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Training and utilization of midwives for rural health services in Myanmar

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One of the key categories of basic health staff for rural health services in Myanmar is a midwife. In this review paper, the history of emergence and further development of midwives since British regime of pre-war days was traced and the important role played by midwives in Myanmar health care system currently is underlined. Without touching in-depth upon the curriculum of midwifery courses, the relevant training programme of midwives preparing for their services is explained. The evolution of a midwife from an accoucher to a “multi-purpose health worker” is also followed through. In this review, the authors attempted to look into changes that took place, in different historical contexts, in training and utilization of midwives for rural health services in Myanmar. It was observed that whenever the government health policy took into serious consideration for improving health of rural people, the role of a midwife became critical and changes in their roles took place. Inherent with these changes were the existence of some gaps in training and service utilization of midwives.

INTRODUCTION

A midwife is internationally defined as a person who, having been regularly admitted to a midwifery educational programme, duly recognized in the country in which it is located, has successfully completed the prescribed course of studies in midwifery and has acquired the requisite qualification to be registered and/or legally licensed to practice midwifery [1].

She must be able to:
• give the necessary supervision, care and advice to women during pregnancy, labour and the postpartum period; and
• conduct deliveries on her own responsibility and to care for the newborn and the infant.

This care includes preventative measures such as the detection of abnormal conditions in mother and child, the procurement of medical assistance and the execution of emergency measures in the absence of medical help. She has an important task in health counseling and education, not only for the women, but also within the family and the community. The work should involve antenatal education and preparation for parenthood and extends to certain areas of gynaecology, family planning and child care. She may practice in hospitals, clinics, health units, domiciliary conditions or in any other service.

Rural Health Sub-Center (RHSC), manned by a midwife, is the most peripheral frontline health facility located deep in the rural community in Myanmar. Midwives are posted at 1452 Rural Health Centers (RHCs) and 5,804 RHSCs all over the country. Midwife/rural population ratio: 2.89/10,000 rural population.

“Midwifery” as defined in Nursing and Midwifery Act 1990 emphasized on three key practices: antenatal care, safe delivery, and post-delivery care including care of the newly born baby. A “midwife” is referred to
a person who has been trained to perform
midwifery and given a license to practice
midwifery. However, the current duties
and responsibilities of a midwife are not
confined only to the three practices men-
tioned. The followings are also included:
- environmental sanitation;
- providing health education and birth
  spacing services;
- providing immunizations to mothers and
  children;
- participation in disease control activities;
- participation in nutrition promotion
  activities;
- providing curative care at health centers;
- supervising health volunteers, particularly
  auxiliary midwives (AMWs) and
  trained traditional birth attendants
  (TTBAs);
- recording and reporting;
- collaboration with local NGOs;
- participation in research activities; and
- providing duties assigned by higher
  authorities.

MATERIALS AND METHODS

An exploration was made to identify how
the change process took place in Myanmar
as regards training and utilization of
midwives for rural health services. It is a
historical research and information was
collected through:
- reviewing available documents and
  records; and
- performing informal interviews with 14
  key informants which included retired as
  well as existing midwifery trainers,
  relevant managers from Departments of
  Health and Medical Sciences and senior
  as well as junior (recently graduated)
  midwives.

RESULTS

Training of midwives in Myanmar (Pre-
independence Period)

Realizing the situation of high maternal and
infant mortality rates in Myanmar, Madras
model of midwifery training of India was
introduced in 1901 at Dufferin Hospital in
Rangoon [2]. Suitable women who had
passed 7th standard (middle school) were
selected by Municipal and District Councils.
At that time there were very few who passed
7th grade. Each training course lasted a year
and after successfully completing the
course, they were recruited by the respective
Councils that selected them after passing the
course. Salary of Kyat 40 per month was
given if a midwife could deliver the
assigned number of babies; if less, a
deduction of Kyat 2 per baby and if
delivered more, an addition of Kyat 2 per
baby was given.

Burma Act No. X of 1922 was passed by the
Lieutenant-Governor of Burma in
Council. It was an Act for the registration
and better training of midwives and nurses
in Burma. Following this, an 18-month
midwifery course was started in 1929 [3].
For selection, an announcement was made
for applicants who passed 7th grade. The
selection procedure, as described by a
trainee of 1938 (now a retired midwifery
tutor) was as follows:

“The announcement said that those who
wanted to apply for the midwifery training
should come to the school they wanted to
attend bringing along with them the
following –
- one dozen of pink longyi (sarong);
- one dozen of white aingyi (traditional
  blouse) with clothen buttons;
- a white belt, a wrist watch, a thermometer
  and a pair of slippers.

“I went to Dufferin Hospital ... the Matron
(a British) checked the items ... she asked
me a few questions in English ... then, I was
told that I was selected to attend the
18-month midwifery course.”

Eighteen-month midwifery training was
given at Dufferin Hospital (Rangoon);
Rama Krishna Hospital (Rangoon);
Mandalay Hospital, and Ellen Mitchel
Memorial Hospital, Moulmein. English was
the medium of instruction and British nurses
were the key trainers. Midwifery training courses using Myanmar as the medium of instruction was initiated on 1-4-37 at township hospitals located in 16 townships in different regions of the country.

Training of midwives in Myanmar (Post-independence Period)

Re-organization of midwifery training schools took place after Independence. The schools were under the control of the Director of Women and Children Welfare Board. This responsibility was again conferred upon the Nursing Division established within the Directorate of Health Services. A Nursing Chief was appointed as head of the Division in 1953. There were only 11 midwifery training schools before 1962.

A midwifery certificate of registration in English language (1952)

A midwifery certificate of registration in Myanmar language (1954)

With an aim of improving accessibility health services to rural people of Myanmar, Rural Health Centers were opened in rural areas of Myanmar in 1953/1954. Rural Health Centers were established in rural areas and Health Assistant training began. An RHC intended to serve a population of 15,000 to 40,000 with one Health Assistant, one Lady Health Visitor, five midwives and one vaccinator. In 1959, an outstanding Myanmar midwifery tutor was sent to New Zealand to attend a 10-month midwifery training course. In 1960, local midwifery tutor course was opened with the assistance of WHO.

