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Acceptability study of protective boots among farmers of Taungdwingyi Township

Tun Pe, *Aye Aye Myint, *Khin Aye Kyu & **Maung Maung Toe*

**Immunology Research Division,*

***Epidemiology Research Division*

Department of Medical Research

****Myanmar Railways Department, Taungdwingyi*

Acceptability of fang-proof protective boots was studied in 180 farmers of Taungdwingyi Township during harvesting season, October 1995. The boots were provided free of charge. Preliminary survey showed that 98% of farmers knew snake-bite could be prevented by working with boots on and only 72% wore them whereas 28% non-users could not afford to buy them. At present 88% wear locally available rubber boots, which could not withstand penetration of Russell's viper's fangs. Following the trial, 99% enjoyed wearing the trial boots. These provide full sense of protection against snakebite. These are light, comfortable and the farmers could work with them on for the whole day either intermittently or continuously. Ninety-nine percent preferred to wear the trial boots in future and intended to use them even if these are not provided free of charge. The boots cost 350 kyats per pair. Ninety-nine percent could afford to buy them at a price of 250 kyats per pair, however 1% preferred a lower price of 150 or 200 kyats per pair. In order to bring down the incidence of snakebite throughout the country, all-out wearing of the boots is needed and it is suggested that health education, selling and distribution of the boots directly to the users at an affordable (subsidised) price of 250 kyats or less per pair should be aimed at.

INTRODUCTION

Russell's vipers usually inhabit in the paddy fields and they are plentiful between October and December, which coincides with the harvesting season [1]. Most snakebites occur while the farmers are at work in the fields harvesting crops, carrying bundles of hay, while caring livestock and on return from work or after visiting friends or video houses after dark on poorly-lit road. Majority of the bites occur during day time (6 am-6 pm) and at work [2]. Although antivenom is the mainstay in treatment of snakebite, there are some delay of at least about 2-4 hours in getting treatment at the nearest health centre. Majority of the effective bites resulted in systemic envenoming while seeking treatment. Routinely-used

tourniquet was found to be ineffective in retarding spread of venom [3]. Although compression immobilization first-aid technique is found to be effective in delaying local spread of venom [4], very few victims applied it, which shows extensive promotion, and health education among farmers is needed.

In spite of energetic antivenom treatment, mortality rate of snakebite remains high ranging from 5-10% in Tharrawaddy [5] and 18% in Taungdwingyi study [2] which has an annual bite of over 300 cases. Majority of the victims are young adults [2], which are the working force of the country. Some of the victims suffered from pituitary insufficiency following recovery [6] and needed permanent replacement therapy.

Since it is a preventable occupational hazard, it is expected that if majority of the farmers wear protective boots at work in the field or on road after dark, the incidence of snakebite and mortality will dramatically decrease and demand for production of expensive antivenom will be reduced. Rubber boots had been introduced to farmers nearly 3 decades ago but because of inconveniences, these did not receive much attention. Few have traditional belief that footwears are prohibited in the paddy field. However some have been using available rubber boots, which do not withstand penetration of snake fangs (personal observations) at work. It is high time that the acceptability of the new fang-proof rubber boots among farmers should be studied.

MATERIALS AND METHODS

It is a concerted effort of Immunology Research Division, Department of Medical Research, Veterinary Section of Myanmar Pharmaceutical Factory, Ministry of Industry 1 and Rubber Shoe Factory 1, Ministry of Industry 1, Yangon. Rubber Shoe Factory 1 manufactured a knee-length protective boot made of synthetic rubber compound. Several modifications in length, design of the boots and preparation of synthetic rubber compound have to be made in order to make rubber which could withstand penetration of Russell's viper's fangs (figure 1).

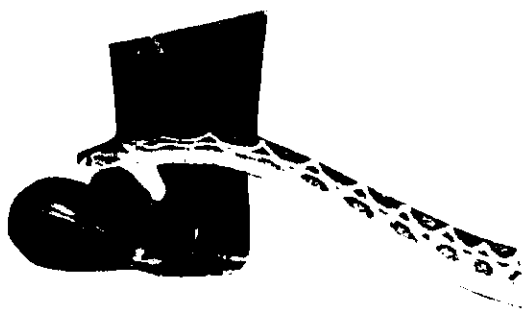


Fig. 1. A Russell's viper biting the trial boot

Testing of the boots, which could withstand penetration of Russell's viper's fangs, was conducted at the Veterinary Section, Myanmar Pharmaceutical Factory. Immunology Research Division carried out acceptability trial of the boots among farmers.

A total of 180 pairs of boots were distributed free of charge to the participant farmers from 5 villages of Taungdwingyi Township (Mahti, Mahti Sanpya, Nyaungbinhla, Pandawgyi and Sathwa) with the help of local elders and health workers. Face-to-face interviews were made by using the two sets of structured questionnaires, before and after distribution of the boots to assess the acceptability of the boots in October 1995 and February 1996 respectively. The questionnaires included demographic characteristic of the boots users, knowledge on the use of protective boots, comfort ability and duration of boot wearing in a day. Affordability of the boots and future intention of continually using them were also enquired.

RESULTS

A total of 180 farmers age ranging from 26-61 years, from 5 villages of Taungdwingyi Township participated in the study. The sex ratio is 163 (91%) male:17 (9%) female. Fifty one percent (92/180) are farm owners and 49% (88/180) daily-wage workers.

The following are results of pre distribution survey on knowledge on the use of protective boots

Ninety-eight percent (177/180) knew snakebite could be prevented by working in the field with protective boots, however, only 72% (129/180) wore them at work in the field. Twenty-eight percent (51/180) of non-users could not afford them. Sixty two per cent (80/129) prefer knee-length boots and 30% (38/129) ankle-length boots. At present, 88% (111/129) wear locally

available rubber boots and could work with them on for 5-9 hours a day in spite of discomfort in 40% (53/131). Eighty-eight percent (114/130) thought that it was convenient to wear the boots and 97% (121/125) mainly wore them during harvesting crops. The price of the local rubber boots is 300 kyats per pair. Ninety-seven percent of non-users could not afford to buy it and 95% (162/171) of them did not take any protective measures against snakebite in the field. Ninety-seven percent (175/180) would like to take part in acceptability boot trial if provided free of charge. In future, 73% (131/180) of non-users would like to wear the trial boot, 65% (32/49), knee-length rubber boots 22% (11/49) and ankle-length rubber boots 8% (4/49).

Responses to questions by the participants following use of the boots (post test)

Ninety-nine percent (179/180) enjoyed wearing the trial boots provided, since these are light and comfortable (6%, 11/180) and provide sense of total protection against snakebite (91%, 164/180). Ninety-eight percent wore the boots during harvesting and 2% (4/180) walking. Eighty-four percent could work with the boots on for 5-9 hours, 86% (156/177) intermittently and 4% (24/177) continuously. Nine percent of the users complained of local pain whereas 91% (159/175) did not.

The main advantages of working with the trial boots are: ability to work for a longer time than before with comfort and the assurance of complete protection against snakebite (100%, 171/171).

On comparing the trial boots and in-use boots, 99% (141/143) prefer to wear the former in future and 1% for the latter. They also intend to continue wearing them (99%, 179/180) after the trial even if these are not provided free of charge. Ninety-nine percent (178/180) could

afford to buy them at a price of 250 kyats per pair and 1% (2/180) prefers a lower price of 150 or 200 kyats per pair.

DISCUSSION

From the study it becomes clear that the boots under trial are superior to the locally available rubber boots. Although the former is slightly more expensive than the latter (350 kyats vs 300 kyats), majority prefer the former because of comfort, ease in wearing and ability to work for the whole day with a minimum discomfort. These provide sense of full protection against snakebite so that the farmers could concentrate on their work and more productive work could be expected. While undergoing the trial, two participants who happened to step on Russell's vipers at work were prevented from being bitten by the snakes.

Although there is a traditional custom of prohibiting wearing footwear in the field, majority of farmers from Taungdwingyi have been wearing boots for decades because of scare of snakebite and its consequences. However, a proportion of farmers (28%), non-users who could not afford to buy boots are still at risk. In order to encourage all-out wearing of the boots, it needs to be subsidized and sell to the users with an affordable price of 250 kyats per pair should be aimed at.

Since majority of the users have an intention of continually using them, they should be encouraged and the price of the boots should be kept to minimum. Since snakebite can occur even before they leave their houses [2] it is suggested that they should wear the boots before leaving the house until coming back home in the evening. No place is safe especially in snake-infested areas.

Health education targeted to the users, selling and distribution of the boots directly to the users at an affordable price will be next

strategies in order to bring down snakebite morbidity and mortality rates of the whole country.

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Studies on the stability of DMR hepatitis B vaccine

**Khin Khin Aye, *Cho Cho Lwin, *Aye Kyaw, *Khin Pyone Kyi,
*Maung Maung Khin & **Than Swe*

*Diagnostics and Vaccine Research Center

**Director-General

Department of Medical Research

The stability of the DMR hepatitis B vaccine at 4°C and room temperature has been followed over a period of one year by monitoring its physical, chemical and biological characteristics bimonthly starting from February 1996. The pH and protein content of the vaccine did not change for both storage conditions throughout the study. The vaccines were found to be sterile and free from pyrogens and passed the general safety test on mice. The HBsAg titer started to decline after eight months of storage at 4°C and after 10 days of storage at room temperature. However, the HBsAg adsorption rate to the adjuvant remained over 95% for both storage conditions.

INTRODUCTION

Myanmar is one of the top ten countries with highest incidence of hepatocellular carcinoma in the world [1]. About 50% of acute viral hepatitis is due to hepatitis B virus (HBV). Myanmar with a population of 40 million is highly endemic for hepatitis B with carrier rate of 10%. Clearly, the most important method of prevention of hepatitis B is active immunization. Myanmar scientists have successfully produced plasma-derived vaccine, which was tested for viral, bacterial, fungal and pyrogen contamination except stability by the WHO standard tests and were found to be satisfactory. The DMR vaccines also passed the chimpanzee safety tests performed by the New York Blood Center. In the present study the stability of the DMR HB vaccine has been followed with respect to physical, chemical and biological characteristics.

MATERIALS AND METHODS

Aliquots of the DMR HB vaccine stored at 4°C

and room temperature were tested bimonthly for (a) protein content (b) pH (c) sterility (d) HBsAg titer (e) non-adsorbed HBsAg titer (f) HBsAg adsorption rate (g) general safety and (h) pyrogenicity. Protein content was determined by Lowry's method [2]. pH was measured by using Universal indicator paper PANPEHA (R) (pH 0-14). Sterility test was performed by inoculating the test material in different culture media for a period of 14 days for evidence of microbial growth using positive and negative controls [3]. The HBsAg titer of the vaccine was determined by reverse passive hemagglutination (RPHA) method using Anthelabs cell Neo test kit manufactured by International Reagents Corporation, Kobe, Japan. The stored vaccine was centrifuged at 2000 g (5000 rpm) for 10 minutes and HBsAg titer was measured on its supernatant by the RPHA method. This value represents the non-adsorbed HBsAg titer. the HBsAg adsorption rate (%) of this product was then calculated from the expression: HBsAg adsorption rate (%) = $(1 - (T/T_0)) \times 100$ where T_0 represents HBsAg titer of the vaccine and T = non-adsorbed HBsAg titer. General safety test and pyrogenicity tests were done by standard

procedures recommended by WHO and Merck & Co., Inc. [3,4].

RESULTS

It was found that the protein concentration (20 µg/ml) and pH (6.0) of DMR HB vaccine did not change in both storage conditions for over a

the HBsAg adsorption rate remained over 95% for a period of one year (Table 1). Table 2 shows the variation of HBsAg titer of DMR HB vaccine during the period of storage at room temperature {13°C (55°F) - 39°C (102°F)}. It was found that the HBsAg titer started to decline after seven days of storage by about four folds and persisted up to a year. However, the HBsAg adsorption rate remained

Table 1. HBsAg titer, non-adsorbed HBsAg titer and HBsAg adsorption rate of DMR hepatitis B vaccine under storage at +4°C

Storage duration (month)	0	2	4	6	8	10	12
TEST							
HBsAg titer	1:1024	1:1024	1:1024	NT	1:512	1:256	1:256
Non-adsorbed HBsAg titer	1:16	1:16	1:16	NT	1:16	1:8	1:4
HBsAg adsorption rate (%)	98.4	98.4	98.4	NT	96.8	96.8	98.4

NT means not tested

Table 2. HBsAg titer, non-adsorbed HBsAg titer and HBsAg adsorption rate of DMR hepatitis B vaccine stored at room temperature [13°C (55°F) - 39°C (102°F)]

Storage duration	Day					Month							
	0	7	10	14	18	1	1½	2½	3½	4½	8½	10	
TEST													
HBsAg titer	1:1024	1:1024	1:256	1:256	1:256	1:256	1:256	NT	1:256	1:256	1:256	1:128	
Non-adsorbed HBsAg titer	1:16	1:4	1:8	1:8	1:8	1:8	1:8	NT	1:8	1:8	1:8	1:4	
HBsAg adsorption rate (%)	98.4	99.6	96.8	96.8	96.8	96.8	96.8	NT	96.8	96.8	96.8	98.4	

NT means not tested

year. For the period under this study, the DMR vaccine remained sterile for both storage conditions. It was found that the HBsAg titer started to decrease at about eight months of storage at +4°C by two fold and then decreased gradually up to the end of the study. However,

over 95% up to the end of the study. Table 3 shows the pyrogenicity test results of HBsAg inactivated by flash heat (i.e. heating the HBsAg at 102°C for 2 min 40 sec.) on three rabbits. There were no significant changes in the rectal temperature (approximately 102°F)

Table 3. Pyrogenicity test results of DMR hepatitis B vaccine
Sample - flash heat inactivated HBsAg, Lot No.03/93 (without adjuvant)
Rabbit - 3 nos. (healthy, weight - 2 kg)

Rabbit No.	Sample No.	Observation period				Test period			
		Rectal temperature (°F)				Rectal temperature (°F)			
		Day 1	Day 2	Day 3	Day 4	0 hr*	1 hr	2 hr	3 hr
R ₁	03	102.8	103	103.2	103.1	102.4	102.6	102.8	102.4
R ₂	03	103	102.4	102	103	102.4	102.8	102.4	102.2
R ₃	03	103	103	103	102.3	102.4	102.2	102.6	102.4

* Pre injection temperature

before and after intravenous injection of the heat inactivated HBsAg in all the three animals. The general safety test results of DMR HB vaccine stored at 4°C and room temperature indicated that all animals survived and gained body weight during the test period (i.e. seven days).

