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Relationship between waist-hip ratio and cardiovascular risk factors among people in South Dagon Township

**Zaw Myint, *Ye Tint Lwin, *Aye Aye Win, *Hay Mar Hpoo, *Mg Mg Myint, *Mya Mya Win, *Lwin Lwin Maw, **Myint Kyi Aye & *Soe Min Thein*

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The objective of the study was to determine the frequency of cardiovascular risk factors (high total cholesterol (≥ 250 mg/dl); low HDL-cholesterol (< 35 mg/dl); and high ratio of TC and HDL-C concentrations (≥ 4.5) in people categorized by two waist-hip ratio (WHR) levels. Total of 370 stored sera (185 each for men and women) from 294 men and 329 women of the previous study were randomly selected. In men, the mean and SD of TC and HDL-C concentrations were 206 ± 46 mg/dl and 28 ± 9 mg/dl, respectively, whereas in women, these values were 222 ± 58 mg/dl and 25 ± 9 mg/dl, respectively. The ratios of TC and HDL-C concentrations in men and women were 8.2 ± 3.2 and 10.2 ± 4.5 , respectively. In men, compared to those with $\text{WHR} < 0.95$ (92 samples), age and life style (physical activity, cigarette smoking) adjusted odds ratios for having high TC, low HDL-C concentrations and high ratio of these two parameters were 3.5 (95% CI=1.8 to 7.1), 1.40 (0.7 to 3.0) and 3.6 (0.7 to 18.2), respectively in those with $\text{WHR} \geq 0.95$ (93 samples). In women, compared to those with $\text{WHR} < 0.92$ (99 samples), these odds ratios were 1.00 (0.6 to 1.7), 2.90 (1.0 to 8.1) and 0.50 (0.1 to 3.1), respectively in those with $\text{WHR} \geq 0.92$ (86 samples). In conclusion, $\text{WHR} \geq 0.95$ could significantly identify men aged 40-60 years in South Dagon Township at increased risk of high total cholesterol concentration whereas $\text{WHR} \geq 0.92$ could significantly identify women at increased risk of low HDL-cholesterol concentration.

INTRODUCTION

In Myanmar, ischaemic heart disease (IHD) is an important priority health problem identified in National Health Plan (1996-2001). Among the modifiable risk factors, obesity and elevated blood lipids are the important risk factors in association with IHD. And the patterning of the body's adipose tissue distribution, independent of total body fat, alters the health risks of obesity. More specifically, ratios of waist-to-hip girth that exceed 0.80 for women and 0.95 for men are associated with an increased risk of death from coronary artery disease and other illnesses [1]. In the previous study, we determined some cardiovascular

risk factors in people in South Dagon Township categorized by various levels of waist-hip ratio. Compared with those with lower waist-hip ratio levels, age and life style (physical activity, cigarette smoking) adjusted odds ratios for having hypercholesterolaemia (≥ 250 mg/dl) and hypertension (systolic pressure ≥ 160 mmHg or diastolic pressure ≥ 95 mmHg) were 2.85 and 2.16, respectively in men with a waist-hip ratio of 0.95-1.00 and 1.16 and 1.54, respectively, in women with a waist-hip ratio of 0.92-0.97. These age and life style adjusted odds ratios were 5.77 and 2.43, respectively, in men with a waist-hip ratio of > 1.00 and 1.49 and 3.02, respectively, in women with a waist-hip ratio of > 0.97 [2].

The distribution of cholesterol among the various lipoproteins is a more powerful predictor of heart disease than the total blood cholesterol (TC). Specifically, elevated levels of high density lipoproteins (HDL) (>35mg/dl is desirable) are associated with a lower heart disease risk. An effective way to evaluate lipoprotein status is to divide TC by HDL cholesterol (HDL-C). This ratio is a superior measure of heart disease risk than either TC or low-density lipoprotein (LDL) levels. A ratio greater than 4.5 indicates a high heart disease risk, whereas a ratio of 3.5 or lower is more optimal [3].

General objective

To verify the relationship between waist-hip ratio and cardiovascular risk factors among people in the South Dagon Township

Specific objectives

1. To measure the fasting serum total cholesterol and HDL-cholesterol concentrations and calculate the ratio of these two parameters among people in the South Dagon Township
2. To determine the relationship between waist-hip ratio and these cardiovascular risk factors among these people

MATERIALS AND METHODS

Study design

A cross-sectional descriptive study

Study population

Apparently healthy, aged 40 to 60 years old people living in South Dagon Township

Study samples

Total of 370 stored sera (185 each for men and women) from 294 men and 329 women of the previous study [2] were randomly selected.

Data collection techniques

Waist and hip circumferences were measured by a steel measuring tape. Waist circumference was measured around the waist, over the abdomen where the girth

is the largest while standing relaxed, not pulling in the stomach. Hip circumference was measured around the hip, over the buttocks where the girth is largest [2]. Fasting serum total cholesterol level and serum HDL-cholesterol level were measured by using Cholesterol RTU test kit [2] and HDL-Cholesterol test kit [4], respectively, based on the enzymatic methods. The ratio of TC and HDL-C was calculated by dividing the concentration of TC by HDL-cholesterol concentration.

RESULTS

Physical and metabolic characteristics of 185 men and 185 women are shown in Table 1. In men, the mean and SD of TC and HDL-C concentrations were 206±46 mg/dl and 28±9 mg/dl, respectively, whereas in women, these values were 222±58 mg/dl and 25±9 mg/dl, respectively. The ratios of TC and HDL-C concentrations in men and women were 8.2±3.2 and 10.2± 4.5, respectively.

Table 1. Physical and metabolic characteristics of study participants

Characteristics	Men (n=185)		Women (n=185)	
	Mean	SD	Mean	SD
Age (years)	49.9	6	49.2	5.8
Weight (kg)	58.5	12.7	51.9	12.9
Height (cm)	162.2	5.9	151.5	5.9
Body mass index (kg/m ²)	22.2	4.4	22.5	4.9
Waist circumference (cm)	83.7	12.9	84.4	11.1
Hip circumference (cm)	89.2	8.2	92.6	11.1
Waist-hip ratio	0.9	0.07	0.91	0.05
Total cholesterol (mg/dl)	206.4	45.5	221.8	57.8
HDL-cholesterol (mg/dl)	27.6	8.6	24.5	8.9
TC/HDL-C ratio	8.2	3.2	10.2	4.5

Table 2 shows the mean serum TC concentration, HDL-C concentration and their ratio of men and women in two different waist-hip ratio levels. The mean values of TC concentration were increased significantly with increased WHR in both men and women.

Table 2. Mean serum TC concentration, HDL-C concentration and their ratio in men and women in different categories of waist-hip ratio

Waist-hip ratio	Numbers	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	TC/HDL-C ratio
		Mean (SE)	Mean (SE)	Mean (SE)
<i>Men</i>		185		
<0.95	92	193.5 (4.1)	27.4 (0.9)	8.0 (0.4)
>/=0.95	93	219.2 (4.9)	27.8 (0.9)	8.4 (0.3)
		(p=0.0001)	(p=0.75)	(p=0.39)
<i>Women</i>		185		
<0.92	99	216.3 (5.4)	25.3 (0.8)	9.5 (0.4)
>/=0.92	86	228.1 (6.6)	23.7 (1.0)	11.0 (0.5)
		(p=0.17)	(p=0.22)	(p=0.02)

Table 3. Prevalence, positive and negative predictions, and sensitivity and specificity of high TC concentration (≥ 250 mg/dl), low HDL-C concentration (< 35 mg/dl) and their high ratio (≥ 4.5) in men by WHR cut-off values

Risk factor	Pre- valence*	Percentage (95% confidence interval)			
		Positive prediction	Negative prediction	Sens- itivity	Speci- ficity
<i>Waist-hip ratio (>/=0.95) (n=93)</i>					
High total cholesterol	15.14	23.7 (15.7- 33.8)	93.5 (85.8- 97.3)	78.6 (58.5- 91.0)	54.8 (46.7- 62.7)
Low HDL-C	81.62	83.9 (74.5- 90.4)	20.7 (13.2- 30.6)	51.7 (43.4- 59.8)	55.9 (38.1- 72.4)
High TC/HDL- C ratio	95.14	97.8 (91.7- 99.6)	7.6 (3.4- 15.6)	51.7 (44.1- 59.2)	77.8 (40.2- 96.1)
One or more risk factors	95.68	97.8 (91.7- 99.6)	6.5 (2.7- 14.2)	51.4 (43.8- 58.9)	75.0 (35.6- 95.5)

*Prevalence of risk factors in total population

Table 3 and Table 4 show prevalence, positive and negative predictions, and sensitivity and specificity of high TC concentration (≥ 250 mg/dl), low HDL-C concentration (< 35 mg/dl) and their high ratio (≥ 4.5) in men and in women, respectively by their WHR cut-off values. All the results for high TC concentration in men and women were comparable to that of previous study [2]. For the other two risk factors, (low HDL-C concentration and ratio of TC and HDL-C concentrations) their prevalence

rates were very high in both men and women. Sensitivity and specificity for identifying risk factors from WHR (≥ 0.95 in men and ≥ 0.92 in women) were between 51% and 75% in men and 47% and 67% in women, respectively with positive prediction varying between 24% and 98% in men and 33% and 97%, respectively in women for individual risk factors. Positive prediction was 98% in men and 99% in women who had one or more risk factors. Negative prediction varied between 8% and 94% in men and 2% and 75% in women for individual risk factors. It was 7% in men and 2% in women who had one or more risk factors.

Table 4. Prevalence, positive and negative predictions, and sensitivity and specificity of high TC concentration (≥ 250 mg/dl), low HDL-C concentration (< 35 mg/dl) and their high ratio (≥ 4.5) in women by WHR cut-off values

Risk factor	Pre- valence*	Percentage (95% confidence interval)			
		Positive prediction	Negative prediction	Sen- sitivity	Speci- ficity
<i>Waist -hip ratio (>I=0.92) (n=86)</i>					
High total cholesterol	28.65	32.6 (23.1- 43.6)	74.7 (64.8- 82.7)	52.8 (38.8- 66.5)	56.1 (47.2- 64.6)
Low HDL-C	87.57	93.0 (84.9- 97.1)	17.2 (10.6- 26.4)	49.4 (41.5- 57.3)	73.9 (51.3- 88.9)
High TC/HDL- C ratio	97.30	96.5 (89.4- 99.1)	2.0 (0.4- 7.8)	46.1 (38.7- 53.7)	40.0 (7.3- 83.0)
One or more risk factors	98.38	98.8 (92.8- 99.9)	2.0 (0.4- 7.8)	46.7 (39.3- 54.2)	66.7 (12.5- 98.2)

*Prevalence of risk factors in total population

In Table 5, odds ratio of men and women having high TC concentration, low HDL-C concentration and their ratios in different categories of WHR adjusted for age, cigarette smoking and physical activity by logistic regression are shown. In men, compared to those with WHR <0.95 , age and life style adjusted odds ratios for having high TC, low HDL-C concentrations and high ratio of these two parameters were 3.5 (95% CI=1.8 to 7.1), 1.40 (0.7 to 3.0) and 3.6 (0.7 to 18.2), respectively in those with WHR ≥ 0.95 .

In women, compared to those with WHR<0.92, the odds ratios were 1.00 (0.6 to 1.7), 2.90 (1.0 to 8.1) and 0.50 (0.1 to 3.1), respectively in those with WHR≥0.92.

Table 5. Odds ratio of high TC concentration (≥250 mg/dl), low HDL-C concentration (<35 mg/dl) and their high ratio (≥4.5) in different categories of waist-hip ratio adjusted for age, cigarette smoking and physical activity in men and women

Waist-hip ratio	Odds ratio (95% confidence interval)		
	High TC	Low HDL-C	HighTC/HDL-C ratio
<i>Men (n=185)</i>			
<0.95	1	1	1
≥0.95	3.50 (1.8-7.1) P=0.0001	1.40 (0.7-3.0) P=0.39	3.60 (0.7-18.2) P=0.12
<i>Women(n=185)</i>			
<0.92	1	1	1
≥0.92	1.00 (0.6-1.7) P=0.91	2.90 (1.0-8.1) P=0.04	0.50 (0.1-3.1) P=0.43

DISCUSSION

The mean values of total cholesterol concentration (206 mg/dl in men and 222 mg/dl in women) in the present study were much higher than those of the studies carried out during 1990s [5, 6, 7], the values of which varied from 170 to 180 mg/dl. The present values were also higher than the result taken at Hlegu Township [8] in 2001 which was 183 mg/dl. Although it can be assumed that serum total cholesterol level may have the upward trend in recent years, it may be due to variations in the studies such as differences in age, sample size, method of measurements etc. Even so, the present data were lower than the defined unit of hypercholesterolaemia which was 250 mg/dl [9]. According to this definition, the prevalence rates of hypercholesterolaemia were 15% in men and 29% in women in South Dagon Township.

For the determination of serum HDL-Cholesterol concentration, there were more variations among various studies. They showed its range between 18 mg/dl and

64 mg/dl [7, 8, 10]. The mean HDL-C concentrations of the present study were 28 mg/dl in men and 25 mg/dl in women and were lower than the cut-off value (<35 mg/dl) for having the higher heart disease risk. Therefore, the prevalence rates were 82% in men and 88% in women and were very much higher than those for hypercholesterolaemia.

Because of this high prevalence of low HDL-C concentration, the prevalence of TC/HDL-C ratio became high among people in South Dagon Township. Because the cut-off values for high TC concentration and low HDL-C concentration are 250 mg/dl and 35 mg/dl, the cut-off value for their ratio should be 7.2. However, the actual cut-off value was defined as 4.5 so that the mean ratio of present study (8.2 in men and 10.2 in women) were very high and their prevalence rates also became 95% and 97%, respectively. Interestingly, according to the Hlegu study [8], that ratio of the all participants was 2.9 (TC=183 mg/dl and HDL-C=64 mg/dl) and was lower than that of the present study. However, another study done in 1994 [11] in which 20 female students of age about 20 years from Institute of Nursing were included showed that the TC/HDL-C ratio was 4.7. Therefore, further study may be needed to find out the actual values of HDL-C and TC/HDL-C ratio for high heart disease risk in Myanmar population.

In South Dagon Township, WHR≥0.95 in men and ≥0.92 in women could identify the cardiovascular risk factors with sensitivity and specificity varying between 47% and 75%. These results were comparable to that of previous study [2] although the confidence interval ranges for specificity was much higher in the present study. Positive predictive values were different for prediction of high TC concentration and other two risk factors. Because of low prevalence rate of hypercholesterolaemia, only one out of four (24%) in men and one out of three (33%) in women could be predicted whereas for low HDL-C concen-

tration and high TC/HDL-C ratio, 8.5 to 9.5 out of ten (84% and 98%) in men and 9 to 9.5 out of ten (93% and 97%) in women could be predicted because of their high prevalence rates. Therefore, the reverse is true for negative prediction of these risk factors i.e. negative prediction for hypercholesterolaemia was very high (94% in men and 75% in women) and it was very low (21% and 8% in men and 17% and 2% in women) for other two risk factors. In the present study, only eight men (4.3%) and three women (1.6%) did not have any risk factors (data not shown).

The present study also showed the closed relationship between WHR and cardiovascular risk factors. In men, hypercholesterolaemia could be significantly identified at 3.5 times the prevalence in the whole population at WHR 0.95. This result further confirmed the previous study [2]. In men having low HDL-C concentration or high TC/HDL-C ratio, these odds ratio were 1.4 and 3.6 times, respectively, although these results were not statistically significant. Moreover, there was no correlation between the two risk factors and WHR in general (data not shown), and no significant difference between two different WHR groups.

Therefore, it can be concluded that relationship between these two risk factors and WHR were not as firm as that for hypercholesterolaemia. In women, low HDL-C concentration (<35mg/dl) could be significantly identified at nearly three (2.9) times the prevalence in the whole population at WHR 0.92. The result for hypercholesterolaemia was comparable to that of previous study [2]. In women having high TC/HDL-C ratio, there was significant difference between two different WHR groups. However, the odds ratio was 0.50 instead of higher than 1.0 (95% CI=0.1-3.1). Therefore, it can be concluded that the relationship between the two risk factors, namely high total cholesterol and high ratio of TC and HDL-C concentrations, and

WHR was not as strong as that for women having low HDL-C concentration.

In conclusion, WHR $\geq$ 0.95 could significantly identify men aged 40-60 years in South Dagon Township at increased risk of high total cholesterol concentration whereas WHR $\geq$ 0.92 could significantly identify women at increased risk of low HDL-cholesterol concentration.

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Serological response of chemoprophylaxis on high risk contacts of new leprosy cases in Nyaungdon Township

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This study was carried out in one of the leprosy endemic townships, Nyaungdon Township, Ayeyawady Division. The aim was to study the serological response of chemoprophylaxis by using a single dose of ROM (rifampicin, ofloxacin and minocycline), which is WHO recommended regime for single lesion of leprosy, on high risk extended contacts of new leprosy cases. The blood samples were collected from extended contacts two times before and six months after the chemoprophylaxis. Indirect NTP-BSA ELISA test was carried out on samples. Determination of the baseline seropositivity rate of extended contacts of new leprosy case was also carried out. Seropositive contacts were assumed as high risk group. In the baseline study, the seropositivity rate is significantly higher in household contacts of multibacillary (MB) leprosy cases. The seropositivity rate of children contacts under 15 years of age is higher than that of adult contacts, $\chi^2 = 31.58$, $p < 0.001$. The difference of mean OD titers in treated group before and after chemoprophylaxis is significantly reduced compared to non-treated group in adult, $p = 0.004$. However, it is not significant in children. The difference of seropositivity rate before and six months after chemoprophylaxis is not significantly reduced in treated group compared to non-treated group.

INTRODUCTION

Leprosy elimination was achieved at the national level at the end of January 2003 in Myanmar (prevalence rate $< 1/10,000$) [1]. In Myanmar, WHO Multi Drug Therapy had been introduced since 1986. At that time the prevalence rate was 59.3/10,000 population. In 1990, the prevalence came down to 27.6/10,000. In 1995, Multi Drug Therapy service reached every village making 100% geographical coverage by integrating into Basic Health Services in Myanmar. From 1997 to now, National Health Plan has been carried out by integrated effort by all departments in Ministry of Health and collaboration with health related departments [2]. Up to June 2003, the new case detection rate (NCDR) was more than 4/10,000 population in 70

townships mainly from Ayeyawady, Bago, Magwe, Mandalay, Sagaing Divisions and Southern Shan State [3]. Although effective elimination program using MDT was carried out, the incidence is still high possibly because of the long incubation period of the disease in the role of healthy carriers in transmission of *Mycobacterium leprae*. ROM (rifampicin, ofloxacin and minocycline) was given as a single dose for adults with single skin lesion in WHO recommended MDT regimen for paucibacillary (PB) leprosy [4].

Household contacts and social contacts of leprosy cases are at high risk to develop the disease within a few years in endemic area. It was found that the risk of developing leprosy increased progressively with increasing phenolic glycolipid-1 antibody (antiPGL-1) levels indicating the risk of

developing leprosy in the next few years [5]. To control leprosy and to accelerate achievement of the goal of leprosy elimination and sustaining of this elimination as a public health problem, chemoprophylaxis would be needed to carry out on high risk group in high prevalence area [6]. Thus, it would be needed to screen the high risk group of healthy carriers for chemoprophylaxis for decreasing the incidence and preventing in transmission of *M. leprae* [7]. Therefore, this study was carried out to assess the efficacy of chemoprophylaxis after six months on high risk extended contacts of new leprosy cases using a single dose of ROM in endemic area of Myanmar.

Objectives

- To determine the baseline seropositivity rate of extended contacts of new leprosy cases in Nyaungdon Township
- To determine the antibody response and seropositivity rate among treated and non-treated groups

MATERIALS & METHODS

This study was a community-based prospective study. Nyaungdon Township, Ayeyawady Division (prevalence rate - 1.52/10,000) was selected for study area. Extended contacts of all new leprosy cases in Nyaungdon Township (n=490) were assumed as study population. Two milliliter of blood was taken out after obtaining informed consent. The samples were transported under cold storage to Immunology Research Division, Department of Medical Research (Lower Myanmar). NTP-BSA ELISA was used to determine antiPGL-1 antibody levels in the subjects' sera. The high risk group of leprosy was identified from cut-off point of controls (apparently healthy blood donors of National Blood Bank, Yangon). After baseline study, all seropositive extended contacts (n=156) were determined as high risk contacts and were enrolled in the chemoprophylaxis study. Subjects with possibility of pregnancy, liver disease, renal disease and subjects who are

taking antiTB treatment were excluded from the chemoprophylaxis study.

Therefore, 152 contacts were enrolled in the study of chemoprophylaxis. These subjects were randomly allocated into treatment and non-treatment of 76 subjects. Each subject in treatment group was given ROM (rifampicin, ofloxacin and minocycline) for >15 years of age (adult) group and RMP (rifampicin alone) with the dose of 25 mg/kg for <15 years of age (children) group. Subjects in non-treatment group were given vitamins as placebo. The forms (capsules, caplet or tablet) and colors of drugs containing vitamins were similar to the drugs containing ROM.

