AIMS OF THE JOURNAL

❖ To serve as an important medium for the publication of original research works 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
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EDITORIAL

It is our pleasure to share and disseminate applicable health research findings to health personnels and those who are interested in health knowledge; young and old alike, far and wide of the whole country through our Myanmar Health Sciences Research Journal.

Being the first issue of 2014, the present one covers a wide range of research works leading to support for solving many national health problems. Under the categories of communicable diseases, non-communicable diseases and other important health issues, it includes HIV, tuberculosis, malaria, dengue haemorrhagic fever, diabetes, traditional medicine, children with cerebral palsy, renal failure, cardiovascular response to exercise, dietary habit, intake of processed meat, verotoxin, pneumococcal infection, and environmental health, etc.

Among the papers included, pneumococcal infection in children has been chosen as the leading article of this issue. It is one of the major childhood illnesses and a common cause of invasive diseases and respiratory tract infections even in developed countries. It is highlighted that nearly 50% of pneumococci infection has been resistant to penicillin due to the uncontrolled usage of medication these days so that the development of the vaccine strategy plays an important role in combating pneumococcal diseases.

Although the article is just to assess the baseline prevalence of pneumococcal infection in Yangon Children’s Hospital, the fact that one million children under five-year-old died of pneumonia and invasive diseases every year is a critical point to be considered since children are the future of our society.

All in all, we are confident that the research findings published in this issue will give the positive and updated impact on Myanmar people to become healthier through application of new knowledge based on them.
Finally, as publishing research journal is the part of Research Knowledge Management and research findings dissemination carried out by the Department of Medical Research (Lower Myanmar), even if the valuable research information of these research papers would be beneficial to the well-being of our Myanmar people at the very least, we will be immensely pleased.
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Short Report:

Evaluation of a Microarray Nanotechnology-based Test for Diagnosis of Tuberculosis

Wah Wah Aung, Thandar Lwin, Phyu Win Ei, Hor Wai Fong, Thiruchelvan Nadarajan, Tin Tin Mar, Wint Wint Nyunt, Nan Aye Thidar Oo & Mi Mi Htwe
Pneumococcal Infection in Children Attending Yangon Children’s Hospital

Mo Mo Win¹, Mar Mar Nyein², Mi Mi Htwe², Than Mya³, Thin Thin Shwe³, Aye Maung Han⁴ & Khin Htwe⁵

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Blood samples were collected from 150 children (73 males and 77 females) with pneumonia (110 cases), septicaemia (22 cases) and meningitis (18 cases) attending Yangon Children’s Hospital from July 2006 to April 2007; ages ranging from 2 months to 12 years. Seventy (46.6%) out of 150 samples yielded bacterial pathogens by blood culture method. Among them, *Streptococcus pneumoniae* (Pneumococci) was isolated from 12 cases (17.14%). They were isolated from 7 cases of pneumonia, 3 cases of meningitis and 2 cases of septicaemia. Gentamicin blood agar plate was used for isolation of *Streptococcus pneumoniae* and for identification, colony recognition and optochin sensitivity test were done. Antibiotic susceptibility test was done by Kirby-Bauer disc agar diffusion method. They were resistant to penicillin (41.6%), gentamicin (41.6%), cotrimoxazole (33.3%), ciprofloxacin, ampicillin and ceftriaxone (16.6%, each) and amikacin (8.3%). The other pathogens isolated were *Haemophilus influenzae* (22 isolates), *Pseudomonas aeruginosa* (12 isolates), *Staphylococcus aureus* (12 isolates), *E. coli* (6 isolates), *Klebsiella pneumoniae* (4 isolates), and *Neisseria meningitidis* (2 isolates). This study highlighted the prevalence of *Streptococcus pneumoniae* and other bacteria pathogens concerning major childhood illnesses.

INTRODUCTION

The pneumococci (*Streptococcus pneumoniae*) are gram-positive diplococci, often lancet-shaped or arranged in chains. They are normal inhabitants of the oral flora and colonize the nasopharynx, particularly in young children from birth and the level of colonization gradually declines with age.¹ Although their isolation from the nasopharynx of the children with respiratory illness does not necessarily represent pneumococcal disease, nasopharyngeal colonization is often the first step in the development of pneumococcal co-infection or secondary bacteria infection may result from organisms that had colonized the nasopharynx. Invasive diseases with meningitis and bacteraemia remain “tip of the iceberg” presentation for pneumococcal disease but *Strept pneumoniae* is infrequently cultured from CSF and blood especially when antibiotics have been given.² An important factor is that pneumococcal diseases will not occur without preceding nasopharyngeal colonization with the homologous strains.

*Strept pneumoniae* is a common cause of invasive diseases and respiratory tract infections in more and less developed countries. Risk groups for diseases caused by pneumococci, such as meningitis, sepsis and pneumonia include young children, elderly people and patients with immunodeficiencies.
Each year, one million children younger than five-year old died from pneumonia and invasive diseases. Community acquired pneumococcal meningitis also has a very high case fatality rate (20% and 50% in more and less developed countries, respectively). Depending on age, 30-60% of survivors developed long-term sequelae including hearing loss, neurological deficits and neuropsychological impairments.¹

Moreover, a high incidence of penicillin-resistant and multidrug-resistant strains of *Strept pneumoniae* among clinical isolates has subsequently been reported. Since the first penicillin-resistant *Strept pneumoniae* was reported in Australia in 1967, the frequency has been increasing around the world. The prevalence of resistance makes choosing an antibiotic for invasive pneumococcal infection difficult.³,⁴

The present study was undertaken to assess the baseline prevalence of pneumococcal infection in Yangon Children’s Hospital.

**MATERIALS AND METHODS**

*Collection of specimens*

A total of 150 blood samples were taken from children (age: from 2 months to 12 years, male: 73, female: 77) suffering from pneumonia (110 cases), septicaemia (22 cases) and meningitis (18 cases) admitted to Yangon Children’s Hospital from July 2006 to April 2007. After taking informed consent, history taking and physical examination, 2 ml of blood were collected into blood culture bottle containing Tryptic Soy Broth and then transported to laboratory.

*Isolation and identification*

All isolates were recognized by their colonial morphology and alpha haemolysis displayed on gentamicin blood agar plates after incubation at 37°C (with 5% CO₂) for 24-48 hours. The isolates were further identified by their gram stain characteristics and sensitivity to optochin (5 µg) and then those isolates were serotyped by the capsular swelling method using commercially available antisera (Statens Serum Institute, Copenhagen, Denmark).

*Antibiotics susceptibility testing*

It was done by Kirby-Bauer disc agar diffusion method. The following antibiotics and concentrations (in bracket) were used: penicillin G (10 unit), ampicillin (10 µg), cotrimoxazole (1.25, 23.75 µg), ceftriaxone (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and amikacin (30 µg).

*Ethical consideration*

This study was approved by Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar) on 15 March, 2006.

**RESULTS**

*Distribution of Strept pneumoniae in children*

Out of 150 blood samples cultured, 70 cases yielded different pathogens. Among them, 12(17.14%) were identified as *Streptococcus pneumoniae* (Fig. 1 a & b).

*Isolation of Strept pneumoniae from invasive infections*

*Strept pneumoniae* were isolated from 7 cases of pneumonia, 3 cases of meningitis and 2 cases of septicaemia (Fig. 2).

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2

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Age distribution of children with Strept pneumoniae infections

Five pneumococcal isolates were found in age group 0-1 year, 5 isolates in 1-2 years age group, 1 isolate each in 3-4 years and 7-8 years age groups (Fig. 3).

Fig. 3. Age distribution of children with Strept pneumoniae infections

Outcome of children with pneumococcal infection

Regarding outcome of 12 cases with pneumococcal infections, all the pneumonia cases (7 cases) and septicaemia (2 cases) had no sequelae and they had complete recovery. In the three meningitis cases, one patient had neurological deficit and one patient died (Table 1).

Table 1. Outcome of children with pneumococcal infections

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pneumonia</th>
<th>Septicaemia</th>
<th>Meningitis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sequelae</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Sequela</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Antibiotics resistance pattern of Strept pneumoniae isolated

They were resistant to penicillin (41.6%), gentamicin (41.6%), cotrimoxazole (33.3%), ciprofloxacin, ampicillin and ceftriaxone (16.6%, each) and amikacin (8.3%) (Fig. 4).

Seasonal variation of pneumococcal infection

Pneumococcal infections were more common in winter (1 case in July, 9 cases from October to January and 2 cases in March were isolated) (Fig. 5).

The other pathogens isolated were Haemophilus influenzae 22 isolates, Pseudomonas aeruginosa 12 isolates, Staphylococcus aureus 12 isolates, Escherichia coli 6 isolates, Klebsiella pneumoniae 4 isolates and Neisseria meningitidis 2 isolates.

DISCUSSION

Pneumococcal infections are the most common cause of invasive bacteria infection in children and major invasive pneumococcal infections are meningitis, septicaemia, pneumonia, otitis media and septic arthritis. Pneumococcal colonization is also an important factor. It may colonize the respiratory tract and spread via aerosolization. Viral respiratory tract infection, malnutrition and local damage to the mucosa may also predispose to pneumococcal infection. In the United States, pneumococcal infection was estimated to cause
200 deaths, 700 cases of meningitis, 1,700 cases of bacteremia annually in children under 5 years of age. According to reports from Invasive Bacteria Infection Surveillance group (1999), case fatality rate of meningitis was 34%, pneumonia 19% and septicaemia 21%.

This study was aimed to evaluate invasive pneumococcal infection causing major childhood illnesses from July 2006 to April 2007. One hundred and fifty blood samples were obtained from children with pneumonia, septicaemia and meningitis. *Streptococcus pneumoniae* was isolated from 12 cases out of 70 culture positive cases. It was mainly isolated from children with pneumonia.

In a study regarding age distribution, the incidence of pneumococcal infection is high in neonates, infants and toddlers, low in adolescents, young adults and then increases again in the elderly. In our study, the majority of cases were found in under 2 years of age group and there was not much sex differences.

The study of the outcome of patients with pneumococcal infection revealed that all pneumonia and septicaemia cases had no sequelae and they recovered completely, whereas one patient with meningitis undergone neurological sequelae and one patient died.

Meningitis is the most severe type of pneumococcal infection. About 5% of children under 5 years of age who contract pneumococcal meningitis will die of the infection and others can have long-term problem such as hearing loss. Many children with pneumococcal pneumonia or bacteremia will need to be hospitalized.

Antibiotic resistance pattern of pneumococcal isolates from this study revealed 41.6% each resistant to penicillin and gentamicin, 33.3% to cotrimoxazole, 16.6% each to ciprofloxacin, ampicillin, ceftriaxone and 8.3% to amikacin. In a report from Taiwan, 82% and 87% of 200 pneumococci were resistant to penicillin and cotrimoxazole, respectively.

Similarly, one study in Memphis reported that pneumococci shown to be resistant to penicillin, also exhibited resistance to cotrimoxazole (95%). Moreover, there were several reports on penicillin-resistant *Streptococcus pneumoniae* in Asia. For example, 69.1% in Hong Kong (1999), 59.3% in Japan (1995) and 68.7% in Korea (1995).

In Taiwan, the first two cases of penicillin-resistant pneumococcal meningitis and extremely high prevalence (71%) of nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among children and a high incidence of multidrug-resistant strains among clinical isolates have been reported.

The study from Thailand in 2003 revealed that 43% of pneumococci were fully resistant to penicillin G. The emergence of penicillin and other antibiotics resistance might be due to the ease of obtaining the drugs from many drug stores, the indiscriminate use of antibiotics and prescribing antibiotics in viral infections, thus, resulted in uncontrolled usage of medication.

In this study, most of the pneumococcal infections were found in winter months which may be due to predisposing viral respiratory tract infection.

**Conclusion**

*Streptococcus pneumoniae* is a notorious bacteria pathogen for infant, children and elderly. Even in the developed countries, despite the availability of excellent antimicrobial therapy and adequate health care systems, respiratory diseases and invasive infections caused by pneumococci still comprise a major health problem. Worldwide emerging resistance to penicillin and other commonly used antibiotics underscores the importance of the development of novel vaccine strategies to combat pneumococcal diseases.

This study emphasized the need for continuous surveillance of *Streptococcus pneumoniae* infection as well as its colonization and changing trends of antibiotic susceptibility.
REFERENCES


Anti-diarrhoeal Effect of Ethanolic Extract of Cuminum cyminum Linn. (ซั้มยูนิ่น) on Castor Oil-induced Diarrhoea in Mice Model

Aung Aung Maw, May Aye Than, Mu Mu Sein Myint, Khin Tar Yar Myint, Win Win Maw, Thandar Myint, Nu Nu Win & Ei Ei Soe

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Department of Medical Research (Lower Myanmar)

This study was to determine phytochemical constituents, acute toxicity and anti-diarrhoeal activity of 80% ethanolic extract of cumin seeds. The phytochemical tests showed alkaloids, flavonoids, glycosides, tannins, steroids, phenols, saponins, and amino acids were present in the extract whereas resins, triterpine and cyanogenic glycoside were not. Acute toxicity test of the 80% ethanolic extract of cumin seeds showed no toxicity in albino mice, even with maximum permissible dose of 16 g/kg body weight. Anti-diarrhoeal activity was evaluated by castor oil-induced diarrhoeal test, enteropooling test and intestinal transit test on mice. Three doses (3, 6 and 9 g/kg body weight) of tested extracts showed significant anti-diarrhoeal effect (% inhibition of defecation) (p<0.01) when compared to control. The extracts of 6 g/kg, 9 g/kg and loperamide showed a significant decrease in the onset of defecation (p<0.05) when compared to control. All doses of tested extracts showed anti-diarrhoeal effects comparable to loperamide (6 mg/kg) as seen by a marked decrease in the number of diarrhoeal stools, from 21.17±4.71 to 5±3.41, 3.67±3.55, 0.5, respectively, and 4.17±0.71 in loperamide at 4 hour (p<0.01). There was also a significant percent reduction in the volume of intestinal content of the test extract (65.46±19.08, 64.93±16.78, 61.15±20.56) and the values were comparable to standard drug loperamide (60.13±12.21). The anti-diarrhoeal index of test extract and standard drug loperamide were 79.16%, 80.91%, 87.83% and 76.65%, respectively. Therefore, it was concluded that the 80% ethanolic extract of cumin seeds possesses significant anti-diarrhoeal activity.

INTRODUCTION

Myanmar is a tropical country with diarrhoea as a common health problem. In Myanmar, traditional medicines are widely used in the treatment of a variety of disorders. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to affordability, accessibility and ancestral experience. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.

Diarrhoea is defined as the production of stool of abnormally loose consistency, usually associated with excessive frequency of defecation and with excessive stool output. Normal stool output is approximately 100 to 200 g/day.¹ Diarrhoea disease is a leading cause of mortality and morbidity, especially among children in developing countries resulting in a major health care problem.² Diarrhoea can cause by a temporary problem, like an infection, or a chronic problem, like an intestinal disease. Diarrhoea commonly results from gastroenteritis caused by bacterial infection, viral infection, food intolerance, parasites, reaction to medicine, intestinal diseases, functional bowel disorders, such as irritable bowel syndrome, in which the intestines do not work normally.³
Many plants available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery. Ayurveda is an ancient form of Indian medicine, which deals with plants and plant products. This indigenous form of medicine uses the active ingredients present in plants for treating diseases.

*Cuminum cyminum* Linn. (Family: Umbelliferae) is widely cultivated, used and consumed in fairly large quantities by Indians. Cumin is widely used in Ayurvedic medicine. Cumin is the seed of a small Umbelliferous plant. Cumin seeds are very useful in digestive disorders like biliousness, sickness, indigestion, atonic dyspepsia, diarrhoea, malabsorption syndrome and flatulent colic. One spoon of cumin seeds is boiled in a glass of water and the concoction is mixed with one teaspoon of fresh coriander leaf juice and a pinch of salt. This decoction can be taken twice daily after meals as medicine for diarrhoea.

The present study was undertaken to investigate the phytochemical activity, the acute toxicity and anti-diarrhoeal activity of the 80% ethanolic extract of *C. cyminum* Linn. on castor oil-induced diarrhoea in albino mice.

**MATERIALS AND METHODS**

*Phytochemical constituents of 80% ethanolic extract of *C. cyminum* Linn.*

Investigation for phytochemical constituents of *C. cyminum* Linn. was carried out by using the method of Harborne,7 Linsted,8 and Central Council for Research in Union Medicine.9

**Acute toxicity test**

The 80% ethanolic extract of cumin seeds was tested by the method of Litchfield and Wilcoxon.10 Mice were randomly divided into 4 groups (I to IV), 10 in each group. They were fasted for 18 hours. The group I mice served as the control orally given with distilled water only. The mice in groups II to IV were orally given with 4 g/kg, 8 g/kg and 16 g/kg body weight of the 80% ethanolic extract of cumin seeds suspended in distilled water, respectively. The animals were observed for the first six hours continuously for the mortality if any and then every 24 hours for two weeks.

**Anti-diarrhoeal activity of 80% ethanolic extract of *C. cyminum* Linn.**

The anti-diarrhoeal effect of 80% ethanolic extract was studied with castor oil-induced diarrhoea test, castor oil-induced entero-pooling test and castor oil-induced small intestinal transit test.

(i) Castor oil-induced diarrhoeal test

Mice were fasted overnight with water *ad lib* and were randomly divided into five groups (I to V) (six animals, each). The mice in group I served as the control and they were orally given only 10 ml/kg b.w of distilled water. Group II received loperamide 6 mg/kg b.w and served as the standard. Group III, IV and V were respectively treated orally with 3, 6 and 9 g/kg b.w of the 80% ethanolic extract of cumin seeds. Diarrhoea was induced by administration of 10 ml/kg of castor oil orally to mice. The animals were then placed in individual cages on a clean paper. The number of the diarrhoeal dropping was counted every hour for 4 hours and the mean number of the stool, passed by the treatment groups was compared with control group.11

(ii) Castor oil-induced entero-pooling test

The accumulation of the intraluminal fluid was determined by the method of Robert, et al.12 Mice were fasted overnight with water *ad lib* and divided into 5 groups (I to V) (six animals, each). Group I which received distilled water (10 ml/kg) orally served as the control. Group II which received loperamide (6 mg/kg b.w) served as the standard. Group III, IV and V received 3, 6 and 9 g/kg b.w, respectively of the 80% ethanolic extract of the dried cumin seed, one hour before the oral administration of castor oil. After one hour, the mice were killed. The small intestines were removed after tying both ends with threads and weighed. The intestine was squeezed
out slowly and its content was collected into a measuring test tube. The volume was measured. Then, the intestine was reweighed and the difference between full and empty intestine was determined. The content of the fecal matter in the intestine was calculated.

(iii) Castor oil-induced small intestinal transit test

Mice were divided into five groups (I to V) (six animals, each). They were fasted for the period of 18 hrs. The mice of group I (10 ml/kg) which received distilled water orally served as the control. Group II received loperamide 6 mg/kg b.w served as the standard. Group III, IV and V received the 80% ethanolic extract of cumin seeds (3, 6, 9 g/kg b.w), respectively, 1 hour before administration of castor oil (10 ml/kg b.w). Marker 10 ml/kg (10% charcoal suspension in 5% gum acacia) was administered orally 1 hr after castor oil treatment. The mice were sacrificed after thirty minutes. The distance travelled by the charcoal plug from pylorus to caecum was determined and measured (cm) and expressed as a percentage of the total length of the small intestine.\textsuperscript{11}

RESULTS

Phytochemical tests on the 80% ethanolic extract of \textit{C. cym\-num} Linn. showed that alkaloids, flavonoids, glycosides, tannins, steroids, phenols, sapo\-nins and amino acid were present whereas resin, triterpine and cyanogenic glycoside were absent. Acute toxicity study on the mice treated with 4, 8 and 16 g/kg doses of the 80% ethanolic extract of cumin seeds were kept under observation for two weeks. It was observed that there was no lethality at this dose level. Therefore, the medium lethal dose (LD\textsubscript{50}) was more than 16 g/kg b.w. The anti-diarrhoeal activity was investigated using 80% ethanolic extract of cumin seeds in castor oil-induced diarrhoea mice models. Anti-diarrhoeal activity was evaluated by castor oil-induced diarrhoea test, entero-pooling test and intestinal transit test. Three doses (3, 6, 9 g/kg b.w) of tested extracts showed significant anti-diarrhoeal effect (% inhibition of defecation) (p<0.01) when compared to control. The extracts of 6 g/kg, 9 g/kg and loperamide showed significantly decreased onset of defecation (p<0.05) when compared to control. All doses of tested extracts showed anti-diarrhoeal effects comparable to loperamide (6 mg/kg) as seen by a marked decrease in the number of diarrhoeal stools (21.17±4.71 to 5±3.41, 3.67±3.55, 0.67±0.82, and 4.17±0.71 at 4 hour (p<0.01) (Table 1).

Table 1. Antidiarrhoeal effect of ethanolic extract of \textit{C. cym\-num} Linn. and loperamide on castor oil-induced dropping in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean number of defecation in four hours</th>
<th>% Inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil+D/W 10 mg/kg (control)</td>
<td>21.17±4.71</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil+Ethanolic extract 3 g/kg</td>
<td>5±3.41**</td>
<td>76.38</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil+Ethanolic extract 6 g/kg</td>
<td>3.67±3.55**</td>
<td>82.66</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil+Ethanolic extract 9 g/kg</td>
<td>0.67±0.82**</td>
<td>96.83</td>
</tr>
<tr>
<td>V</td>
<td>Castor oil+Standard loperamide 6 mg/kg</td>
<td>4.17±0.71**</td>
<td>80.30</td>
</tr>
</tbody>
</table>

\textsuperscript{**}=p<0.01, comparison between different types of treatment and control (castor-oil only)

Fig. 1. Comparative effects of 80% ethanolic extract of \textit{C. cym\-num} Linn. 3 g/kg, 6 g/kg, 9 g/kg, and standard drug lopera\-mide on mice with castor oil-induced diarrhea at various time intervals (Each point represents as mean±SD from the experiments, \textsuperscript{**}=p<0.01)
Comparative effects of 80% ethanolic extract of *C. cyminum* Linn. 3 g/kg, 6 g/kg, 9 g/kg, and standard drug loperamide on mice with castor oil-induced diarrhea at various time intervals are shown in Table 2.

Table 2. Anti-diarrhoal effects of ethanolic extract of *Cuminum cyminum* Linn. and loperamide on castor oil-induced enteropooling in mice

<table>
<thead>
<tr>
<th>Gp</th>
<th>Weight of intestinal content (g)</th>
<th>Volume of intestinal content (ml)</th>
<th>Enteropooling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>%Reduction</td>
<td>Normal</td>
</tr>
<tr>
<td>I</td>
<td>0.73±0.27</td>
<td>0.98±0.09</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.35±0.08**</td>
<td>46.85±23.45</td>
<td>0.33±0.17**</td>
</tr>
<tr>
<td>III</td>
<td>0.40±0.16**</td>
<td>34.63±8.03</td>
<td>0.34±0.15**</td>
</tr>
<tr>
<td>IV</td>
<td>0.43±0.16**</td>
<td>30.27±43.62</td>
<td>0.37±0.18**</td>
</tr>
<tr>
<td>V</td>
<td>0.46±0.09**</td>
<td>31.49±25.08</td>
<td>0.39±1.07**</td>
</tr>
</tbody>
</table>

*Gp=Group, *p<0.05, **p<0.01, comparison between different types of treatment and control (castor-oil only)*

From these results, anti-diarrhoal index of test extract and loperamide were calculated and were found to be 79.16%, 80.91%, 87.83%, and 76.65%, respectively (Table 3).

### DISCUSSION

Preliminary phytochemical tests on some selected Myanmar medicinal plants revealed in some cases the presence of alkaloids and glycosides as the major components where-as in some other plant materials, steroids, tannin, flavonoids and phenolic compounds are mostly found. Anti-diarrhoal properties of medicinal plants were found to be due to tannins, alkaloids, saponins and flavonoids. In this study, the phytochemical constituents of 80% ethanolic extract of *C. cyminum* Linn. revealed the presence of anti-diarrhoal properties compounds (alkaloids, tannins, saponins and flavonoids).

Preliminary studies of the acute toxicity test were carried out before experiments. The acute toxicity test of the 80% ethanolic extract of cumin seeds on albino mice was done. The mice were treated with three doses of (4, 8, 16 g/kg) of this extract. The mice were found to be alive and healthy with maximum permissible dose of 16 g/kg during the observation period of 2 weeks. Therefore, the median lethal dose (LD<sub>50</sub>) was supposed to be more than 16 g/kg. Thus, cumin seeds were found to be less toxic and had no harmful effects.

The 80% ethanolic extract of cumin seeds has shown dose dependent anti-diarrhoal activity in a castor oil-induced model in albino mice. The effect of 80% ethanolic extract of cumin seeds on castor oil-induced diarrhoal test in mice was studied.
This indicated that three doses (3, 6, 9 g/kg b.w) of the extract had similar activity as standard drug loperamide (6 mg/kg b.w) in regard to the dose related effect and wetness of the fecal dropping when compared to control mice.

In enteropooling test, 3, 6 and 9 g/kg b.w of 80% ethanolic extract of cumin seeds significantly reduced the inhibited % reduction in volume of intestinal content compared with control. This indicated that all doses of test extract produced significant anti-secretory effect. In intestinal transist test, significant reduction in reduced motility was found with three doses of the extract and standard loperamide. This indicated that all dose of the extract and loperamide showed significant anti-motility.

The anti-diarrhoeal index of test extract and loperamide were 77.9% to 88% and 77%, respectively. Therefore, these results indicated that the 80% ethanolic extract of cumin seeds was effective as standard drug loperamide.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Director-General, Department of Medical Research (Lower Myanmar) for giving us permission to carry out this research work. We also wish to show our gratitude to all staff of Pharmacology Research Division, DMR (LM) for their kind help and assistance.

REFERENCES

Knowledge of First-year MBBS Students of University of Medicine (Magway) Regarding Human Immunodeficiency Virus Infection

Win Win Maw, Than Than Aye, Swe Swe Hnin, Soemoe Thu, Khin Swe Oo, Nyan Win Tun, San San Myint, Than Than Nu & Thet Nwe Oo

University of Medicine (Magway)

Knowledge about HIV, how it is transmitted, and preventive measures help modify the life style. It is important to assess the knowledge of the medical students on risks of HIV/AIDS. To assess knowledge, self-administered questionnaires were administered to 375 (male-277, female-98) first-year MBBS students of University of Medicine (Magway). Most students (>90%) could indicate the true modes of transmission: sexual contact, contact with contaminated blood, blood transfusion, and mother to child transmission. However, 31 (11.2%) of males and 6 (6.1%) of females had wrong concept that HIV can be transmitted by insects. Twenty-six (9.4%) and 10 (3.6%) of males and 2 (2%) and 3 (3.1%) of females thought people can get infection through causal contact such as hand shaking and sharing eating utensils, toilet and bathroom, respectively. Only small portion (19, 6.9% and 9, 3.2%); answered it is a curable but not a preventable disease. Both male and female students had very good attitude regarding the known patient of HIV/AIDS.

INTRODUCTION

Health educators indicate that education is one of the most effective ways to avoid the continued spread of HIV. Today facts about HIV, its modes of transmission and means to prevent transmission are well understood. These findings can be used to provide knowledge about HIV transmission and behaviours that lower the risk of infection, to correct misperceptions about the risk of transmission. Attention is being paid to educate students as WHO urge school-based education including education on sex, sexually transmitted diseases and HIV as one of the main intervention strategies.