DISCUSSION

From the results of the present study, it seems that the pH and protein content of the vaccine could resist both the storage temperature fluctuations since they remained unchanged throughout the study period. As regards to sterility, the vaccine was free from extraneous contaminants indicating that it meets a requirement for the good manufacturing practice (GMP) standards. The study also showed that the vaccine should be stored at +4°C in order to maintain its anti-genicity. The HBsAg adsorption rate remained over 95% up to a period of one year for both the storage conditions indicating that HBsAg protein remained adsorbed to the adjuvant which may ensure slow release of the HBsAg protein. The general safety test results suggested that there

were no appreciable changes in the composition of the vaccine and the vaccine is safe regardless of its potency. The pyrogenicity test results indicated that the vaccine was free from pyrogens. In general, no significant changes were observed in physico-chemical characteristics of the vaccine except a slight decline in the HBsAg titer after eight months of storage at +4°C. However, the maintenance of adsorption rate of over 90% throughout the study indicated that it may still be effective if given in sufficient quantity to the test subject.

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Role of guardians in adolescents' reproductive health behaviors in rural area

Le Le Win, **San Shwe, *Khin Myint Wai, *Cho Cho Myint,
*Kyi Kyi Mar & *Win Win Mar*

*Health Systems Research Division,
**Epidemiology Research Division
Department of Medical Research
***Department of Health

A cross-sectional community-based study was undertaken in Taikkyi Township during July 1997, to explore the guardians' attitudes and practices relating to adolescents' reproductive health behavior. Altogether 102 guardians who have adolescents between 10 to 19 years of age involved in the study. Majority of the guardians did not favor their adolescents on choosing their fiancées by themselves and having more than one fiancée. While 52% thought adolescents should know about contraceptives before their marriage, 38.2% thought they should not. Most of the guardians approved that dissemination of messages about sex education for the adolescents through mass media was an appropriate way. It was found that most did not allow their teens to discuss such things either in front of them or with others. But some of them (36.3%) said they would respond about those things willingly if or when they were asked. By knowing sex education and reproductive health, about 48% (46.2% of the mothers and 53.6% of the fathers) said adolescents will gain benefits. Regarding information on Reproductive Health, this study highlights the needs for proper education and counselling for guardians, especially on adolescents.

INTRODUCTION

In the last few decades, major changes have taken place, which have had an impact on sexual and reproductive health-related behavior and its consequences among adolescents and youth – those aged between 10 and 24. Youth of age 25 and below comprise more than half of the world's population; 29% are between the ages of 10 and 25, of whom 80% reside in developing countries. This trend is increasing; by the year 2000, it is anticipated that 86% of those below 25 will live in developing countries [1]. At the same time, the world's great cities are rapidly expanding, especially in developing countries. The factors like migration, decline in authority of family and extended multi-generational family of traditional societies, and the exposure to extensively different ways of life in cities are

among the important factors contributing to behavioural changes in young people.

In many countries, these changes have increased the risks of health-related problems including alcohol and drug abuse, cigarette smoking and sexual precocity. In addition, lack of sound information and misinformation among themselves and from peer person lead to increase in high risk of excessive alcohol consumption, hazards of drug use and smoking, unwanted pregnancy, unsafe abortion, sexually transmitted disease and infection with HIV contributing to greater morbidity, mortality and infertility in the young people [1,2].

Furthermore, behavioral changes depend on the nature of their relationships with each other, with their parents or other adults in their families, with schools and with health services.

Among them, parents are the most important persons, because they are more aware of and can control their children's behavior for their better life, especially in the Asian countries. The same scenario is also encountered in our country with the increasing urbanization and opportunities to acquaint the new ways of social life for adolescent population; hence, communication between the adolescents and their guardians should be studied to promote their healthy life. This study will attempt to fulfill the information of role of guardians in adolescents' reproductive health behavior that can be helped to meet the adolescents' needs.

The objective is to explore the attitudes and practices of guardians (parents and elders in the family) relating to adolescents' reproductive health behavior.

MATERIALS AND METHODS

Cross-sectional study was conducted at a randomly chosen township from Yangon Division, namely, Taikkyi Township, during July 1997. Within the chosen township, Gyo-phyu and Yin-taik-kwin villages were selected at random. Altogether, a random selection of 102 households inhabited with adolescents from 10 to 19 years of age involved in the study. One of the parents or elders, who wished to participate from each selected household was interviewed with the pre-tested

questionnaire in order to gather the following variables [3] – socio-demographic characteristics, guardians' attitude and practices toward adolescent reproductive health behavior and type of information needs.

RESULTS

One hundred and two guardians (i.e., mother, father, aunt, sibling and others) involved in the study, of which, mothers and fathers accounted for 63.7% and 27.5% respectively. The majority of the guardians (47.1%) fall in age group of 40-50 years and the mean age was 44.8 years. Most of them (84.3%) were married. Modal education level was found to be lower level (52%), followed by those who can read and write (23.5%) and middle school level (12.7%). Most of them (48%) had own business and 31.3% of them were either skilled labours or odd-job persons.

Regarding the mean ages of adolescent to start interesting in opposite sex and in marriage, most thought that female adolescents started little earlier than males. In response to the question about the age for taking interest in opposite sex, mean ages stated by mother and father for the males were 17.1 years and 17.9 years and for the females were 17.1 years and 15.9 years. Concerning with the statement for suitable age for marriage, those for males were 21.1 years and 21 years and for females were 20.9 years

Table 1. Guardians' attitude toward mean age of adolescent to start interesting in opposite sex and in marriage

	Type of guardian					Total
	Mother	Father	Aunt	Sibling	Others	
Opposite sex						
M Adol*	17.11	17.86	16	16.5	19.33	17.33
F Adol**	17.12	15.86	16	17.25	19.33	17.33
Marriage						
M Adol*	21.14	21	20	21.25	21.67	21.09
F Adol**	20.89	17.89	19.5	20	21.67	20.01
Total	65	28	2	4	3	102

* Male adolescent

** Female adolescent

and 17.9 years respectively (Table 1).

Various attitudes relating to adolescents' reproductive health behaviour were asked, and majority of the guardians disapproved their adolescents on choosing their fiancées by

found for those acceptors according to the type of guardians and type of adolescents in their respective residences. Again, the reason for worth knowing about contraception before marriage was similar to that about dissemination channel. About 87% of

Table 2. Guardians' attitude toward adolescent's reproductive health behaviour (in percentages)

Attitudes	Favor	Against	DK*
Adolescents should be allowed to choose their fiancées by themselves	36.6	62.7	1
Male adolescents should have more than one fiancée before marriage	14.7	84.3	1
Female adolescents should have more than one fiancée before marriage	3.9	91.2	2.9
Sex education should be given to male adolescents	42.2	55.9	2
Sex education should be given to female adolescents	46.1	46.1	2.9
Mass media are appropriate means for disseminating sex education and reproductive health for teens	75.5	18.6	5.9
School-based sex education should be given	59.8	35.3	4.9
Adolescents should know about contraceptives before marriage	52.0	38.2	8.8
Parents and elders can help adolescents for safe sex life	87.3	2.9	6.9

*don't know

themselves and having more than one fiancée. But concerning with giving sex education to the adolescents, there was not much different in their agreement for males and females (42.2% and 46.1%). Regarding the dissemination of messages about sex education through mass media like newspapers, TV, radio, etc., 75.5% said it was the appropriate way. Of which, the majority was mother (63.6%), followed by father (29.9%). Among those who favoured this dissemination channel, 38.9% had only male adolescents in their homes, 25.9% had only females and 35.1% had both male and female teens respectively. Dissemination through mass media could give the adolescents the knowledge and proper ways of thinking was found to be the modal reason given for favouring this channel.

While 52% thought adolescents should know about contraceptives before marriage, 38.2% thought they should not. The same responses as the dissemination through mass media were

guardians accepted that parents and elders can help the teenagers' happy and safe sex life by proper guide (Table 2).

Table 3. Activities performed by guardians toward adolescent's reproductive health behaviour

Activities	Yes		No	
	No.	%	No.	%
Allowed them to discuss about these things in front of me	20	19.6	82	80.4
Allowed them to discuss about these things with others	18	17.6	73	71.6
Talk about these things to adolescents	17	16.7	85	83.3
Asked about these things by adolescents	14	13.7	88	85.3
Willing to respond about such things if/when they were asked	37	36.3	34	33.3
Give them source of information about such things	18	17.6	84	82.4

Most of the guardians did not allow their teens to discuss about sex education, reproductive health and marriage either in front of them or with others. Some (36.6%) of the guardians said they are willing to respond about sex education and reproductive health if or when they were asked. Only 17.6% gave adolescents about the source of information about sex education and reproductive health (Table 3).

By knowing sex education, about 48% said adolescents will gain the benefit on it: 46.2% of the mothers and 53.6% of the fathers. About 34% said it had adverse effect upon them.

Regarding with the sex education for the teens, only 28 (27.5%) said they had enough information. Among 74 guardians who responded as not having enough information, 34 (45.9%) of them still did not want to know about it and the main reason was that they were not interested in it (20.6%). Nearly 18% said they had no free time to spend for it and 14.6% said it was not necessary for them (Table 4).

Table 4. Reasons for not wanting to know about sex education for teens (n=34)

Reasons	No.	%
No interest	7	20.6
No free time	6	17.7
Not necessary	5	14.6
Disgraceful thing	4	11.8
Against tradition	1	2.9
Others	7	20.6
Don't know	4	11.8

DISCUSSION

It was interesting to observe that the findings were controversy. While the guardians had positive aspects on some issues, at the same time they had negative views on some issues. The findings revealed that personal involve-

ment in adolescents' reproductive health was low among the guardians from the study in rural area. The majority had lack of knowledge about sex education and reproductive health for adolescents and they had no desire to know about it. Also, they did not want to give sex education to their teens by themselves and did not allow them for discussions.

But disseminating sex education by other channels, namely, school-based sex education and mass media had been approved. Mass media approach was found to be the highly significant one, especially by the mothers and those who had male adolescents. The guardians also agreed that adolescents are beneficial by knowing sex education and also accepted that they can help for the adolescents' safe sex life. Since they already had some positive attitudes, it needs to encourage them to acquire knowledge of sex education and reproductive health for the adolescents and need to encourage and support the adolescents to promote the healthy life style of the adolescents.

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Russell's viper toxoid: the influence of immunisation schedule and age of the toxoid on antibody response in immunised rabbits and monkeys

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

**Immunology Research Division
Department of Medical Research
**Yangon Zoological Garden*

The influence of immunisation schedule and age of Russell's viper toxoid on antibody response in immunised rabbits and monkeys was studied. Rabbits were immunised subcutaneously with the toxoid at 0, 4 and 8 weeks. For subsequent boosting at 6 wk intervals from 24 to 56 wks, fresh toxoid was used in one group (n=3) and the same batch of aged toxoid in another (n=3). Immunisation of monkeys was carried out up to 40 wks using the latter schedule. Antibody levels peaked at 6-8 wks after the 3rd injection and fell to low level at 24 wks after the first injection. Subsequent boosting at 6 wk intervals from 24 wks onwards resulted in increase in antibody level reaching peak at 12 wk after the 1st booster injection and sustained at its peak throughout the course of immunisation. Antibody response observed in the monkeys was similar to that of the rabbits, however a lower level of antibody was observed. The patterns of antibody response observed following use of both toxoids were comparable. Feasibility and cost effectiveness of boosting at 6 wks intervals in prophylactic active immunisation of farmers at risk with Russell's viper toxoid need to be studied.

INTRODUCTION

The pattern and development of antibody following 3 injections (0, 4, 8 wks) of Russell's viper toxoid was studied up to 6 months in monkeys and human volunteers [1,2] and 64 weeks in rabbits [3]. However, the previous study [3] indicated that boosting with aged toxoid at variable intervals failed to induce antibody response in some instances. This extended study is to find out the influence of immunisation schedule and age of toxoid used in immunisation on antibody response in rabbits and monkeys.

MATERIALS AND METHODS

Russell's viper toxoid was prepared by stepwise formalinisation method [4] and the final

product containing 2 mg protein/ml was aliquoted and stored at 4°C.

Immunisation of rabbits

Two sets of rabbit (3 tests and one control) each weighing 2.5 Kg were injected subcutaneously (s.c.) with 0.1 ml of fresh toxoid at 5 different sites on the back of shaved dorsal skin at 0, 4 and 8 wks. Subsequent immunisations were given at 6 wk intervals from 24 to 54 wks. Controls received adjuvant only. From 24 wk onwards, one set of rabbit was boosted with freshly prepared toxoid and the other with the same batch of toxoid prepared at the beginning of the experiment. Two millilitres of blood were collected at 0, 4, 8 wk before giving the toxoid, then at 2 wk intervals up to 54 wks from all rabbits.

Separated serum was aliquoted and stored at -20°C.

Immunisation of monkeys

The same immunisation schedule was used for immunising 12 monkeys (8 tests and 4 controls) up to 40 wks. Each was separately caged and s.c injection was given at left deltoid region. The same toxoid used for immunisation of the rabbits was used throughout the experiment. It was conducted at the Yangon Zoological Garden. The same serum collection schedule used in the rabbit was followed in the monkey experiment. Controls received preparation containing adjuvant-phosphate buffered saline.

Local reactions such as swelling, ulceration, abscess formation, regional lymph node enlargement and general constitution symptoms such as alertness, eating habit, general well being and limitation of movement of arm were recorded 6 hourly for 24 h and daily for a week after each immunisation. For the rabbit experiments, only local reactions were recorded.

Venom antibody assay

Venom antibody levels were measured by enzyme immunoassay technique [5].

RESULTS

Peak antibody response following 3 monthly s.c. injections of the toxoid in rabbits was observed 4 wks after the 3rd injection and reached low level at 24 wk after the first injection. Further boosting at 6 wk intervals from 24 wk onwards resulted in peaking of antibody response 4 wks after the second booster dose and peak antibody level was maintained throughout the course of immunisation i.e. up to 56 wks. A similar pattern of antibody response was observed in

both experiments using fresh and aged toxoids (Fig. 1). A similar pattern of antibody response was observed in the immunised monkeys, however, the antibody level achieved was low compared to that obtained in the rabbits (data notshown).

