MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

AIMS OF THE JOURNAL

- To serve as an important medium for the publication of original research work in the field of medical science and health research, thus filling gaps in health knowledge for effective utilization of research findings
- To disseminate recent basic, applied and social research findings among health personnel of different strata for enhancing nation-wide health development in Myanmar
- To offer current medical knowledge and updated scientific information obtained from research to health professionals for better and appropriate health care management

EDITORIAL

It is a great pleasure to present this issue of Myanmar Health Sciences Research Journal in welcoming the Golden Jubilee Anniversary of the Department of Medical Research (Lower Myanmar) which was established in June 1963. The MHSR Journal has been published quarterly since April 1989 and a total of 653 scientific articles were published in 59 issues.

The issue of MHSR Journal (Vol. 24, No. 2) consists of ten long articles and one short article on various subjects. The leading article is about the occurrence of *Bacillus cereus* in milk and milk products, and its food poisoning is one of the public concerns. Milk is an important food with many nutrients and a key contributor to improving nutrition and food security in developing countries. *Bacillus cereus* is a food borne pathogenic bacterium which can form heat-resistant spores and produce toxins that cannot be destroyed by pasteurization. Consuming milk contaminated with *Bacillus cereus* or its toxin may cause food poisoning symptoms such as vomiting and diarrhoea. Therefore, it becomes increasingly clear that the implementation of Good Manufacturing Practices (GMP) followed by in-process control, and refrigeration at safe temperature during transportation and storage are essential for milk safety management system. This leading article has mentioned the detailed antimicrobial susceptibility pattern of *Bacillus cereus* in milk and milk products and the findings of this study will be very useful in the management of milk-borne infection related with this organism.

We are most confident that the findings of research articles in this issue will contribute towards sustaining health development and improving health equity in our country. Finally, we would like to thank all the authors and co-authors for their efforts in preparing and submitting the articles for this issue of the MHSR Journal.

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A Study of *Bacillus cereus* in Milk and Milk Products

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The study of *Bacillus cereus* in milk and milk products was conducted from January 2011 to October 2011. The samples examined were milk, yogurt and ice-cream. Total 90 samples of milk and milk products (thirty samples each of milk, yogurt and ice-cream) were tested. The total bacterial count of milk samples, yogurt samples and ice-cream samples ranged from 0 to 10^6cfu/g. Fifty-six percents of milk and milk product samples fell in unsatisfactory category, 35% fell in marginal category and 9% fell in satisfactory category of the total bacterial count. *Bacillus cereus* was isolated from 30% (9/30) of milk [30% (3/10) from pasteurized milk, 10% (1/10) from boiled milk, 50% (5/10) from raw milk], 3.33% (1/30) of yogurt and 50% (15/30) of ice-cream samples. Twelve antimicrobials were tested against 25 isolates of *Bacillus cereus*. Above 75% of isolates were sensitive to chloramphenicol, ciprofloxacin, gentamicin and azithromycin. All isolates of *Bacillus cereus* were resistant to penicillin-G and cefotaxime. The intermediate sensitive patterns were found 44% (11/25) in co-trimoxazole, 4% (1/25) in chloramphenicol, 4% (1/25) in ciprofloxacin, 24% (6/25) in kanamycin, 12% (3/25) in azithromycin, 4% (1/25) in ceftriaxone and 28% (7/25) in erythromycin sensitivity tests. Ninety-six percent (24/25) and 92% (23/25) of isolates were resistant to ceftriaxone and amoxicillin-clavulanic acid, respectively. This study contributes to the understanding of occurrence of milk-borne infection related with *Bacillus cereus* and its antibiotic sensitivity pattern.

INTRODUCTION

Bacillus cereus is one of the causal agents of the foodborne diseases. It is aerobic, but facultative anaerobic, generally mesophilic, motile rod that produces heat-resistant spores. Spores survive in freezing and drying. Some strains require heat activation for spores to germinate and grow. *B. cereus* is ubiquitous and present in soil, vegetation, water and dust. It has been isolated from a large variety of foods, including vegetables, meats (There had been a large outbreak that was traced to beef stew.), cereals, pasteurized milk and powdered milk.[1, 2]

Bacillus cereus is a common contaminant in a wide variety of foods, including milk and dairy products, cereals (especially rice), and food additives.[3] There are only a few outbreaks a year reported by Center of Disease Control (CDC). Between 1972 and 1986, 52 outbreaks of foodborne disease associated with *Bacillus cereus* were reported to CDC.[4] *Bacillus cereus* can cause two types of foodborne illness; a diarrhea syndrome first described by Hauge[5], and an emetic syndrome.[6] Both syndromes have occasionally been associated with dairy products.[7] Food poisoning caused by *Bacillus cereus* is a toxin-mediated disease, rather than infection. It has been increasing in frequency in recent years.[1, 8]

Bacillus cereus food poisoning is one of the public concerns. The current study was carried out because there had not yet been known documented data concerned with *Bacillus cereus* food intoxication outbreaks related to milk and milk products

consumption in Myanmar. This study contributes to the management of foodborne disease by giving the knowledge of occurrence of food poisoning caused by *Bacillus cereus* and its antibiotic susceptibility.

General objective

- To study the occurrence of *Bacillus cereus* in milk and milk products and its antibiotic sensitivity pattern

Specific objectives

- To isolate and identify *Bacillus cereus* from milk, yogurt and ice cream
- To determine the most probable number (MPN) of *Bacillus cereus* per gram of food sample
- To differentiate typical strains of *Bacillus cereus* from other Bacillus species
- To determine antibiotic sensitivity pattern of isolated *Bacillus cereus* and other Bacillus species

MATERIALS AND METHOD

Collection of specimens

50 grams each of dairy product samples were collected in sterile plastic bags. Samples were put in an ice box and delivered to the laboratory immediately. After that the samples were processed at the laboratory.

Total bacteria count of samples

Total bacteria count was determined by surface viable bacteria count, micro method.[9]

Isolation and identification

Under aseptic technique, 50 gm of each sample were weighed, and mixed with 450 ml of Butterfield's phosphate buffer. Serial dilution up to 10^{-3} was done in 90 ml of phosphate buffer solution. For each sample, 1 ml of serially diluted sample was transferred into the tubes containing 9 ml of tryptone-soya-polymyxin broth and then incubated aerobically at 30°C for 18-24 hours. They were subcultured on PEMBA and blood agar. Then they were incubated at 30°C for 18-24 hours. Biochemical tests such as motility test, gelatin hydrolysis, Voges-Proskauer test, nitrate reduction test, carbohydrate fermentation test by using glucose, arabinose, mannitol and xylose, rhizoid growth test and lysozyme test were performed for identification of *Bacillus cereus*.

Antibiotics susceptibility testing

It was done by Kirby-Bauer disc agar diffusion method. The antibiotics and concentrations (in bracket) used were ampicillin (10 μg), cotrimoxazole (1.25, 23.75 μg), penicillin G (10 unit), chloramphenicol (30 μg), kanamycin (30 μg), ciprofloxacin (30 μg), gentamicin (10 μg), azithromycin (15 μg), amoxycillin/ clavulanic acid (20+10 μg), ceftriaxone (30 μg), erythromycin (15 μg) and amikacin (30 μg).

RESULTS

Distribution of total bacterial count category of milk and milk products

The total bacterial count of milk samples, yogurt samples and ice-cream samples ranged from 0 to10^6 cfu/g. There were three bacterial count categories: satisfactory, marginal and unsatisfactory. Satisfactory category was determined if the total bacterial count was less than 10^4 cfu/g. Marginal category was noted if the total bacterial count was less than 10^5 cfu/g. If the total bacterial count was more than or equal to 10^5 cfu/g, it fell in unsatisfactory category.[10]

Table 1 indicates the distribution of total bacterial count category of milk and milk product samples.

Number and percentages of milk and milk product samples with Bacillus species contamination

Bacillus cereus was isolated in 3 samples (30%) from pasteurized milk, 1 sample (10%) from boiled milk, 5 samples (50%) from raw milk, one sample (3.33%) from yogurt and 15 samples (50%) from ice-cream. Other Bacillus species were

Table 1. Distribution of total bacterial count category of milk and milk products

Categories	Milk		Yogurt		Ice-cream		Total	
	No.	%	No.	%	No.	%	No.	%
Satisfactory category	2	6.66	1	3.33	5	16.66	8	8.88
Marginal category	7	23.33	13	43.33	12	40	32	35.55
Unsatisfactory category	21	70	16	53.33	13	43.33	50	55.55
Total	30	100	30	100	30	100	90	100

Satisfactory category = $<10^4$ cfu/g
Marginal category = $<10^5$ cfu/g
Unsatisfactory category = $\geq 10^5$ cfu/g

discovered in 4 samples (40%) from pasteurized milk, 8 samples (80%) from boiled milk, 5 samples (50%) from raw milk, 16 samples (53.33%) from yogurt and 8 samples (26.66%) from ice-cream. There were 3 samples (30%) from pasteurized milk, 1 sample (10%) from boiled milk, no sample (0%) from raw milk, 13 samples (43.33%) from yogurt and 7 samples (23.33%) from ice-cream in which Bacillus species were not seen in Table 2.

Table 2. Number and percentage of milk and milk product samples with Bacillus species

Samples	Contaminated samples							
	Bacillus cereus		Other Bacillus species		Non-Bacillus species		Total	
	No.	%	No.	%	No.	%	No.	%
Milk								
Pasteurized	3	30	4	40	3	30	30	100
Boiled	1	10	8	80	1	10		
Raw	5	50	5	50	0	0		
Yogurt	1	3.33	16	53.33	13	43.33	30	100
Ice-cream	15	50	8	26.66	7	23.33	30	100
Total	25	27.77	41	45.55	24	26.66	90	100

Antibiotics resistance pattern of Bacillus cereus isolated

They are resistant to ampicillin (92%), cotrimoxazole (44%), penicillin G (100%), chloramphenicol (8%), ciprofloxacin (4%), kanamycin (28%), gentamicin (12%), azithromycin (12%), amoxycillin/clavulanic acid (92%), ceftriaxone (96%), erythromycin (20%) and cefotaxime (100%) (Fig. 1).

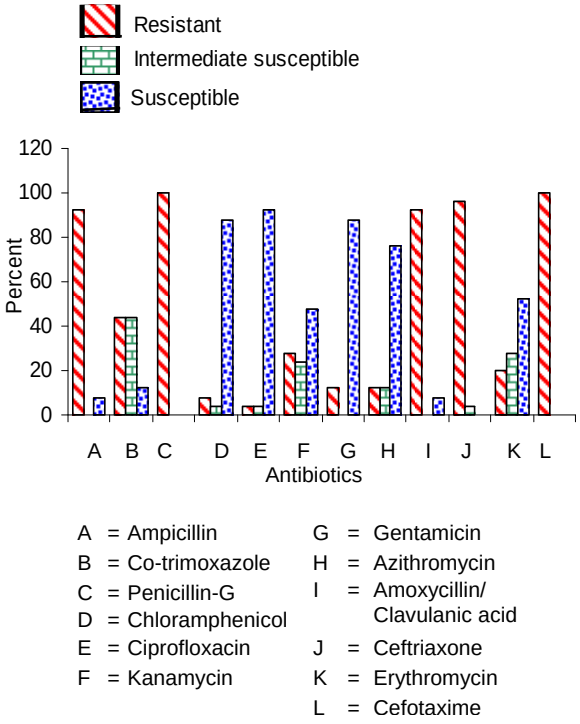

Fig. 1. Percentage of antimicrobial sensitivity pattern of *Bacillus cereus* isolated from milk, yogurt and ice-cream

DISCUSSION

Milk is a good medium for the growth of bacteria. One of the foodborne micro-organisms, *Bacillus cereus,* can be found in dairy products. Due to the heat resistance of *Bacillus cereus*, its pathogenic character, the capability to grow in milk and reported diseases upon consumption of dairy products, the organisms should be considered as hazardous in pasteurized milk.[11] In a large number of foodborne outbreaks caused by *Bacillus cereus,* the levels of *Bacillus cereus* present causing a diarrheal syndrome varied from 1.2×10^3-10^8 organisms per gram; the median value was around 10^7 organisms. Levels of *Bacillus cereus* present causing an emetic syndrome varied from $<10^4$- $>10^7$ organisms per gram, the median value was around 1.0×10^7 organisms per gram.[3]

Bacillus cereus prefers to grow at temperatures between 30°C and 37°C, although it can grow at temperatures up to 55°C and in some cases down to 5°C.

In some situations, *Bacillus cereus* can grow in acidic conditions, down to pH 4.3 at 30-35°C.[12] Sixty percent (54/90) of organisms in this study fell in unsatisfactory category ($\geq 10^5$cfu/g) and this level of contamination had potential to cause both diarrheal syndrome and emetic syndrome.

In this study, the most probable number (MPN) of *Bacillus cereus* isolated from nine milk samples ranged between 43 and >1100/g, from one yogurt sample ranged >1100/g and from 15 ice-cream samples ranged between 75 to >1100/g. Out of 9 *Bacillus cereus* positive milk samples, one (11.11%) sample falls in satisfactory category of MPN of *Bacillus cereus*, eight (88.88%) samples fall in unsatisfactory category. The level of *Bacillus cereus* contamination is less than that of a study which analyzed 458 samples of pasteurized milk and cream from 3 Danish dairies and found the total viable count of *Bacillus cereus* ranging from 10^3 to 3×10^5 cfu/ml.[13] Due to their heat-stable spores, *Bacillus cereus* easily survives the pasteurization process.[14]

The antimicrobials susceptibility test results of this study indicated that almost all isolated *Bacillus cereus* were resistant to ampicillin, amoxycillin/clavulanic acid and ceftriaxone. All isolated *Bacillus cereus* were totally resistant to pencillin G and cefotaxime. The isolates of *Bacillus cereus* were not susceptible to these commonly used antimicrobials. This might be because of high usage of antimicrobials in animal husbandry.

Most of the isolated *Bacillus cereus* of this study were sensitive to chloramphenicol, ciprofloxacin, gentamicin and azithromycin. About half of the isolates of *Bacillus cereus* were susceptible to kanamycin and erythromycin. This observation was relatively similar to that of Bernhard's study.[15]

The antibiotic sensitivity pattern of *Bacillus cereus* to the chloramphenicol and ciprofloxacin in this study is different from the drug sensitivity pattern of Meena's study.[16] There were no antibiotics to which isolates of *Bacillus cereus* were susceptible in hundred percents. The antibiotic susceptibility pattern of *Bacillus cereus* varies according to the different findings of the different studies. There may be different practices of common use of antibiotics in different regions or countries. Occurrence of *Bacillus cereus* isolates with multidrug resistance poses significant public health hazard.

Conclusion

The global burden of foodborne diseases and its impact on development and trade is currently unknown. The absence of reliable data on the burden of foodborne disease impedes understanding about its public health importance and prevents the development of risk-based solution to its management. The observation of *B. cereus* revealed that there were some potential hazards to cause food poisoning from ingestion of milk and milk products. The study of antimicrobial susceptibility pattern of *Bacillus cereus* contributes to the management of the disease caused by it.

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Preliminary Study of Blood Glucose Lowering Effect of *Curcuma longa* Linn. (Turmeric) Rhizomes on Rabbit Model

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The present study was performed to investigate the phytochemical constituents, physicochemical parameter, acute toxicity and blood sugar lowering effect (antihyperglycaemic effect) of *Curcuma longa* Linn. (turmeric) rhizomes. Both aqueous and 50% ethanolic extracts of turmeric rhizomes contained alkaloids, flavonoids, glycosides, tannin, phenol, saponins, resin, triterpene and amino acid. The acute toxicity studies of aqueous and 50% ethanolic extracts of turmeric were done on albino mice. It was observed that median lethal dose (LD_{50}) values of aqueous and 50% ethanolic extracts of turmeric were 8.6 g/kg, (confidence limit 7.54 g/kg-9.8 g/kg) and 11.5 g/kg, (confidence limit 8.46 g/kg-15.64 g/kg), respectively. Blood glucose lowering effect of aqueous and 50% ethanolic extracts of turmeric (2.5 g/kg) were investigated on adrenaline-induced hyperglycemic rabbit model by using oral route. The rabbits were made hyperglycemic by injection with 0.15 ml/kg of adrenaline tartrate subcutaneously. In this study, it was found that the aqueous and 50% ethanolic extracts of turmeric produced a significant decrease in blood glucose level in the rabbit model ($p<0.05$-$p<0.01$). It was found that blood glucose lowering effect of the 50% ethanolic extract of turmeric rhizomes was greater than that of aqueous extract of turmeric. In comparison of antihyperglycaemic effects of turmeric rhizomes extracts and standard drug glibenclamide, it was found that the antihyperglycaemic effect of the extracts of turmeric rhizomes was the same as that of glibenclamide. Therefore, it could be concluded that the two extracts of *Curcuma longa* L. rhizomes possess a significant blood glucose lowering effect on adrenaline-induced hyperglycaemic rabbit model.

INTRODUCTION

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of population all over of the world.[1] Decreased physical activity, obesity, stress and changes on food consumption are responsible for the increasing prevalence of diabetes in the past two decades.[2] Diabetes mellitus is one of the six major priority diseases in Myanmar.[3] Since ancient times, plants have been sources of medicines.[4] Even today, a large number of herbal drugs are being used for the treatment of diabetes mellitus in different regions of the world.[5]

Curcuma longa Linn. is commonly known as "nanwin" in Myanmar and it belongs to the family Zingiberaceae. *Curcuma longa* (turmeric) grows widely in Myanmar, India, South East Asia, China and Africa. It is cultivated extensively throughout the warmer parts of the world.[6, 7] Turmeric has been used in Indian and Chinese systems of medicine as well as Myanmar traditional medicine for a long time. Turmeric was known to be used in several diseases such as flatulence, dyspepsia, menstrual disorder, skin diseases, liver disease, cough, joint pain, inflammation, diabetes mellitus, etc.[8, 9] Many researchers reported that

Curcuma longa Linn. rhizomes extract showed antihyperglycaemic effect in experimental induced-diabetes animal model.[10, 11]

In Myanmar, there is no scientific report of blood sugar lowering effect (antihyperglycaemic effect) of *Curcuma longa* Linn. in experimental animal models as well as in human. Therefore, this study was performed to investigate the antihyperglycaemic effects of *Curcuma longa* Linn. rhizomes on adrenaline-induced hyperglycaemic rabbit model.[10, 11, 12]

Specific objectives

- To perform the aqueous and 50% ethanolic extraction of rhizomes of *Curcuma longa* Linn.
- To investigate the phytochemical constituents and physicochemical properties of crude powder and extracts of the medicinal plant
- To find out acute toxicity of two extracts of turmeric on albino mice
- To find out the blood sugar lowering effect (antihyperglycaemic effect) of two extracts of turmeric on adrenaline-induced hyperglycaemic rabbit model
- To compare the antihyperglycaemic effects of two extracts of turmeric with standard drug glibenclamide.

MATERIALS AND METHODS

Study design

The study design was an experimental study on animals. In acute toxicity study, albino mice were used and control-parallel study design was applied. In antihyperglycaemic activity study, adrenaline-induced hyperglycaemic rabbits were used and cross-over study design was applied.

