They travelled 12.9 miles (4-24 miles) and took 5.35 hours (1-34 h) to get to the hospital.

Locally, swelling, bruising, haematoma, blistering and necrosis were observed. One 290-mm-long snake was responsible for development of systemic complications manifested by incoagulable blood, spontaneous systemic bleeding, hypotension, evidence of increased

Table 1. Details of the antivenom-treated systemic Russell's viper bite cases

ASV given at	Number	Age(yr) range	Pad	ASV 1st dose (ml)	ASV timing (h)	Venom level after 1st dose (ng/ml)	ASV 2nd dose (h)	Systemic complications	Fatality
Village	2	19 (17-21)	+	10	1.00 (030-130)	50 (50)	3.40 (330-345)	0/2	
"	4	27 (16-35)	-	10	1.15 (030-210)	65 (40-60)	2.25 (050-310)	4/4	2
"	2	23 (18-28)	+	10	0.50 (040-100)	55 (50-60)	6.55 (525-830)	0/2	
"	4	26 (16-45)	-	5-50	4.50 (300-650)	63 (30-100)	8.30 (500-1125)	3/4	2
Hospital	6	19 (13-23)	+	40	2.40 (155-320)	68 (50-100)	-	2/6	1
"	10	27 (8-56)	-	40	3.00 (030-400)	58 (30-100)	-	5/10	3
"	6	25 (15-49)	+	40	7.25 (500-1145)	63 (50-80)	-	3/6	1
"	21	33 (14-68)	-	40	8.36 (410-1200)	57 (0-100)	-	9/21	3

capillary perm ability and renal failure.

Systemic cases with pad (n=4) who received one ampoule of ASV at the village within 1 hour after the bite, (irrespective of timing of the second dose) did not develop systemic complications (except one developed oliguria but recovered later) compared to those without pad (n=4). However, systemic cases either with or without pad who received 4 ampoules of ASV either at the villages or on arrival at the hospital, irrespective of timing of ASV have the same chance of developing systemic complications.

Post ASV venom levels of the local and the systemic cases were 23.8 ng/ml (10-50ng/ml) (n=8) and 56 ng/ml (30-80ng/ml) (n=11) (excluding one whose prehospital ASV venom level of 100ng/ml) respectively. Antivenom reaction was in observed 47% (8/17) of the cases.

Two prehospital ASV treated cases who received first dose of ASV as early as 30 minutes after the bite and second dose 2.5 h later and two late arrivals of the same group died of renal failure.

DISCUSSION

The clinical outcome of prehospital and hospital ASV treated Russell's viper bite cases, with or without pad were studied. Since 23% of prehospital and 26% of hospital ASV treated cases had no envenoming, expensive ASV could be spared if proper guidelines for indication of ASV were followed by the local health workers and the medical officers.

The mainstay of treatment in snakebite is early neutralization of incoming venom with specific antivenom in order to prevent systemic complications following envenoming. It had been reported that antivenom given within 2 or 5 hours after the bite might prevent renal failure [5-6]. However, an earlier study at the Tharrawaddy Civil Hospital showed that 8 of 21 cases given antivenom within 4 h after the bite developed renal failure. In two cases, 4 ampoules of ASV was given at 1 h after the bite [7].

A similar observation was made in 7/16 of our cases given with 4 ampoules of ASV within 4 h after the bite. Two of 3 systemic cases who received 1 to 2 ampoules of ASV 30 minutes after the bites and the second dose within 4 h developed systemic complications and renal failure. (One systemic case treated with 4 ampoules of ASV within 50 minutes of the bite developed oliguria but recovered).

However cases who applied first-aid pad and received 1st dose of ASV (one ampoule) at 0.30, 0.40, 1.00, and 1.30 h after the bites and 2nd dose within 4h of the bites did not develop systemic complications (one who received ASV 1.30 h after the bite developed oliguria but recovered). It is speculated that spread of venom from the site of bite (reflected by post ASV venom levels) is delayed following application of the pad which may even lead to some local inactivation of venom components [2].

The circulating venom procoagulant(s) is neutralized by early administration of ASV which might have helped in preventing development of disseminated intravascular coagulation (DIC) and systemic complications.

Renal tubular damage [8-9] and microvascular fibrin occlusion [5,10] in renal vasculature may have already occurred when systemic envenoming with DIC develops in some cases. In these circumstances late antivenom therapy may not be helpful unless it is given before microthrombi occlusion occurs.

It has been reported that one ampoule of intramuscular antivenom given within 2h after

the bite resulted in less number of cases developed DIC and clinical proteinuria [3]. However our study suggested that one ampoule of antivenom given intravenously within 30 minute after the bite to cases without pad did not prevent development of systemic complications.

It is recommended that early ASV with pad is a good combination in preventing development of systemic complications although the number of cases studied is small. One to 2 ampoules of early ASV is sufficient to treat local envenomed cases which further supports our earlier study [10]. Since mean post ASV venom level (56ng/ml) of systemic cases who received one ampoule of prehospital ASV is comparable to those of untreated systemic cases of our earlier studies (60ng/ml), it is suggested that 4 ampoules of ASV will be needed to treat early systemic cases especially bitten by big snakes.

ACKNOWLEDGMENTS

This research was supported by a research grant from the Department of Medical Research.

REFERENCES

1. Tun-Pe, Tin-Nu-Swe, Myint-Lwin, Warrell, D.A. & Than-Win. The efficacy of tourniquets as a first-aid measure for Russell's viper bite in Burma. *Transactions of Royal Society Tropical Medicine & Hygiene* 1987; 81: 403-405.
2. Tun-Pe, Aye-Aye-Myint, Khin-Ei-Han, Thi-Ha & Tin-Nu-Swe. Local compression pad as a first-aid measure for victims of bites by Russell's viper (*Daboia russelli siamensis*) in Myanmar. *Transactions of Royal Society Tropical Medicine & Hygiene* 1995; 89: 293-295.
3. Win-Aung, Tin-Tun, Aye-Kyaw, Tin-Nu-Swe & Hla-Pe. Clinical trial of intramuscular antisnake venom administration as a first-aid measure in the field in management of Russell's viper bite victims. Abstracts of Myanmar Health Research Congress December 1993. Page 68.
4. Tun-Pe, Ba-Aye, Aye-Aye-Myint, Tin-Nu-Swe & Warrell, D.A. Bites by Russell's vipers (*Daboia russelli siamensis*) in Myanmar: effect of the snake's length and recent feeding on venom antigenemia and severity of envenoming. *Transactions of Royal Society Tropical Medicine & Hygiene* 1991; 85: 804-808.
5. Matthai, T.P. & Date, A. Acute renal failure in children following snake bite. *Annals of Tropical Paediactric* 1981; 1: 73-76.
6. Jeyarajah, R. Russell's viper bite in Sri Lanka. A study of 22 cases. *American Journal of Tropical Medicine Hygiene* 1984; 33: 506-510.
7. Myint-lwin, Warrell, D.A., Phillips, R.E., Tin-Nu-Swe, Tun-Pe & Maung-Maung-Lay. Bites by Russell's viper (*Vipera russelli siamensis*) in Burma: haemostatic, vascular, and renal disturbances and response to treatment. *The Lancet* 1985; ii: 1259-1264.
8. Myint-Myint-Than. Changes in the renal tubules in viper envenomation. MSc (Zoology) Thesis. Arts and Science University, Rangoon, 1978.
9. Ratcliffe, P.J., Pukrittayakamee, S., Ledingham, J.G.G. & Dunnill, M.S. Acute renal failure in the isolated perfused kidney, induced by Russell's viper venom. *Clinical Science* 1985; 68: (suppl II) : 39-40P.
10. Aung-Khin. Histological and ultrastructural changes of the kindney in renal failure after viper envenomation. *Toxicon* 1977; 16: 71-75.
11. Tun-Pe, Aye-Aye-Myint, Nu-Nu-Aung, Khin-Aye-Kyu & Tin-Oo. Study of venom neutralising efficacy of monospecific cloudy liquid antivenom. *The Myanmar Health Sciences Research Journal* 1995; 7: 145-152.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Russell's viper bites in Taungdwingyi Civil Hospital: Clinical features and response to antivenom

**Sann Mya, **Tun Pe, **Aye Aye Myint, **Nu Nu Aung, ***Khin Aye Kyu & ***Tin Oo

*Institute of Medicine (1)
**Immunology Research Division
Department of Medical Research
***Taungdwingyi Civil Hospital

One hundred thirty-four Russell's viper (*Daboia russelli siamensis*) bite cases were studies in Taungdwingyi during six months from June to December 1994. Forty percent of the victims were under 20 years of age. Thirty-eight percent showed no evidence of envenoming, 28% had local and 34% systemic features of envenoming. Ten percent of the cases developed local necrosis. Fifteen percent of the victims received antivenom therapy at the villages. Cases presenting with systemic manifestations had spontaneous systemic bleeding (29%); malena (18%), hypotension (22%), oliguria (42%), conjunctival oedema (18%) and renal failure (33%). Twelve deaths (9%) were due to hypotension and renal failure. Spontaneous systemic bleedings were late manifestations and seen from day 2 to 6 after the bite. Thirty-seven percent of the cases with systemic bleeding failed to have normal clot restoration in 6 hours following 40 ml of antivenom administration. Antivenom reactions were observed in 50% of the cases. Fifty two percent of juvenile and 19% of adult viper bites resulted in blank bites and 16% of the former and 31% of the latter bites led to systemic envenoming.

INTRODUCTION

Russell's viper (*Daboia russelli siamensis*) bite is an occupational hazard of our farmers. Snake bite is endemic in six rice-growing divisions of Myanmar. Recent study based on 115 hospitals (1990-91) showed an average annual bite of 4998 cases with a mortality rate of 4.9%. Taungdwingyi Township has the highest incidence of snake bite in Myanmar with an annual bite of over 200 cases and a mortality rate of 10%. Recent reports on geographical variation of biological properties of Russell's viper venom [1-6] in Myanmar and variation in invitro neutralisation of antivenom against Daydaye and Taungdwingyi venoms [4] suggested that clinical features of Russell's viper bite cases from different localities may vary and hence response to antivenom treatment since these antivenoms were raised with venoms from snakes collected from lower Myanmar. This prompted us to study clinical features and response to antivenom treatment of Russell's viper bite cases of Taungdwingyi.

MATERIALS AND METHODS

All snakebite cases admitted to the Taungdwingyi hospital from June to December 1994 were studied. Clinical details of the patients were recorded in standard proforma. Twenty minute clotting test [7] was used to determine the degree of envenoming. A total of 40 ml of monospecific antivenom were given to patients at the discretion of the medical officer. Clotting test was carried out at 2 hour intervals on the cases presenting with clottable blood and 6 hourly after giving antivenom on the cases with systemic features until normal clot restoration occurred. Patients with clottable

blood were observed for 24 hours and antivenom was given if the blood became incoagulable. Two milliliters of blood were taken for the clotting test and one millilitre for venom and antivenom assay. For the latter, serum samples were collected at 0, 1/2, 1,2,3,4,5,6 hour, then 2 hourly up to 12 hour, 4 hourly up to 24 hour and 12 hourly up to 96 hour. Sera were collected onto filter paper strips, air dried, stored in a sealed polythene bag and later transported to the Department of Medical Research once a month. Venom antigen and antivenom levels of the samples were determined by enzyme immunoassay technique [8] at the Immunology Research Division. Both liquid and lyophilised monospecific antivenoms were used.

RESULTS

Table 1. Clinical details of 134 Russell's viper bite cases

Total (134) (45 systemic+38 local+51 non-envenoming)		%
Sex; male : female	99 : 35	74 : 26
Time bite : morning : evening	90 : 42	68 : 32
Age (yr)	27 (7 - 75)	
Activity when bitten; working: walking	90 : 20	82 : 18
Site of bite; lower limb: upper limb	115 :19	86 : 14
First-aid:		
Tourniquet	95	71
Chicken pad	12	9
Rubber pad	45	34
Incision	3	2
Herbal	1	1
Tattoo	1	1
Coagulation	1	1
Distance travelled (miles)	8.2 (0.5-41)	
Bite and admission (h)	4.05 (0.05-46)	
Antivenom pretreatment at village	26/179	15
Time of pretreatment (h)	1.44 (0.25-6.50)	
Time to get to hospital (h)	5.35 (1-34)	
Distance travelled to hospital (miles)	12.9 (4-24)	

A total of 239 snakebite cases were admitted to the Taungdwigyi hospital between June and December 1994, of which 219 were Russell's viper (*Daboia russelli siamensis*), 4 cobra (*Naja Kaouthia*), one green pit viper (*Trimeresurus erythrurus*) and 16 unknown bites. Of the total, 179 cases were available for the study. Forty-five compression pad applied cases were excluded from the study. Of 134 cases, 45 (34%) showed systemic, 38 (28%) local and 51 (38%) no features of envenoming. Only 52/163 (32%) brought the dead snakes for identification.