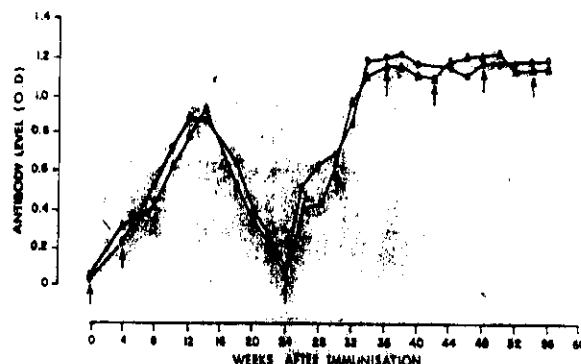


Fig. 1. Pattern of antibody response in rabbits following immunisation with fresh and aged Russell's viper toxoids. ● fresh toxoid ▲ aged toxoid † time of injection of toxoid.

Local reactions

Reactions following immunisation in the rabbits were: local indurations 4/6, 3/6 and 2/6 after the first, second and 3rd injection respectively and local swelling 2/6 and 1/6 after the second and the third respectively.

Reactions following immunisation in the monkeys were: local swelling (3/8), ulceration (1/8) after the first injection and local swelling (1/8), regional lymph node enlargement (1/8) and abscess formation (1/8) after the second. No reactions were observed in subsequent injections. Upset in general constitutional symptoms were not observed.

DISCUSSION

Peak antibody level observed 4-6 wk after the last injection in our study was similar to that observed in the previous study [3] where intramuscular route of immunisation was used. However antibody level is of short-lived and

could not maintain up to 24 wks after the first injection of the toxoid. There was a slow induction of secondary response by both fresh and aged toxoid when the booster doses were given at 24 and 30 wk. Probably intramuscular route of administration induced a quicker antibody response. Boosting at the decline of antibody level was found to induce a more rapid response [3] than that given at a time where antibody level was low as in our present study. Secondary response should induce a more quicker antibody response than primary.

The age of the toxoid has no influence on the pattern of antibody response. The interval of boosting plays an important role in induction and maintenance of peak antibody response. Frequent inoculations at one to 2 weekly intervals with venom or toxoid have been used for active immunisation in man against venom, which yielded high level of neutralising antibody [6,7].

High resting antibody level is essential in immunised individual since immediate neutralisation of injected venom is required when a snake happen to bite a man. It could be achieved by boosting at 6 wk intervals from 8 wk after the 3rd injection of toxoid. The feasibility and cost effectiveness of frequent injections at 6 wk intervals to the risk population needs to be studied.

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Different types of *Shigella* species isolated from Infectious Diseases Hospital during 1996 and their antibiotic susceptibility patterns

*Mar Mar Nyein, *Kyi Lwin Moe, **Tin Ohn & **P. Kyaw Hla

*Bacteriology Research Division
Department of Medical Research
**Infectious Diseases Hospital

Cross sectional descriptive hospital-based study using convenience sampling method was carried out among 53 dysentery cases admitted to Infectious Diseases Hospital from March to September 1996. It consisted of 67.9% males and 32.1% females. Their ages ranged from one year three months to 80 years. The duration of illness before attending the hospital was one to seven days. They passed motions 16 to 20 times per day. Volume of stool output was small in 62.3%, moderate in 28.3%, with mucus in 62.3% and with blood and mucus in 35.9%. Shigellae was isolated from 45.3% of the cases: *Shigella flexneri* 2a (54.2%), *Shigella dysenteriae* A type 1 (29.2%), *Shigella boydii* (12.5%) and *Shigella sonnei* (4.2%). The antibiotic susceptibility pattern revealed that 87.5% of shigellae were resistant to ampicillin, 83.3% to chloramphenicol, 33.3% to cephalothin, 37.5% to furazolidone, 33.3% to nalidixic acid, 83.3% to streptomycin, 91.7% to tetracycline and 33.3% to norfloxacin respectively. It was also noted that 91.7% of shigellae were sensitive to amikacin, 91.7% to augmentin, 83.3% to gentamicin, 58.3% to minocycline, 91.7% to netilmicin, 66.7% to neomycin, 75% to sisomicin and 62.5% to norfloxacin.

INTRODUCTION

Dysentery refers to the frequent passage of bloody mucoid stool discharge, of which shigella infections (shigellosis) are probably the most common bacterial cause. Shigellosis caused by shigella organisms is capable of causing a spectrum of gastrointestinal clinical illness and a number of extra-intestinal complications. The infection occurs worldwide [1] and in developing countries shigella infections account for 5 to 20% of the acute childhood diarrhoea [2-5]. As the organism is everywhere, the socio-economic and environmental status reflect the cause of infection [6]. It is difficult to control since the mode of transmission is by the faecal-oral route [7]. The disease caused by *Shigella dysenteriae* type 1 is particularly severe and may be associated with bacteremia and

disseminated intravascular coagulation and is also the cause of haemorrhagic colitis and haemolytic uraemic syndrome (HUS). It also produces a heat-labile exotoxin that may significantly contribute to neurotoxic and enterotoxic clinical features [8]. Upon autolysin, all shigellae release their endotoxin and major neurological complications are also associated [9]. In Myanmar shigella infections account for 0.6 to 3.0% of diarrhoea cases, 24% of gastroenteritis cases and 22.2 to 49.0% in dysentery cases [10]. According to the report from Infectious Diseases Hospital, 114 dysentery cases admitted to the hospital during 1996. The emergence of drug resistance to commonly used antibiotics in enteric pathogens is also increasing since 1980 [11]. Thus, it is important to monitor the antibiotic resistance of shigella with antibiotic susceptibility testing.

MATERIALS AND METHODS

This study was conducted in Infectious Diseases Hospital from 13-3-96 to 27-9-96. The study includes 53 dysentery cases (36 males and 17 females) before administration of antibiotics and their ages ranged from one year three months to 80 years.

(TSI), Lysine Iron Agar (LIA), Christensen's Urea Agar and Sulphide Indole Motility Agar (SIM). ID kit (Nissui Company, Japan) was used whenever necessary. Then biochemically-confirmed shigella were subjected to slide agglutination using antisera from Denka-Seika Company Limited, Japan.

Table 1. Dysentery cases from Infectious Diseases Hospital (13-3-96 to 27-9-96)

Age groups years	Total		Male		Female	
	No.	Percent	No.	Percent	No.	Percent
1-10	7	13.2	3	42.9	4	57.1
11-20	5	9.4	4	80.0	1	20.0
21-30	13	24.5	8	63.5	5	38.5
31-40	11	20.8	8	72.7	3	27.3
41-50	3	5.7	3	100.0	0	0
51-60	6	11.3	5	83.3	1	16.7
61-70	6	11.3	4	66.7	2	33.3
71-80	2	3.8	1	50.0	1	50.0
Total	53	100.0	36	67.9	17	32.1

Collection of stool samples

Freshly passed stools from patients were collected in clean sterile bottles. Direct plating onto Salmonella-Shigella (SS) agar for detection of shigellae was also done and the plates were transported to the Bacteriology Research Division, Department of Medical Research within three hours.

Bacteriological examination of stool

It was done according to the standard methods described by World Health Organization, 1981 [12]. The stool samples were streaked onto MacConkey (MA) agar and Salmonella-Shigella (SS) agar for primary isolation. Selenite F enrichment both was used for secondary isolation. Non-lactose fermenting bacteria colonies were picked and biochemically tested for further characterization using Triple Sugar Iron Agar

Antibiotic susceptibility testing

It was done according to the method described by Bauer *et al.*, 1966 [13], using Mueller-Hinton broth and agar. The antibiotic discs were obtained from Becton Dickinson Microbiology Systems (BBL) and from Japan. The antibiotic susceptibility patterns were recorded according to the criteria for interpretation of sensitivity to antibiotics.

RESULTS

Characteristics of dysentery patients

Table 1 shows that of the 53 cases tested, 36 cases (67.9%) were males and 17 cases (32.1%) were females. Their age ranges from one year three months to 80 years. During the study period, most of the patients with dysentery who attended the Infectious Diseases Hospital were within the age group of 21 to 30 years (24.5%)

and 31 to 40 years (20.8%). In these cases 13.2% were from under ten years of age and 3.8% were in the age group of 71 to 80 years which reflects that dysentery was common in all the age groups during the study period.

It was observed that 26 cases (49.1%) attended the hospital at the onset of dysentery. The

number of motions was 2-5 times in 20 cases (37.7%), 6-10 times in 21 cases (39.6%), 11 to 15 times in 7 cases (13.2%) and 16-21 times in 5 cases (9.4%) respectively. The stool output of 33 cases (62.3%) was small (less than 50 ml). Concerning the nature of stool, 33 cases (62.3%) passed the stool with mucus and 19 cases (35.9%) with mucus and blood.

Table 2. Distribution of *Shigella* spp. Among dysentery cases in different age groups

Age groups (years)	Tested cases	Shigellosis	Serotypes			
			<i>S. boydii</i>	<i>S. dysenteriae</i>	<i>S. flexneri</i>	<i>S. sonnei</i>
Less than 10	7	3 (42.9)	0	0	3	0
11-20	5	3 (60.0)	0	1	1	1
21-30	13	4 (30.8)	0	2	2	0
31-40	11	3 (27.3)	1	1	1	0
41-50	3	1 (33.3)	0	0	1	0
51-60	6	4 (66.7)	2	0	2	0
61-70	6	4 (66.7)	0	3	1	0
71-80	2	2 (100.0)	0	0	2	0
Total	53	24 (45.3)	3 (12.5)	7 (29.2)	13 (54.2)	1 (4.2)

Figures in parenthesis denote percentages

Table 3. Antibiotic susceptibility pattern of *Shigella* spp. isolated from dysentery cases who attended Infectious Diseases Hospital (in percentages) (n=24)

Antibiotics mcg/disc	Resistant	Intermediate	Moderately Sensitive	Sensitive
Ampicillin Am10	87.5	0	4.2	8.3
Amikacin An30	4.2	4.2	0	91.7
Augmentin AMC30	0	8.3	0	91.7
Chloramphenicol C30	83.3	4.2	0	12.5
Cephalothin CF30	33.3	0	8.3	58.3
Furazolidone Fx 100	37.5	4.2	0	58.3
Gentamicin GM10	16.7	0	0	83.3
Kanamycin K30	12.5	16.7	0	70.8
Minocycline MnO30	25.0	16.7	0	58.3
Nalidixic acid NA30	33.3	4.2	0	62.5
Netilmicin Net 30	4.2	4.2	0	91.7
Neomycin N10	20.8	12.5	0	66.7
Streptomycin S10	83.3	4.2	0	12.5
Sisomicin SIS10	20.8	4.2	0	75.0
Septrin SXT	83.3	0	0	16.7
Tetracycline Te30	91.7	4.2	0	4.2
Norfloxacin NOR 10	33.3	4.2	0	62.5

Distribution of shigella with regard to different serotypes

The distribution of *Shigella* spp. among different age groups with regard to different serotypes is shown in Table 2. It was found that *Shigella* spp. were isolated from 24 cases (45.3%). *Shigella flexneri* was the most prominent species with the isolation rate of 54.2% followed by *Shigella dysenteriae* with 29.2%; *Shigella boydii* with 12.5% and *Shigella sonnei* with 4.2%. From these data it

cephalothin (33.3%), furazolidone (37.5%), nalidixic acid (33.3%), streptomycin (83.3%), septrin (83.3%), and tetracycline (91.7%). They were sensitive to amikacin and augmentin (91.7%) each, gentamicin (83.3%), kanamycin (70.8%), netilmicin (91.7%), neomycin (66.7%), sisomicin (75%), and norfloxacin (62.5%) (Table 3). The resistant pattern of *Shigella* spp. in accordance with different serotypes is shown in Fig. 1. *Shigella flexneri* was found to be more resistant to many antibiotics than other serotypes.

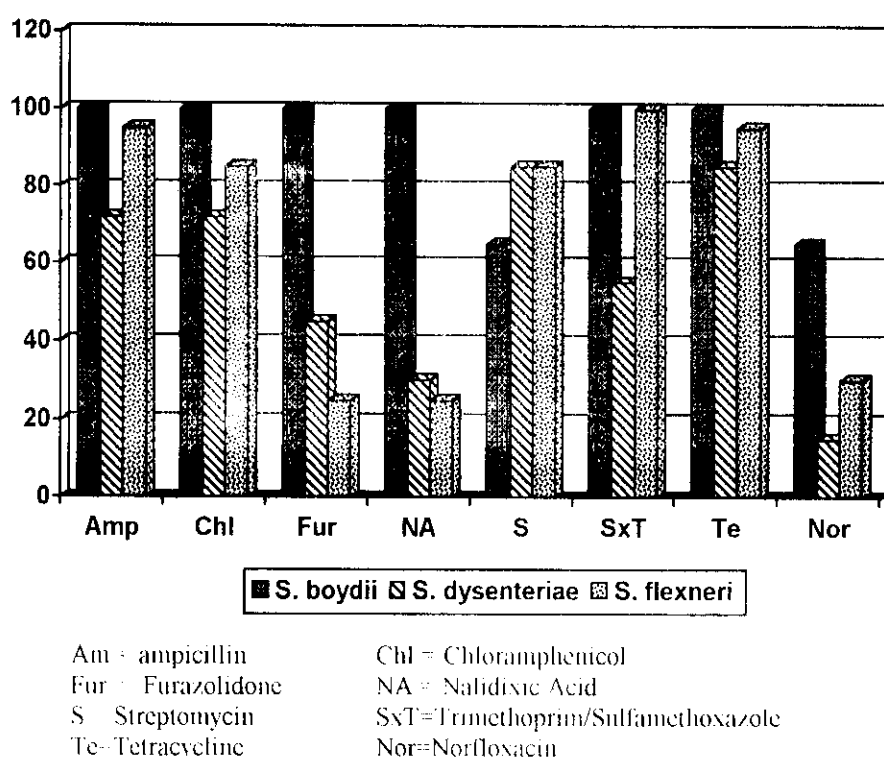


Fig. 1 Resistance pattern of *Shigella* spp. isolated from dysentery cases from Infectious Diseases Hospital

was found that all the four serotypes were isolated from the dysentery cases from Infectious Diseases Hospital.

Antibiotic susceptibility pattern of *Shigella* spp.

The antibiotic susceptibility pattern of *Shigella* spp. revealed that they were resistant to ampicillin (87.5%), chloramphenicol (83.3%),

Environmental status and personnel hygiene among dysentery cases

Table 4 shows that 60.4% used tap water and 69.8% used boiled water. It was also found that, 60.4% used pit type of latrine. They used water in the method of cleaning after defaecation and only 9.4% used soap for hand washing.