Site of study

The study was done at the Pharmacology Research Division, Department of Medical Research (Lower Myanmar).

Collection and identification of plant samples

Dried rhizomes of *Curcuma longa* Linn. (turmeric) were purchased from the herbal market of Yangon. Botanical identification was done in the Department of Botany, Yangon University. Then, they were powdered by grinding machine.

Extraction of Curcuma longa Linn. dried rhizomes

Preparation for aqueous extract

One hundred grams of dried rhizomes of *Curcuma longa* Linn. were put into a 2-litre conical flask and 1 litre of distilled water was added. Extraction was done by heating the flask at 70ºC by a waterbath. After 6 hours extraction, it was cooled down to room temperature. The mixture was filtered by using cheese cloth and the residue was discarded. All the filtrate was poured into a petridish and was evaporated to dry on a boiling waterbath. The dried aqueous extracts obtained were stored in the desiccator.[13]

Preparation for 50% ethanolic extract

One hundred grams of dried rhizomes powder of turmeric were placed into the 2-liter conical flask and 500 ml of 50% ethanol was added into it. Extraction was done in the same procedure as preparation of the aqueous extract.[13]

Phytochemical and physicochemical studies of Curcuma longa Linn. rhizomes

Phytochemical constituents of crude powder, aqueous extract and 50% ethanolic extract of *Curcuma longa* Linn. were tested by using the method of Physicochemical Standards of Unani Formulation.[14] Physicochemical studies of crude powder of turmeric were carried out according to the method of WHO.[15]

Acute toxicity study of aqueous extract and 50% ethanolic extract of C. longa Linn. rhizomes on albino mice

Acute toxicity test was done to determine the symptomatology consequence, degree of toxicity after administration of the drug and to find out the median lethal dose (LD_{50}) by using the method of Litchfield and Wilcoxon.[16]

Fifty albino mice (ddy strain) of both sexes weighing 25-35 gm were used for acute toxicity study of the aqueous extract of turmeric rhizomes. The mice were separated into 5 groups and each group contained 10 mice. Each group was placed in each mouse cage. Food was withheld for a period of 18 hours before the experiment but they were allowed free access to water. One group served as the control and only distilled water was given orally. Four doses of aqueous extract of tumeric (i.e., 6 g/kg, 8 g/kg, 10 g/kg and 12 g/kg) were administered orally with intragastric needle.

After administration of the extracts, each group of mice was kept in separate mouse cages with free access to food and water. The mice were observed for behavior, neurologic, autonomic and other toxic signs continuously for 2 hours and then at 6-hour intervals up to 24 hours. To detect delayed toxicity, the survivours were observed daily for 2 weeks as described in Loomis.[17]

Another fifty albino mice of both sexes weighing 25-35 gm were used for acute toxicity study of 50% ethanolic extract of turmeric rhizomes. Four doses of 50% ethanolic extract of turmeric (i.e., 4 g/kg, 6 g/kg, 8 g/kg and 10 g/kg) were used. The procedure of acute toxicity test was done as described above.

Blood glucose lowering effect of aqueous extract and 50% ethanolic extract of Curcuma longa Linn. rhizomes on adrenaline-induced hyperglycaemic rabbit models

Six adult healthy rabbits of Japanese white strain weighing 2.8±0.12 kg obtained from Laboratory Animal Services Division, Department of Medical Research (Lower Myanmar) were used in this study. The rabbits were fasted for 18 hours before the experiment but water was allowed freely. In the control group, the vehicle (i.e., distilled water) was given orally to each rabbit by using an intragastric tube connected to a plastic syringe containing the calculated volume of distilled water.

Before the vehicle administration, the rabbit was held into a stainless steel rabbit restrainer and immediately 0.2 ml of blood was collected from marginal vein of the ear as the baseline blood sample. Then, distilled water was administered orally and just after administration of distilled water, the rabbit was made hyperglycaemic by injecting adrenaline (0.15 ml/kg) subcutaneously by using the method of Gupta.[18] Similar samples of 0.2 ml of blood were also collected from marginal vein of the ear at 1 h, 2 h, 3 h and 4 h. The results were read on the glucometer which was expressed as mg/dl. Blood glucose levels were determined at 1 h, 2 h, 3 h and 4 h after oral administration of distilled water.

One week after drug free interval, the same 6 rabbits were used again and the rabbits were fasted for 18 hours before the experiment. Only water was allowed. In this experiment, aqueous extract of *Curcuma longa* Linn. rhizomes (dissolved in distilled water) 2.5 g/kg body weight was administered orally to each rabbit. The procedure of administration of aqueous extract of turmeric, collection of blood sample and determination of blood glucose level were done as described above.

One week after drug free interval, the same 6 rabbits were used again. The procedure of administration of 50% ethanolic extract of turmeric (2.5 g/kg body weight), collection of blood samples and determination of blood glucose levels were done as described above. One week after drug free interval, the same 6 rabbits were used again. The procedure of administration of the standard drug glibenclamide (4 mg/kg body weight), collection of blood samples and determination of blood glucose levels were done as described above.

Data management and analysis

The data were expressed as mean ± standard error (S.E). Comparisons were made by using Student's "t" test and ($p<0.05$) was considered as the level of statistical significance.[19]

RESULTS

Phytochemical constituents of crude powder, aqueous extract and 50% ethanolic extract of *Curcuma longa* Linn. rhizomes are shown in Table 1.

Table 1. Phytochemical constituents of *Curcuma longa* Linn.

No.	Constituents	Results		
		Crude powder	Aqueous extract	50% ethanolic extract
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	+
3.	Glycosides	+	+	+
4.	Tannin	+	+	+
5.	Steroids	-	-	-
6.	Phenol	+	+	+
7.	Saponins	+	+	+
8.	Resin	+	+	+
9.	Triterpene	+	+	+
10.	Cyanogenic glycoside	-	-	-
11.	Amino acid	+	+	+

+ =Present – =Absent

Physicochemical tests of crude powder of *Curcuma longa* Linn. rhizomes showed moisture content (12%), swelling index (1.2 cm), foaming index (<100), ash value (8.95%), chloroform extract (13.15%), watery extract (9.45%), ethanol extract (9.50%) and petroleum ether (3.55%).

Acute toxicity studies of aqueous extract and 50% ethanolic extract of C. longa Linn. rhizomes

In the present study, it was observed that the mice given with aqueous extract of turmeric at the doses of 8 g/kg, 10 g/kg and 12 g/kg showed decrease in motor activity, reduction of screen grip, tremor, piloerection and ataxia. They died within 24 hours and within 7 days after oral administration. There were no eye ball abnormalities, lacrimation, salivation, ear and oral mucosa cyanosis, respiration abnormality, micturition and diarrhea in the mice. When the mice died, autopsy examinations were done.

On the examination of the internal organs such as brain, lungs, heart, stomach, intestine, liver, kidneys, spleen and pancreas, they were found to be grossly normal. No gastrointestinal bleeding and perforation were found. For comparative study, five normal mice were sacrificed without giving any drug and the internal organs were examined grossly as the controls.

In this study, no lethality of mice was observed at the dose of 6 g/kg of the aqueous extract of turmeric. It was found that 5 mice out of 10 died, at the dose level of 8 g/kg of the extract. Seven mice out of 10 mice and 10 mice out of 10 died, at the dose level of 10 g/kg and 12 g/kg of the extracts, respectively. Thus, median lethal dose (LD_{50}) of aqueous extract of *Curcuma longa* Linn. rhizomes was found to be 8.6 g/kg and its confidence limit was 7.544 g/kg – 9.804 g/kg.

The mice given with 50% ethanolic extract of turmeric at the doses of 6 g/kg, 8 g/kg and 10 g/kg body weight showed decrease in motor activity, reduction of screen grip, tremor, piloerection and ataxia. Then, they died within 24 hours and within 7 days after oral administration. In this study, no lethality of mice was observed at the dose of 4 g/kg of the extract.

At the dose of 6 g/kg of the extract, it was found that 2 mice out of 10 died. At the dose of 8 g/kg and 10 g/kg of the extracts, 2 mice out of 10 and 3 mice out of 10 mice died, respectively. Median lethal dose (LD_{50}) of 50% ethanolic extract of turmeric rhizomes was found to be 11.15 g/kg and its confidence limit was 8.46 g/kg-15.64 g/kg.

The effect of aqueous extract of C. longa Linn. rhizomes on blood glucose level in adrenaline-induced hyperglycaemic rabbits

Mean blood glucose levels of the rabbits (control group) at 0 h, 1 h, 2 h, 3 h and 4 h after subcutaneous injection of adrenaline tartrate (0.15 ml/kg) were 67.67±3.35 mg/dl, 234.5±31.27 mg/dl, 317.5±24.49 mg/dl, 336.17±18.61 mg/dl and 283.5±21.27 mg/dl, respectively. Significant rises in blood glucose levels were observed at 1 h, 2 h, 3 h and 4 h ($p<0.001$) after subcutaneous

injection of adrenaline tartrate. Mean blood glucose levels of rabbits treated with aqueous extract of turmeric rhizomes (2.5 g/kg) at 0 h, 1 h, 2 h, 3 h and 4 h after subcutaneous injection of adrenaline tartrate (0.15 ml/kg) were 67.17±3.82 mg/dl, 193.5±36.3 mg/dl, 248.17±34.3 mg/dl, 271.5±31.72 mg/dl, 235±32.6 mg/dl, respectively. Significant decreases in blood glucose levels were found at 1 h ($p<0.01$), 2 h ($p<0.01$), 3 h ($p<0.05$) when compared with those of control (Fig. 1).

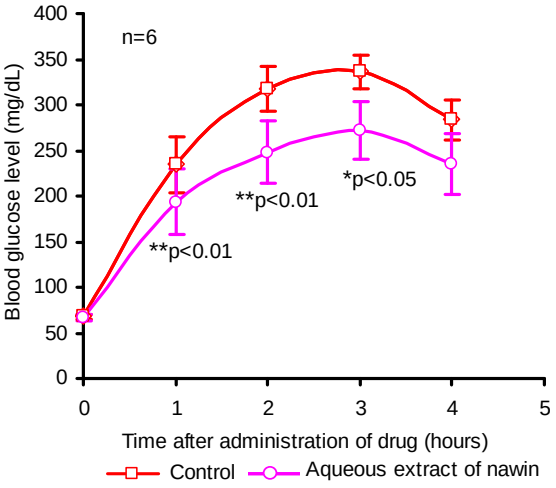

Fig. 1. Time course of the blood glucose lowering effect of aqueous extract of *Curcuma longa* Linn. on adrenaline-induced hyperglycaemic rabbit model (n=6) (The results are shown in mean±S.E)

The effect of 50% ethanolic extract of Curcuma longa Linn. on blood glucose level in adrenaline-induced hyperglycaemic rabbits

Mean blood glucose levels of rabbits treated with 50% ethanolic extract of turmeric rhizomes (2.5 g/kg) at 0 h, 1 h, 2 h, 3 h and 4 h after subcutaneous injection of adrenaline tartrate (0.15 ml/kg) were 69.17±3.29 mg/dl, 207.17±42.83 mg/dl, 248±41.58 mg/dl, 233.83±26.81 mg/dl, 202.5±32.01 mg/dl, respectively. Significant decreases in blood glucose levels were found at 2 h ($p<0.05$), 3 h ($p<0.01$) and 4 h ($p<0.05$) when compared with those of the control (Fig. 2).

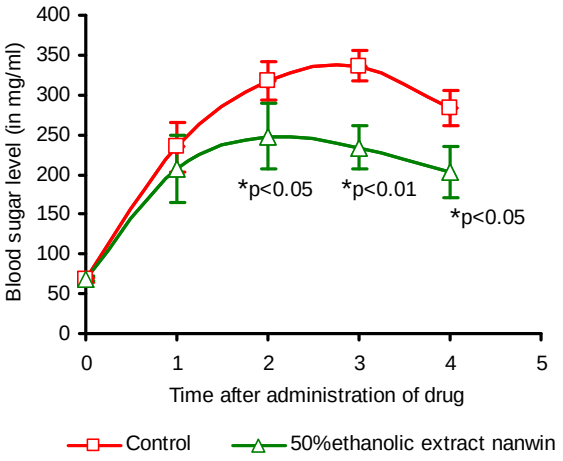

Fig. 2. Time course of the blood glucose lowering effect of 50% ethanolic extract of *Curcuma longa* Linn. on adrenaline induced hyperglycaemic rabbit model (n=6) (The results are shown in mean±S.E)

The effect of standard drug glibenclamide on blood glucose level in adrenaline-induced hyperglycaemic rabbits

Mean blood glucose levels of the rabbits treated with standard drug, glibenclamide (4 mg/kg) at 0 h, 1 h, 2 h, 3 h and 4 h after subcutaneous injection of adrenaline tartrate (0.15 ml/kg) were 73.33±2.93 mg/dl, 226.83±22.62 mg/dl, 275.33±26.72 mg/dl, 250.5±19.83 mg/dl, 224.33±13.33 mg/dl, respectively. Significant decreases in blood glucose levels were found at 2 h ($p<0.01$), 3 h ($p<0.01$) and 4 h ($p<0.05$) after oral administration of glibenclamide when compared with those of controls.

Comparisons of hypoglycaemic effects of aqueous extract, 50% ethanolic extract of Curcuma longa Linn. rhizomes and glibenclamide in adrenaline-induced hyperglycaemic rabbits

In this study, aqueous extract of turmeric (2.5 g/kg) produced a significant blood glucose lowering effect after oral administration of the extract. Percent inhibition of blood glucose levels with aqueous extract of turmeric rhizomes ranged from 25.69±8.21% to 30.23±9.88%. It was found that 50% ethanolic extract of turmeric (2.5 g/kg) produced a significant blood

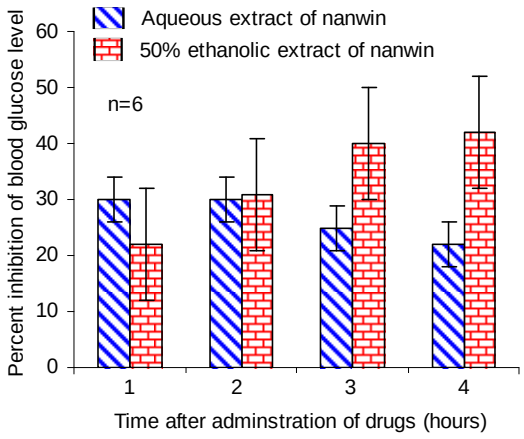

Fig. 3. Comparison between the blood glucose lowering effects of aqueous extract and 50% ethanolic extract of *C. longa* Linn. on adrenaline-induced hyperglycaemic rabbit model (n=6) (The results are shown in mean±S.E)

glucose lowering effect after oral administration of the extract. Percent inhibition of blood glucose levels with 50% ethanolic extract of turmeric rhizomes ranged from 30.89±11.15% to 40.67±10.85%. Percent inhibition of blood glucose level with standard drug (glibenclamide) ranged from 20.18±4.68% to 30.71±7.53% (Fig. 3).

DISCUSSION

Both aqueous extract and ethanolic extract of rhizomes of *Curcuma longa* Linn. were used in this study because the compounds soluble in the aqueous extract and ethanolic extract were different.[20] In the present study, it was observed that phytochemical analysis of crude powder, aqueous extract and 50% ethanolic extract of turmeric showed the presence of the same phytochemical compounds. The toxic plant compound like cyanogenic glycoside was not detected in both crude powder and the extracts of this plant.

In the acute toxicity study, aqueous extract and 50% ethanolic extract of turmeric showed no toxic effect on albino mice.[21] It was reported that aqueous extract of *Curcuma longa* Linn. rhizomes possessed blood glucose lowering effect in rat model.[10] Fifty percent ethanolic extract of *Curcuma longa* Linn. was also reported to possess blood glucose lowering effect in mice model.[12] In comparison between the percent inhibition of blood glucose level with aqueous extract and 50% ethanolic extract of turmeric rhizomes, 50% ethanolic extract produced a significant increase in percent inhibition of glucose level than the aqueous extract ($p<0.05$ - $p<0.01$) in adrenaline-induced hyperglycaemic rabbit model. The antihyperglycaemic effect of 50% ethanolic extract was greater than that of aqueous extract. It was found that the percent inhibitions of both extracts of turmeric and glibenclamide were not significantly different. Therefore, the anti-hyperglycaemic effect of the extracts of turmeric rhizomes was found to be the same as that of glibenclamide. It was reported that plant chemical constituents like flavonoids, tannin, alkaloid, steroid and glycosides could produce antihyperglycaemic activity.[22]

Therefore, in this study, blood glucose lowering effect of the extracts of *Curcuma longa* Linn. rhizomes may be due to one or more compounds like flavonoids, tannin, alkaloids and glycosides contained in these extracts. In conclusion, the results indicate that both extracts of *Curcuma longa* Linn. rhizomes produced significant blood glucose lowering effects on rabbit model.

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Empirical Optimization of Agitation Rate for Industrial Fermentation of *Hansenula polymorpha* Yeast (Recombinant Hepatitis B Vaccine Production)

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Recombinant hepatitis B vaccine was produced by using the HBsAg-expressed *Hansenula polymorpha* yeast cells. The sufficient data of optimized agitation rate for fermentation were not available in production of recombinant hepatitis B vaccine in Myanmar. This study looked into the three lots of fermentation conditions. The yeast fermentation was performed at various agitation rates of 200, 250, 300, 350, and 400 rpm with constant fermentation conditions: temperature of 25˚C, air flow rate of 70 l/min, and vessel pressure of 1.9 bars. Methanol feeding was done to induce more antigen expression and cell growth during fermentation process. The samples were collected eight hourly and were determined for the OD600nm, the cell growth by microscopic examination and the weight of biomass. It was found that the fermentation agitation rate of rpm 250 to 400 in step-wise changing (rpm 250-300-350-400 after every second time of methanol feeding) was the best method for industrial fermentation of *H. polymorpha* yeast in production of recombinant hepatitis B vaccine.

INTRODUCTION

Recombinant hepatitis B vaccine is produced by using the hepatitis B surface antigen (HBsAg)-expressed *Hansenula polymorpha* yeast cells. Product-containing cells are usually generated by a two fermentor cascade, consisting of a 5-L seed fermentor which is used to inoculate the 50-L main fermentor. The whole fermentation process is started from a single vial of working cell bank. In order to improve the production of hepatitis B virus pre-S2 antigen by the methylotrophic yeast *Hansenula polymorpha*, antigen production is done in two steps: first, cultivation of cells in glucose (Seed 1, 2, 3 and seed fermentor), followed by induction of antigen expression with methanol (main fermentation). Agitation is of prime importance in a fermentation process. The effect of agitation on yeast (HBsAg) production is important for the successful progress of the fermentation. Agitating the fermentation broth normally satisfies the oxygen demand of a fermentation process. Agitation is also important for uniform mixing of the medium components within the fermentor (dispersion of cells and nutrients) as well as mass transfer phenomena (e.g., oxygen transfer rates) and heat transfer. It not only assists mass transfer between the different phases present in the culture, but also maintains homogeneous chemical and physical conditions in the culture by continuous mixing.