Students may have knowledge about HIV transmission and behaviours that lower the risk of infection or they may have misperception about the risk of transmission from causal contact and the significance of safer sexual practices. It is important to assess the knowledge, perception on risk of infection, and awareness of seriousness of the HIV/AIDS of the students, what they have already known, what they still don’t know, and to which level they know. Results obtained from these assessments will provide information on students needs and describe the areas of HIV knowledge related to HIV risk that need to be updated in education programs. There is no study conducted to assess knowledge of HIV/AIDS in the students attending medical universities in Myanmar. Therefore, this study was to investigate attitude towards HIV-infected person, the HIV/AIDS-related knowledge on transmission and prevention and control of first-year MBBS students, University of Medicine (Magway). The result of this study will be used to develop and or promote appropriate health education program.

MATERIALS AND METHODS

Subject

The subjects for this study were 375 first-year MBBS students of University of Medicine (Magway): 277(73.86%) males and 98(26.11%) females.
**Instrument**

The survey questionnaire constructed for this study consisted of knowledge about transmission of HIV/AIDS.

**RESULTS**

Most male and female students (265, 95.7%) indicated that sexual intercourse is one of the transmission modes. Other common modes chosen by the students include contact with contaminated blood (267, 96.4% male; 98, 100% female) blood transfusion (259, 93.5% male; 97, 99% female), and mother to child transmission (241, 87% male; 95, 96.9% female). One seventy-five male students (63.2%) and 67 (68.4%) female students accepted that sharing tooth brush and razor is one of the modes of transmission.

<table>
<thead>
<tr>
<th>Table 1. Knowledge on HIV transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=375)</td>
</tr>
<tr>
<td>Male (n=277)</td>
</tr>
<tr>
<td>Female (n=98)</td>
</tr>
<tr>
<td>By inhalation</td>
</tr>
<tr>
<td>13(4.7)</td>
</tr>
<tr>
<td>By ingestion</td>
</tr>
<tr>
<td>Sexual means</td>
</tr>
<tr>
<td>Contact with contaminated blood</td>
</tr>
<tr>
<td>Blood transfusion</td>
</tr>
<tr>
<td>Mother to child</td>
</tr>
<tr>
<td>Casual contact such as hand shaking</td>
</tr>
<tr>
<td>By sharing eating utensils, toilet &amp;</td>
</tr>
<tr>
<td>bathroom</td>
</tr>
</tbody>
</table>

Two hundred and sixty-four (95.3%), 260(93.9%), 263(94.9%), 245(88.4%), 243 (87.7%) male students and 95(96.9%), 98(100%), 95(96.9%), 95(96.9%), 90(91.8%) female students disagreed inhalation, ingestion, sharing eating utensils, toilet and bathroom, casual contact such as hand shaking, by virtue of insect such as fly, mosquitoes are not ways in which HIV/AIDS can be spread, respectively.

Twenty-six (9.4%) and 10(3.6%) of males and 2(2%) and 3(3.1%) of females thought people can get infection through casual contact such as hand shaking and sharing eating utensils, toilet and bathroom, respectively (Table 1).

<table>
<thead>
<tr>
<th>Table 2. Knowledge on HIV prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=375)</td>
</tr>
<tr>
<td>Male (n=277)</td>
</tr>
<tr>
<td>Female (n=98)</td>
</tr>
<tr>
<td>AIDS can be cured</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>(6.9)</td>
</tr>
<tr>
<td>AIDS is an preventable disease</td>
</tr>
<tr>
<td>266</td>
</tr>
<tr>
<td>(96)</td>
</tr>
<tr>
<td>There is an effective vaccine</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>(5.77)</td>
</tr>
<tr>
<td>Can contract disease only by a single</td>
</tr>
<tr>
<td>exposure</td>
</tr>
<tr>
<td>266</td>
</tr>
<tr>
<td>(96)</td>
</tr>
<tr>
<td>Correct use of condom protect against</td>
</tr>
<tr>
<td>HIV infection</td>
</tr>
<tr>
<td>261</td>
</tr>
<tr>
<td>(94.2)</td>
</tr>
<tr>
<td>HIV-positive person cannot donate blood,</td>
</tr>
<tr>
<td>sperm and organ</td>
</tr>
<tr>
<td>224</td>
</tr>
<tr>
<td>(80.9)</td>
</tr>
<tr>
<td>Screening of blood</td>
</tr>
<tr>
<td>HIV is necessary for blood donors</td>
</tr>
<tr>
<td>265</td>
</tr>
<tr>
<td>(95.7)</td>
</tr>
<tr>
<td>No need to change job of HIV-infected</td>
</tr>
<tr>
<td>persons unless nature of the job</td>
</tr>
<tr>
<td>expose their blood to others</td>
</tr>
<tr>
<td>270</td>
</tr>
<tr>
<td>(97.5)</td>
</tr>
<tr>
<td>HIV-infected children can be allowed</td>
</tr>
<tr>
<td>to attend school</td>
</tr>
<tr>
<td>175</td>
</tr>
<tr>
<td>(63.2)</td>
</tr>
</tbody>
</table>

When the respondents were asked whether AIDS can be cured and is a preventable disease, 87%, 96% of male students and 90%, 92.9% of female students said AIDS is incurable but preventable disease, respectively. Only small portion (19, 6.9% and 9, 3.2%) of males and 8(8.2%) and 7(7.1%) of females answered that it is a curable but not a preventable disease.

Sixteen (5.77%) of males and 8(8.2%) of females thought that there is an effective vaccine for prevention of AIDS. However, 261(94.2%) and 90(91.8%) of male and female students, respectively, disagreed vaccine for AIDS.

Most respondents noticed that screening of blood for HIV is necessary for blood donors (95.7% for males, 95.9% for females) and HIV-positive person cannot donate blood, sperm and organ (80.9% for males and 96.9% for females). Eighteen students did not answer this question on organ donation.
and 35(12.6%) of male students did not agree on it.

Ninety-six percent of males and 95.9% of females are aware that they can contract HIV infection even by a single exposure. And 94.2% of male and 93.9% of female respondents answered that the correct use of condom can protect against HIV infection. Ninety-eight percent of both male and female students agreed on no need to change job of HIV-infected persons unless nature of the job expose their blood to others. Ninety-five percent of female students said HIV-infected children can be allowed to attend school, in contrast, only 175 (63.2%) of male students agreed on it (Table 2).

**DISCUSSION**

This study showed an overall good knowledge level of medical students regarding the transmission of HIV and HIV prevention. On average, around 90% of the medical students had knowledge about HIV transmission modes and prevention. This is comparable to other studies. In contrast to the present study where all the participants were first-year students, the study done in Kazakhstan included year 1-7 medical student respondents from the Semipalatinsk State Medical Academy. However, students participated in this study were more knowledgeable than preclinical dental students of Benin City, Nigeria. More than 95% students were aware of the possibility of acquisition of HIV from sexual means, contact with contaminated blood, and blood transfusion. Most of the respondents (88-95%) were aware that HIV cannot be transmitted by inhalation, by ingestion and causal contact such as hand shaking (9.4% for males; 2% for females) and by sharing eating utensils, toilet and bathroom (3.6% for males; 3.1% for females). Students participated in this study were more knowledgeable than those of other studies. In a study from India, Fifteen percent (15.8%) of 300 dental and nursing students believed casual kissing as a route of transmission and 2.5% answered that food sharing can be a mode of HIV transmission.

In a study done on 1081 Chinese college students from 8 colleges in 2000, about half of the sample thought that (or were not sure whether) a person could contract HIV by sharing plates, fork or glass (46%), using a public toilet (49%), being coughed or sneezed on (41%), receiving medical care from someone who has the AIDS virus (51%) or using a public swimming pool (52%). Only about two-thirds of the sample did not think they would be likely to contract the AIDS virus from mosquitoes or other biting insects.

They also mentioned that HIV transmission is not by virtue of insect such as fly, mosquitoes (243, 87.7% for males; 90, 91.8% for females). However, some answered that HIV can be transmitted by casual contact, inhalation, ingestion, sharing utensils, or through mosquitoes. Daily domestic contact was less commonly thought to transmit the disease. Thirty-six (13%) males thought that HIV cannot be transferred from mother to foetus. Two hundred and forty-one (87%) of males and 95(96.5%) of females agreed on the fact that HIV can be transmitted from infected mother to foetus. This was consistent with the findings of a study from China in which 89.9% (n=232) of the participants answered HIV can be transmitted from mother to child.

This confirms the existence of some misconception among the respondents and a pointer that true understanding of the disease is lacking in small percentage of the students. This observation is similar to the finding of surveys among college students in India.

Similarly, a Latin America study revealed that a substantial numbers of dental students had incomplete knowledge of HIV and often lacked confidence on infection control and procedures. When awareness and sources of information about HIV vaccine was
conducted among community population in the Bojanala area, Rustenburg, North West Province, South Africa, less than half (42.7%) (n=150) indicated an awareness of HIV vaccines.\textsuperscript{8}

Compared to that study, most of the respondents possessed correct idea on HIV disease process, prevention and vaccine. Only small portion (8.2-13%) had a wrong idea of AIDS as a curable but not a preventable disease; 16(5.77%) of males and 8(8.2%) of females thought that there is an effective vaccine for prevention of AIDS: 35(12.6%) of male students had an idea of HIV-infected person can donate organs and sperm. Percentage distribution of wrong concepts on cure of AIDS was found to be lower than other study. In a study on nursing students in India, 50% of nursing students thought that both treatment and cure were present for AIDS. Forty percent of nursing students thought that treatment was present for AIDS and 5% of nursing students thought that cure was present for AIDS. Interestingly, 5% gave the answer that neither treatment nor cure was present for HIV.\textsuperscript{1}

\textit{Attitudes toward HIV infected persons}

Attitude towards HIV-infected persons was assumed to be good; most students agreed on HIV-infected persons could continue the job if the nature of the job did not expose their blood to other (98%); HIV-infected children could be allowed to attend school (62% males, 94% females) although 102 (36.8%) of male students did not agree on it.

Awareness and appropriate knowledge may play an important role in preventing the further spread of HIV/AIDS among the general population.

Medical, dental and nursing students are an integral part of the healthcare provider team, responsible for decision making and implementation of many healthcare related practices. In their course of learning and training during the undergraduate course, they are taught the theory and practice of delivering healthcare. Serious and potentially fatal blood-borne infections like HIV and hepatitis B are the front-runners in their occupational diseases profile. Hepatitis B is a highly infectious disease but preventable by its vaccination. Infection with hepatitis B virus (HBV) is a major cause of morbidity and mortality in the South-East Asia region (SEAR).

School-based HIV/AIDS education should focus on the specific student population of each school, while maintaining close links with their parents and the community at large. These links allow for the strengthening of protective influences on young people from both the school and the home; they also help teachers gain support for introducing and sustaining education for HIV/AIDS prevention in school.

Community-based organizations (non-governmental organizations, hospitals, teachers’ unions, religious groups, youth groups, sports clubs, etc.) could provide support, up-to-date information and practical assistance to school-based initiatives on education for HIV/AIDS prevention.\textsuperscript{8}

HIV/AIDS is crucial for health care professionals because of the increasing prevalence of these infections. Occupational risk of these infections is well known in medical and dental workers especially during the professional training period. This accounts for one of the major reasons for delivering knowledge about preventive measures and universal precautions.

REFERENCES


Intake of Nitrites and Nitrates from Processed Meats by Primary School Children

Theingi Thwin, Mya Ohnmar, Sandar Tun, Thuzar Aye, Yin Lynn Myint & Thidar Khine

Nutrition Research Division
Department of Medical Research (Lower Myanmar)

Nitrites and nitrates are used as food additives in the processing of meat products. Nitrite in food is considered primarily to cause health problems because its presence both in food and in the body may lead to the formation of carcinogenic nitrosamines. The nitrates could be reduced into nitrites by the microflora in the oral cavity. To assess the risk of nitrites and nitrates intake from processed meat by primary school children, 378 primary school children, aged 8-10 years attending No. 4, Basic Education High School, Ahlone, were recruited in the study. Patterns of processed meat consumption were determined by 6-day food diary method and their body weights were measured. Nitrites and nitrates contents of ten items of processed meat which were commonly consumed were determined. The mean body weight was 27.2±7.3 kg. During the period of taking 6-day food diary, 164(43.4%) did not consume any processed meat products but 60(28%) consumed them more than 3 days. Nitrites and nitrates contents were 9.44 mg/kg and 67.15 mg/kg in three chicken meat products, 4.31 mg/kg and 23.06 mg/kg in four fish meat products, 5.97 mg/kg and 55.04 mg/kg in two pork meat products and 3.35 mg/kg and 43.5 mg/kg in one crab meat product, respectively. Mean exposure to the nitrite and nitrate (mg/kg body weight/day) from processed meat by children was 0.02±0.02 and 0.16±0.14, respectively. Intake of nitrites in 12(5.6%) children were more than Acceptable Daily Intake (ADI), i.e., 0.06 mg/kg body weight/day. Health education on low intake of processed meat should be encouraged.

INTRODUCTION

Preservation of food products with additives is an ancient practice. In the late 19th century and early 20th century, food preservation was mainly confined to heat sterilization, in combination with the addition of salts and spices. As the society became more and more urbanized, the area of food technology including production, processing, and distribution of food products underwent a revolutionary change. The use of various additives to extend the shelf-life of food products has become widespread in food industry. In order to prevent indiscriminate use, regulations have been developed by many countries limiting the type, purity, uses and amounts of food additives permitted in food.

Nitrite and nitrate are widely used as food additives in the processing of meat products because of their antimicrobial action and their ability to give meat a characteristic pink color, texture and flavor. Cured meat products are the major source of nitrites in human dietary intake. Nitrate and nitrite can also be found in food as naturally occurring compounds, drinking water and vegetables being substantial source of nitrate intake. Interest in the dietary intakes of nitrate and nitrites has arisen from concerns about their possible adverse effect on health.\(^1\)

The nitrate ion has a low level of acute toxicity, but if it is transformed into nitrite, it may constitute a health problem. Reduction to nitrite may take place in contact with metals in the presence of bacteria or enzyme nitrate reductase. It has
been estimated that 5-8% of the nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity.\textsuperscript{2} The acceptable daily intake (ADI) for nitrate is 0-3.7 mg kg\textsuperscript{-1} body weight (as nitrate ion).\textsuperscript{3} Nitrite has higher acute toxicity than nitrate and ADI is 0-0.06 mg kg\textsuperscript{-1} body weight (as nitrite ion).\textsuperscript{4} As an unstable ion, nitrite undergoes a series of reactions as soon as it is added to food. In the acidic environment, nitrite is converted into nitrous acid, which decomposes into nitric oxide. Nitric oxide, being an important product from the standpoint of color fixation in cured meat, reacts with myoglobin to produce a red pigment - nitrosomyoglobin.\textsuperscript{5} The intake of nitrite is normally low compared with the dose that is acutely toxic but nitrite in food is considered primarily to cause health problems because its presence both in food and in the body may lead to the formation of carcinogenic nitrosamines\textsuperscript{5} and clinical symptoms of methaemoglobinaemia.\textsuperscript{6}

Although the food safety monitoring program has been carried out for a decade in Myanmar, there is the scarcity of studies concerning dietary risks due to consumption of nitrites and nitrates from processed meat by school children. Therefore, the aim of this study was to assess the dietary risk connected with the intake of nitrites and nitrates from processed meats by primary school children.

**MATERIALS AND METHODS**

The study was descriptive and primary school children were chosen as the population of this study because they are assumed as the population group which is more likely to have above average consumption of processed meat products. Yangon was purposely selected because various kinds of cured meat products are available from markets and wholesale centers and No. 4, Basic Education High School, Ahlone was randomly selected. A total of 378 primary school children, 8-10 years of age attending the above-mentioned school were included in the study. Total study period was one year.

**Six-day food diary method**

Patterns of processed meat consumption were determined by 6-day food diary method. The children were explained how to fill the food diary. The food items with amount were noted starting from the first food or drink taken after waking up to the last food or drink taken before sleeping. To estimate the amount or portion of food taken, showing food photos with known weights or serving sizes was assisted. The food diaries were filled by the children themselves or with the assistance of their parents.

On next day, the validity of filling food diary was checked by the trained persons by interviewing. Portion sizes and amount of consumed meat products were confirmed by showing food photos. The pretest filling in food diary was carried out before the study and the explanations were made again. The participated students filled the food diaries for six days (including one holiday) and the invalid food diaries were deleted from the analysis. From the data of 6-day food diary, the patterns of processed meat products consumption were analyzed according to names of the food items and frequencies of them. Then, the intakes of processed meat products per day were estimated by showing the photos of them.

**Food sampling and sample preparation**

By the names of food items which were actually consumed by the participated students, altogether twenty processed meat products were identified as commonly consumed. Then, these foods were grouped on the base of meats which were made up of, e.g, chicken, fish, pork and crab meat products, etc. A total of ten items (four fish, three chicken, two pork and one crab) of processed meat products were purchased mostly from the shops of the school canteen and of the area where the students lived (if they were not available in the canteen). Generally, they were local made except fish-
tofu which is imported from Thailand. Food samples were prepared as consumption as soon as possible if they were raw. They were kept at the laboratory with a maximum storage period of 2 days. Prepared foods were frozen until analysis.

**Determination of nitrite and nitrate contents in the selected food samples**

The concentrations of nitrate and nitrite in foods were determined using standard, validated method (ISO/DIS 3091). In summary, 5 grams of homogenized sample were kept in a hot borex solution containing activated charcoal, and proteins were precipitated by potassium ferrocyanide trihydrate and zinc acetate dihydrate. The deproteinated filtrate was required to determine both nitrate and nitrite contents in the sample. The extracted nitrates were reduced by metallic cadmium. A red color was developed by addition of sulphanilamide and N-1 naphthylethylenediamine dihydrochloride to the filtrate and photometric measurement at wavelength of 538 nm was done. Results were expressed as mg kg⁻¹ of sodium nitrate and sodium nitrite. The coefficients of variation (CV) for food matrices were 9.6% for nitrite, and 4.1% for nitrate, respectively. The mean recovery of known amounts of nitrite spiked into food samples was 97.1. Methods for determination of nitrite and nitrate concentrations were corrected for recovery. Nitrite and nitrate contents were determined in a total of 30 samples of ten food items (four fish, three chicken, two pork and one crab).

**Exposure estimate**

Body weights of the participated school children were measured to calculate the exposure of nitrite and nitrate from processed meat products. Weights were measured by means of bathroom scales placing on thin flat surface and zero adjustment was done before measuring.

An individual approach was applied: multiplying the amount (intake) of processed meat products per day and contents of nitrite and nitrate in the particular consumed food items, individual intake of nitrite and nitrate from those foods (expressed in mg day⁻¹) could be calculated. Then, it was divided by the individual body weight to give an exposure in mg kg⁻¹ body weight day⁻¹.

**Statistical analysis**

Data entry and analysis were done by using SPSS 11.5 for Windows. Consumption patterns of processed meat products and percent distributions of nitrite and nitrate intake from those products by primary school children were presented as frequency distributions. Nitrite and nitrate contents in studied samples of each meat were presented as mean±SD values. Acceptable Daily Intake (ADI) of 0.06 mg/kg body weight/day and 3.7 mg/kg body weight/day were used as cut-off points for exposure estimation of nitrite and nitrate, respectively.

**RESULTS**

Table 1 shows the consumption pattern of processed meat product by the studied students. During the period of taking food diary (6 days), among 378 students, 164 (43.4%) did not consume any processed meat products and 154 (40.7%) consumed processed meat products less than three days but 60(28%) consumed them more than 3 days.

<table>
<thead>
<tr>
<th>Days of consumption</th>
<th>Percentage (No. of students)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 days</td>
<td>84.1(318)</td>
</tr>
<tr>
<td>≥3 days</td>
<td>15.9(60)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Means of consumption</th>
<th>Percentage (No. of students)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As snacks</td>
<td>64.0(137)</td>
</tr>
<tr>
<td>As dishes</td>
<td>14.0(30)</td>
</tr>
<tr>
<td>Both</td>
<td>22.0(47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Items of processed meat products</th>
<th>Percentage (No. of students)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One meat product</td>
<td>30.8(66)</td>
</tr>
<tr>
<td>Two meat products</td>
<td>25.7(55)</td>
</tr>
<tr>
<td>Three meat products</td>
<td>30.8(66)</td>
</tr>
<tr>
<td>More than 3 meat products</td>
<td>12.7(27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intake of processed meat products (gm/day)</th>
<th>Percentage (No. of students)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100 g</td>
<td>82.7(177)</td>
</tr>
<tr>
<td>101-200 g</td>
<td>15.4(33)</td>
</tr>
<tr>
<td>201-300 g</td>
<td>1.4(3)</td>
</tr>
<tr>
<td>&gt;300 g</td>
<td>0.5(1)</td>
</tr>
</tbody>
</table>

Table 1. Consumption patterns of processed meat products by primary school children
According to the 6-day food diary, only 214 students consumed processed meat products. Among 214 students, 137 (64%) and 30 (14%) had processed meat products as snacks, as dishes (with meals) and as both, respectively. Time of having these foods was mostly after the day’s teaching. More than one tenth of the students (12.7%) were fond of having processed meat because they did not choose the items of them. The intake of processed meat products was <100 gm/day in the majority of the students (82.7%).

In Table 2, the mean values and standard deviation of nitrite and nitrate content in studied triplicate samples of meat products are shown. The mean nitrite and nitrate contents were 4.31±0.08 mg/kg and 23.06±0.98 mg/kg in four fish meat products, 9.44±0.86 mg/kg and 67.15±3.87 mg/kg in three chicken meat products, 5.97±0.2 mg/kg and 55.04±1.25 mg/kg in two pork meat products, respectively.

Table 2. Mean±SD content of nitrite and nitrate in food samples (mg/kg of food)

<table>
<thead>
<tr>
<th>Items of food samples*</th>
<th>Nitrite (mg/kg/day)</th>
<th>Nitrate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meat products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish slice</td>
<td>7.42±0.15</td>
<td>28.03±1.06</td>
</tr>
<tr>
<td>Fish steak</td>
<td>5.12±0.07</td>
<td>14.02±0.69</td>
</tr>
<tr>
<td>Spicy fish slice</td>
<td>2.89±0.05</td>
<td>17.05±0.98</td>
</tr>
<tr>
<td>Fish-tofu</td>
<td>1.82±0.05</td>
<td>32.87±1.20</td>
</tr>
<tr>
<td>Chicken meat products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced slice</td>
<td>21.72±2.02</td>
<td>136.94±7.5</td>
</tr>
<tr>
<td>Chicken tendon ball</td>
<td>1.33±0.08</td>
<td>39.76±3.9</td>
</tr>
<tr>
<td>Sausage</td>
<td>5.27±0.47</td>
<td>24.75±0.2</td>
</tr>
<tr>
<td>Pork meat products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced slice</td>
<td>1.82±0.09</td>
<td>49.2±0.09</td>
</tr>
<tr>
<td>Sausage</td>
<td>10.31±0.3</td>
<td>62.6±2.4</td>
</tr>
<tr>
<td>Crab meat product</td>
<td>3.35±0.3</td>
<td>43.5±1.3</td>
</tr>
</tbody>
</table>

*=Triplicate samples of each item

Among the studied four fish products, fish slice contained the highest amount of nitrite but the highest amount of nitrate was found in fish-tofu. Minced chicken slice contained higher amount of nitrite and nitrate than those of other two studied chicken products. The nitrite and nitrate contents in pork sausage were the highest among the studied processed meat products.

The grouped data of intake of nitrite and nitrate from processed meat products by 214 school children are shown in Table 3. Intake of nitrite and nitrate from processed meat products was calculated by multiplying the intake amount of processed meat products (gm/day) and the concentration of nitrite and nitrates in the particular products (mg/kg of food). The mean(SEM) of intake of nitrite and nitrate from processed meat products by 214 children were 0.57±0.04 (0.014-3.83) mg/day, and 4.15±0.26 (0.021-26.5) mg/day, respectively.

Table 3. Intake of nitrite and nitrate from processed meat products by primary school children

<table>
<thead>
<tr>
<th>Percent (Number of students)</th>
<th>Nitrite (mg/kg/day)</th>
<th>Nitrate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1-&lt;2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2-&lt;3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>0.04 (0.014-3.83)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percent (Number of students)</th>
<th>Nitrate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>1-&lt;10</td>
</tr>
<tr>
<td></td>
<td>10-&lt;20</td>
</tr>
<tr>
<td></td>
<td>≥20</td>
</tr>
</tbody>
</table>

Exposure of nitrite and nitrate from processed meat products of 214 school children are shown in Table 4. Exposure of nitrite and nitrate (mg/kg/day) was calculated by dividing the intake amount of nitrite and nitrate (mg/day) by body weight in kilogram of particular primary school children. Mean exposure to the nitrite and nitrate (mg/kg body weight/day) from processed meat by children was 0.02±0.02 and 0.16±0.14, respectively.

Table 4. Exposure of nitrite and nitrate from processed meat products of primary school children

<table>
<thead>
<tr>
<th>Percent (Number of students)</th>
<th>Nitrite (mg/kg/day)</th>
<th>Nitrate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>0.01-&lt;0.06</td>
</tr>
<tr>
<td></td>
<td>≥0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.1</td>
<td>0.1-&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>≥1.0</td>
<td></td>
</tr>
</tbody>
</table>

|                             | 40.65 (87)         | 53.7 (115)         |
|                             | 5.6 (12)           | 50.9 (109)         |
|                             | 0.5 (1)            |                     |
Based on current Acceptable Daily Intake (ADI) of 0-0.06 mg kg\(^{-1}\) body weight day\(^{-1}\) (as nitrite ion),\(^6\) 12(5.6\%) of children had higher exposure of nitrite than ADI. However, since the ADI for nitrate is 0-3.7 mg kg\(^{-1}\) body weight day\(^{-1}\) (expressed as nitrite ion),\(^6\) all children of this study were less exposed to nitrate than ADI. Although mean intake of nitrite from meat products was not high, when the body weight was included in calculation of exposure, higher exposure to nitrite than ADI was found. It meant that the children with small body weight frequently consumed the processed meat products with high nitrite contents.

**DISCUSSION**

Six-day food diary method followed by next day recall was used in this study to investigate the consumption patterns and intake of processed meat products. A trained interviewer checked the validity of food diaries by asking the individual students to recall all food and drinks for previous 24 hours. Parents were allowed to assist in writing food diaries and prompts for quantification of portion size by showing of food photos were used. This method could assess current or past diet and be repeated to gain measure of daily variation and improved precision, quick, and inexpensive. Bias caused by errors in memory, perception, conceptualization of food portion sizes was limited. Therefore, this method can be used in primary school children for dietary intake measurement.