Clinical details of 134 Russell's viper bite cases are presented in table 1 and 2.

Table 2. Clinical details of 134 Russell's viper bite cases

Symptoms: (134)		%	Signs: Local & systemic (81)		%
Local pain at site	43	32	Local swelling	54	67
Epigastric pain	35	27	Bleeding at site of bite	3	4
Loin pain	29	22	Local tenderness	44	54
Vomiting	5	4	Local bruising	22	27
Nausea	2	2	Local necrosis	8	10
Drowsiness	12	9	Haematoma	7	9
Difficulty in opening eyes	6	5	Blistering	4	5
			Systemic cases	45	
			Spontaneous systemic bleeding	13	29
			Malena	8	18
			Subconjunctival	2	4
			Haemetemesis	3	7
			Haematuria	6	13
			Hypotension (<80 mmHg)	10	22
			Oliguria (<400 ml/ 24 h)	19	42
			Conjunctival oedema	8	18
			Periorbital oedema	6	13
			Renal failure	15	33
			Fatality	12	27

The average age of the victim is 26 years (7-75yr) and 40% are under 20 years of age. Bites

while in house premises (3%) were also observed.

The sites of bites on upper limb (15%) were 14 fingers (70%), 3 palm (15%), 2 dorsum of hand (10%), 1 head (5%) and on lower limb (85%) were 47 malleolus (33%), 40 dorsum of foot (28%), 29 toes (20.5%), 15 foot (10.5%) and 12 heel/calcaneum (8.4%).

Development of local and systemic features

Local haematoma, bruising and blistering were detected on admission or on day 1-2 and necrosis not until 2 to 10 days after the bite. Bruising also occurs in 11 blank bites.

Systemic cases presented with spontaneous systemic bleeding (29%)., hypotension and oliguria leading to renal failure. Hypotension was presenting feature on admission in 6/10 hypotensive cases and in 4 cases it developed on day 2-3 after the bites. Some died of shock on 15h or 4 days after the bites. Drowsiness, difficulty in opening eyes, conjunctival and periorbital oedema, haematuria were seen in late arrivals on day 2-3 after the bite. Spontaneous systemic bleeding such as haematemesis (day 5), malena (day 2, 5 and 6), subconjunctival haemorrhage (day 4) were late manifestations. Oliguria was usually present on admission and led to acute renal failure in 15/45 (33%) of systemic cases and unresponsive cases died on average 6 days (3 to 10 days) after the bite.

Venom antigen levels

The initial venom antigen levels of local and systemic cases are 26 ng/ml (10-50 ng/ml) (n=28) and 61 ng/ml (30-100ng/ml) (n=30) respectively and that of ASV pretreated local and systemic cases are 24 ng/ml (10-50ng/ml, n=8, excluding 6 antigen negative cases following antivenom therapy) and 60 ng/ml (40-100 ng/ml) (n=12) respectively.

Response to antivenom therapy

In 17/27 (63%) of systemic cases, normal clot restoration occurred 6 hours after 4 ampoules of ASV therapy whereas in 10/27 (37%) cases it took more than 6h (range 12-42h). Antivenom reaction (urticaria, rash, fever and rigor) occurred in 50% (22/44) of the cases.

The Length of snake and the degree of envenoming

A total of 47 snakes (31 juvenile 190-480mm and 16 adult 600-1030mm) brought by the victims were studied. Bites by 52% (16/31) of juvenile and 19% (3/16) of adult snakes resulted in blank bites whereas 16% (5/31) of juvenile and 31% (5/16) of adult bites resulted in systemic envenoming. Thirteen percent (2/16) of adult bites resulted in systemic complications like spontaneous systemic bleeding, hypotension and renal failure. Local envenoming was observed in 32% (10/31) of juveniles and 50% (8/16) of adult bites. Majority (14/23) of bites by juvenile snakes occurred while at work whereas 4/5 adult bites occurred on road.

DISCUSSION

This study shows that Taungdwingyi Township has a high incidence of Russell's viper bites and majority of the victims are farmers bitten while at work. The common site of bite is in foot probably because the bites occurred in the fields while they were harvesting crops.

Fifteen percent of the victims from Taungdwingyi seek antivenom (ASV) therapy from the local health workers compared to none in Tharrawaddy study [9] suggesting that the farmers are aware of the importance of receiving early ASV in snakebite.

Although Magwe hospital is 80 km north of Taungdwingyi, there are some differences in

clinical features of Russell's viper bite cases. Haematemesis (21%), subcojunctival haemorrhage (25%) and local bleeding from the site of bite (34%) are 3 to 8 times more common in Magwe [10] and oliguria (17%) and hypotension (7%) are 2.5 to 3 times more common in Taungdwingyi. Study on biological properties of venom from two close localities, Taungdwingyi and Myehte (66 km south of the former), showed variation in coagulant, haemorrhagic, necrotic and capillary permeability increasing activities [5]. A similar variation in venom properties from two close localities, Tharrawaddy and Letpadan was also reported [6].

Hypotension (35%), haematuria (72%) and local bleeding from the site of bite (31%) are 1.5 to 8 times more common in Tharrawaddy series (380 km south of Taungdwingyi) [9] and local necrosis (10%) and malena (18%) are 2.5 to 5 times more common in Taungdwingyi series. Study on biological properties of venoms from Taungdwingyi and Tharrawaddy localities [5-6] also suggested that the former venom is more necrotic and haemorrhagic than the latter and possess potent defibrinogenating activity which accounts for a higher incidence of local necrosis in Taungdwingyi series.

Studies on neutralisation of biological properties of venoms of Taungdwingyi and Tharrawaddy with a batch of monospecific antivenom suggested that 2 to 40 times more antivenom is needed to completely neutralise different biological properties of venom of Taungdwingyi (unpublished observation). Although coagulant activity of venom of Tharrawaddy is twice potent than Taungdwingyi, it needs 40 times more antivenom to neutralise the latter suggesting qualitative differences in venom procoagulant(s) between them. It may also account for failure of 37% (10/27) of systemic cases treated with 40 ml of antivenom to have normal clot restoration within 6 hours after antivenom (range 12-42h). It also points out that antivenom raised with venom from one locality may have variable neutralising efficacy on venom distant from its origin [11].

A higher incidence of hypotension (35%) in Tharrawaddy compared to Taungdwingyi (22%) may be partly due to increased capillary permeability activity of the former venom. Spontaneous systemic bleeding like malena reflects the haemorrhagic activity of the venom. Local bruising is the result of local injury since it is observed in blank bites.

ACKNOWLEDGEMENT

This research was supported by a research grant from the Department of Medical Research.

REFERENCES

1. Tun-Pe, Nu-Nu-Lwin, Aye-Aye-Myint, Kyi-May-Htwe & Khin-Aung-Cho. Geographical variation of the biological properties of Russell's viper venom: a preliminary report. *The Myanmar Health Sciences Research Journal* 1993; 5: 107-112.
2. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe & Khin-Aung-Cho. Variation in biological properties of venoms from different localities of Ayeyawady Division. *The Myanmar Health Sciences Research Journal* 1993; 5: 121-124.
3. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe, Khin-Aung-Cho & Theingie. Geographical variation of biological properties of Russell's viper (Daboia russelli siamensis) venom: Yangon Division. *The Myanmar Health Sciences Research Journal* 1994; 6: 87-89.
4. Tun-Pe, Sann-Mya, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung-Cho & Theingie. Biological properties of Russell's viper venom of upper and lower Myanmar. *Myanmar Medical Journal* 1994; 39: 1-6.
5. Sann-Mya, Tun-Pe, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung-Cho & Theingie. Geographical variation of biological properties of Russell's viper venom: Magway Division. *Myanmar Medical Journal* 1994; 39: 7-9.
6. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe, Khin-Aung-Cho & Theingie. Geographical variation of biological properties of Russell's viper venom: Bago

Division. *The Myanmar Health Sciences Research Journal* 1995; 7: 92-94.

7. Warrell, D.A., Davidson, N.McD., Greenwood, B.M., Omerod, L.D., et al. Poisoning by bites of the saw-scaled or carpet viper (Echis carinatus) in Nigeria. *Quarterly Journal of Medicine* 1977; 46: 33-62.
8. Tun-Pe, Aye-Aye-Myint, Tin-Nu-Swe & Warrell. D.A.Bites by Russell's vipers (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 Tropical Medicine & Hygiene* 1991; 85: 804-808.
9. Myint Lwin, Warrell, D.A Phillips, R.E Tin-Nu-Swe. Tun-Pe, & Maung-Maung-Lwin. Bites by Russell's viper *(V. russelli siamensis)* in Burma. Haemostatic, vascular and renal disturbances and response to treatment. *Lancet* 1985; ii: 1259-1264.
10. Saw-Naing. Influencing factors on the morbidity and mortality of viper bite cases. Divisional Hospital, Magwe, 1981-82. *Burma Medical Journal* 1985; 31: 214-229.
11. Phillips, R.E., Theakston, R.D.G., Warrell. D.A., Galigedara, Y et al. Paralysis, rhabdomyolysis and haemolysis caused by bites of Russell's viper (Vipera russelli puchella) in Sri Lanka: failure of Indian (Haffkine) antivenom. *Quarterly Journal of Medicine* 1988: 68: 691-716.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Salting and temperature effect on the occurrence of *Salmonella* spp. in hens' and ducks' eggs

Khin Nwe Oo, Hnit San Swe & Myat Thida

Bacteriology Research Division
Department of Medical Research

The study was carried out for obtaining information on the isolation of *Salmonella* spp. from eggs in our community. We also investigated whether the changing of salting and temperature on eggs would alter the isolation rate of *Salmonella* spp. The study was conducted in Yangon from May to October, 1996. The eggs were collected from small markets, street vendors and farms and totally 210 eggs were tested in this study. The *Salmonella* spp. were isolated and identified by standard procedures. They were more isolated from hens' and ducks' egg samples which were bought from farms. They were isolated from samples which were stored for 7 days at room temperature (24° C) and at 4°C. They were not isolated from half-boiled egg and salted egg samples. From this study it was observed that it was safe to eat the eggs after cooking well.

INTRODUCTION

Of the variety of microorganisms responsible for outbreaks of food-poisoning, *Salmonella* spp. are by far the most frequently incriminated. The almost universal presence of these organisms in certain common foods, their ability to grow in a wide variety of foodstuff over a substantial temperature range (4°C-45°C) [1], and the prolonged period of excretion following recovery are the properties which, taken together, distinguish *Salmonella* spp. from other food poisoning organisms. It is because of these characteristics that *Salmonella* spp. are really the only food poisoning organisms in which human beings as carriers pose potential problems as sources of outbreaks.

In Myanmar, *Salmonella* spp. are responsible for 0.9% of acute diarrhoea in children [2]. Salmonella was found in 6.5% of children's stool specimens, 0.8% of cooked foods, 13.4% of flies and 15% of currency note samples [3]. A study on *Salmonella* spp. isolation from raw meat was done in 1974, and 63 salmonella strains covering eight serotypes were isolated from 690 specimens of raw meat including pork, poultry and beef [4], percentage positivity was 9.13%. In the 1994 study, *Salmonella* spp. were found in 5.81% of raw pork and 1.66% of processed pork [5]. There is no available information on the isolation of *Salmonella* spp. from eggs in our community and thus this study was carried out.

Our people usually eat salted ducks' eggs. Some people store the eggs at room temperature (24°C) and also at 4°C for days or weeks. Many people eat half boiled eggs. Therefore this study was carried out to investigate whether the effect of salting and temperature changes would alter the isolation rate of *Salmonella* spp. from eggs.