A sufficient data of optimized agitation for fermentation were not available in production of recombinant hepatitis B vaccine in Myanmar, this study looked into the three lots of fermentation conditions. The yeast fermentation was performed at various agitation rates of 200, 250, 300, 350, and 400 rpm with constant fermentation

conditions: temperature of 25˚C, air flow rate of 70 l/min and vessel pressure of 1.8~1.9 bars. Methanol feeding was done to induce more antigen expression and cell growth during fermentation process. This study shows an improved fermentation process for the production of recombinant hepatitis B vaccine from *H. polymorpha* yeast.

MATERIALS AND METHODS

Cell cultivation process

Working cell banks (WCB) were produced from cultivation of master cell bank (MCB) (original cell bank of HBsAg-expressed *Hansenula polymorpha* yeast). Cell cultivation was started from WCB. Cell cultivation steps (seed 1, 2, 3) were performed in 0.7% Yeast Nitrogen Base (YNB) and 2% glucose media and the incubation conditions were 30˚C, 250 rpm and 24 hours.

Seed fermentation in seed fermentor

Seed fermentation was performed in 5-liter B. Braun seed fermentor. The conditions were 25˚C, 400 rpm, 24 hours, aeration rate 2.25 l/min, inner pressure 0.5 bar (no control) and 2% glucose medium. In the yeast growing, glucose is the single carbon substrate provided in the presence of oxygen in seed fermentation.

Main fermentation process

Main fermentations were performed in industrial 50-liter B. Braun fermentor. B. Braun Bio-stat U-50 L was used for experiment 1 and 2 and B. Braun DCU 400-50 L fermentor was used for experiment 3. The fermentors were the same product of B. Braun and were of comparable mechanisms.

Sterilization of fermentor

Firstly, the fermentor was sterilized at 121˚C for 15 minutes with water for injection (WFI) and full setting of pH, DO probes, and lines. The WFI was discarded and main culture medium including neolin (antifoam) was placed into the fermentor and then kept the temperature at 25˚C. The seed culture was transferred from seed fermentor into the main fermentor by using transfer line. The fermentor was ready to be set for required conditions. The culture conditions were monitored and controlled by computerized control-board. The fermentation condition was set in three ways.

Experiment 1 condition: 25˚C, agitation rate 200 rpm constant for the whole run, 1.8 bar, 1.5 vvm (70 l/min), (B. Braun/Bio-stat U-50 L fermentor)

Experiment 2 condition: 25˚C, agitation rate 200 rpm, changing to 250 after 6 times feeding of methanol, inner pressure 1.8 bar, aeration 1.5 vvm (70 l/min), (B. Braun/Bio-stat U-50 L fermentor)

Experiment 3 condition: 25˚C, agitation rate 250 rpm, 1.8 bar, 1.5 vvm (70 l/min). After every second methanol feeding, agitation rate was increased by 50 (250-300-350-400 rpm). (B. Braun DCU 400-50 L fermentor).

The following items were recorded during the equipments.

(1) Time of culture (2) change of culture temperature (3) change of rpm (4) change of dissolved oxygen (DO), and (5) methanol feeding time.

The dissolved oxygen concentration, aeration rate, pH in medium and agitation rate, temperature and vessel pressure were monitored *in situ* by probe.

Methanol feeding condition

Methanol (0.6%) of total volume/13 minutes. First feeding was started at 30 minutes after seed inoculation. When the DO level reached the highest point, two stopwatches were prepared to record the time and DO level. If DO level did not increase less than 2.0 for 10 minutes, the methanol feeding was started.

Sample collection and tests

During the fermentation process the samples were collected twelve hourly for experiment 1 and 2 and eight hourly for experiment 3.

The samples were determined for the following tests:

Determining cell concentration (cell growth)

The cell growth was determined by OD600nm

Biomass measurement

Twenty milliliters of sample were centrifuged at 3000 rpm for 15 minutes at 4˚C. The supernatant was discarded carefully. The tube was placed upside down for 1 minute on filter paper and then the weight of tube and cell was measured.

Cell contamination

The cell contamination was checked by microscopic examination. After culture was completed, culture broth was harvested and concentration and diafiltration were performed. Then the cell cakes were recovered by centrifugation. The weight of cell cake was recorded and stored at 4˚C for further purification steps.

RESULTS AND DISCUSSION

Table 1 shows the summary of fermentation conditions for the three experiments. Although the culture time of experiment 1 was longer than 2 and 3, it resulted in the smallest amount of cell cake.

Table 1. Culture conditions and results

Condition	Experiment		
	1	2	3
Main initial rpm	200	200	250
Rpm increasing	Constant 200	After 6 feeding	After 2 feeding
Rpm	200	200-250	250-300-350-400
Methanol feeding (frequency)	23 (6900 ml)	24 (7200)	40 (10800 ml)
Aeration (l/min)	60~70	60~70	60~70
Temperature (°C)	25	25	25
Inner pressure (bar)	1.8~1.9	1.8~1.9	1.8~1.9
Methanol feeding rate (ml/min)	300/13	300/13	270/13
Culture time (hours)	143	94	115
Cell cake after harvest (g/l)	1994/51	5609/53	8657/50

The increase of biomass and OD600nm according to the culture time is shown in Table 2. It was found that the biomass increased by increasing the rpm. The biomass of experiment 3 (changing of rpm from 250 to 400) was better than the experiment 2 and 3.

Table 2. Effect of different agitation rates on biomass and OD600nm

Sample collection time (hr)	Biomass (g/l)			OD 600 nm		
	Experiment			Experiment		
	1	2	3	1	2	3
24	30.5	34.2	20.0	45.0	32.0	12.3
48	60.3	72.5	83.6	125.6	121.8	45.7
72	72.0	100.0	142.5	147.0	180.2	75.8
96	75.1	124.5	160.0	170.0	232.8	97.1
115			191.7			157.2
142.3	93.6					

The OD of Exp. 1 slightly decreased at the end of fermentation time due to constant rate of rpm 200. The Exp. 2 showed the initial increase of OD which also increased slightly at the end of culture time. According to the background information on yeast metabolic pathways and the "cybernetic" principle, cells choose to grow at the fastest possible rate.

The Exp. 3 showed the fastest possible growth rate for the whole culture time. In the culture agitated at 200-250 rpm, maximum biomass concentration was obtained after 94 hours of fermentation, while in cultures agitated at 200 rpm, maximum biomass dry weight was observed about 21 hours earlier. The maximum biomass levels of the cultures were 93.56 g/l, 124.5 g/l and 191.65 g/l at the agitation rates of 200 rpm (Exp. 1), 200-250 rpm (Exp. 2) and 250 to 400 rpm (Exp. 3), respectively. The result of experiment 3 showed higher amount of cell cake than the other experiments. It was found that the entire cells were the same cell morphology and free from contamination by microscopic examination and the dissolved oxygen concentration was found to influence the productivity of cell.

The effect of agitation on yeast (HBsAg) production is important for the successful progress of the fermentation. Agitation is important for uniform mixing of the medium components within the fermentor (dispersion of cells and nutrients) as well as mass transfer phenomena (e.g., oxygen transfer rates). Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products/byproducts and oxygen. Thus, agitation results in a better uniform mixing of the fermentation broth, helping to maintain a concentration gradient between the interior and the exterior of the cells. Such a concentration gradient works in both directions; through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other byproducts of catabolism from the microenvironment of the cells.

It was found that agitation speeds of 250-300-350-400 rpm gave satisfied biomass (191.65 g/l). Agitation also favors oxygen supply to the cells that is important for high biomass concentration. The OD of experiment 3 was acceptable in culture time of 115 hours as shown in Table 2. *Hansenula polymorpha* yeast cells can be grown quite rapidly to high cell densities (150 g/l, dry weight) with typical fermentation times of 100-150 hours.[1] The entire cells were of the same cell morphology. The biomass and OD level fall rapidly in fermentations with agitation speeds of 200, 200-250 rpm compared to the agitation speeds of 250 to 400 rpm.

Biomass production increased when the agitation speed was increased from 250 to 400 rpm. Previous study showed that cell growth decreased beyond an rpm of 400.[5] The main reason attributed to shear effects causing decreased adenosine triphosphate (ATP) generation, lower O_2 uptake, and lower specific growth rate of yeast.[2]

In this study, agitation was found to have a significant effect on growth rate because noticeable changes result in changes of bioactive products formation. Agitation creates shear forces, which affect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and also damaging the cell structure.[3] The morphology of the microorganism can strongly influence the product formation, since it affects consequently the mass and heat transfer capabilities of the fermentation broth.[4] In this study, all the cells in experiments were the same morphology.

The aeration (60~70 l/min) could be beneficial to the growth and performance of microbial cells by improving the mass transfer characteristics. The experiment 3 was carried out with higher rpm and higher methanol feeding (10800 ml). The methanol utilization pathway levels provide high productivity and an attractive upstream process design that can be controlled by additions of appropriate amounts of methanol to the culture medium. In *H. polymorpha*, the recombinant viral surface antigen is found to be assembled into yeast-derived lipid membranes similar to the situation in other yeasts, forming 22 nm, 1.17–1.20 g cm^{-3} particles.

Previous study has indicated that this lipoprotein particle structure is essential for the antigenicity of the HBsAg.[5] The *H. polymorpha*-based platform with its methanol pathway promoters and the inclusion of methanol in a fermentation process provides an especially efficient process for a balanced co-production of both vaccine components because membrane proliferation in general is associated with methanol induction.

Conclusion

It was concluded that the fermentation agitation rate rpm 250 to 400 in stepwise increase (rpm 250-300-350-400 after every second time of methanol feeding, total 10800 ml) was the best method for industrial fermentation (condition: temperature of 25˚C, air flow rate of 70 l/min,

and vessel pressure of 1.9 bars) of *Hansenula polymorpha* yeast for the production of recombinant hepatitis B vaccine in Myanmar.

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Learning Styles of Medical Students of Different Genders, University of Medicine (Magway)

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Learning styles are different approaches or ways of learning that differ among individuals. Some learn through seeing while the others learn through listening, moving, doing and touching. The knowledge of learning styles of the students helps educators to identify and solve learning problems among students that in turn help the students to become more effective learners by adapting classroom methods to best fit each student's learning style. To identify the learning styles of medical students of deferent gender of University of Medicine (Magway), the VARK questionnaires were administered to a total of 559 medical students; 182 first-year medical students, 196 third-year medical students and 181 final part II medical students of University of Medicine (Magway) in March 2010. A total of 315 male and 244 female medical students participated in this study. Both males and females preferred multimodal learning style to unimodal learning style (83.2%, 80.7% vs. 16.8%, 19.3%), respectively. In unimodel style, reading/writing mode (38%, 38%) was the most commonly used mode followed by auditory (28%, 36%) and kinesthetic. Visual mode was found to be least commonly used mode in both male (8%) and female students (9%). More male students (26%) used kinesthetics mode than female students (17%). In contrast, more female students (36%) used auditory mode than male students (28%). Most of the male (39.3%) and female (43.7%) students used bimodal learning style. 29.4% of male students used trimodal style and 31.3% quadrimodal. Of the females who preferred multimodal learning style, 35.5% was trimodal user and 20.8% was quadrimodal. Both male and female students used auditory reading (AR) modes more commonly than other combination. Male students showed more preference on auditory kinesthetics (AK) than female students whereas more female students were in favor of visual auditory (VA) modes than male students. Both male (37.7%) and female students (45.7%) used auditory reading (VAR) modes more commonly than other combinations.

INTRODUCTION

People differ consistently from each other in their preferences (e.g., emotional, environmental) for certain ways of information processing. Learning style is the way students begin to concentrate on, process, internalize, and remember new and difficult academic information.[1] There are many thoughts and theories about individual learning styles; Dunn and Dunn, Joseph Renzulli, Howard Gardner, Jung, the Myers-Briggs Type indicator instrument and Kersley's Temperament Sorter, David Kolb and Anthony Gregorc's Type Delineator learning modalities. Learning strengths may also be classified as sensory, perceptual, Cognitive Information-processing, personality type and Personal Talents.[2, 3] Fleming's VARK model is one of the most common and widely-used categorizations of the various types of learning styles.[4, 5]

Visual learners learn best from visual displays including diagrams, illustrated text books, overhead transparencies, videos,

flipcharts and hand-outs. They need to see the teacher's body language and facial expression to fully understand the content of a lesson.

Auditory learners learn through listening. Written information may have little meaning until it is heard. These learners often benefit from reading text aloud and using a tape recorder and learn new information through the process of listening and discussing.

Read-write learners prefer lists, glossaries, textbooks, lecture notes, or handouts; they also prefer printed words and texts as a means of information intake. *Tactile/Kinesthetics persons* learn best through a hands-on approach, moving, doing and touching.[6]

Different students exhibit unique strengths, talents and/or weaknesses. The research on learning styles shows that there are significantly higher learning gains for college students when instructional strategies/resources compliment student learning styles.[7] Therefore, a variety of learning approaches must be provided in every classroom. Understanding the different ways that students learn, interact with and process information can help modify the way of teaching so that all students have an equal opportunity to succeed. The present study is aimed to identify the learning styles of medical students of University of Medicine (Magway) and to determine the learning styles of different genders.

MATERIALS AND METHODS

To determine the preferred mode(s) of learning English version of the VARK questionnaire for young was administered at the end of the first semester (March, 2010) to 182 first-year medical students, 196 third-year medical students and 181 final part II medical students of University of Medicine (Magway). This study was performed at the Department of Microbiology, University of Medicine (Magway) in March 2010. The VARK questionnaire (http://www.vark-learn.com; http:// www. vark-learn. com/ page. asp, questionnaire) (only 16 questions) was used.[8]

Administering the questionnaire

When instructing the students to fill in the questionnaire they were told to make a selection (a, b, c or d) for each question, but they can omit a question or they can choose more than one option if they want to. Information on the meaning of words in the questionnaire and additional contextual or situational information will not be given before they choose their answers as it may bias responses to the questions. They could choose more than one response if they think the context is not clear. Before they complete the questionnaires they will be informed that the results indicate their preferences but are not necessarily their strengths.

RESULTS

In the study group, 315 male and 244 female medical students involved. Figure 1 shows the percentages of male and female students who preferred unimodal versus multimodal learning style. Both males and females preferred multimodal learning style to unimodal learning style, respectively (83.2%, 80.7% vs. 16.8%, 19.3%) (Fig. 1).

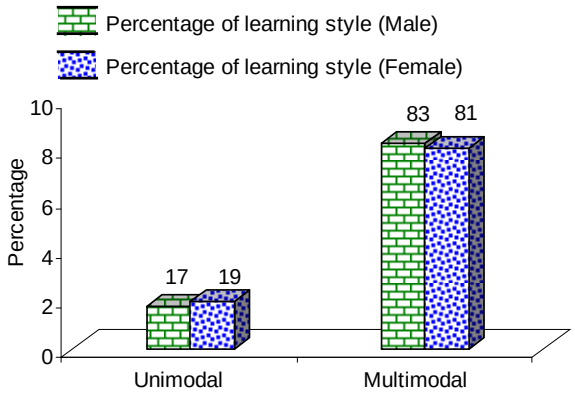

Fig.1. Distribution of learning styles of different genders

Of unimodal students, 37.7% of male students and 38.3% of female students preferred reading/writing mode (R), 28.3%

of male students and 36.1% of female students preferred auditory mode(A), 26.4% of male students and 17% of female students preferred kinesthetics mode (K), and 7.5% of male students and 8.5% of female students preferred visual mode (V) (Fig. 2).

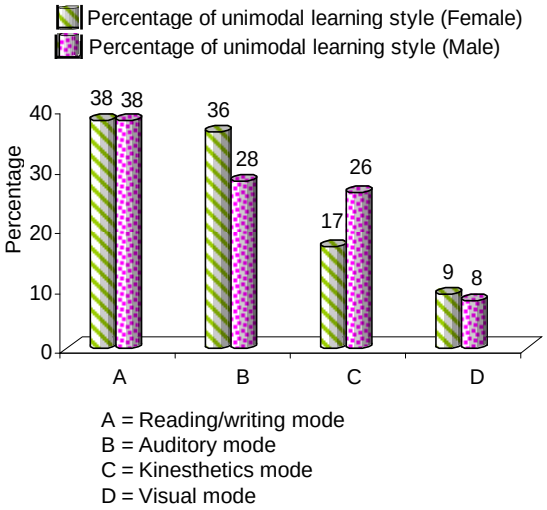

Fig. 2. Percentage distribution of unimodal learning style of different genders

Of the males who preferred multimodal learning style, 39.3% preferred two modes (bimodal), 29.4% of the students preferred three modes (trimodal) and 31.3% of the students preferred four modes (quadri-modal). Of the females who preferred multimodal learning style, some preferred two modes (bimodal, 43.7%), three modes (trimodal, 35.5%) and four modes (quadri-modal, 20.8%).

Table 1. Percentage distribution of bimodal learning styles of different genders

Gender	VK (%)	VA (%)	VR (%)	AK (%)	RK (%)	AR (%)	Total (%)
Male	7 (6.8)	8 (7.8)	21 (20.5)	13 (12.8)	18 (17.6)	36 (35)	103
Female	7 (8.1)	14 (16.3)	11 (12.8)	9 (10.5)	11 (12.8)	34 (39.5)	86

VK = Visual & kinesthetics mode
VA = Visual & auditory mode
VR = Visual & reading/writing mode
AK = Auditory & kinesthetics mode
RK = Reading/writing & kinesthetics mode
AR = Auditory and reading/writing mode

Different modes of bimodal learning style preference are shown in Table 1. Of the students who preferred bimodal learning style, both male and female students (35% and 39.5%) mostly preferred auditory and reading/ writing mode (AR) whereas least preferred visual and kinesthetics (VK-6.8%, 8.1%).

Table 2. Percentage distribution of trimodal learning styles of different genders

Gender	ARK No. (%)	VAK No. (%)	VAR No. (%)	VRK No. (%)	Total
Male	22(28.6)	10(13)	29(37.7)	16(20.8)	77
Female	19(27.1)	6(8.6)	32(45.7)	13(8.6)	70

ARK = Auditory, reading/writing & kinesthetics mode
VAK = Visual, auditory & kinesthetics mode
VAR = Visual, auditory & reading/writing mode
VRK = Visual, reading/writing & kinesthetics mode

Of the students who preferred trimodal learning style, both male and female students had the same pattern of percentage distribution (Table 2).

DISCUSSION

Students receive information in a variety of modes because they are different with unique strength, talent, weakness, learning styles and preferences. Knowledge on the ways they learn and process information will help develop effective instructional strategies and methods which make effective learning. Students learn more in a manner compatible with their own learning preference. Blending of different learning methods is more effective than using one method only.[9]

A total of 315 male and 244 female medical students participated in this study. Both males and females preferred multimodal learning style to unimodal learning style respectively (83.2%, 80.7% vs. 16.8%, 19.3%).