Nitrate and nitrite are present in food naturally, or may be present as a result of the use of fertilizers on crops or from their uses as preservatives. In curing process, nitrate is partly reduced to nitrite by bacteria. Nitrites are essential for the pink color of cooked cured meat products and for the characteristics of cured meat flavor of some of these products. It is also essential for enhancing shelf life of meat products and in protecting against the bacterial spoilage and hazards caused by *Clostridium botulism*. Nitrites and nitrates are widely used in the production of cured meat products and in the preservation of fish in some countries. In most countries, the addition of nitrates and/or nitrites to meat and meat products are governed by legislation. The concentrations which are allowed in the food stuffs differ from country to country. In Canada, the content of nitrites in sausages is 26 mg kg\(^{-1}\) and in ham 24 mg kg\(^{-1}\).\(^8\) In Estonia, the maximum permitted concentrations of residual nitrite (as NaNO\(_2\)) and nitrate (as NaNO\(_3\)) in commercial meat products are 100 and 250 mg kg\(^{-1}\), respectively.\(^9\)

The Estonia food safety monitoring program monitored and analyzed the cured meat products for nitrite and nitrate in 2004. The mean concentrations of sodium nitrite and sodium nitrate in cooked sausage were 30(<10-61) mg kg\(^{-1}\) and 56(<5-160) mg kg\(^{-1}\), respectively.\(^9\) Therefore, the content of nitrate in pork sausage of this study (62.6±2.4 mg kg\(^{-1}\)) was slightly higher than that of Estonia’s study. Moreover, in the Estonia’s study, daily intake of nitrite and nitrate from meat products in 4 to 9 years old children were 0.83 (0.65-0.92) mg/day and 1.65 (1.3-1.8) mg/day, respectively.\(^9\) Therefore, mean nitrate intake from meat products of 4.15±0.26 (0.021-26.5) mg/day in 8 to 10 years old school children of this study was slightly higher than that of Estonian children. The European Directive 95/2/CE (1995) allows 150 ppm of nitrite (if alone) or 300 ppm when combined (nitrite plus nitrate), and the residual values should be less than 50 ppm (if alone) or 250 ppm (if combined). The Food and Drug Administration allows these compounds to be used as food additives as long as they are of food grade and are added only in the amount needed. The maximum amount of nitrite allowed in smoked and cured fish and meat is 200 ppm. There is no regulatory minimum in-going nitrite level for cured products that have been processed to ensure their shelf stability. However, 40-50 ppm nitrite are useful in that it has some preservative effect and color-fixing purpose.
By the time meats are consumed, they contain less than 50 ppm of nitrite. It is that commercially prepared meats in the USA contain about 10 ppm of nitrite when bought in a supermarket.\textsuperscript{10}

In National Food Survey (United Kingdom, 1998), population average intakes (both adults and children) of nitrite and nitrate were 1.8 (1.6-11.9 mg/day) and 3.4 (1.8-3 mg/day, respectively).\textsuperscript{11} In this study, nitrates intake from processed meat products was lower than that of United Kingdom National Food Survey (1998). However, their results were analyzed on Total Diet Study, i.e., all food groups (green and canned vegetables, fruits, dairy products, cereals, and beverages) were included. To estimate total intake, all sources of nitrate and nitrite in the diet should be taken into account. Since our data could only be analyzed on the processed meat products, if all food groups were included, the intake of nitrite could be more.

The Acceptable Daily Intake (ADI) is defined as the amount of a chemical, expressed on mg/kg body weight basis, which can be ingested daily over a lifetime without incurring any appreciable health risk and is based on an evaluation of available toxicological data. If the water concentration data could be included the individual exposure, it was valid to sum the contribution of nitrate water to that from food for each individual. Therefore, it can be said that higher exposure of nitrite from processed meat products than ADI is one of the public health concerns.

In conclusion, this study could not show the dietary risk of nitrite and nitrate at population level, but exposure of nitrite and nitrate from processed meat products in the selected primary school children could be found out. Among them, 5.6% children have exposed nitrates from processed meat products more than Acceptable Daily Intake. Since nitrates and nitrites could be exposed from other dietary sources, e.g. water and vegetables other than processed meats, health education on low intakes of processed meat should be encouraged.

\textbf{ACKNOWLEDGEMENT}

We would like to thank Director-General, Department of Medical Research (Lower Myanmar) for allowing us to carry out this study. We would like to extend our gratitude to the principal and all teachers of No. 4, Basic Education High School, Ahlone. We also wish to express our thanks to primary school children, without whom this study would not be accomplished.

\textbf{REFERENCES}

Nutritional Status of Children with Cerebral Palsy in Cerebral Palsy Clinic, Yangon Children’s Hospital

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¹Clinical Research Division
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³Physiology Research Division

Department of Medical Research (Lower Myanmar)

There has been an increased awareness that significant proportion of children with special needs including cerebral palsy (CP) is undernourished. It can predispose to further morbidities imposing more suffering to the affected child and the family. This study aimed to determine the proportion of malnutrition in children with CP and to find out the association between the clinical factors and malnutrition. It was a cross-sectional descriptive study conducted at CP Clinic, Yangon Children’s Hospital (YCH) from May 2010 to June 2011. Children aged from 1 month to 12 years with all types of CP were included in the study. Data were collected by face-to-face interview with caretakers using the pretested structured questionnaire, measuring weight and height/length and examination to determine type of CP and level of gross motor function of the children. Nutritional status was classified according to Waterlow classification. Among 173 children with CP, 78.6% had undernutrition, 53.8% were wasted and 52% were stunted. Older age (p=0.002) and feeding solid foods (p<0.001) were significantly associated with undernutrition. In children >18 months, those who depended totally on caregivers for feeding (p=0.03) and spastic quadriplegic CP compared to other spastic types (p=0.03) were significantly associated with undernutrition. Malnutrition is quite prevalent in children with CP in YCH and nutritional support should be an integral part of the management of these children.

INTRODUCTION

Cerebral palsy (CP) is a well-known neurodevelopmental condition beginning in early childhood and persisting throughout one’s life span.¹ The average incidence is approximately 2-2.5 per 1000 live births.² This term describes permanent disorder of the movement and posture causing activity limitation due to non-progressive disturbances in the developing brain.³ Throughout the world, parents and doctors have focused on growth determinants of healthy children. In recent years, there has been an increased awareness that significant proportion of children with special needs including CP is undernourished.⁴ This condition may have been once considered as part of the disease, but the importance of nutrition in this population now better recognized.⁵

The incidence of undernutrition and poor growth, which is up to 86% in literature, is related not only with severe forms but also with mild forms of CP.⁶,⁷ This is a very important issue and needs to be addressed. If left untreated, severe nutritional problems may be exacerbated. Therefore, identification of the factors associated with undernutrition is important for the early detection and treatment and for the prevention of late complications in the children's behavior, health, or growth. In Yangon Children’s Hospital (YCH), CP clinic was established
in 2006 and many children with CP register there and are having care. This study aimed to determine the proportion of undernutrition in children with cerebral palsy in CP clinic, to identify the medical problems, feeding characteristics, type of CP, gross motor function and communicating ability in children with CP and to find out the association between different clinical factors and undernutrition in children with CP. Determining the above factors may lead to start timely nutritional rehabilitation, which can significantly improve their nutritional status and quality of life.

MATERIALS AND METHODS

It was a cross-sectional, descriptive study conducted at CP Clinic, YCH from May 2010 to June 2011. Children aged from 1 month to 12 years with all types of CP were included in the study. Patients with any known chronic illness (i.e., cardiac, renal, gastrointestinal, endocrinological or syndromal), any congenital malformation that would independently affect food intake like cleft lip and/or palate and patients who have routine ingestion of any medication (i.e., steroids) which is known to affect growth were excluded from the study. Consecutive sampling method was used.

Data were collected by face-to-face interview with caretakers using pretested structured questionnaire, measurement of body weight and height/length and examination of type of CP and level of gross motor function of the children. Severity of motor impairment in the children with cerebral palsy was graded using the Gross Motor Function Classification System (GMFCS). Five-point ordinal scale was described in levels (I mild to V severe).

Each child’s weight was measured to the nearest 0.1 kg using basinet or standing scales as appropriate for age. Children who could not stand and whose weight had exceeded the range on the basinet were weighed in the arms of the mother. The difference between the combined weight of mother and child and that of mother alone was recorded as the child’s weight.

Height was measured using the stadiometer for patients who were able to stand flat-footed and straight. The recumbent length was measured for subjects who were unable to stand erect by using somatometer. All measurements were recorded to the nearest 0.1 cm. All measurements were performed by the trained investigator, using the same instrument throughout the study. Nutritional status was classified according to Waterlow classification.\(^8\) As for acute malnutrition, weight for height/length 80-90\(^\%\) of median was considered as mild malnutrition, 70-80\(^\%\) as moderate malnutrition and <70\(^\%\) as severe malnutrition. For chronic malnutrition, height for age 90-95\(^\%\), 85-90\(^\%\) and 80-85\(^\%\) was considered as mild, moderated and severe malnutrition. Weight for height <80\(^\%\) was termed wasted and height for age <90\(^\%\) was termed stunted.

Statistical analysis

The data were subjected to statistical analysis using the SPSS 16.0 software package. The data were analyzed to determine the proportion of undernutrition among cerebral palsy and the influences of the various factors. The univariate analysis was performed to identify which variables were predictors of undernutrition. T-test and Chi-square tests were used to compare means and proportions, respectively.

Ethical consideration

This study was approved by the Ethical Review Committee of Department of Medical Research (Lower Myanmar). Written informed consent was obtained from the parents or caregivers of the children before the interview.

RESULTS

Our study included 173 children with cerebral palsy with median age of 18 months (range; from 2 to 144 months). Among them, 59.5\% (103 of 173) were boys and 40.5\% (70 of 173) were girls.
Figure 1 illustrates the results of the anthropometric measures of nutritional status of children with cerebral palsy. According to Waterlow classification, 78.6% (136 out of 173) had malnutrition. Among the children, 53.8% were wasted and 52% were stunted.

As medical problems, 20.2% had seizures, 24.9% had recurrent pneumonia, 46.8% had drooling, 54.9% had constipation and 29.5% had sleep problem. One or more feeding problems were seen in 84.4% of children with choking (26.0%) as the commonest type followed by tongue thrust (19.1%), chewing problem (19.1%), cry/extensor dystonia (10.4%), vomiting/regurgitation (6.9%), swallowing problem (3.5%) and jaw contracture/dystonia (1.7%).

In this study, the commonest type of CP was spastic quadriplegic CP which was 50.9% (88 out of 173) of all type of CP. Spastic hemiplegic, spastic diplegic, spastic monoplegic, dystonic, hypotonic and mixed type of CP were also seen in 13.3%, 2.9%, 1.7%, 17.3%, 13.3% and 0.6%, respectively. Most of the children had Gross Motor Function V(34.9%) followed by GMF IV (26.7%), GMF III(26.7%), GMF II(10.5%) and GMF I(1.2%).

When comparing the groups of children with and without malnutrition, mean age of children with malnutrition was significantly older than that without malnutrition, 28.24±23.43 months in malnutrition group and 17.38±16.10 in well nourished group (p=0.002) on univariate analysis. There was no association between malnutrition and gender or medical problems (Table 1).

Table 1. Comparisons of the bio-demographic characteristics and medical problems of children with CP with and without malnutrition

<table>
<thead>
<tr>
<th>Gender</th>
<th>Yes (n=136)</th>
<th>No (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>78(75.7)</td>
<td>25(24.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Female</td>
<td>58(82.9)</td>
<td>12(17.1)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical problems</th>
<th>Yes (n=136)</th>
<th>No (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizures</td>
<td>28(80.0)</td>
<td>7(20.0)</td>
<td>0.82</td>
</tr>
<tr>
<td>Recurrent pneumonia</td>
<td>35(81.4)</td>
<td>8(18.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Drooling</td>
<td>66(81.5)</td>
<td>15(18.5)</td>
<td>0.39</td>
</tr>
<tr>
<td>Constipation</td>
<td>76(80.0)</td>
<td>19(20.0)</td>
<td>0.62</td>
</tr>
<tr>
<td>Sleep problem</td>
<td>36(70.0)</td>
<td>15(29.0)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Any feeding problem, duration per meal or frequency of meal per day did not differ significantly between children with and without malnutrition. However, as regard for food texture, malnutrition was significantly associated with feeding solid food (p<0.001) (Table 2).

Table 2. Comparisons of feeding characteristics of children with cerebral palsy with and without malnutrition

<table>
<thead>
<tr>
<th>Feeding problems</th>
<th>Yes (n=136)</th>
<th>No (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food texture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid or semisolid</td>
<td>13(50.0)</td>
<td>13(50.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Solid</td>
<td>123(83.7)</td>
<td>24(16.3)</td>
<td></td>
</tr>
<tr>
<td>Duration per meal (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>73(75.3)</td>
<td>24(24.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>&gt;20</td>
<td>62(83.8)</td>
<td>12(16.2)</td>
<td></td>
</tr>
<tr>
<td>Frequency of meal per day (times) (Mean±SD)</td>
<td>2.78±1.17</td>
<td>3±1.86</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* indicates significant difference between two groups
In children older than 18 months, those who depended totally on caregivers for feeding were significantly associated with undernutrition \((p=0.03)\). Children with poor gross motor function (GMF IV and V) had more malnutrition than less severe group (GMF I, II & III) but not statistically significant. Communication ability was not associated with malnutrition (Table 3).

Table 3. Comparisons of functional status in children above 18 months old with and without malnutrition

<table>
<thead>
<tr>
<th>Malnutrition</th>
<th>Yes ((n=136))</th>
<th>No ((n=37))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dependence on feeding</strong></td>
<td></td>
<td></td>
<td>0.03**</td>
</tr>
<tr>
<td>Totally</td>
<td>52(92.9%)</td>
<td>4(7.1%)</td>
<td></td>
</tr>
<tr>
<td>Partially or independent</td>
<td>23(76.7%)</td>
<td>7(23.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Communicating ability</strong></td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>None or voicing or word</td>
<td>65(86.7%)</td>
<td>7(23.3%)</td>
<td></td>
</tr>
<tr>
<td>Sentence</td>
<td>10(90.9)</td>
<td>1(9.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Gross motor function</strong></td>
<td></td>
<td></td>
<td>0.065</td>
</tr>
<tr>
<td>GMF I, II &amp; III</td>
<td>26(78.8)</td>
<td>7(21.2)</td>
<td></td>
</tr>
<tr>
<td>GMF IV &amp; V</td>
<td>49(92.5)</td>
<td>4(7.5)</td>
<td></td>
</tr>
</tbody>
</table>

* indicates significant difference between two groups

Malnutrition was significantly associated with spastic quadriplegic type of CP compared to other spastic types (diplegic, hemiplegic or monoplegic) in children older than 18 months \((p=0.03)\). However, there was no statistically significant difference between spastic quadriplegic or other spastic type and non spastic types (dystonic, hypotonic and mixed).

**DISCUSSION**

This study was conducted in CP clinic in Yangon Children’s Hospital. Although it might not representative of the whole CP population in Myanmar, it can be considered representative of children with CP in a tertiary care center in this country.

In the current study, anthropometric assessment showed higher proportion of malnutrition 78.6% in children with CP, which was comparable to the report from Indonesia \((76\%)^9\) and India \((86\%).^7\) The prevalence of malnutrition was higher than that of studies in developed countries such as Egypt \((15\%),^10\) United Kingdom \((38\%),^11\) Greece \((38\%),\) Turkey \((34.9\%)^13\) and Taiwan \((41.3\%).^4\) The difference might be due to the use of different anthropometric classification in these studies because there are no universally accepted diagnostic criteria to measure the nutritional status of children with CP. We used weight-for-height/length to classify malnutrition in this study because it may be a more reliable indicator of current nutritional status, and this measurement is relatively independent of age and ethnic group. The difference in taking good treatment and compliance, high degree of support for feeding problems and adequate food provision between developed and developing countries may also affect the prevalence of malnutrition in this chronic disease. Some studies found that overweight was also a problem as 5.4% to 18.2% in children with CP.\(^12, 15, 16\) Contrast to this, there was no overweight children in this study reflecting that undernutrition was more important problem in our setting.

In our study, about 52% of children with cerebral palsy were stunted which is slightly lower than 60% of a study from UK\(^17\) but much higher than 9.2% in a study from Nigeria.\(^18\) Stunting is an indicator of chronic malnutrition. According to literatures, stunting has been noted to worsen with advancing age in cerebral palsy.\(^19, 20\)

Comparable to other studies,\(^21, 22\) older children had higher risk of malnutrition \((p=0.002)\). However, it is contrast to one study, which found that children in the youngest age group were most at risk for poor nutritional status and delayed growth.\(^14\) In our country, the prevalence of malnutrition increased with age even in normal children.\(^23\). It may be due to the fact that the practice of reducing the frequency of breastfeeding and inadequacy of complementary feeds either in quantity or quality in the second year of life adversely affected nutritional status of children.

Studies in Turkey, Taiwan and Mexico indicated that CP girls are at high risk for
underweight and argued that there was a gender discrimination against disabled girls in nutrition.\textsuperscript{4, 16, 21} However, there was no association between gender and malnutrition in this study, which may also reflect the non-discrimination between male and female offspring in our sociocultural environment.

Screenings for feeding problem is important because it impairs the child’s ability to safely consume the necessary nutrients and to consider the safe feeding route. Although one or more feeding problem was seen in 84.4\% of children in our study, no association was found between any of the feeding problems and malnutrition, which is similar to other studies.\textsuperscript{4, 24}

We identified feeding problem based on questionnaires, therefore, absence of these complaints from the parents does not mean that it does not exist.\textsuperscript{9} In this study, undernutrition was significantly associated with children mainly having solid food (p<0.001) contrast to finding of a study which showed children who were fed with liquid or soft food tended to be undernourished.\textsuperscript{4} Children with CP should be offered food that they can eat with least frustration.\textsuperscript{7} Solid food is more difficult to eat by CP and it may not provide adequate nutritional requirement for the child. In our study, we cannot measure the caloric intake of the children. However, duration and frequency of feeding were not associated with undernutrition, which is in concordance with Hung et al. study.\textsuperscript{4} Alternative feeding routes should be considered in children with feeding difficulties who cannot have sufficient oral intake to maintain adequate nutritional status.\textsuperscript{25}

We analyzed the functional ability of CP children older than 18 months because most normal children get around easier and have good functional ability at this age. Low functioning CP children (GMF IV and V) tended to be malnourished more than those with mild to moderate impairment but this is not statistically significant. Similar to other studies, we also found that children with severe spastic type (quadriplegic CP) had higher risk to be malnourished compared to other spastic types in older than 18 months old group (p=0.03).\textsuperscript{4} This association may be due to the fact that children with quadriplegic limb involvement may have severe feeding problems compared with mild impairment.

In our study, although communication ability was not found to be related with malnutrition, children who had total dependence on caregivers for their feeding was significantly associated with malnutrition (p=0.03). When the children are unable to assess their food, caretakers are left to be responsible for regulating their intake.\textsuperscript{25} However, caregivers may not meet the caloric intake need because they cannot cope with the lengthy mealtimes and the feeding problem. There are some limitations in this study. We cannot exclude the effect of socioeconomic status of family on nutritional status of children and cannot conduct the proper dietary survey. The prevalence of malnutrition depends on the diagnostic criteria used and it should be cautioned to compare the result of this study with others.

\textit{Conclusion}

Malnutrition and feeding problems are quite prevalent in children with CP at YCH. It is associated with older age, feeding mainly solid food and higher dependence on caregivers for feeding. Nutritional support should be an integral part of the management of these children and nutritional intervention should be provided by a multidisciplinary team to ensure adequate growth, improve quality of life and optimize functional status.

\textbf{ACKNOWLEDGEMENT}

We would like to thank Director-General, Department of Medical Research (Lower Myanmar) for his permission and encouragement to conduct this study. We were also grateful to all the participant children and their caregivers for their kind cooperation in this study.

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Oxidative Stress Marker and Antioxidant Status in Patients with Type 2 Diabetes Mellitus

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The persistence of hyperglycemia in diabetic patients leads to the generation of free radicals. Free radicals in the cells play important roles in the pathogenesis of type 2 diabetes mellitus and in the development of diabetes complications. Alterations in lipid peroxidation and antioxidant defense have been investigated as related with diabetes mellitus. Therefore, this study was aimed to investigate oxidative stress marker and antioxidant status in patients with type 2 diabetes mellitus and controls. For this purpose, 30 type 2 diabetes mellitus patients from diabetic clinic at Mandalay General Hospital and 30 apparently healthy controls were studied. The subjects were females, 35-50 years of age. The plasma malondialdehyde level was represented as oxidative stress marker. The plasma ascorbic acid level was determined as antioxidant vitamin. The mean plasma malondialdehyde level (9.7±4.88 µmol/L) in patients was significantly higher than that of controls (4.09±2.17 µmol/L) (p<0.001). The mean plasma ascorbic acid level in patients (1.08±0.24 mg/dl) was found to be significantly lower than that of controls (1.21±0.27 mg/dl) (p<0.05). Negative correlation was observed between these two parameters in patients (r=-0.47) (p<0.01). Therefore, this study indicated that type 2 diabetes mellitus is associated with enhanced lipid peroxidation and it may be due to impaired antioxidant system.

INTRODUCTION

Diabetes mellitus is not a disease, but rather is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by relative or absolute deficiency in insulin.¹ Type 2 diabetes mellitus usually appears in adults, often in middle age. Family history of diabetes can significantly increase risk of developing it. It is the most common form of diabetes mellitus and usually accounts for 90-95% of total diabetics.²

The persistence of hyperglycemia in diabetic patients leads to the generation of Reactive Oxygen Species (ROS) which causes oxidative damage to carbohydrate, protein, lipid, DNA and is efficiently neutralized by cellular antioxidant defense mechanisms. When antioxidant defenses are not efficient or when free radical formation in the body is increased, this imbalance creates the situation of oxidative stress.³ The possible causes of oxidative stress in diabetes mellitus include free radicals generated by auto-oxidation of sugar and sugar adducts to proteins and by auto-oxidation of unsaturated lipids in plasma and membrane proteins.⁴ Glycation of protein, formation of advanced glycation end-products by ROS and resultant oxidative stress can initiate auto-catalytic cycle of delerious reactions in tissues.⁵ The metabolism of glucose produces reducing equivalents during fuel oxidative phosphor-
rylation in mitochondria, the by-products of which include free radicals, that seemed to be first and key event in the activation of other metabolic pathways (e.g., polyol pathway activation) involved in the pathogenesis of diabetes complications. Diabetes mellitus is associated with increased lipid peroxidation which has been implicated in the pathogenesis of diabetic complications. Lipid peroxidation is an auto-catalytic free radical mediated destructive process whereby polyunsaturated fatty acids (PUFAs) in cell membrane undergo degradation to form lipid hydroperoxides. Within biological system, the evidence of oxidative stress is usually determined by malondialdehyde (MDA) formation from lipid peroxidation. Malondialdehyde (MDA) arises from peroxidation of PUFAs, oxidation of arachidonic acid in cell membrane, and enzymatically during eicosanoid metabolism and is readily metabolized in mammalian tissues. Antioxidant defense is impaired in diabetic condition and further exacerbate oxidative stress shift the homeostatic balance in favor of tissue destruction. Ascorbic acid is the most effective aqueous phase antioxidant in human blood plasma and major important antioxidant defense against diseases and degenerative processes caused by oxidative stress. It can regenerate active tocopherol so that it can also restore the antioxidant properties of oxidized tocopherol, and it offers most effective protection against plasma lipid peroxidation. Therefore, it is important to assess the serum MDA level as the oxidative stress marker and vitamin C level as antioxidant parameter in type 2 diabetes mellitus.

In Myanmar, one study found the relationship between fasting blood sugar (FBS), hemoglobin A1c, serum lipid profile and plasma thiobarbituric acid reactive substances (TBAR-S) level with severity of periodontal disease in non-insulin dependent diabetes mellitus subjects. Moreover, another study reported about lipid profile and lipid peroxidation in diabetes mellitus and its complication (microalbuminuria).

Therefore, this study was aimed to investigate plasma malondialdehyde as oxidative stress marker and plasma ascorbic acid as antioxidant status in patients with type 2 diabetes mellitus in comparison to controls.

**MATERIALS AND METHODS**

**Study design**
Laboratory-based, comparative study

**Study site**
Biochemistry Department, University of Medicine (Mandalay)

**Study period**
From June to December, 2007

**Subject selection**
Cases: 30 patients with type 2 diabetes mellitus

Inclusion criteria
- Between 35-50 years of age
- Female patients
- Patients diagnosed as type 2 diabetes mellitus (FBS ≥126 mg/dl) and were regularly visiting the diabetic clinic in Mandalay General Hospital
- Patients who had blood pressure ≤140/90 mmHg
- Non-smokers

Exclusion criteria
- Patients taking regular vitamins supplements such as vitamin E and C
- Patients with pregnancy
- Patients with other chronic inflammatory diseases

Controls: 30 apparently healthy subjects

Inclusion criteria
- Between 35-50 years of age
- Female subjects
- No history of diseases and physical examinations revealed nothing abnormal
- Subjects who had normal FBS level (80-100 mg/dl)
- Subjects who had blood pressure $\leq$140/90 mmHg
- Non-smokers

**Exclusion criteria**
- Subjects taking regular vitamins supplements such as vitamin E and C
- Pregnant women

**Operational definition**
Non-smoker: one who does not smoke at anytime

**Sample size calculation**
The number of subjects required to provide optimal ability to detect changes in plasma MDA and plasma ascorbic acid were calculated by following formula for a study using unpaired ‘t’ test. Two-sided significance level (1-alpha) was 0.05 and power (1-beta, % chance of detecting) was 80.

$$\text{Standard difference} = \frac{\delta}{\sigma}$$

$\sigma=$ the assumed equal standard deviation of the observations in each group
$\delta=$ the smallest difference in means that is clinically important

Using Altman’s normogram, the line connecting a standard difference and power of 80% cut the sample size axis (N) approximately and the required sample size in each group was obtained by N/2. So, sample size in this study was chosen as 30 subjects for each group (cases and controls).

After taking written consent, subjects were interviewed, and history taking and clinical examinations were done.

**Blood sampling**
After overnight fasting, about 7 ml of blood were collected into two clean and dry test tubes from each subject. For FBS determination, 2 cc of blood were collected in the test tube containing sodium fluoride and potassium oxalate as anticoagulant. For plasma MDA and ascorbic acid determinations, 5 cc of blood were collected in the test tube containing double oxalate as anticoagulant.

**Biochemical methods**
Plasma MDA level was determined by using thiobarbituric acid reaction test. Plasma ascorbic acid level was determined by using phosphotungstate method. FBS level was determined by using direct O’toluidine method.

**Data collection and analysis**
Data were recorded according to the proforma. Results were reported as mean $\pm$SD. Student ‘t’ test (unpaired) was used to observe the significance of difference between above parameters (MDA and ascorbic acid) in type 2 diabetes mellitus patients and controls. The Pearson correlation was determined. Data were analyzed by using Microsoft excel and SPSS 11.0 version.

**RESULTS**
Thirty type 2 diabetes mellitus patients from diabetic clinic at MGH and thirty apparently healthy controls were studied. The subjects were females aged between 35-50 years. The plasma malondialdehyde level and erythrocytes catalase activity in both diabetes mellitus patients and controls were determined. The values were expressed as mean$\pm$SD. Table 1 shows the physical data of type 2 diabetes mellitus patients and controls.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>FBS (mg/dl)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45±3</td>
<td>175.64</td>
<td>131.67</td>
</tr>
<tr>
<td>mellitus</td>
<td>$\pm$14.29</td>
<td>$\pm$10.91</td>
</tr>
<tr>
<td>Controls</td>
<td>75.68</td>
<td>115.67</td>
</tr>
<tr>
<td>42±3</td>
<td>$\pm$11.94</td>
<td>$\pm$9.66</td>
</tr>
</tbody>
</table>

The mean$\pm$SD of the plasma MDA levels in type 2 diabetes mellitus and controls were 9.7$\pm$4.88 $\mu$mol/L and 4.09$\pm$2.17 $\mu$mol/L,
respectively. The mean±SD of the plasma ascorbic acid levels in type 2 diabetes mellitus and controls were 1.08±0.24 mg/dl and 1.21±0.27 mg/dl, respectively. Type 2 diabetes mellitus subjects were found to have significantly higher plasma MDA level than that of controls (p<0.001). The plasma ascorbic acid level in type 2 diabetes mellitus was found to be significantly lower than that of controls (p<0.05), considered as significant.