Table 4. Environmental status and personnel hygiene among dysentery cases who attended Infectious Diseases Hospital during 1996 (in percentages) (n = 53)

Water supply	Type of drinking water	Type of latrine	Cleaning after defaecation	Hand washing
Tap water	Boiled	Reservoir	Water	Water only
60.4	69.8	9.4	79.3	73.6
Surface well	Unboiled	Hand flush	Toilet paper	Soap
11.3	30.2	24.5	7.6	9.4
Artesian well		Pit type	Newspaper	Not done
17.0		60.4	9.4	2.0
Rain water		Surface	Stick	
0		2.0	0	
Pond water			Others	
11.3			3.8	

DISCUSSION

Both developing and developed nations continue to suffer from enteric diseases caused by *Shigella* spp. (shigellosis) [14-17]. It was reported that 6.0% of *Shigella* spp. were isolated from 12709 faecal specimens and of which *Shigella flexneri* (55%) and *Shigella dysenteriae* (29.7%) were identified [18]. They also reported that 15.7% of children under five years of age were positive for *Shigella* spp. Shigellosis is an endemic disease in India [19-20]. These pathogens cause outbreak of disease among individuals who have lowered resistance [21]. Recently, the increase in infections caused by *Shigella flexneri* has been traced to the high attack rate of this disease in patients with Acquired Immune Deficiency Syndrome (AIDS) [22]. The incidence of enteric bacterial disease has been reduced in countries that have achieved sanitary control of drinking water, food and sewage disposal. Most of the strains of shigellae have become resistant to various commonly used antibiotics [23-24]. Gebre-Yohanne in 1984 [25] reported

the changing patterns of drug resistance in *Shigella flexneri* from 1978 to 1982. The sudden increase of multiple drug resistance in *Shigella flexneri* isolates of 1962 was related to an increasing incidence of serotype 2.

In this study group *Shigella* spp. were isolated from all the age groups which accounts for 45.3% of the total samples. The serotypes distributed were also found to be *Shigella flexneri* (54.2%); *Shigella dysenteriae* (29.2%); *Shigella boydii* (12.5%) and *Shigella sonnei* (4.2%). These serotypes were distributed among the study cases; the youngest case was one year four months and the oldest was 80 years old. It was also found that sex differentiation was not an important factor in this study. The most predominant serotype encountered was *Shigella flexneri*. The emergence of drug resistance was also noted especially to common antibiotics such as ampicillin, chloramphenicol, septrin and tetracycline. Some strains were also found to be resistant to norfloxacin and nalidixic acid. The proper use of antibiotics with appropriate dosage and duration will help inhibit drug resistant organisms.

From our study it was also observed that environmental sanitation and personnel hygiene are the most important factors in causing dysentery as reported by other investigators [26, 27]. If the hands are contaminated with faecal coliforms hand washing with water only can not remove them. Unboiled water for drinking may also play an important role as such water may cause diarrhoea due to heavy contamination of organisms and although they used tap water supply and boiled water, water stored before consuming may have been contaminated. It was already reported by various workers [28, 29] that water supply and water sources are an important source of waterborne disease outbreaks, the major cause of diarrhoea and dysentery among cases

directly reflects the environmental sanitation and personnel hygiene.

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Effect of *Azadirachta indica* neem seed kernel extracts on *Anopheles dirus* mosquitoes

*Htay Aung, **Khin Khin Cho, *Than Myat Htay, *Sein Minn,

***Win Myint & ***Aye Than

*Entomology Research Division,

***Pharmacology Research Division

Department of Medical Research

**Zoology Department, Yangon University

Neem oil (seed kernel extract) produced a strong repellent action on *An. dirus* mosquitoes (the major vector of malaria) in Myanmar even at concentrations as low as 0.5 and 1.0 percent. At a concentration of 2 percent no anopheles biting and the protection provided was 100 percent during 10-12 hrs periods. The larvicidal effects of neem oil on *An. dirus* (larvae) were determined by using 3rd instar and above. The LC₉₀ and LC₅₀ were found to be one percent and 0.45-0.5 percent respectively. The residual effect of neem oil under laboratory condition with 2 and 4 percent persisted ca 72 ± 5 hrs. Moreover, under laboratory condition with 2 and 4 percent, the ovicidal effect was found to be 100 percent control.

INTRODUCTION

A number of synthetic insecticides have been developed in agriculture, forestry and households against vectors of human diseases, but these do not fulfill the requirements for integrated pest management [1]. For this reason as well as increasing problems of pest resistance to pesticides, interest in insecticidal botanicals has grown rapidly during recent years.

Essential oils of many plants have mosquito repellent action. These are cassia, camphor, citronella, lemon grass, clove, thyma, pine, geranium, eucalyptus etc. [2]. During the last twenty years, extracts and compounds from the neem tree, *Azadirachta indica*, have attracted the special interest of entomologists and phytochemists all over the world. In recent years some marketable, neem-based products have been developed. It therefore seems opportune to compile and conduct a research on

the effectiveness of plant neem (oil) extract on vector mosquitoes.

Neem *A. indica* A. Juss is a native of Myanmar and India. The tree has extensive distribution in the Asian and African countries. In Myanmar neem tree is found in almost all parts of the country. The tree is locally called Tamar. In Myanmar, the leaves are extensively used as vegetable, and the leaves and other parts of the plant are traditionally used for variety of ailments.

The neem tree products are well-known for insect repellent and antifeedant properties long before the advent of synthetic insecticides. In recent years neem has received worldwide attention for its usefulness in insect control. Neem is characterized by multiple mechanism of action on insect, and use of its several constituents in agriculture have already been reported [3]. Various parts of the tree are being used in India/Myanmar for several millennia for medicinal purposes and Ayurveda regards

the tree as sarva rogo nivarni (cure for all diseases).

Malaria control is besetted with multifaceted problems and it requires simple, non-toxic and cost-effective remedies. In view of this an attempt was made (1) to evaluate the repellent actions of neem seed kernel extracts against mosquitoes (*An. dirus*), (2) to determine ovicidal effect and (3) larvicidal effects of neem seed kernel extracts.

MATERIALS AND METHODS

Source of neem oil extract

Neem oil extract kindly supplied from Pharmacology Research Division, Department of Medical Research (DMR) was used in this study. A 50 per cent emulsified concentration (EC) stock solution of neem oil (methanol extract) was prepared by dissolving it in acetone. From the stock solution, the desired concentrations were prepared by serial dilutions.

Preparation of desired concentrations for laboratory testing

To determine the biological effects of neem oil, the concentrations used were 0.01 percent, 0.05 percent, 0.1 percent, 0.5 percent, 1.0 percent, 2.0 percent and 4.0 percent respectively. The above concentrations were prepared from the 10 percent stock solution.

Repellent tests

Fifty numbers of 4 to 5 day-old *An. dirus* females were used for each concentrations of neem oil tested. Determination of repellent/insecticidal, ovicidal and larvicidal action of neem oil extracts were conducted according to Abdulcada and Sasamanaabu [4] and WHO

Manual on Practical Entomology in Malaria [5].

Larvicidal action of neem oil

Biological effect of neem oil on *An. dirus* was conducted with series of concentrations as mentioned previously. Twenty-five larvae (3rd stage larvae) were exposed to each concentration of neem oil in 200 ml of D/W in enamel bowls. All mosquito larvae were exposed to the neem oil solution continuously.

Each concentration was replicated 10 times with an untreated (only acetone + D/W) control, mortalities were then recorded. Dead larvae and pupae were checked daily and all the dead specimens were kept in 70 percent alcohol for morphological study.

The concentrations LC₅₀ and LC₉₀ values were determined through log concentration and probit mortality [5,6].

Ovicidal effect

Beakers (250 ml) with D/W and filter paper soaked in water were used as ovitraps for mosquito eggs laying. Ovitrap with different concentrations of neem oil (dissolved in acetone EC 50%) extract were placed in cages with known number of (50 to 100 female *An. dirus* mosquitoes) fully gravid females. Two ovitraps with only pure D/W were placed inside each cage as control. After 24-48 hours ovitraps were removed and the number of eggs laid were counted and recorded.

RESULTS

Mosquitoes which landed on and bit treated and untreated human baits were caught, counted and recorded.

Table 1. Percent protection from mosquito bite on human volunteer

Neem oil % Mixed in coconut oil	Mosquito alighted/caught		
	Neem oil in coconut oil	Coconut oil alone (Control)	Percent Protection
	(N _t)	(N _c)	(PP)
0.1	24	32	25.00
0.2	18	28	35.71
0.5	15	31	51.61
1.0	3	35	91.14
2.0	0	32	100.00
4.0	0	31	100.00

Table 1 shows volunteers treated with 1, 2 and 4 percent concentrations of neem oil gave 100 percent protection from mosquito bites. Mosquito caught from each replica was pooled and percent protection was calculated using the formula.

$$PP = [(N_c - N_t)/N_c] \times 100$$

Where, PP = Percent protection

N_c = number of mosquitoes
collected on coconut oil

N_t = number of mosquitoes
collected on neem oil

This table shows the results of percent protection from the mosquito bites. Neem oil concentrations 0.1, 0.2, 0.5, 1.0, 2.0 and 4.0 per cent gave 25.0, 35.71, 51.61, 91.14, 100 and 100 per cent protection respectively.

Table 2. Effects of neem oil extracts on oviposition of *An. dirus* females

Neem oil conc. %	Nos. of females tested	Nos. of eggs laid	Remarks
0.01	50	11	Even at
0.05	50	6	the low
0.10	50	4	conc. no
0.50	50	0	egg
1.00	50	0	hatching
2.00	50	0	was
4.00	50	0	observed
Control	50	1652	

It was also observed that more number of eggs were collected in control beaker with distilled water only. It reveals that the application of neem oil treated ovitrap reduced oviposition of the gravid females. However, a good number of *An. dirus* eggs (1652 eggs) were collected from untreated control (Table 2). Any increase in neem oil concentrations showing significant difference in reduction of the mosquito egg laying was observed obviously.

The mortality of *An. dirus* larvae when exposed to different concentrations of neem oil in acetone is shown in Table 3. The persistence effect of neem oil was also determined. Neem oil 2 percent and above persisted more than 72 hours. However, lower concentration of 1 percent could control only 50 percent for only 28 hours (Table 4).

Table 3. Larvicidal effect of neem oil on immature stages of *An. dirus* mosquitoes

Neem oil conc. %	Nos. of larvae tested	No. effected	% kill	Probit kill
0.01	25 x 10	97	38.8	4.51
0.05	25 x 10	119	47.6	4.92
0.10	25 x 10	127	50.8	5.10
0.50	25 x 10	180	72.0	5.71
1.00	25 x 10	228	91.2	6.30
2.00	25 x 10	247	98.9	7.40
4.00	25 x 10	250	100.0	7.40
Control	100 x 10	10	0.1	-

Table 4. Persistancy effect of neem oil EC₅₀ applied in enameled bowls on immature stages of *An. dirus* mosquitoes

Neem oil conc. (%)	Effect of neem oil EC ₅₀ on <i>An. dirus</i> larvae (% died in hrs)						Remarks
	24 hrs		48 hrs		72 hrs		
	T	C	T	C	T	C	
0.5	26.5	0.0	4.0	0.0	0.0	0.0	Neem
1.0	91.0	0.0	31.3	0.0	0.0	0.0	EC ₅₀ 2%
2.0	100.0	0.0	100.0	0.0	64.0	0.0	persists
4.0	100.0	0.0	100.0	0.0	81.0	0.0	till 72hrs.
T = Test C = Control							

T - Test C = Control

DISCUSSION

The primary objective of the present study is to gain more knowledge on mosquito control with synthetic insecticides extracted from plant ingredients. During the last 20 years extracts and compounds from the neem tree, *Azadirachta indica*, have attracted the special interest of entomologists and phytochemists all over the world [1]. Thus, neem plant was selected and its seed kernel extract was tested against *An. dirus*, the primary vector of malaria in Myanmar.

Essential oils like lemon grass, thyme, citronella, camphor, eucalyptus, pine etc. have mosquito repellent action. The fractionation of these essential oils has been done and the repellent effect of these fractions has been studied. Often many unrelated plants have similar repellent constituents. Eucalyptus, alpine, geraniol, citronella, camphor and linaloor were the most commonly used natural oils as mosquito repellent before the artificial compounds became available [7]. The problem with the natural oil is that they provide protection against mosquitoes only for a short duration of ten not exceeding a few hours. However, the present study indicated that neem oil is somewhat superior and gave a better results. Regarding neem oil 2 percent persisted for 10h 20min $\pm$ 34min period.

Moreover, a number of synthetic repellents have been developed. Some promising ones are dimethyl phthalate (DMP), a mixture called 6-2-2 and diethyl toluamide (DEET). The discovery of diethyl toluamide or N, X-diethyl-3-methyl benzamide commonly known as DEET in 1954 has ushered a new era in repelling mosquitoes. DEET is colorless, only liquid with slight colour. There has not been any significant development since the advent of DEET and it is the most popular repellent. It is effective against a variety of insect species, ticks, mites and other insects and lasts longer in

comparison to other repellents. The protection provided by DMP for 3 hours and DEET for 5-7 hours. However, neem oil lasted more than 10 hours. Mostly these compounds are used during dusk and dawn to prevent mosquito bites on exposed parts such as ankles, legs, arms, neck and ear lobes.

The present studies agreed well with the findings of Sexana [8]. Neem oil produced a strong repellent action on *An. culicifacies* (the major vector of rural malaria in India) and other nuisance anopheline mosquitoes even at concentrations as low as 0.5 and 1.0 percent. At a concentration 2 percent there was no anopheline biting and the protection provided was 100 percent during 11hrs period which is longer than the present study on *An. dirus*.

The present study showed that 2 percent neem oil is a good barrier for *An. dirus* female to lay her eggs. The study agreed well with many scientists [9,10], who all suggested that neem oil has an antiovipositional and ovicidal toxicity against insects like *Callosobruchus* spp.

Yadav [9] suggested that neem oil of 30 mg/10g concentration in green gram seeds was anti-ovipositional to the bruchid, *Callosobruchus maculatus* and of 10mg/kg concentrations to *C. anolis* and *C. chinensis*.

Neem alkaloids are safe to humans and neem oil is edible after processing [10]. It can be mixed in coconut or any other oil for application on the skin to repel mosquitoes.