In unimodel style, reading/writing mode was the most commonly used mode followed by auditory and kinesthetics. Reading/writing learners learn best by reading printed materials. Reading/writing mode type of learner benefits from writing out important information again and again, reading notes silently, organizing any

diagrams into statements, rewriting the ideas and principles in other words, making flashcards of words and concepts that need to be memorized, instructors who use the blackboard to accent important points or provide outlines of the lecture material.[12]

Auditory learners are individuals who has the ability to retain and learn new information through the process of listening and discussing. Tapes, audio, lectures, discussion, debate, games, questions and answer sections are effective for auditory learners. They tend to benefit most from traditional teaching techniques and lectures. Auditory learners also gain benefit from reading aloud, interviewing, debating, participating on a panel and giving oral reports. Discussion, questions, answers, debate and verbal activities are required to engage and support an auditory learner and so will enhance the auditory learner's classroom experience.[11] They also benefits from attending lectures and tutorials, discussing topics with teachers and other students, putting summarized notes on tape and listen to them, joining a study group, tape recording lectures, and when recalling information or solving problems, talking out loud.[12]

Visual mode was found to be least commonly used modes in both male and female students. However, more male students (26%) used kinesthetics mode than female students (17%). In contrast, more female students (36%) used auditory mode than male students (28%). Most of the male (39.3%) and female (43.7%) students used bimodal learning style commonly, 29.4% of male students used trimodal style and 31.3% quadrimodal. Of the females who preferred multimodal learning style, 35.5% was trimodal user and 20.8% was quadrimodal.

Both male and female students used auditory reading (AR) modes more commonly than other combination. Male students showed more preference on auditory kinesthetics (AK) than female students whereas more female students were in favor of visual auditory (VA) modes than male students. Both male (37.7%) and female students (45.7%) used auditory reading (VAR) modes more commonly than other combination.

Visual learners remember things best by seeing something written. Things written down in a handout, text or on the overhead such as drawing, maps, pictures, diagrams, demonstrations, graph, chart, flow diagrams, display, computer graphics, cartoons and film will benefit visual learners who prefer to see. Visual learners as well as kinesthetic learners will value to-do lists, assignment logs, and written notes.[10]

Teaching learning methods that can be used for visual learners include replacing words with symbols or initials, translating concepts into pictures and diagrams, underline or highlighting notes or textbooks with different colors, practicing turning visuals back into words, and making flashcards of key information with words, symbols, and diagrams.

Learning strategies for the kinesthetic learners include role play, practical class, drama, things to build, drawing, playing board games, making dioramas, making models, movement, sports and physical games, tactile experience or hands on experience, field trips, visiting museums, studying with others, setting up experiments, using memory games and using flash cards to memorize promote deep learning for kinesthetic learners as they prefer doing, manipulation and moving to strengthen short and long-term memory. They will do best in answering definitions, fill-ins and multiple choice.[13, 14]

Learning strategies for the kinesthetics/ tactile learning style include sitting near the instructor in classroom situations, reading out loud from textbook and notes, copying key points onto large writing surfaces (i.e. chalkboard or easel board), copying key points using word processing software, listening to audiotapes of notes while exercising, taking in information through

field trips, laboratories, trial and error, exhibits, collections, and hands-on examples summary, recalling experiments and role-play, and using pictures and photographs that illustrate an idea.[12]

Students of University of Medicine (Magway) represent a diversity of age, experience, culture, ethnicity and learning preference. There will be problems when students with one mode of learning exposed to instruction that required other modes of learning. Therefore, it is important to provide a variety of learning to meet for the educational needs of all students to motivate and improve performance of them and also make students to adapt to other modes of learning in addition to preferred mode. Methods that intentionally combine information processing across learning styles may have the greatest potential for supporting academic success for more students.

Therefore, the most successful teaching technique is one that involves a variety of different methods in order to accommodate every student's unique learning style.

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Community-Based Cervical Cancer Screening in Bago and Indagaw Suburban Areas

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Cervical cancer is one of the cancers that can be readily cured if early detection is accessible. Estimated 95% of women in developing countries have never been screened for cervical cancer. Pap smear (cytology) is the test accepted to be the most appropriate for cervical cancer screening if sufficient resources exist. A community- and laboratory-based study was conducted from August to September, 2007 with the aim of early detection of cervical cancer in married women with or without gynaecological symptoms residing in suburban military community in Bago and Indagaw areas. Total 170 women, 75 from Bago and 95 from Indagaw, aged 19-55 years included in this study. Each was allocated to have a Pap smear using conventional cytology which was performed in Department of Medical Research (Lower Myanmar). In Bago area, most of the women, (74.67%) had inflammatory smear, 16% had normal smear, 6.67% had mild dyskaryosis, 1.33% had moderate dyskaryosis and 1.33% had squamous cell carcinoma. In Indagaw area, 69.47% had inflammatory smear and 24.21% had normal smear; 4.21%, 1.05% and 1.05% had mild dyskaryosis, moderate dyskaryosis and severe dyskaryosis, respectively. This study highlighted that most of the women in these communities had inflammatory lesions of cervix, some had precancerous lesions of cervix, and one case of cancer cervix was detected. So, this study suggests that National Screening Programme for cervical cancer is urgently needed for the control of cancer cervix in Myanmar.

INTRODUCTION

Cervical cancer is one of the leading causes of cancer death in women in the developing world. Estimated 95% of women in developing countries have never been screened for cervical cancer. Over 80% of women newly diagnosed with cervical cancer live in developing countries; most are diagnosed when they have advanced disease. The cure rate for invasive cervical cancer is closely related to the stage of disease at diagnosis and the availability of treatment. If left untreated, cervical cancer is almost always fatal.

Due to its complexity, cervical cancer control requires a team effort and communication between healthcare providers at all levels of the healthcare system for early diagnosis and treatment.[1] According to the WHO South-East Asia region (SEARO) report, cervical cancer incidence and mortality were estimated 188,000 new cases and 102,000 deaths respectively, in 2008.

In Myanmar, estimated cervical cancer incidence in 2008 was 26.4% (6434 cases per 100,000).[2] Over the past 30 years (1976-2006), it was shown that among 56097 total commonest female cancers, cervical cancer accounts for 13181 cases (23.5%) which always being either the commonest or the second commonest female cancers all along.[3] This is due to the fact that the majority of women in the world do not have access to cervical screening, which can prevent up to 75% of cervical cancer.[4]

The primary underlying cause of cervical cancer is infection with one or more high-risk types of the human papillomavirus (HPV), a common virus that is sexually transmitted. Most new HPV infections resolve spontaneously; if it persists, infection may lead to the development of precancer which left untreated, can lead to cancer. As it usually takes 10-20 years for precursor lesions caused by HPV to develop into invasive cancer, most cervical cancers can be prevented by early detection and treatment of precancerous lesions.[1]

Prophylatic human papillomavirus (HPV) vaccines have been developed against types 16 and 18, based on virus-like particles and which induce high titres of neutralizing antibodies, could potentially prevent 70% of cervical cancer worldwide. These vaccines are more than 90% effective at preventing type 16/18 associated cervical intraepithelial neoplasia (CIN), although overall efficacy against all types CIN 2/3 in all vaccine recipients, irrespective of current infection is considerably less. The optimal age group to vaccinate is pre-adolescent girls. The rationale of prophylatic HPV vaccines is based on the necessity of HPV infection in cervical carcinogenesis, by preventing this primary event, secondary changes which result in cytological abnormalities will also be prevented.[5]

Studies have shown that those countries with formal screening programmes and wide population coverage experienced substantial drops in incidence and mortality while neighboring countries with limited population screening did not. The success of cervical cytology screening lies in its relative simplicity, low cost and non-invasive nature. Annual screening reduces the probability of developing invasive carcinoma by over 95%. Most cases of invasive cervical carcinoma occur because they are not screened at an appropriate interval or there is inadequate follow-up for an identified abnormality.[6]

The National Health Service Cervical Screening Programme (NHS CSP) in the United Kingdom is a fully integrated programme, extending across the screening age range and connecting all the professional disciplines involved. In England, screening commences at 25 years and continues until 64, screening is done every 3 years until 50, and every 5 years thereafter. All cervical cytology in the United Kingdom is reported using the British Society of Cervical Cytology (BSCC) classification. The Bethesda System is widely used internationally, and the two systems can be roughly translated, though there are subtle differences.[7]

In WHO recommendations of comprehensive cervical cancer control (2006), new programmes should start screening women aged 30 years or more, and include younger women only when the highest-risk group has been covered. In the age group of 25-49 years, a three-year interval can be considered if resources are available. For women over 50 years, a five-year screening interval is appropriate. Annual screening is not recommended at any age. Screening is not necessary for women over 65 years, provided the last two previous smears were negative.[1]

Conventional cytology can detect up to 84% of precancer and cancer. However, its sensitivity can be as low as 38% under poor conditions. The specificity is usually over 90%. Liquid-based cytology (LBC) was introduced in the mid-1990s and is increasingly used in high-resource settings. LBC is more sensitive than Pap smear and has almost the same specificity. Cytology is recommended for large-scale cervical cancer screening programmes, if sufficient resources exist. Other tests include visual inspection: with acetic acid (VIA) or Lugol's iodine (VILI) and HPV DNA testing show promise but there is as yet no comparable evidence on their effectiveness. Large studies are still underway.

These visual methods (VIA and VILI) are relatively simple and can be taught to nurses, nurse-midwives and other health workers. The tests are likely to be less costly than

other approaches in routine use. When detection of HPV is used as a primary screening test, the sensitivity for detection of precancer and cancer varies from 50% to 95%, with most studies reporting high sensitivity of 85% or more. The specificity ranges from 50% to 95%, with an average of 84%. The combination of cytology and HPV testing has very high sensitivity and negative predictive values approaching 100%. The high cost and the need for molecular laboratory present major challenges in low-resource settings.[1]

Effective screening programmes have been introduced in many industrialized countries. However in developing countries, Pap smear programme is limited to testing women attending primary healthcare and other health clinics (opportunistic screening). Nearly all programmes in developing countries have no organized efforts to reach the high-risk women or to ensure that those found to have an abnormal smear receive effective follow-up and treatment. Existing programmes in these countries are failing to achieve a major impact.[8]

Regardless of the test used, the key to an effective programme is to reach the largest proportion of women at risk with quality screening and treatment. Organized screening programme designed and managed at the central level to reach most women at risk are preferable to oppotunistic screening. Screening is only effective if there is a well-organized system for follow-up and treatment. This study was carried out with the aim of early detection of cervical cancer by means of cervical cytology screening in the community.

General objective

- To study the community-based cervical cytology screening in married women residing in suburban military community of Bago and Indagaw areas

Specific objectives

- To find out the prevalence of abnormal cervical cytology cases
- To determine the background characteristics regarding gynaecological history of the study population
- To study the association between abnormal cervical cytology cases with risk factors

MATERIALS AND METHODS

It was a community- and laboratory-based, cross-sectional descriptive study. A total of 170 consented married women with or without gynaecological symptoms residing in suburban military community of Bago and Indagaw areas were screened to detect cervical cytological abnormalities from August to September, 2007.

The nature of the study and procedure of cervical smear was explained thoroughly to the women attending the screening places of Bago and Indagaw suburban areas. After getting informed consent, thorough history taking and physical examination were done and the findings were recorded in the proforma.

Then, they were examined in a private room with the third person and placed in dorsal position and speculum examination was performed under good light source. By using a sterile disposable Cusco's speculum without lubrication, Pap smears were taken by disposable Ayre's wooden spatula at the transformation zone (TZ) of the cervix. The long tip of the spatula is inserted into the cervical os and rotated through a full circle (360°). Pap smear specimens were immersed immediately in 95% absolute alcohol (ethanol) for 30 minutes and then removed and allowed to air dry. Papanicolaou method, (i.e., Gill's haematoxylin V stain, Orange G stain, OG-6 and multiple polychrome stain, EA-50) was used for the staining of Pap smear. After staining, cytopathology diagnosis was given by the pathologist using Bethesda classification.

One hundred and seventy medical records of women were reviewed and the prevalence was calculated. Coded data obtained from the study were cleaned and entered into Microsoft Excel. After checking the range

and consistency, prevalence of abnormal Pap smears in the study population was calculated.

Comparison of the BSCC and Bethesda classification systems[7]

BSCC classification	Bethesda classification
Inadequate	Unsatisfactory for evaluation
	Negative for intraepithelial lesion or malignancy
	Differential diagnosis
	Organisms
	Trichomonas vaginalis
	Fungal organisms morphologically consistent with Candida species
	Shift in flora suggestive of bacterial vaginosis
	Bacteria morphologically consistent with actionmyces species
	Cellular changes consistent with herpes simplex virus
Negative	Other non-neoplastic findings
	Reactive cellular changes associated with inflammation (includes typical repair) radiation
	intrauterine contraceptive device
	Glandular cells status posthysterectomy
	Atrophy
Borderline nuclear change	Atypical squamous cells of undetermined significance (ASC-US)
	Atypical squamous cells cannot exclude HSIL (ASC-H)
	Atypical endocervical, endometrial or glandular cells (Not otherwise specify or specify)
	Atypical endocervical or glandular cells favour neoplasia
Mild dyskaryosis	Low-grade squamous intra-epithelial lesion SIL)
Moderate dyskaryosis	High-grade squamous intraepithelial lesion (HSIL)
Severe dyskaryosis	High-grade squamous intraepithelial lesion (HSIL)
Malignant	Squamous cell carcinoma
Glandular neoplasia?	Endocervical carcinoma *in situ*
	Adenocarcinoma
	Endocervical
	Endometrial
	Extra uterine
	Not otherwise specify

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RESULTS

In this study, 170 consented married women with or without gynaecological symptoms residing in suburban military community of Bago and Indagaw areas, 75 from Bago and 95 from Indagaw were screened for cervical abnormalities from August to September, 2007. The most common age group for cervical cancer screening in this study was 36-45 years, constituting 48.23% (82 cases) and the second most common was 26-35years, constituting 30% (51). The other age groups as 46-55 years, 16-25 years and >55 years were 15.30% (26), 5.88% (10) and 0.59% (1), respectively. Most of women i.e., 54.12% (92) had primary education, 30% (51) had middle education, 12.35% (21) had high education, 2.94% (5) were graduates and only 0.59% (1) was illiterate. Regarding age of marriage, the most common age group was 15-19 years, constituting 81 cases (47.64%) and the second most common was 20-24 years constituting 66 (38.83%). The less common age groups were 25-29 years constituting 18 (10.59%), 30-34 years, constituting 3 (1.76%) and 35-39 years, constituting only 2 (1.18%).

Regarding the marital status, 91.76% (156) were married once and 8.24% (14) were married twice. Most of the study population had 2-3 children, constituting 77 cases (45.29%) and the second most common group had 4-5 children, constituting 53 (31.17%). The others in this study had 0-1 child, constituting 29 (17.06%), 6-7 children, constituting 9 (5.29%) and the least common group had >8 children, constituting 2 (1.18%) (Table 1).

Table 1. Background characteristics of the study population

Characteristics	Frequency	Percentage
Age group(years)		
16-25	10	5.88
26-35	51	30.00
36-45	82	48.23
46-55	26	15.30
>55	1	0.59
Education		
Illiterate	1	0.59
Primary school	92	54.12
Middle school	51	30.00
High school	21	12.35
Graduate	5	2.94
Age of marriage (years)		
15-19	81	47.64
20-24	66	38.83
25-29	18	10.59
30-34	3	1.76
35-39	2	1.18
Marital status		
married once	156	91.76
Twice	14	8.24
Parity (numbers)		
0-1	29	17.06
2-3	77	45.29
4-5	53	31.17
6-7	9	5.29
>8	2	1.18

Regarding the history of white discharge (Table 2), this study revealed 39.41% (67) had history of white discharge and 60.59% (103) had no history of white discharge. Among them, 91.04% (61 cases) were milky discharge and 8.95% (6) were yellowish; 50.75% (34) had offensive and 33 cases (49.25%) had non-offensive discharge. Regarding the amount of white discharge, 40.30% (27) were moderate, 32.83% (22) were scanty and 26.87% (18) were profuse. And, 38.81% (26) had history of association of pruritus with white discharge and 61.19% (41) had not.

Table 2. Distribution of history of white discharge in study population

History of white discharge	Frequency	Percentage
Yes	67	39.41
No	103	60.59
Amount		
Scanty	22	32.83
Moderate	27	40.30
Profuse	18	26.87
Color		
Milky	61	91.04
Yellowish	6	8.95
Odour		
Offensive	34	50.75
Non-offensive	33	49.25
Association with pruritus		
Yes	26	38.81
No	41	61.19

Regarding the history of abnormal vaginal bleeding (Fig.1), this study showed that 14.71% (25) of normal population had history of abnormal vaginal bleeding such as postcoital bleeding 1.18% (2), perimenopausal bleeding 3.53% (6), and intramenstrural bleeding 10% (17) but there was no history of postmenopausal bleeding. And 89.29% (145) had no history of abnormal vaginal bleeding.

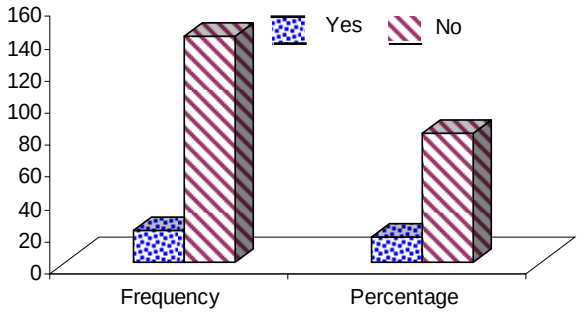

Fig.1. Distribution of abnormal vaginal bleeding in study population

Table 3. Cytological diagnosis of conventional Papanicolaou smear in study population

Bethesda system	Cytology report	Bago No (%)	Indagaw No (%)	Total population No (%)
Normal	Normal	12 (16.00)	23 (24.21)	35 (20.59)
NILM*	Inflammatory	56 (74.67)	66 (69.47)	122 (71.76)
LSIL**	Mild dyskaryosis	5 (6.67)	4 (4.21)	9 (5.29)
HSIL***	Moderate dyskaryosis	1 (1.33)	1 (1.05)	2 (1.18)
HSIL***	Severe dyskaryosis	0	1 (1.05)	1 (0.59)
SCC****	Invasive carcinoma	1 (1.33)	0	1 (0.59)
Total		75 (100)	95 (100)	170 (100)

* =Negative for intraepithelial lesion or malignancy
**=Low-grade squamous intraepithelial lesion
***=High-grade squamous intraepithelial lesion
****=Squamous cell carcinoma

The cytological diagnosis of conventional Papanicolaou smear of the total study population is shown in Table 3. Among total 75 women from Bago, 74.67% (56) had inflammatory smear, 16% (12) had normal smear, 6.67% (5) had mild dyskaryosis (LSIL), 1.33% (1) had moderate dyskaryosis (HSIL), and 1.33% (1) had squamous cell carcinoma. Among total 95 women from Indagaw, 69.47% (66) had inflammatory smear 24.21% (23) were normal, 4.21% (4) had mild dyskaryosis (LSIL), 1.05% (1) had moderate dyskaryosis (HSIL) and 1.05% (1) had severe dyskaryosis (HSIL). Therefore, among the total of 170 women participating cervical cancer screening in this study, 71.76% (122) had inflammatory smear, 20.59% (35) were normal, 5.29% (9) had mild dyskaryosis (LSIL), 1.18% (2) had moderate dyskaryosis (HSIL), 0.59% (1) had severe dyskaryosis (HSIL) and 0.59% (1) had squamous cell carcinoma. Among 9 cases of LSIL, 3 cases were accompanied with koilocytosis (cytologically consistent with human papilloma virus infection).