Fig. 1. The mean plasma MDA in diabetes mellitus and controls

Fig. 2. The mean plasma ascorbic acid in diabetes mellitus and controls

Figure 1 shows the mean plasma MDA levels in patients with type 2 diabetes mellitus and controls. The mean plasma MDA level in patients with type 2 diabetes mellitus was found to be significantly higher than that of controls (p<0.001).

Figure 2 shows the mean plasma ascorbic acid levels in patients with type 2 diabetes mellitus and controls. The mean plasma ascorbic acid level in patients with type 2 diabetes mellitus was found to be significantly lower than that of controls (p<0.05).

Fig. 3. The correlation between plasma MDA and ascorbic acid level in type 2 diabetes mellitus

Figure 3 shows the negative correlation between plasma MDA and ascorbic acid levels in type 2 diabetes mellitus patients (r=-0.47) (r^2=0.22). The correlation was significant at p<0.01 level (y=-9.54x+19.98).

DISCUSSION

Diabetes mellitus has been known to be a state of excessive generation of free radicals contributed by several mechanisms, including hyperglycemia and impaired antioxidant status, causing oxidative stress. This oxidative stress exacerbates the development and progress of diabetes and its complications. Oxidative stress can be measured by several blood markers that typically reflect the tissue peroxidation. Lipid peroxidation is one of the important oxidative stresses induced by the reactivity of oxygen free radicals. Many methods have been described to assess some of the chemical stages of the oxidative degradation of an unsaturated fatty acid including measurement of MDA.  

In the present study, the mean plasma MDA level of controls was found to be 4.09±2.2 μmol/L. It was comparable with
the values mentioned in the other studies in which the same method was used (3.92±0.66 and 3.11±0.17 μmol/L). Mean plasma MDA level of type 2 diabetic patients was 9.7±4.9 μmol/L. It was comparable with the values mentioned in the other studies (11.39±1.78 and 10.28±0.41 μmol/L).

In the present study, the mean plasma MDA level in patients with type 2 diabetes mellitus was found to be significantly higher than that of controls (p<0.001). In the study from Germany, it was found that the generation of ROS is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress.

A group of researchers from France mentioned that type 2 diabetes mellitus patients had significantly higher plasma oxidative stress marker than control subjects (p<0.001). In Myanmar, a study found that mean plasma MDA level was significantly higher in diabetes mellitus (p<0.01). There was correlation between lipid peroxidation and diabetes nephropathy.

These studies strongly suggested that enhanced oxidative stress is present in type 2 diabetes mellitus. Variation of values in these studies and in the present study may reflect different methods and procedures. In the present study, duration and complications of diabetes, and the physiological variations like life style, stress, and exercise were not taken into account.

The present study revealed that there is highly significant association of oxidative stress with type 2 diabetes mellitus probably due to increase production of ROS. Therefore, there is enhanced lipid peroxidation indicating increased tissue oxidative stress and it may contribute to diabetic complications.

There are efficient and sophisticated antioxidant systems in living cells. The non-enzymatic antioxidants and enzymatic defenses can offer an indication of the antioxidant status of an individual. In the present study, the mean plasma ascorbic acid level in controls was 1.21±0.27 mg/dl. It was comparable with the values mentioned in other studies (1.23±0.26 and 1.38±0.13 mg/dl). Mean plasma ascorbic acid in patients with type 2 diabetes mellitus was 1.08±0.3 mg/dl. It was comparable with the value mentioned in one study from Nigeria (1.03±0.09 mg/dl).

In the present study, the mean plasma ascorbic acid level was significantly lower in diabetic patients than controls (p<0.05). The observed value in the present study was in agreement with reports of other investigators. A group of researchers from United Kingdom reported that lower plasma ascorbate levels in patients with type 2 diabetes mellitus who consumed adequate dietary vitamin C than controls were found (p<0.001).

In the study from India, the mean plasma ascorbic acid level in all diabetic patients, i.e., those with and without retinopathy was markedly lower than normal controls (p<0.001). So, they suggested that low ascorbate levels in diabetes appear to be a consequence of the disease itself and not due to inadequate dietary intake of vitamin C. Therefore, lower plasma ascorbic acid level in diabetic patients may be caused by increased urinary excretion of the vitamin, defective transport across the cell membrane, and impaired ascorbic acid metabolism along with increased oxidation of ascorbic acid to dehydro-ascorbic acid which may promote diabetic process.

These studies confirmed that compared to apparently healthy persons, ascorbic acid levels are significantly depressed in plasma of diabetic patients. Therefore, diabetic patients have significant defects in antioxidant defense which may increase vulnerability to oxidative damage and progression of the disease. Diabetic patients showed a significant negative correlation between mean plasma MDA and ascorbic acid levels (r=-0.47) (p<0.01). Similar correlation was found in the studies of other investigators.
Therefore, diabetes may cause disorders in ascorbic acid metabolism; ascorbic acid deficiency may provoke disorders in diabetes. Therefore, increase in oxidative stress may be due to decrease in the antioxidant defense.

Conclusion

The present study revealed that diabetes mellitus is associated with enhanced tissue oxidative stress and alterations in antioxidant. Antioxidant therapy to combat the progression of free radical production may also be beneficial. Therefore, monitoring oxidative stress and antioxidant parameters in type 2 diabetes mellitus patients could be important in the progression of diabetes mellitus and in the prevention of diabetic complications.

Moreover, further studies on the measurement of other antioxidant parameters should be required to elucidate their roles in type 2 diabetes mellitus. It is suggested that these studies may help to develop effective strategies for therapeutic approaches to the treatment of diabetic complications.

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REFERENCES


Nephroprotective Effect of Watery Extract of Alternanthera pungens (Myae-khat-kyet-mauk) in Albino Rats Using Cisplatin-induced Acute Renal Failure

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The present study was conducted to investigate the possible potential nephroprotective activity of watery extract of whole plant of Alternanthera pungens in cisplatin-induced nephrotoxic rats. Nephrotoxicity was induced in Wistar strain albino rats by single intraperitoneal (i.p) administration of cisplatin 5 mg/kg (n=6). Effect of concurrent administration of Alternanthera pungens watery extract at the doses of 800 mg/kg, 600 mg/kg and 3,200 mg/kg body weight (n=6 in each group), given by oral route was determined using serum creatinine, serum urea and change in body weight as indicators of kidney damage. Cystone, polyherbal renal protective drug, was used as standard drug for nephroprotective activity. At the dosages of 1,600 mg/kg and 3,200 mg/kg body weight of the watery extract of Alternanthera pungens showed significant decrease in elevated serum urea and creatinine (p<0.001) (one-way ANOVA, followed by Dunnetts test) when compared to that of toxic group. Both groups showed significant protective activity in cisplatin-induced nephrotoxic rats. At the dose of 1,600 mg/kg watery extract and cystone standard were found to normalize the serum urea and creatinine levels when compared to normal control. It was observed that the watery extract of Alternanthera pungens significantly protected the kidneys from injury. The current study revealed that the watery extract of Alternanthera pungens had promising nephroprotective activity and was comparable to cystone in the animal model.

INTRODUCTION

Nephrotoxicity is of critical concern during the early stages of drug development when selecting new drug candidates. Because of its unique metabolism, the kidney is an important target of the toxic effect of drugs, xenobiotic and oxidative stress.1 Cisplatin (Cis-diaminedichloro platinum II, CDDP) is a potent antitumor agent, extensively used for treatment of several cancers like testicular and lungs cancer. Unfortunately, the gracious drug cisplatin is conjoined with a brutal side effect since it induces nephrotoxicity.2 The injection of cisplatin produced proximal and distal tubular necrosis, mainly in the corticomedullary region and intratubular casts in the outer stripe of the outer medulla. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules.3 Cisplatin decreases antioxidants and antioxidant enzymes4, 5 leading to enhanced generation of reactive oxygen metabolites and lipid peroxidation. A large number of herbs have traditionally been used to treat drug or toxin induced renal diseases.6 It is reported that many Indian medicinal plants show beneficial effects against renal injury.7 Alternanthera pungens is used traditionally against dysentery, venereal diseases, cholera, and many parasitic diseases and also this plant showed antioxidant property.8 It is
interesting and important to verify whether their traditional uses are supported by scientific data or merely based on folklore.9

The present study was conducted to investigate the possible potential nephroprotective activity of watery extract of whole plant of Alternanthera pungens in cisplatin-induced nephrotoxic rats.

MATERIALS AND METHODS

Drugs

Cisplatin (Platinex) (Khamdelwal Laboratories Pvt. Ltd, India) was used to induce nephrotoxicity. Cystone syrup (Himalaya Drug Company, Bangalore, India) was used as standard positive control drug.

Chemicals

For the evaluation of nephroprotective activity, serum urea and creatinine were estimated by the use of commercial kits from Hospitex Diagnostics (Italy). For testing antioxidant activity, 1, 1-diphenyl -2-picryl-hydrazyl (DPPH), ascorbic acid (Vitamin C) and distilled water were used.

Analytical instruments

- Balance-AW 220, Shimadzu, Japan
- UV Spectrophotometer, 1601(Shimadzu)
- Vortex mixer
- Biochemical analyzer & Standard accessories: Screen Master Touch, Hospitex Diagnostics (Italy)

Plant material

Whole plants of Alternanthera pungens were collected from Amarapura, Mandalay Region in March, 2012. The plants recommended by local traditional practitioners to treat renal disease were taxonomically authenticated at the Department of Botany, University of Mandalay.

The collected plants were carefully washed and dried under continuous ventilation in the laboratory until total dryness was obtained. The samples were then powdered with a grinder and stored at room temperature until use.

Experimental animals

Forty healthy albino mice (ICR strain) of both sexes with average weight of 25-30 gm were used for acute toxicity test. Thirty-six healthy albino rats (Wistar strain) of both sexes with an average weight of 180-250 gm were used for testing nephroprotective activity. The animals had free access to standard pellet diet and water ad libitum.

Preparation of the extract

One-hundred grams of dried coarse powder were extracted with 1,500 ml of distilled water in a boiling water bath for 6 hours at 60°C.10 Then, the extract was concentrated in vacuum under reduced pressure and evaporated at 50°C until solid residue was obtained.

Preliminary phytochemical analysis

The watery extract of the whole plant of Alternanthera pungens was subjected to preliminary phytochemical analysis for detecting various phytoconstituents.11

1,1-Diphenyl-2-picryl-hydrazyl(DPPH) radical scavenging assay

Determination of radical scavenging activity of DPPH method is based upon the change on absorbance of watery extract solution in various concentrations. The principle is that, in the presence of a stable free radical (DPPH), antioxidant donates a hydrogen atom to quench the stable free radical. Due to the ethanolic component, DPPH solution appears violet and at 517 nm, the color of which changes upon neutralization by free radical to pale yellow.12

Acute oral toxicity study

Acute oral toxicity study was done to determine the median lethal dose (LD₅₀) according to the method of Litchfield and Wilcoxon.13 Thirty albino mice in three groups (10 mice/ group) were orally given watery extract of Alternanthera pungens, at the doses of 1,600 mg/kg body weight, 3,200 mg/kg body weight and 6,400 mg/kg body weight, respectively. The control group (10 mice/ group) received 10 ml/kg body
weight of distilled water. All the animals were kept under observation for screening of toxic symptoms for 2 weeks.

Nephroprotective activity

The rats were divided into six groups, each containing six animals for this study.

Group I
Animals received distilled water for 10 days as normal control.

Group II
Animals received distilled water from day 1 to day 10 and received cisplatin, 5 mg/kg, i.p., single dose on day 11 as toxic control.

Group III
Animals received cystone syrup, 5 ml/kg body weight once daily (o.d) orally from day 1 to day 10 and cisplatin, 5 mg/kg, i.p., single dose on day 11 as standard.

Group IV, V & VI
Animals received watery extract of *Alternanthera pungens* 800 mg/kg body weight, 1,600 mg/kg body weight, and 3,200 mg/kg body weight orally o.d, respectively, from day 1 to day 10 and cisplatin, 5 mg/kg, i.p., single dose on day 11 as the test groups for nephroprotective activity.

All experimental groups had free access to standard pellet diet and water ad libitum from day 1 to day 15. On day 16, animals were anaesthetized by chloroform and sacrificed. Blood samples were collected by cardiac puncture for its biochemical parameters. Kidneys were dissected out immediately and transferred into 10% formalin for further histopathological studies.

Parameters assessed for renal toxicity

Body weight

The body weight (in grams) of the animals were recorded on the first and last day of experiment and the percent reduction was calculated.

Serum urea and creatinine

Urea and creatinine levels in serum were determined by enzymatic method using Hospitex Diagnostics kits (Italy).

Histopathological studies

Formalin preserved samples of kidneys from various experimental groups were studied for both gross and histopathological changes during experiment. Then, weights of the kidneys of all groups were noted immediately after blood collection. Sections of kidneys, stained with haematoxylin and eosin, were observed under standard micro-technique.

Statistical analysis

Data were statistically analyzed by Student ‘t’ test and all values were expressed as Mean±SE. Data were also analyzed by one-way ANOVA, followed by Dunnett’s comparison and p values <0.001 were considered as significant.

RESULTS

Botanical investigation of “Myae-khat-kyet-mauk” confirmed it as *Alternanthera pungens*. Yield percentage of the watery extract of the whole plant of *Alternanthera pungens* was 17.6 (w/w) and the results of phytochemical tests are shown in Table 1.

Table 1. Phytochemical screening of watery extract of *Alternanthera pungens*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reagent</th>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendroff’s</td>
<td>Dragendroff’s</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Dil: HCl, Zn or Mg</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>10% Lead acetate</td>
<td>Lead acetate</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>Conc: H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;, CHCl&lt;sub&gt;3&lt;/sub&gt;, acetic anhydride</td>
<td>Liebermann</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>10% FeCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>=Naphthol, Conc:H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Molisch</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Water</td>
<td>Foam</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>1%FeCl&lt;sub&gt;3&lt;/sub&gt;, dil: H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Tri-terpene</td>
<td>Conc: H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;, CHCl&lt;sub&gt;3&lt;/sub&gt;, acetic anhydride</td>
<td>Liebermann</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>10% NaOH, 3% CuSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Protein</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>=amino acid, Ninhydrin</td>
<td>Ninhydrin</td>
<td>-</td>
</tr>
<tr>
<td>Resin</td>
<td>Acetic anhydride, H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Benedict’s solution</td>
<td>+</td>
</tr>
</tbody>
</table>

The free radical scavenging activity expressed as 50% Inhibitory Concentration (IC<sub>50</sub>)
of the watery extract *Alternanthera pungens* was 0.09 µg/ml and that of ascorbic acid was 0.49 µg/ml. Acute toxicity test did not show mortality of animals in any doses tested. Therefore, it was concluded that median lethal dose (LD₅₀), when administered orally, was supposed to be more than 6,400 mg/kg body weight. The percent reduction of body weight, kidney weight, serum creatinine and urea data are shown in Table 2.

**Table 2. Nephroprotective effect of watery extract of *Alternanthera pungens* on different physical and biochemical parameters**

<table>
<thead>
<tr>
<th>Gps</th>
<th>Treatment</th>
<th>Physical parameters</th>
<th>Biochemical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reduction of B.W (%)</td>
<td>Kidney weight (g)</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>7.2</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.13</td>
<td>±0.13</td>
</tr>
<tr>
<td>II</td>
<td>Cisplatin 11th day (toxic)</td>
<td>23.4</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.06*</td>
<td>±0.06</td>
</tr>
<tr>
<td>III</td>
<td>Cystone+cisplatin (standard)</td>
<td>1.2</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.19</td>
<td>±0.19</td>
</tr>
<tr>
<td>IV</td>
<td>Watery extract 800 mg/kg B.W+</td>
<td>11.1</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>cisplatin</td>
<td>±0.19</td>
<td>±0.19</td>
</tr>
<tr>
<td>V</td>
<td>Watery extract 1,600 mg/kg B.W+</td>
<td>3.1</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>cisplatin</td>
<td>±0.12</td>
<td>±0.12</td>
</tr>
<tr>
<td>VI</td>
<td>Watery extract 3,200 mg/kg B.W+</td>
<td>14.2</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>cisplatin</td>
<td>±0.04*</td>
<td>±0.04*</td>
</tr>
</tbody>
</table>

Gps=Groups, B.W=Body weight, *=Compared to control, **=Compared to cisplatin

The effect of cisplatin and watery extract of *Alternanthera pungens* on serum urea and creatinine are shown in Fig. 1 a & b. Data were statistically analyzed by Student ‘t’ test and all values were expressed as Mean±SEM for 6 animals in each group.

**Fig. 1 a & b. Effect of cisplatin and watery extract of *Alternanthera pungens* on serum urea and creatinine (All values are expressed as mean±SEM (n=6); *p<0.001 compared to control, **p<0.001 compared to cisplatin.)**

**Fig. 2. Histopathologic evaluation of rat kidneys**

A = Normal control kidney showing normal glomeruli and tubules
B = Toxic group (11th day, cisplatin) kidney showing congestion of glomeruli capillaries and stroma congestion
C = Standard group (cystone+cisplatin) kidney showing normal glomeruli and tubules
D = Watery extract (800 mg/kg B.W+cisplatin) kidney showing normal glomeruli and tubules
E = Watery extract (1,600 mg/kg B.W+cisplatin) kidney showing normal glomeruli and tubules
F = Watery extract (3,200 mg/kg B.W+cisplatin) kidney showing mild congestion of stroma of tubules
Data were also analyzed by one-way ANOVA, followed by Dunnett's comparison and p values <0.001 were considered as significant: *p<0.001 compared to control, **p<0.001 compared to cisplatin. Kidney weights were significantly decreased (p<0.001) in toxic and 3,200 mg/kg body weight dose groups when compared with control group. Glomerular congestion of capillaries was found in some rats of group II. Mild congestion of tubular stroma was found in some rats of group VI. The photomicrograph of tissue sections are presented in Fig. 2.

DISCUSSION

Cisplatin is a potent anticancer agent used in solid tumours of testes, ovary, breast, lungs, bladder etc. However, its clinical use is limited by its renal toxicity. Cisplatin accumulates in the renal tubular cells approximately 5 times its extracellular concentration, causing the impairment of kidney. Therefore, it is recognized as the main side effect and the dose limiting factor associated with its use. The mechanism of cisplatin-induced nephrotoxicity is complex and involves oxidative stress, apoptosis, inflammation and fibrogenesis.

Nephrotoxicity is an increasingly common and potentially catastrophic complication in hospitalized patients. Early observational studies from the 1980’s and 1990’s established the general epidemiologic features of acute kidney injury. Kidney is mainly affected by many chemicals and drugs. Drug-associated nephrotoxicity accounts for 18-27% of all acute kidney injury cases in US hospitals. There is no specific treatment to reverse the nephrotoxicity, but it may be reduced with the symptomatic treatment.

Present findings of significant increase in serum urea and creatinine levels (p<0.001) and the decrease in body weight in toxic group when compared to the normal control, indicated the induction of nephrotoxicity with cisplatin. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules. Because this renal damage occurs in the first hour after administration, it is important that the protective agent needs to be present in sufficient concentrations in renal tissues before the damage occurs. This is the rationale behind the prophylactic treatment.

In this study, prophylactic activity of watery extract of Alternanthera pungens was tested with three different doses. At the dosages of 1,600 mg/kg and 3,200 mg/kg body weight of the watery extract of Alternanthera pungens, significant decreases were observed in cisplatin-induced elevated serum urea and creatinine (p<0.001) when compared to those of toxic group. Both groups showed significant protective activity in cisplatin-induced nephrotoxic rats.

At the dose of 800 mg/kg body weight of the watery extract of Alternanthera pungens, no significant decrease in elevated serum urea and creatinine was observed when compared to that of toxic group. Cystone was reported to protect against cisplatin-induced toxicity and protection may be mediated through its ability to inhibit lipid peroxidation. In this study, cystone (standard) showed significant decrease in the elevated serum urea and creatinine (p<0.001) when compared to that of toxic group.

The result of 1,600 mg/kg watery extract of Alternanthera pungens was found to decrease in serum markers when compared with cystone (standard) but the difference was not statistically significant. Both groups of 1,600 mg/kg watery extract and cystone standard were found to normalize the serum urea and creatinine levels when compared to normal control. The results of present study revealed the promising significant prophylactic activity by 1,600 mg/kg body weight dose of watery extract of Alternanthera pungens.

The biochemical results were also supported by histopathological features of the kidney. Normal glomeruli and tubules were observed.
in 800 mg/kg, 1,600 mg/kg body weight and control groups, respectively. Only mild congestion of tubular stroma was observed at 3,200 mg/kg dose of watery extract of Alternanthera pungens in some rats and also mild congestion of glomeruli capillaries were observed in cisplatin treated group.

In conclusion, our study showed that the whole plant of Alternanthera pungens possess marked nephroprotective activity at the dose of 1,600 mg/kg body weight and thus, it played a promising role in the treatment of acute renal injury. The exact mechanism of protection cannot be determined by the present study. However, the ability of the constituents of phenol and tannin in the watery extract to scavenge free radicals may possibly involve in the protection mechanism. However, caution should be exercised in its use especially at high dose of the extract, 3,200 mg/kg body weight due to the possibility of toxicity. Further study for isolation of active components of Alternanthera pungens and evaluation of its nephroprotective activity in chronic renal failure model should be performed.

ACKNOWLEDGEMENT

We would like to thank Daw Sandar Lin and staff of Laboratory Animal Services Division for provision of experimental mice and rats. We are very grateful to Daw Nwè Nwè Yi, Associate Professor, Department of Botany, University of Mandalay for her identification and confirmation of the plant specimens and Dr. Naw Mu Lah Eh Min, Pathologist (retired), Institute of Medicine, Mandalay for her kind examination of histopathology specimens and giving expert opinion.

REFERENCES

The Use of Herbal Medicines and Traditional Medicine Formulations (TMFs) for Hypertension at Traditional Medicine Hospital, Yangon

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¹Pharmacology Research Division
²Epidemiology Research Division
³Department of Medical Research (Lower Myanmar)
Department of Traditional Medicine

This study was done to determine the use of herbal medicines and Traditional Medicine Formulations (TMFs) for treating hypertension at Outpatients’ Department (OPD) of Traditional Medicine Hospital, Yangon. This study was a cross-sectional, descriptive study in 140 hypertensive patients attending the OPD of that hospital. Data were collected from the hypertensive patients by reviewing the prescriptions and records as well as by interviewing the patients and traditional medicine practitioners (in-charge) of the OPD with preformed questionnaires. The data collected were used to evaluate the number and type of herbal drug therapy, the most commonly prescribed herbal drugs and dosage schedules for treatment of hypertension. In overall patients, it was observed that there were male (35%) and female hypertensive patients (65%) with the mean age of 57.74±10.04 years and mean blood pressure of 148.71/93.93 (±12.34/6.65) mm/Hg. The most commonly prescribed herbal drug for hypertension was TMF-17 (63.57%) followed by TMF-23 (57.86%), TMF-27 (42.86%), TMF-12(B) (38.57%) and TMF-33 (31.43%). Single drug therapy was used in 4.29% of hypertensive patients and combination drug therapy was used in 95.71% of the patients. The most commonly used two-drug combination therapy was TMF-17+TMF-23 (22.73%). The most commonly used three-drug combination and four-drug combination therapies were TMF-23+TMF-17+TMF-12B (16.95%) and TMF-17+TMF-33+TMF-27+TMF-23 (22.73%), respectively. In conclusion, it was found that the different types of combination drug therapy were used more than single drug therapy in the treatment of hypertensive patients. This study showed the first research findings for the use of herbal medicines and TMFs for treating hypertension at the OPD of Traditional Medicine Hospital, Yangon and the findings may be beneficial for future research on efficacy and safety of these herbal medicines in hypertensive patients.

INTRODUCTION

Hypertension is an important public health problem both in developing and developed countries. It has been reported that estimated total number of adult hypertension in the year 2000 was 972 millions worldwide. The number of adult hypertension in 2025 was predicted to increase about 60%, a total of 1.56 billion worldwide.¹ Hypertension is a common cause of death in cardiovascular diseases.² Management of hypertension becomes a challenge to the medical profession. There has been a continuous search for a remedy which produces least side effects and cost effectiveness. Nowadays, people commonly use medicinal plants for treating diseases.
because of lower cost and fewer side effects. In Myanmar, traditional medicine and Indian system of medicine, there are many medicinal plants which have been known to have antihypertensive activity.\(^3\)

In Myanmar Traditional Medicine Formulary (1989), some Myanmar Traditional Medicines Formulations (TMFs) have been recommended for the treatment of hypertension. In Traditional Medicine Hospital, Yangon, traditional medicine practitioners widely use herbal preparations and TMFs for the treatment of hypertension.\(^4\)

Drug utilization studies are important for optimization of rational drug therapy and have received a great attention in recent years. Most of the information on drug use patterns has been derived from studies on modern western medicines. In China, there are some studies on drug utilization pattern of Chinese herbal medicines for diseases.\(^5,6,7\)

However, there were very few studies on drug use pattern of Myanmar herbal medicines. Moreover, there is no scientific report of drug use pattern of herbal medicines for treating hypertension in patients at Traditional Medicine Hospital, Yangon. The present study was conducted to investigate the use of herbal medicines and TMFs for treatment of hypertension at the Traditional Medicine Hospital, Yangon.

**MATERIALS AND METHODS**

*Study design*
Hospital-based, cross-sectional descriptive study

*Site of study*
Outpatients’ Department of Traditional Medicine Hospital, Yangon

*Study population*
Hypertensive patients attending the OPD of the Traditional Medicine Hospital, Yangon, (From January 2011 to June 2012)

*Sample size determination*
The required sample size was calculated by using the following formula.
\[ n = \frac{pq (Z_{\alpha}/d)^2}{} \]

The required sample size was 140: 70 new hypertensive patients (first-visit patient) and 70 old hypertensive patients (follow-up patients).

*Ethical consideration*
Ethical approval for this study was obtained from Institutional Ethical Review Committee of Department of Medical Research (Lower Myanmar).

*Study procedure*
Firstly, treatment guidelines for hypertension with herbal medicines and TMFs as well as checklist of herbal medicines used for hypertension were taken from Medical Superintendent of the Traditional Medicine Hospital, Yangon.

*Patient selection*
Patients who met with the inclusion criteria were selected from the OPD of Traditional Medicine Hospital.

*Inclusion criteria*
- Known hypertensive patients with all stages of hypertension (i.e., blood pressure was 140/90 mmHg and above), applying JNC7 blood pressure classification, 2003
- Adult patients (Age - 18 years and above) of both sexes
- Patients who gave consents after explaining the written information about research work

*Exclusion criteria*
- Patients who did not fulfill the above criteria
- Patients who were taking other medicines for co-morbidities such as cancer, tuberculosis and HIV infection

*Data collection*
Data, regarding use of traditional medicines were collected from hypertensive patients attending the OPD of Traditional Medicine
Hospital, Yangon by reviewing prescription books and interviewing the patients with preformed questionnaires (client exit interview). The data were recorded by using Proforma. The data collected were evaluated by using some of the drug use indicators described in WHO (1993).  