Sharma [7] also stated that chemical control research is systematically developing. employing neem oil is the same as using an insecticide/larvicide, though with different characteristics and mode of action. Unlike other existing insecticides, neem oil is not a conventional pesticides. It does not produce the non-discriminatory rapid, directly non-toxic (not toxic to human, instead they are edible)

effects that are associated with traditional medicine.

In Mon, Kayin and Tanintharyi, the southern part of Myanmar that wells (both used and unused) support (heavy) *An. dirus* breeding has already been reported [11,12,13 & 14]. Application of neem oil into these well could control *An. dirus* breeding for the entire breeding season in the area.

The use of simple and cheap neem products such as crushed neem seed kernels, seems promising for treatment of temporary water pools in towns and villages in developing countries during the rainy season, for prevention of breeding of mosquitoes.

The overall results indicate that neem oil is an effective repellent against insects (both medical and agriculture pests) in areas where insecticides are not sprayed for administrative and financial reasons, and where there is no effective method for protection against mosquitoes vectors. Information on night biting activity of *An. dirus* and other potential *anopheline* mosquitoes in Myanmar is 1st and 2nd quarters of the night. Moreover, the application of neem oil (2 percent) could be recommended as prophylactics against the bites of (*An. dirus* and *An. minimus*) mosquitoes in Myanmar.

Thus, in the present context of malaria situation, application of neem oil as mosquito repellent, larvicides or antifeedant is simple and may be very useful as it is indigenous in nature and acceptable by the communities, non-toxic and cost effective for controlling mosquitoes breeding in stored water i.e., domestic water containers like metal drums, earthen pots, ceramic jars etc., (for *An. aegypti* DHF vector) and wells (for *An. dirus* malaria vector).

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Pathological study of coronary artery disease in Yangon General Hospital

Aung, **Ne Win, *Hla Htay, **David Kyaw & ***Le Le Win*

*Yangon General Hospital
**Pathology Research Division
***Health System Research Division
Department of Medical Research
****Institute of Medicine 1, Yangon

A descriptive, cross-sectional, histopathological study of coronary artery disease was conducted among 95 autopsied subjects (66 males, 29 females), with age ranging from 11 to 82 years. Fatty streaks and fibrous plaque were observed in the second and the third decade respectively. More severe atherosclerotic lesions were seen in males. The left anterior descending artery is the commonest artery to have coronary stenosis. The presence of significant stenosis was noted more in those who had myocardial infarction, hypertension, cerebrovascular diseases than those without these diseases. Likewise the weight of the heart was greater in those with coronary artery disease and hypertension. Sixty six percent of patients who died of tuberculosis, and 25% of those who died of cirrhosis of liver were found to have coronary artery stenosis.

INTRODUCTION

Coronary artery disease (CAD) is one of the diseases of the cardiovascular system, which is common in Myanmar and is a major cause of hospital morbidity and mortality [1,2]. The prevalence of CAD among adults in Yangon Division was 21/1000 [3]. However, community surveys, based on ECGs, and standard questionnaires, may not reflect the actual prevalence since a large number of CAD may remain asymptomatic and ECG diagnosis of CAD has low specificity [4]. Since severe atherosclerosis is the anatomical basis for CAD [5], a study of the extent of atherosclerosis of coronary arteries at autopsy, would compliment the available prevalent data on CAD based on clinical and ECG findings. Not only large scale multicentre studies are needed, they must also be well designed and organized in order to obtain information reliable enough to address this issue. This pilot study was therefore conducted as an exercise before embarking on a much more wider scale.

MATERIALS AND METHODS

Study design

A descriptive cross-sectional study of a selected population within a study period of three months from May 1993 to July 1993.

Subjects

All cases of both gender, brought dead to the Yangon General Hospital, or died within 24 hours of admission during the study period. Irrespective of the cause of death, it is the policy of the hospital to autopsy all cases that died within 24 hours of admission and those dying outside the hospital from accidents including suicides. In our material, the difficulty of autopsy data of not being able to fulfil the principle of random selection, was to a large extent overcome by including all types of deaths and nearly all deaths occurring within the study period.

Methods

The coronary arteries were dissected, prepared, fixed in a standardized way, placed in plastic bags with formalin. The vessels were identified only by the tag number enclosed in the bag which corresponded to the accession number on the basic data form WHO 5004 [6]. Grading of atherosclerotic lesions was then made, by gross examination with the naked eye, based on the WHO Technical Report Series No. 143[7], and by microscopic examination, of slides stained with hematoxylin and eosin[8] by a pathologist, in another department blinded to the basic data. The heart was also weighed (in grams) according to the method described by Kagan and Uemura, 1976 [9].

Data analysis

Data obtained were entered on the basic data form, the form for detailed study of pathological findings and the form for visual grading and transferred to a coded form for storage in a disc. the degree and extent of CAD were assessed in terms of relation between age, sex, and disease using a standard software for analysis. Patient's characteristics and comparability of subgroups were tested by Student's 't' test and χ^2 test.

RESULTS

Sample population was 95 (66 males and 29 females) with age ranging from 11 to 82 years. It was divided into even groups according to age in years (Table 1).

Atherosclerosis in subjects who died from violent causes which includes accidents, homicides, suicides and not from diseases related to atherosclerosis or capable of influencing its course can be considered as the least in extent and its level is an indication of the basic level of atherosclerosis in the population concerned [10,11]. For these

reasons, those subjects dying from violent causes were grouped separately and shown in Table 2.

Table 1. Distribution atherosclerosis by age and sex

Age (yrs)	Sex	No. of cases	Cases with AS	% of AS (+)
10-20	Male	7	0	0.0
	Female	3	0	0.0
21-30	Male	14	1	7.2
	Female	5	0	0.0
31-40	Male	13	1	7.6
	Female	7	2	28.6
41-50	Male	15	7	46.7
	Female	4	1	25.0
51-60	Male	7	5	71.4
	Female	1	1	100.0
61-70	Male	7	6	85.5
	Female	5	3	60.0
>70	Male	4	4	100.0
	Female	3	3	100.0
Total	Male	67	24	35.8
	Female	28	10	35.7
Grand Total		95	34	35.8

Table 2. Distribution atherosclerosis by age and sex among violent deaths

Age (yrs)	Sex	No. of cases	Cases with AS	% of AS (+)
10-20	Male	6	0	0.0
	Female	2	0	0.0
21-30	Male	14	1	7.1
	Female	2	0	0.0
31-40	Male	7	0	0.0
	Female	5	1	20.0
41-50	Male	8	3	37.5
	Female	0	0	0.0
51-60	Male	2	0	0.0
	Female	0	0	0.0
61-70	Male	1	1	100.0
	Female	1	1	100.0
Total	Male	38	5	13.1
	Female	10	2	20.0
Grand Total		48	7	14.6

Age group and sex distribution of coronary stenosis in the sample population are shown in figure 1 and figure 2 respectively. All of the cases in age group >70 years have coronary stenosis. Seventy-five per cent of cases in the age group 51-70 years are associated with stenosis. The incidence is lowest among the youngest age group 21-30 years. Coronary stenosis was involved in more than one area in 35 cases in which female population is two and a half times of male population. Sex distribution is not significantly different in cases of stenosis of one vessel.

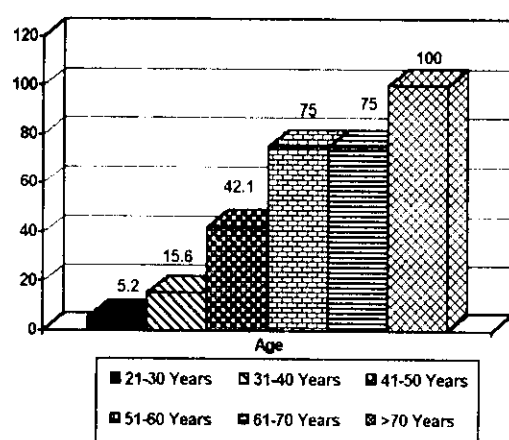


Fig. 1. Distribution of coronary stenosis by age among the sample population

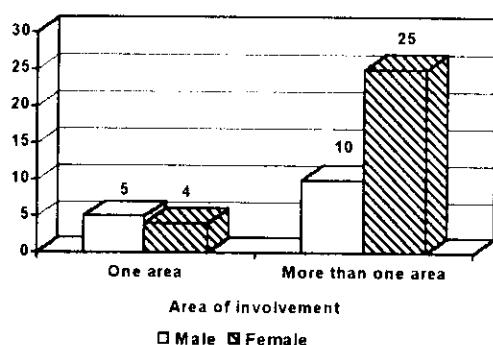


Fig. 2. Distribution of coronary stenosis by sex among the sample population

Mean weights of the heart are shown in figure 3. The highest mean weight (372 g) was observed in cases with myocardial infarction; lowest mean weight (240 g) in cases without

hypertension and CAD. The mean weight of the heart in cases with hypertension is 278 g.

DISCUSSION

Differing degree of severity of atherosclerosis among the inhabitants of different countries were first noted in 1919, when it was found that among the inhabitants of Germany from 18 to 20 years of age, atherosclerosis of the coronary arteries was encountered almost twice as often as in the inhabitants of Switzerland of the same age [12]. The Seven Countries Study found that CAD was less common in Japan and Mediterranean countries than in United States, Finland and the Netherlands, despite equally high levels of economic development [13].

It was also noted that migrant population tend to acquire the disease levels of adopted population culture [14]. These observations indicate the operation of powerful environmental factors on CAD and is not therefore, the inevitable consequence of either ageing or affluence. The difficulties of comparing data on the severity of atherosclerosis have long been known. However within certain limits, comparison with data obtained in the atherosclerosis of the aorta and coronary arteries in Five Towns, a European study [4] and the present study is possible because the same technique was used and the assessment of atherosclerosis in the two studies was identical.

The prevalence and extent of coronary atherosclerosis quite clearly increased with age in this study (Fig. 1) as well as in others [15]. Vaneck (1976) pointed out in his study that its development occurred in stages, starting in the early ages with fatty streaks, followed by fibrous plaques, in young adults with or without the appearance of all the major atherosclerotic changes (complicated lesions, calcified lesions, stenosis).

The next stage in the Middle Ages is characterized by a sharply rising frequency of major atherosclerosis changes and also of stenosis while in older age groups, although the prevalence of atherosclerotic lesions continued to increase, calcified lesions were the most frequent. However, as mentioned initially, differing degrees of severity of atherosclerosis exist among countries.

Fatty streaks were observed by Stary (1983) in his work of series of children and young adults in the West, as early as 10 years [16]. However in this study, the earliest age with fatty streak was in a 21-year-old who died from a road traffic accident. Smooth muscle proliferation of the right coronary of a 13-year-old male who died of drowning was too nonspecific to be labeled as due to early atherosclerosis in this study.

Fibrous plaques and coronary stenosis was found only in second and third decade in this study, while it was detected in all the coronaries even in the 10-14-year age group in the European study [4]. The age-standardized values of coronary stenosis for any coronary artery in Europe were 26% for males and 13% for females [4]. Twenty-five percent of females has coronary stenosis involving more than one area in contrast to 10% of males ($p < 0.05$). However, complicated lesions were seen only in three males in this study. Vaneck (1976) reported that there were more males with complicated lesions in his study [4]. A more detailed study involving a larger population would shed much light on this matter.

In the European study, the artery most susceptible to stenosis was anterior descending artery [4]. It is also true in this study.

Atherosclerosis of the coronaries was seen more in persons who had died from various manifestations of atherosclerosis [4]. In this

study coronary stenosis was found in 85% of patients who died of myocardial infarction while in the studies of 320 persons who had died of myocardial infarction at the Mjasnikov Institute of Cardiology of the Academy of Medical Sciences of the USSR, 82% had coronary stenosis of one of the arteries [17]. Coronary stenosis was also found in 60-80% of men and 50-75% of women with myocardial infarction in Europe. The figures are close to those of the International Atherosclerosis Project Study, in which stenosis was found on the average in 60% of persons who had died of coronary artery disease [11]. These findings indicate that irrespective of gender, race, or environment, those who die from myocardial infarction usually have severe coronary artery disease.

In this study out of seven patients who died of myocardial infarction only one female patient had a rupture of the heart. It is generally accepted that the frequency of rupture of the heart is higher in women than in men [4,16,17]. The greater frequency of myocardial rupture in women has been ascribed to the weak development of collateral blood circulation because of the less marked nature of coronary atherosclerosis [18]. Another important factor is considered to be the presence of hypertensive disease, which is more frequent in women [19].

Kagan (1976) pointed out in a study of five towns in Europe that coronary stenosis occurred most frequently in those who had myocardial infarction than those who died of cerebrovascular accidents (cerebral infarcts and cerebral hemorrhage) [20]. In this study although the subgroup sample population is small, patients dying from myocardial infarction has significantly more coronary stenosis ($p < 0.05$) than those dying from strokes.

Sixty-six percent of males and 60% of females subjects with hypertension have fibrous plaque

in the left anterior descending artery compared to 98% of men and 91% of women with hypertension in the European study [21]. The apparent lower figures in this study may reflect the generally lower prevalence of other risk factors for coronary artery disease in this country [3]. Stamler (1962) found that the occurrence of ischemic heart disease in hypertensives is low in countries where IHD is not widespread [22]. The same is true of the prevalence of coronary stenosis in hypertensive disease, stenosis being more frequent in those European towns where coronary heart disease is present [21].

The predominance of atherosclerosis in men over women has been reported by a number of authors [23,24]. Possibly, the effects of hormonal factors, including the inhibitory effect of female sex hormones on atherosclerosis is offset by hemodynamic factors [24,25]. As expected because of hypertrophy caused by high blood pressure [21] the mean heart weight in those with hypertension (excluding those with coronary stenosis) is greater than the mean weight of the heart in those without hypertension and CAD. However the mean weight of the heart with acute myocardial infarction even without hypertension is 372 g in this study (Fig. 3).

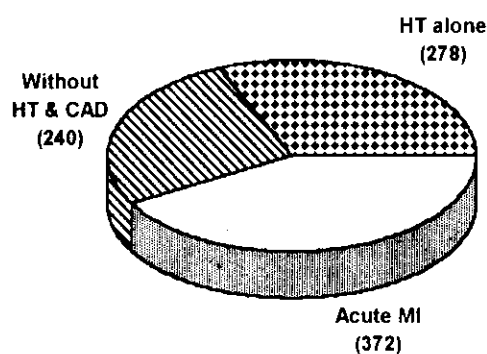


Fig. 3. Mean weight of heart in gram (G) in subjects dying from acute myocardial infarction (MI), hypertension alone (HT) and without hypertension and coronary artery disease (CAD)

This finding was also reported by others [21]. Vihert (1976) suggested that the insufficiency

of coronary blood flow, *per se* stimulated the increase in muscle mass [20].