MATERIALS AND METHODS

Sample collection

The eggs were bought from small markets and street vendors in Botataung Township, Yangon.

They were collected from May to October, 1996. Eighteen each of raw hen and duck eggs which were bought from market and also 18 each from farm were tested. Twelve each of them were stored at room temperature for 3 and 7 days and studied. Another [illegible] each of them were stored at 4°C for 3 and [illegible] days and tested the cool temperature effect. Eighteen hen eggs were tested as half-boiled eggs to study the hot temperature effect. Twenty four salted duck eggs were tested to study the salting effect. A total of 210 eggs were bought and tested in this study.

Isolation and identification of salmonellae

Egg sample was put into (W/V of 1:4) peptone water and incubated at 37°C for 24 hours. Peptone water was used as a pre-enrichment medium for increased recovery of salmonellae [6]. On the next day, one milliliter of suspension from the pre-enrichment medium was transferred into 10 ml of Selenite-F broth. The tube was incubated at 43°C aerobically for increased recovery of salmonellae [7]. After 24 hours, it was subcultured onto Salmonella-Shigella agar and incubated at 37°C aerobically for 24 hours. Suspicious colonies were picked and biochemically tested. Those which showed typical biochemical characteristics of salmonellae were tested with salmonella polyvalent antisera "O" and group antisera "A-E".

Antibiotic sensitivity test

The antibiotic sensitivity test was performed on Mueller-Hinton medium by the agar disk diffusion method [8]. The antibiotic disks such as chloramphenicol, gentamicin, septrin, amoxicillin, ampiclox and tetracycline disks were used in this study. The zone diameter was compared with the zones of the control strain.

RESULTS

Altogether 210 egg samples were collected from small markets, street vendors and farm. All samples were received at the laboratory in good condition and therefore isolation and identification of *Salmonella* spp. were carried out. Twenty four each of raw hens' and ducks' eggs were tested as raw. Among them *Salmonella* spp. were isolated from samples which were bought from farm. It was not isolated from samples which were collected from small markets and street vendors (Table 1).

Table 1. *Salmonella* spp. isolation from raw egg samples

Tested eggs	No. of eggs tested	No. of eggs from which *Salmonella* spp. isolated	Serogroups of isolated *Samonella* spp.
Raw hen eggs (from market)	12	0	-
Raw hen eggs (from farm)	12	4	C_1, E_1
Raw duck eggs (from market)	12	0	-
Raw duck eggs (from farm)	12	3	E_1, E_3

Table 2. *Salmonella* spp. isolation from egg samples which were bought from markets and were stored at room temperature (RT) and at 4°C

Tested eggs	No. of eggs tested	No. of eggs from which *Salmonella* spp. isolated	Serogroups of isolated *Samonella* Spp.
Hen eggs at RT for 3 days	12	4	E_1, E_3
Hen eggs at RT for 7 days	12	6	E_1, E_3
Hen eggs at 4°C for 3 days	12	2	E_1
Hen eggs at 4°C for 7 days	12	2	E_3
Duck eggs at RT for 3 days	12	0	-
Duck eggs at RT for 7 days	12	4	E_1, E_3
Duck eggs at 4°C for 3 days	12	0	-
Duck eggs at 4°C for 7 days	12	6	E_1, E_3

As shown in Table 2 it was isolated from samples which were stored at room temperature and at 4° C for 3 days and 7 days. It was not isolated from half boiled egg samples and salted duck egg samples.

All of the isolated strains were sensitive to gentamicin, chloramphenicol, septrin, ampiclox, amoxicillin and tetracycline in this study.

DISCUSSION

Salmonellosis is a zoonotic disease that can be transmitted to humans. It is a global problem representing 60% to 80% of all reported cases of food-borne diseases in countries with operational health surveillance systems. Epidemiologists believe, however, that globally the number of persons infected by salmonellae from animal products each year is about four million [9].

Non typhoidal salmonellosis is an animal disease. Eggs from farms can be contaminated with *Salmonella* spp. from excreta of hens and ducks. These eggs can also be contaminated from the hands of handlers and utensils during the process of transportation and from the hands of sellers. However, there was no isolation of salmonella from the eggs which were bought from markets and street vendors.

From this study it is known that the egg shells can be contaminated with *Salmonella* spp. and thus hand washing is needed after handling the eggs. Other wise the inner part may again be contaminated and give infection if it is eaten raw.

In this study it was isolated from eggs which were stored at room temperature and at 4° C. It was more isolated from eggs which were stored for 7 days than 3 days. Salmonellae grow at temperature between 4° C and 45° C [1]. Thus raw eggs were not safe to be eaten even after storage at 4° C refrigerator if they are originally contaminated.

The salmonella is sensitive to heat and will not survive at temperature above 70° C [1]. It was not isolated from half boiled eggs and from samples which were mixed with hot coffee and thus it is safe to eat half-boiled eggs either with water or coffee.

Also it was not isolated from salted eggs. It may be due to that there is no contamination of salmonellae on these tested eggs originally. Thus it would be needed to study the survival of *Salmonella* spp. in salted eggs.

All of the isolated salmonellae were sensitive to chloramphenical and septrin. These drugs are drugs of choice which are usually used in non typhoidal salmonellosis in this community.

From this study it was observed that the eggs bought from the farm were more contaminated with salmonellae that can survive even at 4° C. But they can be readily destroyed by heat used in normal cooking methods. Therefore it is safe to eat the eggs after cooking well.

REFERENCES

1. WHO. Report of a WHO expert committee on salmonellosis control: the role of animal and product hygiene. *WHO Technical Report Series,* No. 774, Geneva, 1988.
2. Tin-Aye, Mar-Mar-Nyein, Kanemasa, Y. & Hayashi, H. Etiological agents responsible for acute diarrhoea in children in an urban community in Burma. *Microbiology and Immunology* 1983; 27(6): 55.
3. Khin-Nwe-Oo, Myint-Myint-Lwin & Aung-Mon. Isolation of salmonellas from stool, foods, flies and currency notes. *The Myanmar Health Sciences Research Journal* 1989; 1(2): 48-50.
4. Khin-Khin-Soe & Khin-Khin-Aye. Salmonella isolation from raw meat. *Abstract. Burma Res. Cong.* 1974; 151.
5. Aye-Aye-Oo. Bacteriological evaluation of raw and processed pork. M.Med.Sc. (Micro) Thesis, IM (1), Yangon, 1994-1995.

6. Thomason, B.M., Dodd, D.J. & Cherry, W.B. Increased recovery of Salmonella from environment samples enriched with buffered peptone water. *Applied Environmental Microbiology* 1977; 34: 370-373.
7. Harvey, R.W.S. & Thomson, S. Optimum temperature of incubation for isolation of salmonellae In: A system for detecting salmonellae in meat and meat products by Georgala, D.L. & Boothroyd, M. *Journal of Applied Bacteriology* 1965; 28(1): 206-39.
8. National Committee for Clinical Laboratory Standards, Approved Standard: M2-M3. Proformance standards for antimicrobial disk susceptibility tests, 3rd ed. National Committee for Clinical Laboratory Standards, Vilanera, Pa. 1989.
9. WHO Bulletin. Hygiene in animal production. An important tool to prevent human salmonellosis. 1988; 66(6): 784-5.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

The association between faecal leukocytes and pathogens present in stool of children with diarrhoea and dysentery

*Mar Mar Nyein, **Khin Myat Tun, *Moe Moe Win,
***Than Saw & ***Aye Than

*Bacteriology Research Division
**Clinical Research Division
***Parasitology Research Division
Department of Medical Research

A total of 378 cases of children with diarrhoea and dysentery admitted to Yangon Children's Hospital from March to mid-September, 1996 were studied. The study population comprised 233 males and 145 females. Faecal leukocytes were identified under a high power field microscope by wet mounting of methylene blue staining method. Those cells clearly identified with either round nucleus or as polymorphonuclear were noted and degenerated cells that could not be clearly identified were ignored. The bacterial pathogens and protozoal pthogens were identified by standard methods. Faecal leukocytes were present in 31.2 % of total cases (diarrhoea 28.5%, dysentery 45.8%). The association between the presence of faecal leukocytes and isolated pathogens from the stools was analysed. It was found that faecal leukocytes were seen in stools which were associated with *Shigella* (25%), *Shigella* and *Entamoeba histolytica* (71.4%), *Entamoeba histolytica* (29.6%), enteropathogenic *Escherichia coli* and *Entamoeba histolytica* (41.7%), enteropathogenic *Escherichia coli* (9.8%), *Klebsiella* spp. (50%) and *Vibrios* (50%).

INTRODUCTION

Acute infectious diarrhoea illness has been divided into two major types on the basis of their pathogenesis (a) Inflammatory and (b) non-inflammatory [1]. Inflammatory processes usually arise from the colon by a mechanism of mucosal invasion by pathogens such as *Shigella, Salmonella* and invasive *Escherichia coli* which may produce a classic dysenteric stool with blood and pus [1]. In contrast, non inflammatory processes often arise from the action of an enterotoxin in the upper small bowel which results in a watery diarrhoea.

Microscopic examination of stool has long been recognized as an important part of diagnostic tool for patients with acute diarrhoeal illness. The diagnostic potential of faecal leukocytes in acute diarrhoea was also demonstrated. Because of consuming factor in identifying enteric pathogens, and the limited indications for antibiotics in the treatment of diarrhoea, examination of faecal leukocytes will play an important role as possible aid towards early diagnosis [2-7].

MATERIALS AND METHODS

Study population

Random samples of stools from children with diarrhoea/dysentery admitted to Yangon Children's Hospital were included in this study. Out of 378 cases tested (231 males and 147 females), 319 cases had diarrhoea and 59 cases had dysentery. The age of children ranged from one month to 10 years. All patients attending the hospital were interviewed by a trained personnel or a

physician for symptoms, date of onset of disease and any previous treatment. Patients having 3 or more loose stools within 24 hours without abdominal pain and cramps, and without the presence of mucus/blood in the stool were defined as having watery diarrhoea. Those having 3 or more loose stools within 24 hours with or without abdominal pain and cramps, and with visible mucus and/or blood in stool, were defined as having dysentery. Also, 30 stool samples from apparently healthy children (17 males and 13 females) were tested to serve as controls.

Bacteriological examination

Rectal swabs collected in Cary Blairs' transport media were plated onto MacConkey Agar (MA), Salmonella-Shigella (SS) Agar and Thiosulphate- Citrate- Bile- Sucrose (TCBS) media and incubated at 37°C for 24 hours. *Escherichia coli, Salmonella, Shigella* and *Vibrio* species were identified by standard methods as described in the WHO Manual [8].

Campylobacter was isolated from Brucella agar with vancomycin (10 mg/L), Cephalothin (15 mg/L) and 5 per cent sheep blood after 48 hours of anaerobic culture using gas generation kit (Oxoid, BR-56).

Parasitic examination

Fresh stool samples were also examined for the presence of motile trophozoites, larvae and eggs of parasites by standard methods.

Faecal leukocyte examination

Stool specimen from each patient was examined by placing a small fleck of mucous or stool on a clean glass slide and mixed thoroughly with two drops of Loffler's methelyne blue stain [9]. The number of white blood cells present in about 8-10 random fields under the high power objective (40 x) were counted and the average numbers expressed as <25 or >25 per high power field. Those cells clearly identified as having either round nucleus or as polymorphonuclear were noted and degenerated cells that could not be clearly identified were ignored.

RESULTS

Among diarrhoea cases, leukocytes were present in 28.3% and 28.9% of males and females respectively. So also in dysentery cases, leukocytes were found in 45 per cent of males and 47.4% of females (Table 1). It was noted that the presence of leukocytes does not play an important role with regard to different age groups.