- Average number of herbal medicines prescribed per patient encounter
- The dose and dosage regimen
- Percent of patients prescribed with single drug therapy and combination drug therapy
- Most commonly prescribed herbs and TMFs for treatment of hypertension
- Other co-prescribed herbal drugs along with antihypertensive medications
- Percent of patients with associated use of western antihypertensive drugs

Data were also collected from 3 traditional medicine practitioners (in-charge) at the OPD of Traditional Medicine Hospital by interviewing with preformed questionnaires to get the following information.

- Stages of hypertension used by the traditional medicine practitioners for herbal drug therapy for hypertension
- Types of herbal medicines and TMFs used for various stages of hypertension
- Types of therapy used for hypertension at the OPD of that hospital
- Commonly prescribed herbs and TMFs for treatment of hypertension

Data management and analysis

The data processing and analysis were done by using Microsoft Office Excel, 2007 software. The data were shown in mean± standard deviation (SD) and percentage.

RESULTS

A total of 140 hypertensive patients (70 newly visited patients and 70 follow-up patients) with mean age (mean±SD) of 57.74 ±10.04 years were included in this study. In overall patients, duration of hypertension of the patients ranged from 1 month to 20 years. Thirty-five percent of patients were males and 65% of patients were females. Mean systolic blood pressure and mean diastolic blood pressure were found to be 148.71±12.34 mmHg and 93.93±6.65 mmHg, respectively. The results are shown in Table 1 & 2.

Table 1. Demographic characteristics of hypertensive patients attending OPD of Traditional Medicine Hospital, Yangon

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=70)</th>
<th>Total (n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Newly visited</td>
<td>Follow-up</td>
</tr>
<tr>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29  41.43</td>
<td>20  28.57</td>
</tr>
<tr>
<td>Female</td>
<td>41  58.57</td>
<td>50  71.43</td>
</tr>
<tr>
<td>Age (year)</td>
<td>57.37±9.59</td>
<td>58±10.53</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>57.74±10.04</td>
<td>57.74±10.04</td>
</tr>
<tr>
<td>Age range</td>
<td>40-78</td>
<td>37-78</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>16  22.86</td>
<td>24  34.29</td>
</tr>
<tr>
<td>Middle</td>
<td>17  24.29</td>
<td>17  24.29</td>
</tr>
<tr>
<td>High</td>
<td>23  32.86</td>
<td>22  31.43</td>
</tr>
<tr>
<td>University</td>
<td>14  20</td>
<td>7   10</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Own Job</td>
<td>14  20</td>
<td>26  37.14</td>
</tr>
<tr>
<td>Employee</td>
<td>9   12.86</td>
<td>5   7.14</td>
</tr>
<tr>
<td>House wife</td>
<td>7   10.5</td>
<td>5   7.14</td>
</tr>
<tr>
<td>Dependent</td>
<td>24  34.29</td>
<td>18  25.71</td>
</tr>
<tr>
<td>Retired</td>
<td>16  22.86</td>
<td>16  22.86</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10  14.29</td>
<td>15  21.43</td>
</tr>
<tr>
<td>Arthritis/Arthritis</td>
<td>21  30</td>
<td>23  32.86</td>
</tr>
<tr>
<td>History of stroke</td>
<td>9   12.86</td>
<td>10  14.29</td>
</tr>
<tr>
<td>Gastritis</td>
<td>2   2.86</td>
<td>1   1.43</td>
</tr>
</tbody>
</table>

Table 2. Blood pressure at the day of survey, stages of hypertension and associated use of western antihypertensive drugs

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=70)</th>
<th>Total (n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Newly visited</td>
<td>Follow-up</td>
</tr>
<tr>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Systolic BP (mmHg) Mean±SD</td>
<td>146.57±11.41</td>
<td>150.86±12.94</td>
</tr>
<tr>
<td></td>
<td>140-180</td>
<td>140-180</td>
</tr>
<tr>
<td>Diastolic BP (mmHg) Mean±SD</td>
<td>94.14±6.7</td>
<td>93.71±6.63</td>
</tr>
<tr>
<td></td>
<td>90-110</td>
<td>90-110</td>
</tr>
<tr>
<td>JNCT(2003) Blood pressure classification</td>
<td>Stage I</td>
<td>60  85.71</td>
</tr>
<tr>
<td></td>
<td>Stage II</td>
<td>10  14.29</td>
</tr>
<tr>
<td>Associated use of western antihypertensive drug</td>
<td>Yes</td>
<td>41  58.57</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>29  41.43</td>
</tr>
</tbody>
</table>

Regarding the prescribed drugs, in overall patients, the average number of traditional

43
medicines prescribed per patient encounter was found to be 3.36±1.07 (ranged: 2-6). It was observed that the patients receiving single drug therapy was 6(4.29%) and patients receiving combination drug therapy for treatment of hypertension was 134 (95.71%). Combined herbal preparation for treatment of hypertension was used in 9(6.43%) of the patients. The results are shown in Table 3.

Table 3. Use of herbal preparations and Traditional Medicine Formulations (TMFs) as well as types of herbal drug therapy for treatment of hypertension during survey

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=70)</th>
<th>Newly visited</th>
<th>Follow-up</th>
<th>Total (n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td><strong>TMFs for hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMF-17</td>
<td>43</td>
<td>61.43</td>
<td>46</td>
<td>65.71</td>
</tr>
<tr>
<td>TMF-23</td>
<td>43</td>
<td>61.43</td>
<td>38</td>
<td>54.29</td>
</tr>
<tr>
<td>TMF-27</td>
<td>30</td>
<td>42.86</td>
<td>30</td>
<td>42.86</td>
</tr>
<tr>
<td>TMF-12(B)</td>
<td>27</td>
<td>38.57</td>
<td>27</td>
<td>38.57</td>
</tr>
<tr>
<td>TMF-33</td>
<td>21</td>
<td>30</td>
<td>23</td>
<td>32.86</td>
</tr>
<tr>
<td>TMF-21</td>
<td>10</td>
<td>14.29</td>
<td>12</td>
<td>17.14</td>
</tr>
<tr>
<td>TMF-15</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>11.43</td>
</tr>
<tr>
<td>TMF-16</td>
<td>5</td>
<td>7.14</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>TMF-43</td>
<td>1</td>
<td>1.43</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td><strong>Type of therapy for hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single drug therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination drug therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined herbal medicine preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>4.29</td>
<td>6</td>
<td>8.57</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>95.71</td>
<td>64</td>
<td>91.43</td>
</tr>
<tr>
<td><strong>Types of herbal medicine used</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lime juice</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4.29</td>
</tr>
<tr>
<td>Citric juice</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>Hydrocotyle asiatica</td>
<td>2</td>
<td>2.86</td>
<td>1</td>
<td>1.43</td>
</tr>
<tr>
<td>Average number of drugs prescribed per patient encounter (Mean±SD) (range)</td>
<td>3.47±1</td>
<td>3.26±1.14</td>
<td>3.36±1.07</td>
<td>(2-6)</td>
</tr>
</tbody>
</table>

Details of commonly used TMFs for drug therapy for hypertension as well as types of co-prescribed drugs used along with antihypertensive medication at the day of survey are shown in Table 4 & 5.

Table 4. Details of commonly used TMFs for single drug therapy and combination drug therapy for hypertension

<table>
<thead>
<tr>
<th>Type of therapy</th>
<th>Patients</th>
<th>Newly visited</th>
<th>Follow-up</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Single drug therapy</td>
<td>(n=4)</td>
<td>(n=2)</td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>TMF-23</td>
<td>1  25%</td>
<td>1  50%</td>
<td>2  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-12(B)</td>
<td>1  25%</td>
<td>1  50%</td>
<td>2  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-17</td>
<td>1  25%</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>TMF-33</td>
<td>1  25%</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>Combination drug therapy</td>
<td>(n=22)</td>
<td>(n=22)</td>
<td>(n=44)</td>
<td></td>
</tr>
<tr>
<td>Two-drug combination therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-23</td>
<td>10 45.45%</td>
<td>-  -</td>
<td>10 22.73</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-27</td>
<td>2  9.1%</td>
<td>4  18.18%</td>
<td>6  13.64</td>
<td></td>
</tr>
<tr>
<td>TMF-23+TMF-27</td>
<td>3  13.64%</td>
<td>2  9.1%</td>
<td>5  11.36</td>
<td></td>
</tr>
<tr>
<td>Three-drug combination therapy</td>
<td>(n=30)</td>
<td>(n=29)</td>
<td>(n=59)</td>
<td></td>
</tr>
<tr>
<td>TMF-23+TMF-27+</td>
<td>5  16.67%</td>
<td>5  17.24%</td>
<td>10 16.95</td>
<td></td>
</tr>
<tr>
<td>TMF-12(B)</td>
<td>4  13.33%</td>
<td>-  -</td>
<td>4  6.78</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-23+</td>
<td>3  10%</td>
<td>-  -</td>
<td>3  5.08</td>
<td></td>
</tr>
<tr>
<td>TMF-12(B)</td>
<td>3  10%</td>
<td>-  -</td>
<td>3  5.08</td>
<td></td>
</tr>
<tr>
<td>Four-drug combination therapy</td>
<td>(n=12)</td>
<td>(n=10)</td>
<td>(n=22)</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-33+</td>
<td>3  25%</td>
<td>2  20%</td>
<td>5  22.73</td>
<td></td>
</tr>
<tr>
<td>TMF-27+TMF-23+</td>
<td>1  8.33%</td>
<td>-  -</td>
<td>1  4.55</td>
<td></td>
</tr>
<tr>
<td>TMF-33+TMF-12(B)</td>
<td>-</td>
<td>-  -</td>
<td>1  4.55</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-27+</td>
<td>1  8.33%</td>
<td>-  -</td>
<td>1  4.55</td>
<td></td>
</tr>
<tr>
<td>TMF-23+TMF-12(B)</td>
<td>-</td>
<td>-  -</td>
<td>1  4.55</td>
<td></td>
</tr>
<tr>
<td>Five-drug combination therapy</td>
<td>(n=1)</td>
<td>(n=5)</td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-23+</td>
<td>1  100%</td>
<td>3  60%</td>
<td>4  66.67</td>
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</tr>
<tr>
<td>TMF-27+TMF-33+</td>
<td>-</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>TMF-12(B)</td>
<td>-</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-33+</td>
<td>-</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>TMF-27+TMF-33+</td>
<td>-</td>
<td>-  -</td>
<td>1  16.67</td>
<td></td>
</tr>
<tr>
<td>Six-drug combination therapy</td>
<td>(n=1)</td>
<td>(n=2)</td>
<td>(n=3)</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-23+</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-33+TMF-21+</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-15+TMF-27</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-17+TMF-23+</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-27+TMF-33+</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
<tr>
<td>TMF-12(B)+TMF-21</td>
<td>-</td>
<td>-  -</td>
<td>1  33.33</td>
<td></td>
</tr>
</tbody>
</table>

Regarding the combination drug therapies in overall patients, the following results were found.

- 44(31.43%) patients received two-drug combination therapy;
- 59(42.14%) patients received three-drug combination therapy;
- 8(5.71%) patients received four-drug combination therapy;
- 8(5.71%) patients received five-drug combination therapy;
- 2(1.43%) patients received six-drug combination therapy.
- 22(15.71%) patients received four-drug combination therapy;
- 6(4.29%) patients received five-drug combination therapy;
- 3(2.14%) patients received six-drug combination therapy.

They gave treatment for hypertension based on these 3 causes. They used single drug therapy only for mild hypertensive patients and combination drug therapy was used for moderate to severe hypertensive patients. According to information taken from 3 traditional medicine practitioners (In-charge) at OPD of Traditional Medicine Hospital, Yangon, all traditional medicine practitioners at the OPD used WHO, 1999 blood pressure classification and Myanmar Traditional Medicine way for diagnosis of hypertension. They started to give treatment when the patient’s blood pressure was 140/90 mmHg and above. There are 3 underlying causes of hypertension such as ən faca, əp (wol ə) əv yə əf əp (fəm, av ə dp (Odr) according to Myanmar Traditional Medicine.

They use TMFs and method of treatment for hypertension stated in standard treatment guidelines of Myanmar Traditional Medicines and Traditional Medicine Formulary. According to traditional medicine practitioners (In-charge), main TMFs which can be used in treatment of hypertension were TMF-23 (aq ;əv yə əf, TMF-17 (əo əq ;əD ju ə ) and TMF-27 ( yn y ə v kəfo ə ). Co-administered TMFs for treatment of hypertension were TMF-12(B) (p u ə v yə y ək vəq ;), TMF-21 (q əq ;əl), TMF-15 (t yəhə ə wəkəq ;), TMF-16 (t yə əfəv əq ;), TMF-43 (t u ə yə ləgəq ; re f ); TMF-11 (nəw u əq ;) and TMF-35 (zəv ə wəv ə). There were no specific TMFs described for each stage of hypertension in Treatment Guideline of Myanmar Traditional Medicine.

**DISCUSSION**

It was found that in all these patients, the most commonly prescribed TMFs for hypertension was TMF-17 (əo əq ;əD ju ə ) (63.57%) followed by TMF-23 (aq ;əv yə əf ) (57.86%), TMF-27 (yn yə v kəfo ə ) (42.86%), TMF-12(B) (p u ə v yə y ək vəq ;) (38.57%) and TMF-33 (t prəu ə v yə ək vəq ;) (31.43%). Single drug therapy for hypertension was used in 4.29% of the patients and combination drug therapy was used in 95.71% of the patients. In both categories of patients, the combination drug therapy was used more than single drug therapy. Commonly used combination therapies were two- to three-drug combination therapy followed by four-drug combination therapy.

In the present study, single drug therapy was used in stage 1 hypertensive patients and combination drug therapy was used in both stage 1 and stage 2 hypertensive patients. Among patients receiving combination drug therapy, the most commonly used two-drug combination therapy was TMF-17+TMF-23 (22.73%).
drug combination and four-drug combination therapies were TMF-23+TMF-17+TMF-12(B) (16.95%) and TMF-17+TMF-33+TMF-27+TMF-23 (22.73%), respectively. So, it was observed that there were different types of combination therapy depending on severity of blood pressure and underlying causes of hypertension described in traditional medicine point of view.

In some hypertensive patients, sugar and herbal preparations like lime juice, citric juice as well as Hydrocotyle asiatica Linn. leaves (Myin-khwa) decoction were combined with TMFs in order to be more effective in treatment of hypertension. Sugar was used in combination with TMFs like TMF-17, TMF-23, TMF-33 and TMF-15.

Diabetes mellitus (17.86%) and arthralgia/arthritis (31.43%) were the most frequent co-morbidities. In patients with diabetic mellitus, commonly used TMFs were TMF-17 (68%), TMF-23 (64%) and TMF-27 (44%). It was found that TMF-23 (64%), TMF-27 (44%) and TMF-17 (31.43%) could be used to treat both in diabetes mellitus and hypertension.

It was observed that TMFs used in treatment of hypertensive patients are in powder form except TMF-12(B) which is in tablet form. The prescribing TMFs were not expressed in strength except TMF-12(B) (300 mg tablet) but prescribed in dose and dosage regimen. Teaspoon was commonly applied for measurement of the dose for prescribing. These TMFs were prescribed in one teaspoonful dose for each time orally (one to three times per day). TMF-12(B) was prescribed in 5 tablets single oral dose per day at bed time. The patients were advised to take TMF-17, TMF-27, TMF-23, TMF-33, TMF-28 and TMF-35 with warm water while the other TMFs were used to take with water.

Regarding the combination therapy, two to three-drug combinations were prescribed at the same time while the remaining drugs were given in different time intervals of a day. If the TMF was used as single drug therapy, one teaspoonful dose for each time was used while in combination therapy, the patients were advised to mix the prescribed TMFs in equal amount and then to take in one teaspoonful dose for each time. If the hypertensive patients had history of taking treatment with western antihypertensive drugs, they were advised to take one hour interval from traditional medicines to prevent drug interaction. In this hospital, all the TMFs were prescribed (free of charge) to the patients. In the present study, the use of traditional medicines for treatment of hypertension was according to Standard Treatment Guidelines of Myanmar Traditional Medicines (2008).

In conclusion, it was found that the different types of combination drug therapy were used more than single drug therapy in the treatment of hypertensive patients. This study showed the first research findings for the use of herbal medicines and TMFs for treating hypertension at the OPD of Traditional Medicine Hospital, Yangon and the findings may be beneficial for future research on efficacy and safety of these herbal medicines in hypertensive patients.

ACKNOWLEDGEMENT

We would like to thank Director-General and Board of Directors of DMR (Lower Myanmar) and Director-General of Department of Traditional Medicine for allowing us to perform this research work. We would also like to thank the Traditional Medicine Practitioners at OPD of Traditional Medicine Hospital (Yangon) for their kind help throughout this study.

REFERENCES


2. Boon NA, Fox KAA & Bloomfield P. Cardiovascular disease. In: *Davidson’s Principles and Practice of Medicine*, 19th Ed,


Detection of Verotoxic *Escherichia coli* in Street Vended Grilled Meat

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\(^2\)Biological Toxicology Research Division  
\(^3\)Bacteriology Research Division  
Department of Medical Research (Lower Myanmar)

Verotoxin producing *Escherichia coli* (VTEC), particularly O157: H7 serotype is more significant than other well-recognized food-borne pathogens and its contamination is known to be largely associated with raw and undercooked meat. This study was aimed to detect VTEC in street vended grilled meats. A total of 75 grilled meat samples (25 samples each for chicken, pork and mutton) were collected from street vendors in Latha Township and tested for coliforms, fecal coliforms and *Escherichia coli* by standard microbiological analysis procedures and verotoxin production was detected by Reverse Passive Latex Agglutination (RPLA) test. Out of 75 samples tested, coliforms were isolated from 58 samples (77.33%), fecal coliforms in 45 samples (60%) and *Escherichia coli* in 29 samples (38.67%). Coliform, fecal coliform and *Escherichia coli* counts were ranging from 3 to >1,100 MPN/g. Verotoxin 1 (VT1) was detected in 3 isolates (1 isolate from grilled chicken and 2 isolates from grilled pork), Verotoxin 2 (VT2) in 3 isolates (2 isolates from grilled chicken and 1 isolate from grilled pork) and both VT1 and VT2 in 3 isolates (all from grilled pork). Among the isolated *Escherichia coli*, 2 isolates were found to be of O157K+ serotype (1 from grilled pork and 1 from grilled chicken). As VTEC can infect even with as low as 10 organisms and causes severe complications like Hemorrhagic Colitis and Hemolytic Uremic Syndrome, the presence of VTEC strains in the tested samples is of considerable risk to the health of consumers and highlights the importance of food safety interventions.

**INTRODUCTION**

*Escherichia coli* (*E. coli*) was established as a food-borne pathogen in 1971 when imported cheese contaminated with an enteroinvasive strain of serogroup O124 caused illnesses in nearly 400 individuals in 14 American states.\(^1\) Pathogenic *E. coli* are classified into 6 categories by means of their virulence features: Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), entero-invasive *E. coli* (EIEC), enteroaggregative *E. coli* (EaggEC), and diffusely adhesive *E. coli* (DAEC).\(^2\) Among pathogenic *E. coli* that cause food-borne illnesses, EHEC are more significant than other *E. coli* in recent years because public health problems with EHEC are being recognized throughout the world.\(^3\)

All EHEC strains produce either verotoxin 1 (VT1) or verotoxin 2 (VT2) or both. The ability to produce verotoxin was acquired from a bacteriophage, presumably directly or indirectly from *Shigella*. These toxins destroy cells by inhibition of the protein biosynthesis. They have affinity for the lining of the colon and the renal glomeruli and tend to initiate life threatening illnesses like Hemorrhagic Colitis (HC) and Hemolytic Uremic Syndrome (HUS), respectively, especially in
those with immune deficiency, young children and the elderly. 

Among *Escherichia coli* serotypes that produce verotoxin, O157:H7 serotype is the best known and implicated in food-borne outbreaks worldwide and also responsible for 85-95% of HUS cases. Other EHEC serotypes that are reported occasionally include O111, O26:H11, O103:H2 and O113:H21. The main sources of infection are raw or undercooked meat, alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, game meat, cheese curds, raw milk, fresh fruits and vegetables. 

As urbanization changed the life-styles, the habit of eating outside at road-sidestall food is common among the urban population nowadays. The habit of consumption of grilled meat has also been increasing. As VTEC is highly incriminated in undercooked meat, this study was carried out to detect contamination of VTEC in street vended grilled meat.

**MATERIALS AND METHODS**

It was a cross-sectional, descriptive laboratory-based study conducted from October 2008 to September 2009. A total of 75 grilled meat samples (25 each for grilled chicken, grilled pork and grilled mutton) were collected randomly from street vendors in Latha Township of Yangon Region.

**Collection of samples**

All the samples were collected aseptically, placed in sterile containers, kept at 4°C and then transferred to the laboratory.

**Enumeration of coliforms, fecal coliforms and *Escherichia coli***

According to standard microbiological analysis procedures, 50 grams of the samples were mixed with 450 ml of Butterfield’s Phosphate Buffer and blended for 2 minutes and made three consecutive decimal dilutions and inoculated into triplicate tubes containing Lauryl Tryptose (LT) broth to detect the growth of coliforms. LT-positive tubes were transferred into EC medium and examined for the growth of fecal coliforms. A loopful of suspension from EC-positive tubes were streaked on sorbitol MacConkey agar for isolation of *Escherichia coli* and confirmed by performing biochemical tests (Indole, Voges Proskauer test, methyl-red test and utilization of citrate test).

**Toxin detection**

VT1 and VT2 were detected by Reverse Passive Latex Agglutination test (VTEC-RPLA kit, Oxoid, TD 960).

**Serological characterization**

Confirmed *Escherichia coli* isolates were identified for O157 serotype by slide agglutination with O157K+ antiserum (Denka-Seika Company Limited, Japan).

**RESULTS**

Among 75 grilled meat samples tested, coliforms were isolated from 58 samples (77.33%), fecal coliforms in 45 samples (60%) and *Escherichia coli* in 29 samples (38.7%) (Table 1).

<table>
<thead>
<tr>
<th>Tested samples</th>
<th>Present (%)</th>
<th>Absent (%)</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td>17(68)</td>
<td>8(32)</td>
<td>4.72</td>
<td>0.09</td>
</tr>
<tr>
<td>Grilled pork</td>
<td>23(92)</td>
<td>2(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td>18(72)</td>
<td>7(28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td>15(60)</td>
<td>10(40)</td>
<td>0.33</td>
<td>0.846</td>
</tr>
<tr>
<td>Grilled pork</td>
<td>16(64)</td>
<td>9(36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td>14(56)</td>
<td>11(44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td>11(44)</td>
<td>14(56)</td>
<td>0.79</td>
<td>0.675</td>
</tr>
<tr>
<td>Grilled pork</td>
<td>8(32)</td>
<td>17(68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td>10(40)</td>
<td>15(60)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no statistically significant relationship between the percentage of contamination of coliform, fecal coliform and *Escherichia coli* among grilled chicken, grilled pork and grilled mutton samples.
Coliform counts of 44 samples (75.9%) were 3-210 MPN/g, 6 samples (10.3%) were 211-500 MPN/g and 8 samples (13.8%) were 501->1,100 MPN/g. Fecal coliform counts of 38 samples (84.4%) were 3-210 MPN/g, 1 sample (2.2%) was 211-500 MPN/g and 6 samples (13.3%) were 501->1,100 MPN/g. Twenty-four samples showed Escherichia coli count of 3-210 MPN/g and 5 samples were 501->1,100 MPN/g (Table 2).

Table 2. Coliform, fecal coliform and Escherichia coli counts of grilled meat samples

<table>
<thead>
<tr>
<th>Tested samples</th>
<th>No. of counts of grilled meat samples</th>
<th>&lt;3</th>
<th>3-210</th>
<th>211-500</th>
<th>501-&gt;1,100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coliform count (MPN/g) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td></td>
<td>9(62.9)</td>
<td>2(11.8)</td>
<td>6(35.3)</td>
<td></td>
</tr>
<tr>
<td>Grilled pork</td>
<td></td>
<td>20(67.0)</td>
<td>2(8.70)</td>
<td>1(4.3)</td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td></td>
<td>15(83.3)</td>
<td>2(11.1)</td>
<td>1(5.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fecal coliform count (MPN/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td></td>
<td>8(53.3)</td>
<td>1(6.7)</td>
<td>6(40)</td>
<td></td>
</tr>
<tr>
<td>Grilled pork</td>
<td></td>
<td>16(100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td></td>
<td>14(100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Escherichia coli count (MPN/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken</td>
<td></td>
<td>6(54.5)</td>
<td>0</td>
<td>5(45.5)</td>
<td></td>
</tr>
<tr>
<td>Grilled pork</td>
<td></td>
<td>8(100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grilled mutton</td>
<td></td>
<td>10(100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

VT1 was detected in 3 isolates (1 isolate from grilled chicken and 2 isolates from grilled pork), VT2 in 3 isolates (2 isolates from grilled chicken and 1 isolate from grilled pork) and VT1+VT2 in 3 isolates (all from grilled pork) (Fig. 1).

![Fig. 1. Percentage of verotoxin producing Escherichia coli](image-url)

Among the isolated Escherichia coli, 2 isolates were found to be of O157K+ serotype (1 from grilled pork and 1 from grilled chicken).

**DISCUSSION**

This study showed that among 75 grilled meat samples tested, E. coli was isolated from 29 samples (38.7%) and it is in accordance with the finding of a Thailand study where 38.6% of high heat food samples collected from food vendors was found to be contaminated with E. coli. According to New Zealand standard of microbiological reference criteria for food, the recommended microbiological limits of coliforms and E. coli for cooked food including meat are 500 and <3 MPN/g, respectively.

In this study, 8 samples (12.1%) showed coliform count of >500 MPN/g and all 29 samples from which E. coli was isolated showed E. coli count of >3 MPN/g which exceeded the recommended limits. High coliform and E. coli counts of the tested samples indicate unsatisfactory hygienic and sanitary standard. Thick sliced meat was found to be more contaminated with bacteria than thin sliced meat probably due to the heat applied being not sufficient to kill the bacteria in the inner part of the meat.

Among 29 E. coli isolates, 9 isolates were found to produce verotoxin (VT1 in 3 isolates, VT2 in 3 isolates and VT1+VT2 in 3 isolates) and 2 isolates of VT producing E. coli were of O157K+ serotype. O157 serotype has been implicated in many food-borne outbreaks worldwide and is the primary cause of HC and HUS. The infectious dose of EHEC O157 is as low as 2-2,000 cells.

To be fully pathogenic, apart from toxin production, VTEC also require the presence of other virulence markers such as eae chromosomal gene for attachment and plasmid-encoded enterohemolysin. Although these markers could not be identified, the occurrence of VTEC in this study is of considerable risk for the health of consumers because the morbidity and mortality associated with several outbreaks of VTEC disease have highlighted the threat of these organisms pose to public health.
EHEC O157 strain represents a challenging problem in practice. Its low infectious dose in combination with disease severity makes successful prevention strategies to be focused on reducing or eliminating the presence of microorganisms, rather than on prevention of pathogen growth. This focus is particularly more important for raw products that may not be thoroughly cooked before consumption (e.g. meat) or ready-to-eat products that do not receive a definitive treatment that assures elimination of E. coli O157:H7 (e.g. fermented sausages, apple cider). 