Among 76 subjects of each group, there were 42 of each in adult group and 34 in children group, respectively. To study the serological response of chemoprophylaxis, blood samples were collected again after six months. Among them, 31 subjects dropped out from the study. Sixteen dropouts of the remaining subjects were from the treatment group and 15 subjects were from non-treatment group. Among 121 subjects, totally 9 subjects, 3 subjects from treatment group and 6 subjects from non-treatment group, were still seropositive.

Statistical analysis

Frequency distribution and cross tabulation of variables were constructed by using SPSS (version 11.5). The high risk individuals (seropositive) were compared in study Rural Health Centers. Seropositivity rate and mean antibody titer among exposed (treatment) and non-exposed (non-treatment) groups were analyzed using Wilcoxon Signed Rank Test and Chi Square Test. A comparison was made on all these groups for the study period.

RESULTS

Twenty-one new leprosy cases including 13 multibacillary (MB) and 8 paucibacillary (PB) were detected in Nyaungdon Township. Among 490 extended contacts (65 households and 425 neighbours), 306 were

contacts of MB cases and 184 were that of PB cases. Baseline seropositivity of these overall contacts was 31.84% (Fig.1). The seropositivity rate in children contacts was higher than that of adult contacts (50.72% vs. 24.43%, $\chi^2=31.58$, $P<0.001$). The seropositivity rate was not different by gender (29.41% vs. 34.13%).

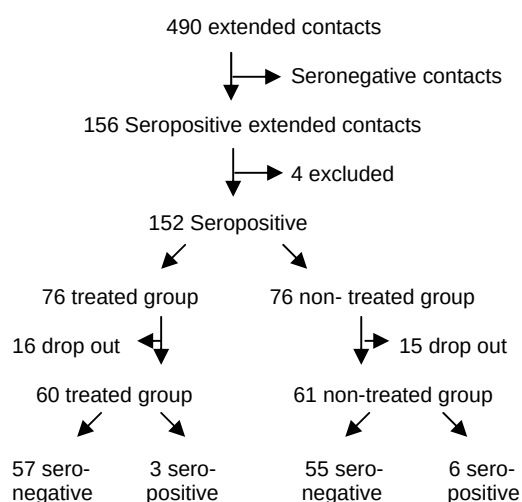


Fig.1. Serological response of extended contacts to chemoprophylaxis

The seropositivity rates of overall contacts of MB and PB cases were 36.27% and 24.46%, respectively. The seropositivity rates of household contacts of MB and PB cases were 42.50% and 40%, respectively, and that of neighbour contacts of MB and PB cases were 35.34% and 22.01%, respectively. The seropositivity rate of household contacts of MB cases was the highest in all different types of contacts and followed by the household contacts of PB cases, the neighbour contacts of MB cases and the last of neighbour contacts of PB cases. Number of seropositive extended contacts distributed by age group was the highest in the age group of 11 to 20 years (Fig. 2).

The mean OD titers were significantly reduced after the chemoprophylaxis in both treatment and non-treatment groups for both adults and children (Table 1). The difference of mean OD titers before and after chemoprophylaxis in treatment group was significantly reduced compared to non-treatment

group adults, but was not significant in children (Table 2).

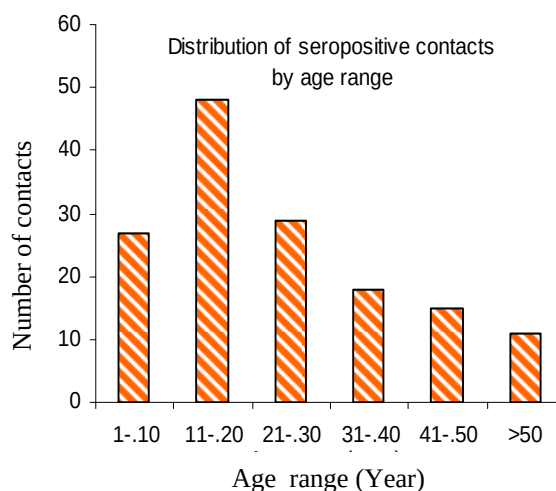


Fig. 2. Number of seropositives among extended contacts of new cases of leprosy by age range in Nyaungdon Township

Table 1. Mean OD titers before and after chemoprophylaxis of treatment and non-treatment groups

Type of contacts	Optical density titer (Mean±SD)					
	Treatment group			Non-treatment group		
	Before	After	p value	Before	After	p value
Adult (n=35)	0.28	0.13	0.005*	0.27	0.17	0.007*
	±	±		±	±	
	0.08	0.06		0.06	0.06	
Child (n=25)	0.29	0.15	0.005*	0.27	0.16	0.006*
	±	±		±	±	
	0.08	0.06		0.08	0.06	

*Wilcoxon Signed Rank Test, *P<0.05 = significant

Table 2. The difference of mean OD titers before and after chemoprophylaxis in treatment and non-treatment groups

Type of contacts	Mean optical density titer difference between before and after			
	Treatment group		Non-treatment group	p value
Adult (n=35)	Mean OD	0.15	0.1	0.004
	SD	0.09	0.08	
Child (n=25)	Mean OD	0.14	0.11	0.18 (NS)
	SD	0.09	0.08	

*Wilcoxon Signed Rank Test, *P<0.05 = significant, NS = not significant

DISCUSSION

The epidemiological significance of presumably many people who are infected with

leprosy but without clinical symptoms is still to be investigated. Improving their understanding of the natural history of leprosy, and ability to recognize infection might ultimately have important implications for leprosy control, particularly if it would permit the definition of targets in the population for immunoprophylactic and therapeutic strategies [8].

Therefore, in this study, to reduce the subclinical infection leading to decrease new case detection rate of leprosy, the high risk group of extended contacts of new cases in Nyaungdon Township, Ayeyawady Division, was determined using NTP-BSA ELISA test and chemoprophylaxis was carried out using ROM on these high risk group of contacts.

It has been suggested that the screening of the sera of apparently healthy individuals may permit the identification of subclinical disease. There have been few prospective studies in which antibody levels to PGL-1 have been related to the subsequent risk of leprosy. If such cases could be detected and treated, it is theoretically possible that their infectivity for others would be curtailed, with a significant impact on the overall incidence of leprosy [5]. From July 1997 to October 1998, two rounds of screening and chemoprophylaxis using ROM were carried out in Kiribati, Pacific Ocean. About 92% of the population has been screened and there is 85% reduction in number of new cases [9].

In Federated States of Micronesia, new case detection rate was significantly reduced after chemoprophylaxis consisted of ROM for adults and rifampicin alone for children <15 years [10]. These observations suggested that subclinical infection with *M. leprae* is common in endemic communities and that PGL-1 seropositivity is a marker of subclinical infection. Detection of subclinical infection may confound control strategies that use screening tests to identify asymptomatic highly infectious cases for earlier therapy [8].

In this study, household contacts of MB cases are the highest risk. Age is found to be a potential risk factor for contacts to develop leprosy. Among the household contacts of MB cases, the risk for children under 14 years of age was substantially higher than that for adults [11]. The seropositivity rate was higher in children than adult contacts in the present study. When considering gender, there have been conflicting findings. Vijayakumaran *et al.*, found no gender differences which is consistent with the study of Rao *et al.* [12]. In Malawi, it was found that the risk was significantly greater for males than for females [11]. The sero-positivity rate had no difference by gender in this study. The difference of seropositivity rate before and after six months of chemoprophylaxis was not significantly reduced in treatment group compared to non-treatment group. The mean OD values were significantly reduced after six month of chemoprophylaxis. The difference of mean OD values (antibody titer) in treatment group was significantly reduced compared to non-treatment group in adults after six months.

This study revealed that ROM treated adult contacts have significant reduction of mean OD value of antiPGL-1 level. However, a significant reduction of seropositive rate in treatment group was not clearly observed. Evidence-based clinical trial could be recommended for the importance of chemoprophylaxis in interrupting the transmission of subclinical leprosy.

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**The phytochemical constituents and the antioxidant effects
of different extracts of *Thea sinensis* Linn. (Tea) leaves**

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Antioxidants may play a major role in the prevention of diseases, including cardiovascular and cerebrovascular diseases, some forms of cancer and effective to be long life and anti-aging. Thus, the aim of this study was to evaluate phytochemical constituents and the antioxidant activity of different extracts of *Thea sinensis* Linn. (လတ်ဖတ်) leaves. It was found that leaves of *Thea sinensis* Linn. contained alkaloids, α -amino acids, basic compounds, flavonoids, phenolic compounds, reducing sugars, saponins, steroids and terpenoids. The chloroform, ethanol, petroleum ether extracts of tea leaves were tested for their antioxidant activity by using thiocyanate method (the inhibition of linoleic acid autoxidation to detect lipid oxidation) in comparison with the synthetic antioxidant butylated hydroxy anisole (BHA). The chloroform, ethanol, petroleum ether extracts and BHA significantly lowered the autoxidation of linoleic acid when compared with that of control ($p < 0.01$ - $p < 0.0005$). The % inhibition of autoxidative activity of the chloroform, ethanol, petroleum ether extracts and BHA were 75.97%, 87.06%, 59.10% and 85.34%, respectively, after 14th day incubation.

INTRODUCTION

Antioxidants are substances that retard oxidation by atmospheric oxygen at moderate temperatures (autoxidation). An important characteristic of antioxidants is that they significantly inhibit or delay oxidative processes at very low concentrations [1]. Antioxidants may reduce the energy of the free radicals, stop the free radicals from forming in the first place or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals [2].

There are two types of antioxidants namely synthetic and natural. Dietary antioxidants (natural) such as α -tocopherol, ascorbic acid, carotenoids, flavonoids, and other phenolics may be effective in protection from oxidative damage. The synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), tertabutyl hydroquinone (TBHQ) have been

developed but their use has begun to be restricted due to their carcinogenicity [3]. Vitamin E (α -tocopherol) is an effective natural antioxidant but has limited usage. As a result, there is considerable interest in preventive medicine in the development of natural antioxidants from plants [4].

Recent developments in biomedical point to the involvement of free radicals in many diseases [5]. Free radicals attack the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation [6]. Free radicals also attack DNA and cause mutation leading to cancer [7]. For these reasons, antioxidants are of interest for treatment of any kind of cellular degeneration [8]. Recently, antioxidants have been found to play an important role in prevention of oxidation-related diseases such

as cardiovascular and cerebrovascular diseases, and some forms of cancer. Flavonoids are antioxidant molecules found in plant sources such as fruits, flowers, roots, stems, tea, wine, grains and vegetables. They may be regarded as a semi-essential food related to human health. They act as antioxidants by directly scavenging free radicals or by inhibiting the lipid peroxidation cascade [9].

Biological organs contain many polyunsaturated fatty acids (PUFA), such as linoleic, linolenic and arachidonic acids, mainly in the form of esters with phospholipids, triglycerides, or with cholesterol. These PUFA can undergo lipid peroxidation which can be interrupted by antioxidants by the donation of electrons. Lipid peroxidation is initiated by active oxygen species attacking unsaturated fatty acids, and is propagated by a chain reaction cycle involving lipids, peroxy radicals and lipid hydroperoxides. Superoxide anion, hydrogen peroxide and hydroxyl radical actively participate in the initiation of lipid peroxidation. These peroxides cause damage and dysfunction in cell and organelle membranes [10].

Phenolic compounds including flavonoids are known to be major antioxidative compounds in various herbs and spices [11]. Among them, tea leaves (*Thea sinensis*, *Camellia sinensis* (L.) O. Kuntze) are rich in phenolic compounds. In spite of the uses of plants as antioxidants, no study on antioxidant activity of tea leaves has so far been reported in Myanmar. Therefore, the aim of this study was to evaluate the phytoconstituents and antioxidative activities of its extracts.

MATERIALS AND METHODS

Plant material

The tea leaves were purchased from Pin Pyo Ywet Nu warehouse, Yangon and botanically identified and authenticated by a taxonomist from Botany Department, Yangon University. It was confirmed as *Thea*

sinensis Linn. The leaves were made to fine powder and stored in air-tight glass bottle.

Determination of phytochemical constituents of Thea sinensis Linn. (tea) leaves

Both ethanolic extracts of *Thea sinensis* were tested qualitatively for the presence of alkaloid, flavonoid, glycoside, tannin, steroid, phenol, saponin, resin and amino acid by using the method of Physicochemical standards of Unani formulations [12].

Successive extraction of plant material by various solvents

The powder of tea leaves (100g) was percolated with 1 liter of petroleum ether (60-80°) for 3 days at room temperature. Petroleum ether soluble portion was filtered and then evaporated to dryness by rotary evaporator to get petroleum ether (PE) extract (1.0 g, 1.0%). The residue was further extracted with chloroform for 3 days at room temperature. Filtration and then evaporation of chloroform soluble portion were performed to obtain chloroform extract (1.5 g, 1.5%). Finally, the residue was further extracted with 95% ethanol for 3 days at room temperature. Ethanol soluble portion was filtered and evaporated to obtain ethanol extract (10.0 g, 10.0%).

Screening of anti-oxidative activities of various crude extracts

The antioxidative activities of petroleum ether, chloroform and ethanol extracts from *T. sinensis* were determined by thiocyanate method [13, 15]. Two milligrams of each extract sample were added to a 2.53% linoleic acid solution consisting of 99.5% ethanol (8.1 ml), 0.05 M phosphate buffer (pH 7.0, 8.0 ml), and distilled water (3.9 ml) in a screw-top vial (36 mm i.d.×75 mm). A solution without the sample was used as a negative control and synthetic antioxidant BHA was used as positive control. Duplicate vials were prepared for each sample. Each vial was incubated at 40°C for 14 days in the dark. During the 0, 3, 5, 7, 10, 14th day incubation, 100 µl of each vial was mixed with 75% ethanol (9.7 ml) and 30%

ammonium thiocyanate (100 µl), 0.02 M ferrous chloride (100 µl) was added and the mixture was vigorously shaken. Absorbance of the generated red color was measured at 500 nm after 3 minutes. Absorbance measurements were done in triplicate and average values were taken to calculate % inhibition by following equation:

$$\% \text{ Inhibition} = [(A_c - A_b)/A_c] \times 100$$

A_c and A_b are absorbance of control at 500 nm and absorbance of sample at 500 nm, respectively.

The data were expressed as mean $\pm$ SE of six determinations and statistical analysis was performed according to the unpaired student 't' test.

RESULTS AND DISCUSSION

Extraction from the tea leaves

Petroleum ether extract (1 gm, 1.0%), chloroform extract (1.5 gm, 1.5%) and ethanol extract (10 gm, 10.0%) from the leaves of *Thea sinensis* Linn. were obtained by successive extraction method, respectively.

Phytochemical constituents of *Thea sinensis* Linn. (tea) leaves

The results of preliminary phytochemical examination are shown in Table 1. It was found that leaves of *Thea sinensis* Linn. contained alkaloids, α -amino acids, basic compounds, flavonoids, phenolic compounds, reducing sugars, saponins, steroids and terpenoids.

Antioxidative activities of crude extracts by thiocyanate method

This method bases on the inhibition of autoxidation of linoleic acid and measures the absorbance at 500 nm after a colouring reaction with ferrous chloride and thiocyanate at intervals during incubation. According to this method, low absorbance that results in high % inhibition indicates high antioxidative activity at constant concentration. The results of antioxidative activity in absorbance and % inhibition (mean $\pm$ SE) of

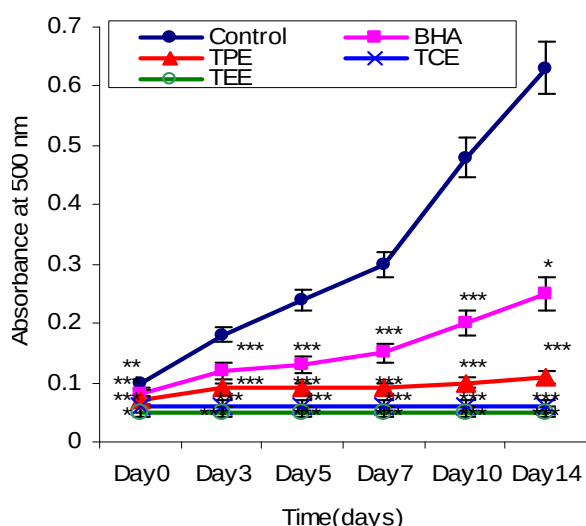
Table 1. Preliminary phytochemical examinations of *Thea sinensis* Linn. samples

No.	Constituent	Extract	Reagent used	Observation	Result
1	Alkaloids	DW	Mayer's reagent	white ppt	+
			Dragendroff's reagent	orange ppt	+
			Sodium picrate	yellow ppt	+
			Wagner's reagent	reddish-brown	+
2	α -amino acids	DW	Ninhydrin reagent	purple color	+
3	Acidic or basic compounds	DW	Bromocresol green	light bluish color	basic compound
4	Carbohydrates	DW	α -naphthol and conc: H_2SO_4	red ring	-
5	Flavonoids	70% EtOH	Mg turning & conc: HCl	pink color	+
6	Glycosides	DW	10% lead acetate	white ppt	-
7	Phenolic compounds	DW	1% $FeCl_3$ & 1% $K_3Fe(CN)_6$	deep blue	+
8	Reducing sugars	DW	Benedict's solution	red ppt	+
9	Saponins	DW	Distilled water	frothing	+
10	Terpenoids /steroids	P.E	Acetic anhydride & conc: H_2SO_4	greenish color	+
11	Tannins	DW	2% NaCl & 1% Gelatin	white ppt	+

+ = Presence

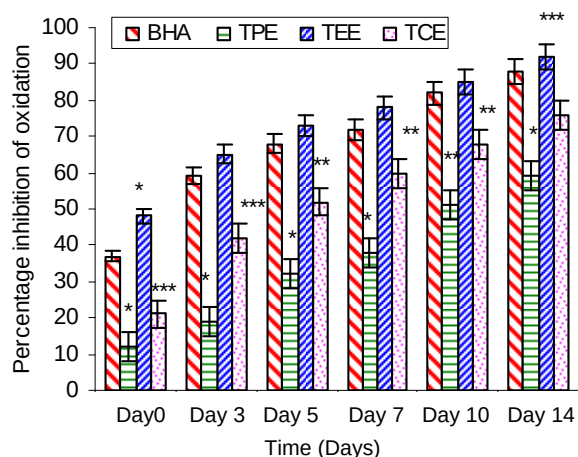
- = Absence

various extracts at various times in days are illustrated in Fig.1 and Fig. 2. It was also found that three extracts had significantly lower absorbance than that of control ($p < 0.1-0.0005$) and significant increased % inhibition than that of control ($p < 0.1-0.0005$) after 14th day incubation (Table 2). However, while the antioxidative activity of ethanol extract was significantly higher % inhibition than that of BHA, petroleum ether extract and chloroform extract activities were significantly lower % inhibition than that of BHA ($p < 0.1-0.0005$) for every reaction time in days at constant concentration (2 mg/ml). Among these three extracts, the antioxidative activity of ethanol extract was higher than that of chloroform extract, which was in turn higher than that of petroleum ether extract, when compared to that of BHA, for every reaction time in days at constant concentration (2 mg/ml). The antioxidative activities of three extracts from the tea



BHA=Butylated hydroxy anisole
TPE=Petroleum ether extract of *T. sinensis* Linn.
TCE=Chloroform extract of *T. sinensis* Linn.
TEE= Ethanol extract of *T. sinensis* Linn.
Concentration (0.02% or 2 mg/ml)
SE=Standard Errors, n=6
Significantly different from control; *p<0.01,
p<0.005, *p<0.0005

Fig. 1. Antioxidative activities of extracts from tea leaves and BHA on autoxidation of linoleic acid by thiocyanate method



BHA=Butylated hydroxy anisole
PE=Petroleum ether extract of *T. sinensis* Linn.
TCE=Chloroform extract of *T. sinensis* Linn.
TEE=Ethanol extract of *T. sinensis* Linn.
Concentration (0.02% or 2 mg/ml)
SE=Standard Errors, n=6
Significantly different from BHA; *p<0.01,
p<0.005, *p<0.0005

Fig. 2. Antioxidative activities (in % inhibition) of extracts from tea leaves and BHA on autoxidation of linoleic acid by thiocyanate method

Table 2. Antioxidative activities of extracts from tea leaves as measured by the thiocyanate method after 14th day incubation

Sample ¹	Absorbance at 500 nm	% inhibition ²
Control	0.619 ± 0.017	0
Butylated hydroxy anisole	0.091 ± 0.003***	85.340 ± 0.445
Petroleum ether extract	0.252 ± 0.007*	59.108 ± 1.410***
Chloroform extract	0.148 ± 0.004**	75.970 ± 1.019***
Ethanol extract	0.080 ± 0.003***	87.055 ± 0.529

1=Concentration (0.02% or 2 mg/ml)

2=A high inhibition percent indicates a high antioxidative activity.