Training of midwives for use in multi-purpose health work in Myanmar

The health policy of the Government in 1962 was “to narrow the gap in health between urban and rural areas” and emphasis was paid on further enhancing rural health services operating through rural health centers. There were 16 midwifery training schools in 1976. A 4-year People’s Health Plan (PHP) was drawn and implemented in 1977/1978 [4]. PHP was practical realization of Primary Health Care (PHC) concepts using Country Health Programming (CHP) methodology. One of the objectives of Primary Health Care and Basic Health Services project of PHP states:

• to improve the efficiency and effectiveness of BHS through:
  ➢ extending the functions and roles of basic health staff to those of multipurpose health workers through in-service training and revision of basic training curricula of these categories of health workers;
  ➢ deploying special disease control project workers (TB, Leprosy, Malaria, Trachoma, etc.) after appropriate training into BHS at the peripheral level so that they may function as multipurpose workers for all these diseases.

In order to fulfill the service requirements, training curriculum of midwives changed and the followings are some of the new topics included in the new curriculum:
• Community health nursing Parts I, II and III; and
• Introduction to midwifery research.

One of the learning objectives of community health nursing Part I states that the student will demonstrate competencies in knowledge, skills and attitudes (KSA) being able to: describe the health care system based on PHC approach in Myanmar. One of the learning objectives of community health nursing Part III states that the student will be able to develop KSA to allow them to function as an effective health team member, including participation with school health team and other special campaigns. During field training, students are required to complete studying special disease control programmes and their integration in the work of the RHC. In spite of the change in curricular content, training duration of 18 months remained unchanged. Total number of midwifery training schools (as of 2005) is 20, and yearly production of midwives is about 1,100.

Current practice of selecting candidates for midwifery training (since 2004) is that applications are called among those who passed tenth grade in each State/Division where the applicant passed tenth grade. Then, lists in order of merit are made in each State/Division according to the total marks of tenth grade in Myanmar, Mathematics and English. Finally, selection of the tops made according to the quota provided for each State/Division.

A midwifery certificate of registration (1994)

Prospects and perspectives of midwives

The career prospects of a midwife nowadays can be considered not so poor. She can become a nurse and work in a hospital setting, or can remain in public health work after becoming a Lady Health Visitor and then a Health Assistant. A nurse can also become a Health Assistant. The next steps for a Health Assistant are Health Assistant-1 and Township Health Assistant. The career ladder of a midwife is shown in Fig.1. Among the rural health team human resources for health, midwives constitute the only category having a demand from the private sector. Attrition rate for midwives is about 3% in 2004.

Fig.1. The career ladder of a midwife

The new organizational set-up of Subcenters with 1 midwife and 1 PHS-2 was introduced in 1988. As of 2005, there are 1337 RHCs still with the old staffing pattern (Fig. 2).

Fig. 2. Staffing pattern of a rural health centre

We would like to make a few quotes of some midwives interviewed as regards their
opinions on their jobs. These quotations reflect existence of some gaps in training and service utilization of midwives for rural health services. This situation has also been elicited in other studies [5, 6].

“I am quite happy with what I am doing ... we have been trained for disease control activities when we were students, so we regard these as our duties and responsibilities. However, I want to become a Nurse and I will try for it.” (a new graduate recently posted to a Rural Health Center in Hlegu Township)

“We want to pay more attention to MCH works ... we can keep the UCI duties as these are very much linked to MCH care ... other responsibilities for disease control should be handed over totally to PHS-2.” (a senior midwife from Bago Township)

“While I was a student, 80% of our training was on midwifery and 20% on other health care services .... After joining the service I came to realize that only 20% of our work is on midwifery and 80% on other health service activities.” (a midwife of 10-month service from a Sub-center in Sittway Township)

**DISCUSSION**

In this review, we attempted to look into changes that took place, in different historical contexts, in training and utilization of midwives for rural health services in Myanmar. It was observed that whenever the government health policy took into serious consideration for improving health of rural people, the role of a midwife became critical and changes in their roles took place.

Two major impacts on training and utilization of midwives for rural health services in Myanmar:

- implementation of rural health centers in 1953/1954
- implementation of National Health Plan in 1977/1978

During the last few decades several projects had been implemented in Myanmar according to the main programmes of PHC had significantly increased the workload of the RHC staffs over the whole country. Some activities were new to the RHC staffs and a series of training had to be given to the implementers. Following, programme managers are concerned with the huge amount of workload borne by the RHC staffs, particularly midwives. There were complaints that midwives being overburdened with various project activities so much so that they have not sufficient time to perform their main task of MCH care. Midwives complained that they would prefer MCH services including immunization rather than doing disease control jobs. The new idea to place a midwife and a PHS-2 in duo at Sub-centers can be a possible solution for transferring some of the midwives’ workload to the shoulders of PHS-2. What is more important than increasing numbers of any category of human resources for health (HRHs) is the ways these HRHs are being managed. Management and supervisory practices at township levels should be critically reviewed and appropriate revisions be made. Another important point is that long-term solutions like increasing the numbers of production and employment are, in general, resource-based. Without actually changing staff numbers, an effective staff increase can be achieved by changing personnel and operating policies that lead to higher productivity, efficiency and motivation of rural health staff. These are the considerations to be made in future policy formulations in attempting to improve rural health services of Myanmar through rural health teams in which midwives are members.

**ACKNOWLEDGEMENT**

We are grateful to all the informants who provided valuable information on historical accounts on training of midwives.
in Myanmar. We also would like to express our thanks to Dr Nilar Tin, Director (Planning) of the Department of Health and Dr U Ye Htut, Director (Administration) of the Department of Medical Research (Lower Myanmar) for assisting us in filling key information gaps for this paper.