The data from Five Towns in Europe do not support the view that the presence of tuberculosis means a general decrease in the amount of atherosclerosis [26]. In this study also, 62% of subjects dying from pulmonary TB had fibrous plaque of the coronary arteries, compared to 15% in those dying from violent deaths. A valid explanation could not be given as data on other risk factors that can give rise to CAD in these subjects were not available.

In this study 25% (one out of four) dying of cirrhosis of liver had coronary stenosis, in comparison to 4.2% of subjects dying from violent deaths. This is in contrast to the findings of Vaneck (1976) [15]. He pointed out that male and female subjects with cirrhosis of the liver had significantly less extensive raised lesions than subjects in the standardized average atherosclerosis group which includes violent death but mostly more extensive than the low atherosclerosis group. Study of relationship between liver cirrhosis and atherosclerosis is confused by the question of what effect alcoholism may have on the development of extension of this condition. Most studies have suggested that atherosclerosis is less extensive in subjects with cirrhosis and that myocardial infarction is less frequent [27,28]. The evidence presented by Hirst *et al.* (1956) indicates that coronary atherosclerosis or its complications were less frequent only where chronic alcoholism had been accompanied by cirrhosis [29]. However the mechanism whereby cirrhosis acts as such a factor is also unclear [29,30].

In conclusion, a rough idea of the extent of atherosclerosis among a selected population in Yangon can be obtained from this study and a similar study conducted on a larger scale, throughout the country would provide more definite information on the prevalence of atherosclerosis in this country.

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Effects of beta-thalassaemia trait on pregnancy and foetal outcomes

*Ne Win, Hla Hla May, Myat Mon Oo, Thoung Hla, Khin Saw Aye
& Soe Soe*

Pathology Research Division, Department of Medical Research

Ten cases of pregnant mothers with confirmed beta-thalassaemia trait were studied for any complications occurred during pregnancy and labour and after birth. All cases were found to have no complications during pregnancy and labour giving normal full-term vaginal delivery. all the newborns were also found to have normal birth weight and have no complications. These findings indicate that beta-thalassaemia trait has no deleterious effects on pregnancy and foetal outcomes. No active interventions are required during antenatal care throughout pregnancy in beta-thalassaemia trait.

INTRODUCTION

Beta-thalassaemia has been recognized in Myanmar for many years [1]. Usually carriers or traits are not seriously anaemic in adults of both sexes. But the anaemia usually worsens at the time of clinical stress, commonly pregnancy. Indeed, female patients may present or be diagnosed for the first time in pregnancy as refractory hypochromic anaemia [2]. thalassaemia trait is the most common maternal genetic abnormality associated with pregnancy. But there are only a few studies well reported and there is still uncertainty regarding the clinical course, complications and management [3]. More importantly, there is no evidence as to whether or not maternal beta-thalassaemia is associated with an increase in fetal or maternal morbidity. To investigate these problems we studied 10 pregnant mothers who are found to have beta-thalassaemia trait after screening of 150 normal pregnant mothers attending to antenatal care unit, Central Women Hospital.

MATERIALS AND METHODS

For a period of 18 months with the effect from June 1994, 150 cases of pregnant mothers

attending to antenatal care clinic at out-patient department of CWH were selected for thalassaemia screening. All these cases have low Hb concentration of <10.5 gm/dl according to their A-N care record. Patients' consent is taken after explanation of aims of study and of thalassaemia trait.

Diagnosis of beta-thalassaemia trait was based on the association of hypochromia, microcytosis and confirmed by finding a raised Hb-A2 concentration of values above 3.5% of total Hb concentration [3].

Peripheral blood films were examined to observe hypochromia as well as other morphological changes. Hb-A2 was determined by cellulose acetate electrophoresis followed by elution method [4].

Thus obtained 10 cases of beta-thalassaemia traits among pregnant mothers were looked for any associated abnormal conditions or complications throughout pregnancy, during labour and upto after birth.

RESULTS AND FINDINGS

All cases of beta-thalassaemia trait with pregnancy had high Hb-A2 contents ranging

from 3.8% to 6.5%. Case No. 3 is a known case, mother of 10-year-old boy with thalassaemia major who is under regular blood transfusion at Day Care Unit, YCH. Remaining 140 cases had Hb-A2 content less than 3.5%.

Table 1. Total Hb concentration and Hb-A2 content in % among thalassaemia trait pregnant mothers

Case no.	Gestation (Weeks)	Total Hb (Gm/100 dl)	Hb-A2 % (% of total)	Remarks
1	24	10.0	3.8	-
2	28	9.8	6.5	-
3	20	9.6	6.1	Known case
4	26	10.3	5.6	-
5	22	10.0	4.3	-
6	24	8.8	5.1	-
7	20	9.5	5.6	-
8	26	9.7	4.9	-
9	24	9.9	5.3	-
10	28	8.5	6.2	-

Note: period of gestation in weeks and total Hb concentration were taken from patients' antenatal care record

Peripheral blood films of these 10 cases revealed mild to moderate hypochromic microcytic anaemia, anisocytosis and microcytosis, target cells and prominent basophilic stippling. There were no significant changes in white blood cells and platelets series.

All thalassaemia trait cases under this study had no associated complications throughout pregnancy in both mother and foetus. They had normal full term vaginal delivery of normal newborns.

DISCUSSION

This study clearly demonstrates that maternal beta-thalassaemia trait has no deleterious effects on pregnancy as well as on foetus. And

also there does not appear to have any effect on foetal growth and development. Moreover, there is no increase in maternal morbidity or complications such as the rate of cesarean section, pre-eclampsia, antepartum haemorrhage, postpartum haemorrhage and so on.

All cases under this study showed no symptoms and signs of anaemia and others. Anaemia associated was very mild or absent clinically and the condition was almost entirely without recognizable feature. All of these 10 cases except in one case were newly diagnosed during this study as beta-thalassaemia trait.

It has been suggested that beta-thalassaemia trait with pregnancy cases presenting with anaemia should be given oral iron and physiological dose of folic acid throughout pregnancy as iron deficiency is common in such cases [3]. Iron and folic acid supplements are also given to pregnant mothers presenting with anaemia at AN care unit, CWH.

The findings in this study suggest that pregnant mothers with beta-thalassaemia trait should receive normal antenatal care with no active intervention to increase the haemoglobin concentration. Once thalassaemia has been diagnosed in pregnant mothers, screening of thalassaemia should be performed to the rest of the family including her husband so as to do genetic counseling if necessary.

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Bacterial pathogens and drug susceptibility pattern: from diarrhoea cases of children

**Mar Mar Nyein, **Than Nu Shwe, **Khin Mu Mu Kyaw, *Saw Thandar &
**Thein Thein Myint*

***Bacteriology Research Division
Department of Medical Research
*Yangon Children's Hospital**

Stool specimens from 200 cases of children with ages of less than five years with history of acute diarrhoea from Yangon Children's Hospital were proceeded for isolation of bacterial pathogens. Enterotoxigenic *Escherichia coli* ETEC (15.5%), enteropathogenic *Escherichia coli* EPEC (19%), Shigellae (3.5%), Salmonellae (2.5%), ETEC + EPEC (6.5%), ETEC+Shigellae (1.0%) were isolated. Antibiotic susceptibility pattern of EPEC revealed that they were resistant to ampicillin (96%), tetracycline (90%), streptomycin (82%), chloramphenicol (80%) and septrin (61%); but were sensitive to nalidixic acid (100%), furazolidone (92%), sisomycin (90%), gentamicin (88%), norfloxacin (70%), and cephalothin (66%). For Shigellae they were resistant to ampicillin (100%), cephalothin, chloramphenicol, furazolidone, streptomycin (66.7%) but were 100% sensitive to sisomycin, minocycline, norfloxacin, gentamicin; tobramycin (89%), neomycin (67%) and septrin (56%). For Salmonellae they were resistant to ampicillin (100%), chloramphenicol and tetracycline (80%) but were 100% sensitive to cephalothin, furazolidone, gentamicin, nalidixic acid, norfloxacin, septrin and sisomycin. For ETEC they were resistant to ampicillin (100%), tetracycline (79%), chloramphenicol (53%) and septrin (53%). They were 100% sensitive to cephalothin, furazolidone, gentamicin, nalidixic acid, neomycin, norfloxacin, septrin and sisomycin.

INTRODUCTION

Diarrhoea is a leading cause of illness and death among children in developing countries where an estimated 1.3 thousand million episodes occur each year in those under five years of age, with an average of 3.3 episodes of diarrhoea per year. According to the results from the survey conducted in 1995, diarrhoea accounts for 16% of total mortality in children of less than five years of age [1]. According to the priority ranking of disease based on score system in the National Health Programme 1993-1996 diarrhoea stood in the fourth position. Though there were reports regarding the aetiology of acute diarrhoea in Myanmar

[2,3,4], the distribution of bacterial pathogens in diarrhoea in relation to antibiotic susceptibility testing will explain the emergence of drug resistance. Moreover, the antibiotic susceptibility pattern with changing pattern needs to be explored by monitoring periodically to assist clinicians in the management of these cases.

MATERIALS AND METHODS

Place of study and selection of cases

The study was conducted in Medical Units 1, 2, 3, Neonatal Unit and Diarrhoea Training Unit

(DTU) of the Yangon Children's Hospital, Yangon, Myanmar. A total of 200 children aged below five years of both sexes with a history of acute diarrhoea which is defined as an increase in frequency of defaecation more than three times per day and or in liquidity of the stool were included.

A history was taken from parents or from the relatives to elicit the present illness as well as personal, social and environmental factors. History of present illness includes duration of illness before attending the hospital; number of motions per day; volume of stool per motion; nature of stool; history of taking antibiotics. For personal, social and environmental factors; source of drinking water, food taking habits, type of latrines, method of cleaning after defaecation and hand washing after defaecation were included.

Specimen collection and detection of pathogenic organisms

Stool specimens were collected in a sterile bottle as well as rectal swabs of these children were taken and plated onto Salmonella-Shigella (SS) agar directly for the isolation of *Shigella* spp. The swabs were then placed immediately in Cary-Blair transport media. Detection of pathogenic organisms was done according to the method described by World Health Organization, 1981 [5], using Salmonella-Shigella (SS) agar, Xylose-Lysine-Dextrose (XLD) agar, MacConkey Agar (MA) and Thiosulphate Citrate Bile Sucrose (TCBS) agar. Selenite F and alkaline peptone water were also used as an enrichment media. Biochemical tests, gram staining, oxidase reaction were also performed for the confirmation of the organisms.

For *Shigella* and *Salmonella* serotyping, the antisera were obtained from Denka-Seika Company Limited, Japan. For *Vibrio* 01 (Inaba and Ogawa) and 0139, the antisera were supplied by Professor Y. Takeda, Tokyo, Japan and typing of *Escherichia coli* 'O' and 'OK' antisera [6,7]

was done as described in Ewing, 1986 [8]. For heat labile toxin, Biken test [9] was utilized and for detection of heat stable toxin, a test kit for the detection of enterotoxin (ST) in culture filtrate or supernatant was used (*Escherichia coli* ST, EIA toxin detection kit, TD 700, Oxoid Laboratories).

Antibiotic susceptibility testing

The method described by Bauer [10], was followed using Mueller Hinton broth and agar. The antibiotic discs were obtained from Becton Dickinson Microbiology Systems (BBL). The antibiotic susceptibility pattern was recorded as according to the criteria for interpretation of sensitivity to antibiotics.

RESULTS

The isolation of bacterial pathogens from diarrhoea cases of children is shown in Table 1.

Table 1. Distribution of bacterial pathogens from diarrhoeal case from the Yangon Children's Hospital

Pathogens	Number of cases (%)	Sex	
		Male (%)	Female (%)
<i>Escherichia coli</i>			
LT	6 (6.1)	4 (66.7)	2 (33.3)
ST	17 (17.2)	11 (64.7)	6 (35.3)
STLT	8 (8.1)	4 (50.0)	4 (50.0)
All ETEC	31 (31.3)	19 (61.3)	12 (38.7)
EPEC	37 (37.4)	23 (62.2)	14 (37.8)
ETEC + EPEC	13 (13.1)	10 (76.9)	3 (23.1)
ETEC + Shigellae	2 (2.0)	2 (100)	0
Shigellae	7 (7.1)	5 (71.4)	2 (28.6)
Salmonellae	5 (5.1)	2 (40.0)	3 (60.0)
Vibrios	0	0	0
Klebsiellae	4 (4.0)	2 (50.0)	2 (50.0)
Total cases with pathogens	99 (49.5)	63 (63.6)	36 (36.4)
Cases with no pathogens	101 (50.5)	61 (60.4)	40 (38.6)
Tested cases	200	124 (62.0)	76 (38.0)

Figures in parenthesis denote percentages

Table 2. Age distribution in relation to bacterial pathogens isolated from diarrhoea cases of children from Yangon Children's Hospital (n= 99)

Age Group months	Pathogens / Tested	ETEC	EPEC	Shigellae	Salmonellae	Klebsiellae	ETEC + EPEC	ETEC + Shigellae
< 6	29/52	8 (27.6)	15 (51.7)	1 (3.5)	1 (3.5)	1 (3.5)	3 (10.4)	0
7-12	29/63	6 (20.7)	10 (34.5)	3 (10.4)	2 (6.9)	3 (10.4)	4 (13.8)	1 (3.5)
13-18	19/36	8 (42.1)	4 (21.1)	2 (10.5)	1 (5.3)	0	3 (15.8)	1 (5.3)
19-24	10/23	5 (50.0)	3 (30.0)	0	1 (10.0)	0	1 (10.0)	0
25-36	7/16	2 (28.6)	3 (42.9)	1 (14.3)	0	0	1 (14.3)	0
37-60	5/10	2 (40.0)	2 (40.0)	0	0	0	1 (20.0)	0
All age	99/200 (49.5)	31 (31.3)	37 (37.4)	7 (7.1)	5 (5.1)	4 (4.0)	13 (13.1)	2 (2.0)

Figures in parenthesis denote percentages

From 99 cases that yielded bacterial pathogens, ETEC (31.3%); EPEC (37.4%); Shigellae (7.1%); Salmonellae (5.1%), *Klebsiella* spp. (4.0%), ETEC + EPEC (13.1%), ETEC + *Shigella* (2.0%) were identified. *Vibrio* species were not isolated. It was observed that 63.6% of males and 36.4% females yielded bacterial pathogens.