Each value represents the mean ± SE (n=6)

Statistically significant; *p<0.01, ** p<0.005,

*** p<0.0005

leaves after 14th day incubation are listed in Table 2. These results indicated that ethanol extract showed more antioxidative activity than chloroform extract, which in turn showed more activity than petroleum ether extract. In addition, antioxidative activity of ethanol extract was found to be higher than that of BHA. From the results it was expected that the compounds possessing antioxidative activity may contain in ethanol extract and chloro-form extract, respectively.

Caffeine, from the leaves of tea (*Thea sinensis*) and coffee (*Coffea arabica*) were shown to have antioxidative activity (in a linoleic acid oxidation test) comparable to that of BHA and BHT [16]. Thus, these results were in agreement with results obtained in this present study. The mechanism of their action could be related to their antioxidant function since vascular disorders may be caused by oxidative damage of cell membranes. Polyunsaturated fatty acids, present in cell membranes, are easily oxidized both by enzymatic and autoxidative peroxidation via free radical chain reactions. Lipid peroxidation can be inhibited by flavonoids acting as strong O_2^- scavengers and $^1O_2$ quenchers. The autoxidation of linoleic acid leads to the formation of four hydroperoxide isomers: 13-hydroperoxy-9-*cis*, 11-*trans*-octadecadienoic; 9-hydroperoxy-10-*trans*, 12-*cis*-octa-

deca dienoic; 9-hydroperoxy-10-*trans*, 12-*trans*-octadecadienoic acids. The addition of phenolic compounds such as flavonoids partially inhibited the formation of 13-*trans*, *trans* and 9-*trans*, *trans* isomers. The antioxidative activity of flavonoids is related to an inhibition of the formation of *trans*, *trans* hydroperoxide isomers. The inhibition of the formation of *trans*, *trans* isomers by flavonoids showed that these compounds act as H-atom donors to the peroxy radical, thus inhibiting the autoxidation of fatty acids by chain radical termination [17].

The results of this study indicated that tea leaves are indeed a rich source of natural anti-oxidants and have ability to scavenge O₂:-OH and peroxy radicals. Oxygen-derived free radicals are known to play a significant role in the pathophysiology of many diseases, cardiovascular diseases such as ischemic heart diseases, arrhythmias, stroke, and brain damage; lung injury such as adult respiratory distress syndrome; liver damage; cancer; influenza; malaria and many more [18]. Leaves of tea (*Thea sinensis* Linn.) should be used as "anti-aging herb" or in the formulation of "longevity medicine", owing to their high antioxidative activities.

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**Preliminary study on hypoglycemic effect of
Phyllanthus niruri Linn. (Taung-ze-phyu) on rabbit model**

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The aim of this study is to determine phytochemical constituents, acute toxicity and the hypoglycemic effect of aqueous and 95% ethanol extract of whole plant of *Phyllanthus niruri* Linn. (Taung-ze-phyu). Phytochemical results showed that crude powder, aqueous extract and 95% ethanol extract contained alkaloids, flavonoids, tannins, saponins, steroids, amino acids and polyphenols. Acute toxicity study of crude powder, aqueous extract and 95% ethanol extract were evaluated in mice. Both crude powder and 95% ethanol extract showed no evidence of toxicity up to the maximum feasible dose level of 3 gm/kg body weight. In contrast, the maximum feasible dose level of aqueous extract was 6 g/kg body weight. Therefore, the median lethal dose (LD₅₀) of crude powder and 95% ethanol extract was observed to be more than 3 gm/kg body weight. The median lethal dose (LD₅₀) of aqueous extract was observed to be more than 6 gm/kg body weight. Evaluation of hypoglycemic effect of 95% ethanol extract (1.5 gm/kg body weight) and aqueous extract (3 gm/kg body weight) was carried out on adrenaline-induced diabetic rabbits. It was found that 95% ethanol extract significantly lowered the blood glucose levels at 2hr ($p < 0.05$). Aqueous extract also lowered the blood glucose level at 1hr, 2hr, 3hr and 4hr ($p < 0.005 \sim p < 0.05$) respectively when compared with those of the control. Hypoglycemic effect of standard drug glibenclamide (4 gm/kg body weight) was also studied to compare with that of the plant extracts. Between the two extracts, the effect of aqueous extract (3 gm/kg body weight) when compared with that of glibenclamide was not significantly different.

INTRODUCTION

Phyllanthus niruri Linn. (Taung-ze-phyu) is a small annual herb growing widely in the rainy season of Myanmar. It belongs to the family Euphorbiaceae. It is also a well known medicinal plant for hepatitis, diabetes mellitus and hypertension. Decoction of fresh whole plant was taken for diabetes mellitus and hepatitis [1]. Many researchers have observed its hepatoprotective, hypotensive, hypoglycemic and diuretic effects in laboratory animals [2, 3, 4, 5 & 6]. There have been some reports on the hypoglycemic effect of *Phyllanthus niruri* Linn. which varies in regard to the plant species, the part of the

plant used and in the preparation of extracts as well as the animal models. But there has been no systematic research on the hypoglycemic effect of *P. niruri* locally. Hence, this study was conducted.

MATERIALS AND METHODS

This study was done at the Pharmacology Research Division, Department of Medical Research (Lower Myanmar), Yangon. Cross-over study design was used. Experimental animals were obtained from Laboratory Animal Services Division, DMR (Lower Myanmar).

Collection and identification of Phyllanthus niruri Linn.

Fresh whole plants growing in the suburban of Yangon were collected. The plants were identified and authenticated at the Botany Department, Yangon University. A voucher specimen was deposited at the herbarium of Pharmacology Research Division, Department of Medical Research (Lower Myanmar).

Preparation of whole plant extracts of P. niruri Linn.

Aqueous and 95% ethanol extracts of whole plant *P. niruri* Linn. were prepared by the standard method [7]. One hundred grams of whole plant powder was extracted with either 1 litre of distilled water or 95% ethanol on water bath at 60°C for 6 hours. It was filtered through cheese cloth and the filtrate was evaporated to dryness on a boiling water bath. The extract obtained was dissolved in distilled water before administration to respective animals.

Preliminary phytochemical analysis of crude powder, aqueous and 95% ethanol extracts of P. niruri Linn.

Phytochemical analysis of crude powder, aqueous extract and 95% ethanol extract of whole plant was carried out according to the standard methods [7, 8 & 9].

Acute toxicity tests of P. niruri Linn.

Acute toxicity tests of crude powder, aqueous and 95% ethanol extracts were performed by the method of Litchfield and Wilcoxon [10]. Adult healthy albino mice, both male and female, weighing 30-35 gm were selected. They were divided into 5 groups of 10 mice each. Each group of mice was housed separately in mouse cages. They were fasted for 18 hours prior to the experiment, allowing access to water only. Each group received orally crude powder of whole plant in the doses of 0.5, 1, 2 and 3 gm/kg body weight, respectively. One group, served as control, was given 0.1 ml/10 gm body weight of distilled water. Food and water were supplied as usual after administration of test sample. They were

kept under close observation for 24 hours. Any toxic symptoms and mortality found within 24 hours were recorded. Careful observation was continued up to 14 days in order to detect delayed effects. Acute toxicity tests of aqueous and 95% ethanol extracts were also done. The procedure is same as above. The tested doses for aqueous extract and 95% ethanol extract are 1, 2, 3 and 6 gm/kg and 0.5, 1, 2 and 3 gm/kg body weight, respectively.

Hypoglycemic effect of aqueous extract on adrenaline-induced hyperglycemic rabbit models

Six adult healthy rabbits of Japanese White strain, weighing 2.5-3.0 kg were used. They were deprived of food for 18 hours before the experiment. On the experiment day, 0 hr blood glucose level of all rabbits was determined with Glucometer (Ascensia ELITE XKL, Bayer Corporation, U.S.A) by taking blood samples from the marginal ear vein with blood glucose test strips (Ascensia ELITE XKL, Bayer Corporation, U.S.A). It was then immediately followed by oral administration of distilled water (10 ml/kg body weight), for control, by using a Ryle's tube No. 6. After administration of distilled water, all rabbits were induced to mimic hyperglycemia by injecting them subcutaneously with 0.2 mg/kg body weight of adrenaline tartrate B.P. using the method of Gupta *et al.* [11].

Serial blood glucose levels were measured at 1, 2, 3 and 4 hours after administration of adrenaline. And then all the rabbits were allowed to rest for a week. After a week's rest, the fasting blood glucose levels (0 hr) of all the rabbits were determined. The aqueous extract of whole plant 3gm/kg body weight was administered orally instead of distilled water. Adrenaline tartrate was injected subcutaneously as in control group. The blood glucose levels at 1, 2, 3 and 4 hours after adrenaline injection were recorded. The mean blood glucose levels of distilled water treated group (control group) and aqueous extract treated group (test group) were then compared and analyzed by the student 't' test.

The above procedure was repeated after a week's rest of wash out period using standard drug glibenclamide (4 mg/kg body weight) orally instead of aqueous extract. The mean blood glucose levels of control group and glibenclamide treated group (standard group) were compared and analyzed as above.

Hypoglycemic effect of 95% ethanol extract on adrenaline-induced hyperglycemic rabbit models

A new group of six adult healthy rabbits of JW strain weighing 2.5-3.0 kg were used. The test procedure used was the same as described above. Crossover study design was also used. The tested dose of the extract was 1.5 gm/kg body weight.

Statistical analysis

Data were expressed as mean $\pm$ SE (Standard Error) and the mean differences calculated using Student 't' test. $p < 0.05$ was chosen as a significant level.

RESULTS

Phytochemical constituents of crude powder, aqueous extract and 95% ethanol extract of *Phyllanthus niruri* Linn. were determined (Table 1).

Table 1. Phytochemical constituents of *Phyllanthus niruri* Linn.

No.	Phytochemical constituents	Results		
		Crude powder	Aqueous extract	95% ethanolic extract
1	Alkaloides	+	+	+
2	Flavonoids	+	+	+
3	Glycosides	+	+	+
4	Saponins	+	+	+
5	Tannins	+	+	+
6	Phenols	+	+	+
7	Steroids/Terpenes	+	+	+
8	Amino acid	+	+	+
9	Reducing sugar	+	+	+
10	Cyanogenic glycosides	-	-	-

Acute toxicity tests of crude powder, aqueous extract and 95% ethanol extract

showed that no lethality of the mice was observed up to 14 days even with the maximal permissible doses. Therefore, LD₅₀ for the crude powder, aqueous extract and 95% ethanol extract are more than 3 gm/kg, 6 gm/kg and 3 gm/kg body weight, respectively.

Hypoglycemic effects of aqueous and 95% ethanol extract of *Phyllanthus niruri* Linn. were observed. In adrenaline-induced hyperglycemic rabbits, aqueous extract (3 gm/kg body weight) could effectively reduce the blood sugar levels at 1 hr ($p < 0.005$), 2 hr ($p < 0.01$), 3 hr ($p < 0.05$) and 4 hr ($p < 0.05$), respectively, after drug administration (Fig.1). It was highly significant at 2 hours after drug administration.

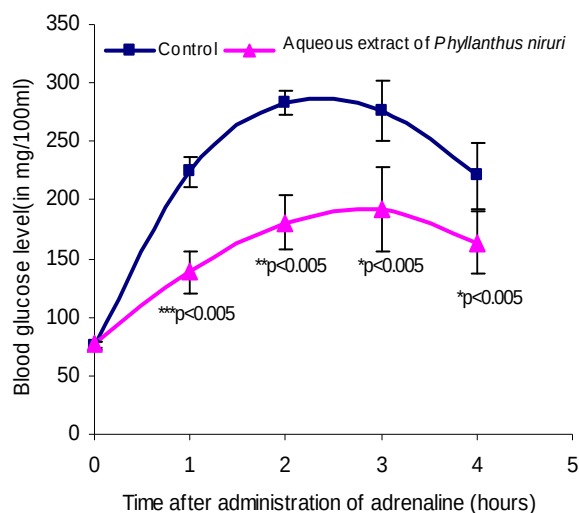


Fig 1. Times course of the effect of aqueous extract of *Phyllanthus niruri* on adrenaline-induced hyperglycemic rabbit model

Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

An oral hypoglycemic drug, glibenclamide (4 mg/kg body weight) was observed to lower the blood sugar level significantly at 2 hrs ($p < 0.05$) and 3 hr ($p < 0.05$) and 4 hrs ($p < 0.01$), respectively. It was highly significant at 4 hr after drug administration (Fig. 2). The extent of hypoglycemic effect produced by the aqueous extract when compared with that of glibenclamide was not significantly different.

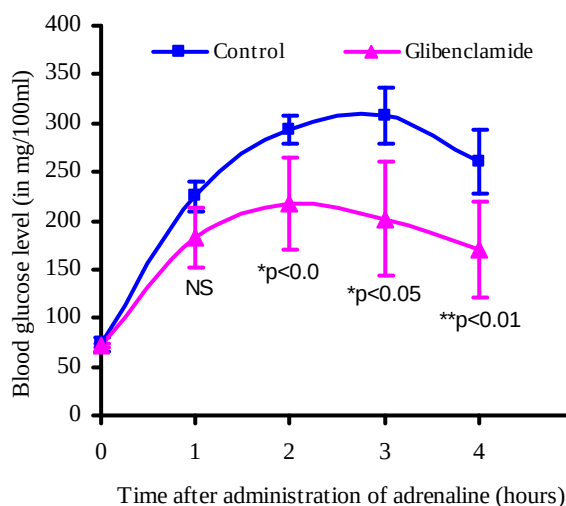


Fig 2. Time course of the effect of glibenclamide on adrenaline-induced hyperglycemic rabbit model

Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

In 95% ethanol extract treated test, 1.5 gm/kg body weight of ethanol extract significantly lowered the blood glucose level at 2 hour ($p<0.05$) after drug administration (Table 2). It was not effective as glibenclamide or aqueous extract (data not shown). This may be due to short duration of action.

Table 2. Effect of 95% ethanolic extract of *Phyllanthus niruri* on blood glucose levels (Mean $\pm$ SE) of adrenaline-induced hyperglycemic rabbits model

Test sample	Blood glucose level (mg/100ml)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control (n=6)	73.6 $\pm$ 4.5	181.3 $\pm$ 17.5	277.8 $\pm$ 16.5	238.2 $\pm$ 46.4	250.5 $\pm$ 32.7
Ethanolic extract (n=6)	76.5 $\pm$ 2.9	164.8 $\pm$ 17.5	220.3 $\pm$ 28.5*	228.8 $\pm$ 40.2	212.5 $\pm$ 41.4

*= $p<0.05$

DISCUSSION

Chemical constituents of the aqueous and 95% ethanol extract of *P. niruri* indicated the presence of alkaloids, flavonoids and saponins. Some plants that contain these alkaloids have been reported to have hypoglycemic activity [12], so the hypoglycemic effect caused by these extracts may be attributed to the

presence of alkaloids and flavonoids. These are mainly phenolic compounds, which have been reported to have antidiabetic effects [13]. The acute toxicity studies showed that LD₅₀ of the aqueous extract and 95% ethanol extract were more than 6 gm/kg and 3 gm/kg body weight.

In this study we observed that the administration of aqueous extract (3 gm/kg) and 95% ethanol extract (1.5 gm/kg body weight) of *P. niruri* to adrenaline-induced hyperglycemic rabbits caused a significant decrease in blood glucose level. This confirms the claims by traditional medical practitioners and herbalists that the *P. niruri* has blood glucose lowering properties. The precise mechanism by which these extracts lower blood glucose is, however, not clear. It may be due to increased insulin secretion arising from pancreatic stimulation and probably increased utilization of peripheral glucose [13]. It is also believed that some of these hypoglycemic plants perform this function by removing the insulin-inactivating compounds through the SH groups in these inactivating compounds. Nicotinic acid is known to be insulin's inhibitor [14].

Similarly, other hypoglycemic plants containing anthocyanocides appear to act by improving vascularization of the pancreas. Others act by blocking oxidative enzyme of the Krebs cycle (succinic dehydrogenase and cytochrome oxidase), thus increasing anaerobic glycolysis and decreasing gluconeogenesis and entailing an increased rate of transfer of glucose from the blood to the tissue [12]. Nwanjo also reported that the aqueous crude extract of *P. niruri* may have hypoglycemic effect in streptozotocin-induced diabetic Wistar rats and that there was no evidence of hepatotoxicity [5]. Thus, it can be concluded that these observations showed the aqueous and 95% ethanol extracts of *Phyllanthus niruri* Linn. may have hypoglycemic effect in adrenaline-induced hyperglycemic rabbits.

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Acute effect of onion (*Allium cepa*) on blood glucose level of diabetic patients

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To determine the acute effect of onion on blood glucose levels of diabetic (NIDDM) patients, a self-controlled study was done. It included 20 diabetic patients whose fasting plasma glucose concentration exceeded 126 mg/dl. After taking the fasting plasma glucose sample, 50 g of oral glucose load was given to the patients and the plasma glucose levels at thirty-minute time intervals up to two hours were taken again (OGTT). Then, the same procedure was done after one week at which glucose load and onion (50 g) were administered. The results were analyzed by using paired 't' test. The mean fasting plasma glucose concentrations of diabetic patients were 147.35 ± 17.18 mg/dl vs 149.6 ± 19.76 mg/dl, ($p=0.199$), respectively. When the glucose and onion were administered, the plasma glucose levels were found to be decreased when compared to those levels after giving glucose only; (225.60 ± 27.25 mg/dl vs 214.40 ± 33.39 mg/dl at 30 min; $p=0.099$), (282.55 ± 31.67 mg/dl vs 229.40 ± 37.61 mg/dl at 60 min; $p=0.0001$), (270.20 ± 22.48 mg/dl vs 194.45 ± 37.26 mg/dl at 90 min; $p=0.0001$) and (248.75 ± 20.13 mg/dl vs 161.65 ± 30.50 mg/dl at 120 min; $p=0.0001$), respectively. This study shows that onion has an acute effect of lowering the plasma glucose levels which could be useful in the management of patients with diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's systems. The dietary components beneficial in the prevention and treatment of diabetes have not been clearly defined, but it is postulated that medicinal/culinary herbs may play a role [1]. The ancient physicians used onion to treat diabetes and once again modern studies proved that onion does indeed play a part in reducing blood sugar level [2]. One of the active constituents of onion is APDS (allyl propyl disulphide) and it has been shown to block the breakdown of

insulin by the liver and possibly to stimulate insulin production by the pancreas, thus increasing the amount of insulin and reducing sugar levels in the blood [3, 4]. In preliminary study of healthy male volunteers, administration of 25-50 gm of raw or boiled onion lowered blood sugar levels in people with diabetes [5]. Onion does not reduce blood sugar levels in healthy non-diabetic people [6].

In Myanmar, onion is eaten as one of the daily food ingredients in meals. Study relating to the acute effect of raw onion supplementation on blood glucose level of NIDDM patients has not been scientifically done yet. The present study was aimed to determine that whether onion can be used as a remedial ingredient for acutely lowering blood glucose levels in the management of

diabetic (NIDDM) patients. Objectives of the study were;

- To determine the blood glucose levels of NIDDM patients before and after glucose administration at specific time intervals by doing Oral Glucose Tolerance Test (OGTT).
- To determine the blood glucose levels of NIDDM patients before and after glucose with onion administration at specific time intervals.
- To compare the blood glucose levels at specific time intervals before and after glucose only and glucose with onion administration in NIDDM patients.

MATERIALS AND METHODS

Study design

A self-controlled study was used.

Subjects

Both males and females of 40-60 years old attending a private clinic in Thingangyun Township whose fasting plasma glucose concentration exceeded 126 mg/dl (WHO criteria) were recruited for the study. A total of 20 (ten males and ten females) were included in this study. Those taking insulin and/or medicine for other health conditions; those with allergic reaction to onion ingestion and those who refused to participate in the study were excluded.

Method

After taking the baseline fasting plasma glucose sample, 50 gm of oral glucose load dissolved in 200 ml of water was given to the patients and the plasma glucose levels at thirty-minute time intervals up to two hours were taken again (OGTT). Then, the same procedure was done after one week at which glucose load and onion (50 gm) were administered. Venous plasma glucose level was determined by enzymatic colorimetric method [7].

Statistical analysis

All the blood glucose levels at baseline fasting and at 30-minute intervals were expressed as mean±standard deviation (SD) and analyzed by using paired 't' test (two-tailed). Differences were considered significant at $p<0.05$ levels.