REFERENCES


Comparison of efficacy and safety of different brands of oral artemesine plus mefloquine in uncomplicated falciparum malaria in adults

*Khin Phyu Pyar, *Win Win Myint, **Myat Phone Kyaw, ***Thaw Zin , *Khin Nyo, *Than Htut & ****Marlar Than

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**Experimental Medicine Research Division  
***Department of Medical Research (LM)  
****Clinical Research Unit (Malaria)  
Defence Services General Hospital

The efficacy of artemisinin derivatives in treatment of falciparum malaria is well established and a variety of different brands are now available, assumingly to be identical in composition and efficacy. A hospital-based, double blind randomized, controlled study to find out the efficacy and pharmacokinetics of different brands of artesunate when combined with mefloquine in Myanmar population was carried out in CRU (Malaria) DSGH, to establish the relative merits of the different brands, provide data for consumer choice and for better future utilization of these combinations in the chemotherapy of malaria. A total of 120 adult uncomplicated falciparum malaria patients were randomized, to receive 4 different brands of artesunate 200 mg OD for 3 days. A single brand of mefloquine (Helm-Germany) 500 mg OD for 3 days was used as the combination drug in all four regimens. Artequin TM600/1500 combi-pack (Mepha-Switzerland) was used as the control drug. The Mean Fever Clearance Times (FCT) were 11.15 hrs, 13.69 hrs, 11.8 hrs, 11.68 hrs and 12.88 hrs. The Mean Parasite Clearance Times (PCT) were 46.12 hrs, 50.51 hrs, 46.88 hrs, 42.45 hrs and 51.22 hrs in the Plasmotrim Lactab TM 200 mg (Mepha-Switzerland), Falcinate Tab 50 mg (Aurocham-India), Dawnasunate Tab 50 mg (MPF, Myanmar), Artenmed Tab 50 mg (Vietnam) and Control drug. Artequin TM600/1500 combi-pack (Mepha-Switzerland) respectively. The 14 days Adequate Clinical and Parasitological Response (ACPR) was 100 % in all the five groups. There were no adverse, clinical, hematological, biochemical and ECG changes in all the groups. The different brands of artemesine available are comparable in efficacy for the treatment of uncomplicated falciparum malaria in adults.

**INRODUCTION**

Many previous studies in Myanmar have demonstrated the efficacy of artemisinin derivatives and its great potential to fight against falciparum malaria. National antimalarial drug policy recommended 3 days course of Artemisinin Combination Therapy (ACT) in uncomplicated falciparum malaria. A variety of oral artemesine are now available in Myanmar, but whether they are identical in composition and efficacy has not yet been confirmed. The present study aimed to test the qualitative and quantitative composition of five locally available commercial brands of artemesine and to compare the efficacy of each when combined with one single brand of mefloquine given in the recommended dosages for three days in adult uncomplicated falciparum malaria patients, using Artequin TM a fixed dose combination pack of artemesine + mefloquine as a control, so as to provide data on the relative merits of the different brands and to provide data for consumer choice.
Objectives

- To compare the therapeutic efficacies, safety & tolerability of 5 commercial tablet formulations of artesunate.
- To verify the composition of active compounds present in them.

PATIENTS AND METHODS

Study design

A hospital-based, double blind randomized controlled study

Acute symptomatic uncomplicated falciparum malaria patients admitted to Clinical Research Unit (Malaria) DSGH were recruited. Patients were categorized as symptomatic if they were febrile or with one of the following symptoms: headache, feeling ill, aches and pains, nausea or vomiting.

Inclusion criteria

- Both sexes
- The age group between 10 - 60 years
- Positive peripheral blood film for trophozoite forms of pure *P. falciparum* with the count ranging from 1000/ul up to 250,000/ul
- Patients who had no evidence of severe and complicated falciparum malaria
- Patients who had not been treated with artemisinin or mefloquine within the past 14 days
- Patients who were willing to give informed consent for treatment and were able to remain hospitalized for 14 days

Exclusion criteria

- Patients with mixed infection (falciparum malaria + vivax malaria)
- Asymptomatic patients
- Patients requiring parenteral treatment
- Patients with fever due to causes other than malaria e.g. TB, etc.
- Patients with other concomitant diseases like diabetes mellitus, etc.
- Pregnant women in 1st trimester of pregnancy

Withdrawal criteria

- Patient's request
- Any serious adverse effects to drugs
- Serious or repeated noncompliance with protocol specifications

Sample size

A total of 120 patients completed the study: 30 patients each for Plasmotrim Lactab (Mepha-Switzerland), Falcinate (India) and Artenmed (Vietnam), 20 patients for Dawnasunate (MPF, Myanmar) and 10 patients for Artequin TM 600/1500 (Mepha-Switzerland) combi-pack.

Study period

14 months

Materials

Study drugs and control drugs

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug</th>
<th>Manufacture/Country</th>
<th>Batch no.</th>
<th>Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plasmotrim Lactab</td>
<td>Mepha-Switzerland</td>
<td>250512</td>
<td>1 tab OD for 3 days</td>
</tr>
<tr>
<td>2.</td>
<td>Falcinate Tab 50 mg</td>
<td>Aurocham-India</td>
<td>S-297</td>
<td>4 Tabs OD for 3 days</td>
</tr>
<tr>
<td>3.</td>
<td>Dawnasunate Tab 50 mg</td>
<td>MPF - Myanmar</td>
<td>4 tabs OD for 3 days</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Artenmed Tab 50 mg</td>
<td>Vietnam</td>
<td>30903</td>
<td>4 tabs OD for 3 days</td>
</tr>
<tr>
<td>5.</td>
<td>Mefloquine 250 mg</td>
<td>Helm - Germany</td>
<td>165703</td>
<td>2 tabs OD for 3 days</td>
</tr>
<tr>
<td>6.</td>
<td>Artequin 600/1500 mg</td>
<td>Mepha - Switzerland</td>
<td>390009 (l+2) tabs</td>
<td>on Day 1,2,3</td>
</tr>
</tbody>
</table>

Procédure

Eligible patients were subjected to the following procedure; Routine history taking, clinical examination and relevant investigations were done and recorded in the standard proforma. Patients were randomized to 5-drug regimens by means of sealed envelopes. Drug administrations were observed in all patients and if vomiting occurred in less than 30 min, drug administration with full dose were repeated. If vomiting occurred between 30-60 mins, half the dose was repeated. No re-treatment was given if vomiting occurred after 60 minutes.
Clinical assessment

Symptoms review, adverse effect review (according to the checklist) and physical examination were made on Days 0, 1, 2, 3, 4, 7 and 14. Body temperature were recorded 4 hourly until normal for 24 hours and then daily up to Day 14.