The association of abnormal cervical cytology cases with risk factors is shown in Table 4. This study showed that most abnormal cases (12) were 36-55 years of age, age of marriage were 15-19 years, and

Table 4. Association of abnormal cervical cytology cases with risk factors

Abnormal cervical cytology cases	Frequency	Percentage
Age group		
36-45	7	53.85
46-55	5	38.46
>55	1	7.70
Age of marriage		
15-19	13	100
Marital status		
Married once	12	92.30
Twice	1	7.70
Parity		
3-4	7	53.85
5-6	6	46.15
Vaginal bleeding		
Yes	4	30.76
No	9	69.23
White discharge		
Yes	9	69.23
No	4	30.76

they had 3-6 children. Among them, 30.76% had history of vaginal bleeding i.e., 15.38% of postcoital bleeding and 15.38% of peri-menopausal bleeding and 69.23% had history of white discharge.

DISCUSSION

Cervical cancer is the second most common cancer in women following breast cancer worldwide. In Myanmar, cervical cancer is the most common female genital cancer and has high mortality and morbidity. A study among high-risk patients coming to the Gynaecological Out-patient Department and in patients of the gynaecological wards of Mandalay General Hospital revealed 0.67% of invasive cancer, 12% of dysplasia and 59.3% of infection.[9]

In one study by liquid-based cytology and conventional Papanicolaou smear for screening of cervical neoplasia among patients coming to the Gynaecological Out-patient Department of Central Women's Hospital, Yangon and Mandalay, conventional papanicolaou smear found that 40% of patients had inflammatory smear, 35% had CIN I, 8.3% had CIN II, 1.7% had CIN III, 1.7% had atrophic smear and 13.3% had normal.[10] In a Nigerian study, among unscreened population of sexually active women attending gynaecological clinics at the Federal Medical Centre, Globe, inflammation was the commonest abnormal-lity in 52.4% while cervical intraepithelial neoplasia was seen in 17.9%, CIN I, II and III accounting for 12.4%, 3.4% and 2.1%, respectively.[11]

In this study, the most common age group who participated in cervical cancer screening was 36-45 years (48.23%). Most of the women had primary education (54.12%). Most women (47.64%) in this study population were married between 15 to 19 years of age and 91.76% of women were married once. And most (45.29%) had 2-3 children and 31.17% had 4-5 children. There was no family history of cervical cancer.

Regarding the history of abnormal vaginal bleeding, this study showed that 14.71% of study population had abnormal vaginal bleeding such as postcoital bleeding (1.18%), perimenopausal bleeding (3.53%), and intramenstrural bleeding (10%).

This study found out that 39.41% of study population had history of vaginal discharge. Among them, most of women (40.3%) had moderate amount of vaginal discharge, 91.04% had milky discharge, 50.75% had offensive discharge and 38.8% had history of association with pruritus.

Regarding the cytological diagnosis of conventional Papanicolaou smear, this study found that most of the women (71.76%) in study population had inflammatory smear. There were 13 cases of precancerous lesions of cervix, mild dyskaryosis (5.29%), moderate dyskaryosis (1.18%), severe dys-karyosis (0.59%), and squamous cell carcinoma (0.59%). The remaining 20.59% were normal. This study highlighted that most of the women i.e., nearly three fourth in apparently normal population had inflam-matory smear and 7.65% had precancerous lesions of cervix by conventional Pap smear. Among the total study population, one fifth had normal smear. A study among non-pregnant married subjects presenting with discharge and abnormal vaginal bleeding at

Central Women's Hospital, Yangon in 1999 showed that there was an association between the risk of cancer cervix and young age at marriage, number of marriages, family history of cancer cervix and history of sexually transmitted diseases. Age greater than 30 years, age at marriage lesser than 20 years, parity more than 2, marital partner more than 1, and family history of cancer cervix were found to have increased risk for having abnormal histology.[12]

Regarding the association of abnormal cervical cytology cases with risk factors, this study found out that abnormal cervical cytology cases were 36-55 years of age. Age of marriage ranged from 15 to 19 years, and they had 3-6 children. Among them, 30.76% had history of abnormal vaginal bleeding and 69.23% had history of vaginal discharge. Thus, this study showed that age greater than 35 years, age at marriage less than 20 years, parity more than 3 and history of abnormal vaginal bleeding and vaginal discharge were found to have increased risk for having abnormal cervical cytology. Thus, these findings were consistent with other studies. This study highlighted that most of the women in these communities had inflammatory lesions of cervix and some had precancerous lesions of cervix and one case of cancer cervix was detected. So this study recommends that National Screening Programme for cervical cancer is urgently needed for the control of cancer cervix in the community.

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Feeding Practices of Mothers with Less Than Two Years Old Children during Child's Illness and Diarrhea

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The study was conducted in Theiktookan Village of Thonegwa Township to explore the child feeding practices of rural mothers during child's illness and diarrhea. It was a cross-sectional study and used both qualitative and quantitative methods. Initial qualitative research was carried out to develop the structured questionnaire. Four Focus Group Discussions (FGDs) comprising 8 lactating mothers in each FGD (two FGDs with mothers of low-income families and two FGDs with mothers of high-income families) were held. A total of eighty-nine lactating mothers were interviewed with semi-structured questionnaires including socio-demographic background, nutrition knowledge, breast feeding practices and feeding practices during diahrrhoea and illness of children. Mean age of children was 12.7±6.9 months and the range was 0.5-23 months. All of the children were breastfed and 78 children (87.6%) were still breastfed. Seventy-eight children (87.6%) have already been introduced with complementary foods. Eight children (8.9%) and 25 children (28.1%) never got illness and diarrhea, respectively. It was found that inappropriate feeding practices were present in mothers residing in the rural area. Among 78 children, 10 children (11.2%) were fed with breast milk alone during illness and diarrhea. Twenty-seven (38.5%) and 18 (33.9%) children were fed with breast milk alone during illness and diarrhea, respectively, and 3 (4.3%) and 9 (17%) children were fed with breast milk only with chicken soup during illness and diarrhea, respectively. Therefore, nutrition education on feeding practices of mothers needs to be improved.

INTRODUCTION

Adequate nutrition during the first two years of life is important to ensure optimal physical and mental development. Nutritious complementary foods and appropriate complementary feeding practices could reduce malnourished children. Malnutrition has been responsible directly or indirectly for sixty percent of 10.9 million deaths annually of under five children.[1] Appropriate complementary feeding includes the introduction of foods other than breast milk at about six months of age, adequate energy density and frequency of feeding of these complementary foods, and satisfactory nutrient density of these food.[2]

Repeated bouts of common illnesses - such as diarrhea, respiratory infections, malaria, or measles effect the overall nutritional status of infants and young children, which in turn effect their immunity. Deficiencies of key micronutrients for immunity such as vitamin A and zinc weaken the body's protective mechanisms against infection. A cycle of illness and malnutrition can be deadly for vulnerable children, particularly those under two. Appropriate feeding both during and after illness is critical not only for recovery from a current illness but also to prevent a child from succumbing to this vicious cycle over time. Optimal feeding during and after illness is a cluster of behaviors that includes quantity of food,

quality of food, frequency of feeds, duration of attention, and care.[3]

Mothers around the world recognize that a child who is sick will have little or no appetite. Mothers decreased amount of food fed to their children and prohibited certain kinds of foods including nutritious foods during illness and diarrhea in spite of additional need of foods. Infants and children need additional foods during and after an illness for catch-up growth. Complementary feeding practices of mothers with less than two years old children was presented and it was observed that foods were introduced too early or too late, and faulty complementary feeding practices were found.[4] The aim of this study was to explore the child feeding practices of mothers during child's illness and diarrhoea.

General objective

To assess feeding practices of mothers with less than two years old children during child's illness and diarrhea in the selected rural area of Yangon.

Specific objectives

- To explore current complementary feeding practices of mothers with less than two years old children.
- To find the food choices of mothers with less than two years old children during child's illness and diarrhea.
- To find the reasons of mothers for giving certain kinds of food to their children during the child's illness and diarrhea.

MATERIALS AND METHODS

Study design

The study was a cross-sectional descriptive study.

Study place and period

The study was done in Theiktookan Village of Thonegwa Township in Yangon Division from March to October 2007.

Study population

For qualitative study

Focus group discussions - Four FGDs (with 32 mothers)

In-depth interviews - Four (with 2 grand-mothers, one lactating mother and one woman from Myanmar Maternal and Child Welfare Association)

For quantitative study

Eighty-nine mothers of under two years old children were interviewed with semi-structured questionnaire.

Sample size determination

Using the data on prevalence of in-appropriate and inadequate complementary practices of 70%, sample size would be 41, calculated as follows:

$$n = \frac{1.96 \times 1.96 \times (1-P)}{P \times e^2} = 41$$

(Confidence level 95% and relative precision of 20%)

With the assumption of 10% non-respondent rate, a total of 50 households need to be selected from each urban and rural area of Yangon Division. Taking the effect size of 1.5, the sample size was 75.

Sampling method (quantitative)

From the sixteen states and divisions of Myanmar, Yangon Division was purposely chosen for convenience of data collection. From Yangon Region, Thonegwa Township was chosen by simple random sampling method. From the list of villages from Thonegwa Township, Thiketookan Village was chosen again. Ninety households of mothers with under two years old children were also chosen. Total eighty-nine mothers included in this study.

Data collection

Both qualitative and quantitative methods were used for this study and the data included practices of mothers about child feeding practices during illness

and diarrhea. Qualitative research was carried out to develop the structured questionnaire and to explore belief and reasons of child feeding pattern during illness and diarrhea. Child feeding patterns of mothers during illness and diarrhea were asked with structured questionnaires including background characteristics of mothers and children, current complementary feeding practices, foods fed to children, and foods prohibited to children during the child's illness and diarrhea.

For qualitative data collection, four FGDs were performed and, 8 lactating mothers participated in each FGD. Two FGDs were done with lactating mothers of high income families and two FGDs with those of low income families. In-depth interviews were also done with 2 grandmothers, one lactating mother and one woman from MMCWA.

Data processing and analysis

Focus group discussions and in-depth interviews

Tape records of FGDs and in-depth interviews were transcribed, translated, and transcripts were coded into major themes that were generated manually. Matrix analysis was done manually according to main themes and sub-themes.

Structured questionnaire

Data were checked and data entry was done with Epi Info version 6.0 and data analysis was done with Epi Info 6.0 and SPSS 11.5 software.

Ethical consideration

The protocol was approved by Institutional Ethical Committee, Department of Medical Research (LM) for ethical clearance.

RESULTS

Background characteristics of mothers and children

Lactating mothers who had children younger than 2 years participated in the study. Table 1 summarizes the background characteristics of the mothers and children. Half of the mothers were housewives and, but at the time of interview, majority of them could not work because of taking care of their children. Mean age of children was 12.7±6.9 months with minimum 0.5 month and maximum 23 months.

Table 1. Background characteristics of children and mothers

Characteristics	Number n=89	Percent
Sex of the children		
Boys	50	56.2
Girls	39	43.8
Age group of children (months)		
<6	19	21.3
6 - 8	12	13.5
9 - 11	9	10.1
12 - 24	49	55.1
Education level of mothers		
Illiterate	6	6.8
Read and write	2	2.2
Less than primary school level	39	43.8
Less than middle school level	27	30.3
Middle school passed	7	7.9
High school passed	2	2.2
Graduate	6	6.8
Occupation of mothers		
Government employee	2	2.2
Skilled laborer	8	9.0
Common laborer	15	16.9
Farm owner	12	13.5
Farmhand	7	7.9
Housewife	45	50.5

Breast feeding practice

All of the children were breastfed. Among them, 11 children (12.4%) had already been stopped breast feeding but 78 children (87.6%) were still breastfed. Almost all of the children were not exclusively breastfed except two children of four and five months old. Mothers gave either plain water or boiled water to their infants when they felt that their children were thirsty.

Complementary feeding practices

Seventy-eight children (87.6%) have already been introduced with complementary foods. Table 2 shows types of complementary foods were given according to age groups. Almost all of the children had been fed with solid foods. All under 6 months old babies were introduced with solid supplementary food too early. Among forty-nine 12-23 months old children, about 74% of children were fed with family foods.

Table 2. Current complementary feeding practices of mothers according to age groups

Type of complementary foods	Age groups (months) <6 (%)	6-8 (%)	9-11 (%)	12-23 (%)	Total (%)
Cereal powder (Local-made)	-	1(9.1)	-	-	1(1.3)
Rice with mother Milk	-	1(9.1)	-	-	1(1.3)
Rice with milk powder (Red Cow)	8(80)	4(36.4)	-	-	12(15.4)
Rice with salt and oil	2(20)	4(36.4)	3(37.5)	4(8.1)	13(16.6)
Rice with vegetable	-	-	-	1(2.1)	1(1.3)
Rice with fermented fish paste	-	-	-	1(2.1)	1(1.3)
Rice with fish/meat/ egg/ pulses	-	1(9.1)	2(25)	7(14.3)	10(12.8)
Family foods			3(37.5)	36(73.4)	39(50)
Total	10	11	8	49	78

About 12.5% and 15.5% of 9-11 months and 12-23 months old children, respectively, were fed with supplementary food appropriately based on the child's age. None of the 6-8 months old children was fed with appropriate complementary foods, amount and frequency according to the guideline (Fig. 1).

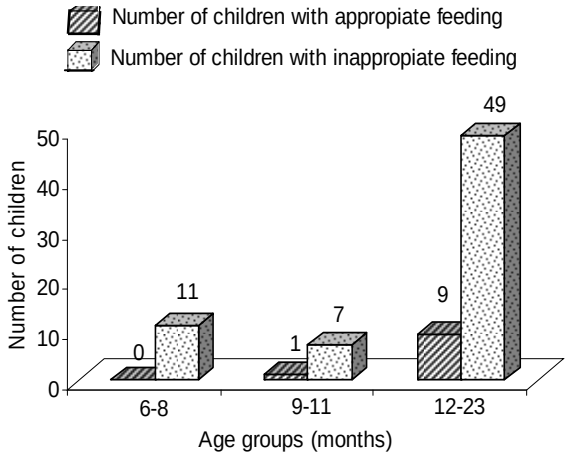

Fig. 1. Frequency distribution of children with appropriate and inappropriate feeding practices in type of foods, amount and frequency by age groups

Qualitative data findings

Most of the interviewed mothers of over one year old children fed their children with family food 2-3 times per day. Majority of the mothers of under one year old children fed with sieved rice or chewed rice with milk powder or salt and oil 1-2 times per day. Few mothers of under 6 months old children started complementary feeding at the age of 6 months. Ready-made weaning foods were rarely fed to children and few mothers from high-income families used local-made weaning foods branded with Golden Cow or Gold Power. Snacks like chicken biscuit and other biscuits, were given to children 1-2 times per day.

"Mostly I fed my child with chewed rice with salt and oil because my mother did not want to feed curry because of worm infestation".

(a mother of 11 months old baby)

"I fed my child the foods we ate such as rice and land crab curry about one cup".

(a mother of 1year and 9 months old child)

"I fed three spoons of Dumex cereal powder one time per day to my child".

(a mother of 5 months old child from well-to-do family)

There were different methods in preparing the complementary foods:

- cooked rice was sieved through a clean-washed thin cloth and the sieved rice was placed in a small bowl and kept in a pot of cooked rice for a while which was still on stove.
- cooked rice was chewed and fed to the baby.
- cooked rice was meshed by using hand or spoon.

The prepared rice was mixed with salt and oil or milk powder (Red Cow was used by mothers with low income and infant formula Dumex was used by mothers from well-to-do family) and fed to babies. All children over one year were fed by family foods. Majority of mothers did not feed vegetables and, meat/fish to their children daily.

Child feeding practices of mothers during illness and diarrhea

Among 89 children, eight children (8.9%) and 25 children (28.1%) never got illness and diarrhea. Ten children (11.2%) were fed with breast milk alone during illness and

diarrhea among 78 children who had been already fed complementary foods. In Table 3, foods which were fed to the children during illness and diarrhea are shown.

Table 3. The frequency distribution of mothers based on mode of feeding their children during illness and diarrhea

Types of foods	During illness* n=70 (%)	During diarrhoea # n=53 (%)
Breast milk alone	27(38.6)	18(33.9)
Breast milk and chicken soup	3(4.3)	9(17.0)
Breast milk and foods other than rice (eg. biscuit, bread, coffee, quaker)	18(25.7)	6(11.3)
Breast milk and rice gruel/ rice gruel with fish/ chicken	17(24.3)	9(17.0)
Breast milk with rice with milk powder/ biscuit/ salt and oil/fish/chicken	5(7.1)	11(20.8)

*8 children had never got illness.
#25 children had never got diarrhea.

Qualitative data findings

Most of the mothers withheld feeding of solid foods during illness with various reasons: rising temperature, surfeited with food and children did not want to eat. Majority of the interviewed mothers fed rice gruel/ chicken soup/ biscuits to their babies during illness. They did not feed rice to their children being afraid of rising temperature. They fed snacks like chicken biscuit, other biscuits or bread because children refused to eat rice. None of the mothers mentioned that extra food was needed during illness.

All of the mothers answered that they gave ORS to their children when they had diarrhea. Majority of the mothers fed breast milk with chicken soup alone during diarrhea because they thought that chicken soup is nutritious. Commonly withdrawn solid foods included rice, fruits, vegetables, meat products and beans products. Avoidance of feeding certain food items such as orange, sour fruits, vegetable soup cooked with tamarind juice was mentioned by some mothers with a reason that these foods would increase loose motion (Table 4).

Table 4. Foods prohibited for children during illness and diarrhea and the reasons

During illness	Reasons*			
	Surfeited with food	Rising temperature	Can cause coughing	Can cause bloating
*Types of prohibited food to children**				
Rice	1	47	-	-
Vegetables (Roselle, bitter gourd, tomato, cabbage, egg plant)	7	3	-	2
Fruits (banana, mango)	-	48	-	-
Legumes/ potato	5	5	-	-
Sour foods	-	4	-	-
Sweet foods	1	2	2	-
During diarrhoea	**Reasons***			
	Loss of appetite	Increased motion	Can cause abdominal pain	Can cause bloating
*Types of prohibited food to children**				
Rice	3	28	2	-
Pork/ beef/ fish	-	12	3	-
Vegetables	2	6	5	-
Water convolvulus	-	8	3	-
Tomato	-	3	2	-
Fruits (mango/ banana)	-	9	2	-
Sour foods	-	8	2	-
Legumes	-	3	1	3
Bread/ potato/ rice noodle with fish soup	-	9	2	-

*Mothers answered more than one kind of foods and reasons

"My daughter fed rice gruel to my grandchild when he got illness because rice gruel is easy to digest and causes sweating and she fed biscuit while he got diarrhea because the child refused to eat rice".

(a grandmother of one year old child)

"Sayama advised me not to feed rice to baby but to feed rice gruel while rising temperature".

(a mother of 7 months old child)

"When my baby got diarrhea, I fed chicken soup and fried chicken to be strong".