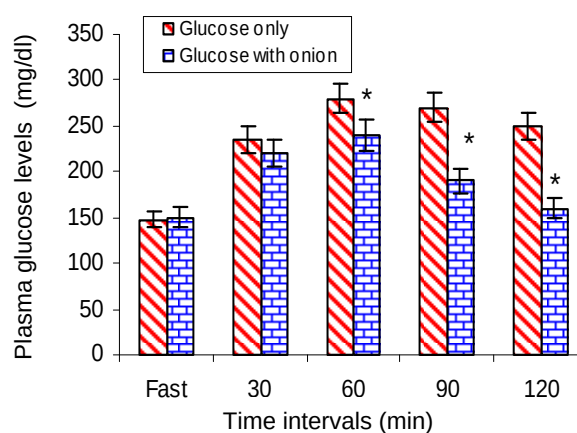
Ethical consideration

Ethical clearance was obtained from Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar) to conduct the study.

RESULTS AND DISCUSSION

An acute effect of onion on blood glucose level was determined on 20 (ten males and ten females) NIDDM patients whose mean age was 50.9 ± 4.037 years.

Figure 1 shows the acute effect of onion on blood glucose levels of NIDDM patients at different time intervals.



*= $p<0.05$

Fig.1. Comparative effect of glucose load only and glucose with onion on plasma glucose levels of NIDDM patients

The mean fasting plasma glucose concentrations at the baseline level were same for all NIDDM patients: 147.35 ± 17.18 mg/dl vs 149.60 ± 19.76 mg/dl, ($p=0.199$). At thirty minutes, the effect of onion was not found as the difference between the plasma glucose levels of the two groups (glucose only and

glucose plus onion) was statistically not significant (225.60 ± 27.25 mg/dl vs 214.40 ± 33.39 mg/dl; $p=0.099$). After that, the plasma glucose levels were found to be significantly decreased in those patients who received glucose and onion when compared to those with glucose only: 282.55 ± 31.67 mg/dl vs 229.40 ± 37.61 mg/dl at 60 min, ($p=0.0001$); 270.20 ± 22.48 mg/dl vs 194.45 ± 37.26 mg/dl at 90 min, ($p=0.0001$) and 248.75 ± 20.13 mg/dl vs 161.65 ± 30.50 mg/dl at 120 min, ($p=0.0001$), respectively. So, oral administration of onion had blood glucose lowering activity acutely in NIDDM patients.

Traditional treatments for diabetes mellitus have mostly disappeared in occidental societies, but some are prescribed by practitioners of alternative medicine or taken by patients as supplements to conventional therapy. However, plant remedies are the mainstay of treatment in underdeveloped regions. A botanical substitute for insulin seems unlikely, but traditional treatments may provide valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts [2].

Several uncontrolled human studies [3, 5] and at least one double-blind clinical trial [4] have shown that large amounts of onion can lower blood sugar levels in people with diabetes. Two sets of compounds make up the majority of onion's known active constituents – sulfur compounds, such as allyl propyl disulphide (APDS), and flavonoids, such as quercetin. APDS has been shown to block the breakdown of insulin by the liver and possibly to stimulate insulin production by the pancreas, thus increasing the amount of insulin and reducing sugar levels in the blood. However, onion does not reduce blood sugar levels in healthy non-diabetic people [6]. It has also been shown that garlic and onion juices exerted antioxidant and anti-hyperglycemic effects and consequently alleviated liver and renal damage caused by alloxan-induced diabetes [8]. Most human studies that have shown an effect from onions used at least 25 gm per day and often two to four times that amount [3,4]. Though

some studies have found cooked onions acceptable, several studies suggest that onion constituents are degraded by cooking and that fresh or raw onions are probably most active [4, 9, 10]. Therefore, the use of 50 gm of raw onion in this study can be sufficient enough to lower the blood glucose levels in diabetic patients.

In conclusion, onion has an acute effect of lowering the plasma glucose levels which could be useful in the management of patients with diabetes mellitus as a simple dietary adjunct or a remedial ingredient.

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Anti-inflammatory and anti-plaque activity of Ponna yeik (*Ixora coccinea* Linn.) leaves extract used as a mouthwash on chronic gingivitis patients

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Ponna yeik (*Ixora coccinea* Linn.) is locally claimed to be useful in treatment of toothache and oral diseases as a mouthwash in Myanmar. In Myanmar, 80% of school children had gingivitis and 18% of them had periodontal destruction. Bacterial plaque in oral cavity is regarded as the primary local etiological factor in inflammatory disease. Preventing and controlling of periodontal disease would prevent the microbial colonization of plaque on the teeth and gingiva. There are varieties of antiseptic mouthwashes in modern dental practice, but chlorhexidine gluconate is the most effective anti-plaque mouthwash, which is not cheap and easily available. This study with the aim to evaluate the efficacy of Ponna yeik mouthwash, which was easily available at low cost, was conducted at the Institute of Dental Medicine, Yangon. The study design was a randomized controlled clinical trial and chlorhexidine gluconate was used as a positive standard drug. Twenty patients with typical chronic gingivitis who participated in this study were randomly divided into two groups, 10 patients for 0.2% watery extract of Ponna yeik mouthwash and 10 patients for 0.2% chlorhexidine mouthwash two times a day for 4 weeks. The plaque score, bleeding on probing supra-gingival plaque formation, staining effect and severity of gingivitis were examined prior to the clinical trial, as baseline and 4 weeks after trial. Both chlorhexidine and Ponna yeik mouthwashes showed significant effectiveness in plaque score, bleeding on probing and severity of gingivitis when compared to before treatment. Staining effects were observed in patients who used chlorhexidine but not in patients who used Ponna yeik mouthwash. There were no significant differences between two groups in all scores except staining score after 4 weeks of treatment. It was concluded that Ponna yeik mouthwash revealed anti-inflammation and anti-plaque activity without staining.

INTRODUCTION

In the priority ranking of disease based on scoring system (National Health Plan, 1996-2001) [1], oral diseases are placed at 27th rank in Myanmar. In Myanmar, 80% of school children had gingivitis and 18% of them had periodontal destruction [2]. Baseline oral health survey in Myanmar (1990) showed 22.6% of 12 to 13 years old children were free from gum disease and

2.6% of people aged 65 years and above were free from periodontal disease [3]. Among the oral diseases, periodontal disease is the principle disease that affects the morbidity of the dentition. Periodontal disease can be divided into two categories, disease affecting gingival tissue and disease affecting periodontium. Gingivitis, an inflammatory response of the gingiva without destruction of supporting tissue [4], is the common form of periodontal disease

and can be considered as infectious disease. The common form of gingival disease is chronic gingivitis.

Bacterial plaque in oral cavity is regarded as the primary local etiological factor in gingivitis. When uncontrolled, plaque may mature and extend subgingivally leading to periodontitis and eventual loss of teeth [5]. Preventing and controlling of periodontal disease would prevent the microbial colonization of plaque on the teeth and gingiva. Among the varieties of antiseptic mouthwashes in modern dental practice, chlorhexidine gluconate is the most effective antiplaque mouthwash and it is used as standard adjunctive periodontal treatment [6, 7], which is not cheap and easily available in our country. Nowadays, there is a wider area of interest in herbal medicine so that research activities have been done to find out the new, traditional medicine that would be effective and easily available for herbal mouthwash. There are numerous indigenous plants, which are reputed to be effective against the diseases of bacterial origin [8].

Ponna yeik (*Ixora coccinea* Linn.) is locally claimed to be useful in treatment of toothache and oral diseases as a mouthwash in Myanmar. In conditions of toothache, gum swelling in palate is relieved by warm decoction of leaves of Ponna yeik [9]. Ponna yeik is easily available and preliminary study showed the same as chlorhexidine gluconate's anti-bacterial activity on *Staphylococcus aureus*. The present study was, therefore, aimed to find out a new and effective herbal mouthwash in adjunctive periodontal treatment and to compare the effect of *Ixora coccinea* L. (Ponna yeik) mouthwash with 0.2% chlorhexidine solution on chronic gingivitis.

MATERIALS AND METHODS

Collection of medicinal plants

The specimens used in this study were collected from Department of Medical

Research (Lower Myanmar), Yangon. The plants were identified according to the description given in the characterization literature regarding taxonomy [10, 11]. The collection time of the leaves was from November to January after the rainy season and full-grown leaves in nearly uniform size were plucked from the branches.

Preparation of Ponna yeik mouthwash

The fresh leaves were weighed and cleaned with water and then dried at room temperature under the shade. The leaves were grinded into powder form by using electric grinder. Crude watery extracts were prepared by refluxing 50 gm of air-dried leaves powder in a round-bottomed flask on a boiling water bath using 500 ml of distilled water for 6 hours. It was filtered using cheesecloth and the filtrate was evaporated to dryness on a boiling water bath. It was serially diluted to 0.2% solution for mouthwash (solution A).

Preparation of chlorhexidine gluconate mouthwash

Five percent chlorhexidine gluconate stock was serially diluted up to 0.2% solution (solution B). It was used as a positive standard drug.

Experimental design

This study was a randomized controlled clinical trial.

Patients selection

After obtaining the ethical clearance from Institute of Dental Medicine, Yangon, 20 patients within the age of 17 to 22 years from the Institute of Dental Medicine, having typical chronic gingivitis with all anterior teeth and at least one molar tooth present at each quadrant, not taking any antibiotics for the past three months and who received periodontal treatment during the last three months preceding the study were selected after taking written informed consents that they were willing to participate in the trial. Those who had advanced periodonitis or any acute gingival

condition (e.g. acute necrotic ulcerative gingivitis, acute haemorrhagic gingivitis, gingival abscess), enamel hypoplasia or dental fluorosis, taken antibiotics for other reasons during the clinical trial, periodontal problem due to other causes and impacted last molar during the clinical trial were excluded.

Trial procedure

All selected patients were randomly divided into two equal groups and explained about the clinical trial, its objectives and procedure. The degree of plaque accumulation, bleeding on probing and gingival condition of the subjects were examined by only one trained dental surgeon throughout the study. Before starting the study, the examiner was trained by an expert periodontologist with using true pressure sensitive probe, to measure and record the scoring system as expertly and exactly as possible. The proportion agreement was calculated to measure the degree of agreements in both intra-observer and inter-observer variations. The intra-observer calibration was done at 12 anterior teeth of patients with chronic generalized marginal gingivitis of all scoring system used in this study for two consecutive days.

The degree of plaque accumulation, bleeding on probing and gingival condition of the subjects were examined and recorded at baseline and after 4 weeks of treatment. Twenty milliliters of solution were allowed to hold in the mouth for one minute and rinsed with plain water for 3 times before breakfast and before bedtime daily. Every tooth present in the mouth was examined except crowned teeth and abutments of the bridges. The severity of gingivitis was assessed according to noninvasive modification of Loe and Silness index [12], bleeding on probing was assessed according to Sulcus Bleeding Index [13], degree of plaque accumulation was assessed according to Turesky modification of the Quigley-Hein Index 1970 [14] and staining was assessed according to the modification of the Lobene Index 1968 [15].

The scoring units of Sulcus Bleeding Index were used to assess the bleeding on probing. They were as follows:

- 0 = Healthy appearance with no bleeding on probing
- 1 = Healthy appearance with no color or contour changes but bleeding on probing
- 2 = Bleeding on probing and color changes in tissue; but no swelling
- 3 = Bleeding on probing, color changes and slight swelling of the gingival unit
- 4 = Bleeding on probing, obvious swelling, with or without colour changes
- 5 = Spontaneous swelling, bleeding on probing, color changes and significant swelling with or without ulceration

Ethical consideration

Ethical approval for this research proposal was obtained from Institutional Ethical Review Committee of University of Dental Medicine, Yangon. Written and oral information was given to all eligible patients before obtaining their informed consents.

RESULTS

A total of 20 chronic gingivitis patients (10 males and 10 females) participated in this study and their baseline demographic and clinical characteristics are shown in Table 1.

Table 1. Baseline demographic and clinical characteristics of chronic gingivitis patients participating in the study

Total patients evaluated (n=10)	Ponna yeik	Chlorhexidine	p value
Sex (males: females)	1:1	1:1	NS
Age (years) Mean $\pm$ SE	19 $\pm$ 2.2	19 $\pm$ 1.7	NS
Height (cm) Mean $\pm$ SE	136.7 $\pm$ 3.4	149.1 $\pm$ 2.8	NS
Weight (kg) Mean $\pm$ SE	96.3 $\pm$ 6.9	96.1 $\pm$ 6.7	NS
Baseline clinical data (scores) Mean $\pm$ SE			
Severity of gingivitis	1.14 $\pm$ 0.04	1.2 $\pm$ 0.03	NS
Bleeding on probing	0.6 $\pm$ 0.052	0.67 $\pm$ 0.03	NS
Degree of plaque accumulation	1.58 $\pm$ 0.01	1.6 $\pm$ 0.008	NS
The staining effect			
Intensity	0.65 $\pm$ 0.01	0.65 $\pm$ 0.01	NS
Areas	0.6 $\pm$ 0.05	0.65 $\pm$ 0.01	NS

NS = not significant

It shows that 2 treatment groups were comparable. All patients gave no history of betal chewing and smoking.

Table 2 shows the comparison of patients' response for all scores to Ponna yeik and chlorhexidine gluconate in the two groups tested. After 4 weeks, both Ponna yeik and chlorhexidine gluconate treatments were significantly different from the baseline data scores in all different parameters ($p < 0.01$ to $p < 0.0005$). Both Ponna yeik and chlorhexidine gluconate treatments were significantly different from the baseline data scores in severity of gingivitis ($p < 0.05$ & $p < 0.0005$), but there was no statistically difference between two groups. Comparison of bleeding on probing (Sulcus Bleeding Index) between Ponna yeik and chlorhexidine gluconate mouthwash showed significant reduction after treatment, when compared with the baseline scores in both groups ($p < 0.01$ & $p < 0.005$). But bleeding Index scores after treatment were not significantly different between 2 groups. Comparison of degree of plaque accumulation between Ponna yeik and chlorhexidine gluconate mouthwashes, after 4 weeks of treatment showed no significant difference between two treatments but they were significantly reduced after treatment when compared with before treatment ($p < 0.01$ & $p < 0.005$) (Table 2 & Fig. 1).

Table 2. Comparison of patients' response to different mouthwashes for all scores

	Mean scores $\pm$ SEM					
	Ponna yeik mouthwash			Chlorhexidine mouthwash		
	Before TM	After TM	p value	Before TM	After TM	p value
Severity of gingivitis	1.14 $\pm$ 0.04	0.93 $\pm$ 0.04	$P < 0.05$	1.2 $\pm$ 0.03	0.87 $\pm$ 0.02	$P < 0.0005$
Bleeding on probing	0.6 $\pm$ 0.052	0.34 $\pm$ 0.04	$p < 0.01$	0.67 $\pm$ 0.03	0.36 $\pm$ 0.03	$p < 0.005$
Degree of plaque accumulation	1.58 $\pm$ 0.01	1.5 $\pm$ 0.01	$p < 0.01$	1.6 $\pm$ 0.008	1.5 $\pm$ 0.02	$p < 0.005$
Staining effects Intensity	0.65 $\pm$ 0.01	0.65 $\pm$ 0.01	NS*	0.65 $\pm$ 0.01	0.087 $\pm$ 0.01	$p < 0.005$
Staining effects Areas	0.6 $\pm$ 0.05	0.6 $\pm$ 0.05	NS*	0.65 $\pm$ 0.01	1 $\pm$ 0.01	$p < 0.005$

TM= Treatment, * NS = not significant

Chlorhexidine gluconate treatments showed significant increased staining effect in both area and intensity scores when compared with the baseline data scores ($p < 0.005$). Ponna yeik treatments showed no significant increased staining effect in both area and intensity scores when compared with the baseline data scores (Table 2). Comparison between Ponna yeik and chlorhexidine gluconate treatments at 4 weeks was not significantly different in all different parameters except staining scores ($p < 0.05$) (Fig. 1).

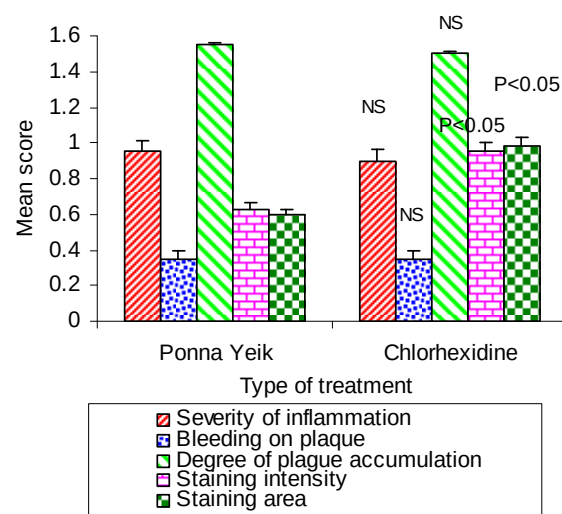


Fig 1. Comparison between effectiveness of Ponna yeik and chlorhexidine mouthwashes after 4 weeks of treatment

DISCUSSION

Since 1960, the role of plaque in the etiology of chronic gingivitis became understood. Periodontal disease can be prevented by either inhibiting the formation of the plaque on the tooth surface or by completely removing the plaque before inflammatory changes occur in the periodontal tissue [16]. There is a direct correlation between the presence and amount of dental plaque on the tooth surface and the presence and severity of periodontal disease. Therefore, plaque control is the most effective method for all inflammation in periodontal tissue. Prevention of plaque formation, removal of plaque by inhibition of calcification of bacteria occurred and elimination of specific pathogenic organism in the plaque are all goals of

chemical therapy by means of anti-plaque mouthwashes.

Current techniques for the assessment of gingival inflammation index system [17], degree of plaque index system [18], bleeding on probing system [17] and probing force investigation were using true pressure sensitive probe and each method had its advantages. One of the significant indices used in epidemiologic research is the Community Index of Periodontal Treatment Needs (CIPTN)(E) which was developed by the World Health Organization [19]. This index assesses the periodontal treatment needs in the community, not simply the level of disease. A study of Lang suggested a weakness in this CIPTN system; it may overestimate the need of treatment. The present method was chosen because it was the most sensitive and having the least error due to use of true pressure sensitive probe [15].

Chlorhexidine gluconate was chosen as a positive control because of its established efficacy and safety [20, 21, 22]. In 1970, Loe & Schiott showed that by rinsing for 1 minute twice a day with 10 ml of 0.2% chlorhexidine gluconate, plaque deposition and gingival inflammation would be completely prevented even in the absence of oral cleaning [23].

In this study, the findings showed that Ponna yeik mouthwash, when used twice a day in conjunction with usual daily oral hygiene measures, significantly reduced gingival inflammation, bleeding on probe and degree of plaque accumulation as good as chlorhexidine gluconate. This finding suggested that the active principle in both rinses (Ponna yeik and chlorhexidine gluconate) effectively control the chronic gingivitis. Therefore, Ponna yeik was evaluated to possess anti-plaque and anti-inflammation activities.

Scientific information on the pharmacologic activity of Ponna yeik for antidiarrhoea, antidyentery, inferility and central nervous system depressant activity had been reported by other researchers [10, 24, 25]. It was also

reported that Ponna yeik (*Ixora coccinea*) root and bark contain glycoside, octadecadienoic acid, mannitol and myristic acid [26]. Anti-plaque and anti-inflammation activities have not yet been reported in the available literature before this study. Therefore, our finding is the first report for the tested activity of Ponna yeik which is cheaper, easily available throughout the country, and easier to make a mouthwash than chlorhexidine which is imported from other countries spending lots of foreign currency.

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Determination of dengue antibody among the children admitted to Mandalay Children Hospital

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Myanmar belongs to the disease endemic countries (DEC) of dengue virus infection. In order to explore the dengue antibody status among the children, a hospital-based study was first introduced in Mandalay in the years 2006 and 2007 by the Virology Research Division, Department of Medical Research (Upper Myanmar) jointly with the Mandalay Children Hospital. Blood samples were collected from the patients who gave informed consent, and were sent to the Department of Medical Research (Upper Myanmar) for the virological studies. A total of 570 blood samples were obtained during dengue seasons of two consecutive years (from May 2006 to August 2007) from 484 clinically-diagnosed cases of dengue haemorrhagic fever (DHF), comprising 484 acute phase samples (S1) and 86 convalescent phase samples (S2). Paired sera were obtained from 86 patients (17.7% of patients from whom S1 was taken). A panel of samples (182 in number) was tested by PanBio Dengue Duo IgM and IgG ELISA tests. Among the tested samples, 153 samples showed positive result for dengue antibody (either IgM alone or both IgM and IgG) which in turn indicated 84% laboratory-confirmed cases of dengue infection. This study also paves the way to explore more about the seroepidemiology and molecular epidemiology of dengue viruses circulating in Mandalay and Upper Myanmar.

INTRODUCTION

Dengue is a continuing and growing global health issue especially in the tropical countries. Dengue virus infection is endemic in many parts of Asia, the Pacific and the Americas [1]. A significant proportion of the world dengue cases comes from South East Asia [over 38,000 cases in Indonesia in 2005 and up to 15,000 cases of dengue haemorrhagic fever (DHF) reported each year in Myanmar].