Parasitological assessment

The Giemsa stained thick and thin blood smears were examined 6 hourly until negative for 24 hours and daily up to 3 consecutive negatives were resulted, and parasite counts were also noted, then weekly up to Day 14, whenever it is indicated e.g., reappearance of fever.

Haematological measurements

Hb%, PCV, T & DC, platelets were done on Day 0, 3 and 7. Biochemical tests; Blood urea, sugar, serum bilirubin, SGOT, SGPT and Alkaline Phosphatase were checked on Days 0, 3 and 7. ECG was measured on Days 0, 1, 3 and 7, and to be repeated on Day 14 if found abnormal in first week.

Thin Layer Chromatography (TLC)

Quality assurance of artesunate in different brands was done by Thin Layer Chromatography using methanol & ethyl acetate as mobile phase on SiOz TLC plates. Movement of compounds present in different brands was recorded as retention factors on TLC plates. Chromatograms were developed by using iodine as visualizing reagent. Authenticity of artesunate in each brand used was checked by observing similarity in Rf values.

Fourier Transform Infrared Spectrometer (FT-IR)

KBr pellet method was used to detect quantitatively the artesunate content in mixed content powder from different brands & blank (KBr) in ratio of 1:200 and checked with specified reference spectrum of a artesunate with regards to the functional group using infrared absorption spectrum. Close resemblance between spectrum of extracted material and specified reference spectrum achieved indicated authenticity of artesunate present in each brand tested.

Therapeutic response

Therapeutic response was assessed according to WHO criteria.

Indicators for drug efficacy were:

- Parasite Clearance Time (PCT)
  Time from initiation of therapy to the first negative blood film that remained negative for 48 hours
- Percent clearance of parasitaemia at 24-48 hours
- Fever Clearance Time (FCT)
  Time from initiation of therapy to time the temperature reached normal (37°C) and remained so for 24 hours
- Early Treatment Failure (ETF) rate
- Late Treatment Failure (LTF) rate
- Adequate Clinical and Parasitological Response (ACPR)

Definitions (WHO, 2003)

Early treatment failure (ETF)

- Development of danger signs or severe malaria on Day 1, Day 2, or Day 3, in the presence of parasitaemia
- Parasitaemia on Day 2 higher than Day 0 count irrespective of auxiliary temperature
- Parasitaemia on Day 3 with axillary temperature +/- >37.5°C
- Parasitaemia on Day 3 +/- > 25% count on Day 0

Late Treatment Failure (LTF)

It is divided into Late Clinical Failure and Late Parasitological Failure.

Late Clinical Failure (LCF)

- Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of Early Treatment Failure
- Presence of parasitaemia and axillary temperature +/- >37.5°C on any day from Day 4 to Day 14 (Day 28*), without previously meeting any of the criteria of Early Treatment Failure
Late Parasitological Failure (LPF)

- Presence of parasitaemia on Day 14 (or Day 28*) and axillary temperature <37.5°C, without previously meeting any of the criteria of Early Treatment Failure or Late Clinical Failure

Adequate Clinical and Parasitological Response (ACPR)

- Absence of parasitaemia on Day 14 (or Day 28*), irrespective of axillary temperature, without previously meeting any of the criteria of Early Treatment Failure, Late Clinical Failure or Late Parasitological Failure (* if followed up for 28 days.)

Retreatment of failure

ETF and LTF cases were retreated with standard seven-day course of oral quinine sulphate (10 mg of salt/ kg three times/ day) and tetracycline (4 mg/kg four times / day).

Statistical evaluation

Statistical evaluation comparing the regimens was done by computer using EPI-INF0 software. Categorical data were compared by calculating the chi-square value with Yate's correction or by Fischer's exact test. Normally distributed continuous data were compared by the Student's t-test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test. PCT, FCT, Symptom clearance times and the resolution of other signs (anemia, Hb%), hepatomegaly, splenomegaly and the risk of treatment failure were evaluated by survival analysis with cumulative incidences and calculated by the product limit method and compared by the Mantel-Haenzellog rank test.

Ethical considerations

The protocol was approved by the Ethical Committee, Research and Development Committee, Directorate of Medical Services, Ministry of Defense. Informed written consent was obtained from all the patients.

RESULTS

Baseline characteristics like age, sex, weight, initial temperature, initial parasite count, Hb% between groups were comparable (Table 1).

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Plasmodron</th>
<th>Falcinate</th>
<th>Dawna-sunate</th>
<th>Arten-med</th>
<th>Arte-quin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>29</td>
<td>29</td>
<td>20</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>Age (Year)</td>
<td>±9.8</td>
<td>±8.3</td>
<td>±10.8</td>
<td>±7.4</td>
<td>±5.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.3</td>
<td>161.2</td>
<td>163.5</td>
<td>165.9</td>
<td>160.7</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>±6.7</td>
<td>±7.3</td>
<td>±4.1</td>
<td>±5.1</td>
<td>±7.9</td>
</tr>
<tr>
<td>BMI</td>
<td>51.6</td>
<td>47.3</td>
<td>49.7</td>
<td>49.9</td>
<td>47.3</td>
</tr>
<tr>
<td>Initial temp. (°C)</td>
<td>±7.7</td>
<td>±6.4</td>
<td>±6.2</td>
<td>±5.3</td>
<td>±10.3</td>
</tr>
<tr>
<td>BMI</td>
<td>18.8</td>
<td>18.1</td>
<td>18.7</td>
<td>18.0</td>
<td>18.1</td>
</tr>
<tr>
<td>Total WBC</td>
<td>±2.8</td>
<td>±2.2</td>
<td>±2.3</td>
<td>±1.7</td>
<td>±2.4</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>38.5</td>
<td>38.5</td>
<td>38.4</td>
<td>38.5</td>
<td>38.5</td>
</tr>
<tr>
<td>(g/dl)</td>
<td>±0.8</td>
<td>±1.0</td>
<td>±1.0</td>
<td>±0.8</td>
<td>±0.9</td>
</tr>
<tr>
<td>Initial parasite count (π/cu.mm)</td>
<td>±24548.6</td>
<td>7503</td>
<td>13296.0</td>
<td>6814.3</td>
<td>3528.9</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>±5.1</td>
<td>±5.2</td>
<td>±7.1</td>
<td>±3.4</td>
<td>±5.2</td>
</tr>
<tr>
<td>Urea</td>
<td>24.8</td>
<td>25.7</td>
<td>26.5</td>
<td>26.5</td>
<td>25.5</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>±14.8</td>
<td>±5.2</td>
<td>±7.1</td>
<td>±3.4</td>
<td>±5.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>110.5</td>
<td>108.2</td>
<td>115.9</td>
<td>106.6</td>
<td>113.4</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>±10.8</td>
<td>±7.5</td>
<td>±11.4</td>
<td>±8.4</td>
<td>±10.0</td>
</tr>
</tbody>
</table>

Five various commercial tablet formulations of artesunate are all effective equally (Fig. 1) and spectrum of each formulation is comparable with standard drug.