Recommendations
- Cook meat thoroughly including the inner part of the meat. Internal temperature should be 160°F.
- Avoid drinking unpasteurized milk and fruit juices. Wash fresh fruits and vegetables thoroughly before eating raw or cooked.
- Separate raw and cooked food and utensils in order to avoid cross-contamination. Keep the food in refrigerator if they are not going to be eaten within 4 hours.
- Health education on the principles of safe food preparation and personal hygiene should be given to general public and food handlers including street vendors.
- Constant monitoring of food and improving diagnostic procedures for detection of VTEC in clinical specimens as well as in foods such as meat and dairy products should be done.
- Cooperation between government, food industries, sellers and the consumers can improve the food safety hygiene.

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REFERENCES

Effect of Health Education on Changes in Dietary Habit and Cardiovascular Risk Factors among Sedentary Workers

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The study was done to assess the effect of health education on readiness to change dietary habit and change in cardiovascular risk factors among employees. The study was pre-, post-test study and conducted in Myanmar Timber Enterprise (Head Quarter), Kyokone, Insein Township, Yangon during 2011. A total of 196 employees underwent the screening procedures for cardiovascular risk factors (measurement of height and weight, waist circumstances, blood pressure, fasting plasma glucose and lipids profile). Among them, 36.7%, 11.7%, 17.8% and 19.4% of employees had hypertension, diabetes, hypercholesterolemia and obesity, respectively. After screening procedures, 50 employees with one or more cardiovascular risks (obesity/diabetes/hypertension/hypercholesterolemia) gave consents to attend bimonthly health education sessions (promotion of healthy dietary habits and physical activity) for six months. After six months of intervention, the end-line assessment on body weight, waist circumstance, blood pressure, fasting plasma glucose and lipid profile was done on 33 participants. Change in dietary habit was interviewed with the structured questionnaires before and after health education. At the end-line assessment, 32 employees had changed their dietary habit but one employee could not change his dietary habit in spite of increasing knowledge. Mean body mass index, waist circumference, fasting blood sugar, mean total cholesterol before and after intervention were 30±4.2 vs. 29.8±4.3, 91.8±9.1 vs. 91.2±9.2 cm, 110.9±48.1 vs. 107.5±38.6 mg%, 188.4±56.5 vs. 191.5±37.4 mg%, respectively. These findings could not support obvious effect on changes in cardiovascular risk factors. It might be due to short duration between two assessments.

INTRODUCTION

Noncommunicable diseases (NCDs) are the biggest cause of death worldwide according to the WHO Global Status Report on NCDs, 2010. More than 36 million people died from NCDs in 2008, mainly cardiovascular diseases (48%), cancers (21%), chronic respiratory diseases (12%) and diabetes (3%). More than 9 million of these deaths occurred before the age of 60 and could have largely been prevented. Premature deaths from NCDs ranged from 22% among men and 35% among women in low-income countries to 8% among men and 10% among women in high-income countries.¹ Common, preventable risk factors underlie most NCDs. These risk factors are a leading cause of the death and disability burden in nearly all countries, regardless of economic development. The leading risk factor globally for mortality is raised by blood pressure (responsible for 13% of deaths globally), followed by tobacco use (9%), raised blood glucose (6%), physical inactivity (6%), and overweight and obesity (5%).²

Noncommunicable Diseases Country Profiles (2011) showed that 20% of total death in all
Cardiovascular disease is the major cause of death in many countries. The major causal risk factors for cardiovascular disease are smoking, high blood pressure, high plasma cholesterol, low HDL cholesterol and high plasma fasting glucose. An aggressive primary prevention in high-risk individuals gives the highest risk reduction, but they constitute only a small fraction of the population. Therefore, a broader approach with long-term prevention in individuals at moderate risk of cardiovascular disease is desirable.

Obesity, physical inactivity, and unhealthy eating habits are major risk factors for chronic disease, disability, and premature death. The combination of eating a balanced, reduced calorie, diet and regular physical activity has a stronger effect on long-term weight loss than either strategy alone; therefore, both strategies are needed. A better understanding of the relationships among multiple behavioral risk factors is important for the design of individual, clinical, and public health interventions, particularly cost-effective interventions to target high-risk individuals and population subgroups.

From a behavioral sciences perspective, the stages of change from the Transtheoretical Model suggest that individuals can be at different stages of readiness to change for different behavioral risk factors. Each behavioral risk factor has its own set of knowledge, attitudes, intentions, decisional balance, and self-efficacy. Motivational readiness described in the stages of change model has been used to tailor interventions to an individual’s level of motivational readiness to change behavior. Interventions tailored to match level of motivational readiness outperform standard interventions.

With lifestyle behavioral choices contributing to a significant proportion of chronic diseases globally, evidence-based strategies to improve behavioral risk factors such as healthier eating and regular physical activity should be considered in a variety of settings. Recent research has shown that effective worksite health promotion programmes were those that offered multiple risk-factor interventions combined with group participation and individualized risk reduction counselling to high-risk employees. These programmes were found to produce positive clinical and cost outcomes such as increases in health awareness, risk reduction, disease prevention and a reduced demand for health services.

Workplaces are considered to be a key channel for the delivery of interventions to reduce chronic diseases. The workplace offers several advantages in that a substantial number of the working population can be reached and multiple levels of influence on behavior can be targeted. Workplace health program can identify and prevent major chronic disease risks to an extent that decrease clinical risk costs in health system and improves overall economic output. National and international data consistently demonstrate that the investment of workplace health program delivers a rate of return on investment or cost/benefit ratio of about 1:5.

A healthy workplace offers the ideal setting for introducing health promotion programs: since the majority of the adult population spends approximately one-third of their daily life at work, the workplace offers an excellent environment for promoting health. If neglected, the work environment can have extremely negative consequences for workers’ health, causing stress, injury, illness, disability and death. A healthy workplace promotes the overall success of the organization: a healthy workplace can result in changes that are beneficial to the
long-term survival and success of an organization. Benefits include improved worker health status, increased job satisfaction, enhanced morale and work productivity and cost savings (e.g. reduced absenteeism and employee turnover, lower health care and insurance costs). There were no intervention studies before to assess the effect of health education on changes in cardiovascular risk factor in workplace setting. Therefore, this study aimed to promote healthy lifestyle by improving dietary habit and reduction of cardiovascular risk factors among employees in selected workplace.

MATERIALS AND METHODS

Study design
Pre-, post- test design was used. The design is depicted in figure below.

\[ X \quad O_1 \quad \text{Time} \quad O_2 \]

Where, X=A program intervention
O1=A baseline measurement
O2=An end line measurement
Time= intervention period (6 months)

The study was started with identification of employees with cardiovascular risk factors. Then, the risk persons were counseled how to practice healthy eating and be more physically active through small group education session. After six-month intervention, these risk factors were reassessed.

Study area
The study was conducted in Myanmar Timber Enterprise (Head Quarter), Kyokone, Insein Township.

Study population
Employees of Myanmar Timber Enterprise (Head Quarter)

Inclusion criteria for identification of cardiovascular risk factors
- Employees of Myanmar Timber Enterprise (Head Quarter) with the age more than 30 years
- Both sexes

Exclusion criteria for identification of cardiovascular risk factors
- The employees who plan to retire during the intervention period
- Pregnant women and lactating mothers

Inclusion criteria for intervention
The employees who have body mass index ≥30.0 with or without systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or total cholesterol ≥220 mg/dl or fasting glucose ≥110 mg/dl

Sampling method
Sampling method was nonprobability sampling method. A convenience sample was recruited from Myanmar Timber Enterprise (Head Quarter) in Yangon. Employees from Myanmar Timber Enterprise (Head Quarter) were selected as the studied population because majorities of them are middle-aged adults and their nature of work is sedentary. The authorities from this department were willing to participate in this intervention after advocating. After giving an explanation about the purpose, the study procedures, risks and benefits of participating, a total of 200 employees who met the inclusion criteria gave consents to participate actively in the study. Interviewer training and validation of data collecting tools were also carried out before actual survey was conducted. Identification of the employees and personal medical history were asked with the structured questionnaires.

For the screening of cardiovascular risk factors among employees, body weight, height and blood pressure were measured. After 10-hour overnight fast, 3 ml of venous blood samples were taken for determination of plasma lipid profile and fasting plasma glucose. Fasting plasma lipid profile and plasma glucose were determined by standard laboratory procedure at Nutrition Research Division, Department of Medical Research (Lower Myanmar).

After screening procedures, the participants with one or more cardiovascular risk factors
were chosen as subjects for the intervention which promotes the health status of them through healthy eating and more physical activity. Among 200 employees, only 59 employees (30.1%) did not have risk factors and 137 employees (69.9%) had one or more risk factors. Employees with risk factors undergone ECG and consulted with physician for further treatment. After screening procedures, 50 employees with one or more cardiovascular risks (obesity/diabetes/hypertension/hypercholesterolemia) gave consents to attend bimonthly health education sessions (promotion of healthy dietary habits and physical activity) for six months.

After six education sessions (third month of intervention), interim assessments on 35 employees were done by using the structured questionnaire for readiness to change the dietary habit and promotion of physical activity. Only 33 employees participated in the end-line assessment. Health education talk entitled “Healthy Lifestyle and Prevention of Noncommunicable Diseases” by Professor Tint Swe Latt was also conducted after interim assessment. After six months of intervention, the end-line assessment on body weight, waist circumstance, blood pressure, fasting plasma glucose and lipids profile was done on 33 participants. Change in dietary habit and physical activity was interviewed with the structured questionnaires.

Data entry and analysis
Data entry and analysis was done with SPSS 11.0. Univariate categorical data were presented with frequency and percent tables and those of continuous variables were presented with mean±SD. Difference between means before and after health education was calculated by paired ‘t’ test. The level of significance was set at p value <0.05.

Ethical considerations
The proposal was submitted to the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar) for approval.

RESULTS
A total of 196 employees participated in screening of cardiovascular risk factors. Among them, 29.6% were males and 70.4% were females with mean age of 44.3±7.8 with minimum 30 years and maximum 58 years. Larger proportion of employees were clerical staffs and 16.3% and 6.1% of employees were officers and workers/drivers, respectively (Table 1).

Table 1. Background characteristics of employees participated in screening procedure

<table>
<thead>
<tr>
<th>Age group(years)</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>12(18.8)</td>
<td>52(81.3)</td>
<td>64(32.7)</td>
</tr>
<tr>
<td>40-49</td>
<td>16(21.6)</td>
<td>58(78.4)</td>
<td>74(37.8)</td>
</tr>
<tr>
<td>≥50</td>
<td>27(46.6)</td>
<td>31(53.4)</td>
<td>58(29.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education level</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than high school</td>
<td></td>
<td>4(100)</td>
<td>4(12.1)</td>
</tr>
<tr>
<td>High school level</td>
<td></td>
<td>13(100)</td>
<td>13(39.4)</td>
</tr>
<tr>
<td>Graduate</td>
<td>2(12.5)</td>
<td>14(87.5)</td>
<td>14(48.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rank</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Officer</td>
<td>12(37.5)</td>
<td>20(62.5)</td>
<td>32(16.3)</td>
</tr>
<tr>
<td>Clark</td>
<td>40(26.3)</td>
<td>112(73.7)</td>
<td>152(77.6)</td>
</tr>
<tr>
<td>Worker/Driver</td>
<td>6(50)</td>
<td>6(50)</td>
<td>12(6.1)</td>
</tr>
<tr>
<td>Total</td>
<td>58(29.6)</td>
<td>138(70.4)</td>
<td>196</td>
</tr>
</tbody>
</table>

Table 2 shows the overall prevalence of overweight, obesity, hypertension, diabetes and hypercholesterolemia among employees. Prevalence of overweight, obesity, hypertension, diabetes and hypercholesterolemia among employees were 36.7% (M-41.4%, F-34.8%), 19.4% (M-8.6%, F-23.9%), 36.7% (M-41.4%, F-34.8%), 11.7% (M-12.0%, F-11.7%) and 17.8% (M-12.1%, F-20.3%), respectively.

Among 33 employees who participated in the intervention programme, only 2 employees were males and the rest were females. Mean age of employees was 48.2±6.2 years with minimum 37 years and maximum 57 years. Seven employees were officers and the rest were clerical staffs. Five (15.2%), 12(36.4%), 13(39.4%) and 3(9.1%) employees had one, two, three and four risk factors, respectively. The persons with only one risk factor were obese persons.
Table 2. Prevalence of risk factors of cardiovascular disease in employees participated in screening procedure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (cm)</td>
<td>81.2</td>
<td>13.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>25.8</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (BMI &lt;18.5)</td>
<td>9</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (BMI 18.5-24.9)</td>
<td>77</td>
<td>39.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight (BMI 25-29.9)</td>
<td>72</td>
<td>36.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI ≥30)</td>
<td>38</td>
<td>19.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>124</td>
<td>63.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>36.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.5</td>
<td>17.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.8</td>
<td>10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>171</td>
<td>87.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting blood sugar (mg%)</td>
<td>96.4</td>
<td>35.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>161</td>
<td>81.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>17.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total serum cholesterol (mg%)</td>
<td>177.3</td>
<td>45.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparison of cardiovascular risk factors before and after health education

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Health education</th>
<th>Mean difference in changes (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Mean weight (lb)</td>
<td>151.8 ±25.3</td>
<td>151.2 ±26.3 (-1.21 to 2.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean BMI</td>
<td>29.9 ±4.2</td>
<td>29.8 ±4.3 (-0.23 to 0.48)</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean waist circumference (cm)</td>
<td>91.8 ±9.1</td>
<td>91.2 ±9.1 (-0.88 to 2.12)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mmHg)</td>
<td>138.8 ±16.7</td>
<td>128.2 ±21.4 (5.15 to 16.07)</td>
<td>0.00*</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (mmHg)</td>
<td>87.8 ±8.9</td>
<td>84.5 ±3.3 (-6.29 to 12.38)</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean fasting blood glucose (mg %)</td>
<td>110.9 ±48.1</td>
<td>107.4 ±38.6 (-6.17 to 8.64)</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean total serum cholesterol (mg%)</td>
<td>188.4 ±56.5</td>
<td>191.5 ±37.4 (-20.51 to 14.3)</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean LDL-cholesterol (mg%)</td>
<td>122.3 ±45.3</td>
<td>136.5 ±40.2 (-32.26 to 3.93)</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean HDL-cholesterol (mg%)</td>
<td>42.4 ±12.9</td>
<td>46.0 ±14.3 (-9.12 to 19.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean triglyceride (mg%)</td>
<td>133.9 ±52.7</td>
<td>112.1 ±41.8 (4.83 to 38.96)</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

*Statistically significant

Table 4. Means systolic and diastolic blood pressure, fasting blood sugar, total cholesterol level and mean difference of employees by categories

<table>
<thead>
<tr>
<th></th>
<th>Mean ±SD</th>
<th>Mean difference of change</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant with no hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline systolic</td>
<td>125</td>
<td>±7.6</td>
<td></td>
</tr>
<tr>
<td>Endline systolic</td>
<td>115</td>
<td>-10.0</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±14.1</td>
<td>(-1.8 to 21.8)</td>
<td></td>
</tr>
<tr>
<td>Participant with hypertension</td>
<td></td>
<td></td>
<td>0.003*</td>
</tr>
<tr>
<td>Baseline systolic</td>
<td>143.2</td>
<td>±16.5</td>
<td></td>
</tr>
<tr>
<td>Endline systolic</td>
<td>132.4</td>
<td>-10.8</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±21.8</td>
<td>(4.2 to 17.4)</td>
<td></td>
</tr>
<tr>
<td>Participant with no hypertension</td>
<td></td>
<td></td>
<td>0.285</td>
</tr>
<tr>
<td>Baseline diastolic</td>
<td>81.2</td>
<td>±3.5</td>
<td></td>
</tr>
<tr>
<td>Endline diastolic</td>
<td>77.5</td>
<td>-3.8</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±8.9</td>
<td>(-3.9 to 11.4)</td>
<td></td>
</tr>
<tr>
<td>Participant with hypertension</td>
<td></td>
<td></td>
<td>0.133</td>
</tr>
<tr>
<td>Baseline diastolic</td>
<td>90</td>
<td>±9.1</td>
<td></td>
</tr>
<tr>
<td>Endline diastolic</td>
<td>86.8</td>
<td>-3.2</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±9.9</td>
<td>(-1.05 to 7.4)</td>
<td></td>
</tr>
<tr>
<td>Participant with no hypercholesterolemia</td>
<td></td>
<td></td>
<td>0.05*</td>
</tr>
<tr>
<td>Baseline total cholesterol</td>
<td>163.08</td>
<td>±35.6</td>
<td></td>
</tr>
<tr>
<td>Endline total cholesterol</td>
<td>179.91</td>
<td>±33.5</td>
<td>(4.39 to 33.61)</td>
</tr>
<tr>
<td>Participant with hypercholesterolemia</td>
<td></td>
<td></td>
<td>0.402</td>
</tr>
<tr>
<td>Baseline</td>
<td>218.7</td>
<td>±62.8</td>
<td></td>
</tr>
<tr>
<td>Endline</td>
<td>205.3</td>
<td>-13.39</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±38.1</td>
<td>(-19.8 to -46.6)</td>
<td></td>
</tr>
<tr>
<td>Participant with nondiabetes</td>
<td></td>
<td></td>
<td>0.127</td>
</tr>
<tr>
<td>Baseline</td>
<td>87.7</td>
<td>±12.7</td>
<td></td>
</tr>
<tr>
<td>Endline</td>
<td>90.8</td>
<td>±3.09</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±7.3</td>
<td>(-7.13 to 0.95)</td>
<td></td>
</tr>
<tr>
<td>Participant with diabetes</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Baseline</td>
<td>183.4</td>
<td>±45.7</td>
<td></td>
</tr>
<tr>
<td>Endline</td>
<td>161</td>
<td>22.12</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>±46.4</td>
<td>(17.8 to 62.2)</td>
<td></td>
</tr>
</tbody>
</table>

* = Statistically significant

The participants were stratified into normal and hypertension, diabetes and hypercholesterolemia; the mean difference was more pronounced in participants with hypertension, diabetes and hypercholesterolemia than normal participants (Table 4). Percentages of employees with reduction in intake of rice, fatty food, salt, sweet foods and
The prevalence of overweight and obesity in this study was higher than those in risk factor studies in Yangon Region (2004) and nationwide survey (2009) (36.7% vs. 23.8% vs. 29.9% and 19.4% vs. 7.9% vs. 6.8%). This high prevalence may be due to the sedentary nature of work of employees.

Prevalence of hypertension in present study (36.7%, M-41.4%, F-34.8%) was similar with those of raised blood pressure in high-, low-, lower-middle and upper-middle-income countries all having rates of around 40% for both sexes. Prevalences of diabetes mellitus and hypercholesterolemia among employees, i.e., 11.7% and 17.8% were higher than those of age-standardized adult diabetes study in which prevalences were 9.8% among men and 9.2% among women in 2008. In comparison with the global prevalence of raised total cholesterol in 2008 (38%), the prevalence of hypercholesterolemia in present study was only 17.8% and this may be due to higher cut-off point than global prevalence (high cholesterol > 190 mg%).

Changes in cardiovascular risk factors after health education showed that systolic blood pressure and triglyceride level were significantly reduced (p<0.05). Similar finding was found in worksite health promotion programme in Kuala Lumpur, Malaysia. There were reductions in body mass index, weight, diastolic blood pressure and fasting blood sugar but not statistically significant.

In this study, mean HDL-cholesterol level became higher after health education. This may be due to combined effect of dietary changes and physical activity. Physically active employees had higher mean HDL-cholesterol level than physically inactive employees (42.5±9.9 vs. 47.1±15.4) but not statistically significant. Mean levels of total cholesterol and triglyceride were increased which were similar to the findings in Malaysian study. Mean difference of risk factors between before and after intervention in the present study was comparable to Malaysian study in which intervention period was 2 years.

Reduction in mean total cholesterol (-13.39) was found in employees with hypercholesterolemia but increasing mean cholesterol level of 16.86 (95%CI; from 4.39 to 33.61), within normal limit, was seen in employees with no hypercholesterolemia. This reduction may be due to the additional effect of reduction of fatty foods and medication. Reduction of fatty food intake was found in employees with hypercholesterolemia than employees with no hypercholesterolemia (100% vs. 64.7%). Similar finding was found in employees with diabetes and reduction of mean fasting blood sugar (-22.12) may be due to the effect of avoidance of sweet food in addition to medication effect (avoidance of sweet foods is 100% in employees with diabetes). Although reduction of salt intake was more prevalent in employees with hypertension, the means in systolic and diastolic blood

![Diagram](image-url)
pressures both in nonhypertensive and hypertensive employees were not much different. The study done at New York in 2004 found that changes in dietary fat intake and fruit and vegetables changes were related.\textsuperscript{19}

In the present study, such relationship was not found between the variables but statistically significant relationship was found between diabetes and increasing vegetable consumption (p=0.04). Reduction in rice consumption was observed only in employees with diabetes than nondiabetes employees (50% vs. 37.5%) and did not find in employees with overweight/obesity, hypertension and hypercholesterolemia.

In regard with readiness of changing diet and physical activity, 32 employees had changed their dietary habit but one employee could not change his dietary habit in spite of increasing knowledge at the endline assessment. Thirty-one employees had active stage indietary changes. Among 33 employees: 8, 18 and 7 employees had no change, ready to change and active stage in physical activity changes, respectively.

The employee with no change in dietary habit did not change his physical activity and his biochemical parameters were not markedly changed after health education. One employee with ready to change dietary habit was in the stage of ready to change physical activity. Physically active employees had lesser waist circumference, body weight and body mass index than non-active employees (94.8 vs. 90.1 cm, 31.7 vs. 29.2 and 170.8 lb vs. 144.9 lb, respectively).

\textit{Conclusion and recommendations}

All of the risk factors except total cholesterol and triglycerides showed some extent of changes after health education in the intervention population. But more different parameters between two assessments were found in employees with hypertension, diabetes and hypercholesterolemia than normal employees.

Our study demonstrated a moderate improvement in cardiovascular risk reduction following health education about healthy eating and physical activity. To achieve a greater impact of worksite health promotion, future strategies should aim at providing a more conducive environment to facilitate individual behaviour change. In this study, there was not much improvement in self-reported exercise or physical activity. In order to encourage exercise among the participants, structured exercise programmes should be implemented within the working hours. The data suggested that a worksite approach in health promotion programs on cardiovascular risk factors can be implemented.

Future research like randomized controlled trial is needed to show the effectiveness of multiple risk factor interventions and can be compared within and between groups. Furthermore, a critical issue is how health-care providers can reach and motivate patients who need to change behaviors, but are unwilling to communicate. This was the first study that observed the effect of health education to change modifiable risk factors of cardiovascular diseases in work place setting. There were several limitations in the study. It was a pilot study, the sample size was relatively small, the intervention period is short and the subjects were not randomly selected.

\textbf{ACKNOWLEDGEMENT}

We gratefully acknowledged the Director-General, Department of Medical Research (Lower Myanmar) for allowing to conduct this project. Also, our sincere gratitude were to authorities from Myanmar Timber Enterprise (Head Quarter) for their keen interest and support to carry out this research project. We mentioned our heartfelt thanks to the employees from Myanmar Timber Enterprise (Head Quarter) for their active participation in screening procedure and intervention research. The financial support of the World Health Organization was also gratefully acknowledged.
REFERENCES

Determination of Histamine Content in Commonly Consumed Fish-head

Thin Thin Wah, Tin Tin Htwe, May Than Htay, Nilar, Myo Myo Kyaw, Phyu Phyu Zin, Kyaw Kyaw San & Thaung Hla

Biological Toxicology Research Division
Department of Medical Research (Lower Myanmar)

Histamine poisoning is one example of the food poisoning. The aim of the study was to determine histamine content in commonly consumed fish. A total of five different types of fish-head and five samples of each: Clarias batrachus (Nga-khu), Channa stnata (Nga-yant), Cirrhinus marigala (Nga-gyin), Silonia silondia (Nga-myin) and Panganius hypophthalmus (Nga-tan) were tested. Each fish-head was divided into three portions to be tested as raw, after processing and after cooking. Histamine was extracted from 75 samples (25 as raw, 25 after processing and 25 after cooking). Histamine content was tested by using simple and convenient method of visual colorimetry on a short silica-gel column cartridge. Histamine was detected in total 23 samples: 2(8.7%) from raw, 13(56.5%) from after processing and 8(34.8%) from after cooking. Out of 23 samples, histamine content were found to be more than 100 ppm in 2 samples (Nga-yant from processing, Nga-tan from after cooking), between 50-100 ppm in 4 samples (Nga-gyin from processing and Nga-myin, Nga-gyin, Nga-tan from after cooking) and less than 50 ppm in 17 samples. The maximum level of histamine content safe for consumption is 50 ppm according to the recommendation of American Food and Drug Administration and any fish with histamine content of above 50 ppm have to be discarded. In this study, 6 samples were found to contain the dangerous level of histamine.

INTRODUCTION

The safety of food is essential for effective prevention of food poisoning and other food-inducing illnesses. Histamine poisoning is an example of the food poisoning. Histamine poisoning (HA) is also known as “scombroid fish poisoning” although non-scombroid fish species are often implicated. Free histamine is produced by means of bacteria having histidine decarboxylase act on histidine in these fishes. The usual level of histamine in fresh fish is 0.1 mg/100 g. Even before a fish smells bad, high level of histamine indicating decomposition can be detected.

When fish containing high level of histamine is consumed, it can cause transient food poisoning. This food poisoning is characterized by allergy-like symptoms such as flushing, sweating, and burning of the mouth, nausea, vomiting, and diarrhea, dizziness, swelling of the tongue and face and abdominal pain. The reported number of histamine poisoning cases is relatively small. A large number of unreported cases are expected, however, because of the transient tendency of the histamine poisoning. Histamine is water-soluble and relatively stable to heat, thus, not decomposable for removal in the ordinary cooking process. Monitoring the histamine content in those foods will be the most effective measure for prevention of the histamine poisoning.

Nowadays, fish-head is the popular cuisine in almost every restaurant. Most of the people can afford to consume it. Fish-head consumption becomes increasing. Higher bacterial contamination was associated with the gills, gut and skin than blood and meat. Histamine content had been tested in fish
meat 5, 6, 7, 8 but not in fish-head. Thus, this study was conducted to determine the histamine content in commonly consumed fish-heads.

**MATERIALS AND METHODS**

**Collection of samples**

Heads of five different types of fish: *Clarias batrachus* (Nga-khu), *Channa stnata* (Nga-yant), *Cirrhinus marigala* (Nga-gyin), *Silonia silondia* (Nga-myin) and *Panganius hypophthalmus* (Nga-tan), five samples of each, were collected in plastic bags and delivered to the laboratory of Biological Toxicology Research Division, DMR(LM). Each fish-head was divided into three portions immediately to be tested as: raw, after processing and after cooking. After that, the samples were processed at the laboratory.

**Extraction of histamine**

The extraction of histamine from fish samples was carried out as follows:

Five grams of samples were homogenized with 40 ml of 5% trichloroacetic acid (TCA), then diluted to 50 ml as the final volume with the same solvent. Subsequently, the homogenate was transferred into a test tube and centrifuged (11,200 g) at 4°C for 10 minutes. The supernatant was stored in a 50 ml-plastic bottle in a refrigerator until testing.