(a mother of 15 months old child)

"I did not feed foods that would cause diarrhea such as orange and sour foods while getting diarrhea".

(a mother of one year old child)

While asking about the source of advice on feeding during illness and diarrhea, most of the mothers answered that the elders, peers and nurses were the source of advice. Nurses were also source of advice on solid food restriction/withdrawal during illness and diarrhea. They were usually advised by these health care providers to restrict solid foods while light, easy-to-digest foods such as rice gruel and chicken soup were given during illness and diarrhea.

DISCUSSION

Breastfeeding was almost universally practiced among the mothers who participated in this study but not exclusively breast-fed.

Among all children who had been fed with complementary foods, only 10 (12.8%) children were fed with supplementary food appropriately (appropriate foods with amount and frequency) based on the child's age. Among 68 children (who were older than 6 months), only 49 children (62.8%) were fed with appropriate complementary foods but amount and frequency did not meet with feeding guideline. Complementary foods should be varied and include adequate quantities of meat, poultry, fish or eggs, as well as vitamin A rich fruits and vegetables every day. About 50% of under 6 months old children had been started with complementary feeding with sieved or chewed rice.

According to WHO complementary feeding guideline for breastfed children, complementary feeding should be started at the age of six months of children.[1] According to the guideline, semi-solid or pureed foods are needed at first, until the ability for "munching" (up and down mandibular movements) or chewing (use of teeth) appears. Small amounts of food should be started at six months of age, and the quantity increases as the child gets older, while maintaining frequent breastfeeding. Infants can eat pureed, mashed and semi-solid foods beginning at six months of age.[1]

About 75% of 12-23 months old children were fed with family foods. By 12 months, most children can eat the same types of foods as consumed by the rest of the family.[1] It is found that, 27 mothers (34.6%) and 18 mothers (23.1%) fed with breast milk alone during diarrhea and illness. About 68% of children were fed with liquid diet (breast milk alone/ breast milk with chicken soup alone/ rice gruel) during diarrhea and illness. Decreasing solid food during diarrhea episode has been observed in other studies done in India (83%)[5] and Pakistan (65%).[6]

Majority of mothers reported that certain kind of foods especially solid foods were prohibited during illness and diarrhea. Such withdrawal was based on the ideology of the disease in the child. Rice was omitted in 47 children and 28 children with the reasons of rising temperature and increased motion, respectively. Vegetables, fruits, legumes, meat/ fish, sour foods were also prohibited to feed children during illness and diarrhea being afraid of rising temperature and increased motion.

Mothers restricted their children's diet when they had illness and diarrhea. Experts now believe that children should continue their regular diet when they have illness or diarrhea. In fact, the American Academy of Paediatrics states that most children should continue to eat a normal diet including formula or milk while they have mild diarrhea.[7] And the CDC recommends that children receiving semi-solid or solid foods should continue to receive their usual diet during episodes of diarrhea.[8]

The mothers' knowledge about and attitudes towards complementary feeding practices and feeding practices during child's illness and diarrhea were not consistent with healthy practices. It seems that lack of knowledge in population has led to faulty beliefs in relation to the mode of feeding during illness and diarrhea. Many cultures hold strong beliefs about feeding of appropriate and nutritious foods during illness. The cultural reasons cited include

feeding practices passed from generations and also traditional belief that some foods should be withdrawn during illness and diarrhea.

Conclusion

The findings of this study indicate that lack of knowledge in population has led to faulty beliefs in relation to the mode of feeding during illness and diarrhea. Beliefs concerning the relationship between illness and diarrhea and the consumption of particular foods (notably rice, vegetables and fruits in this study) are strongly cited in rural mothers. All the obtained results are evident that improper beliefs are the result of insufficient knowledge in community.

Recommendations

The results indicate a need for the health education program to promote increased fluid intake and continued normal feeding during illness and diarrhea. Food-based complementary feeding guide-line should be developed with locally available nutritious foods, preparation methods with appropriate meal frequency according to age and appropriate amount by commonly used utensils (eg. bowl or cup or spoon).

There is a need to target on appropriate health and nutrition education programs that would improve caregivers and mothers' knowledge and help in developing positive attitude and healthy practices which will eventually help to reduce morbidity and mortality commonly associated with childhood illness and diarrhea.

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Application of Energy Dispersive X-ray Fluorescence (EDXRF) Method for Determination of Multi-elements in Cosmetics

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Cosmetics which are widely used to cleanse, perfume, protect and change the appearance of skin contain minerals, metallic, non-metallic additives and preservatives. As claimed effects and safety of cosmetics depend upon its raw ingredients being absorbed through the skin and lips. Determination of elemental concentration of ingredients in cosmetics for consumer safety becomes a necessity. Selected well-known brands of make-up cakes and lipstick samples, bought from 7 cosmetic shops and 3 beauty salons in Yangon area were coded and prepared along standard methods for testing. Photographic recording and documentation were done under strict confidentiality and all samples were subjected to analysis by Energy Dispersive X-ray Fluorescence (EDXRF) technique. The measuring time for each sample was 99 seconds and its minimum detectable level was 0.001ppm. There was wide variation in elemental concentrations without significant detection of impurities. High concentrations of titanium (Ti: 6150.9±4937.3 ppm in make-up cakes and 6640±4741 ppm in lipsticks) and iron (Fe: 2317.6±1802.2 ppm in make-up cakes and 1584.5±1028.5 ppm in lipsticks) were detected in all samples. Other elements significantly detected were calcium (118-267 ppm), sulphur (632-1865 ppm) and copper (30-45 ppm) in lipstick samples. In make-up samples, significantly detected elements were calcium (195-13336 ppm) and copper (28-104 ppm). Toxic heavy metals like lead, mercury and arsenic were below the limit of detection. In conclusion, selected brands tested were of acceptable quality but a wider study was recommended on cheaper, less-popular cosmetics for likelihood of counterfeit brands and low-quality ones for detection of hazardous substances, impurities and adulterants.

INTRODUCTION

The use of lipstick and cosmetics dated back to the times of ancient Egyptians, who used henna or crushed insects like carmine, cochineal beetles and ants to obtain different shades of colors.[1] Cosmetics used today are mixtures of surfactants, oil and ingredients, widely used to cleanse, perfume, protect and change the appearance of the skin. Although lipstick has become the most commonly used cosmetic product in the world, consumers generally have no idea that beauty products may contain harsh chemicals and potentially harmful ingredients.[2] Contrary to previous beliefs that skin was impermeable, it was now well known that skin easily absorbed chemicals and that regular application of lipstick can lead to ingestion up to 4 pounds in one lifetime.[3] Environmental Working Group (EWG) of WHO has estimated that 98% of all chemicals used in lipsticks and cosmetics have not been tested for human safety.[4]

The Consumer Protection Act came into effect in 2009 to promote and advance the social and economic welfare of consumers; urged the testing of all cosmetics for consumer safety.[2] Cosmetics also contain mineral, metallic and non-metallic additives,

which increase the potential for raw materials to be contaminated with small amount of lead as well. Although not intentionally used as cosmetic ingredients, recent reports of Campaign for Safe Cosmetic (CSC) indicated the presence of lead in more than half of 33 brands of lipsticks in Europe with lead levels ranged from 0.03 to 0.65 ppm, but none of them was listed lead as an ingredient. One-third had higher level of more than 0.1 ppm.[5]

In Australia, National Industrial Chemical Notification and Assessment Scheme (NICNAS) called for information on lead contamination of cosmetic products manufacturing industries in May, 2007.

Although permissible concentration of lead in candy is 0.1 ppm according to the Food and drug Administration (FDA), there is no safety limit for lipstick.[6] As claimed effects and safety of cosmetics depend upon its raw ingredients being absorbed through the skin and lips, determination of elemental concentration of ingredients in cosmetics for consumer safety becomes a necessity.

Elemental concentration in cosmetics can be identified and quantified by using Energy Dispersive X-ray Fluorescence (EDXRF) technique which can be used for different sample types of solid, powder and liquid forms. It is relatively simple, rapid, low cost and can perform multi-elements determination from ppm to high-weight percent of elements.[7]

General objective

- To promote consumer safety through evaluation of multi-elements in lipsticks and make-up cakes using EDXRF technique

Specific objectives

- To determine the toxic element lead (Pb) in various cosmetic samples
- To determine the presence of other elements in various cosmetic samples
- To compare the potential hazard of elemental concentrations among cosmetic samples

MATERIAL AND METHODS

This study was conducted in collaboration with National Poison Control Centre, DMR (LM) and Department of Physics, Yangon University (YU) and Universities' Research Centre (YU). A laboratory-based, analytical study was carried out from January to December, 2010.

Selected well-known brands of lipstick and make-up cake samples, 10 each, were bought from 7 cosmetic shops and 3 beauty salons in Yangon area. Samples were coded and prepared along standard methods for testing. Photographic recording and documentation were done under strict confidentiality.

Sample preparation

The collected lipstick samples were cut into slices by using cutting machine. The volume of each slice was 0.45 cm^3 (1.5 cm x 1 cm x 0.3 cm). The collected make-up cake samples were grounded in order to get fine powder**.** The grinder produced samples that were tested to be fine enough to meet the conditions for homogeneous dense materials. Each powder sample was poured into a steel container and pressed into a pellet using 3-ton weight of hydraulic press. The diameter of each pellet was 2.5 cm.

Sample analysis

All samples were subjected to analysis by Energy Dispersive X-ray Fluorescence (EDXRF) technique.[7] The prepared samples were placed in a sample holder of EDXRF machine and covered with a cylindrical-shaped cover. The chambers were pumped up to vacuum, using EDX-700 software, selected for the required measurement condition. After measuring, unknown peaks for each sample were received. Each unknown peak was identified by using library file in EDX-700 software to get quantitative and qualitative concentrations in the test samples. The measuring time for each sample was 99 seconds and its minimum detectable level was 0.001 ppm.

RESULTS

Wide variation in elemental concentrations was detected in all samples without significant detection of impurities. Fig. 1 and 2 show the samples of EDXRF spectrum for lipstick and make-up cake samples, respectively.

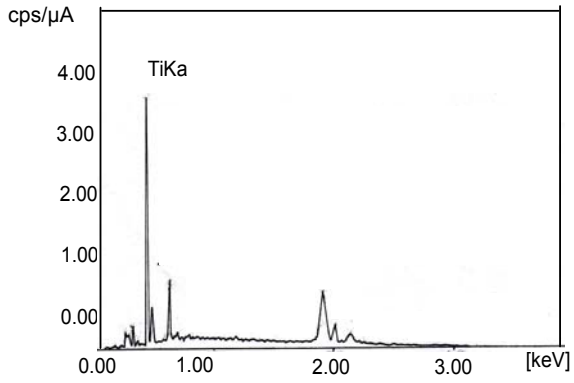

Fig. 1. EDXRF spectrum for lipstick samples

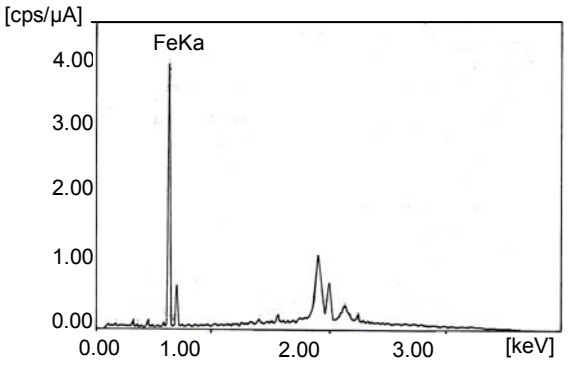

Fig. 2. EDXRF spectrum for make-up cake samples

The different elemental concentrations detected in lipstick samples are shown in Table 1. In the lipstick samples, high concentrations of titanium (Ti) 6640± 4741 ppm and iron (Fe) 1584.5±1028.5 ppm were detected. Other elements significantly detected include calcium (Ca) (118-267 ppm), sulphur (S) (632-1865 ppm) and copper (Cu) (30-45 ppm). Toxic heavy metals like lead, mercury and arsenic were below the limit of detection (0.001 ppm). The different elemental concentrations in make-up cake samples are shown in Table 2. In the make-up cake samples, high concentrations of titanium (Ti) 6150.9±4937.3 ppm and iron (Fe) 2317.6±1802.2 ppm were detected. Other elements significantly detected include silicon (Si) (54445-214521 ppm), calcium (Ca) (195-13336 ppm), copper (Cu) (28-104 ppm) and potassium (K) (289-3867 ppm). Toxic heavy metals like lead, mercury and arsenic were below the limit of detection (0.001 ppm).

DISCUSSION

Every day people consume, use or contact many different chemicals (natural and synthetic) contained in lipstick and cosmetic products generally assuming them as tested by FDA to be safe. Even though permitted levels of FDA are available, repeated daily exposure to least toxic chemicals can still lead to gradual build up within the body over time.[8] Mercury compounds permitted by FDA in eye makeup have been known to reach concentrations up to 65 ppm and accumulation of lead has been linked to attention and learning problems, permanent brain damage, hyperactivity and aggression. [9]

In addition to lead and mercury, the cosmetic industry is known to use many chemicals like artificial colors and dyes and preservatives to extend the shelf life of the products. In spite of being assumed as safe, these ingredients include petroleum-based products, which, after repeated prolong daily application, accelerate the aging process, and promote cancer and unhealthy estrogen levels.[10] The present study used the EDXRF which is employed in many fields such as environmental control and research concerning agriculture, geology, archaeology, product quality control, and also health and disaster management.[11]

Results highlight its usefulness in screening of elemental concentration in non-biological samples for health purposes but it has the limitation of quantifying very low levels of toxic chemicals like lead and mercury. However, it has indicated high concentration of titanium and iron especially in locally produced lipstick and make-up

Table 1. Elemental concentration (ppm) of lipstick samples (L 01- L 10)

Elements	Concentration of elements (ppm)									
	L 01	L 02	L 03	L 04	L 05	L 06	L 07	L 08	L 09	L 10
Si	BDL	29967.6	19929.4	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Ti	21783.2	12094.7	3836.1	10293	13488.4	972.8	147	3309.3	235.9	247.7
Ca	241.1	138	147.5	142.1	266.7	122.6	118.7	151.4	200.5	210.4
S	1332.6	BDL	632.2	1325.2	1864.1	829.1	BDL	803.7	1113.8	927.1
Br	BDL	BDL	BDL	BDL	BDL	BDL	BDL	321.2	336.8	326.9
Fe	5775.6	674	475.4	806.9	2830.3	4722	295.5	BDL	126.8	138.9
Cu	39.3	30.7	45	32	33.8	BDL	30.4	31.4	37.4	34.4
Zn	33.7	31.1	BDL	34	BDL	BDL	BDL	BDL	29.9	29.8
Mn	BDL	BDL	27.3	BDL	BDL	BDL	BDL	1719.5	BDL	29.3
K	BDL	1968.2	946.8	BDL	BDL	BDL	BDL	97.4	BDL	BDL
Sr	BDL	30.5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
P	BDL	BDL	BDL	BDL	BDL	BDL	BDL	5948.3	BDL	BDL
Cl	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1511.4	BDL	BDL
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
As	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Hg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL=Below detection limit (0.001ppm)

Table 2. Elemental concentration (ppm) of make-up cake samples (M 01-M 10)

Elements	Concentration of elements (ppm)									
	M 01	M 02	M 03	M 04	M 05	M 06	M 07	M 08	M 09	M 10
Si	87017.7	142825	55974.8	93616.1	117083.4	60485.2	214520.9	147049.9	161554.8	54445.2
Ti	1965.9	8171.6	7405.2	12066.2	3975.9	2890.6	14268.1	9914.4	63.8	787
Ca	195.1	BDL	244.9	7179	259.5	9539.3	13335.1	12945	BDL	1011.9
S	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Br	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Fe	828.3	2240.1	1218.5	2812.6	1330.5	2419.7	6913	2608.3	2323.1	482
Cu	42.8	BDL	BDL	31.9	28.2	BDL	103.9	39.3	BDL	36.3
Zn	164.7	BDL	651.7	BDL	BDL	BDL	211.4	BDL	880.4	167.7
Mn	BDL	BDL	BDL	BDL	BDL	BDL	151.2	BDL	BDL	26.3
K	931.4	3866.4	1589.4	BDL	3496.2	2402.2	2914.8	BDL	BDL	289.8
Sr	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
P	BDL	BDL	BDL	3298.3	BDL	BDL	BDL	BDL	BDL	BDL
Cl	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
As	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Hg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL= Below detection limit (0.001ppm)

samples. Although there is no safety limit for cosmetics, the permissible level for lead in candy is 0.1 ppm according to FDA in Australia (2007). The permissible values of copper and titanium in food for daily intake recommended by the FAO/WHO (2001) are 4 mg/100g and 0.8 mg/100g, respectively.

Iron, calcium and sulphur were not hazard for consumers. Other elements significantly detected include calcium and copper, both being assumed as non-toxic at the levels detected. It has yet to be studied whether these are added for specific purposes or contaminated while adding of coloring agents or preservatives. Toxic heavy metals like lead, mercury and arsenic are below the limit of detection. Further confirmation is necessary by Atomic Absorption Spectrophotometer (AAS).

In conclusion, selected brands tested are of acceptable quality but an in-depth study using more sensitive analytical instruments was needed to clarify these findings. In addition, a more extended study is recommended especially on cheaper, less-known cosmetics, for likelihood of counterfeit brands and low-quality ones, and for detection of hazardous substances, impurities and adulterants, especially lead and mercury.

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Standardization and Acute Toxicity Study of *Premna integrifolia* Linn. (Taung-Tan-Gyi)

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The aim of this study was to evaluate the acute toxicity study, standardization and quality control assurance of various parts of *Premna integrifolia* Linn. (leaves, stem bark and roots). The botanical name *P. integrifolia* Linn. was confirmed as Myanmar name: Taung-Tan-Gyi. Phytochemical constituents of alkaloids, glycosides, steroids/terpene, flavonoids, polyphenols, tannins reducing sugar, carbohydrate were found in all parts of plant samples. Thin layer chromatogram and physicochemical standardization of various parts of plant sample were done according to WHO guideline. In the elemental analysis test, potassium (K), calcium (Ca), iron (Fe) and strontium (Sr) were found in all parts of plant samples. The LD_{50} values of 70% EtOH extracts of various parts of plant samples were supposed to be more than 20g/kg body weight according to acute toxicity study in albino mice.

INTRODUCTION

Traditional Medicine in Myanmar has been an important health care service throughout history and can be regarded as a priceless national asset unique to the Myanmar people. In addition to histological evidence, scientific research has repeatedly proven its effectiveness and usefulness in health care. [1] The most common reason for using traditional medicine is that it is more affordable and being more closely corresponding to the Myanmar's culture and ideology. Regardless of why an individual uses it, Traditional Medicine undoubtedly provides an important health care service, both with and without geographic or financial access to modern medicine, and in all areas of health care, whether it is promotion, prevention, treatment, or rehabilitation.[2]

Premna integrifolia Linn. is used as hypoglycaemic, colic, fever, cordial in India.[3] In Myanmar, there has been a conflict in the botanical identity of agnimantha and *Clerodendrum phlomidis* Linn. (Ta-Pa-Say). It is used as folklore medicine for diabetes treatment and is included in the Traditional Medicine Formulation No. 27 (TMF-27, Pyi-Lon-Chan-Thar). The cause of confusion with Taung-Tan-Gyi (*P. integrifolia* Linn.) is because these two plants were reputed for similar activities, having same Hindi and Sanskrit names, but with different botanical names.[3, 5, 6] Thus, this issue needs to be clarified. Therefore, this study was done for acute toxicity, standardization and quality control assurance of *P. integrifolia* Linn.