Fig.1. Percentage parasite clearance of five different brands

Authenticity of artesunate in each brand used checked by TLC has similarity in Rf values and by Fourier Transform Infrared Spectrometer (FT-IR) method showed close resemblance (Fig. 2).
There was no early treatment failure (ETF) and adequate clinical and parasitological response (ACPR) was 100% in all groups. There were no adverse clinical effects and laboratory and ECG abnormalities. Drug tolerance was similar in frequency and severity. Dawnasunate from Myanmar Pharmaceutical factory is well tolerated, safe and equally effective compared to that of four other imported brands.

DISCUSSION

The quality of commercially available drugs varies greatly among countries. Due to lack of regulations and poor quality control practices in some countries, the amount of the active ingredient can be inconsistent. Poor formulation techniques can affect the release of active ingredients from a tablet, with some tablets releasing very little amount of drug. Some drugs may be contaminated with other substances. Poor storage conditions, especially in warm and humid tropical environments may contribute to chemical degradation of many pharmaceuticals.

Artesunate is a semi-synthetic derivative of artemisinin, a naturally occurring sesquiterpene endoperoxide. It is difficult to detect and identify by standard spectrophotometric methods. The standard method used to determine artesunate in tablets involves high performance liquid chromatography (HPLC). In many countries, such equipment is not available.

Modern electronic technology is rapidly approaching the state at which it can reliably and affordably provide much greater assurances that a drug product was manufactured safely and distributed under conditions that did not compromise its potency. FDA has concluded that this approach is a much more reliable direction for assuring the legitimacy of a drug than paper record keeping requirements, which are more likely to be incomplete or falsified, and that it is feasible for use by 2007.

Radiofrequency Identification (RFID) tagging of products by manufacturers, wholesalers, and retailers appears to be the most promising approach to reliable product tracking and tracing. Significant feasibility studies and technology improvements are underway to confirm that RFID will provide cost-reducing benefits in areas such as inventory control, while also providing the ability to track and trace the movement of every package of drugs from production to dispensing. Most importantly, reliable RFID technology will make the copying of medications either extremely difficult or unprofitable. FDA is working with RFID product developers, sponsors, and participants of RFID feasibility studies to ensure that FDA's regulations facilitate the development and safe and secure use of this technology. FDA is also working with other governmental agencies to coordinate activities in this area.

In Myanmar, relatively simple and inexpensive methods such as TLC and FT-IR methods are used extensively as quality assurance procedures. These methods, if used together with some form of sample pretreatment such as solvent extraction, can become powerful techniques for identifying and detecting compounds and impurities. The present study is the first time that different brands of artemesunate available in the market have been subjected to such tests.
The study supported the high pharmaceutical quality of Dawnasunate from MPF, in attaining similar clinical efficacy on patients with uncomplicated *P. falciparum* malaria.

**Conclusion**

Different brands of artesunate available in Myanmar are comparable in efficacy and equally effective. All are safe and tolerable. Dawnasunate from (Myanmar Pharmaceutical Factory Myanmar) has equal efficacy like other imported brands. The TLC and FT–IR methods provided a practical and cost-effective means of detecting the artesunate content and can thus, be useful for screening of counterfeit and substandard drugs in the market.

**REFERENCES**

Women’s awareness of common female cancers in selected peri-urban townships

*Myo Myo Mon, *Mon Mon,** Kyu Kyu Than,
*Khin Sandar Oo,***San San Aye, *Kyaw Oo &**** Soe Aung

*Medical Statistics Division
**Epidemiology Research Division
***Health Systems Research Division
****Department of Medical Research (Lower Myanmar)
*****Medical Oncology, Yangon General Hospital

With the objectives of estimating perceived magnitude of female cancer problem and assessing community awareness of those cancers, a community based study was conducted employing both qualitative and quantitative-approaches. Triangulation of research methods was done to validate the findings. Four peri-urban townships from Yangon Division; Hlaing Thar Yar, Shwe Pyi Thar, South Dagon and East Dagon Townships were selected purposely. For quantitative survey, 400 women were interviewed using a structured questionnaire. Six focus group discussions were done for the qualitative assessment. Mean age of sampled women was 48.4 years and majority were married, dependent and primary school passed. Cancer breast, uterus and larynx were mentioned as the most common problem according to their perceived magnitude. Almost all (99.3%) were aware of breast cancer and only 69.5% were aware of cervical cancer. Breast lump was considered as a condition that will later develop into cancer by 38.8% and 41.5% mentioned that it was painless in early stage. Abnormal bleeding per vagina and white discharge were mentioned as main symptoms of cervical cancer (76.3%, 63.3%). Regarding the risk of cervical cancer, female hygiene was indicated by 88.5% and number of sexual partners by 77.0%. Almost all of them said that both diseases can be cured at early stage and surgery was mentioned as a main treatment option. Relatives/ friends were stated as their main source of information (90.5%) and only 29.0% mentioned health staff. Government hospitals/ clinics were identified as a main treatment centre available for cancer patients (97.3%).

INTRODUCTION

Cancer is a public health problem worldwide. More than 10 million people are diagnosed with cancer every year and it accounts for 13% of deaths worldwide that is 7 million deaths every year. Cancer is the second most common cause of death in developed countries, and similar epidemiological transition has been followed in developing countries [1]. Cervical cancer is the second most common female cancer worldwide but commonest in developing countries and eighty percent of new cases and deaths occur in these areas [2] Breast cancer is the most common cancer of women, comprising 23% of all female cancers, and it is still the leading cause of cancer mortality in women worldwide that is 14% [3].