**Fabrication of HA cartridge**

First, a small amount of cotton was stuffed inside the bottom of 1 ml disposable syringe to retain packing materials. Fifty milligrams of silica-gel 60 were put on top of the cotton. The silica-gel was “sandwiched” by inserting another layer of cotton. Finally, the material inside the syringe was compacted by tamping with a thin rod.

**Assay with HA cartridge**

One milliliter of TCA extract was mixed with 250 µl of 1 M sodium hydroxide. Then, the whole mixture was passed through the HA cartridge; subsequently, the cartridge was washed with 200 µl of 0.1M phosphate buffer (pH 6), followed by 1000 µl of distilled water. Finally, 200 µl of 1.0 mM 2, 3-Naphthalenedicarboxaldehyde (NDA) solution was passed through the HA cartridge slowly, and the color inside the HA cartridge was observed after 3 minutes and the concentration of histamine present was estimated by conforming the color tone with those of the standards.

**Preparation of histamine standard solutions with HA cartridges**

To be used as the control for this experiment, histamine standard cartridges were separately prepared to make concentrations of 0, 25, 50, 100, 500 and 1000 mg kg⁻¹. Then, 1mM NDA solution was passed through each HA cartridge and the color changes inside the HA cartridges were observed after 3 minutes.

**RESULTS**

Five different types of fish-head: *Clarias batrachus* (Nga-khu); *Channa stnata* (Nga-yant); *Cirrhinus marigala* (Nga-gyin); *Silonia silondia* (Nga-myin) and *Panganius hypophthalmus* (Nga-tan) were tested. Each fish-head was divided into three portions to be tested as raw, after processing and after cooking. Out of total 75 samples (25 samples as raw, 25 samples after processing and 25 samples after cooking), histamine was detected in total 23 samples.

Out of 23 samples, 2(8.7%) were from raw, 13(56.5%) were from after processing and 8(34.8%) were from after cooking (Table 1).

<table>
<thead>
<tr>
<th>Types of fish-head</th>
<th>Raw No.</th>
<th>Processing No.</th>
<th>After cooking No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clarias batrachus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Channa stnata</em></td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>Cirrhinus marigala</em></td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>Silonia silondia</em></td>
<td>-</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>Panganius hypophthalmus</em></td>
<td>-</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total (%)</td>
<td>2(8.7)</td>
<td>13(56.5)</td>
<td>8(34.8)</td>
</tr>
</tbody>
</table>
The resulting color tones of histamine standard solution and samples of histamine content solution in fish (Fig. 1 & 2). Regarding histamine content, 2 samples had more than 100 ppm (Channa stnata from processing, Panganius hypophthalmus from after cooking), 4 samples had between 50-100 ppm (Cirrhinus marigala from processing and Silonia silondia, Cirrhinus marigala, Panganius hypophthalmus from after cooking) and 17 samples had less than 50 ppm (Fig. 3).

In this study, histamine was not detected in all three portions of the fish head of Channa stnata (Nga-khu). The maximum level of histamine safe for consumption recommended by American FDA is 5 mg/100 g (50 ppm), and any fish containing histamine above this level have to be discarded.

**DISCUSSION**

The bacteria contamination promotes the development of histamine in fish. The possibility of cross-contamination during handling and processing of fish in the fish industry has been demonstrated. It is well-known that the most susceptible part of fish to bacterial colonization is the gill, followed by the outer skin and the slime of fish. The gill was identified as the source of M. morganii in mackerel and sardine, and its presence was found in most of the fish tested.

The reported number of histamine poisoning cases is relatively small. A large number of unreported cases are expected, however, because of the transient tendency of the histamine poisoning. The maximum level of histamine safe for consumption recommended by American FDA is 5 mg/100 g (50 ppm), and any fish containing histamine above this level has to be discarded. Histamine levels causing illness in fish are mostly above 20 mg/100 g (200 ppm). Levels above 50 mg/100 g (500 ppm) are hazardous for consumption. Freezing may inactivate the enzyme-forming bacteria. Both the enzyme and the bacteria can be inactivated by cooking.
However, once histamine is formed, it cannot be eliminated by heat or freezing. After cooking, recontamination of the fish with the enzyme-forming bacteria is necessary for additional histamine to form. For these reasons, histamine development is more likely in raw, unfrozen fish. After forming histamine, it cannot be destroyed by freezing, cooking, smoking and canning. Therefore, histamine content in fishes should be tested and HA method is quick and accurate method for detection of histamine. The types of fish tested in this study are commonly consumed by people. Histamine was detected in 23 samples, among them, 6 samples contained the dangerous level of histamine even after processing and cooking.

Conclusion
Histamine content in fish is responsible for allergy-like food poisoning and this study revealed the presence of histamine in fish-head samples and showed the need for proper handling of fish to prevent introduction and cross-contamination of prolific histamine formers in fish and proper processing methods.

REFERENCES
Deltamethrin Treated Clothes for Personal Protection on Malaria among Temporary Migrant Workers in Rubber Plantation, Mon State, Myanmar

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\textsuperscript{2}Department of Medical Research (Lower Myanmar)  
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\textsuperscript{5}Township Medical Officer, Thanbyuzayat Township, Mon State  
\textsuperscript{6}Vector Borne Diseases Control, Department of Health

Malaria transmission is provoked by man-vector contact and vector density. Generally, rubber plantation workers work from dusk to dawn coinciding with peak biting time of \textit{Anopheles} vectors and protect from wind and cold by wearing of hats, shirts, longyi, jackets, jeans and sweaters, etc. There is a need to introduce locally available, convenient and innovative measure for those temporary migrant workers. Three rubber plantation villages, Thatkot and Weayet villages as intervention and Kyonekan Village as control areas of Thanbyuzayat, were selected to undertake a quasi-experimental study from November 2010 to June 2011. Fifty, each of migrant rubber plantation night-time workers with no history of malaria previously were recruited from the above areas. Appropriate clothes of 100 workers from Thatkot and Weayet villages were impregnated with deltamethrin (50 mg/meter) bi-monthly. Blood films were taken monthly for six months. The results showed that only one malaria positive case was found in Thatkot Village (intervention area) while there were 4-6\% of monthly malaria positive cases found in Kyonekan Village (non-intervention area) (p<0.05). Spleen positivity was found 24\%, 22\% and 26\% of 2-9 years old school children in Thatkot, Weayet and Kyonekan villages, respectively. Infant parasite rate was none in all areas. More than 90\% of the workers wore deltamethrin impregnated clothes regularly. Nearly all workers (98\%) had willingness to impregnate their cloths regularly. The study revealed that deltamethrin impregnated clothes are very effective (98-100\% protection) to prevent mosquitoes bite and malaria transmission to rubber plantation workers in Mon State.

INTRODUCTION

Malaria, a tropical human disease caused by protozoan parasite belonging to genus plasmodium, is one of the most important infectious diseases in the World. Its global burden and economic cost are still enormous, and it caused about 250 million cases resulting in nearly one million deaths in 2006.\textsuperscript{1} The recent UNHCR report in 2009 estimated the number of migrants to be 15.2 million and 26 million internally displaced people globally.\textsuperscript{2} The prevalence of malaria has not changed significantly yet. Malaria has been the first and second priority publication health problem in Myanmar and there are 600,000 malaria cases annually. Morbidity rate in 2006 was 9.1/1000 and mortality rate was 2.97/100000 population.\textsuperscript{3} The poor are the highest burden of malaria, they are at a higher risk of becoming infected with malaria, because they live in dwellings that offer little protection from mosquitoes yet they may not afford protection methods like insecticide-treated nets (ITNs) from
mosquitoes. In Myanmar, 71% of 55 million populations are residing in malaria-risk areas of various hilly, coastal and plain areas of the country. In developing areas of Myanmar, new agricultural techniques are introduced alongside with increase in employment opportunities.

In this context, temporary migration from other parts of the country is common to improve their livelihood thereby increasing in population mobility. Depending upon the immunity level, local travelers from other parts of the country visiting these endemic areas can contract malaria. Besides, shady habitats in Myanmar are favourable for *Anopheles dirus* close to human dwellings resulting in increased malaria transmission. Well-breeding *Anopheles dirus* are prevalent in Mon State and Tharinthayi Division.

Treatment of mosquito nets with synthetic pyrethroids like permethrin or deltamethrin enhances the protection provided by the mosquito net in various community level settings and is recommended as a malaria control measure. However, it may not be practical for mobile military troops as soldiers from borders and night-time workers at rubber plantation to carry mosquito nets with them. Various protective modifications include impregnation of curtains, treatment of clothes, sheets and temporary shelters with insecticides.

It is well known that one of the elements for successful malaria prevention requires reduction in man-vector contact. Migrant workers often do their work in 3D characteristics (difficulties, dirty and distance) making malaria prevention difficult. In addition, many of the refugees have no source of income and rely almost completely on assistance from government support agencies working in the area. Therefore, the malaria prevention methods used must be appropriate for a migrants’ life style (economics, organization). Normally, migrant populations in rubber plantations do their job at night time from dusk to dawn. This specific time coincides with peak biting of *Anopheles* vectors. Usually, both women and men cover themselves to protect from wind and cold by wearing of cover cloths, hats, jackets, jeans, longyi, shirts and sweaters, etc. There is a need to introduce locally available, convenient and innovative measure for this particular hard-to-reach temporary migrant workers.

Therefore, the study aimed to evaluate the effect of innovative personal protection on malaria transmission among temporary migrant workers in rubber plantation in Thanbyuzayat Township, Mon State, Myanmar.

**MATERIALS AND METHODS**

*Study area*

Three rubber plantation villages: Thatkot and Weayet as intervention areas and Kyonekan as control area, in Thanbyuzayat Township, Mon State from November 2010 to June 2011.

*Study population*

Fifty migrant workers (who were working in rubber plantation), each were recruited from both Thatkot and Weayet villages as intervention areas, and 50 migrant workers from Kyonekan Village as control area.

*Study design*

Quasi-experimental study design was undertaken in both control and intervention areas in Thanbyuzayat Township, Mon State.

*Methodology*

Fifty each of night-time rubber plantation migrant workers who have not history of malaria previously were randomly selected from above intervention and control areas. First, finger prick blood samples were taken from all workers from both areas, at the same time night-time used clothes (wearing clothes; cover cloths, hats, shirts, longyi, jackets, jeans and sweaters, etc.) from volunteers of intervention groups were impregnated with deltamethrin at the rate of 50 mg/meter square. All impregnated materials were dried under shady place. Bimonthly deltamethrin impregnation to...
clothes was done in intervention groups during the study period. The volunteers indicated that they washed their night-time working clothes about 2-3 times a month.

**Finger prick blood collection**

Monthly finger prick blood samples were collected from all volunteers from both control and intervention areas for six months. If volunteers missed to come to the recruited house to give their finger prick blood samples, they were followed up to get blood films.

**Microscopic identification**

Finger prick blood were taken on grease-free-glass slides and thick and thin blood films were made and dried in room temperature. Dried slides were stained with 10% Giemsa’s stain for 10 minutes. After staining, slides were washed with buffer water. Malaria parasites were examined under oil emersion lens with compound microscope.

**Impregnating the clothes in intervention areas**

Clothes (cover cloths, hats, jackets, jeans, longys, shirts and sweaters) of eligible 100 migrant subjects appropriate to use at night-time rubber plantation work were impregnated with deltamethrin at the rate of 50 mg/meter squire by dipping method bimonthly for 6 months carried out by volunteers. All impregnated materials were dried under the shade. In control area, there was not done any control measure.

**Spleen positive rate and infant parasite rate**

Spleen positive rate was examined among 2-9 years old school children from primary school of each village and infant parasite rate was detected under 2 years children in migrant families of the study areas according to the methods of Bruce-Chwatt to determine the malaria endemicity and cause of transmission in study areas.

**Knowledge, attitude and feasibility about malaria, mosquitoes and intervention**

All the volunteer workers were interviewed with same questions about malaria, vectors, control measures, for knowledge, attitude and feasibility of malaria, mosquitoes and intervention. The responses in the questionnaires were compiled, coded, entered in computer using Microsoft Excel and the determinants of malaria, disease occurrence, knowledge were analyzed using Excel software.

**Mosquito susceptibility test**

Mosquito susceptibility test was done with 1, 2, 3, 4 and 5 times washed and non-washed deltamethrin impregnated clothes according to WHO cone test method. Ten wild caught Anopheles mosquitoes were exposed on deltamethrin impregnated clothes surface with the help of cone for 5 minutes. At the end of each exposure time, the mosquitoes were transferred to the paper cup and kept 24 hours with a piece of cotton wool soaked with 10% sugar solution attached to the nylon mesh. Motility counts were made at the end of 24-hour observation period.

**Analysis of data**

Field data were analyzed by using Microsoft EXCEL. Parasite positive rate, spleen positive rate and infant parasite rate were calculated. KAP questioner’s data were calculated by percentage.

**RESULTS**

A total of 150 migrant volunteers participated in the study (Table 1).

Table 1. Details of the study areas

<table>
<thead>
<tr>
<th>Study village</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control)</td>
<td>22</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>(Intervention area)</td>
<td>(18-71)</td>
<td>(18-61)</td>
<td>(18-71)</td>
</tr>
<tr>
<td>(Control)</td>
<td>22</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>(Intervention area)</td>
<td>(19-68)</td>
<td>(18-63)</td>
<td>(18-68)</td>
</tr>
<tr>
<td>(Control)</td>
<td>28</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>(Intervention area)</td>
<td>(18-69)</td>
<td>(18-64)</td>
<td>(18-69)</td>
</tr>
</tbody>
</table>

Yrs = Years

Among the migrant participants, highest population (56) was found from Ayeyawady Region followed by Bago Region (47) and very rare from Chin (Fig.1). In intervention areas only one malaria positive case was found in Thatkot Village in initial month.
(January) of study period and, in Weayet Village, malaria positive case was not observed throughout the study period while there were 4-6% of monthly malaria positive cases in non-intervention area of Kyonekan Village (Fig. 2).

Spleen positive rate was found 24%, 22% and 26% in 2-9 years old school children in Thatkot, Weayet and Kyonekan, respectively. Infant parasite rate was observed zero in all villages. Most of the workers (95% and 99%) wore deltamethrin impregnated cloths regularly and 98% of workers had willingness to impregnate their clothes regularly.

The knowledge about malaria transmission was very high; 86%, 92% and 92% of the migrant workers knew malaria is transmitted by mosquito bite and 54%, 66% and 30% knew the transmission was caused by Anopheles mosquitoes, respectively. They had at least 1-3 mosquito nets in a family, 76, 88 and 66 ordinary mosquito nets and 23, 40 and 42 insecticide treated nets (ITNs) in Thatkot, Weayet and Kyonekan, respectively, and in the resting days, 98-100% of the night-time workers slept in mosquito nets.

Regarding the knowledge of preventive measures, 68-98% of the population made protection from mosquito bite by wearing long dress at night and using mosquito coils and repellents. Over 90% of the plantation workers knew that when malaria transmitted to man, they suffered rising high temperature, chill and rigor. Over 90% of the workers knew to go to clinic when they suffered malaria infection.

Susceptibility status of repeated washed deltamethrin treated cloths found to be 90-100% Anopheles mosquito mortality on 4 times washed impregnated clothes in 5, 10, 15 minutes exposure time (p<0.001) within 24 hours.

**DISCUSSION**

Malaria places an enormous economic burden on affected countries and has a highly detrimental effect on economic and social development. One of the factors contributing to reemergence of malaria is human migration.

In intervention areas, only one malaria case was found in Thatkot Village in initial month (January) of the study and in Weayet Village, malaria positive cases were not observed throughout the study period. Transmission of malaria was found monthly in non-intervention area of Kyonekan Village but in intervention areas of Thatkot and Weayet villages were found to be 98 to 100% recovery from malaria transmission in night-time rubber plantation migrant workers using deltamethrin impregnated clothes. The use of deltamethrin treated curtains and clothes resulted 92% reduction in slide positive rate and 95.5% reduction in malaria cases in Delhi.
Deltamethrin treated nets and icon residual spray were very effective malaria control tools through reducing man vector contact in a study of ten villages in Oktwin Township, and malaria positivity rate and infant parasite were significantly reduced from pre-intervention to post-intervention period. In the present study, spleen positive rate was observed nearly 30% in 2-9 years old children, it meant that the areas are mesoendemic areas and infant parasite rate was 0% so that it indicated that transmission was not occurring in villages. It was an external transmission according to Bruce-Chwatt.

Malaria endemicity was high in forest foot hill area of Oktwin Township, Bago Division. The report of International Organization for Migration (2010) revealed that malaria endimicity of Thanbyuzayat Township was declined from hyperendemicity to mesoendemicity but Bhayathounsu village remained hyperendemicity (unpublished data 2010). The use of insecticide treated clothes reduced both malaria infection rates and indoor mosquito density significantly in Dadaab refugee camp, North Eastern Province of Kenya. The present study found that deltamethrin treated clothes effectively control malaria transmission in migrant rubber workers in Thanbyuzayet Township, Mon State. All migrant workers had 1 to 3 mosquito nets and they slept in net when they rested at home. Over 92% of the workers wore deltamethrin treated clothes regularly and over 95% of the workers wanted to impregnate their clothes regularly. Similar results have been found in army populations of India and Iran and refugee’s population of Dadaab refugee camps, in North Eastern Province of Kenyan.

Susceptibility status of repeated washed deltamethrin treated cloths found to be 90-100% mortality against Anopheles mosquito on 4 times washed impregnated clothes in 5, 10, 15 minutes exposure time (p<0.001). Of the four fabrics: cotton, nylon, polyethylene and jute, cotton was the best on the basis of median lethal dose (LD$_{50}$) and 90% lethal dose (LD$_{90}$) values and persistence of insecticide. The present study revealed that no side effects were observed among the participants from the use of deltamethrin treated clothes throughout the study period. Human toxicity studies of different researchers reported that deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin treated nets and clothes were safe to impregnators and users. Deltamethrin was 3.9 and 4.6 times more effective than lambdacyhalothrin and cyfluthrin, respectively, against Ae. aegypti, Cx quinquefasciatus and Anopheles stephensi. The cost of insecticide treated clothes may be much lower than the cost of ITNs, although insecticide impregnation by self may be much lower than insecticide treated clothes. The cost of deltamethrin treatment was 30.5 kyats per cloth.

All the participants in the treatment groups said the deltamethrin treatment was advantageous. Among the advantages given were that use of the treated cloths reduced the mosquito bites and that other insects like head lice in women were reduced. The workers also said that they were not feeling as followed by mosquitoes densely when they were working in night time after wearing deltamethrin treated cloths. Treated clothing is also effective against blood feeding arthropods, biting flies, ticks, and body lice.

Most of the rubber plantation workers from Thatkot, Weayet and Kyonekan villages had good knowledge about transmission of malaria (86-92%), clinical symptoms of malaria (over 90%), and how to prevent mosquitoes (68-92%), although a study revealed that majority of forest related workers in hard-to-reach areas of PynOoLwin Township expressed the inadequate understanding of malaria transmission and symptoms of malaria. Over 90% of the participants accepted it as cheap, cost effective (30.5 kyats/cloth) and easy malaria control
method for personal protection. Over 98% of the population of the study areas wanted to impregnate their cloths with deltamethrin insecticide. Similar result was found in northeastern Thailand and Orissa, India using permethrin treated military uniforms and cloths for personal protection against malaria.\textsuperscript{31,32}

At the onset of the study, the migrant rubber plantation community was very enthusiastic about the study and everyone accepted the study as cost-effective malaria control method. The deltamethrin treated clothes act quickly and repel or kill biting insects and mosquitoes. They are long-lasting and to some extent withstand weathering, sunlight and washing in cold water. They are more pleasant to use (little or no odor, color or greasiness). They are safe and do not irritate human skin if applied at the correct doses. They do not affect plastic products. They are cheaper than repellents and mosquitoes nets, only infrequent applications of small amounts being required.

\textit{Conclusion & Recommendation}

This strategy be considered for the use in the control of malaria and nuisance caused by mosquito bites in vulnerable communities like night time plantation workers, night time migrant workers, and poor communities living in malaria endemic areas as poor people and migrant families are less likely to afford ITNs. Insecticide treated cloths could complement the ITNs.

The study also recommended that the migrant communities especially during influx of migrants to malaria prone region as some migrants may not have immunity to malaria. The use of deltamethrin insecticide-treated clothes prevent malaria transmission and has potential as an appropriate method for malaria control among night-time migrant workers and plantation workers in malaria endemic areas.

This study showed that deltamethrin insecticide treated clothes are protective against malaria among night-time rubber plantation workers who were contacted with high density of vector mosquitoes in working hours. Clothing treated with deltamethrin can remain toxic to insects and mosquitoes for several weeks or months, depending on wear and exposed to washing and rain. Treated clothing remains effective after up to 10 rinses with cold water and soap.

\textbf{REFERENCES}


Cardiovascular Responses in Upper and Lower Extremities Exercises

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Department of Sports and Physical Education

This randomized controlled study was done to determine the cardiovascular responses in upper (arm) and lower (leg) extremities exercises. The study was conducted on thirty male soccer players, aged from 16 to 20 years from the Institute of Sports and Physical Education, Yangon. The subjects participated in both arm cranking and leg cycling tests at three different workloads (25±1, 50±1 and 75±1 watts) with bicycle ergometer on different days in the same environmental conditions. The heart rate and blood pressure of the subjects were determined before, during and after the exercise. The significant increases in heart rate from 61.2±9.1 beats.min⁻¹ to 89.3±10 beats.min⁻¹ at load I to 108.9±14 beats.min⁻¹ at load II and to 136.2±19 beats.min⁻¹ at load III (p<0.001) in arm exercise and from 62.3±8 beats.min⁻¹ to 80.6±8.2 beats.min⁻¹ at load II and to 105.3±9.3 beats.min⁻¹ at load III (p<0.001) in leg exercise were noted. Systolic blood pressure increased significantly from basal level of 104.3±7.2 mmHg to 112.5±7.2 mmHg at load I to 127.3±7.4 mmHg at load II and to 135.1±8.3 mmHg at load III (p<0.001) in arm exercise and from basal level 103.0±7.5 mmHg to 110.3±8.9 mmHg at load I, to 114.0±21.4 mmHg at load II and to 130.3±11.2 mmHg at load III (p<0.01) in leg exercise. The increment in heart rate due to arm exercise was significantly greater than that due to leg exercise (28.1±8.5 beats.min⁻¹ vs. 18.3±6 beats.min⁻¹ at load I, 47.7±12.4 beats.min⁻¹ vs. 29.8±7.0 beats.min⁻¹ at load II and 75.0±17.3 beats.min⁻¹ vs. 43.0±8.9 beats.min⁻¹ at load III, respectively). The study clearly pointed out that upper extremity exercise elicited greater cardiovascular response than lower extremity exercise.

INTRODUCTION

Exercise is synonymous with physical activity. Although exercise is defined ultimately in terms of muscular contraction, exercise affects every organ in the body. Furthermore, different forms of exercise evoke quite different responses in various organ systems. These responses depend on whether exercise involves small or large muscle mass, rhythmic or isometric, acute or chronic (training), intense or mild and of long or short duration.

Cardiorespiratory fitness was defined as "the ability to continue or persist in strenuous task involving large muscle groups for an extended period of time."¹ By attaining aerobic fitness, the circulatory and respiratory systems are able to adjust quickly to and recover from moderate to vigorous activities, such as running, swimming, cycling and brisk walking. Cardio-respiratory fitness also offers protection from a myriad of health disorders, including cardiovascular disease, stroke, hypertension, diabetes mellitus and obesity.²

The role of circulatory system in exercise is to augment the delivery of metabolic substrates and oxygen required for ATP generation. Continuous removal of the
Carbon dioxide and hydrogen ions generated by aerobic and anaerobic metabolism also is necessary to maintain an intracellular pH for muscle contraction and glycolytic enzyme functions. These are achieved by increasing cardiac output and altering the distribution of blood flow to various vascular beds. They also revealed that in the performance of dynamic exercise, cardiac output increases from resting level of 4-6 L/min to maximal levels as high as 36 L/min in well-trained athletes.

The difference in circulatory response to arm and leg exercises was pointed out by Christensen in 1931. In larger groups of subjects the higher heart rate in arm work than in leg work at a given level of oxygen uptake and the lower mechanical efficiency in arm work have been documented since then. Upper body exercise is common and is often a predominant activity for many individuals.

For example, wheelchair users use the upper body musculature for locomotion. In addition, within the past several years, arm-crank exercise that predominantly activates the upper body musculature has been recommended for the rehabilitation of individuals who have suffered a myocardial infarction or undergone bypass surgery.

Upper body exercise however, causes a greater strain on the cardiovascular system compared to exercise with the lower body. A greater maximal oxygen uptake (VO$_2$ max) response is found at a given constant power output utilizing an arm crank than leg cycling. Upper extremity exercise also elicits higher systolic and mean arterial blood pressures, equal or lower cardiac output and lower stroke volume at a given sub-maximal power output as compared to lower extremity exercise.

At a given sub-maximal workload, arm exercise is performed at a greater physiological cost than leg exercise. At a given power output heart rate, systolic and diastolic pressure, oxygen consumption, respiratory exchange ratio and blood lactate concentration are higher while stroke volume and anaerobic threshold are lower during leg exercise. Sub-maximum arm exercise produced higher heart rate, blood pressure and pulmonary ventilation than comparable intensities of leg exercise.

By understanding the differences in physiological response between arm and leg exercises, professionals can formulate exercise programmes for specific diagnosis and/or training. Thus, the present study aimed at determining cardiovascular responses in the same person for exercises of upper and lower extremities.

**MATERIALS AND METHODS**

Thirty apparently healthy male soccer players aged from 16 to 20 years were recruited randomly from Institute of Sports and Physical Education, Yangon. The subjects underwent medical examination including history taking, physical examination and ECG recording. Those with acute illness and ECG abnormalities were excluded from the study.

**Experimental procedure**

Written informed consent was obtained from each subject after explaining about the procedure. The experiment was performed at 08:00 a.m. The subjects were asked to wear shorts and short sweat shirt in order to have free movement when they were exercising. They were instructed not to make any other physical activity on that day and not to take coffee or tea for 2 hours before the test.

All subjects, participated in both arm cranking and leg cycling tests, performed 3 loads of discontinuous test on different days. Monark bicycle-ergometer was used for both arm cranking and leg cycling. All studies were performed in a comfortable laboratory environment with a mean temperature of 21°C and within the range of 18-23°C.

Each test was preceded by a 10-minute rest in seated position, after which baseline
measurements of heart rate and blood pressure were made. Three workloads selected were 25±1 watts as the first load, 50±1 watts as the second load, and 75±1 watts as the third load for all subjects. For an arm cranking, the bicycle-ergometer was placed and secured on the table. The subject sat in front and the axle was placed at shoulder level. The position was adjusted to be the same for all subjects.

Then, the subject was instructed to crank 50 rpm and the workload was raised to 0.5 kg (25 watts). The subject worked for 3 minutes. During this period, the heart rate was recorded every minute with polar heart rate monitor. Blood pressure was measured immediately at the end of 3 minutes and the subject was allowed to take rest for 5 minutes.