About 2.5 billion people are currently living in areas of risk and it is expected that this number will increase as transmission spreads to neighboring geographical regions. Symptomatic dengue infection is one of the leading

causes of hospitalization among children in endemic areas. Each year, estimated 100 million cases of dengue fever (DF) occur, and between 250,000 and 500,000 cases of DHF are reported to the WHO. The severe end of the disease spectrum, dengue shock syndrome (DSS), is associated with significant mortality with case fatality rates of between 1 and 5% [2]. As many as 2,000 patients with dengue virus infection are admitted to Mandalay Children Hospital (MCH) each year with very few deaths [3].

The first major epidemic of DHF occurred in Myanmar in 1970. Nowadays, DHF occurred throughout the country except in Chin State. Almost 80% of cases are reported from three

divisions (Yangon, Bago and Mandalay) and one state (Mon), with over 50% of cases recorded from Yangon Division only [4].

Major epidemics occurred in 1975, 1987, 1990, 1991, 1994, 1998, 2001, 2002, 2005 and 2007. The disease is spreading in rural areas, and morbidity trend is going up. Although mortality has declined, case fatality rate is still high. An extensive review on Dengue/DHF prevention and control programme in Myanmar has been conducted in June, 2003 by an International External Review Team. They recommended further improvement in disease surveillance, case management, laboratory services to support surveillance and case management, and multi-sectoral and community participation in dengue prevention and control.

In this connection, this study has been designed to collect the baseline data on dengue, and to explore the dengue antibody status among the children admitted to Mandalay Children Hospital who are diagnosed clinically as dengue virus infection. Mandalay Division is one of the divisions with the highest DHF endemicity in Myanmar, but only a few number of dengue serosurveys have been conducted in this area. Therefore, this study was conducted to establish the serological surveillance system for dengue/DHF in Mandalay Children Hospital, being the largest paediatric care centre of Upper Myanmar. It also served as the first serosurveillance on dengue/DHF in the Mandalay City area.

MATERIALS AND METHODS

Study design

Hospital and laboratory-based, cross-sectional descriptive study

Places of study

Mandalay Children Hospital, and Virology Research Division, DMR (Upper Myanmar)

Duration of study

March 2006-September 2007, covering the dengue seasons of two consecutive years

Subjects

Children admitted to Mandalay Children Hospital, for the complaint of fever of unknown origin, and being diagnosed as dengue virus infection according to the WHO criteria [5].

Inclusion criteria

- Children of both sexes
- Those presenting with an acute febrile illness with two or more of the following manifestations: rash, haemorrhagic manifestations, leukopenia, thrombocytopenia, evidence of plasma leakage, headache, retro-orbital pain, myalgia, arthralgia
- Whose parents/guardians give their written informed consent to participate their children in the study

Exclusion criteria

- Children with fever of known origin (apart from viral aetiology)

Procedures

Children with the complaint of fever of unknown origin who were admitted to the Paediatric Wards I, II and III of Mandalay Children Hospital (MCH) were clinically screened out to diagnose dengue virus infection according to the WHO criteria.

Those who met the inclusion criteria were enrolled into the study after explaining the nature of the study and procedures to parents/guardians and getting the written informed consents. Blood 3 ml was withdrawn from each child, and clinical data were obtained.

Sera were temporarily stored at 4°C for few hours in MCH, and subsequently transferred to the Virology Research Division of the Department of Medical Research (Upper Myanmar) situated in PyinOoLwin. These sera were separated by low-speed centrifugation, and stored at -20°C until further procedures. The second blood sample (convalescent sample) of the same volume was taken from the child, two weeks after the first sample, during the follow-up visit. A

panel of sera was tested for the presence of dengue antibodies (both IgM and IgG) by ELISA-based detection system, using the commercially available Dengue Duo IgM and IgG ELISA test kits (PanBio Pty Ltd, Brisbane, Australia), according to the manufacturer's instructions.

RESULTS

A total of 570 blood samples were obtained during dengue seasons of two consecutive years (from May 2006 to August 2007) from 484 clinically-diagnosed cases of DHF, comprising 484 acute phase samples (S1) and 86 convalescent phase samples (S2). Paired sera were obtained from 86 patients (17.7% of patients from whom S1 was taken). A panel of samples (182 in number) was tested by PanBio Dengue Duo IgM and IgG ELISA tests. Among the tested samples, 153 samples showed positive result for dengue antibody (either IgM alone or both IgM and IgG) which in turn indicated 84% laboratory-confirmed cases of dengue infection. Among 182 clinically-diagnosed dengue samples, 153 (84%) samples were found to be positive by dengue IgM ELISA, out of which 119 samples were also positive for dengue IgG capture ELISA. Four samples revealed IgM negative but IgG positive. Fifteen samples were negative for both IgM and IgG. Another 10 samples revealed equivocal results for either IgM or IgG.

Table 1 shows the clinical manifestations of 137 serologically-confirmed dengue cases in Mandalay Children Hospital. Fever was encountered in all patients. Other common features among the cases were hepatomegaly (98.54%), positive tourniquet test (84.84%), reduced blood pressure (76.64%), increased pulse rate (72.99%), vomiting (51.82%), bleeding (32.85%), increased packed cell volume (25.54%), haematemesis and malaena (24.82%), epistaxis (18.25%), thrombocytopenia (16.06%), skin rash (3.78%) and lymphadenopathy (mainly axillary glands) (2.27%). This scenario reflected the real image of dengue patients seeking medical treatment at the hospital as in-patients.

Table 1. Clinical manifestations of 137 serologically-confirmed DHF cases in Mandalay Children Hospital

Clinical manifestation	Cases	Percentage
Fever	137	100
Hepatomegaly	135	98.54
Tourniquet test positive	112	84.84
Hypotension	105	76.64
PR >115/min	100	72.99
Vomiting	71	51.82
Bleeding	45	32.85
PCV >42%	35	25.54
Haematemesis & malaena	34	24.82
Epistaxis	25	18.25
Thrombocytopenia	22	16.06
Skin rash	5	3.78
Lymphadenopathy	3	2.27

Fig. 1 reveals the days of fever on admission of dengue patients to Mandalay Children Hospital. Most of the patients (66 in number) were admitted on day 4 of fever.

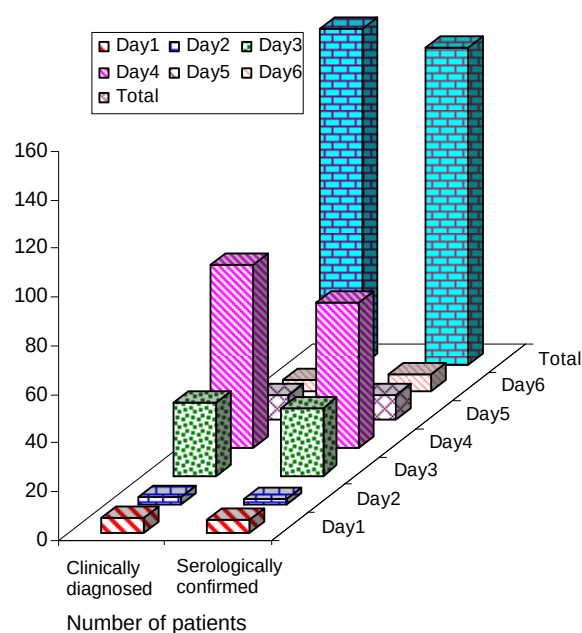


Fig 1. Days of fever on admission of dengue patients

According to the MCH Hospital Registry, there were total dengue admissions of 427 cases in year 1999, 130 cases in 2000, 1183 cases in 2001, 2118 cases in 2002, 326 cases in 2003, 406 cases in 2004, 2431 cases in 2005, 1764 cases in 2006, and 854 cases in 2007, respectively. Table 2 shows the dengue admissions to MCH from year 2002

to 2007, according to age groups, as well as the mortality and case fatality rate (CFR).

Table 2. Dengue admissions to Mandalay Children Hospital (2002-2007), according to age groups with mortality and case fatality rate (CFR)

Year	<1 Year	1-5 Years	>5 Years	Total	Death	CFR
2002	112	862	1144	2118	5	0.23%
2003	12	163	151	326	2	0.61%
2004	9	149	248	406	3	0.73%
2005	64	854	1513	2431	5	0.20%
2006	91	761	912	1764	20	1.13%
2007	21	355	478	854	5	0.58%

Table 3 shows the dengue admissions to MCH from year 2002 to 2007, according to disease severities (DHF grade I and II, and DSS).

Table 3. Dengue admissions to Mandalay Children Hospital (2002 to 2007), according to disease severities

Year	DHF G I & II	DSS	Total
2002	1947	171	2118
2003	249	77	326
2004	291	115	406
2005	1598	833	2431
2006	1267	497	1764
2007	660	194	854

There were 317 DHF grade I & II cases and 167 DSS (DHF grade III & IV) cases among 484 clinically-diagnosed dengue cases from whom the blood samples were collected. Twenty-two children were under 1 year of age (12 males and 10 females), 202 children were between 1 and 5 years of age (104 males and 98 females), and 260 children were above 5 years of age (113 males and 147 females), respectively. The youngest child was 5 months old, and the eldest was 12 years old.

Although the MCH was established in 1998, laboratory confirmation of the admitted dengue cases to MCH was made possible only in the year 2006 due to the sero-surveillance activities conducted by the Virology Research Division, Department of Medical Research (Upper Myanmar) with

the collaboration of the paediatricians and hospital staffs.

Total number of dengue admissions in year 2006 was 1764 patients (852 male and 912 female) with the peak of 383 patients in the month of August. The number of monthly admissions were 29 patients in January, 9 in February, 12 in March, 24 in April, 53 in May, 206 in June, 320 in July, 383 in August, 322 in September, 240 in October, 137 in November and 29 in December, respectively. There was a marked reduction in number of dengue admission in 2007 with a total of 854 patients. The patients of dengue admissions were 2 in January, 2 in March, 5 in April, 13 in May, 39 in June, 162 in July, 171 in August, 131 in September, 197 in October, 98 in November and 34 in December, respectively. No dengue admission in February 2007 was noted.

DISCUSSION

Although this study was entitled as “Determination of dengue antibody among the children admitted to Mandalay Children Hospital”, this is, in fact, the first ever sero-epidemiological study on dengue virus infection which was conducted in Mandalay and Upper Myanmar in the hospital-based setting. Previous literatures on dengue virus studies in the areas other than Yangon city were mainly concerned with the outbreak investigations. The first serosurveillance study on dengue viruses outside Yangon city was conducted in Pyinmana, Central Myanmar, in the year 2004 for the whole year [6].

Present study provides the laboratory proven (evidence-based) baseline data on dengue virus infection prevailing in Mandalay and Upper Myanmar. In addition, by studying the molecular epidemiology of the new dengue virus strains isolated from this particular area in Myanmar, it can elucidate the changing epidemiology of dengue virus infection in Myanmar. It can also predict the future dengue outbreaks with its prevailing

serotype. This kind of genetic information may also be helpful in predicting the direction(s) of dengue epidemics not only in Myanmar but also in the neighboring countries at the regional level.

The periodicity and frequency of the dengue epidemics, in other words, the interval between two epidemics can also be estimated. With regards to the dengue epidemics in Mandalay, recent data showed that large-scale epidemics occurred for two consecutive years, e.g. in the years 2001 and 2002, as well as in years 2005 and 2006. This scenario of “coupling epidemics” or “sequential epidemics” warrants the immediate needs of interrupting dengue transmission cycle between the vector mosquitoes and the host men.

It is noted that small dengue outbreaks occurred in Sagaing Division in year 2006, particularly in Wet Let Township and Shwe-Bo Township, and a considerable number of dengue patients from these areas came to MCH for the necessary treatment. So also, pockets of cases were reported in PyinOo-Lwin Township in the year 2007.

With regard to the gender issue, the male to female ratio in Mandalay study in 2006 was 1:1.07 (852 vs 912). There was no significant difference. More or less similar finding was observed in the 1998 dengue outbreak in Taunggyi as the male to female ratio was 1:0.9 [9], also in the 2004 dengue outbreak in Pyinmana was 1:1.09 [6]. Dengue admissions to Mandalay Children Hospital under 1 year age group were 5.3% and 5.1%, 1 to 5 years age group were 40.7% and 43 %, above 5 years age group were 54% and 51.9% in 2002 and 2006, respectively. Therefore, no significant difference was found in all age groups. However, the admission data for above 5 years age group in Lashio in 1994, Mawlamyaing in 2001 and Pyinmana in 2004 shifted to the right during the respective dengue seasons [6, 7, 8, 10].

Concerning the disease severity among admitted dengue patients, total number of DHF grade I and II patients against DSS was

1947 vs 171 for the year 2002, 1598 vs 833 for the year 2005 and 1267 vs 497 for 2006. Hence, the pattern of disease severity directed towards the more severe forms in recent years. Based upon the above mentioned data, we can not underestimate the possibility of huge dengue outbreak in Mandalay area in the near future. The effects of increased urbanization, population migratory movement and water supply system may definitely play as key factors in future episodes.

In connection with the antibody study, a panel of serum samples was brought to the Institute of Tropical Medicine, Nagasaki University, Japan, and was undergone the molecular studies there. Dengue virus isolation attempts were made by inoculating the sera into C6/36 mosquito cell lines. Infected culture fluids were subjected to the dengue antigen ELISA tests, as well as the RT-PCR tests to detect the dengue virus genome. Among the tested samples, three new dengue virus strains from Upper Myanmar were successfully isolated for the first time. These strains included one strain of dengue serotype 3 and two strains of dengue serotype 4 [11]. Therefore, this study also paves the way to explore more about the seroepidemiology and molecular epidemiology of dengue viruses circulating in Mandalay and Upper Myanmar.

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Smoking as a risk factor for pulmonary tuberculosis in adults

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Tobacco use, particularly smoking, is widely recognized by the medical community as well as the general public as a major public health problem. The aim of this study was to determine tobacco smoking as a behavioral risk factor associated with pulmonary tuberculosis in adults. A case-control study design was used. The study subjects were 100 new pulmonary TB patients (cases) and age-sex matched 100 non-TB cases and healthy subjects (controls) attending Township TB Centre in Kyimyindine Township, Yangon from November 2006 to June 2007. Data were collected by face-to-face interview using questionnaires. Among the cases, proportion of non-smokers, current active smokers and ex-active smokers were 39%, 41% and 20%, respectively. Among the controls, they were 60%, 27% and 13%. It was found that current active smoking was associated with development of pulmonary TB (OR=2.15, 95% CI=1.05-4.38). Moreover, active smokers who started smoking at ≤ 20 years of age (OR= 4.12, 95% CI=1.7-9.99), or had a duration of >10 yrs (OR=4.18, 95% CI=1.63-10.73), or smoked more than 10 cigarettes/day (OR=3.13, 95% CI=1.47-6.66), were at a higher risk of pulmonary TB compared to non-smokers. Therefore, an effective anti-smoking campaign is needed to have a positive repercussion on TB incidence.

INTRODUCTION

Tobacco use, particularly smoking is widely recognized by the medical community as well as the general public as a major public health problem. The risk from tobacco smoke is not limited to the smoker alone but also affects those around the smoker [1]. Smoking is prevalent worldwide. The USA exports 194 billion cigarettes yearly, mainly to the developing world where the level of smoking habit is largely unrecorded. However, the annual rise in the number of smokers is estimated to be 2.1% [2]. Tuberculosis has re-emerged to become the world's leading cause of death from a single infectious agent, accounting for a quarter of the avoidable adult deaths in the developing world [3]. Nearly one third of world's population is infected with *Mycobacterium*

tuberculosis and 3 million people die due to TB every year [4]. Both smoking and TB primarily affect the lungs as they enter the lung through inhalation route. Long-term exposure to smoking has an adverse effect on the lung's defense mechanisms [5].

The prevalence of pulmonary tuberculosis among men aged 15 years and over is 2-4 times higher than in women of the same age. Interestingly, there is no difference in prevalence between two sexes below 15 years but the rates diverge above this age [6]. This sex difference may be due to either biological or behavioral (health related) changes occurring at about the age of 15 in one or both sexes. The tobacco smoking habit starts in men around the age of 15. So, it is possible that there is an association between tobacco smoking and higher rates of TB in men.

Although smoking is one of the literature-cited factors, the association between smoking and pulmonary tuberculosis has been assessed in very few studies and none in Myanmar. Using WHO guidelines, there are effective TB control programs and highly effective treatment is available, however, if people are not aware of its seriousness and prevention, especially behavioral risk factors, it will remain a public health problem. For these reasons, it is important to determine smoking as a risk factor associated with TB manifestation in adults. This knowledge can be utilized to develop a program for TB prevention and control and also for antismoking campaigns.

General objective

- To determine tobacco smoking as a behavioral risk factor associated with pulmonary tuberculosis in adults

Specific objectives

- To identify smoking status related to pulmonary tuberculosis
- To find out the association of smoking habits with pulmonary TB among active smokers

MATERIALS AND METHODS

Study design

A clinic-based case-control study was carried out.

Study area

Secondary health centre (Township TB Centre) in Kyimyindine Township, Yangon Division was selected.

Study population

A total of 200 subjects were recruited. Study subjects were 100 new pulmonary TB patients (cases) and age-sex matched 100 non-TB cases and healthy subjects (controls).

Exclusion criteria

- Known cases of diabetes mellitus
- Known cases of HIV-positive patients

- Patients who currently had an immunosuppressive drugs
- Those who had evidence of any other lung diseases

Working definitions

Case

A new pulmonary TB case, aged ≥ 15 years, diagnosed at Tuberculosis Center (Yangon Division) and referred to Township TB Centre for subsequent treatment and never had a treatment for TB (new patients who have taken anti-TB drugs for less than 4 weeks will be included). The TB criteria are at least two sputum specimens positive for acid fast bacilli (AFB) by microscopy, or with at least one sputum specimen positive for AFB and radiographic abnormalities relevant to pulmonary tuberculosis.

Control

Non-TB cases and healthy subjects, aged ≥ 15 years who came to the secondary health centre for various reasons (e.g. medical check-up). They did not have any history of TB. Controls were matched for sex and for age within 10 years interval (15-24 years, 25-34 years, 35-44 years, 45-54 years and ≥ 55 years).

Non-smoker

Any person who has never smoked (non-active smoker) or who has never or less than 3 times/week been exposed to tobacco smoked by others at home, work, or in public places (non-passive smoker).

Current active smoker

Any person who smoked a tobacco product at the time of the study or persons who used to smoke but had stopped smoking < 6 months before the interview.

Ex-active smoker

Any person who used to smoke and had stopped smoking ≥ 6 months before the interview.

Passive smoker

Any non-smoker who was exposed to tobacco smoke > 3 times/week, either at home, work, or in public places.

Sample size determination and sampling

Assuming that prevalence of smoking in control population 40%, OR=2.5, confidence level=95% and power 80%, desired sample size was 85 in each group, for case:control rate of 1:1. Taking non-responses into account, 100 each for cases and controls were recruited for the study.

Consecutive sampling method was used to recruit cases, followed by selection of controls matched for age and sex.

Data collection

Data collection was done from November 2006 to June 2007. All subjects enrolled in the study were interviewed by the trained personnel. Informed consent was taken prior to the interview. Following data were collected from cases and controls using a pretested semi-structured questionnaire;

- Background characteristics
- Smoking status (non-smoker/ current active smoker/ ex-active smoker/ passive smoker)
- Age at which smoking started
- Duration of smoking
- Type of tobacco smoked
- Quantity of item smoked per day
- Data on potential confounding factors
 - Alcohol drinking
 - History of TB in the family
 - House environment
 - Body mass index (BMI)

Statistical analysis

Background characteristics of case and control groups were compared using chi square test. Smoking status and habits were compared among the cases and controls. The univariate analysis and multiple logistic regression methods (adjusting for potential confounding factors) were used to find out the effect of smoking on TB. The crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using stata 8 software. The level of significance was set at 0.05. Due to the small number of passive smokers, we merged this category

into category of non-smokers for the analysis.

Ethical consideration

This study was approved by the Institutional Ethical Review Committee of Department of Medical Research (Lower Myanmar).

RESULTS

In the case and control groups, the number of persons in each sex and age group were more or less similar. The majority of cases and controls were males between 15-34 yrs, married and had family members between 4-6. There was a significant difference between two groups regarding the education. The control group was found to have higher education status than the cases. Occupations were more or less similar between the two groups (Table 1).

Table 1. Background characteristics of case and control groups

Variable	Case (n=100)		Control (n=100)		p value*
	Number	Percent	Number	Percent	
Sex					
Male	89	89	89	89	1.00
Female	11	11	11	11	
Age (years)					
15- 34	53	53	49	49	0.62
35- 54	39	39	39	39	
≥ 55	8	8	12	12	
Education					
Primary school	35	35	13	13	<0.001
Middle school	40	40	30	30	
High school	18	18	25	25	
College	7	7	32	32	
Occupation					
Government employee	7	7	17	17	0.07
Employee at private business	7	7	7	7	
Own business	29	29	36	36	
Unskilled labour	35	35	28	28	
Dependent	22	22	12	12	
Marital status					
Single	19	19	24	24	0.64
Married	70	70	64	64	
Others	11	11	12	12	
Number of family members					
1-3	23	23	21	21	0.9
4-6	51	51	53	53	
≥7	26	26	26	26	

* Chi square test

Table 2 shows the association of other factors (confounding factors) with pulmonary TB in the study population. Body mass index (BMI) had a significant difference between case and control groups. Thus, education and BMI were found to be potential confounders in this study and controlled for in the final analysis.