In Myanmar, the incidence of cancer in general has been progressive over the past decade, as mentioned in the National Health Plan (1996-2001) [4]. According to data from Yangon Cancer Registry (1993-2000), three most common cancers for women are
cancer cervix (Ca cervix), cancer breast (Ca breast) and cancer lung (Ca lung). The rising trend of two most common female cancers was observed according to the cancer registry during the past decade. The incidence of Ca cervix and Ca breast during 1993 and 2001 was 27.4 to 30.7%, 25.3 to 30.6% respectively [5].

At least one third of all cancer cases are preventable and another one third permits the early detection and effective treatment. Early detection and diagnosis can then greatly increases the chances for successful treatment particularly relevant to common female cancers like Ca breast and Ca cervix. Increased awareness of possible warning signs of these cancers among general public is a necessity. Therefore, a community-based survey was carried out to assess the awareness of women regarding these cancers.

**Objectives**

- To estimate the perceived magnitude of female cancer as a problem
- To assess the community awareness of common women cancers
- To recommend the programme for successful implementation of cancer control programmes for better programmatic implication

**MATERIALS AND METHODS**

**Quantitative survey**

The following 4 townships from Yangon Division were selected purposely to cover the criteria of peri-urban townships namely Hlaing Thar Yar, East Dagon, South Dagon and Shwe Pyi Thar Township. Assuming that the proportion of women aged 40 and above who had satisfactory level of knowledge on women cancers is 50% and accuracy level of 0.05, the calculated sample size was 100 at 95% confidence level for each township.

Within each township, 5 wards were selected randomly from the list of wards. Then 20 households were chosen randomly from each ward. A total of 400 women aged 40 years and above were interviewed by well trained interviewers using a pre-tested structured questionnaire. Data cleaning, coding and analysis were done using SPSS 11.5 software.

**Qualitative survey**

FGD guide which was pre-tested in a township of non-studied area was used. Respondents were women aged 40 years and above residing in the study areas. A total of 6 FGD sessions was done and 56 women participated in these sessions.

Female cancers which were prevalent in their community were explored by free-listing and ranking methods. Their knowledge about the risk factors, signs and symptoms, treatment options and prevention of cancer cervix and breast was discussed.

After completion of all fieldworks, transcripts were transcribed on a day by day basis, manual edition for inconsistencies and similarities were conducted. Matrix analysis was done after the transcription.

**RESULTS**

Table 1 shows that mean age of the respondents was 48.4 years and most of them were married (65.8%). Regarding occupation, majority (67.8%) depend on other family members for their living and about one fifth (22.3%) did odd jobs. Most of the respondents finished primary school (40.3%) and secondary school level (21.3%). Only a few (2.5%) attended university. Family income ranged from 6000 Kyats to 250000 Kyats per month and median family income was 35000 Kyats.

**Perceived magnitude of female cancers**

During the quantitative assessment, open question regarding the most common female cancers was described as Ca breast, Ca uterus and Ca larynx without any probing. Others include Ca cervix, Ca lungs, Ca liver, Ca stomach and haematological cancer. Ranking of common female cancers
Table 1. Background socio-demographic characteristics of the respondents

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49 years</td>
<td>250</td>
<td>62.5</td>
</tr>
<tr>
<td>50-59 years</td>
<td>115</td>
<td>28.8</td>
</tr>
<tr>
<td>60 years and above</td>
<td>35</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Mean age</strong> - 48.4 ± 8.1 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>11</td>
<td>2.8</td>
</tr>
<tr>
<td>Married</td>
<td>263</td>
<td>65.8</td>
</tr>
<tr>
<td>Divorced</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Widowed</td>
<td>98</td>
<td>24.5</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dependent</td>
<td>271</td>
<td>67.8</td>
</tr>
<tr>
<td>Odd jobs</td>
<td>89</td>
<td>22.3</td>
</tr>
<tr>
<td>Government / private servant</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td>Own business</td>
<td>25</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Education status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>23</td>
<td>5.8</td>
</tr>
<tr>
<td>Read and write</td>
<td>85</td>
<td>21.3</td>
</tr>
<tr>
<td><strong>Primary school passed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school passed</td>
<td>90</td>
<td>22.5</td>
</tr>
<tr>
<td>High school passed</td>
<td>31</td>
<td>7.8</td>
</tr>
<tr>
<td>University/ Graduate</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Monthly family income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25000 kyats</td>
<td>84</td>
<td>21</td>
</tr>
<tr>
<td>25000-&lt;50000 kyats</td>
<td>189</td>
<td>47.3</td>
</tr>
<tr>
<td>&gt; 50000 kyats</td>
<td>127</td>
<td>31.8</td>
</tr>
<tr>
<td><strong>Median income</strong>- 35000 Kyats</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

was also conducted in the FGD sessions and Ca uterus was ranked as first followed by Ca breast and Ca cervix consecutively. According to the above findings, Ca breast Ca uterus were the most common and female cancers mentioned by the respondents.

**Awareness of common female cancers**

According to the above figure, almost all (99.3%) were aware of breast cancer and only 69.5% of the respondents were aware of cancer cervix. There were about one third (30.5%) of the respondents who were not aware of the cervical cancer. This finding coincides with the qualitative finding.

**Cervical cancer and breast cancer**

**Cervical cancer**

Regarding cervical cancer, abnormal bleeding per vagina was mentioned as the most common symptom followed by white discharge, 76.3% and 17.6% respectively. During the FGD sessions, dynamicity of group discussion was very alive in describing the symptoms and majority mentioned these two common symptoms.

“If the woman experienced abnormal and irregular bleeding, she may suffer from cervical cancer.”

(54 years old, married, having 5 children, primary school passed)

Over eighty percent of the respondents highlighted that female hygiene was an important risk factor for cancer cervix. Having multiple sexual partners was described as a risk by 77.0% of the respondents. Other risk factors like sexual exposure at young age and use of oral contraceptive pills were mentioned by about half of the respondents (57.2%, 51.8%) whereas smoking and number of children were stated by some respondents (21.6%, 33.5%). Majority of the participants in the qualitative study also discussed these risk factors.