Then, the subject was asked to crank again at 5 rpm and workload was raised to 1 kg (50 watts). The subject worked for 3 minutes, and heart rate was recorded every minute with polar heart rate monitor. Blood pressure was measured immediately at the end of 3 minutes and the subject was allowed to rest for 5 minutes.

The same procedure was repeated again with the workload of 1.5 kg as 3rd workload (75 watts). From beginning to end of the experiment, the subject was not allowed to take food and water.

For leg cycling exercise, appropriate seat, height was selected for each subject and position was standardized. The same workload and the procedure were followed as in arm cranking, but on a separate day.

Heart rate was measured by Polar Heart Rate Monitor. Transmitter was wrapped around the chest at nipple line and receiver was attached to the wrist. Heart rate was directly read as digital number from screen of receiver. The instrument was standardized with the measurement of the radial pulse by palpation method for sixty seconds. Blood pressure was measured over the right brachial artery using mercury sphygmomanometer according to WHO method (1978).

RESULTS

Table 1 shows general characteristics of the subjects. Mean age, height and weight of the subjects were more or less similar. Cardiovascular status such as resting heart rate, systolic and diastolic blood pressure were more or less comparable.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Test group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>17.43±1.25</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.91±6.65</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.27±4.37</td>
</tr>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>61.20±9.17</td>
</tr>
<tr>
<td>Blood pressure (mmHg) SBP</td>
<td>104.3±7.27</td>
</tr>
<tr>
<td></td>
<td>DBP</td>
</tr>
<tr>
<td>SBP=Systolic blood pressure</td>
<td></td>
</tr>
<tr>
<td>DBP=Diastolic blood pressure</td>
<td></td>
</tr>
<tr>
<td>n=Number of subjects</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows cardiovascular responses to arm and leg exercises. The heart rate and the systolic blood pressure increased significantly from basal level in all 3 workloads in both arm and leg exercises.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Test group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
</tr>
<tr>
<td>Arm 61.2±9.1</td>
<td>89.3±9.7***</td>
</tr>
<tr>
<td>Leg 62.3±7.9</td>
<td>80.5±8.2***</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Arm 104.3±7.2</td>
<td>112.5±7.2***</td>
</tr>
<tr>
<td>Leg 103.0±7.4</td>
<td>110.3±8.8***</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Arm 68.0±5.5</td>
<td>70.6±6.5***</td>
</tr>
<tr>
<td>Leg 68.6±5.0</td>
<td>71.6±5.9*</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Arm 36.3±6.6</td>
<td>41.8±9.1***</td>
</tr>
<tr>
<td>Leg 34.3±5.6</td>
<td>38.6±8.6**</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Arm 80.1±5.3</td>
<td>84.6±5.3***</td>
</tr>
<tr>
<td>Leg 80.1±5.4</td>
<td>84.2±5.9***</td>
</tr>
<tr>
<td>HR=Heart rate, SBP=Systolic blood pressure</td>
<td></td>
</tr>
<tr>
<td>DBP=Diastolic blood pressure, PP=Pulse pressure</td>
<td></td>
</tr>
<tr>
<td>MAP=Mean arterial pressure</td>
<td></td>
</tr>
<tr>
<td>p&lt;0.05 means statistically significant</td>
<td></td>
</tr>
<tr>
<td>*indicates p&lt;0.05 from baseline value</td>
<td></td>
</tr>
<tr>
<td>**indicates p&lt;0.01 from baseline value</td>
<td></td>
</tr>
<tr>
<td>***indicates p&lt;0.001 from baseline value</td>
<td></td>
</tr>
<tr>
<td>****indicates p&gt;0.05 from baseline value</td>
<td></td>
</tr>
</tbody>
</table>
Although the diastolic blood pressure increased significantly in the 1st workload of leg exercise, that of arm exercise was not significantly increased. The diastolic blood pressure was not significantly increased in the 2nd and the 3rd workloads of both arm and leg exercises. In the case of pulse pressure, changes were significant in both arm and leg exercises. Mean arterial pressure changes were statistically significant in all 3 workloads of both arm and leg exercises.

Heart rate changes of both arm and leg exercises showed significant increase from basal level. The greater the increment of the workload, the larger the changes in the heart rate were noted. The increment in the heart rate due to arm exercise was significantly greater than that of leg exercise.

A significant increase in the systolic blood pressure was observed in all 3 work sessions in both arm and leg exercises. In addition, the change of the systolic blood pressure due to arm exercise after the 2nd workload was found to be significantly greater than that of leg exercise.

There were no significant changes in the diastolic blood pressure in all 3 work sessions of arm exercise. In leg exercise, a significant increase in the diastolic blood pressure in the 1st workload was observed but no significant changes of diastolic blood pressure were found in the 2nd and the 3rd workloads. There were no significant differences in the diastolic blood pressure changes between arm and leg exercises.

**DISCUSSION**

In this study, the heart rate increased in all 3 workloads of both arm and leg exercises; the greater the increment of the workload, the larger the changes in the heart rate. The heart rate initially increased at any given workload and if the exercise was sustained, it remained more or less steady at the raised values. When this steady state, value for the heart rate was measured at several workloads, interrupted by rest periods, there was a linear relation between the heart rate and given workload. The heart rate increment due to arm exercise was significantly greater than that of leg exercise. These findings were in agreement with previous observations.

They explained that exercise with the arm evoked higher heart rate than the leg exercise at a given workload. This could be due to the increased sympathetic tone resulting from static contraction of the muscles which stabilize posture. However, the differences in physiological variables remained between arm and leg exercises even when the light workloads were used to minimize the effect of the static component during arm exercises.

In the present study, the same workload was given in both arm and leg exercises. The legs have greater muscle mass than arms, so that at the same level of comparable load, arm exercise may induce much higher metabolic workload as well as muscle spindle activity and consequently stimulation of chemoreceptors and mechoreceptors than does leg exercise. The lactate concentration in arterial blood was higher during arm work, reflecting a more pronounced metabolic acidosis, and evoked higher pulmonary ventilation, which in turn had an effect on heart rate and cardiac output.

During arm cranking, the afterload on the heart is greater than that of leg cycling because area of vascular bed dilation is lesser than that of constriction in arm cranking leading to increased total peripheral resistance. In addition, during arm cranking the venous return is less facilitated which may result in relatively low stroke volume.

During upper extremity exercise, increased sympathetic output elevates the peripheral resistance and heart rate. An elevated heart rate may maintain a high level of cardiac output. The mean arterial pressure (MAP) was increased with increased work load. The changes were statistically significant in both arm leg exercises.

There was significant increase in the systolic blood pressure (SBP) observed in all 3 work sessions in both arm and leg exercises.
Increased systolic blood pressure generally reflects increased cardiac output. The increase in cardiac output is due to an increase in heart rate or stroke volume or both. In the present study, increased heart rate in both arm and leg exercises were noted. There might also be increased stroke volume because arm cranking and leg cycling exercise were isotonic in nature and the heart rate was below 180 beats/min. Increase in stroke volume contributes to increase in cardiac output up to 40-45% of VO$_2$ max. Further increases in cardiac output were due to increase in the heart rate.\textsuperscript{18}

In both arm and leg exercises, the diastolic blood pressure increased in the 1\textsuperscript{st} workload but decreased in the 2\textsuperscript{nd} and the 3\textsuperscript{rd} workloads. The diastolic blood pressure generally reflects the total peripheral resistance. There was a generalized sympathetically mediated vasoconstriction in exercise that is opposed in active tissue by a metabolically induced vasodilatation.\textsuperscript{19} This can be observed in the changes of diastolic blood pressure in this study. At the 1\textsuperscript{st} workload, it increased probably because of a generalized vasoconstriction of exercise stress but when exercise was continued with the 2\textsuperscript{nd} and the 3\textsuperscript{rd} workloads, decrease in the diastolic blood pressure was noted probably due to accumulation of vasodilator metabolites in the active muscle.

The changes in mean arterial pressure of arm exercise were greater than those of leg exercise although statistically significant change was found only at the 2\textsuperscript{nd} workload. This finding was in agreement with the previous studies.\textsuperscript{20} They described that at a given maximal oxygen uptake, the systolic and diastolic blood pressures are considerably higher when work is performed with upper extremities than that with the lower extremities.\textsuperscript{20} Another study stated that with exercise, the resistant vessels dilate in the active muscles by a local mechanism, and constriction in the inactive muscles was due to an increase in sympathetic vasoconstrictor activity.\textsuperscript{21} It is, therefore, possible that a higher arterial pressure will ensure if the work is performed with small muscle groups when only a small part of the vascular bed dilates compared to the large remaining vascular bed in which a constriction of the resistance vessels will occur.\textsuperscript{21} On the other hand, the higher blood pressure for a given oxygen uptake during arm exercise compared to leg exercise indicates a higher sympathetic outflow.\textsuperscript{15}

The changes of systolic blood pressure due to arm exercise after the 2\textsuperscript{nd} workload was found to be significantly greater than that of leg exercise. Although there were not statistically significant differences of systolic blood pressure after the 1\textsuperscript{st} and the 3\textsuperscript{rd} workloads, exercise indicated a higher sympathetic outflow.

The changes of systolic blood pressure in arm exercise after the 2\textsuperscript{nd} workload was found to be significantly greater than that of leg exercise. Although there were not statistically significant differences of systolic blood pressure after the 1\textsuperscript{st} and the 3\textsuperscript{rd} workloads, systolic blood pressure in arm exercise was found to be greater than that of leg exercise. When compared between arm and leg exercises, the increment of the heart rate was significantly greater in arm exercise, so systolic blood pressure in arm exercise was also greater than that in leg exercise.

**Conclusion**

Heart rate increased significantly from the basal level in both arm and leg exercise. The greater the increment of the workload, the larger the changes in heart rate were noted. The increment in heart rate changes due to arm exercise was significantly greater than that of leg exercise.

A significant increase in systolic blood pressure, pulse pressure and mean arterial blood pressure was observed in all 3 work sessions in both arm and leg exercises. There was no significant change in diastolic blood pressure in all three work sessions of arm exercise. In leg exercise, a significant increase in diastolic blood pressure was observed in the 1\textsuperscript{st} workload but not in the 2\textsuperscript{nd} and the 3\textsuperscript{rd} workloads. There were no
significant differences in diastolic blood pressure changes between arm and leg exercises.

Recommendations

Since the study in agreement with previous studies, clearly pointed out that upper extremity exercise elicited greater cardiovascular response than lower extremity exercise;

- Exercise regimen prescribed to patient with ischaemic heart diseases should be based on leg exercise rather than arm exercise.
- In cases of lower body injuries requiring prolonged rest, arm exercise should be prescribed to maintain cardiovascular fitness of the patients. This is very important for athletes.

REFERENCES

A Laboratory Investigation on Oviposition Response of Aedes aegypti to Larvicide Treated Domestic Water

Sai Zaw Min Oo, Pe Than Htun, Thaung Hlaing, Sein Thaung, Khin Myo Aye, Yi Yi Myint & Thuzar Nyein Mu

Medical Entomology Research Division Department of Medical Research (Lower Myanmar)

Investigation on oviposition response of dengue vector Aedes aegypti to larvicides was conducted in the laboratory. Larvicides used in ovitraps were Abate (temophos 1% sand granules), Bti (Bacillus thuringensis israelensis) and pyriproxyfen (insect growth regulator). Laboratory-reared Aedes aegypti female gravid mosquitoes were exposed to larvicide-treated ovitraps and equal numbers of control ovitraps in order to lay eggs. Oviposition response was measured by oviposition activity index (OAI) in terms of counting the number of eggs from each treated ovitrap and compared with non-treated control group. A total of four hundred female mosquitoes were used in this experiment and the mean number of eggs laid per mosquito was 52.6±19.18 eggs and there were no survival of larvae and pupae from treated ovitraps. OAI for Abate, Bti and pyriproxyfen were observed as -0.03, +0.23 and -0.21, respectively. There were no significant differences detected among larvicides treatments (p=0.08 to 0.85). According to oviposition activity index, OAI of +0.3 and above are considered as attractants and -0.3 and below were considered as deterrents. The number of eggs laid varied depending on types of water as mosquito has visual and olfactory cues to assess water before laying eggs. In conclusion, commercially available and currently used larvicides Abate, Bti and pyriproxyfen have neither oviposition attractant nor oviposition deterrent property. Hence, all these tested larvicides had no influence on egg laying behavior of dengue vector Aedes aegypti and can be used for DHF vector control programme.

INTRODUCTION

Dengue fever is one of the most prevalent vector-borne diseases in the world and poses a heavy economic cost to health systems and societies. Depending on geographical and climatic conditions, spread and incidence as well as severity of dengue fever (DF) and dengue hemorrhagic fever (DHF) are increasing in tropical and subtropical regions including Southeast Asia. In Myanmar, dengue is one of the major public health problems causing high morbidity and mortality especially in children. The first case of DHF was reported in 1969 and the first epidemic followed in 1970 in the capital Yangon where 1651 cases with 90 deaths were reported. The morbidity and mortality of DHF in Myanmar 2009 were 24285 and 181, respectively. The main vector, Aedes aegypti, usually breeds in domestic water containers around human dwellings. Oviposition (laying eggs) is an important component of most mosquito-borne diseases and selection of oviposition sites by gravid female mosquitoes is a crucial event for the survival of the species. This container inhabiting mosquito species is known to follow visual or olfactory cues to appropriate water containers and then use both chemical and physical factors in the water before making decision to lay their eggs and then selecting it for oviposition. Depending on the quality and infusion substances of water, oviposition attractants
or deterrents (avoidance) can be present at breeding sites. Over many years, dengue control has mainly been based on the reduction of breeding sites and chemical control targeting on immature stages and adult stage due to the lack of vaccine for dengue.\(^5\)

In Myanmar, especially in Yangon and Ayeyawady Regions, wide-scale applications of larvicide (especially Abate) have been carried out after Cyclone Nargis in 2008.\(^6\) Currently available and used larvicides in Myanmar are:

(i) Chemical larvicide Abate (temephos 1% sand granules)
(ii) Bio-larvicide Bti (Bacillus thuringiensis israelensis) and,
(iii) Insect growth regulator pyriproxyfen

This study aimed to learn about the possible influence of these larvicides on the egg laying behavior of dengue vector Aedes aegypti and to support the effectiveness of further vector control activities in the programme.

**MATERIALS AND METHODS**

**Study site**

This study was conducted in the laboratory of Medical Entomology Research Division, Department of Medical Research (Lower Myanmar).

**Mosquito**

*Aedes aegypti* mosquitoes required for this experiment were obtained from the Insectary, Medical Entomology Research Division. *Ae. aegypti* colonies were maintained under controlled temperature and relative humidity at 25°C to 30°C and 80% to 85%, respectively. Mosquitoes were fed on 10% glucose-soaked cotton pads and later females were fed on laboratory white mice for blood meal and allowed to develop eggs and to become gravid.

**Oviposition activity bioassay**

Laboratory glass containers (250 ml) were used as ovitraps to attract female mosquitoes for laying eggs and inner surface of the container was covered by filter paper to collect eggs. Two hundred milliliters of domestic water was filled in each ovitrap. Then, Abate (temephos 1% sand granules), Bti (Bacillus thuringiensis israelensis) and pyriproxyfen (insect growth regulator) were added in concentration of 1 gm temephos/10 L, 2 gm Bti/L and 2 mg pyriproxyfen/10 L according to the WHO standard doses in each respective ovitrap.\(^7,8\)

For control group, only domestic water (water source from DMR-LM) was provided. These different larvicide-treated ovitraps and equal number of control ovitraps were placed in the rounded mosquito bed net by placing randomly to avoid positional bias. Thereafter, blood-fed gravid female mosquitoes were released in the bed net and exposed to ovitraps in order to lay eggs.

All ovitraps were collected one day after exposure to gravid female mosquitoes in the bed net. Then, filter papers from all ovitraps were removed and the number of eggs from the filter paper of each ovitrap was counted. And then, all the filter papers were placed back to the respective ovitraps and numbers of adult mosquitoes emerged from each larvicide-treated ovitrap and control ovitrap were observed and recorded. For one experiment, 16 ovitraps and 40 gravid female mosquitoes were used at the same time and this experiment was replicated ten times. Hence, in total, 400 gravid female mosquitoes and 160 ovitraps were used throughout the experiment.

**Data and Statistical Analysis**

Oviposition Activity Index (OAI) and Student ‘t’ test were used to analyze and determine the data. OAI means in terms of counting the number of eggs from each treated ovitrap and compared with non-treated control group.\(^9\) According to these authors, OAI of +0.3 and above are considered as attractants and -0.3 and below are considered as deterrents. Microsoft Excel was applied for data entry.
Oviposition Activity Index (OAI) = \frac{\text{NT} - \text{NC}}{\text{NT} + \text{NC}}

\text{NT} = \text{Number of eggs laid in treated water container}
\text{NC} = \text{Number of eggs laid in control water container}

**RESULT AND DISCUSSION**

This experiment was conducted from April to October 2011 and a total of 400 gravid female mosquitoes were used to lay eggs in the laboratory controlled temperature and relative humidity. Number of eggs laid per each experiment was not concerned with different seasons and mean numbers of eggs per mosquito was 52.6±19.18 (Table 1).

<table>
<thead>
<tr>
<th>Total no. of mosquitoes used</th>
<th>Total no. of eggs</th>
<th>Mean no. of eggs per mosquito</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>21059</td>
<td>52.6±19.18</td>
</tr>
</tbody>
</table>

This experiment also showed the larvicidal action of all three larvicides according to their WHO recommended doses. There were no survival of larvae and pupae from all larvicides treated ovitraps but there was 95% survival rate from non-treated ovitraps (Table 2). It shows the doses used for this experiment were totally effective to all larva and pupae.

Table 2. Total number of survived mosquitoes from different treatments and different total number of eggs per ovitrap

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total no. of eggs per ovitrap</th>
<th>Total no. of survived mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abate</td>
<td>4763</td>
<td>0</td>
</tr>
<tr>
<td>Bti</td>
<td>7974</td>
<td>0</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>3289</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>5033</td>
<td>4781(95%)</td>
</tr>
</tbody>
</table>

Differential oviposition activities of *Aedes aegypti* mosquito for three larvicides were measured by comparing the mean numbers of eggs per each treated ovitrap and control ovitrap. The comparison of different mean number of eggs per each treatment is shown in Table 3.

Mean±SD of number of eggs for Abate, Bti and pyriproxyfen were 119±82, 217±130 and 82.2±48.2, respectively, and the control mean±SD was 126±87. Mean number of eggs in Abate treated ovitrap was close to the mean number of eggs in control ovitrap. On the other hand, mean numbers of eggs in Bti and pyriproxyfen ovitraps varied from the mean number of eggs in control ovitrap. However, there were no significant differences of mean number of eggs between larvicide-treated ovitraps and control ovitrap (p value ranged from 0.08 to 0.85).

![Fig. 1. Oviposition Activity Indices (OAI) of Abate, Bti and pyriproxyfen deviating from control](image)

Furthermore, according to oviposition activity index, there were no OAI of less than -0.3 and greater than +0.3 (Fig. 1). Therefore, all these larvicides have neither oviposition deterrent nor attractant property.

If oviposition attractant and deterrent properties of *Aedes* mosquito exist in all these tested larvicides, vector control...
activities using these larvicides should be concerned about their properties. If attractant larvicides are used, doses of larvicides should be applied according to the manufacturers’ field recommended doses in order to prevent the occurrence of tolerance and resistance of mosquitoes to these larvicides. If deterrent larvicides are used, every possible breeding source for mosquitoes should be applied. If not, mosquitoes can avoid that larvicides-treated water and can choose the non-treated sources. Therefore, detection of oviposition response of mosquitoes is important for successive vector control programme.

In summary, this study showed that there were no evidences that the oviposition (eggs laying behavior) of *Aedes aegypti* female mosquitoes are influenced by the currently available and commercially used larvicides. Therefore, all these tested larvicides can be safely used in vector control programme as all these larvicides do not affect the bionomics of the mosquitoes.

REFERENCES


SHORT REPORT

Evaluation of a Microarray Nanotechnology-based Test for Diagnosis of Tuberculosis

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Department of Medical Research (Lower Myanmar)
2 National Tuberculosis Program, Myanmar
3 Olipro Biotechnology Sdn. Bhd., Malaysia

Active tuberculosis (TB) is diagnosed by detecting Mycobacterium tuberculosis bacilli in specimens from the respiratory tract or in specimens from other body sites. Although acid-fast bacilli (AFB) microscopy and culture using Lowenstein-Jensen media are still the routine conventional standard methods to diagnose TB, there are many extra-pulmonary, paediatric or paucibacillary TB cases that are difficult to diagnose by these routine conventional methods. Thus, the new rapid diagnostic methods play an important role and their uses are needed to be evaluated in clinical settings.

Biochips are analytical devices which can perform specific binding between microarray target element (substrate) and probe molecules (solution) and produce microscopic target elements spots.1 TB Protein Biochip test is a multi-antigenic assay which is made up of nitrocellulose filter membrane immobilized with specific M. tuberculosis antigenic markers namely lipoarabinomannan (LAM), polyclonal 38 KDa, polyclonal 16 KDa, Early Secretory Antigenic Target (ESAT) 6 and Culture Filtrate Protein (CFP) 10 by microarray nanotechnology. It can detect IgG antibody to coding antigens of M. tuberculosis in the sample of human serum and provided rapid qualitative diagnosis of TB infection.

This study was carried out to evaluate the validity of TB Protein Biochip test for diagnosis of tuberculosis in clinical setting and to determine the presence and relevance of TB specific antigenic protein markers among TB patients in Myanmar. Sputum and blood specimens were collected from new pulmonary TB patients (aged ≥12 years) attending TB Center, Yangon Division during 2010-2011. Two sputum specimens were inoculated onto Lowenstein-Jensen (LJ) culture media and incubated at 37°C for 6-8 weeks at National TB Reference Laboratory, National TB program, Myanmar. Mycobacterium tuberculosis isolates were identified by its growth rate and pigmentation as described in Bacteriological Methods in Laboratory Diagnosis of Tuberculosis by Tuberculosis Research Centre (ICMR) Chetput, Chennai.2

The serum specimens were tested by TB Protein Biochip Diagnostic Kit (Olipro Biotechnology Sdn. Bhd.) to detect IgG antibody to Mycobacterium tuberculosis antigens at Bacteriology Research Division, Department of Medical Research (Lower Myanmar). The reacted chip was read under Olipro Scanner and the result was analyzed using the software.

This study was approved by the Ethical Committee on Medical Research Involving Human Subjects, Department of Medical
Research (Lower Myanmar). TB protein Biochip test kits, Olipro scanner and technical assistance were provided by Olipro Biotechnology Sdn. Bhd., Malaysia.

The results of TB Protein Biochip test were compared with those of *M. tuberculosis* cultures which were used as gold standard. The new cut-off value was calculated based on the Myanmar healthy group of sample. The biochip results of 527 serum samples (244 TB positive and 283 TB negative) were determined by the 1.5 SD cut-off values. The new cut-off values among Myanmar population were calculated as 10.43545, 5.49000, 3.1617, 3.6286 and 2.9495 for LAM, 38 KDa, 16 KDa, ESAT-6 and CFP-10 antigens, respectively.

The study population showed reactivity towards all five antigenic markers (LAM, 38 KDa, 16 KDa, ESAT-6 and CFP-10) at a different rate individually. Among these antigenic markers, the clinical samples showed more reactivity to the LAM antigen as this antigen is a major glycolipid component of present on the cell wall of *M. tuberculosis*.

The sensitivity, specificity, positive predictive value and negative predictive value of TB Protein Biochip test were 60.25%, 67.84%, 62% and 66.44%, respectively (Table 1). False negative results could be due to immune compromised cases or those whom immune system that do not respond well to the mycobacterium antigen panel and false positive result could be due to latent TB or extrapulmonary TB.

This study indicated the presence of tested five antigenic markers among Myanmar TB population besides its relevance in detecting or screening of TB patients from healthy individuals among the Myanmar population. Moreover, it also suggested the inclusion of new markers/antigens which will increase the sensitivity of the tested in-house TB protein Biochip.

### Table 1. Sensitivity and specificity of TB Protein Biochip test

<table>
<thead>
<tr>
<th>Gold standard</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB protein biochip</td>
<td>147</td>
<td>91</td>
<td>238</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>97</td>
<td>192</td>
<td>289</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>283</td>
<td>527</td>
</tr>
</tbody>
</table>

Sensitivity=60.25%, Specificity=67.84%
Diagnostic index=28%
Positive predictive value=62%
Negative predictive value=66.44%

### REFERENCES

NOTICE TO CONTRIBUTORS

The Myanmar Health Sciences Research Journal publishes original articles, review articles, short reports and correspondences in the field of biomedical and health sciences. All scientific papers are reviewed by referees with expertise in the field of research work related to the paper.

**Original Articles**
These should be headed with the title, the names of the authors (not more than 9), and the address(es) where the work was done. They should be accompanied by an abstract of not more than 250 words, which will precede the main text of the paper and should convey its scope. The articles are usually divided into Introduction, Materials and Methods, Results, Discussion, Acknowledgement and References. In principle, an original article should not exceed 3500 words (i.e., 5 printed pages) including the abstract, tables, figures and references.

**Review Articles**
These should largely cover the current review concerning the medical and health research publications pertaining to Myanmar. They should not be more than 5000 words.

**Short Reports**
These should be similarly headed, but do not need an abstract nor divisions into sections. They should not be longer than one printed page or 1000 words, including references (not more than 5) and one illustration or figure if necessary.

**Correspondences**
Letters to the Editor on general topics of interest related to health, comments on papers published in this Journal, and, if appropriate, replies to comments are welcome. Letters should generally not exceed 350 words; tables and figures are not accepted. References (not more than 2) may be given only if essential.

**Submission of Manuscripts**
Manuscripts should be sent through the respective Directors General. They are also advised to send an advance copy in duplicate (i.e. the original with one good copy) to: The Editor, The Myanmar Health Sciences Research Journal, Department of Medical Research (Lower Myanmar), No. 5, Ziwaka Road, Dagon Township, Yangon 11191, Myanmar.

A letter signed by all authors that it is submitted solely to this Journal must accompany manuscripts. They should be typewritten on one side only with double-spacing throughout (including references) and liberal margins. Contributions must be written in English clearly and concisely.

Illustrations (photographs, drawings, graphs, tables) should not be more than 4. Tables and figures should each be numbered consecutively. Line drawings should be of high resolution and high contrast. Black-and-white or color photographs may be accepted in high quality. They should be provided as computer graphic files after the manuscript is accepted for publication in the final form. Details of results presented in this way should not be repeated in the text.

**References**
Reference should be cited as outlined by International Committee of Medical Journal Editors. The number of references should be kept to a minimum; only those that are indispensable to substantiate a statement or as sources of further information should be included. Citation of published literature in the text should be numbered consecutively in the order in which they are first mentioned in the text.

The following form may be used:
- Dengue is a major public health problem in tropical and subtropical countries.\(^1,2\)

All published work referred to should be listed in numerical order at the end of the text on a separate sheet.

References to articles should contain the following: name(s) and initial(s) of author(s), title of the article, full name of the journal, year of publication, volume no., first and last pages. Examples:-


(c) **Book**: Thaw Zin. Role of Analytical Toxicology Laboratory in the prevention, control and management of poisoning: Principle and guidelines. In: *Guidelines on Poison Prevention, Control and Management*. Department of Medical Research (Lower Myanmar), Ministry of Health, Dec 2003; 76-98.


Acceptance and publication of paper will be expedited if these instructions are carefully followed.