Table 2. Association of other factors (confounding factors) with pulmonary TB in the study population

Variable	Case (n=100)		Control (n=100)		p value*
	Number	Percent	Number	Percent	
<i>Alcohol</i>					
No	51	51	45	45	0.24
Yes	49	49	55	55	
<i>Family history of TB</i>					
No	79	79	85	85	0.18
Yes	21	21	15	15	
<i>BMI (Body Mass Index)</i>					
Under weight	51	51	24	24	< 0.001
Normal	38	38	61	61	
Over weight	11	11	15	15	

* Chi square test

Table 3 shows the association of smoking status with adult pulmonary tuberculosis. It was found that among the cases, 41% were current active smokers and 20% ex-active smokers, whereas in control group 27% were current active smokers and 13% ex-active smokers. Current active smoking was associated with development of pulmonary TB.

Table 3. Association of smoking status with adult pulmonary TB

Smoking status	Case (n=100) (%)	Control (n=100) (%)	Crude OR (95% CI)	Adjusted OR ^a (95% CI)	p value
Non-smoker (Ref)	39	60	1	1	
Current active smoker	41	27	2.34 (1.24-4.39)	2.15 (1.05-4.38)	0.03
Ex-active smoker	20	13	2.37 (1.06-5.3)	2.03 (0.79-5.2)	0.14

^aAdjusted for education and BMI

Ref = Reference category

Moreover, active smokers who started smoking at ≤ 20 years of age, or had a duration of >10 years, or smoked more than 10 cigarettes/day were at a higher risk of

pulmonary TB compared to non-smokers (Table 4).

Table 4. Association of smoking habits with pulmonary TB among active smokers

Smoking status	Case (n=100) No. (%)	Control (n=100) No. (%)	Crude OR (95%CI)	Adjusted OR ^a (95%CI)	P value
Non-smoker (Ref)	39	60	1	1	
Active smokers					
n=61 n=40					
Age of start smoking					
<20 yrs	34	11	4.75 (2.16-10.48)	4.12 (1.7-9.99)	0.002
>20 yrs	27	29	1.43 (0.74-2.77)	1.33 (0.62-2.82)	0.46
Duration of smoking					
<10 yrs	33	31	1.64 (0.87-3.09)	1.45 (0.69-3.03)	0.32
>10 yrs	28	9	4.79 (2.04-11.22)	4.18 (1.63-10.73)	0.003
Number of cigarette smoked per day					
1-10	16	19	1.29 (0.59-2.82)	1.11 (0.47-2.67)	0.33
>10	45	16	3.30 (1.71-6.36)	3.13 (1.47-6.66)	0.02

^a Adjusted for education and BMI

Ref = Reference category

DISCUSSION

This study showed that current active smoking was associated with an increased risk for developing active pulmonary TB in adults with relative odds increased to 2.15 times. This result corresponded with other studies, but the risk estimated for TB was different. Alcidine *et al*, in a case-control study, showed the risk estimated for TB was higher (OR=3.8, 95% CI=1.5-9.8) for active smokers among young adults between 15 to 24 years) [7]. In Niorn *et al*'s report of a case-control study, multiple logistic regression analysis showed OR of 2.7 (95% CI=1.04-6.97) for current active smokers [8]. The adjusted OR of both studies was higher compared to the present study because of the difference in study design, study population and sample size.

This study found the number of cigarettes/cheroots smoked per day and duration of smoking were strongly associated with active pulmonary TB in active smokers. The results corresponded with other studies.

Peng *et al.* found that persons who smoke >20 cigarettes a day had 2.5 times (95% CI=1.11-5.6) more risk of pulmonary TB compared to non-smokers [9]. Yu *et al.* also found that relative risk of heavy smokers (≥ 400 cigarettes a year) compared with non-smokers was 2.17 (95% CI=1.29-3.63) [10]. Regarding the duration of smoking, Baskin *et al.* reported that persons smoking for ≥ 20 years had 2-3 times higher risk than never smokers [11].

These findings were consistent with the understanding that long-term exposure to cigarettes smoke had an adverse effect on the lung's defense mechanism, namely clearance of potential pathogens, such as *M. tuberculosis* [5]. Cilia are tiny hair-like projections which help to sweep dirt and waste products out of the lungs. When impaired by exposure to cigarette smoke, the cilia can not perform this cleansing process [12]. The acute inhalation of cigarette smoke results in ciliostasis and reduced mucociliary clearance by converting ciliated epithelium to non-ciliated epithelium, by altering the amount and character of the mucus products, and by inflammatory and emphysematous narrowing of the airways [13, 14].

Smoking affects the normal function of alveolar macrophages which eliminate micro-organisms, including *M. tuberculosis*, from the distal airway and keep the alveoli sterile. *M. tuberculosis* can survive within the macrophage, particularly if the immune system is not operating adequately [15]. The occurrence of tuberculosis is thought to be linked to altered immune response, multiple defects in macrophage/monocyte immune responses and CD4 lymphopenia [16]. Tobacco smoke could alter native and acquired resistance to *M. tuberculosis*. Exposure to tobacco smoke also results in morphological and functional changes in the alveolar macrophages [17]. All of these factors, in combination, may contribute to increased susceptibility of an individual to tuberculosis infection and occurrence of the disease.

Conclusion

The findings of the study highlighted effects of smoking on pulmonary tuberculosis in adults. Therefore, an effective anti-smoking campaign is needed to have a positive repercussion on TB incidence in the country.

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Bacteriological evaluation of dried prawn powder and pickled fish available from some local markets in Yangon

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Bacteriological analysis and the assessment of the effect of heat treatment on decontamination of bacterial pathogens were done on 20 samples of dried prawn powder and pickled fish collected from local markets in Yangon from July 2007 to March 2008. Coliform count, thermotolerant coliform count, viable bacterial count and detection of contaminated pathogenic bacteria were carried out according to standard microbiological techniques. Coliform count of 46 to 540 most probable number (MPN)/100 ml and thermotolerant/ faecal coliform count of 23 to 70 MPN/100ml were detected in all tested dried prawn powder samples. Coliform count of 240 to >2400 MPN/100 ml and thermotolerant coliform count of 17 to >2400 MPN/100 ml were detected in all tested pickled fish samples. All dried prawn powder and pickled fish samples had viable bacterial counts of 10^3 colony forming unit (CFU)/ml to uncountable numbers ($>10^5$ CFU/ml). Coliform, thermotolerant coliform and viable bacterial counts were again detected after heating the dried prawn powder samples up to 70-80°C for 3 minutes and steaming the pickled fish samples at 90°C for 15 minutes. After heat treatment, in all tested samples, the coliform count was reduced to <2 to 8 MPN/100ml and thermotolerant coliform count was reduced to <2 MPN/100 ml. Bacterial pathogens such as Enteropathogenic *Escherichia coli*, *Klebsiella spp.*, *Citrobacter freundii*, *Proteus spp.* and *Staphylococcus spp.* were isolated from the 60% of dried prawn powder samples and 85% of pickled fish samples. After heat treatment, the pathogenic bacteria were not isolated in all contaminated dried prawn powder samples and in 88.2% of contaminated pickled fish samples. This study highlighted the poor microbiological quality of dried prawn powder and pickled fish available in local markets in Yangon and advantage of heat treatment in decontamination of pathogenic bacteria in food.

INTRODUCTION

Food plays a major role in transmission of diseases and food-borne illness is one of the major health problems. The food-borne illness is characterized by disturbance in the gastrointestinal tract including abdominal pain, diarrhoea and sometimes vomiting. Common food-borne diseases are diarrhoea, dysentery, cholera, typhoid, hepatitis and food poisoning [1]. Common bacterial pathogens which have been identified as the cause of food-borne diseases include *Bacillus cereus*, *Brucella species*, *Campylo-*

bacter jejuni, *Clostridium botulinum*, *Clostridium perfringens*, enteropathogenic *Escherichia coli* (EPEC), enteroinvasive *Escherichia coli* (EIEC), enterotoxigenic *Escherichia coli* (ETEC), enterohemorrhagic *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Salmonella species*, *Shigella species*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* [2].

Current statistics for food-borne illnesses in various industrialized countries showed that up to 60% of cases were due to poor food

handling techniques in food service establishments. In Myanmar, the notifiable food-borne diseases include diarrhoea, dysentery, typhoid and paratyphoid fevers. It has been estimated up to 70% of diarrhoea cases were due to contaminated food [3].

Microbiological quality of ready-to-eat food is determined by: viable colony count and detection of indicator organisms in a particular food. Viable colony count is a count of bacteria which includes those that occur naturally in most food and those present through contamination. The count increases significantly over time in response to poor temperature control of a product. It indicates the quality and potential keeping quality (freshness) of the product.

Indicator bacteria are usually present in faeces of human and other warm-blooded animals. Detection of these organisms demonstrates the contamination of faecal matter and the possibility of presence of intestinal pathogens in food. Coliforms mainly consist of microorganisms of faecal origin and those containing in soil and vegetation. Thermo-tolerant (Faecal) coliforms include mainly the genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*. Measurement of coliform count, faecal coliform count, total bacterial count and detection of pathogenic microorganisms are necessary in determining the safety of food [4].

Dried prawn powder is widely used among Myanmar people and an ingredient in preparation of various kinds of salads (Let-thoke). Most of the sellers use ready-made dried prawn powder sold by vendors of local markets where poor standard of environmental and personal hygiene exists. Pickled fish is a favorite side dish in Myanmar meals. It is a kind of fermented salted fish and usually sold by vendors and road side food stalls in markets. Most people used to prepare it as it is or after washing with water and eat as salad after mixing with onion, chilli, garlic, oil and fish sauce. The profile of pathogenic bacterial contamination in dried prawn powder and pickled fish needs to be explored as they can act as one of the

vehicles for transmission of food-borne diseases.

Bacteria are killed if exposed to temperatures above 62.8°C for long enough [5]. As heat can kill majority of microorganisms, heated samples are needed to be tested to determine whether heating can reduce the contaminated microorganisms. Thus microbiological analysis of dried prawn powder and pickled fish sold in local markets in Yangon and the assessment of the effect of heat treatment on decontamination of bacterial pathogens may provide valuable data in formulating strategies to prevent the occurrence food-borne illnesses.

MATERIALS AND METHODS

Twenty samples of dried prawn powder and pickled fish were collected from the local markets in South Okkalapa Township, North Okkalapa Township, Latha Township, Dagon Township and Yankin Township from July 2007 to March 2008. They were collected in sterile plastic bags and transported to Bacteriology Research Division, Department of Medical Research (LM) to perform laboratory procedures. Each sample was thoroughly mixed, homogenized and divided into 2 parts to perform laboratory tests in two sets for heated and unheated samples.

Laboratory tests for unheated samples:

Determination of viable bacterial count

A 20-gram sample was mixed with 180 ml of sterile phosphate buffered saline and then homogenized. A ten-fold serial dilution in normal saline was done by using 1 ml of homogenized suspension. Viable bacterial count was determined by surface spread method [6].

Detection of indicator bacteria

Coliform and thermotolerant/faecal coliform counts were determined by multiple tube method according to the WHO guidelines for drinking water quality, 1997 [7]. The most probable number (MPN) of bacteria present

in the samples was statistically interpreted by Mc Crady Table.

Isolation of pathogenic bacteria in unheated samples

An approximately 60 ml of homogenized samples was centrifuged at 0°C for 15 min and primary isolation was done on Nutrient agar, MacConkey agar, Salmonella-Shigella agar, Manitol Salt agar and Thiosulphate Citrate Bile Salt Sucrose agar. Simultaneously, Selenite F broth and alkaline peptone water were used for secondary isolation. Suspected colonies were inoculated onto short set of biochemical reaction for identification using Triple Sugar Iron agar, Lysine Iron agar, Urea agar and Sulphide Indole Motility agar [8].

Laboratory tests for heated samples

Another set of dried prawn powder and pickled fish samples were treated by heating the dried prawn powder samples at 70-80°C for 3 min and steaming the pickled fish samples at 90°C for 15 min. Viable bacterial count, coliform, faecal coliform count and detection of pathogenic bacteria were performed by the same laboratory procedure as described above.

RESULTS

Total viable bacterial count

Total viable counts of unheated and heated 20 dried prawn powder samples and pickled fish samples are shown in Table 1. It was found that all unheated samples had total viable bacterial count in uncountable numbers ($>10^5$ CFU/ml). Heating reduced the count 10^3 to 10^4 lower than the count in unheated samples.

Coliform and thermotolerant coliform count of unheated and heated samples

Coliform count and faecal coliform count from 20 unheated and heated samples, expressed in MPN/100 ml are shown in Table 2 and 3. In dried prawn samples, coliform bacteria ranging from 22 to >2400 MPN/100 ml were detected in all unheated

Table 1. Total viable bacterial count CFU/ ml of dried prawn powder and pickled fish samples

Township	Sam-ple	Dried prawn samples		Pickled fish samples	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	UC	5×10^2	UC	5×10^3
	2	7×10^4	1.5×10	UC	6×10^2
	3	3×10^4	2×10^2	UC	7×10^2
	4	5×10^5	1×10^3	UC	9×10^2
North Okkalapa	5	UC	7×10^2	UC	1×10^2
	6	UC	1×10^2	UC	6×10^2
	7	UC	2×10^3	UC	3×10^2
	8	6×10^4	1×10^2	UC	2×10^3
Latha	9	UC	1×10	UC	1.5×10^3
	10	7×10^4	3×10^2	UC	1×10^2
	11	4×10^4	2×10^2	UC	9×10^2
	12	7×10^4	1×10^2	UC	4×10^5
Yankin	13	UC	1.5×10	UC	1×10^3
	14	UC	5×10^3	UC	5×10^3
	15	UC	5×10^2	UC	5×10^2
	16	UC	7×10^4	UC	7×10^4
Dagon	17	UC	2×10^3	UC	2×10^3
	18	UC	7×10^2	UC	3×10^2
	19	2×10^4	1×10^2	UC	1×10^2
	20	3×10^5	1×10^3	UC	1×10^3

UC= Uncountable ($>10^5$ CFU/ml)

samples and thermotolerant coliforms ranging from <2 to 70 MPN/100 ml were detected in 80% (18/20) of unheated samples. After heating at 70-80°C for 3 min, the coliform counts were reduced to $<2-8$ MPN/ml and thermotolerant coliform counts were reduced to $<2-7$ MPN/100ml.

In pickled fish samples, coliforms and thermotolerant coliforms were detected in high count in all unheated samples. After steaming, the coliform count reduced to $<2-240$ MPN/100 ml and faecal coliform count reduced to $<2-23$ MPN/100 ml.

Isolation of contaminated pathogenic bacteria

Among 20 samples of unheated dried prawn powder samples, the pathogenic bacteria were isolated from 60% (12/20) of tested samples; *Escherichia coli* (4/20), *Escherichia coli* and *Citrobacter freundii* (1/20),

Table 2. Coliform and thermotolerant coliform counts of tested unheated and heated dried prawn powder samples (n=20)

Township	Sample	Coliforms MPN/100ml		Thermotolerant coliforms MPN / 100ml	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	46	<2	2	<2
	2	240	8	70	4
	3	110	8	6	<2
	4	140	4	12	<2
North Okkalapa	5	350	<2	2	<2
	6	350	4	24	<2
	7	220	4	7	<2
	8	130	8	9	<2
Latha	9	>1800	8	23	7
	10	540	<2	33	<2
	11	540	8	4	<2
	12	920	8	<2	<2
Yankin	13	540	<2	23	<2
	14	130	<2	7	<2
	15	350	4	4	<2
	16	79	<2	4	<2
Dagon	17	33	<2	<2	<2
	18	22	<2	<2	<2
	19	49	<2	2	<2
	20	94	<2	2	<2

Escherichia coli and *Staphylococcus albus* (1/20), *Klebsiella* spp. (4/20), *Proteus mirabilis* (1/20) and *Staphylococcus aureus* (1/20).

The pathogenic bacteria were not isolated in all contaminated dried prawn powder after heating the samples at 70-80°C for 3 min. Among 20 samples of unheated pickled fish samples, pathogenic bacteria were isolated from 85% (17/20) of tested samples; *Escherichia coli* (6/20), *Citrobacter freundii* (2/20), *Escherichia coli* and *Citrobacter diversus* (1/20), *Klebsiella* spp. (3/20), *Proteus mirabilis* (2/20), *Serratia* spp. (1/20), *Staphylococcus aureus* (1/20) and *Bacillus* spp. (1/20). Some 88.2% (15/17) of pickled fish samples which showed pathogenic bacterial contamination were found to be free of pathogenic bacteria after steaming at 90°C for 15 min. One *Bacillus* spp. contaminated sample and one *Klebsiella* spp. contaminated sample were found to be still contaminated with these bacteria after steaming.

Table 3. Coliform and thermotolerant coli form counts of tested unheated and heated pickled fish samples (n=20)

Township	Sample	Coliforms MPN/100gm		Thermotolerant coliforms MPN / 100gm	
		Unheated	Heated	Unheated	Heated
South Okkalapa	1	>2400	350	>2400	13
	2	>2400	7	220	<2
	3	>2400	9	350	<2
	4	>2400	3	170	<2
North Okkalapa	5	>2400	<2	23	<2
	6	>2400	2	44	<2
	7	>2400	<2	23	<2
	8	>2400	3	37	<2
Latha	9	>2400	240	>2400	23
	10	>2400	4	26	<2
	11	>2400	2	70	<2
	12	240	2	22	<2
Yankin	13	1600	9	23	<2
	14	>2400	34	240	11
	15	540	<2	11	<2
	16	1600	7	23	<2
Dagon	17	920	5	17	<2
	18	350	3	14	<2
	19	>2400	240	110	11
	20	1600	9	26	<2

Serotyping of isolated *E. coli* strains

Serotyping was done on 6 *E. coli* strains isolated from dried prawn powder samples and 7 *E. coli* strains isolated from pickled fish samples. In those of dried prawn samples, 2 isolates were ETEC (serotype O8 K25 and O25K+) and one isolate was EPEC (serotype O86 K61). In those of pickled fish samples, one isolate was EPEC (serotype O1 K51), two isolates were EIEC (serotype O124 K72 and O114 K90) and two isolates were ETEC (serotype O6 K15, O159 K+).

DISCUSSION

Microbiological analysis of food consists of determining coliform and thermotolerant/ faecal coliform count, total viable count and isolation of pathogenic bacteria from the representative samples. Satisfactory microbiological quality means when food is free from pathogens and the indicator bacteria are within the permissible range.

In the present study, coliform count of 46 to 540 MPN/100 ml and thermotolerant coliform count of 23 to 70 MPN/100ml were detected in all tested dried prawn powder samples. Coliform count of 240 to >2400 MPN/100 ml and thermotolerant coliform count of 17 to >2400 MPN/100 ml were detected in all tested pickled fish samples. All dried prawn powder and pickled fish samples had viable bacterial count of 10^3 CFU/ml to uncountable numbers ($>10^5$ CFU/ml).

The average level of bacterial count which can give rise to illness is about $>10^5$ CFU/ml. Some virulent bacteria eg. *Salmonella* spp. causing typhoid can cause disease when 10 CFU/ml of organisms are ingested. It showed that dried prawn powder and pickled fish can be a potential source of food-borne illness if they are contaminated with infectious dose of pathogenic bacteria.

Bacteria were killed if exposed to temperatures above 62.8°C for long enough. Non-spore forming bacteria like enterobacteriaceae, salmonella, shigella, staphylococcus spp. were killed at 62.8°C for 3 min [5]. After heating the dried prawn powder samples up to 70-80°C for 3 min, the coliform count reduced to <2 to 8 MPN/100ml and thermotolerant coliform count reduced to <2-7 MPN/100 ml. After steaming the pickled fish at 90°C for 15 min, the coliform counts were reduced to <2-240 MPN/100 ml and faecal coliform count reduced to <2-23 MPN/100 ml.

All unheated samples had total viable bacterial count in uncountable numbers ($>10^5$ CFU/ml) to ($>10^3$ - 10^4 CFU/ml). Heating of the samples reduced the total viable bacterial count to 10^3 - 10^4 lower than the count in unheated samples. According to the microbiological limits for various food determined by the New Zealand Standard 1998, recommended total standard plate count is 10^4 to 10^5 CFU/ml and thermotolerant coliform count is zero for ready-to-eat food. Thus, heating the dried prawn powder at 70-80°C for 3 min and steaming the pickled fish at 90°C for 15 min can

reduce the bacterial count and make the food quality to a fairly safe level.