Table 1. Presence of leukocytes in stool of children with diarrhoea and dysentery (leukocytes/ cases)

Age groups (yrs)	Diarrhoea			Dysentery		
	Male	Female	Total	Male	Female	Total
<6	12/51 (23.5)	10.36 (27.8)	22/87 (25.3)	3/7 (42.9)	0/1 (0)	3/8 (37.5)
7-12	27/77 (35.1)	11/56 (19.6)	38/133 (28.6)	0/3 (0)	2/3 (66.7)	2/6 (33.3)
13-24	13/48 (27.1)	13/22 (59.1)	26/70 (37.1)	9/15 (60.0)	3/6 (50)	12/21 (57.1)
25-36	1/8 (12.5)	2/11 (18.2)	3/19 (15.8)	3/5 (60)	1/5 (20)	4/10 (40)
37-48	1/3 (33.3)	1/1 (100)	2/4 (50)	0 -	0/1 (0)	0/1 (0)
49-60	0/2 (0)	0/2 (0)	0/4 (0)	1/4 (25)	0 -	1/4 (25)
60>	0/2 (0)	0 -	0/2 (0)	2/6 (33.3)	3/3 (100)	5/9 (55.6)
Total	54/191 (28.3)	37/128 (28.9)	91/319 (28.5)	18/40 (45)	9/19 (47.4)	27/59 (45.8)

Figures in parenthesis denote percentages

Table 2. Association between faecal Leukocytes and pathogens present in stool of diarrhoea and dysentery cases

Pathogens	Diarrhoea	Leukocyte	Dysentery	Leukocytes	Total	Leukocytes
Shigella alone	17	5	7	1	24	6
	(5.3)	(29.4)	(11.9)	(14.3)	(6.4)	(25.0)
Shigella + *E. his.*	8	5	13	10	21	15
	(2.5)	(62.5)	(22)	(76.9)	(5.6)	(71.4)
E. his.	43	10	11	6	54	16
	(13.5)	(23.3)	(18.6)	(54.6)	(14.3)	(29.6)
EPEC + *E. his.*	8	1	4	4	12	5
	(2.5)	(12.5)	(6.8)	(100)	(3.2)	(41.7)
EPEC	38	3	3	1	41	4
	(11.9)	(7.9)	(5.1)	(33.3)	(10.9)	(9.8)
Klebsiella spp.	5	2	1	1	6	3
	(1.6)	(40)	(1.7)		(1.6)	(50)
Vibrios	9	4	1	1	10	5
	(2.8)	(44.4)	(1.7)		(2.7)	(50.0)
Vibrio + *E. his.*	5	0	0	0	5	0
	(1.6)				(1.3)	
Klebsiella spp. + *E. his.*	0	0	1	0	1	0
			(1.7)		(0.3)	
EPEC + *Shigella*	1	0	0	0	1	0
	(0.3)				(0.3)	
EPEC + *Vibrios*	1	0	0	0	1	0
	(0.3)				(0.3)	
With athogens	135	30	41	24	176	54
	(42.3)	(22.2)	(69.5)	(58.5)	(46.6)	(30.7)
No pathogens	184	61	18	3	202	64
	(57.7)	(33.2)	(30.5)	(16.7)	(53.4)	(31.7)
Total	319	91	59	27	378	118
		(28.5)		(45.8)		(31.2)

Association between faecal leukocytes and pathogens present in stool of diarrhoea and dysentery cases is shown in Table 2. It was found that pathogens were isolated from 42.3% and 69.5% of diarrhoea and dysentery cases respectively. Leukocytes were found in 25% of cases with the isolation of *Shigella*, 71.4% in cases with *Shigella* and *Entamoeba histolytica*, 29.6% in cases with *Entamoeba histolytica* and 9.8% in enteropathogenic *Escherichia coli* cases. Among the cases without isolation of pathogens, 33.1% and 16.7% of cases were present with leukocytes among diarrhoea and dysentery groups respectively. Of the cases with identified pathogens, leukocytes were present in 22.2% and 58.5% of diarrhoea and dysentery respectively. However, leukocytes were found from one case out of 30 cases (3.3%) of the control specimens.

Moreover, leukocytes were obtained more frequently in mixed infection of *Shigella* and *E.histolytica* (71.4%) than infection with *Shigella* species alone (25%).

DISCUSSION

Patients usually reporting to the treatment centre at varying stages of the disease may influence the number of cells shed in the faeces and also because even apparently healthy individuals in the tropics may have some degree of inflammation in the intestine [10,11]. In our study, only 3.3% from the control group,

shed leukocytes and this is significant compared with the tested groups. Thus, it might be useful to correlate the inflammation due to infections.

The presence of >25 white blood cells (WBC) per high power field (hpf) was suggested as a significant cut-off level and it was claimed that the best prediction of shigellosis was the presence of > 25 wbc/hpf together with the presence of red blood cells [12]. In our findings, among the 20 cases of dysentery with *Shigella,* leukocytes were identified from 25% from cases with *Shigella* and 71.4% from cases with mixed infection of *Shigella* and *Entamoeba histolytica.* The reason that some cases infected with *Shigella* did not shed WBC may be that these patients were in the early phase of shigellosis before colonic inflammation developed or that they were passive carriers and that dysentery was due to some other pathogen. In our case shigellosis accompanied by amoebic dysentery, shed more wbc, that is 62.5% in the diarrhoea cases and 76.9% among dysentery cases.

The examination of diarrhoeal stools for faecal leukocytes has not reached widespread acceptance as a diagnostic tool despite its reported value in detecting invasive bacterial pathogen [4,9,13].

Diagnosis of bacterial diarrhoea by clinical feature is often difficult [14]. Physicians are therefore in difficulty to either initiate antibiotic therapy on the basis of reliable clinical features or withhold antibiotic until the isolation of causal pathogens. Thus, accurate methods in the early recognition of bacterial diarrhoea are needed. In our case, the presence of leukocytes among dysentery cases is significant when compared with diarrhoea cases in association with *Shigella* isolation. In our country most of the children suffer from multiple infections and the presence of leukocytes may be significant in cases without identified pathogen. However, the absence of leukocytes may be a rapid and reliable way to identify patients with viral or nonspecific diarrhoea for which antibiotic therapy is not indicated.

Thus, faecal leukocytes by methylene blue wet mount is a useful test in naturally acquired enteric disease. It is quick, simple and require only a light microscope. The demonstration of inflammatory diarrhoea by this technique leads one to consider the organisms that are known to produce inflammatory processes namely *Shigella, Salmonella* or invasive *Escherichia coli* and ulcerative or allergic colitis. Many authors feel the stool leukocytes test remains of value in indicating the presence of colitis [15-17].

Recently, the detection of faecal leukocytes as a useful diagnosis predictor for invasive diarrhoea with mucobloody stools was demonstrated [18]. To confirm this the faecal LFLA (anti-lactoferrin latex bead agglutination) assay distinguishes inflammatory from noninflammatory diarrhoea, which may provide new information on mild inflammatory processes, and is useful as a rapid test in infection diarrhoeal illness [19].

Our study has shown that this method is very useful but it is very important to mount the slides by placing a small fleck of mucous if present in stool. A direct stool sample is also preferable to a rectal swabs.

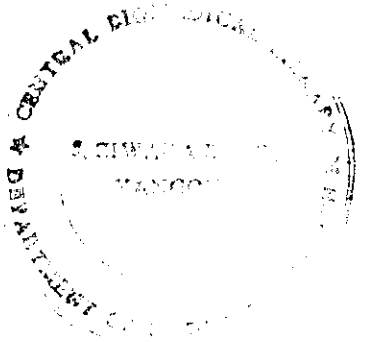
ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Director-General Dr. U Than Swe (retired) and Deputy Director-General Dr. U Myint Lwin for their encouragement and suggestions. We are also grateful to the staff of Clinical Research Division and Bacteriology Research Division of Department of Medical

Research and Yangon Children's Hospital for their cooperat on. Last but not the least to the Superintendent of Yangon Children's Hospital and Professor Dr. Thein Thein Myint for allowing us to carry out our research on the children under their care.

REFERENCES

1. Callender, G.R. The differential pathology of dysentery. *American Journal of Tropical Medicine and Hygiene* 1993; 14: 207-233.
2. Wilimore, J.G. & Shearman, C.H. On the differential diagnosis of the dysenteries. The diagnostic value of the cell-exudate in the stools of acute amoebic and bacillary dysentery. *Lancet* 1918; 2: 200-206.
3. Graham, D. Some points in the diagnosis and treatment of dysentery occurring in the British Salonika force. *Lancet* 1918; i: 55-56.
4. Anderson, J. A study of dysentery in the field with special reference to the cytology of bacillary and its bearing on early and accurate diagnosis. *Lancet* 1921; ii: 998-1002.
5. Wolff, H.L. The faecal smear in the therapy of diarrhoea. *Tropical and Geographical Medicine* 1969; 21: 427-434.
6. Harris, J.C., Dupont H.L. & Hornick, B. Faecal leukocytes in diarrhoeal illness. *Annals of Internal Medicine* 1972; 76: 697-703.
7. Bercoviz, Z. Studies in the cellular exudate of bowel discharges (1). The diagnostic significance of cellular exudate studies in chronic bowel disorders. *Annals of Internal Medicine* 1994; 14: 1323-1340.
8. WHO. Manual of diagnostic laboratory procedures for acute enteric infection, Simplified methods. 1981, Geneva: World Health Organization.
9. Harris, A.H. & Coleman, M.B. (eds.). Diagnostic procedures and reagents. 4th edition, American Public Health Association Incorporation, New York, 1963; p. 43.
10. Stoll, B.J., Glass, R.L., Banu, H., Huq, M., Khan, U. & Ahmed, M. Value of stool examination in patients with diarrhoea. *British Medical Journal* 1983; 286: 2037-2040.
11. Mathan, M.M. & Mathan, V. I. Rectal mucosal morphological abnormalities in normal subjects in southern India: a tropical colonopathy. *Gut* 1985; 26: 710-717.
12. Hossain, M.A. & Albert, M.J. Effect of duration of diarrhoea and predictive values of stool leukocytes and red blood cells in the isolation of different serogroups or serotypes of shigella. *Transactions of the Royal Society of Tropical Medicine Hygiene* 1991; 85: 664-666.
13. Peirce, J.E., Dupont, H.I. & Lewis, K.R. Acute diarrhoea in a resident institution for the retarded: usefulness of faecal leukocytes examination. *American Journal of Diseases in Childhood* 1974; 128: 772-775.
14. Knox, J.D.E., MacNaughtan, G., Laurence, A.R. et al. Diagnosis of diarrhoea in general practice, bacteriologic "self-help". *Lancet* 1967; ii: 1392-1394.
15. Pickering, L.K., Dupont, H.L., Olarte, J., Conklin, R. & Erricson, C. Faecal leukocytes in enteric infections. *American Journal of Clinical Pathology* 1977; 68: 562-565.
16. Korzeniowski, O.M., Barada, F.A., Rouse, J.D. & Guerrant, R.L. Value of examination for faecal leukocytes in the early diagnosis of shigellosis. *American Journal of Tropical Medicine and Hygiene* 1979; 28: 1031-1035.
17. Ise, T., Tanebe, Y., Sakuma, F., Jordon, O., Serrate, E. & Pena, H. Clinical evaluation and bacterial survey in infants and young children with diarrhoea in the Santa Cruz district, Bolivia. *Journal of Tropical Paediatrics* 1994; 40: 369-374.
18. Galli, F., Gyr, K., Pulwitt, A. & Meier, R. Etiology, diagnosis and course of infectious diarrhoea in the Liestal Canton hospital (5 year- retrospective stusy). Schweiz Medical Wochenschr 1994; 124: 1489-1494.
19. Miller, J.R., Barrett, I.J., Kotloff, K. & Guerrant, R.L. A rapid test for infectious and inflammatory enteritis. *Archives of Internal Medicine* 1994; 154: 2660-2664.