“If the woman is unhygienic of her private part and if she has multiple sexual partners, she might be at risk of Ca cervix.”

(54 years old, married, having 5 children, primary school passed)

“If a woman is young and if she bears a lot of children she is at risk.”

(40 years old, single, graduate)
Table 2. Knowledge of respondents on cancer cervix and breast

<table>
<thead>
<tr>
<th>Symptom/Condition</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cervical cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White discharge</td>
<td>176</td>
<td>63.3</td>
</tr>
<tr>
<td>Abnormal bleeding</td>
<td>212</td>
<td>76.3</td>
</tr>
<tr>
<td>Bleeding after coitus</td>
<td>117</td>
<td>42.1</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>121</td>
<td>43.5</td>
</tr>
<tr>
<td><strong>Risk of cancer cervix</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual exposure at young age</td>
<td>159</td>
<td>57.2</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>214</td>
<td>77.0</td>
</tr>
<tr>
<td>Use of oral contraceptive pills</td>
<td>144</td>
<td>51.8</td>
</tr>
<tr>
<td>Smoking</td>
<td>60</td>
<td>21.6</td>
</tr>
<tr>
<td>No. of children</td>
<td>93</td>
<td>33.5</td>
</tr>
<tr>
<td>Female hygiene</td>
<td>246</td>
<td>88.5</td>
</tr>
<tr>
<td><strong>Curability of cancer cervix</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be cured at early stage</td>
<td>262</td>
<td>94.2</td>
</tr>
<tr>
<td>Can’t be cured</td>
<td>12</td>
<td>4.3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Main treatment option</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>257</td>
<td>92.4</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>199</td>
<td>71.6</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>186</td>
<td>66.9</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be prevented</td>
<td>182</td>
<td>65.5</td>
</tr>
<tr>
<td>Can not be prevented</td>
<td>51</td>
<td>18.3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>45</td>
<td>16.2</td>
</tr>
<tr>
<td><strong>Breast cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chance of cancer for breast lump</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>155</td>
<td>39</td>
</tr>
<tr>
<td>No</td>
<td>232</td>
<td>58.4</td>
</tr>
<tr>
<td>Don’t know</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Pain in early stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>183</td>
<td>46.1</td>
</tr>
<tr>
<td>No</td>
<td>166</td>
<td>41.8</td>
</tr>
<tr>
<td>Don’t know</td>
<td>48</td>
<td>12.1</td>
</tr>
<tr>
<td>Curability of cancer breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be cured at early stage</td>
<td>387</td>
<td>97.5</td>
</tr>
<tr>
<td>Can’t be cured</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Main treatment option</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>377</td>
<td>95.0</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>302</td>
<td>76.1</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>278</td>
<td>70</td>
</tr>
<tr>
<td><strong>Breast self-examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be done</td>
<td>370</td>
<td>93.2</td>
</tr>
<tr>
<td>Can not be done</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be prevented</td>
<td>223</td>
<td>56.2</td>
</tr>
<tr>
<td>Can not be prevented</td>
<td>79</td>
<td>19.9</td>
</tr>
<tr>
<td>Don’t know</td>
<td>95</td>
<td>23.9</td>
</tr>
</tbody>
</table>

Almost all of the respondents (94.2%) said that the disease can be cured at early stage. Surgery was mentioned as a main treatment option (92.4%) followed by chemotherapy and radiotherapy (71.6% and 66.9%). More than half (65.5%) said that it was preventable.

Majority in the FGD sessions also mentioned surgery as a main treatment option because they thought only surgery could save the life.

“If cervical cancer patient doesn’t have her cervix removed surgically, she may die.”

(48 years old, married, having 4 children, primary school passed)

**Breast cancer**

Table 2 shows that breast lump was considered as a condition that will later develop into cancer by 38.8% and about two fifth (41.8%) mentioned that it was painless in early stage. Breast cancer was also stated as a curable disease in early stage (97.5%). Surgery was regarded as a main treatment option (95.0%) and more than half (56.2%) thought that it was preventable. Majority of the sampled women (93.2%) agreed that breast self-examination can be done to detect the breast lump.

During the qualitative assessment, most of the participants actively discussed and they mentioned all treatment options for breast cancer. They said that chemotherapy and radiotherapy can be given if the disease is in early stage and surgery was considered if the disease is in advanced stage. Although many participants said that breast cancer can be prevented, some of the preventive measures they mentioned were incorrect such as not wearing tight bodice.

“Breast cancer can be prevented by giving breast feeding in married women.”

(45 years old, married, having one child, high school passed)

“Women shouldn’t wear tight bodice to prevent the occurrence of breast cancer”

(43 years old, married, having 2 children, graduate)
**Perceived risk on survival of cancer**

Regarding the survival of cancer, breast cancer was considered as a most favourable cancer for survival by most of the respondents (87.5%) followed by cancer in general and cervical cancer (68.0%, 56.0%) (Fig. 2).

Fig. 2. Perceived risk on survival of cancer mentioned by respondents

To show their opinion about chance of survival during the FGD sessions, the words “dead” and “survive” were written on separate papers and told them to put one stone to either paper according to their will. It was done separately for both cancers. Group dynamic was very alive at that time and they put stones on either paper. Stones on the “survive” paper were much more than “dead” in case of cancer breast. It was equivocal in case of cervix and reversed in case of cancer in general. From this process it was found out that all participants thought breast cancer had the greatest chance of survival compared with cervical cancer and other cancers. But there were some negative thoughts about survival also present in them.

“It’s quite sure that cancer patient has no chance for survival and are sure to die.”
(56 years old, married, having 4 children, primary school passed)

“Breast cancer patients have more chance of survival if operated in time.”
(40 years old, single, high school passed)

**Source of information and choice of treatment centre**

According to Table 3, it can be seen that respondents’ source of information was mainly from lay persons like relatives/ friends/ patients (90.5%). Health staff was mentioned by only 29.0%. Other sources they mentioned were radio/ TV/ video, journal/ magazine, pamphlet and health talks.