Age distribution of cases that were associated with bacterial pathogens is shown in Table 2.

It was observed that among the age group of <6 months ETEC (27.6%); EPEC (51.7%); Shigellae, Salmonellae, and Klebsiellae (3.5%) respectively were isolated. The serogroups obtained among the children is shown in Table 3.

The most prominent serogroups present were six cases each of O125 K70 and O126 K71; five cases each of O86 K61, K62, O114 K90, O128 K67 and O159 K+. Moreover O157 K+ was also isolated from one case. The distribution of ETEC is shown in Table 4.

Table 3. Distribution of *Escherichia coli* serotypes from children with diarrhoea from Yangon Children's Hospital

Serotypes	Total	Male	Female
O1 K51	2	1	1
O8 K25	1	0	1
O8 K40	1	1	0
O25 K+	3	1	2
O27 K+	1	0	1
O28 K73	2	1	1
O78 K80	2	2	0
O86 K61	3	1	2
O86 K62	2	1	1
O114 K90	5	3	2
O119 K69	1	1	0
O125 K70	6	6	0
O126 K71	6	4	2
O127 K63	2	1	1
O128 K67	5	4	1
O136 K78	1	1	0
O148 K+	1	0	1
O157 K+	1	0	1
O159 K+	5	5	0
Total	50	33	17

Table 4. Distribution of enterotoxigenic *Escherichia coli*

Age (months)	ETEC	LT	ST	STLT
<-6	11 (23.9)	4 (36.4)	5 (45.5)	2 (18.2)
7-12	11 (23.9)	2 (18.2)	7 (63.6)	2 (18.2)
13-18	12 (26.1)	2 (16.7)	6 (50.0)	4 (33.3)
19-24	6 (13.0)	0	5 (83.3)	1 (16.7)
25-36	3 (6.5)	1 (33.3)	1 (33.3)	1 (33.3)
37-60	3 (6.5)	0	3 (100)	0
Total	46	9 (19.6)	27 (58.7)	10 (21.7)

Figures in parenthesis denote percentages

ETEC = Enterotoxigenic *Escherichia coli*

LT = Heat labile

ST = Heat stable

In this study, 46 cases (23.0%) were associated with ETEC. It was noted that 23.9% each were from less than six months and 7 to 12 months; 26.1% were from 13 to 18 months; 6.5% each

were from 25 to 36 months and 37 to 60 months age groups respectively. Among these include heat labile toxin LT (19.6%); heat stable toxin ST (58.7%), and STLT (21.7%). It was apparent that ETEC was isolated more from children of less than 18 months old than compared with the age groups of 19 to 60 months old. The heat stable toxin producing cases were more prominent than heat labile cases. However the ETEC cases were distributed in all the age groups.

The antibiotic susceptibility pattern of EPEC, ETEC, Shigellae and Salmonellae is shown in Table 5.

Of the 40 cases of EPEC, the organisms were resistant to ampicillin (96%), tetracycline (90%), streptomycin (82%), chloramphenicol (80%), septrin (64%) and norfloxacin (24%). They were sensitive to nalidixic acid (100%), furazolidone (92%), sisomycin (90%), gentamicin (88%), polymyxin B (86%), amikacin and norfloxacin (70%), cephalothin (66%). Out of nine cases of shigellae they were resistant to ampicillin (78%); cephalothin,

Table 5. Antibiotic susceptibility pattern of bacterial pathogens in percentages

Antibiotics potency (mcg/disc)	EPEC			Shigellae			ETEC			Salmonellae		
	R	I	S	R	I	S	R	I	S	R	I	S
Amikacin AN-30	4	26	70	0	0	9	37	53	10	40	60	0
Ampicillin AM-10	96	4	0	78	0	22	100	0	0	100	0	0
Cephalothin CF-30	26	8	66	67	0	33	0	26	74	0	0	100
Colistin CL-10	0	0	100	0	0	100	0	0	100	0	0	100
Chloramphenicol C-30	80	14	6	67	11	22	53	0	47	80	0	20
Furazolidone Fx100	4	4	92	67	11	22	0	0	100	0	0	100
Gentamicin GM-10	6	6	88	11	0	89	0	0	100	0	0	100
Minocycline* Mn030	NT			0	0	100	NT			NT		
Nalidixic acid NA-30	0	0	100	0	22	78	0	0	100	0	0	100
Neomycin N-30	8	50	42	0	33	67	0	0	100	0	0	100
Norfloxacin NOR-10	24	6	70	0	0	100	0	0	100	0	0	100
Polymyxin B P-300	4	10	86	22	0	78	NT			0	0	100
Sisomycin S-10	10	0	90	0	0	100	0	0	100	0	0	100
Streptomycin S-10	82	0	18	67	0	33	63	11	26	40	0	60
Tetracycline T-30	90	8	2	56	22	22	79	0	21	80	0	20
Tobramycin NN-10	12	2	86	11	0	89	0	5	95	0	20	80
Trimethoprim/ Sulfamethoxazole SXT	64	2	34	44	0	56	53	0	47	0	0	100

R=Resistant

I=Intermediate

S=Sensitive

*=Japan

chloramphenicol, furazolidone and streptomycin (67%); tetracycline (56%) and septrin (44%). They were sensitive to minocycline, norfloxacin and sisomycin (100%); gentamicin and tobramycin (89%); polymixin B (78%), neomycin (67%). Five cases of salmonellae were resistant to ampicillin (100%), chloramphenicol and tetracycline (80.0%). They were totally sensitive to cephalothin, furazolidone, gentamicin, nalidixic acid, neomycin, norfloxacin, polymixin B, septrin and sisomycin. Of the 46 cases of ETEC, they were resistant to ampicillin (100%), tetracycline (79%), streptomycin (63%), chloramphenicol and septrin (53%). They were totally sensitive to colistin, furazolidone, gentamicin, nalidixic acid, neomycin, norfloxacin and sisomycin.

DISCUSSION

In this study, the age distribution of diarrhoea cases revealed that 57.3% were less than one year age group and 42.7% were between one to five years old, where the majority of affected children were during crawling period and were first exposed to heavy infection. It was also noted that 49.5% of cases detected were with bacterial pathogens and 50.5% were not with bacterial pathogens. In these non-bacterial pathogen cases, the pathogenic organisms could probably due to some other viral or protozoal infections.

The EPEC, ETEC are still major aetiological agents responsible for acute diarrhoea in children of less than five years of age followed by shigellae (4.5%) and Salmonellae (2.5%). The trend of the isolation of bacterial pathogens remains constant since 1980 after the recognition of the role of ETEC in Myanmar. The bacterial pathogens isolated were similar with other countries [11-14]. For antibiotic susceptibility pattern it was noted that most of

these strains were resistant to commonly used antibiotics and even 24% of cases were found to be resistant to norfloxacin in EPEC cases. It was also noted that 90% of the cases were multidrug resistant with at least two drugs. The emergence of the drug resistant strains is quite alarming. A study in 1980 [3] revealed that EPEC organisms were resistant to ampicillin (18%), tetracycline (26%), chloramphenicol (17%) and cephaloridine (14%). In 1986 the resistance rate increased as ampicillin (89%), tetracycline (47%), chloramphenicol (59%), septrin (27%) in Myanmar [15]. In this study EPEC isolated were resistant to ampicillin (96%), tetracycline (90%), chloramphenicol (80%) septrin (64%) and cephalothin (36%).

From this study it was also apparent that environmental sanitation and personel hygiene are the important factors in causing diarrhoea as reported by various authors [16-18]. Among diarrhoea cases in this study, 80.5% used pit type of latrines; 16% of cases used hand flush and 2% only used reservoir type of latrines. Inquiring the method of cleaning after defaecation among diarrhoea cases revealed that 97% of the cases used water only after defaecation. Hand washing was done with water in 57.5% of the cases and very few (4%) used soap and 38.5% had not done hand washing. Water supply was from tap water (38%), artesian well (37%), surface well (11.5%). From those cases 68.5% drank water without boiling.

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Anthropometrical characteristics and dietary intake of omnivorous and vegetarian postmenopausal women in two rural communities

**Theingi Thwin, **Khin Mar Aye, **Htay Htay & *Khin Maung Naing*

*Nutrition Research Division
Department of Medical Research
**Institute of Medicine (1), Yangon

To compare the anthropometry and three-day dietary intake of omnivorous and vegetarian postmenopausal women, a community-based, comparative study was carried out at two communities in Thaton District, Mon State. Apparently healthy 54 vegetarians and 50 omnivorous postmenopausal women aged fifty to seventy years were studied. Height, weight, skin-fold thickness (biceps, triceps, subscapular, suprailiac) were measured. Body mass index and percent body fat were calculated. Three-day dietary intake of carbohydrate, protein, fat and fiber of randomly selected subsample (15 vegetarians and 15 omnivores) were measured by weighing method. The duration of taking vegetarian diet was 25.9 ± 10.8 years. Weight, height, body mass index and percent body fat were not statistically different. The vegetarians consumed significantly more carbohydrate ($p < 0.05$) and fiber than did the omnivores ($p < 0.05$). Energy, protein and fat consumption were not statistically different between two groups. Unlike western postmenopausal women, the prolonged consumption of vegetarian diet did not produce a significant change in the anthropometry of Myanmar postmenopausal women.

INTRODUCTION

Anthropometry is an easy, cheap and non-invasive method of assessing nutritional status. Correlation has been found between anthropometrics and biochemical indices of nutrition. Anthropometrical indices have also been reported to be of prognostic value in predicting mortality. References of anthropometry for the elderly are available for Caucasians and Chinese [1,2]. However, information is sparse on anthropometrics indices in Myanmar postmenopausal women, and thus, there is a need to establish the reference values.

As dietary habit appears to influence the adiposity or anthropometry, vegetarian women have lower body weight or adiposity than non-

vegetarian women [3]. Since adipose tissue is the major source of estrogen formation in postmenopausal women, vegetarian postmenopausal women have lower adiposity and lower estrogen production than do non-vegetarian postmenopausal women. Thus, it can be assumed that there is the lower mortality rate of vegetarian postmenopausal women from breast and endometrial cancers than that of non-vegetarian women [4,5]. Very few studies have focused on anthropometry and dietary habits of Myanmar postmenopausal women. Therefore, the objective of this study is to compare the weight, height, body mass index, percent body fat, dietary intake of carbohydrate, protein, fat and the content of fiber of the omnivorous and vegetarian postmenopausal women.

MATERIALS AND METHODS

A community-based, comparative study was done in two rural communities (*Tharyargone and Alantayar Villages*) of Thaton District, Mon State. A group of 54 vegetarians and another group of 50 omnivores, apparently healthy postmenopausal women, aged 50-70 years, were studied. The criteria for entry into this study were: (1) menopausal period of at least five years, (2) on vegetarian diet of at least three years duration, and (3) subjects being capable of taking self-care and leading a normal active life.

After taking informed consents, thorough history taking and physical examination were done to exclude the presence of metabolic and endocrine diseases by the principal investigator. Identification of subjects, age of menopause, habit of smoking, and use of types of cooking oil were recorded during interview by a trained technician. Weight and height were measured with a bathroom scale and stadiometer by the standard method. Weight and height were measured to the nearest of 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was derived from dividing of weight in kilograms by square of height in meters (kg/m^2).

Biceps, triceps, subscapular and suprailiac skinfold thickness were measured with Holtain calipers in a scale of millimeters. Percent body fat was calculated using the formula of Siri [6]. Three-successive-day dietary intake of the randomly selected 15 women of each group were measured by weighing method. The nutritive value of individual intake was calculated by using the food composition table of Burmese foods [7] and East Asian foods [8]. Unpaired Student's "t" test was employed to detect differences in mean values of two groups, i.e., height, weight, BMI, percent body fat, three-day dietary intake of carbohydrate,

protein, fat and fiber. All tests were considered to be significant at the $p < 0.05$ levels.

RESULTS

The pattern of vegetarian is lacto-vegetarian with a mean duration of 25.9 ± 10.8 years. Although the ages of the two groups were not statistically different, age of menopause of vegetarians was significantly earlier than that of omnivore ($p < 0.05$). The number of regular smokers in vegetarians was less (74% vs. 88%) but the duration of smoking was significantly longer than that of the omnivores ($p < 0.05$). More than half of the subjects in both groups used palm oil but one fourth of the subjects used groundnut oil or palm oil indiscriminately (Table 1).

Table 1. Demographic and behavioural characteristics of the omnivorous and vegetarian postmenopausal women

Variables	Omnivores (n=50)	Vegetarians (n=54)
Age in years		
Mean $\pm$ SD	58.7 ± 6.5	59.1 ± 7.5
Age at menopause (years)		
Mean $\pm$ SD	46.7 ± 4.7	$44.3 \pm 5.4^*$
History of smoking		
Smoking habit	88%	74%
Duration	23.7 ± 6.2	$29.6 \pm 6.4^*$
Distribution of daily consumption of oil		
Palm oil	56%	68%
Groundnut oil	14%	7%
Non-specific oil	30%	25%

*Significantly different from omnivores, $p < 0.05$

Table 2 shows there is no significant difference between the height, weight, body mass index, skin-fold thickness and percent body fat of the two groups.

Table 2. Anthropometric characteristics of the omnivorous and vegetarian postmenopausal women

Variables	Omnivores (n=50)	Vegetarians (n=54)
Weight(kg)		
Mean $\pm$ SD	41.0 $\pm$ 8.8	48.0 $\pm$ 7.9
Height(cm)		
Mean $\pm$ SD	142.4 $\pm$ 5.6	141.1 $\pm$ 5.9
Body mass index		
Mean $\pm$ SD	20.5 $\pm$ 4.1	20.7 $\pm$ 3.3
Skin-fold thickness(mm)		
Triceps	4.5 $\pm$ 3.7	3.7 $\pm$ 1.2
Biceps	4.6 $\pm$ 2.3	4.2 $\pm$ 1.9
Subscapular	7.3 $\pm$ 2.9	8.7 $\pm$ 3.3
Suprailiac	6.1 $\pm$ 3.4	6.5 $\pm$ 2.2
Sum of 4 skin-fold thickness	21.8 $\pm$ 8.5	20.6 $\pm$ 7.4
% Body fat	15.3 $\pm$ 4.9	14.6 $\pm$ 4.6

Table 3. Mean values of the characteristics and dietary pattern of the omnivorous and vegetarian postmenopausal women (Three-day dietary study)

Variables	Omnivores (n=15)	Vegetarians (n=15)
Characteristics of study subjects		
Age (years)	58.9 $\pm$ 7.6	62.9 $\pm$ 8.2
Age at menopause (years)	47.3 $\pm$ 3.2	47.2 $\pm$ 4.7
Body mass index	21.1 $\pm$ 4.2	20.6 $\pm$ 2.7
Dietary pattern		
Energy (kcal)	1717.7 $\pm$ 418.9	1925.7 $\pm$ 495.3
Protein(g)	44.1 $\pm$ 13.2	45.1 $\pm$ 15.7
% Energy	10	9
Carbohydrate (g)	189.9 $\pm$ 86.2	386.9 $\pm$ 141.7*
% Energy	69	80
Fat (g)	36.5 $\pm$ 21.7	26.8 $\pm$ 13.8
% Energy	19	16
Fiber (g)	30.0 $\pm$ 1.3	39.8 $\pm$ 2.4*

*Significantly different from omnivorous women, $p < 0.05$

The mean age, age of menopause, and body mass index between the two groups were comparable. The vegetarians consumed

significantly more carbohydrate and more fiber than those of omnivorous women ($p < 0.05$). The percentage of calories, contributed by protein, carbohydrate, and fat were 10%, 69% and 19% for omnivores and 9%, 80% and 16% for vegetarians respectively (Table 3).