The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

Importance of plasma protein binding of antituberculosis drugs on the response to short-course chemotherapy

*Thaw Zin, * May Aye Than, **Malar Myint, ***Khin Myo Myint
*Thida Hmun, *Nwe Nwe Yin & ***Htay Kyi

*Pharmacology Research Division
Department of Medical Research
**Institute of Medicine (1), Yangon
***Yangon General Hospital

Recently, there has been an increasing concern over the consequences of altered plasma protein binding of drugs in disease states, malnutrition and concomittant drug use on the therapeutic efficacy and toxicity of many antituberculous drugs. The increasing awareness of the problems associated with altered plasma protein binding necessitates studying the plasma protein binding profile of drugs used in short-course therapy for tuberculosis since such a study has not been done regardless of the fact that malnutrition and multiple drug use is often unavoidable and where drug resistance and hepatotoxicity has been a major issue. A total of 5 clinically healthy volunteers and 18 smear-positive tuberculosis patients, who were undertaking a short-course chemotherapy at the Union Tuberculosis Institute, were recruited and the plasma protein level and their binding property to rifampicin, isonazid and pyrazinamide at steary-state were studied. The findings showed that although the total plasma protein level was not significantly reduced, there is decrease in the mean albumin level in relation to the globulin level in tuberculous patients. The protein binding profile showed rifampicin to be highly bound (74-87%) and pyrazinamide to be moderately bound (22-40%) to plasma proteins but no significant binding was seen with isoniazid (0.7-2.4%). All 3 drugs achieved adequate serum concentration well above the MIC throughout the study accompanied by rapid clinical improment and sputum conversion to negativity in 88.9% of patients within one month of treatment. No sign of hepatic toxicity was seen in the study. Two patients (11.1%) had relapse after completion of therapy. The study highlights the importance of plasma protein binding of drugs and its influence on the metabolism and interaction between drugs used concomittantly in short-course chemotherapy. The study also showed that addition of pyrazinamide in short-course chemotherapy is very effective and well tolerated but increase in dosage of pyrazinamide during the twice-weekly phase may be necessary to prevent relapse.

INTRODUCTION

The usefulness of pyrazinamide as a potent antituberculous drug was first reported in 1952 but was used only as a secondary choice drug for cases with refractory tuberculosis and/or relapsed cases which are resistant to all other antituberculous drugs. This is because of rather high incidence of liver impairment after the use of regimens with pyrazinamide [1], but it was later revealed that adverse effects, in recommended doses was found to be lower than previously suggested [2]. Studies have shown that hepatotoxicity is not increased when pyrazinamide is added to the initial phase of short-term chemotherapy containing rifampicin and isoniazid [2-5]. In addition, pyrazinamide has the unique ability to enter and kill the tubercle bacilli in the bacilli-ingested macrophages under the environment

of acidic pH inside the phagosome of the cells, so-called persisters, and in areas of acute inflammation, both of which favours the acid-fast bacilli to stay in a dormant state and proliferate very slowly. This sterilization action, which is deficient in other drugs, has recently spotlighted pyrazinamide once again as a pivotal and first line agent for the 6-month short-course chemotherapy of tuberculosis [6]. But controversy exists as to the cause of hepatotoxicity, which is considered secondary to a direct and dose-related toxic effect of pyrazinamide and which may be increased by other drugs like isoniazid and rifampicin or *vice versa* [7-9].

Plasma protein binding of drugs has been found to vary widely with disease, concomi-tant drug therapy, age and many other factors. Such variability is inherent to certain clinical situations or therapeutic modalities since it is the free form of drug that gives rise to drug efficacy as well as side effects [10,11]. But reports on the plasma protein binding of pyrazinamide is still not yet available in the literature and as it is increasingly used in Myanmar, the present study was conducted with the aim:

(1) To map out the plasma protein binding profile of pyrazinamide, rifampicin and isoniazid in healthy subjects and tuberculosis patients undertaking short-course chemotherapy,
(2) To find out whether alteration of such profile is associated with plasma protein level, age, concomittant drug therapy, efficacy and adverse effects.

SUBJECTS AND METHODS

A total of 18 newly diagnosed pulmonary tuberculosis patients, aged 18-50 years, who came to the OPD of Union Tuberculosis Institute for treatment were recruited into the study after being considered eligible for short-course chemotherapy according to the following criteria:

(1) Sputum positive, culture positive or
(2) Sputum positive, culture negative, X-Ray positive with or without cavity lesions.

A standard history, clinical examination and appropriate laboratory investigations were done to exclude having any organic disease or dysfunction. Six clinically healthy volunteers of comparable age and body weight were also selected for the study. The selected healthy volunteers and patients were given, under supervision, rifampicin 600 mg, isoniazid 300 mg and pyrazinamide 1500 mg orally, daily before breakfast for 1 week to achieve a steady-state plasma level. On day 7, blood samples (20 ml) of all subjects were collected immediately, before drug administration, for measurement of free and bound drug concentrations. The serum was separated, of which; 5 ml was used to obtain protein-free ultrafiltrate using an Amicon ultrafiltration set with PM 30 filter. Both the serum and the protein-free filtrate were stored at -20°C until analysis, which was done within 2 months of blood collection.

For tuberculosis patients, the chemotherapy was continued with the same dose of the above drugs for 2 months, and then twice weekly up to 9 months. A second blood sample was again collected on day 30 for measurement of free and bound drug concentrations to see the effect of enzyme induction by rifampicin and for routine biochemical and haematological tests as done previously. Clinical assessment of the subjects for signs of improvement as well as adverse effects of the drugs was done during the weekly follow-up period up to 9 months.

ANALYTICAL METHODS

The total rifampicin and pyrazinamide concentration in the serum (both free and bound form) and the protein-free filtrate (free

form) were measured by HPLC using the method of Guillamount *et al.* [12] and Brouard *et al* [13] respectively while the total isoniazid concentration was measured using the improved microflurometric method of Miceli *et al.* [14]. The percentage of drug bound to plasma proteins was calculated from the difference between total drug concentration in the serum and the free drug concentration in the ultrafiltrate. Statistical comparison between the patient groups on day 7 and 30, and between the patients and healthy volunteers was done by Student's "t"test with the p value of 0.05 as the minimal level of significance.

RESULTS

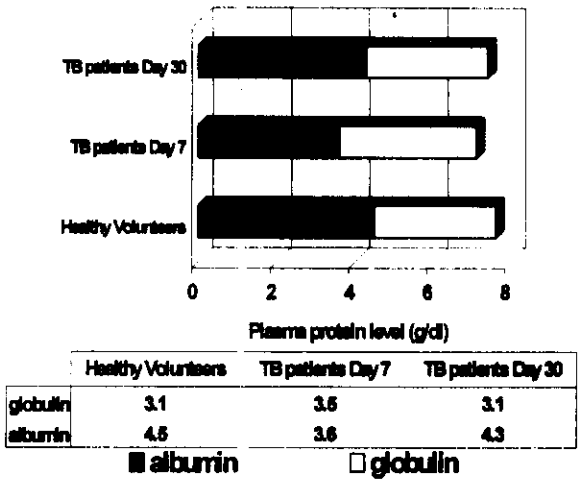
A total of 6 clinically healthy volunteers (3 males and 3 females) and 18 smear-positive tuberculosis patients (12 males and 6 females) took part in the study and their baseline clinical and laboratory data shown in Table 1 were in line with the criteria for selection of subjects and comparability. All patients gave history of cough with expectoration, evening rise in temperature, loss of weight and chest pain, the duration of symptoms ranging from 20 to 180 days. Six patients had history of haemoptysis. Sputum for AFB was positive in all patients. Culture and sensitivity was positive in 13 patients of which 2 were later revealed resistant to streptomycin. All patients were diagnosed as pulmonary tuberculosis on chest X-ray, 9 as moderately advanced and 9 as fully advanced stage of tuberculosis. Laboratory results did not show any significant difference between healthy volunteers and patients and between baseline and after 30 days of treatment in tuberculosis patients. Although there was a slight rise in serum bilirubin and liver enzymes after 1 month of treatment, the levels were still within the normal limits. There were also no reports of any adverse or clinical signs indicating hepatic involvement.

Table.1. Comparative demographic, clinical and laboratory data of healthy volunteers and tuberculosis patients.

Patients	Healthy Volunteers (n=5)	Tuberculosis (n=18)	
Demographic data	29.2±2.7	30.5±1.7	
Age (years)	51.6±1.8	43.6±1.6	
Wight(kg)			
Clinical data			
Cough±expection			
Fever		all	
Chest pain			
Haemoptysis		6 pts	
Duraton of Symptoms (days)		54.7±9.2 (range 20-180)	
Laboratory data		Day-7	Day-30
Haemoglobin(g/dl)	12.6±0.4	10.2±0.5	
Total proteins(g/dl)	7.6±0.2	7.1±0.2	7.4±0.2
albumin(g/dl)	4.5±0.1	3.6±0.1	4.3±0.1
globulin(g/dl)	3.1±0.1	3.5±0.4	3.1±0.2
Urea(mg/dl)	8.7±0.6	15.3±1.5	13.4±1.3
Liver function tests			
Total serum bilirubin (μmol/l)		6.1±0.4	7.3±0.8
Alkaline phosphatase (IU/l)		52.2±2.5	48.1±2.1
SGOT (IU/l)		7.9±0.6	11.2±1.3
SGPT (IU/l)		9.6±1.2	10.5±0.8
Chest X-Ray			
Moderately advanced		9 pts	
Fully advanced		9 pts	
Sputum culture			
Positive		13 pts	
Sensitivity		2 strep. resist.	

Figure 1 shows the serum protein levels of the healthy volunteers and patients. No significant difference was seen between healthy volunteers and tuberculosis patients and between day 7 and day 30 of tuberculosis patients. Although tuberculosis patients did have a lower body weight and haemoglobin levels than healthy

volunteers, no signs of protein malnutrition was seen even though they were in the advanced stage of tuberculosis as seen in the chest X-ray.



	Healthy Volunteers	TB patients Day 7	TB patients Day 30
globulin	3.1	3.5	3.1
albumin	4.5	3.6	4.3

Fig.1. Plasma protein level of healthy volunteers and tuberculosis patients

Table 2 shows the clinical history and response of the patients to antituberculous therapy. Two out of 18 patients had relapse within 2-4 months after completion of therapy. The patients who had relapse were chronic smokers who came to the OPD for having haemoptysis for 1-2 months duration and their chest X ray revealed fully advanced pulmonary tuberculosis of both lungs with cavitation. Their sputum culture was positive and sensitivity revealed a total resistance to streptomycin and only relatively sensitive to isoniazid. Seventeen out of 18 patients successfully completed the therapy out of which 13 showed marked improvement in their re-check X-rays. The remaining who failed to show satisfactory response were changed to alternative regimen containing ethambutol and streptomycin.

Figure 2 and table 3 show the comparison of plasma protein binding of rifampicin, isoniazid and pyrazinamide among healthy volunteers and tuberculosis patients.

Table 2. Clinical history and response of tuberculosis patients to antituberculosis therapy

	Tuberculosis patients	
	Responders (n=16)	Non-responders/ Relapse (n=2)
Clinical history		
Duration of symptoms (days)	55.3±9.4 (range 20-180 days)	45.0±5.0 (range 30-60 days)
Haemoptysis (no. of pts.)	4	2
Chest X-ray on admission		
Lesion (no. of pts.)		
- Moderately advanced	9	0
- Fully advanced	7	2
- Involvement of both lungs	9	2
- Cavitation	3	2
- Pleural effusion	1	0
Culture & sensitivity		
Positive (no. of pts.)	9	2
Sensitivity		
- Rifampicin	9(s)*	2(s)*
- Isoniazid	4(rs),5(s)*	2(rs)*
- Streptomycin	3(s),5(rs),1(r)*	2(r)*
Completion of therapy		
Completed (no. of pts.)	15	2
Relapse	0	2
Defaulted	1	-
Recheck X-ray on completion		
Improved (no. of pts.)	13	-
Not improved/ static	2	-
Deteriorated	-	2

The percentage of plasma protein binding of pyrazinamide was higher in tubrculosis patients as compared with healthy volunteers ($p<0.05$), but both the total and the free pyrazinamide concentration were significantly lower on day 30 than on day 7 in tuberculosis patients ($p<0.01$).