This finding is comparable with a study carried out on bacteriological profiles of mohinga (rice noodles with fish soup) which showed significant reduction of the number of bacteria contaminated in mohinga noodles after mixing with hot fish soup [9]. However, there is risk of bacteria recontamination of samples even after heating. Thus, proper processing, handling and storage of food before consuming play a major role in prevention of food-borne diseases. The pathogenic bacteria were isolated from 60% (12/20) of dried prawn samples and 85% of pickled fish samples.

Mensah *et al.* described that the container in which the food was served was also important and the use of paper and leaves increased the risk of contamination [10]. Khin Nwe Oo *et al.* reported that enteric bacteria were isolated from flies, cooked children's foods, drinking water, currency notes and vegetables [11].

In this study, all the samples were taken from local markets with poor hygienic condition where food is sold without covers; the microorganisms can be contaminated from improper handling, uncleaned utensils and containers, contaminated water, dusts, insects and exposure to flies.

Serotyping of isolated *Escherichia coli* strains showed O8 K25, O25 K+, O86 K61, O1 K51, O124 K72, O159 K+, O114 K90 and O6 K15 strains which related to two EPEC, two EIEC and four ETEC. These pathogenic types of *E. coli* can cause serious type of diarrhoea in children and adults.

All dried prawn powder samples and 88.5% of pickled fish samples which showed pathogenic bacterial contamination in unheated samples were found to be free of pathogenic bacteria after heat treatment.

One *Bacillus* spp. contaminated sample and one *Klebsiella* spp. contaminated sample were found to be still contaminated with these bacteria after steaming at 90°C for

15 min. Although heating at 62.8°C for long enough can kill non-spore forming bacteria, spore forming bacteria like *Bacillus* spp. can resist the steaming temperature. One sample which showed *Klebsiella* spp. contamination even after steaming was found to have heavy initial bacterial load, thus steaming cannot kill all the pathogenic bacteria. This study highlighted the poor microbiological quality of dried prawn powder and pickled fish available in markets in Yangon and the findings contribute the scientific information on the benefits of proper heating of food before consuming.

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**Reproductive health communication
between parents and adolescents in North Okkalapa Township**

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Adolescents usually get reproductive and sexuality information mainly from their friends and media, mainly popular print and electronic media. In fact, the family members, especially parents, are often influential sources of knowledge, attitudes and values related to reproduction and sexuality. However, they tend to shy away from actively communicating with adolescents mainly because they lack accurate information about adolescent reproductive issues and skills to communicate these sensitively with their children. The objective of the study was to provide the description of level of communication and identify some associated factors of reproductive health communication between their parents and their adolescents. The face-to-face interviews were done with 91 parents who have at least one adolescent (10-19 years old) child. They were interviewed about their communication on reproductive health and their knowledge, perception and practices related to reproductive health. Eighty-seven percent of the parents communicated on reproductive health issues at least 1 time with their adolescent child during last 6 months but only 30% of the adolescent children communicated on reproductive health issues 4 times and more with their parents during that period.

INTRODUCTION

According to United Nations Demographic Year Book, youths aged 15-24 years constitute about 19% of the total population in Myanmar [1], and 10-19-year-old adolescents form about 20.5% of the population. In Myanmar, the importance of the reproductive health of adolescents and young people is well acknowledged by the government and highlighted in the 'Myanmar Reproductive Health Policy'. There is increasing awareness of the importance of sexual and reproductive health in adolescents worldwide. They are likely to encounter a variety of developmental issues. Among them, reproductive health is one of such major concerns. It is essential that teenagers are equipped with basic knowledge on their reproductive health so that they would be able to make meaningful choices and decisions on matters

concerning their future. Adolescents are vulnerable to sexually transmitted diseases (STDs) including HIV/AIDS and they usually lack accurate information on reproductive health. The Reproductive Needs Assessment (1999) indicated deficiency in RH information among Myanmar adolescents [2].

Most adolescents usually get reproductive and sexuality information mainly from their friends and, to some extent from the media, mainly popular print and electronic media. Nowadays in Myanmar, adolescent reproductive health information and services are being given by various organizations, both private and public, and in most cases the main approach used is peer education. There is a consensus that, to promote the reproductive health, it is needed to address at multiple levels: individual, relational (partner, family), community/institutional and structural (legal, political, economic) [3].

Programme interventions for adolescent reproductive health usually focus on the individual level to increase the knowledge and behaviour change. However, the family level is often overlooked or not properly addressed by the programmes. Expanding the programmatic approach from the individual level to family level may be a more holistic approach, covering the issues like social and relational factors which may be missed when only the individual level is focused.

In fact, the family members, especially parents, are often influential sources of knowledge, attitudes and values related to reproduction and sexuality. Therefore, there is increasing focus on the parents' involvement and their role in adolescent reproductive health. Strong and supportive relationship between youth and parents is essential for emotional and social development of youth. Moreover, parents are role models who shape young people's perception of gender roles and influence the choices that youths make about their own sexual behaviour [4].

However, they feel uncomfortable and tend to shy away from actively communicating with adolescents mainly because they lack accurate information about adolescent reproductive issues and the skills to communicate them sensitively with their children. Conventionally, open discussions do not usually take place between adolescent children and parents/guardians, and parents do not feel free to initiate conversations on sexual and reproductive health even if they think it is necessary to talk with their children regarding these matters. In a study conducted in Myanmar in 1997 on the role of guardians in adolescent reproductive health behaviours, most of the guardians approved of sex education for the adolescents through media, but they did not allow their teens to discuss these matters in front of them or with others [5]. Some 36% were willing to talk with their children about sexual and reproductive health if they were asked. Another study exploring the adolescents reproductive

health needs showed that youths were unlikely to communicate with or receive support from parents [6].

There is some evidence that teens who live in stable family environments and are close to their parents are more likely to remain sexually abstinent, postpone intercourse, have fewer partners, and use contraception [7, 8, 9, 10]. Teens who feel they can talk to their parents in general are more likely to delay sexual intercourse [11].

So, a parental intervention study was carried out in North Okkalapa Township in 2007 to assess the effectiveness of adolescent reproductive health training of parents on reproductive health communication between adolescents and their parents. The main component of the intervention was ARH training of parents mainly covering ARH information, communication skills and parenting skills.

Based on the findings of the preintervention assessment of the parental intervention study, this study tries to describe the level and patterns of reproductive health communication between parents and adolescents and identify some factors associated with this communication.

General objective

- To describe the level of communication and identify some associated factors of reproductive health communication between parents and adolescents in North Okkalapa Township

Specific objectives

- To determine the level of knowledge of adolescent reproductive health among the parents of adolescents
- To explore attitudes of parents to communication about reproductive health matters with adolescent children
- To describe level of reproductive health communication between parents and adolescents
- To identify some associated factors of reproductive health communication between parents and adolescents

MATERIALS AND METHODS

Study design

A cross-sectional study was performed among the parents of adolescents in North Okkalapa Township.

Sample size

This study was the pre-intervention (baseline) assessment of an intervention study for parents. The sample size calculation was originally done for assessing the intervention. We used the proportion of parents who communicate with their adolescent child (ren) on reproductive matters as the variable of main interest for assessment. We assume conservatively that this main variable before the intervention, that is, P_1 is 0.50 because we do not find information on the proportion of parents who communicate with their adolescent children on reproductive matters and that proportion will at least 25% higher at the comparison area at the post test (that is $P_2=0.75$). P will be 0.615 $[(0.75+0.5) / 2]$. If we take alpha error as 5% and beta error as 10%, $Z\alpha$ will turn out to be 1.96 and $Z\beta$ 1.28. Assuming non-response rate as 15%, the required sample size was 91.

Subject recruitment

We recruited parents for the study from 12 wards of North Okklapa Township, who had at least one adolescent child aged 10 to 19 years. In each ward, we randomly selected 8 households from the list of households with at least one adolescent child. Then from the selected households we recruited into the study either parent (father or mother) who agreed to participate in the study. When we could not get required number of parents in a ward, we recruited more parents from the nearest selected ward. Finally we recruited 91 parents into the study.

Data collection

Data were collected by the trained interviewers during July 2007, using a pre-tested structured questionnaire. The trained interviewers collected data on background characteristics, knowledge of reproductive

health, communication on RH matters between parents and adolescents and attitudes of parents to RH communication between parents and adolescents.

Data management and analysis

The completed questionnaires were checked daily for completeness and consistency by the supervisors during data collection and errors were corrected accordingly. Data from the completed questionnaires were entered into the computer using data checking system. The background characteristics were described with proportions. Knowledge level of adolescent reproductive health of parents, attitudes to reproductive health communication with adolescent children and communication of reproductive health matters between parents and adolescents were calculated using proportions. Knowledge level of adolescent reproductive health of parents was compared between sexes, educational status and income levels.

RESULTS

Background characteristics

There were 10 fathers (11%) and 81 mothers (89%) participating in the study. About half of the parents were in their forties and most respondents were mothers over 40 years old. Most respondents were middle or higher school level and housewives. Monthly household income was shown in quintiles (one-fifths of population arrayed in ascending order of income amount) and most respondents' household earn lower than 100,000 kyats monthly. The parents in 1st income quintile had monthly household income of 15,000-60,000 kyats and those in 5th income quintile had monthly household income of 150,001-900,000 kyats.

Knowledge of adolescent reproductive health

Table 1 shows knowledge level of 3 main areas of reproductive health among parents by selected socioeconomic characteristics. High level of knowledge of pubertal changes, contraceptive and HIV/STIs is defined as

Table 1. Knowledge level of adolescent reproductive health (ARH) among the parents by their education and income level, North Okkalapa Township, 2007

	High knowledge of puberty (%)	High knowledge of contraceptive (%)	High knowledge of HIV and STIs (%)	Overall high knowledge of ARH* (%)
<i>Education</i>				
Primary (n=14)	2 (14.3)	1 (7.1)	1 (7.1)	1 (7.1)
Middle (n=30)	6 (20.0)	3 (10.0)	6 (20.0)	4 (13.3)
Higher (n=39)	12 (30.8)	9 (23.1)	4 (10.3)	6 (15.4)
University (n=8)	1 (12.5)	1 (12.5)	1 (12.5)	0 (00.0)
<i>Income quintile</i>				
1st (n=27)	6 (22.2)	1 (3.7)	2 (7.4)	1 (3.7)
2nd (n=11)	3 (27.3)	3 (27.3)	2 (18.2)	3 (27.3)
3rd (n=24)	6 (25.0)	5 (20.8)	2 (8.3)	2 (8.3)
4th (n=15)	2 (13.3)	2 (13.3)	3 (20.0)	2 (13.3)
5th (n=14)	4 (28.6)	3 (21.4)	3 (21.4)	3 (21.1)
Total (n=91)	21 (23.1)	14 (15.4)	12 (13.1)	11 (12.1)

correct answers to over half of 33, 11 and 13 questions on pubertal changes, contraceptive and HIV/STIs, respectively. High level of overall ARH knowledge is defined as correct answers to over half of 57 reproductive health knowledge items. Only a few parents had high level of knowledge on reproductive health. There is no definite linear trend of increase in knowledge level along increasing trend of education level or income quintile. The difference in knowledge levels between varying education levels and income quintiles is not statistically significant at 0.05 level.

Attitudes to communication of reproductive health

Almost all parents in the study thought that it is a good thing to communicate about reproductive health matters with adolescent children. Most parents believed that their adolescent children would come to them for information on reproductive health matters they want to know. But self-efficacy level of parents in communicating RH matters with adolescents was low (35%) (Table 2).

Table 2. Attitudes to RH communication between parents and adolescents, North Okkalapa Township, 2007

	Fathers (%) (n=10)	Mothers (%) (n=81)	All parents (%) (n=91)
I agree that we should discuss with our adolescent children on reproductive health matters	10 (100.0)	79 (97.5)	89 (97.8)
I think my adolescent children will come and discuss with me if they want to know about some RH matters.	5 (50.0)	64 (79.0)	69 (75.8)
I can discuss well with my adolescent child on RH matters.	4 (40.0)	28 (35.0)	32 (35.2)

Table 3. Communication of parents about reproductive health with adolescent children, North Okkalapa Township, 2007

Communication pattern	Not at all in previous 6 months (%)	1-3 times in previous 6 months (%)	4+ times in previous 6 months (%)
RH communication with adolescent sons (n=62)	36 (58.1)*	20 (32.3)	6 (9.7)
RH communication with adolescent daughters (n=69)	12 (17.4)*	36 (52.2)	21 (30.4)
RH communication with adolescent children (n=91)	12 (13.2)	51 (56.0)	28 (30.8)

*Chi-squared =23.3, P=0.0001

Parent-adolescent communication

Table 3 shows the communication of parents about reproductive health matters with adolescent children in previous 6 months. Over 80% of the parents talked at least one time with their adolescent children. But level of communication between parents and their adolescent daughters (82.6%) was much higher than that between parents and their adolescent sons (42%). This finding may partly be affected by the fact that mothers made up the great majority of the study population.

Topics of parent-adolescent communication

The most frequently communicated topic with adolescent sons was HIV and STIs followed by romantic love, practice of abstinence, condom use, reproductive organs and contraceptives. The most frequently com-

municated topic with adolescent daughters was menstruation followed by HIV and STIs, romantic love, pubertal changes and reproductive health organs. In fact, more important topics for preventive behaviour like condom use and contraceptive use were not common ones for communication between parents and adolescents.

Table 4. Associated factors for parental communication with adolescent children, North Okkalapa Township, 2007

Characteristics	Number of parents who communicated RH matters with adolescent children (%)	p value
Sex		
Fathers (n=10)	8 (80.0)	0.50
Mothers (n=81)	71 (87.7)	
Age (years)		
30-39 (n=13)	11 (84.6)	0.53
40-49 years (n=51)	46 (90.2)	
50+ years (n=27)	22 (81.5)	
Education		
Primary (n=14)	14 (100)	0.16
Middle (n=30)	26 (86.7)	
Higher (n=39)	31 (79.5)	
University (n=8)	8 (100)	
Income quintile		
1 st (n =27)	25 (92.6)	0.52
2 nd (n =11)	10 (90.9)	
3 rd (n =24)	19 (79.2)	
4 th (n =15)	12 (80)	
5 th (n =14)	13 (2.9)	
Knowledge of reproductive health		
Low (n=80)	69 (86.3)	0.6
High (n=11)	10 (90.9)	
Self-efficacy to communicate with adolescent children		
Low (n=59)	47 (79.7)	0.006
High (n=32)	32 (100)	

Associated factors for parental communication with adolescent children

Table 4 shows association between parental communication with adolescent children and some selected characteristics. Age, sex, education level, income level and knowledge level of reproductive health have no significant association. But high level of self-efficacy of parents to communicate with

adolescent children was significantly associated with high level of communication with adolescent children on reproductive health matters.

DISCUSSION

In Myanmar society, parents and children rarely discuss on reproductive health issues as this is perceived as an inappropriate thing to talk and is culturally sensitive. This study tried to find out the level of communication between parents and their adolescent children on reproductive health, as part of an intervention to promote the RH communication among the family members. The findings indicated that most parents in the study talked with their adolescent children about reproductive health matters. They talked more with adolescent daughters on reproductive health matters than with adolescent sons. This finding is partly attributable to the fact that most parents in the study were mothers. Maybe one explanation is that mothers think daughters are more vulnerable to bad consequences of inappropriate reproductive decisions and are more likely to communicate reproductive health issues with daughters.

Traditionally, mothers talk with their daughters about nature of menstruation, how to keep clean and to make it unseen and unknown to male members of the family. And this seems to be the least sensitive RH issue to talk between mother and daughter. Expectedly, this study also found out that menstruation is the most frequently discussed topic with the adolescent girls (84.2%) followed by the topic on HIV/AIDS (26.3%). With the adolescent boys, HIV/AIDS is the most discussed RH issue followed by romantic love and abstinence.

The high overall level of parental communication about reproductive health (over 80%) is mainly due to the discussion of HIV/STIs in boys and menstruation in girls. Level of communication about other important topics like condom and contraceptive use and pubertal changes was low. So their

reproductive health communication did not cover all important areas of adolescent reproductive health. A study conducted in Mandalay between father-son pairs in 2005 also revealed that only 23% of fathers talked about condom during sex to their sons [12].

All parents felt the need that they should discuss with their adolescent children on reproductive health matters. However, their knowledge of reproductive health and their self-efficacy about communicating with adolescent children was low. Only 12.9% of the study population had high ARH knowledge, however, the study revealed that there is no association between the level of knowledge and communication. Majority of parents with low level of knowledge are found to be communicating with adolescents on RH issues.

These findings indicate that parents need informational support and communication skills building to successfully communicate correct reproductive messages to their adolescent children. So, it is clear that it is necessary to introduce innovative parental interventions that equip them with essential adolescent reproductive health information and communication skills with adolescents to contribute to healthy and happy development of adolescents.

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Cloning of the hepatitis B surface antigen gene (1): extraction and transformation of the recombinant HBsAg gene containing plasmid into the *E. coli* DH5 alpha cells

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The majority of currently available recombinant hepatitis B (HB) vaccines in the market have been produced by using appropriate expression plasmids in *Saccharomyces cerevisiae* and *Hansenula polymorpha* yeast cells as host systems. Recently, attempts were made to use *Pichia pastoris* as an alternative and more productive host for cloning of yeast expression plasmid carrying the hepatitis B surface antigen (HBsAg) structural gene for production of recombinant HB vaccines in the future. DNA cloning involves separating a specific gene or DNA segment from a larger chromosome, attaching it to a small molecule of carrier DNA, and then replicating this modified DNA thousands or millions of times through both the increase in cell number and the creation of multiple copies of the cloned DNA in each cell, followed by expression of desired protein. In this study, the HBsAg gene was first extracted and purified from chromosomal DNA of *H. polymorpha* transformant cells. It was then ligated with pGEM-T vector followed by transformation into the competent *E. coli* DH5 alpha cells which had already been prepared in our laboratory. The results from each and every steps were confirmed by direct PCR identification, restriction enzyme analysis followed by agarose gel electrophoresis determination and DNA sequencing analysis. The nucleotide sequences of the HBsAg gene, extracted and purified from the final transformant *E. coli* were found to be totally identical to that of the HBsAg gene which had been initially extracted and purified from *H. polymorpha* cell. Research works on amplification and ligation of the HBsAg gene and yeast plasmid in *E. coli* DH5 alpha cells for further transformation of the recombinant HBsAg gene containing plasmid into the *P. pastoris* yeast cells are in progress at the Research and Development Centre of Pharmaceuticals, Institute of Science and Technology, CJ Corporation, Ichon City, Republic of Korea.

INTRODUCTION

‘Genes’ are the fundamental units of genetic information in living system. ‘DNA’ is the chemical basis of hereditary and is organized into genes. Biochemically, a ‘gene’ is defined as a segment of DNA (or, in few cases, RNA) that encodes the information required to produce a functional biological product. The final product is usually a protein. Therefore, genes control the synthesis of various types of protein [1]. Advances in molecular genetics and nucleic acid chemis-

try have made possible to identify genes coding for biologically active substances, to analyze them in detail, to transfer them within and between organisms, and to obtain gene expression under controlled conditions with efficient synthesis of the encoded product [2]. To clone means to make identical copies. Cloning of DNA from any organism entails five general procedures; cutting of DNA at precise location by restriction endonuclease (RE), joining two DNA fragments covalently by DNA ligase to construct the recombinant DNA, selecting a

small molecule of DNA capable of self-replication, moving recombinant DNA from the test tube to a host cell, and selecting or identifying host cells that contain recombinant DNA. A gene that codes for a specific product can be isolated and propagated by insertion into a suitable vector with the aid of highly specific RE enzymes which cleave the vector DNA at predetermined sites, and ligases which join the gene insert to the vector. The vector can then be introduced into host organisms and individual clones that carry the desired gene can be selected and propagated in mass culture [3].

For development of currently available recombinant hepatitis B (HB) vaccines, hepatitis B virus (HBV) recovered from plasma of a hepatitis B carrier has been used to prepare viral DNA. That DNA has been cloned in *Escherichia coli* and the gene coding for hepatitis B surface antigen (HBsAg) has been isolated. This gene has been inserted into yeasts by means of appropriate expression vectors. Purified HBsAg proteins expressed from transfected yeast cells have been formulated into HB vaccines and shown to induce protective antibody response in human [2]. The majority of currently available recombinant hepatitis B (HB) vaccines in the market have been produced by using appropriate expression plasmids in *Saccharomyces cerevisiae* and *Hansenula polymorpha* yeast cells as host systems. In this study, an attempt was made to extract the HBsAg gene from the transformed *Hansenula polymorpha* yeast cells containing it which was then transformed into the competent *E. coli* cells for further transformation into final host, *Pichia pastoris* yeast cells with an aim to produce recombinant HB vaccine in the near future by using as an alternative and a more productive strain.