Objective

- To evaluate the acute toxicity and quality assurance of *P. integrifolia* Linn.

MATERIALS AND METHODS

Leaves, stem bark and roots of *Premna integrifolia* Linn. were collected during the summer season from Magway Division.

Botanical identification of P. integrifolia L.

Plant samples were subjected to taxonomic identification at Botany Department, Yangon University.[7, 8, 9]

Chemical investigation of plant samples

Raw materials of plant samples such as leaves, stem bark and roots were tested for preliminary phyto-chemical investigation by Herborne method and Unani method.[10, 11] Physico-chemical standardization and thin layer chromatogram of various parts of *Premna integrifolia* Linn. (leaves, stem bark and roots) were determined by Herborne method, Unani method and WHO guide-lines.[10, 11, 12]

Two grams of dried powder of each part of *Premna integrifolia* Linn. (leaves, stem bark and roots) were placed in each test tube separately. Ten milliliters of methanol were added and then mixed for 15 minutes with vortex mixer. Thin layer chromatography was employed to detect the phyto-constituents of the methanolic extracts of plant samples as well as to find the best solvent system for toluene : EtOAc (4.5:0.5) for lipophilic compounds and EtOAC:MeOH:H_2O (5:0.6:0.5) for polar compounds.[13]

Qualitative elemental analysis of plant samples by Energy Dispersive X-rays Fluo-rescence Spectrometer (Shimadzu EDX-700) was done at University of Research Center, Yangon. X-ray spectrometry has long been recognized as a powerful method for multi-elemental analysis.[14] The general advantages of ED-XRF are: simultaneous analysis, faster multi-element screening, detection of unexpected elements, high X-ray tubes or low beam currents or radioisotope excitation and little sample damage by radiation.[15]

Preparation of plant extracts

One hundred grams of each part of dried powder of *Premna integrifolia* Linn. (leaves, stem bark and roots) were separately percolated with petroleum-ether (40-60°C) about two weeks for removal of fat and wax and then filtered. The residue was extracted with 70% ethanol on waterbath at 50°C for 6 hours and then filtered and the filtrate was evaporated on waterbath at 100ºC until to dry residue.[10]

Acute toxicity study of plant extracts

Acute toxicity of defatted 70% EtOH extract of various parts (leaves, stem bark and roots) from *Premna integrifolia* Linn. was tested on mice by Litchfield and Wilcoxan Method.[16] Acute toxicity is the toxicity produced by a pharmaceutical when it is administered in one or more doses during a period not exceeding 24 hours. One hundred healthy albino mice (ddy strain) of 25-30 g body weights were used for this study. They were separated into 10 groups, each containing 10 mice and they were allowed free access to water and pellet diet for a week. On the experiment day, all groups of mice were fasted overnight and only water was given. Three doses of test extracts (12 g/kg, 16 g/kg, and 20 g/kg) were administered orally. The control group received distilled water only. The general toxicity signs were recorded 1 hourly up to 6 hours and daily up to two weeks.

RESULTS AND DISCUSSION

Botanical identification

Myanmar name	: Taung-Tan-Gyi
Botanical name	: *Premna integrifolia* Linn.
Family name	: Verbenaceae
Hindi name	: Arni
Sanskrit name	: Agnimantha
Synonym	: *Premna obtusifolia* Linn., *Premna corymbosa* auct. Non Rottl & Willd. *Cornutia corymbosa* Burm. f.

Some authors described two types of *Agnimantha viz, Brithadagnimantha* (i.e., the big variety of Agnimantha) which is considered by many as *P. integrifolia* Linn. and the *Kshudra* or *Llaghu agnimantha* (the smaller variety) which is considered to be C*lerodendrum phlomidis* Linn.[3]

Two kinds of *Agnimantha* are described in Ayurvedic texts *viz, Laghu* and *Vridha*. In *Charaka Samhita,* both are mentioned as a separate entity, *Agnimantha* and *Tarkari*, which according to some Ayurvedists are *P. integrifolia* Linn. and *C. phlomidis* Linn., respectively. However, in Ayurvedic Formulary Part I, published by the Goverment of India, *C. phlomidis* Linn. has been accepted as *Agnimantha,* whereas *P. integrifolia* Linn. was considered as a substitute.[17]

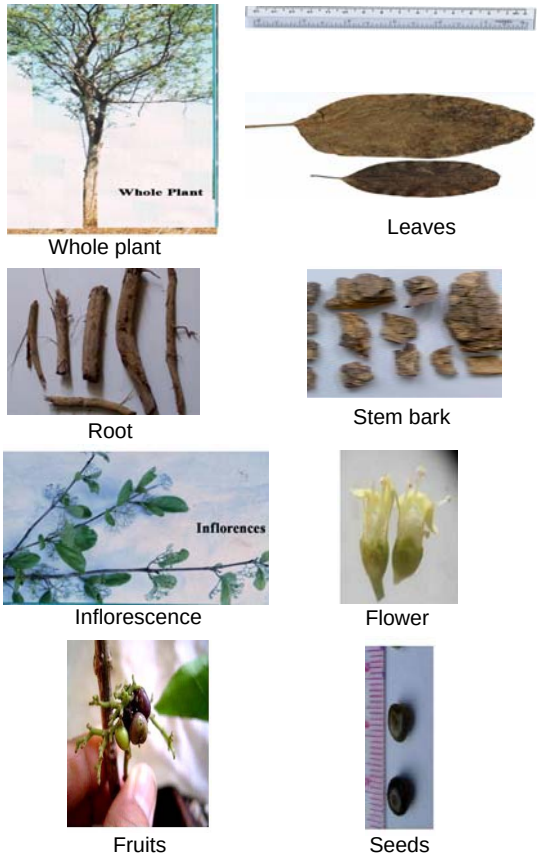

Fig. 1. Photographs of every part of *Premna integrifotia* Linn.

The family Verbenaceae as defined consists of about 100 genera and 2600 species, of pantropical distribution; only a limited number of species occur in temperate regions, the largest genera are Clerodendrum (400), Verbena (250), Vitex (250), Lippia (200), Premna (200), and Lantana (150). Shrub or tree, 1-4 (0.8) m tall, erect branchlets pale yellow, lenticellate, densely pubescent when young, subglabrescent. Petiole 0.3-5 cm, puberulent, leaf blade papery to subleathery, oblong, broadly ovate, oblong-ovate, obovate to suborbicular, 3-15x2.5-9.5 cm, base broadly cuneate, rounded, or truncate, apex acute to rarely acuminate or obtuse, margin entire, slightly undulate, subglabrous only along veins on both surfaces. Inflorescences 1.5-15 cm x 2.5-24 cm, Peduncle 0.8-3 cm, Bracts up to 6 mm, puberulent. Calyx cupular, 1.5-3 mm, 2-lipped, lower lip subentire to shortly 3-dentate, upper lip longer than lower lip and 2-dentate, abaxillary puberulent and yellow glandular. Corolla axillary glandular, lobes subequal or middle lobe slightly longer and broader. Ovary glabrous, apically glandular, style 3.5-4 mm. Fruit globose or obovoid, 2-4 mm in diameter, sparsely yellow glandular (Fig. 1).

Phytochemical and physico-chemical standardization

Preliminary phytochemical investigation of leaves, stem barks and roots of *Premna integrifolia* Linn. was done by test tube method.[10, 11] The phytoconstituents of various parts of plant samples (leaves, stem bark and roots) are shown in Table 1.

Table 1. Results of preliminary phytochemical investigation of leaves, stem barks and roots of *P. integrifolia* Linn.

Constituents	Reagents	Observation	Results		
			Leaves	Barks	Roots
Alkaloids	Dragendroff's reagent	Orange ppt	+	+	+
	Mayer reagent	White ppt	+	+	+
	Sodium picrate solution	Yellow ppt	+	+	+
Steroid/ Terpenes	Acetic anhyd-ride and conc: H_2SO_4	Green blue	+	+	+
Flavonoid	conc: HCl/Mg	Red	+	+	+
Polyphenol	10% $FeCl_3$ solution	Deep blue	+	+	+
Tannin	1% Gelatin solution	White ppt.	+	+	+
Saponin	distill H_2O	Frothing	+	+	+
Cyanogenic glycoside	Picric paper	Brown	-	-	-
Amino acid	Ninhydrin regent	Violet	+	+	+
Glycoside	10% lead acetate	White ppt	+	+	+
Reducing sugar	Fehling solution	Brick red	+	+	+
Carbo-hydrate	α-Naphthanol sol:	Red ring	+	+	+

(+) = Present (-) = Absent

Secondary metabolites (alkaloids, glycosides, steroids/terpene, flavonoids, polyphenols, tannins) possessed pharmacological effect. Alkaloids were found as major constituent of the stem back, but the roots had polyphenol as a major constituent. Results of physico-chemical standardization of *Premna integrifolia* Linn. are shown in Table 2.

Table 2. Results of physico-chemical standardization of various parts of *P. integrifolia* Linn.

Quality control parameters	Results		
	Leaves	Stem barks	Roots
Swelling index (cm)	0.1	0.1	0.2
Foaming index	<100	<100	<100
Total ash value (%)	11.8	4.6	6.8
Water soluble ash (%)	9.9	3.2	5.9
Acid insoluble ash (%)	0.5	0.8	0.8
Moisture content (%)	8.0	6.8	8.2
Extract values			
Watery extract (%)	22.1	8.0	10.1
Ethanolic extract (%)	7.2	5.8	4.7
Chloroform extract (%)	5.2	4.2	4.0
Pet-ether extract (%)	1.6	4.4	1.8

TLC screening of methanolic extract of various parts of plant samples under UV-254 nm and UV-365 nm are shown in Table 3.

Table 3. Results of TLC screening of methanolic extract of various parts of *P. integrifolia* Linn**.**

Sample	Solvent system	Detection (nm)	R_f value
Methanolic extract of leaves	EtOAc:MeOH:H_2O 5:0.6:0.5	UV 254	-
		UV 365	0.96, 0.92, 0.78, 0.68, 0.6, 0.14
	Toluene:EtOAc 4.5:0.5	UV 254	0.57, 0.44
		UV 365	0.98,0.57, 0.44, 0.2
Methanolic extract of stem barks	EtOAc:MeOH:H_2O 5:6:0.6	UV 254	-
		UV 365	0.95, 0.93, 0.15, 0.12
	Toluene:EtOAc 4.5:0.5	UV 254	0.8, 0.72
		UV 365	0.48, 0.09, 0.03
Methanolic extract of roots	EtOAc:MeOH:H_2O 5:0.6:0.5	UV 254	-
		UV 365	0.38
	Toluene:EtOAc 4.5:0.5	UV 254	-
		UV 365	0.47, 0.09, 0.03

Elemental analysis

The qualitative elemental analysis was performed by ED-XRF. Potassium (K), calcium (Ca), iron (Fe) and strontium (Sr) were found in all parts of plant samples. Leaves were found to be rich in calcium (Ca) and potassium (K). Calcium (Ca) was present as a major element in the stem bark whereas phosphorus (P) and calcium (Ca) were abundant in roots.

Potassium is a special consideration among the elements for the diabetics. Potassium supplements help to support sugar balance in the body i.e., converting blood glucose to glycogen (stored carbohydrates that make up the body's priority fuel reserves). Calcium increase in cell promotes the secretion of insulin. Insulin is secreted from the β-cells of the islets into the blood by a complex process; it requires Ca^{2+} which is important in the action of insulin.[18]

Acute toxicity study

Defatted 70% EtOH extract of leaves (15.5%), defatted 70% EtOH extract of stem barks (27.3%), defatted 70% EtOH extract of roots (35%) of *Premna integrifolia* Linn. were tested for acute toxicity on albino mice. At the end of the test period, all of the test animals were healthy and alive. Therefore, these extracts showed no lethal effect when tested for acute toxicity (LD_{50}) up to maximum doses of 20 g/kg.

Conclusion

Thus, the study confirmed the folklore medicinal plant by the Myanmar name Taung-Tan-Gyi and botanical name *Premna integrifolia* Linn. These 70% EtOH extracts of various parts (leaves, stem bark and root) of *Premna integrifolia* Linn. showed no lethal effect when tested for acute toxicity (LD_{50}) up to maximum doses of 20 g/kg, so it was practically non-toxic.

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Determination of Rifampicin and Isoniazid in Different Formulations from Market in Yangon, Myanmar

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In treatment of tuberculosis, four-drug fixed-dose combination (4FDC) (rifampicin, isoniazid, pyrazinamide and ethambutol) and two-drug fixed-dose combinations (2FDC) (rifampicin, isoniazid) were replaced by individual drug formulation because of more patient compliance and less expense. The WHO-supplied 4FDC from the National Tuberculosis Program (NTP) was compared with other available formulations/brands of anti-TB drugs currently available in the market in terms of qualitative and quantitative profiles of active substances (RIF and INH) and presence of impurities. Analytical parameters include uniformity of weight, pH value, identification of active ingredients and disintegration. It was carried out from February to December 2004. Some samples were received from NTP and some were bought from registered drug companies and private drug shops. The findings indicated the uniformity of weight within 5%, disintegration to be <30 minutes and pH value ranging from 6.5 to 7.2. Qualitative test of RIF and INH were determined by UV Spectrophotometer and Fourier Transform Infra-Red Spectrophotometer. Quantitative analysis of RIF and INH was done by High Performance Liquid Chromatography (HPLC) and fluorescence spectrophotometer, respectively. The content of active substance was expressed as percentage of the amount claimed to be present in the formulation. The RIF contents of 4FDC and 2FDC used by the NTP were 130% and 105%, respectively. Other drug combinations from market showed that the RIF content was found to range from 78-112%. The content of INH was also variable, and ranged from 90-107%. It was concluded that the 4FDCs supplied by the WHO and used by the NTP are of acceptable quality.

INTRODUCTION

Tuberculosis stands third in prioritized health problem in the National Health Plan and is also a second major cause of hospital deaths in Myanmar.[1] The burden inflicted by tuberculosis on the health sector is the rising incidence and spread of MDR-TB, especially in the poor but also economically, in the most productive age group of 15-50 years.[2] Drugs play an important role in improving human health and promoting well being.

However, to produce desired effect, they have to be safe, efficacious and of acceptable quality and have to be used rationally.[3] The use of ineffective and poor quality drugs will not only endanger therapeutic treatment but also erodes public confidence in using drugs.[4] Four standard anti-tuberculosis drugs (rifampicin, isoniazid, pyrazenamide and ethambutol) have been used in Myanmar as first-line drugs in tuberculosis since 1966. WHO has recommended the use of short-course chemotherapy to achieve 85% cure rate in smear-positive pulmonary tuberculosis cases.[5]

Nowadays, various combinations of drugs have been used for tuberculosis, however, drugs resistance is becoming a major health

problem.[6] Long duration of treatment, poor case holding, poor drug supply, poor quality drugs and poor patient compliance are major issues. Irregular taking of drugs has led to development of resistant strains.[5] Many brands and formulations including fixed-dose combinations are available and have been used with assumption that they are of similar efficacy and bioequivalence.[7, 9] Therefore, assurance of drug quality is of prime importance in affecting a cure as well as prevention of MDR-TB.

MATERIALS AND METHODS

Study design

Laboratory-based analytical study

Study site

Pharmacology and Pharmaceutical Toxicology Research Division, DMR (LM)

Sample size

Fifteen different brands of anti-TB drugs available as individuals and fixed dose combinations in the market in Yangon as well as those used in National Tuberculosis Control Programme (NTP) and code names were used for anti-TB drugs tested.

Study procedure

Fifteen different brands of anti-TB drugs available as individuals and combinations were tested for their uniformity of weight, disintegration and pH value by British Pharmacopeia method (2001). Identification of the two drugs in the formulations was done by Thin Layer Chromotography (TLC) and Fourier Transform Infra-Red (FT-IR) Spectrophotometric method. Extraction of drug samples and standard powder was done by using HPLC grade methanol and 5% trichloroacetic acid and they were stored at -20˚C until analysis.

Qualitative analysis of rifampicin and isoniazid

Thin Layer Chromotogram (TLC)

Rifampicin (RIF) and isoniazid (INH) from fixed-dose combinations and standard drug were soluble in methanol to obtain 1mg/ml concentration. Test samples and standard drug were loaded as the spots on silica gel plate (GF_{254}). TLC plate was immersed in the solvent system of chloroform and methanol (9:1) in TLC tank. After development of the chromatogram, it was air-dried for 10 minutes. For the visualization of the compounds, thin layer chromatograms were checked under the UV light of 254 nm and 365 nm wavelengths and sprayed with iodine vapour. The R_f values of the sample spots were compared with that of standard drug.[8]

UV Spectrophotometric method

0.1 g of rifampicin was dissolved in 80 ml of methanol and the solution was filtered with Whatman filter paper. Two milliliters of the filtrate was diluted with 100 ml of phosphate buffer (pH=7.4) and measured at 475 nm by ultra violet spectrophotometer (B.P-2001). The spectrum was compared with optical density of standard.

Fourier Transform Infra-Red Spectrophotometer (FT-IR) method

A quantity of the contents of the capsules containing 0.2 g of isoniazid was dissolved in 10 ml of methanol, filtered, and the filtrate was evaporated to dryness. Isoniazid was determined by Fourier Transform Infrared Spectrophotometer (FT-IR 8400). The infrared absorption spectrum of the test sample was compared with that of standard drug in pharmaceutical library.

Quantitative determination by HPLC and fluorometric method

All samples were analyzed quantitatively by standard methods for INH[10] and High Performance Liquid Chromatography (HPLC) for rifampicin.[11]

RESULTS

Physico-chemical properties of each sample are shown in Table 1. pH of standard rifampicin is between 4.5 to 6.5. The findings indicated the variability in weight to be <5%, disintegration to be <30 minutes and pH ranging from 4.96 to 7.10 in all the samples

Table 1. Physico-chemical properties of drugs

Sample	Batch no.	Uniformity of weight (%)	Disintegration (min)	pH value (1% sol)
Rifampicin 300 mg (Unicef)	CTJ2302	3.8(7.5)	16	4.96
Rifampicin 150 mg (Unicef)	63502	6.4(10)	23	6.4
Rifampicin 300 mg (NTP/WHO)		1.4(5)	2	6.8
Isoniazid 75 mg (NTP/WHO)		2.9(10)	15	6.9
Isoniazid 100 mg (MPF)		1.7(7.5)	5	7.1
ATB-1 (Rifampicin 450 mg Isoniazid 300 mg)	B41344	2.3(5)	1	6.9
ATB-2 (Rifampicin 450 mg Isoniazid 300 mg)	CR157	1.13(5)	5	7.02
ATB-3 (Rifampicin 450 mg Isoniazid 300 mg)	E2001	2.4(5)	3	6.3
ATB-4 (Rifampicin 450 mg Isoniazid 300 mg)	1074	2.6(5)	1	6.5
ATB-5 (Rifampicin 450 mg Isoniazid 300 mg)	X3085	2(7.5)	11	6.7
Two-drug fixed-dose combination (Rifampicin 150 mg Isoniazid 75 mg) (NTP/WHO)	40001	2(5)	3	6.2
ATB-6 (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400 mg Ethambutol 275 mg)	X4005	1.2(5)	2	5.2
ATB-7 (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400mg Ethambutol 275 mg)	FT404	3.2(5)	4	5.1
ATB-8 (Rifampicin 450 mg Isoniazid 300 mg Pyrazinamide 750 mg Ethambutol 800 mg)	X3065	5.2(7.5)	3	7.0
4FDCS (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400 mg Ethambutol 275 mg) (NTP/WHO)		3.2(5)	4	5.04

tested. Qualitative analysis using UV spectrophotometry and FT-IR confirmed the presence of rifampicin and INH without significant detection of impurities (Fig.1). This was further subjected to analysis by TLC for confirmation (Fig.2). Quantitative analysis of rifampicin and INH was done by using HPLC and fluorescence spectrophotometer, respectively.