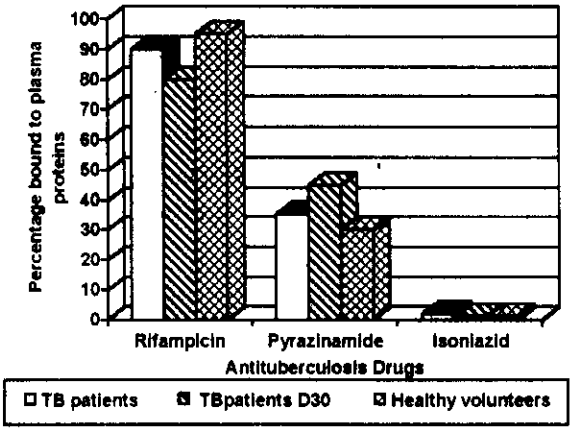


Fig. 2. Plasma protein binding profile of rifampicin, pyrazinamide and isoniazid

Table 3. Comparison of plasma protein binding of rifampicin, isoniazid and pyrazinamide among healthy volunteers and tuberculosis patients

	Drug concentration (μg/ml)			
	Total conc.	Bound conc.	Free conc.	% Bound
Rifampicin				
Healthy volunteers	8.0±0.4	6.6±0.2	1.4±0.1	86.8±0.4
TB patients				
Day7	8.2±0.5	6.7±0.4	1.4±0.1	81.5±1.2
Day30	7.9±0.3	5.9±0.2	1.5±0.1	74.8±1.9
Isoniazid				
Healthy volunteer	6.2±0.4	0.1±0.1	6.1±0.3	0.7±0.2
TB patients				
Day7	6.4±0.2	0.2±0.1	6.2±0.3	0.9±0.3
Day30	5.4±0.3	0.1±0.0	5.3±0.2	0.8±0.2
Pyrazinnamide				
Healthy volunteers	39.4±0.5	8.7±0.5	30.7±1.8	22.2±2.6
TB patients				
Day7	39.3±2.9	12.2±1.0	26.8±2.4	31.8±1.9
Day30	26.7±3.2	10.0±1.1	16.7±2.3	39.7±1.9

The protein binding profile showed rifampicin to be highly bound (74-87%) and pyrazinamide to be moderately bound (22-40%) to plasma proteins but no significant binding was seen with isoniazid (0.7-2.4%).

Table 4 shows the comparison of plasma protein binding of rifampicin, isoniazid' and pyrazinamide among tuberculosis patients who did and did not have relapse after completion of short-course antituberculous therapy. No significant difference was seen between the 2 groups for all three drugs although there was a trend towards a lower concentration of pyrazinamide in patients who did not show satisfactory response to the short-course therapy.

Table 4. Comparison of plasma protein-binding of rifampicin isoniazid and pyrazina-mide among responsive and non-responsive tuberculosis patients

	Drug concentration (mg/ml)			
	Total conc.		% Bound	
	Day 7	Day 30	Day 7	Day 30
Rifampicin				
Responsive patients	8.2±0.5	7.9±0.3	81.5±1.2	74.8±1.9
Non-responsive patients	8.1±0.7	7.3±0.9	84.1±1.6	76.2±2.8
Isoniazid				
Responsive patients	6.4±0.2	5.4±0.3	2.3±0.9	0.8±0.2
Non-responsive Patients	7.4±0.8	6.2±1.4	3.8±1.1	1.2±0.4
Pyrazinamide				
Responsive patients	39.3±2.9	26.7±3.2	31.8±1.2	39.7±1.9
Non-responsive patients	33.8±4.7	21.9±4.9	32.7±1.6	39.4±2.8

DISCUSSION

Drug administration has been placed on a more rational basis with the understanding of pharmacokinetic-dynamic relationship. Patients often vary widely in their dose re quirements of a particular drug, and great strides in optimising a dosage regimen of an individual

patient have come with an understanding as to how the sources of variability influence pharmacokinetic and/or pharmacodynamic processes [15]. Most drugs bind to plasma and tissue proteins but to a variable extent, which can vary widely with many factors. Since only the unbound (free) drug capable of diffusing across membranes, interacting at metabolic and transport sites, and interacting at an effector site to produce a response, understanding the relationship between altered protein binding and pharmacokinetics and pharmacodynamics is important. It has also been shown that even for a given drug, protein binding can vary widely with disease, concomitant drug therapy, age and many other factors [16,17]. Tuberculosis is a disease which is often associated with hypoalbuminaemia and as drugs has to be given in combination to prevent or delay resistence, it is important to find out whether such factors influence the protein binding and interaction between drugs, as rifampicin is also a potent hepatic enzyme inducer. It has been reported that in states of malnutrition, the protein binding of PAS, ethionamide and streptomycin decreases [11,18]. In the present study such is not seen with pyrazinamide probably because of insignificant difference of plasma protein level between healthy volunteers and patients. The percentage of protein binding of pyrazinamide and rifampicin seem unaltered which probably indicates that drug binding displacement may be insignificant. All three drugs were found to achieve adequate serum concentration well above the MIC, having a slightly lower pyrazinamide level in the 30 day as compared to the other two drugs.

Previous authors have reported that the peak serum level of pyrazinamide occurring at 2 hours is about 35 µg/ml after 1500mg [18,19]. The present study showed pyrazinamide concentration to be 39.3 µg/ml for responders and 33.8 µg/ml for non-responders, at steady state, both of which are much higher than the MIC of less than 20 µg/ml, that was reported for tuberculous bacilli [13]. The protein binding of rifampicin and isoniazid was also similar to a regime containing ethambutol [22] and studies have shown that interation between antituberculous drugs given in combination is of little signicicance if given in standard moderate dosage [3,7,23].

Lack of difference in the percentage of binding between the day-7 and day-30 samples showed that the drugs had indeed achieved a steady-state within 7 days. But the significant decrease in pyrazinamide concentration, unlike to the other 2 drugs may indicate increased pyrazinamide metabolism due to microsomal enzyme induction by rifampicin. It has been well established that rifampicin accelerates the metabolism of many drugs through hepatic enzyme induction and possibility through interfering with hepatic uptake [20,21]. The rate limiting factor for pyrazinamide metabolism has been shown to be the hepatic microsomal enzyme, pyrazinamide deaminase, which converts pyrazinamide to pyrazinoic acid, which is then further oxidised by xanthine oxidase to 5-hydroxy pyrazinoic acid [12,21]. Since it is the free drug that is metabolised, this may be the cause of a significant decrease in the free pyrazinamide in relation to the bound form seen in the study. Thus, this may be of clinical significance if the amount of free drug is increased in hypoproteinaemia, which further needs to be studied. In addition, it has also been reported that isoniazid increase the volume of distribution and clearance of pyrazinamide [20]. But the serum level of pyrazinamide persisted above minimum effective concentration , and therefore, unlikely to affect it's therapeutic status. However, the decrease in pyrazinamide concentration in non-responsive patients may be of clinical importance since the concentration that is measured in the study is during the initial daily regimen and it is likely that the concentration during the biweekly phase may reach subtherapeutic levels

favouring development of drug resistance. Thus, increased dosage may be required during the twice weekly phase, although further studies on bioavailability needs to be done to support this.

Total eradication of microbe is indispensable for the chemotherapy of tuberculosis, and the potency of a certain antituberculous agent could be assessed by the availability of negative conversion of bacilli in sputum by culture within two months of administration of the drug. Another important aspect to assess the potency of a drug is to observe the relapse rate after the termination of therapy with the drug. The addition of PZA to the ordinary regimen consisting of INH, RIF and SM/ETB has been reported to raise the negative conversion rate of bacilli from 60-70% by the regimen without PZA to as high as 80-90% by the regimen with PZA [4]. In addition, studies have shown that there was no difference in the relapse rate of about 0-2% within 2 years after the termination of chemotherapy between the two regimens; 6 months with PZA and 9 months without PZA [2-4]. The present study showed the sputum conversion of becilli from 88.9% within 1 month and 100% winthin 2 months of therapy, supporting the high efficacy of the present regime. Although the relapse rate of 11.1% may seem high, the cases studied are all in advance stage of tuberculosis and a lower relapse rate is to be expected in newly infected cases where resistance to antituberculous drugs would be low [30].

Thus, the addition of PZA in the primary stage of short-course therapy for advanced pulmonary tuberculosis in Myanmar has been found to be very affective and without signs of hepatic toxicity. But one should bear in mind of the consequences of increased pyrazinamide metabolism due to hepatic enzyme induction by rifampicin especially in patients who do not show satisfactory response to antituberculous therapy.

REFERENCES

1. Girling, D.J. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle* 1978; 59(1): 13-32.
2. Coombs, D.L., *et al.* USPHS tuberculosis short-course chemotherapy trial 21: effectiveness, toxicity and acceptability; the report of final results. *Annals of Internal Medicine* 1990 ; 112: 397-406.
3. Garling, D.J. The role of pyrazinamide in primary chemotherapy for pulmonary tuberculosis. *Tubercle* 1984; 65: 1-4.
4. Pretet, S., Liard, R & Perdrizet, S. Pyrazinamide is a major antituberculosis drug. *Lancet.* 1982; 1(8268): 399.
5. Steele, M.A. & Des-Prez, R.M. The role of pyrazinamide in tuberculosis chemotherapy. *Chest* 1988; 94(4): 845-50.
6. Mandell, G.L.& Sand, M.A. Drugs used in the chemotherapy of tuberculosis and leprosy. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th Ed. Pergamon Press, 1990, pp 1154-1155.
7. Singh, J., Arora A., Garg, P.K., Thakur, V.S. Pande, J.N. & Tandon, R.K. Antituberculosis treatment-induced hepatotoxicity: role of predictive factors. *Postgraduate Medical Journal* 1995; 71: 359-62.
8. Ali, J. Hepatotoxic effects of tuberculosis therapy. A practical approach to a tricky management problem. *Postgraduate Medical Journal* 1996; 99(5): 217-20,230-1, 235-6.
9. Durand, F., Bernuau, J., Pessayre, D., Samuel, D., *et al.* Deleterious influence of pyrazinamide on the outcome of patients with fulminant or subfulminant liver failure during antituberculous treatment including isoniazid. *Hepatology* 1995; 21(4): 929-32.
10. Mackichan, J.J. Pharmacokinetic consequences of drug displacement from blood and tissue proteins. *Clinical Pharmacokinetics* 1984; 9,suppl.1: 32-41.
11. Levy, R.H. & Moreland, T.A. Rationale for monitoring free drug levels. *Clinical Pharmacokinetics* 1984; 9,suppl.1: 1-9.
12. Guillamount. M., Leclercq, Y., Frobert, Y., Guiss, B. & Harf, R. Determination of rifampicin, disacetylrifampicin, isoniazid and acetyl isoniazid by HPLC: application to human serum extracts, polymorphonucleocytes and alveolar macrophages. *Journal of Chromatology* 1982; 232: 369-376.

13. Brouard, A., Barreteace, H., Merdjam, H., Paillet, M., & Fredj, G. Rapid determination of pyrazinamide in biological fluids by HPLC. *Journal of Chromatology* 1985; 345: 453-456.
14. Miceli, J. N., O son, W.A. & Webeer, W.W. An improved microspectrofluorometric assay for determining isoniazid in serum. *Biochemical Medicine* 1975; 12: 348-355.
15. Rowland, M. Protein binding and drug clearance. *Clinical Pharmacokinetics* 1984; 9, suppl.1: 10-17.
16. Blaschke, T.F. Protein binding and kinetics in liver disease. *Clinical pharmacokinetics* 1977; 2: 32-44.
17. Jusko, WJ. & Gretch, M. Plasma and tissue protein binding and pharmacokinetics. *Drug Metabolism Review* 1976; 5: 43-140.
18. Buchanan, N. & van der Walt, L.A. the binding of antituberculosis drugs to normal and Kwashiorkor serum. *South African Medical Journal* 1977; 52: 522-525.
19. Lacroix, C., Phan-Hoang, T., Nouveau, J., Guyonnaud, C. *et al.* Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects. *European Journal of Clinical Pharmacology* 1989; 36: 395-400.
20. Holdiness, M.R. Clinical pharmacokinetics of antituberculosis drugs. *Clinical Pharmacokinetics* 1984; 9: 511-544.
21. Salfinger, M. *et al.* Pyrazinamide and pyrazinoic acid activity against tubercle bacilli in cultured human macrophages and in the BACTEC System. *Journal of Infectious Diseases* 1990; 162: 201-207.
22 Marlar-Myint, Khin-Myo-Myint, Thaw-Zin, Htay-Kyi & Win-Maw. Influence of serum albumin on serum protein-binding of rifampicin and its clinical importance. the sixth Medical Specialities Conference, MMA, 1993. Abstract of papers, pp 41.
23. Miguet, J.P., Mavier, P., Soussy, C.J. & Dhumeaux, D. Induction of hepatic microsomal enzymes after brief administration of rifampicin in man. *Gastroenterology* 1977; 72: 924-926.
24. Venkatesan, K. Pharmacokinetic drug interactions with rifampicin. *Clinical Pharmacokinetics* 1992; 22: 47-65.
25. Roboz, J., Suzuki, R. & Yu, T.F. Mass fragmentographic determination of pyrazinamide and its metabolites in serum and urine. *Journal of Chromatography* 1978; 147: 337-347.
26. Koch-Weser, J. & Sellers, E.M. Binding of drugs to serum albumin. *New England Journal of Medicine* 1975; 294: 311-316, 526-531.
27. Lima, J.N., Boudoulas, H. & Bloanford, M. Concentration dependence of disopyramide binding to plasma protein and its influence on kinetics and dynamics. *Journal of Pharmacology and Experimental Therapeutics* 1981; 219: 741-747.
28. Reidenberg, M.M. & Drayer, D.E. Alteration of drug protein binding in renal disease. *Clinical Pharmacokinetics* 1984; 9 suppl. 1: 18-26.
29. Singhal, K.C. & varshney, M.K. Effect of simultaneous isoniazid administration on pharmacokinetic parameters of pyrazinamide. *Journal of Indian Medical Association* 1991; 89(8): 227-9.
30. Than-Lwin, Tin-Nu-Swe, Khin-Nwe-Oo, Khin-Myat-Tun, Thaw-Zin & Ti-Ti. Drug resistannce pattern in sputum positive treatment-failure cases admitted to Aung San Tuberculosis Hospital. Abstract of papers, Myanmar Health Research Congress, Yangon, December 1995, pp. 41.