MATERIALS AND METHODS

HBsAg structural gene

The HBsAg gene was extracted and purified from the Master Cell Bank (MCB) con-

taining the structural gene coding for the HBsAg protein, integrated in the genomic DNA of the *H. polymorpha* transformant yeast cells for production of recombinant HB vaccine. It was provided by the CJ Pharmaceutical Corporation, Republic of Korea. The HBsAg gene consists of 678 base pairs, coding the hepatitis B surface antigen (S) protein with 226 amino acids [4].

Host strain

E. coli DH5 alpha strain, grown in Luria-Bertani (LB) media supplemented with ampicillin and stored at -20°C was used in cloning process for plasmid amplification. This media was prepared under the clean bench by addition of 10 gm tryptone, 5 gm yeast extract, 10 gm sodium chloride, 15 gm agar and 1 ml ampicillin solution (stock concentration of 100 mg/ml) into deionized water to make a final volume of 1 liter, followed by plating [5].

Expression vectors

The pGEM-T vector (3003bp) with terminal thymidine to both ends containing ampicillin resistance gene was commercially available from the Promega Co, USA [6].

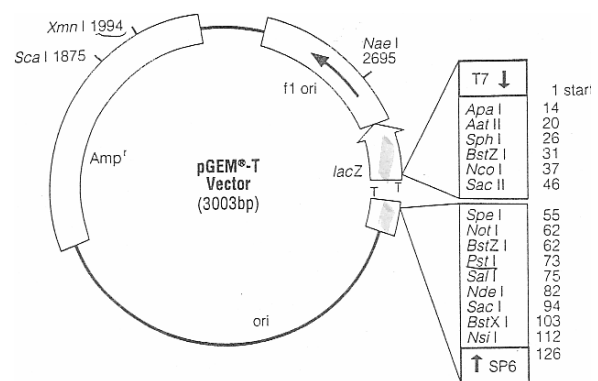


Fig. 1. Physical map of plasmid pGEM- T(3003 bp)

Extraction and purification of the HBsAg gene from *H. polymorpha* cell

First, the HBsAg coding DNA segment, integrated in the chromosomal DNA of *H. polymorpha* transformant cell from Master Cell Bank (MCB) was extracted by using the QIAGEN test kit, USA [7]. The extracted DNA segment containing the gene

of interest was amplified by Thermocycler using; PCR reaction buffer, dNTPs, DNA template, polymerase enzyme, double distilled water, and specific primers; AY21 F (5'-ATG GAG AAC ATC ACA TCA GGA-3' and CTR (5'-CTC TTT GTT TTG TTA GGG T-3'), with an annealing temperature of 45°C and repeated cycles of 30. The DNA segments were purified by using PCR purification test kit from QIAGEN. The purified HBsAg gene of 678 base pairs was confirmed by agarose gel electrophoresis and DNA sequencing analysis by using the ABI Prism 3100 Genetic Analyzer [8].

Ligation of the HBsAg gene and plasmid pGEM-T

To obtain the plasmid-insert DNA construct, the above purified HBsAg gene was ligated to the commercially available plasmid pGEM-T vector which had already been linearized with *EcoRV* at base 51, by using T4 ligase enzyme at 16°C for 3 hours followed by 4°C for overnight [9].

Preparation of competent E. coli DH5 alpha cells

Just before transformation, the competent *E. coli* DH5 alpha cells were prepared by calcium chloride method [10]. For efficient transformation, it is essential that number of viable cells should not exceed 10^8 cells per milliliter for which *E. coli* is equivalent to a OD of 0.35–0.45 at which these cells are competent for an efficient transformation.

Transformation of the HBsAg gene containing plasmid into E. coli cells

The recombinant product, plasmid-insert DNA construct, was then transformed into competent *E. coli* DH5 alpha cells by using the heat-shock procedure. i.e 42°C for 90 seconds without shaking [9]. It is well known that only transformed cells containing the ampicillin resistance gene (*AMP*) on the plasmid vector can survive on an indicator plate containing LB media with ampicillin. In our study, transformants containing recombinant genes were identified by the appearance of white colonies whereas non-

transformed colonies showed blue colour with a relatively smaller in size on the indicator plates. The closed circular recombinant plasmid of about 3700 base pairs was then extracted by using AccuPrep plasmid extraction kit (Bioneer) [11] and confirmed by RE analysis with *EcoRI* followed by agarose gel electrophoresis. The presence of the HBsAg structural gene in this plasmid-insert DNA construct was also detected by direct colony PCR identification (using primers AY21F and CTR) followed by purification with PCR purification QIAGEN test kit [7]. The purified HBsAg gene of 678 base pairs was confirmed by agarose gel electrophoresis determination and DNA sequencing analysis by using the ABI Prism 3100 Genetic Analyzer [8].

RESULTS

Electrophoretic identification of the HBsAg gene, extracted from the chromosomal DNA of *H. polymorpha* transformant (MCB of CJ Corporation) followed by PCR purification, is shown in Fig. 2.

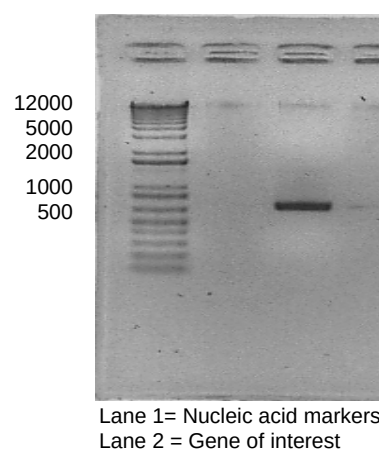


Fig 2. Electrophoretic identification of the HBsAg gene, extracted from the genomic DNA of *H. polymorpha* transformant

The distinct DNA band was observed at 700 base pairs level and consistent with the number of base pairs identified in the HBsAg gene. Fig. 3 illustrates the nucleotide sequences of the DNA segment, extracted from the chromosomal DNA of *H. polymorpha* transformant, analyzed by using the ABI

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ATGGAGAACA TCACATCAGG ATTCCTAGGA CCCCTGCTCG TGTTACAGGC
GGGGTTTTTC TTGTTGACAA GAATCCTCAC AATACCGCAG AGTCTAGACT
CGTGGTGGAC TTCTCTCAAT TTTCTAGGGG GAACCTACCGT GTGTCTTGGC
CAAAATTCGC AGTCCCCAAC CTCCAATCAC TCACCAACCT CCTGTCTCC
AACTTGTCCT GGTATCGCT GGATGTGTCT GCGGCGTTTT ATCATCTTCC
TCTTCATCCT GCTGCTATGC CTCATCTTCT TGTGGTTCT TCTGGACTAT
CAAGGTATGT TGCCCGTTG TCCTCTAATT CCAGGATCTT CAACTACCAG
CACGGGACCA TGCAGAACCT GCACGACTCC TGCTCAAGGA ACCTCTATGT
ATCCCTCCTG TTGCTGTACC AAACCTTCGG ACGGAAATTG CACCTGTATT
CCCATCCCAT CATCTGGGC TTTCCGAAAA TTCCTATGGG AGTGGGCCTC
AGCCCGTTTC TCCTGGCTCA GTTTACTAGT GCCATTGTGT CAGTGGTTGC
TAGGGCTTTC CCCACTGTG TGCTTTTCAG TTATATGGAT GATGTGGTA1
TGGGGGCCAA GTCTGTACAG CATCTTGAGT CCCTTTTAC CGCTGTTACC
AATTTTCTTC TGTCTTTGGG TATACATT

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Fig. 3. Nucleotide sequence of the HBsAg gene extracted from *H. polymorpha* transformant (678 base pairs)

Prism 3100 Genetic Analyzer. The nucleotide sequences obtained were identical to that of the HBsAg gene. On determination of the optical density of cultured *E. coli*, it was found to be 0.38, indicating the competency of *E. coli* cells for an efficient transformation. Electrophoretic identification of the extracted plasmid-insert DNA construct, transformed into *E. coli* cell, is demonstrated in Fig. 4.

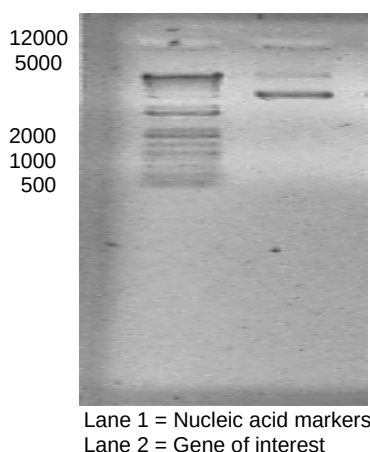


Fig. 4. Electrophoretic determination of the extracted plasmid-insert DNA construct in *E. coli* cell

The DNA band was observed at the level of about 3700 base pairs, indicating the formation of cloned DNA in the transformant. Direct colony PCR identification of the HBsAg DNA segment, transformed into

E. coli cell by using the plasmid pGEM-T vector in agarose gel electrophoresis, is depicted in Fig. 5.

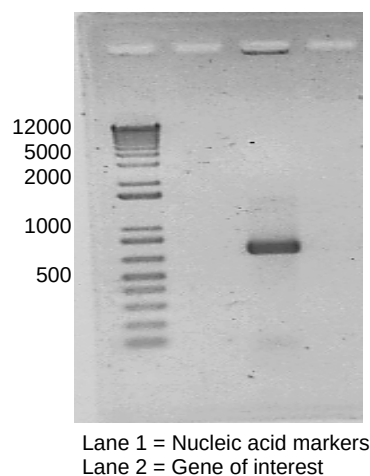


Fig. 5. Direct colony PCR identification of the HBsAg coding DNA segment in the *E. coli* transformant

The distinct DNA band was detected at the expected level of 700 base pairs, confirming the presence of the DNA of interest in the transformant. The nucleotide sequence of the purified HBsAg segment, extracted and purified from transformant *E. coli* cell, was analyzed by using the ABI Prism 3100 Genetic Analyzer. The nucleotide sequences obtained were found to be identical to that of the HBsAg gene initially extracted and purified from chromosomal DNA of *H. polymorpha* cell as illustrated in Fig. 2.

DISCUSSION

E. coli are prokaryotic unicellular, gram-negative bacilli, and usually grow readily on ordinary culture media. They are non-pathogenic in their habitat. The length of a typical *E. coli* cell is 2 μm and its chromosome is a single double-stranded circular DNA molecule. The *E. coli* is the first organism used for recombinant DNA work and still the most common host cell. *E. coli* has many advantage: its DNA metabolism and many other biochemical processes are well understood, many naturally occurring cloning vectors and plasmids associated with *E. coli* are well characterized and effective

techniques are available for moving DNA from one bacterial cell to another [1]. Yeasts are eukaryotic, unicellular, microscopic and usually spherical in shape with diameter of 3-15 μm . Plasmids are circular DNA molecules that replicate separately from the host chromosome. They can be introduced into bacterial cells by a process called transformation [10].

Pichia pastoris is one of the methylotrophic yeast cells, capable of metabolizing methanol as its sole energy and carbon source. It is also well documented that *P. pastoris* has many of the advantage of higher eukaryotic expression system such as protein procession, protein folding and post translational modification. The heterologous expression in *Pichia* can be either intracellular or secreted. The major advantage of expressing heterologous protein as secreted protein is that it secretes very low level of native proteins. Therefore, the secreted heterologous protein comprises the vast majority of the total protein in the medium and serves as the first step in purification of protein [12, 13, 14, 15]. However, the yeast plasmid amplification can never be achieved in yeast cells, an initial plasmid construction was performed in *E. coli* cells before transfer of plasmid-insert DNA construct (recombinant HBsAg gene) into *P. pastoris* [1].

In our study, we extracted and purified the HBsAg gene from genomic DNA of *H. polymorpha* transformant cell. It was then ligated with pGEM-T vector followed by transformation into the competent *E. coli* DH5 alpha cells which had already been prepared. The results from each and every steps were confirmed by direct PCR identification, RE analysis followed by agarose gel electrophoresis determination and DNA sequencing analysis. It was found that the nucleotide sequences of the HBsAg gene, extracted and purified from final transformant *E. coli* were identical to that of the HBsAg gene which had been initially extracted and purified from chromosomal DNA of *H. polymorpha* cell, indicating the successful transformation of the HBsAg

gene into the *E. coli* cells for further cloning process .

This study described the possible use of *E. coli* DH5 alpha cells as an intermediate host for cloning of the HBsAg gene containing plasmid prior to the transformation into an ultimate host, *P. pastoris* for expression of HBsAg protein. Research works on amplification and ligation of the HBsAg gene and yeast plasmid in *E. coli* DH5 alpha cells for further transformation of the recombinant HBsAg gene containing plasmid into the *P. pastoris* yeast cells are in progress at the Research and Development Centre of Pharmaceuticals, Institute of Science and Technology, CJ Corporation, Ichon City, Republic of Korea.

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A clinicopathological study of myelodysplastic syndromes in YGH

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The clinical and haematological studies of 36 cases of myelodysplastic syndromes (MDS), none of which had previously received chemotherapy or radiotherapy were studied. They were classified according to FAB classification. There were 19 males and 17 females in age ranging from 22 to 87 years. Among them, there were 24 cases of refractory anemia (RA), 4 cases in each of refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-t) and chronic myelomonocytic leukaemia (CMML). Symptoms of anemia were present in 97.2%, infective manifestations in 55.6%, bleeding manifestations in 38.9%, hepatomegaly in 11.2%, splenomegaly in 8.4% and lymphadenopathy in 5.6% of patients. Haematological findings showed that pancytopenia in 61.1%, combination of anemia and thrombocytopenia in 22.2%, combination of anemia and leucopenia in 11.1% and anemia only in 5.6% of MDS patients. In the bone marrow examination, hypercellularity in 27 cases, hypocellularity in 9 cases and dyshaemopoiesis involving two or more cell lines were seen in all cases.

INTRODUCTION

The term myelodysplastic syndromes was introduced in 1975 by a group of French, American, and British haematologists (FAB group) to describe a group of disorders with characteristic abnormalities of peripheral blood and bone marrow morphology and impaired bone marrow function, which tend to evolve into acute myeloid leukaemia (AML) [1]. MDS arises in two distinct settings: Idiopathic or primary MDS which, occurring mainly in patients over age 50, often develops insidiously and therapy related MDS (TR-MDS) or secondary MDS which is a complication of previous myelo-suppressive drug or radiation therapy, usually appears 2 to 8 years after exposure [2]. Although the patients present with infections, bleeding, bruising, progressive fatigue, dyspnoea on exertion, many patients present without symptoms but with anemia, thrombocytopenia, leucopenia, or a combination of these findings on routine laboratory evaluation [3]. The MDS can be

diagnosed only by a haematologist, primarily on the basis of characteristic full blood count indices, morphological abnormalities on the peripheral blood film, and characteristic bone marrow appearances [4]. The FAB classification has been widely used since 1982. It is useful in predicting rates of survival and transformation to AML. There are five subtypes of MDS in FAB classification [5] (Table 1).

This classification allows to set delineate prognosis and adequate stratification of the majority of patients with these subgroups. A precise diagnosis and the determination of prognostic indicators are central to the establishment of a treatment plan [3]. Treatment options in MDS are limited. In younger patients, allogeneic bone marrow transplantation offers some hope for reconstitution of normal haemopoiesis and long-term survival. Older patients with MDS are treated supportively with antibiotics and blood products transfusions. The median survival in primary MDS varies from 9 to 29 months, but some individuals in good

Table 1. FAB classification of MDS

Morphological subtype	Prevalence(%)	Peripheral blood	Bone marrow
Refractory anemia (RA)	12-43	Blasts <1%	Blasts <5%
Refractory anemia with ring sideroblasts (RARS)	14-37	Blasts <1%	Blasts <5%; >15% of NRBC are ring sideroblasts
Refractory anemia with excess blasts (RAEB)	13-43	Blasts <5%	Blasts 5-19%
Refractory anemia with excess blasts in transformation (RAEB-t)	4-27	Blasts >5% or Auer rods present	Blasts 20-29% or Auer rods present
Chronic myelomonocytic leukaemia (CMML)	1-22	As any of above but with $>1 \times 10^9/L$ monocytes	As any of above with or without an increase in monocytes or promonocytes

prognostic groups may live for 5 years or more. Overall progression to AML occurs in 10-40% of individuals. The overall median survival of TR-MDS is only 4 to 8 months and progress rapidly to AML [2]. There have been a lot of international literature regarding MDS since many decades ago. MDS is not uncommon in Myanmar. However, there were a few studies on MDS in our country [6, 7, 8]. Therefore, this study was conducted with an aim to determine the clinical and haematological features of patients with MDS classified according to the FAB classification.

MATERIALS AND METHODS

This was a hospital-based descriptive study which was conducted at General Medical Wards and Department of Clinical Haematology, Yangon General Hospital from August 2003 to July 2004. Prior to the study, an approval from the institutional ethical committee, University of Medicine 1 was obtained. A total of 36 patients with MDS were included in this study. Careful history taking, clinical examination, evaluation of laboratory investigation and bone marrow aspiration were performed on each patient, and trephine biopsy was done in those patients with hypocellular MDS and recorded as in proforma.

RESULTS AND DISCUSSION

During the one-year study period, there were 36 newly diagnosed cases among hospitalized patients in General Medical Wards and Department of Clinical Haematology in YGH. The mean age $\pm$ SD was found to be 49.8 ± 16.4 (range 22-87 yrs). Generally, more than half of the patients (20 cases) were between 20-50 years. In the present study, affected age group was more common in younger rather than old age group. However, there was no apparent sex preponderance with a ratio of (male : female -1.1:1). Out of 36 cases, 24 cases (66.4%) were RA, and 4 cases (11.2%) each were RAEB, RAEB-t and CMML (Table 2).

Table 2. Distribution of MDS according to FAB classification

Morphological subtype	No. of patients
RA	24 (66.4%)
RAEB	4 (11.2%)
RAEB-t	4 (11.2%)
CMML	4 (11.2%)
Total	36

There was no case of RARS in the present study. Among 24 cases of RA, 5 cases were bilineage MDS, and 19 cases were trilineage MDS. There was no bilineage MDS in other types of MDS in this study.

On studying the clinical manifestations, 36 patients (100%) presented with symptoms of anemia, 20 patients (55.6%) presented with infective manifestations and 14 cases (38.9%) presented with bleeding manifestations. Hepatomegaly was seen in 11.2%, splenomegaly in 8.4% and lymphadenopathy in 5.6% (Table 3). Regarding the haematological manifestations, pancytopenia was present at the time of diagnosis in 61.1%, combination of anemia and thrombocytopenia in 22.2%, combination of anemia and leucopenia in 11.1% and anemia only in 5.6% (Table 4). In this study, bone marrow aspiration was done in all cases and trephine biopsy was done in only one case. Hypercellularity of bone marrow was seen

Table 3. Clinical manifestations in different types of MDS

Types	Anaemia (%)	Infection (%)	Bleeding (%)	Hepatomegaly (%)	Splenomegaly (%)	Lymphadenopathy (%)	Total
RA	24 (100)	13 (54.2)	9 (37.5)	-	-	-	24
RAEB	4 (100)	3 (75)	-	1 (25)	-	-	4
RAEB-t	4 (100)	3 (75)	2 (50)	1 (25)	1 (25)	1 (25)	4
CMML	4 (100)	1 (25)	3 (75)	2 (50)	2 (50)	-	4
Total	36 (100)	20 (55.6)	14 (38.9)	4 (11.2)	3 (8.4)	1 (5.6)	36

Table 4. Haematological findings in different types of MDS

Types	Pancytopenia	Anemia & thrombocytopenia	Anemia & leucopenia	Isolated anemia
RA	15	5	2	2
RAEB	2	-	2	-
RAEB-t	4	-	-	-
CMML	1	3	-	-
Total	22 (61.1%)	8 (22.2%)	4 (11.1%)	2 (5.6%)

in 27 patients (75%) and hypocellularity was seen in 9 patients (25%). Dyserythropoiesis was seen in 34 cases (94.4%), dysgranulopoiesis in 33 cases (91.7%) and dysmegakaryopoiesis in 33 cases (91.7%). In the present study, 86.1% of cases presented with trilineage MDS (dyserythropoiesis, dysgranulopoiesis and dysmegakaryopoiesis) and 13.9% of cases presented with bilineage MDS (dyserythropoiesis with dysgranulopoiesis or dysmegakaryopoiesis).

In conclusion, the present study described the clinicopathological findings of MDS in Myanmar according to FAB classification, based on the percentage of blast cells and ring sideroblasts in the bone marrow and presence or absence of a raised peripheral blood monocyte count [9]. It is a well-known fact that the most significant independent variables for determining outcome for both survival and AML evolution are marrow blast percentage, number of

cytopenias, and cytogenetics [10]. Although cytogenetic study is compulsory, at present, it is inaccessible in our country. Therefore, cytogenetic analysis of MDS cases are recommended for further study in Myanmar. Besides, study on the natural course of MDS should be carried out in the future. However, it is expected that the present data obtained from this preliminary study might be valuable for clinicopathological field in our country.

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