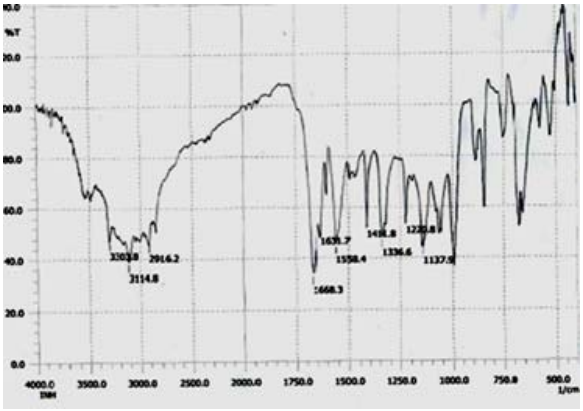

Fig. 1. FT-IR spectrum of isoniazid

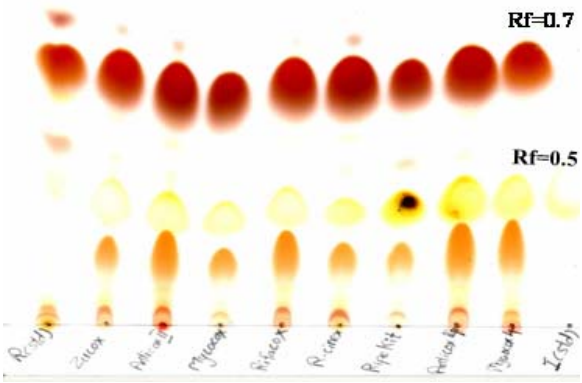

Fig. 2. Identification of rifampicin and isoniazid in two-drug fixed-dose combinations by using TLC method

The content of active substance was expressed as percentage of the amount claimed to be present in the formulation per tablet/capsule. The rifampicin and INH contents of 4FDC currently used by NTP were 130% and 103%, respectively. Rifampicin and INH contents in two-drug combinations used by the NTP were 105.9% and 102%, respectively, while in other drug combinations (ATB-6, 7, 8), the rifampicin content was found to range from 91-111%. The contents of INH of the combination drugs (ATB-1 to ATB-8) were also variable, and ranged from 91-107% (Table 2). It was concluded that the 4FDCs supplied by the WHO and used by the NTP are of acceptable quality and further bioavailability in patients should be done.

Table 2. Quantitative assay of rifampicin and isoniazid in all samples

Sample	Batch no.	Rifampicin assay by HPLC (%)	Isoniazid assay by fluorescence spectrophotometer (%)
Rifampicin 300 mg (Unicef)	CTJ2302	104.02	
Rifampicin 150 mg (Unicef)	63502	130.00	
Rifampicin 300 mg (NTP/WHO)		103.4	
Isoniazid 75 mg (NTP/WHO)			98
Isoniazid 100 mg (MPF)			92
ATB-1 (Rifampicin 450 mg Isoniazid 300 mg)	B41344	78.46	91
ATB-2 (Rifampicin 450 mg Isoniazid 300 mg)	CR157	78.06	93
ATB-3 (Rifampicin 450 mg Isoniazid 300 mg)	E2001	85.22	95
ATB-4 (Rifampicin 450 mg Isoniazid 300 mg)	1074	86.61	97
ATB-5 (Rifampicin 450 mg Isoniazid 300 mg)	X3085	79.76	99
2FDC (NTP/WHO) (Rifampicin 150 mg Isoniazid 75 mg)		105.93	102
ATB-6 (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400 mg Ethambutol 275 mg)	X4005	95.19	96
ATB-7 (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400 mg Ethambutol 275 mg)	FT404	111.13	107
ATB-8 (Rifampicin 450 mg Isoniazid 300 mg Pyrazinamide 750 mg Ethambutol 800 mg)	X3065	91.46	94
4FDCS (NTP/WHO) (Rifampicin 150 mg Isoniazid 75 mg Pyrazinamide 400mg Ethambutol 275 mg)		130	103

DISCUSSION

Nowadays, fixed-dose combinations administered under direct observation by NTP have been recommended by the WHO to help overcome the problem of treatment failure due to drug availability, irrational prescribing as well as quality assurance (drug bioavailability). In Myanmar, drugs are now dispensed free of charge by the NTP/WHO DOTS program at the TB centers which prevent patients from buying cheaper substandard drugs as well. Among anti-TB drugs, rifampicin and INH are the most important drugs in the treatment of TB and resistance to at least these two has been termed as multidrug-resistant tuberculosis (MDR-TB). MDR-TB has now become a worldwide problem and this occurs as a result of:

- Pharmaceutical factors: Counterfeit and substandard drugs
- Prescription factors: Irrational and irregular use which include inappropriate dose, dosing interval and duration of treatment

Since the WHO has stressed the need for screening and quality control, the project was carried out as part of a WHO/TDR/RCS project on 4FDC and comparing its quality with other anti-TB drugs available and used by patients. The present study indicated that uniformity in weight, identification and disintegration tests were acceptable for all preparations. The 4FDC supplied by the WHO contained adequate active ingredient (rifampicin 130%) in contrast to other 4FDC including ATB-6, 7, 8 (rifampicin range, 91.46 - 111.13%). With the 2FDC, only that supplied by the WHO contained adequate active ingredient (rifampicin 105.93%), while others (ATB-1 to 5) contained less than claimed (rifampicin range, 78.06%-86.61%). Individual drug preparations of rifampicin from NTP and UNICEF were also acceptable (rifampicin 103.4%-130%). INH present in drug combinations can be assumed adequate (INH range, 91% - 107%).

Therefore, pharmaceutical study on quality assurance carried out on 4 FDC supplied by WHO and other available combinations in Myanmar indicated that the 4FDC supplied by WHO contained adequate active ingredients (rifampicin130% and isoniazid 103%) without significant detection of impurities and are considered to have acceptable quality to be used by NTP. Finally, one must also bear in mind that the results of the

tested drugs may also vary from batch to batch, and also from difference in the date of manufacture and storing conditions (temperature, humidity, etc.).

In conclusion, although the study is a preliminary one, it has highlighted the quality assurance of TB drugs available in Myanmar, and the likely variability that is to be expected.

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A Pilot Study on Anti-streptolysin O Levels in Normal Medical Students, University of Medicine (Magway)

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ASO titers were determined in 30 third-year MBBS students of University of Medicine, Magway. ESR and blood for complete picture, CP and ECG pattern of participants were also determined to exclude acute rheumatic fever applying Jones' criteria. All were apparently normal without scarlet fever and rheumatoid arthritis. All students were free from acute rheumatic fever according to Jones' criteria. The mean titre of ASO was 235 IU, slightly raised compared with a reference level. Mean ASO titre for female was 235.2 IU/ml and for male was 234.9 IU/ml.

INTRODUCTION

Anti-streptolysin O antibodies are produced in Group A β-haemolytic infection. Increase in ASO titer generally occurs 1-4 weeks after infection with Group A β-haemolytic streptococci. The ASO latex test is a rapid latex agglutination test for the qualitative and semiquantitative determination of anti-streptolysin O antibodies (ASO) in serum. Although isolation of Group A β-haemolytic streptococci from throat of an individual may indicate carrier or infection, throat culture is positive in only 11% of patients with acute rhumetic fever (ARF). Increased ASO titer is indicative of acute Group A β-haemolytic streptococcal infection and provides supportive evidence in diagnosis of sequelae of GAS infection.[1] Recently ARF become increased in adult population.[2]

Normal ASO level in preschool children is less than 100 IU/ml but the level rises with the age, peaking in school age and decreasing in adult. ASO titers can also vary depending on the geographic location, seasonal and the climatic conditions.[3] Therefore, knowledge on titer of ASO in normal adult is important in diagnosis of post streptococcal diseases. The aim of the study was to determine the level of ASO in apparently normal third-year MBBS students.

MATERIALS AND METHODS

Because streptococcal antibody titres may vary in some conditions: in person with a history of rheumatoid arthritis, scarlet fever, current tonsillitis or history of tonsillitis 1 month before, several strepto-coccal infections and with history of taking antibiotics two weeks before sample collection, students with a history of above factors were excluded from the study. Students who fulfill criteria of acute rheumatic fever i.e., Jones' Criteria: Major criteria: Migratory polyarthritis, carditis, chorea, subcutaneaous nodules, erythema marginatum; Minor criteria: fever, arthral-gia, abnormal ESR were also excluded from the study.

History, blood tests (ESR and CP) and ECG were obtained for Jones' criteria. ASO levels were measured semiquantitatively by latex ASO test. This study was done at the Department of Microbiology, University of Medicine (Magway) from June to December, 2011.

Collection and transport of specimen

Blood samples (2 ml) from thirty volunteer students who fulfill the above criteria were collected aseptically after 6 hours of fasting and sera were separated out, centrifuged and stored at -20°C until used. Frozen samples were totally thawed and brought to room temperature before testing. Turbid samples, contaminated samples, lipemic samples and haemolyzed samples were discarded.

Procedure for ASO titres

A qualitative method and semiquantitative method were used according to manufacturer's instruction. ASO latex (ASO latex agglutination slide test) manufactured by Mediclone Biotech Pvt. Ltd, India was used to detect ASO levels.

A drop of serum (50 ul, neat and/ or diluted) was placed on one section of slide. A drop of latex reagent was placed next to the drop of serum, and the drops were mixed. The slide was gently rotated manually for 2 minutes, and the presence or absence of agglutination was then determined. Positive and negative controls were also tested simultaneously.

In semiquantitative method, dilutions of serum with normal saline: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 etc were used according to manufacturer's instruction.

Interpretation of result

Value**:** The ASO level is less than 160 Todd units per milliliter or 200 IU/ml.
Serum ASO concentration was calculated approximately by multiplying the dilution factor by the detection limit.

RESULT AND DISCUSSION

In considering the nonsuppurative complication of Group A streptococcal infection, an increase in ASO level is used as an indicator of Group A streptococcal infection. The absolute value of ASO is of diagnostic and provides a guideline to physicians. Clinical microbiology laboratories often use criteria suggested by manufacturers of commercial ASO test kits to interpret the results. ASO titres can vary depending on the geographic location, age group of the study population, and the climatic conditions. There are no age-specific ASO value in Myanmar children and adult.

To determine the level of ASO in apparently normal third-year MBBS students, a cross-sectional study (pilot study) was done. A total of asymptomatic "normal" 30 third-year MBBS students of UMMG, aged 19-22 years, were investigated for ASO level. (Table 1)

Table 1. ASO titer in third-year MBBS students by latex agglutination test

Sex	Sera tested No. (%)	ASO titers (IU)					
		< 200 No. (%)	250 No. (%)	300 No. (%)	350 No. (%)	400 No. (%)	500 No. (%)
Male	13 (43.33)	10 (50)	1 (10)	1 (7.6)	-	-	1 (7.6)
Female	17 (56.67)	10 (50)	2 (20)	5 (29.4)	-	-	0
Total	30 (100)	20 (100)	3 (100)	6 (20)	-	-	1 (3.33)

History, blood tests (ESR and CP) and ECG were obtained for Jones' criteria. ASO levels were measured semiquantitatively by latex ASO test. Neutralization assay is a gold standard test. However, it is expensive, time-consuming, and not as simple as latex agglutination test. The LA test has a sensitivity of 91% and specificity of 86%.

All participants did not meet/ fulfill Jones criteria indicating that they were free from acute rheumatic fever. Antibiotic treatment may reduce the response to streptococcal antigen. Students with a history of current antibiotic treatment *i.e.,* in past 2 weeks were excluded from the study. Venous blood was collected for ASO detection.

In normal person i.e. a person without clinical features of rheumatism, the ASO level is less than 160 Todd units per milliliter or 200 IU/ml. False positive result may be obtained in conditions such as

rheumatoid arthritis, scarlet fever, recent tonsillitis, several streptococcal infections and healthy carriers. All participants did not have above diseases and infections.

Among them, 13 (43.33%) were male and 17 (56.67%) were female students. Mean age is 19 years. Most of them (10 males and 10 females) have normal ASO titre (<200IU): Only 10 (33.33%) of students have had a titers over 200 units. One male student had ASO levels 250 IU and 1 had 300 IU, 5 female students had ASO titre of 300 IU/ml and 2 had 250 IU/ml.

One male student (1 in 30, 3.33%) with a history of arthritis and tonsillitis had ASO titre of 500 IU although ESR and blood for complete picture CP and ECG showed normal. However, he did not have recent sore throat. A throat swab culture was not done. Arthritis might be reactive arthritis.

The mean ASO titer was 235 IU, slightly raised compared with a reference level. Mean ASO titre for female is 235.2 IU/ml and for male is 234.9 IU/ml. ASO titre more than 200 IU/ml is considered elevated for adult. ASO titre is increased 1-4 weeks post GBS infection and returns to normal within six months after infection subsides. A high titre indicates a recurrent infection or chronic infection.

In this study, all participants gave no history of recent throat infection. We can consider this 235 IU/ml presumptively as baseline ASO titre although this value is not likely representative of the whole population of this age group. Additional studies on ASO levels in large group and in different seasons should be done for establishment of standard value (normal level) to assist correct interpretation.

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SHORT REPORT

Knowledge on Danger Signs and Ante-natal Care Visits Made by Third Trimester Pregnant Women in Shwepyitha Township

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Safe motherhood is one of the components of essential reproductive health packages. It is supported by four pillars namely: family planning, antenatal care (ANC), clean and safe delivery and essential obstetric care. The World Health Organization (WHO) indicated that a minimum of 4 visits covering the entire period of pregnancy should be targeted.[1] The ANC coverage is extremely high in the industrialized countries, with 98% of women having at least one visit. In the developing world, antenatal care use is around 68% and the lowest level is in South Asia, where only 54% of pregnant women have at least one antenatal care visit. In Myanmar, ANC coverage by trained persons was 73% according to Myanmar MDG report 2006.

When properly conducted, ANC is very effective in reducing maternal mortality through provision of health knowledge as well as early detection of danger signs of pregnancy. In our country, research studies concerning with antenatal care are limited. This may lead to missing the importance and advantages of antenatal care. This study was conducted with the objectives to determine the knowledge of pregnant women in third trimester regarding danger signs in pregnancy, and to find out the utilization of antenatal care compared with standard frequency. A community-based cross-sectional descriptive study was carried out in Shwepyitha Township from September to November 2011. The list of all pregnant women were recorded and among 461 women, all 172 pregnant women in third trimester were invited to participate and a total of 165 respondents were interviewed (response rate 96%).

Face-to-face interview was conducted using a pretested structured questionnaire and antenatal cards were reviewed to determine the ANC visit times of the pregnant mother. Data analysis was done by SPSS 16.0. Chi-square test was used to detect the differences in knowledge level by education. Significance level was set at 0.05.

Regarding danger signs, they mentioned bleeding per vagina from any cause (47.9%), odema of limbs (46.7%), fits (21.2%), watery discharge (21.2%), headache due to hypertension (16.4%) and weakness (13.3%) during pregnancy. Knowledge scores for danger signs are categorized into low (0-2 marks) which is less than or equal to median score and high (3 and above marks) which is greater than median score out of 9 correct answers.

Most of respondents (75.76%) had low knowledge about the danger signs of pregnancy. There was no statistically significant association between the age of respondents and knowledge on danger signs. However, high knowledge level was significantly associated with high school

and above education levels (Chi square= 10.6, p=0.02, Table 1).

Table 1. Association between education of respondents and knowledge on danger signs

Education of respondents	Knowledge on danger signs			
	Low no. (%)	High no. (%)	Total no. (%)	P value
Primary school & below	35(81.4)	8(18.6)	43(100)	0.02
Middle school	54(84.4)	10(15.6)	64(100)	
High school	27(64.3)	15(35.7)	42(100)	
Graduate/ college	9(56.2)	7(43.8)	16(100)	
Total	125(75.8)	40(24.2)	165(100)	

Approximately 61% of respondents correctly knew that ANC should be taken at least 4 and more times. However, nearly 73% had taken less than 4 ANC. There was no significant association between the age of women and frequency of antenatal care. It was also noted that about 74%, 62%, 49% and 56% of lower education, middle school, high school and graduate/college, respectively, had taken less than 4 antenatal care visits but not statistically significant. The knowledge on danger signs had no association with frequency of ANC taken: 52.6% of those who had high knowledge took <4 ANC and 63.8% in low knowledge category had <4 ANC.

Apparently, education status was the significant predictor for high knowledge level of danger signs of pregnancy. Their knowledge about the danger signs during pregnancy was less than 50% in this study. In one hospital-based study among 128 pregnant women in Thingangyun in 2003, 16.9% knew anaemia, 25.4% knew abortion and 8.5% knew ante-partum haemorrhage as danger signs of pregnancy.[2]

In Reproductive Health Baseline Community Survey, 64.5% knew the complications related to pregnancy as severe morning sickness (38%), abortion (30.5%), swelling of the feet (26.3%) and bleeding in early pregnancy (25.5%).[3] In most cases of maternal deaths in developing countries, this was due to the fact that pregnant women, their families and the community, in general, did not know about the danger signs that can occur during pregnancy, delivery or the puerperium.[4] The women who did not seek for ANC during pregnancy were decreasing according to the data shown by FRHS 1997 and 2001.[5]

In our study, 6.7% of pregnant mothers were not seeking for antenatal care. Only 5.5% of pregnant women who were admitted to Thingangyun Sanpya Hospital did not take antenatal care.[2] Therefore in our study, the women who did not seek ANC were more than in Thingangyun study probably due to the community-based nature of our study. In hospital-based studies, those who were admitted to hospital were likely to be cared by a healthcare provider during the pregnancy, and referred for hospital care. However, the reasons of not using ANC should be further explored by community-based studies.

As an overall, the number of mothers taking ANC is to be considered high, but their knowledge on danger signs needs much improvements. Emphasis should be made by health care providers on health education about the danger signs during antenatal care taking by mothers. The reasons for pregnant women who sought no antenatal care or less ANC visits should be further investigated.

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