The Myanmar He alth Sciences Research Journal, Vol. 10, No. 1, 1998

Glutaraldehyde gelification time - a highly acceptable bedside tool in the assessment of children tuberculosis

** Ne Win, **Khin Swe, ***Thein Thein Myint & ****Saw Mya Yi*

*Pathology Research Division
Department of Medical Research
**Institute of Medicine 1, Yangon
***Yangon Children's Hospital
****Professor (Retired), Institute of Medicine 2

Glutarldehyde gelification time (GGT) was determined three times in fifty-three children with the clinical diagnosis of tuberculosis supported by CxR evidence, once before the initiation of treatment (GGT0) and twice within six months of antituberculous treatment, at three months interval (GGT3 and GGT6 respectively). The values of GGT0 are shortened in 46 cases (86.8%). GGT3 and GGT6 revealed shorten in 27 cases (50.9%) and 6 cases (11.3%) respectively. This significant change of GGT during treatment could be used as a supportive evidence to clinical judgment in the assessment of response to treatment in childhood tuberculosis.

INTRODUCTION

The diagnosis and assessment of tuberculosis (TB) is difficult due to lack of an ideal infallible laboratory test, the obscurity of clinical symptoms and radiological features, the rising incidence of its a typical forms and well known occurrence of drug resistant cases. At present the diagnosis as well as assessment of TB is based on clinical judgment. Many attempts are still progress for reliable and preferable assessment parameter in the management of TB. Clinical, radiological, erythrocyte sedimentation rate (ESR) and bacteriological examinations play essential role to assess a case of TB under treatment. But inadequacies of those methods are still unresolved.

Some of the studies have pointed out that glutaraldehyde gelification time (GGT) test has a very high potential to be used as a reliable screening test in the diagnosis of TB [1,2,3,4]. But there is no report on GGT correlated with anti-TB treatment. This study was conducted to find out the changes in GGT during anituberculosis therapy in children.

MATERIALS AND METHODS

This study was conducted at Yangon General Hospital for one year period effective May 1994. Total 53 children under twelve years of age were diagnosed as primary complex or other types of TB based on clinical and radiological evidences. GGT was determined three times throughout the study, once before, after 3 months, and after 6 months of antiTB treatment. Thus obtained GGT values were recorded as GGT0, GGT3, and GGT6 respectively.

GGT test

The GGT test was done as described elsewhere [1,2,3,4]. In brief, to 1 ml of 2.5% glutaraldehyde in normal saline, 1 ml of whole blood in EDTA was added and gently mixed in

a sterile test tube or bottle. The formation of a gel was then observed every minute for at least 30 minutes. GGT test is regarded as positive if it is shorter than 12 minutes since cut-off point for TB in children is 12 minutes [3].

To avoid personnel variation in reading the end point (i.e. gel formation), the test was done only by an assigned investigator throughout the study. A symptom-free child with no history of TB contact was chosen as negative control for that day of GGT determination entering into the study. Altogether 30 apparently normal healthy children were involved as negative controls.

RESULTS

Table 1 reveals the rate of positive and negative GGT test in the study population. GGT0 was positive in 46 cases (86.8%) and negative in 7 cases (13.2%). GGT3 was positive in 27 cases (50.9%) and negative in 26 cases (49.1%). GGT6 was positive in 6 cases (11.3%) and negative in 47 cases (88.7%).

Table 1. Distribution of GGT among the study population in three successive determinations (n=53; three months interval)

Serial GGT	Total cases	Positive cases	Negative cases	significance p<0.0002)
GGT0	53	46	7	0.0000(vs GGT6)
GGT3	53	27	26	0.0001(vs GGT0)
GGT6	53	6	47	0.0002(vs GGT3)

There was a significant difference between GGT0 and GGT3 (p value 0.0001). The difference was highly significant GGT0 and GGT6 (p value 0.0000). The difference between GGT3 and GGT6 was also significant (p value 0.0002).

The shortest GGT observed in this study was 1 minute. GGT in normal control cases ranged from 15 minutes to more than thirty minutes. There was no control case with GGT less than 15 minutes.

GGT0 was normal in 7 cases. GGT3 and GGT6 of these cases were also normal; never became abnormal or positive throughout the study.

Figure 1 shows a trend line of mean GGT values in minutes against the duration of treatment. (GGT 0 6.2±3.4; GGT3 14.3±2.8; GGT6 22.6±4.7). The line moves upward from positive to negative (i.e. from abnormal to normal), along with the anti-TB treatment.

DISCUSSION

The finding of changes in GGT in this study are encouraging for development of a rapid, reliable, easy and economical bedside test in the assessment of response to treatment of

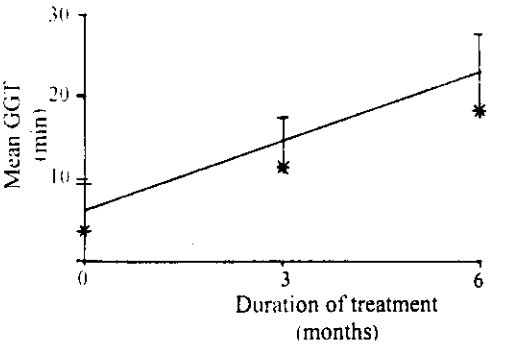


Fig.1. GGT against duration of treatment

tuberculosis in children. GGT appears to reflect the activity of the tuberculous infection and it also mirrors the response or recovery of the patients. An objective evaluation of the activity of the disease and of the patient's progress thus seem possible by the use of GGT changes.

GGT test is relatively much easy to perform, and requires a very few skills and laboratory facility. It is also less time consumed and highly economical. As reported earlier, specificity and sensitivity of the test in TB is considerably high [1,2,3,4] and thus reproducible and reliable. It should be emphasized that, at present, all the available methods applied in the assessment of TB are having many inadequacies and disadvantages.

The only factor influencing the GCT is globulin level, mainly gamma globulin and other is an acute phase protein, fibrinogen, to a lesser extent [5].

In conclusion, although GGT alone may not provide efficient value in the assessment of treatment response, it can be used easily, simply, reliably and cheaply as an adjunct to clinical findings and judgments in monitoring the childhood tuberculosis.

REFERENCES

1. Larssons, P., Preliminary study on an easy and inexpensive method for detection of tuberculosis in cattle using the glutaraldehyde test. *Journal of Nepal Medical Association.* 1988; 26: 9-17.
2. Larssons, P., Upadhayay, MP & Sherestha, KB. The glutaraldehyde test as a rapid screening method for tuberculosis; a preliminary report. *Annals Tropical Medicine and Parasitology.* 1990; 84 (2): 111-121.
3. Ei-Ei-Khin. Glutaraldehyde gelification time test as a rapid screening test for tuberculosis. *Dissertation. M. Med. Sc(Child Health).* Institute of Medicine 1. Yangon. 1993.
4. Tin-Htay. A study of glutaraldehyde gelification time as a screening test for pulmonary tuberculosis. *Thesis. M. Med. Sc(Pathology).* Institute of Medicine 1. Yangon. 1993.
5. Hopwood. D., Callen, CR., McLabe, M., *et. al.* The relation between glutaraldehyde and various proteins. *Histochemical Journal* 1970; 2(2): 137-150.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

SHORT REPORT

Clinical features and response to antivenom of Russell's viper bite cases of Kyaukse, Mandalay Division

*Aung Myint, **Tun Pe, **Aye Aye Myint & **Nu Nu Aung

*Kyaukse Civil Hospital
**Immunology Research Division
Department of Medical Research

Russell's viper (*Daboia russelii siamensis*) (RV) bite is endemic in 6 rice-growing divisions of Myanmar. Recent report on geographical variation of biological properties of RV venom from upper and lower Myanmar [1] and variation in clinical features of RV bite cases from Taungdwingyi and Thayawady [2-3] suggested that more studies on RV bite cases from different endemic localities are needed. In this communication, the findings of the study on RV bite cases from Kyaukse, Mandalay Division are reported.

All snakebite cases admitted to Kyaukse hospital from June to October 1995 were studied. Clinical details of the patients were recorded in standard proforma. Twenty minute clotting test was used to determine the degree of envenoming. A total of 40 ml of monospecific antivenom was given to patients at the discretion of the medical officer. Clotting test was carried out at 2 hour intervals on cases presenting with clottable blood and 6 hourly after given antivenom on cases with systemic manifestations until normal clot restoration occurred. Sera were collected onto filter paper strips, air dried, stored in a sealed polythene bag and later transported to the Immunology Research Division, Department of Medical Research for venom antigen assay [4].

Clinical details of 24 RV bites and 20 envenomed cases are shown in table 1 and 2 respectively.

Table 1. Clinical details of 24 Russell's viper bite cases

Total (24) 15 systemic + 5 local +4 non-envenoming		%
Sex: Male:Female	20:4	83:17
Time bite: Day (6am-6pm) night (6pm-6am)	11:13	46:54
Age (yr) (median)	24(1 3-60)	
Activity when bitten: working: walking: at home	16:4:3	70:17:13
Site of bite: lower limb:upper limb	20:4	83: 17
Number of dead snake brought	7	29
Length of the snake (cm) median	62(26-102)	-
Distance travelled (miles) median	8(1/8-23)	-
Bite and admission (h) median	2.40 (0.20-21.30)	-
First-aid:		
Tourniquet	22	92
Immobilisation	8	33
Suction	14	58
Incision	6	25
Herbal	5	21
Tattoo	14	58

Of 24 bites, 62%(15) had systemic, 21%(5) local and 17%(4) no features of envenoming. Antivenom was given to all cases. Clot restoration time after antivenom therapy took more than 6 hours in 53%(8/15). Pyrogenic reactions were observed in 67%(16/24) cases: 14 liquid (4 with precipitates) and two lyophilised antivenoms.

Table 2. Clinical details of 20 envenomed Russell's viper bite cases.

Symptoms(20)		%	Signs:local: systemic(20)		%
Local pain	20	100	Local swelling	19	95
Epigastric pain	11	55	Local bruising	2	13
Vomitting	11	55	Local necrosis	2	13
Drowsiness	1	7	Local blackening	1	7
Syncope	2	13	Lymph node enlargement	1	7
			Local blistering	1	7
			Systemic(15)		
			Spontaneous systemic bleeding	7	47
			Haematuria	3	20
			Malena	4	27
			Hypotension (<80mmHg)	4	27
			Oliguria (<400ml/24h)	9	60
			Conjunctival oedema	2	13
			Periorbital oedema	2	13
			Renal failure	4	27
			Fatality	3	13

Venom antigen was not detected in 17%(4/24) of the total bites. Antigen level of local envenomed cases (n=5) ranges from 10-40 ng/ml (mean 24ng/ml) and that of systemic cases (n=15) 55-100ng/ml (mean 71ng/ml).

11/15 wound treated cases(tattooing, incision and suction) did not develop local lesion and only 4/15 were associated with local lesion. Although 25-58% of the victims carried out initial wound treatment, local lesion was found to be not associated with it.