DISCUSSION

The age limit of 45 to 55 years might be regarded as the 95% confidence interval for the age of the menopause [9]. The age of menopause of the non-vegetarians lies within normal accepted range. The age of menopause of smoker is 1 to 2 years earlier than non-smokers [10]. However, in the present study, there is no significant correlation ($r = 0.21$, $p > 0.05$) between the age of menopause and the duration of smoking in vegetarians. Therefore, the reason for earlier age of menopause in these women cannot be explained and this might be a potential scope for study.

No significant differences in anthropometrical measurements in two groups were found and it was similar to that of the study of Armstrong and coworkers [5]. However, in the study of Barbosa and co-authors [11], the thigh skin-fold thickness and the sum of skin-fold thickness of the non-vegetarian postmenopausal women were significantly greater than those of the vegetarians, ($p < 0.01$ and $p < 0.05$, respectively). They described that the greater adiposity contributed the higher peripheral estrogen effect in non-vegetarians.

Racial differences in anthropometrical measurements, even among Asian women were found. The BMI of this study is lower than that of elderly Chinese women ($n = 106$) [2]. The 75th percentile of BMI for Myanmar 60-64 years and 65-69 years women (24.4 and 21.9) corresponded with the 50th percentile of BMI for respective age group of Chinese women (24.3 and 22.2). This might be partially

explained by the fact that the Chinese study was conducted in an institutionalized community and their activities were assumed to be lower than those who were actively involved in household activities in the present study.

4. Daily nutrient intakes vary in different regions of the world. Myanmar postmenopausal women consume more carbohydrate and fiber but less protein and fat than American post-menopausal women. Therefore, required energy is achieved mainly

Table 4. Daily nutrients intake of omnivorous and vegetarian postmenopausal Myanmar and American women

Dietary pattern	Myanmar (Present study)		American study (11)	
	Omnivores (n=15)	Vegetarians (n=15)	Omnivores (n=15)	Vegetarians (n=15)
Energy(kcal)	1718	1926	1334	1425
Protein(g)	44	45	54	47
% Energy	10	9	16	13
Carbohydrate (g)	290	386*	166	216
% Energy	69	80	50	61
Fat (g)	37	27	54	50
% Energy	19	16	36	32
Fiber (g)	30	39*	13	24*

* Significantly different from omnivorous women, $p < 0.05$

Energy requirement depends on age, body size, body composition, physical activity, and lean body mass. According to the FAO/WHO Expert Consultation, daily energy requirement for women over sixty years is 1400-2000 kcal and the safe intake of protein for women over the age of 60 years is 30-56 g/day [12]. Thus, the energy requirement and protein intake of the subjects was found to be sufficient. In the present study, insignificant different amount of protein consumption between the two groups might be due to high protein content of preserved bean paste and bean meat (6.8 g and 49.1 g in 100 g of food, respectively) [7]. In 1994, FAO/WHO Expert Committee recommended that the daily dietary fat should supply 15%-35% of the total energy [13]. In this study, only 19% and 16% of calories were contributed by fat in omnivorous and vegetarian postmenopausal women.

Nutrient intakes of Myanmar and American postmenopausal women are compared in Table

from carbohydrate in Myanmar women whereas dietary fat and protein mainly fulfill the energy requirement of American women.

The enzymatic, hydrolytic reaction to starch of fruits might be a cause of more carbohydrate consumption in both Myanmar and American vegetarians. Like the present study, American vegetarians consumed more fiber than non-vegetarians did. It was said that the coronary arterial diseases and large bowel cancers could be protected by the high fiber diet [14].

Therefore, a good habit of taking high fiber diet in Myanmar vegetarians should be encouraged. In conclusion, according to WHO/FAO recommendations on daily energy, protein and fat requirements of respective age groups, intakes of both omnivores and vegetarians were found to be adequate. Unlike the western postmenopausal women, the prolonged consumption of vegetarian diet in Myanmar

postmenopausal women has not decreased the adiposity.

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SHORT REPORT

Clinical features and response to antivenom of Russell's viper bite cases of Yegyi, Ayeyawady Division

*Tint Lwin, **Tun Pe, **Aye Aye Myint, **Nu Nu Aung & *Kyaw Kyaw

*Yegyi Civil Hospital

Department of Health

**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 reports on geographical variation of biological properties of RV venom [1-2] and clinical features of RV bite cases from Taungdwingyi and Tharrawaddy [3-4] prompted us to study more RV bite cases from different endemic localities. In this communication, study on clinical features of RV bite cases from Yegyi, Ayeyawady Division is reported.

All snakebite cases admitted to Yegyi hospital during 1995-1996 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 total of 40 ml of monospecific antivenom was given to patients at the discretion of the medical officer. The clotting test was carried out at 2 hourly intervals on the cases presenting with clottable blood and 6 hourly after giving antivenom on systemic cases until normal clot

Table 1. Clinical details of 34 Russell's viper bite cases

Total (34)	26 systemic + 5 local + 3 non envenoming	%
Sex: Male: female	32:2	94:6
Time bite: Day (6am-6pm):night (6pm-6am)	20:14	59:41
Age (yr) (median)	30 (14-48)	
Activity when bitten: working: walking	25:9	74:26
Site of bite: lower limb: upper limb	31:3	91:9
Number of dead snake brought	18	53
Length of the snake (cm) (median)	53 (20-66)	
Distance travelled (miles) (median)	5.5 (1-14)	
Bite and admission (h) (median)	4.55 (0.20-18.45)	
Antivenom pretreatment at village	10	29
Time of pretreatment (h) (median)	1 (0.5-7.00)	
First-aid: Tourniquet	31	91
Immobilisation	10	29
Suction	4	12
Incision	3	9
Herbal	2	6
Tattoo	5	15

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 [5].

Clinical details of 34 RV bites and 31 envenomed cases are shown in Table 1 and 2 respectively.

Table 2. Clinical details of 31 envenomed Russell's viper bite cases

Symptoms (31)	%	Signs:local+systemic (31)	%
Local pain	29 85	Local swelling	30 88
Epigastric pain	6 23	Bleeding at site of bite	5 19
Loin pain	4 15	Local bruising	6 23
Vomiting	11 42	Local necrosis	1 4
Drowsiness	5 19	Local blackening	1 4
Dyspnoea	1 4	Lymph node enlargement	4 15
		Systemic (26)	
		Spontaneous systemic bleeding	14 54
		Haematuria	9 35
		Haematemesis	6 23
		Malena	3 12
		Epistaxis	2 8
		Subconjunctival	1 4
		Haemoptysis	1 4
		Gingival	1 4
		Albuminuria	20 77
		Hypotension ($<80\text{mmHg}$)	5 19
		Oliguria ($<400\text{ml}/24\text{h}$)	9 35
		Conjunctival oedema	3 12
		Periorbital oedema	3 12
		Renal failure	4 15
		Fatality	3 9

Of 34 bites, 76% (26) presented with systemic, 15% (5) with local and 9% (3) with no features of envenoming.

Antivenom was given to all cases. Clot restoration time after antivenom therapy took more than 6 hours in 67% (16/24) of the cases. Twenty-nine percent of the cases received pre-hospital antivenom treatment within one hour (median) after the bite. Forty-two percent of the systemic envenoming cases were given 50-120 ml antivenom and in one systemic with spontaneous systemic bleeding, 300 ml was given.

Venom antigen was not detected in 9% (3/34) of the total bites. Antigen level of local envenomed cases ($n=5$) ranges from 10-30 ng/ml and that of systemic cases ($n=26$) 50-100 ng/ml.

Clinical features of Russell's viper bite cases of Yegyi differs from Danubyu which is 120 Km away from each other in Ayeyarwaddy Division. More cases from Yegyi are systemic envenomed (76%) following the bites but less number of them developed spontaneous systemic bleeding (54%) compared to 67% in Danubyu, probably early administration of high dose of antivenom (50-120ml) may account for it.

It also shows that the snakes (20-66 cm) responsible for the bites in Yegyi are quite effective in injecting venom into the victims since 76% of the bites resulted in systemic envenoming compared to 44% in Tharrawaddy [4], 34% in Taungdwingyi [3] and 58% in Danubyu (unpublished observations). Spontaneous systemic bleeding such as haemoptysis (44%), gingival bleeding (28%), epistaxis (17%), and hypotension (28%) are more common in Danuphyu (unpublished observations) and malena (12%) and albuminuria (77%) in Yegyi bites. Variation in composition and biological properties of venom and early entivenom therapy may have help in development of milder spontaneous systemic bleeding observed in Yegyi series. A similar variation in properties of RV venom from two

close localities, Danubyu and Nyaungdon [1] has been reported. The russell's viper venom from Yegyí probably possessed nephrotoxic properties since 77% of the bites developed albuminuria and 35% haematuria.

Since clot restoration time took more than 6 hours in 66% of the cases, the antivenom used was not effective in correcting clotting defect which was expected in antivenom raised with limited pooled antigen [6].

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SHORT REPORT

Haemoglobin electrophoresis by Iso-electric Focusing (IEF) in thalassaemia major children

*Ne Win, *Hauk Kying, **Than Swe, *Khin Saw Aye & *Soe Soe

*Pathology Research Division
 **Director-General
 Department of Medical Research

Haemoglobin may be separated by electrophoresis on paper, cellulose acetate (CA), starch gel, agar gel, and acrylamide gel or starch block. Each method has advantages and disadvantages. For routine work, electrophoresis on paper or CA is simple and is generally satisfactory for distinguishing the common types of haemoglobin [1].

The technique of IEF is an electrophoretic one but instead of the separation being carried out at a constant pH, the separation is carried out in a pH gradient, which is established between two electrodes and is stabilized by carrier ampholytes. In this technique, proteins migrate until they align themselves at their pI (Isoelectric point) at which point a protein possesses no net overall charge and will therefore concentrate at this point as migration ceases. IEF is therefore an equilibrium technique in which the effects of diffusion are overcome. It is the electrophoretic technique with the highest resolution, in which components that differ by 0.001 of a pH unit, or less, can be resolved. The pI point of a protein defines a physical parameter of the protein and has great significance [2].

Haemoglobins are probably the group of proteins that have been most studied by Isoelectric Focusing (IEF). Haemoglobin is a relatively small (65,000 M.Wt.) and highly charged molecule. Therefore it focuses easily

and quickly. It has been suggested that IEF is an ideal method for screening for Hb variants.

Diagram 1. Bands of *normal* haemoglobins by IEF [3]

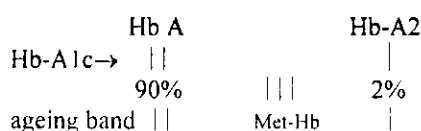


Diagram 2. Bands of Hb-F and some *abnormal* haemoglobins [3]

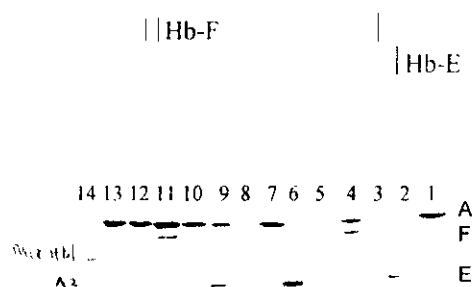


Fig. 1. Haemoglobin electrophoregram by IEF in 11 thalassaemia major (channel 1 to 11), 2 normal adults (channel 12 & 13) and cord blood (channel 14)
 Channel 1, 7, 8. = Hb-A, Hb-E
 Channel 2, 4, 5, 9. = Hb-A, Hb-F, Hb-E
 Channel 6. = Hb-F, Hb-E
 Channel 3, 12, 13. = Hb-A
 Channel 14. = Hb-A, Hb-F, Met-Hb, Hb-A2

We have done haemoglobin electrophoresis by IEF on 67 cases of known thalassaemia major who are taking regular blood transfusion. From these cases we expect thick Hb F bands. Cord blood samples and normal Hb are also included as controls so as to compare with thalassaemia major cases. Finally the films of H bands of different types are obtained. The bands obtained are shown in Fig. 1.

It reveals different Hb bands in 11 thalassaemia major cases (channel 1 to 11), 2 normal adults (channel 12 & 13) and one sample of cord blood (in channel 14). The bands encountered are normal Hb-A, Hb-F, Hb-A2 and abnormal Hb-E. Hb-A2 and Hb-E bands appear much close to each other, even difficult to differentiate. The bands of channel 1, 3, 7, 8 and 10 are A, A2 and very faint F and therefore consistent with Hb-A thalassaemia major. Channel 2, 4, 5, 9 and 11 are cases of Hb-AE heterozygous beta-thalassaemia since there are A, E and F bands. Bands in channel 6 is consistent with Hb-E homozygous beta-thalassaemia because there are prominent E and F bands only.

Out of 67 cases under study 20 cases are Hb-A beta-thalassaemia major, 39 cases are Hb-AE heterozygous and 8 cases are Hb-E thalassaemia major.

IEF have some advantages over CAE such as the band separation between A and F is more marked, less time consumed, can run 14 samples at the same time, can see the bands clearly throughout the whole procedure and does not require any staining solutions. We conducted this study using IEF electrophoresis kits and its instruction manual enclosed [3], supplied by Japanese counterpart of collaborative project, "Hepatocarcinogenic studies in Myanmar Thalassaemics".

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