Although Kyaukse, Mandalay Division and Myinmu, Sagaing Division are situated 79 miles apart in mid-land, the patterns of envenoming and clinical features of RV bite case from the two localities differ from each other. Although antivenom is routinely given to all snake bite cases in both places, only 14% of the bites from Myinmu have systemic envenoming compared to 62% in Kyaukse and 13% of the former developed spontaneous systemic bleeding compared to 47% in the latter (to be published). The small biting snakes (mean length 29cm), lower mean admission venom level (61 ng/ml) of systemic cases, routine administration of variable doses of antivenom and practice of immobilisation (70%), probably all helped in reducing degree of envenoming in Myinmu compared to that of Kyaukse. However, the incidence of local blackening due to direct venom effect is twice more common in Myinmu probably variation in venom composition may account for it.

Seventeen percent of antivenom given could have been saved if it was withheld from giving to 4 non envenomed cases. Five cases with local feature could have been managed with 10-20ml rather than 40ml antivenom and 3 cases with systemic features with 40ml rather than 60-70ml. Unnecessary use of antivenom could have been prevented if the guidelines for antivenom indications were followed.

It is interesting to know that none of the victims from Myinmu carried out initial wound treatment whereas 21-58% of the victims from Kyaukse are still carrying out the harmful first-aid measure which are no longer recommended nowadays. Extensive health education is still needed to promote compression immobilisation first-aid techinique [5] and to abandon harmful first-aid treatment. Since 13% of the total

bites occurred in the premises of houses, wearing of protective boots should start before leaving the house for work.

The study also confirms that epigastric pain and vomiting are the common symptoms of systemic envenoming of RV bites case. However, hypotension, oliguria, conjunctival oedema and renal failure are not encountered in Myinmu (to be published). A larger samples size is needed to clarify the present observation.

Spontaneous systemic bleeding: gingival, haematemesis, epistaxis observed in Tharrarwaddy study [3] were not found in Kyaukse. But malena and subconjunctival haemorrhages were 2 to 4 times more common in Kyaukse than Tharrawaddy and haematuria and increased capillary permeability activity were 2 to 3 times more common in Tharrarwaddy. It is likely that variation in venom properties may account for the differences. It has been shown that the antivenoms used including precipitated preparations are not effective in correcting clotting defect in 6h in 53% of the cases. This is expected in antivenoms raised with limited pooled antigens from lower Myanmar [6] and in precipitated liquid antivenom [7]. The latter has poor neutralising efficacy and gives antivenom reactions therefore should no longer be used for injecting into humans.

ACKNOWLEDGMENTS

This research was supported by a research grant from the Department of Medical Research.

REFERENCES

1. Tun-Pe, Sann-Mya, Aye-Aye-Myint, Kyi-May-Htwe, Khin-Aung-Cho & Theingie. Biological properties of Russell's viper venom of upper and lower Myanmar. *Myanmar Medical Journal* 1994; 39: 1-6.
2. Sann-Mya, Tun-Pe, Aye-Aye-Myint, Nu-Nu-Aung, Khin-Aye-Kyu & Tin-Oo. Russell's viper bites in Taungdwingyi Hospital: clinical features and response to antivenom. Myanmar Health Research Congress Abstract 1996, p.38.
3. Myint-Lwin, Warrell, D.A., Phillips, R.E., Tin-Nu-Swe, Tun-Pe & Maung-Maung-Lay. Bites by Russell's viper (*V. russelii siamensis*) in Burma: haemostatic, vascular and renal disturbances and response to treat-ment. *Lancet* 1985; ii: 1259-1264.
4. Tun-Pe, Ba-Aye, Aye-Aye-Myint, Tin-Nu-Swe & Warrell, D.A. Bites by Russell's vipers (*Daboia russelli siamensis*) in Myanmar: effect of the snake's length and recent feeding on venom antigeniamia and severity of envenoming. *Transactions of Royal Society Tropical Medicine & Hygiene* 1991; 85: 804-808.
5. Tun-Pe, Aye-Aye-Myint, Khin-Ei-Han, Thi-Ha & Tin-Nu-Swe. Local compression pad as a first-aid measure for victims of bites by Russell's viper (*Daboia russelii siamensis*) in Myanmar. *Transactions Royal Society Tropical Medicine and Hygiene* 1995; 89: 293-295.
6. Tun-Pe, Aye-Aye-Myint & Kyi-May-Htwe. Potency assay of antivenom: neutralisation of biological properties of three Russell's viper venoms by a monospecific antivenom. *The Myanmar Health Sciences Research Journal* 1994;6: 118-120.
7. Tun-Pe, Aye-Aye-Myint, Nu-Nu-Aung, Khin-Aye-Kyu & Tin-Oo. Study of venom neutralising efficacy of monospecific cloudy liquid antivenom. *The Myanmar Health Sciences Research Journal* 1995; 7: 149-152.

The Myanmar Health Sciences Research Journal, Vol. 10, No. 1, 1998

SHORT REPORT

Clinical features and response to antivenom of Russell's viper bite cases of Nyaunglaybin, Bago Division

*Min Than, **Tun Pe, **Aye Aye Myint & **Nu Nu Aung

*Nyaunglaybin Civil Hospital
**Immunology Research Division
Department of Medical Research

Russell's viper *(Daboia russelii siamensis)* (RV) bite is endemic in 6 rice-growing divisions of Myanmar. Because of the intradivisional variation of venom properties in Bago Division [1] and variable performance of antivenom against venom from different localities of Myanmar [2], it is worth studying the clinical picture of Russell's viper bite cases from different localities of Bago Division . In this communication, the findings of the study on RV bite cases from Nyaunglaybin are reported.

All snakebite cases admitted to Nyaunglaybin Civil Hospital from June 1995 to August 1996 were studied. Clinical details of the patients were recorded in standard proforma. Twenty minute clotting test was used to determine the degree of envenoming. A monospecific antivenom was given to patients at the discretion of the medical officer. A clotting test was carried out at 2 hour intervals on the cases presenting with clottable blood and 6 hourly after giving antivenom on the cases with systemic features until normal clot restoration occurred. All sera were collected onto filter paper strips, air dried, stored in a sealed polythene bag and later transported to the Immunology Research Division, Department of Medical Research for venom antigen assay [3].

Clinical details of 28 RV bites and 17 envenomed cases are shown in table 1 and 2 respectively.

Table 1. Clinical details of 28 Russell's viper bite cases

Total (28) 15 systemic + 2 local +11 nonenvenoming		%
Sex: Male: female	22: 6	89: 11
Time bite: Day(6am-6pm) : night (6pm-6am)	22: 6	79: 21
Age (yrs)(median)	30(13-60)	
Activity when bitten: working: walking: in house	18: 8: 2	64:29:7
Site of bite: lower limb: upper limb: neck	26: 1: 1	93:3.5:3.5
Number of dead snake brought	8	29
Length of snake(cm) (median)	30(10-50)	-
Distance travelled (miles) (median)	5(1-10)	-
Bite and admission (h) (median)	4 (0.15-6.30)	-
First-aid: Tourniquet	23	82
Immobilisation	17	61
Suction	7	25
Incision	7	25
Herbal	3	11
Tattoo	5	18

Of 28 bites, 54% (15) had systemic, 7% (2) local and 39% (11) no features of envenoming. Forty millilitres of antivenom were given to all cases. Clot restoration time after antivenom therapy took more than 6 hours in 53% (8/15) of the cases. Pyrogen reactions were observed

in 22% of the cases (n=23). 6/11 antigen negative and two local envenomed cases were given 40ml of antivenom. 4/7 systemic with spontaneous systemic bleeding are treated with 40ml antivenom and 18%(5/18) died of renal failure and shock.

Table 2. Clinical details of 17 envenomed Russell's viper bite cases.

Sysmtoms (17)		%	Signs:local+ systemic (17)		%
Local pain	17	100	Local swelling	13	76
Epigastric pain	7	47	Bleeding at site of bite	2	12
Loin pain	4	27	Local bruising	1	6
Vomiting	10	67	Local necrosis	1	6
Drowsiness	4	27	Local blackening	1	6
Dyspnoea	2	13	Systemic (15)		
Syncope	1	6	Spontaneous systemic bleeding	7	47
Ptosis	1	6	Haematuria	1	7
			Malena	1	7
			Gingival	2	13
			Albuminuria	4	27
			Hypotension (80 mmHg)	7	47
			Oliguria (400ml/24h)	6	40
			Conjunctival oedema	4	27
			Periorbital oedema	2	13
			Facial oedema	4	27
			Renal failure	6	40
			Fatality	5	18

Venom antigen was not detected in 39% (11/28) of the total bites. Mean admission venom antigen level of local envenomed cases (n=2) is 20ng/ml and that of systemic is (n=15) 64ng/ml (30-80ng/ml).

Clinical features of Russell's viper bite cases of Nyaunglaybin differ from Tharrawaddy [4], although both are from Bago Division. The incidence of hypotension (47%), oliguria (40%) and evidence of generalised increase capillary permeability (27%) are common presenting features observed in both studies. However, 72% of the latter developed microscopic haematuria whereas only 2% of the former developed haematuria. Vomiting (67%) and epigastric pain (47%) are 2 to 6 times more common in this study probably differences in venom properties may account for it. Haematemesis, subconjunctival haemorrhage and epistaxis are not observed in this study.

The harmful first-aid measures such as application of herbal medicine, tattooing, suction and incision (11-25%) which are no longer recommended were still practised among farmers, showing some poor dissemination of health education at the grassroots level. However, it is encouraging to note that immobilisation was carried out in 61% of the victims.

Since 4 ampoules of antivenom were routinely given to all snakebite cases, the non envenomed cases (39% of the total) also received antivenom which could have been saved if guidelines for antivenom administration were followed.

An unknown bite occurred to a 33-year old male in the neck while he was asleep on the paddy ground at mid-night. He developed no signs of envenoming. He might have been bitten by a Chinese krait (*Bungarus muticinctus*) which usually bites at night [5]. Although 40 ml of monospecific Russell's viper (RV) antivenom was given to him, retrospective immunodiagnosis showed the RV antigen was not detected in the patient's serum before antivenom administration.

The incidence of local blackening (6%) due to local venom effect is comparable to that of Kyaukse, Mandalay Division (to be published) which is not related to first-aid treatment. Clot restoration time after 4 ampoules of antivenom was more than 6 hours in 53% compared to 25% in the Tharrarwaddy study, showing a poor performance of antivenom in restoring normal clotting. This is expected in antivenom raised with limited pooled antigen [2].

Pyrogenic reactions (22%) were observed as in Tharrarwaddy (100%) and in other studies (to be published).

ACKNOWLEDGMENT

This research was supported by research grant from the Department of Medical Research.

REFERENCES

1. Aye-Aye-Myint, Tun-Pe, Kyi-May-Htwe, Khin-AungCho & Theingie. Geographical variation of biological properties of Russell's viper venom: Bago Division. *The Myanmar Health Sciences Research Journal* 1995; 7: 92-94.
2. Tun-Pe, Aye-Aye-Myint & Kyi-May-Htwe. Potency assay of antivenom: neutralisation of biological properties of three Russell's viper venoms by a monospecific antivenom. *The Myanmar Health Sciences Research Journal* 1994; 6: 118-120.
3. Tun-Pe, Ba-Aye, Aye-Aye-Myint, Tin-Nu-Swe & Warrell, D.A. Bites by Russell's vipers (*Daboia russelli siamensis*) in Myanmar: effect of the snake's length and recent feeding on venom antigeniamia and severity of envenoming. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991; 85: 804-808.
4. Myint-Lwin, Warrell, D.A., Philips, R.E., Tin-Nu-Swe, Tun-Pe & Maung-Maung-Lay. Bites by Russell's viper (*V. russelii siamensis*) in Burma: haemostatic, vascular and renal disturbances and response to treatment. *Lancet* 1985; ii: 1259-1264.
5. Tun-Pe, Tin-Myint, Aung-Htut, Than-Htut, Aye-Aye-Myint & Nu-Nu-Aung. Envenoming by Chinese krait (*Bungarus multicinctus*) and banded krait (*B. fasciatus*) in Myanmar. *Transactions of the Royal Society of Tropical Medicine and Hygiene 1997;: 91: 686-688.*

Tokyo Offset/5... 02-421