Table 3. Source of information and choice of treatment centre mentioned by the respondents

<table>
<thead>
<tr>
<th>Source of information</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatives/ friends</td>
<td>362</td>
<td>90.5</td>
</tr>
<tr>
<td>Health staff</td>
<td>116</td>
<td>29</td>
</tr>
<tr>
<td>Health talks from NGO/ INGO</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Radio/ TV/ Video</td>
<td>31</td>
<td>7.8</td>
</tr>
<tr>
<td>Journal/ Magazine</td>
<td>27</td>
<td>6.8</td>
</tr>
<tr>
<td>Pamphlet</td>
<td>17</td>
<td>4.3</td>
</tr>
</tbody>
</table>

**Treatment centre**

<table>
<thead>
<tr>
<th>Treatment centre</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Government hospital/ clinic</td>
<td>389</td>
<td>97.3</td>
</tr>
<tr>
<td>Private hospital/ clinic</td>
<td>115</td>
<td>28.8</td>
</tr>
<tr>
<td>Traditional medicine hospital/ clinic</td>
<td>84</td>
<td>21</td>
</tr>
<tr>
<td>Quacks</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Government hospitals/ clinics were the most frequently mentioned treatment centre for cancer (97.3%) and private hospitals/ clinics as second most common choice for cancer (28.8%). Some (21.0%) mentioned of traditional medicine hospital/ clinic.

During the FGD sessions, majority stated government hospitals and not few stated going to traditional healers of various kinds such as monks, traditional medicine clinics, taking traditional herbs and going to “Outlan Sayar” (lower spirit medium). Majority went there with the hope of cure, relief and lesser cost compared to western medicine.

Some of their responses were,

“Cancer patient should go to hospital/ clinic as soon as possible.”
(44 years old, married, having 6 children, primary school passed)

“A monk who lived in Hle-gu could treat cancer patient and accept donation only if the patient had money. He used injection
and did not accept the patient after receiving radiotherapy.”

(60 years old, married, having one child, middle school passed)

**DISCUSSION**

Most common female cancers were ranked by the respondents according to their perceived magnitude as follows: breast cancer, uterus cancer, larynx cancer, cervical cancer, lungs cancer, liver cancer, stomach cancer and haematological cancer. Although the cervical cancer is the most common cancer in female, it was ranked only fourth by the respondents. It might be due to the fact that some women could not differentiate between uterus and cervix exactly. They could not distinguish these two as different types of cancer. Findings from both quantitative and qualitative assessment highlight that two commonest female cancers as described by the respondents were Ca breast and Ca uterus. Regarding awareness, it was quite alarming that many respondents were not aware of cervical cancer. Study done in Kenya revealed that about half of the respondents were aware of cervical cancer [6]. And in a study done in South Africa, one-fifth of the women had not heard of breast and cervical cancers [7].

Abnormal bleeding per vagina and white discharge were recognized by most of the respondents as the common presentations of cervical cancer. These two were most significant symptoms that could easily be aware by the women. Study done in Vietnamese women found out that about two-fifth could mention the symptoms [8]. Therefore it can be said that respondents from this study had higher level of awareness about the symptoms of cervical cancer. It was a positive finding for the promotion of health education activities aiming on early detection.

Concerning risk factors for cervical cancer, female hygiene was considered as most important by majority of the respondents. Sexual behaviours like multiple sexual partners and sexual exposure at young age were also stated as the risk factors. In the study done in British population regarding risk factors for cervical cancer, only forty-one percent of respondents mentioned factors relating to sex [9]. In the Vietnamese study, three-fourth did not know that having multiple sexual partners was a risk factor [8]. Knowing these important risk factors by the respondents was also a positive finding for encouraging prevention activities. However, smoking and number of children were not considered as the risk factors by most of them. So health education activities should also stressed on these risk factors.

Regarding breast cancer, only one third of the respondents mentioned that the breast lump could be a sign of cancer. Only some respondents from the KAP study of Nigerian women could mention painless breast lump as a common presentation [10]. Breast self-examination was accepted as a method that can detect the breast lump at early stage.

Both cancers were considered as preventable by half of the respondents. However, some preventive measures they mentioned were unclear and vague. Some traditional beliefs were still prevalent in the community. Regarding the source of information, majority mentioned the lay persons such as relatives, patients and only few mentioned health staff as their source. In Tunisian study, women get most of their information about gynaecological cancers from their colleagues [11]. More than half of the women from the Indian study also obtained information regarding cervical cancer from relatives and neighbours [12]. It was a negative finding highlighting that health staffs should be encouraged to provide health information concerning common female cancers whenever they have a chance.

The above findings highlight that even though cervical and breast cancer were perceived as common female cancers, information regarding prevention and
treatment procedures still need to be promoted for the health of all women.

Recommendations

1. Awareness raising activities concerning commonest female cancer like cervical cancer should be promoted in peri-urban townships as there were about one third of the respondents who were not aware of cervical cancer.

2. Many said that both cancers can be prevented. However, some preventive measures they mentioned were unclear and vague. Therefore, health education activities should focus on prevention aspect of common female cancers.

3. Proper breast self-examination technique should be incorporated along with health education activities within the community as almost all respondents agreed that breast lump can be detected by breast self-examination.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Director-General (DMR-LM) for allowing us to conduct this study. We also wish to extend our thanks to WHO/APW for giving financial support. Last but not the least, we must not forget to give our sincere thanks to Township Medical Officers and all the participants from the study townships.

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4. National Health Plan (1996-2001), Ministry of Health, Myanmar,


Smoking in an urban community: prevalence, associated factors and behavior among adult males in Kyimyintine Township


*Clinical Research Division
Department of Medical Research (LM)
**Department of Medical Research (Lower Myanmar)

Smoking is the single most important preventable cause of diseases and premature death in the world today. It is a major public health problem in developing countries. The objectives of this study were to estimate the prevalence, to find out the associated factors of smoking, and to determine the behavior of adult male smokers in Kyimyintine Township. A cross-sectional survey was conducted among 486 adult males, aged 15 years and above in March 2006. Overall prevalence of current smokers was 46.1%. The factors associated with smoking were presence of paternal smoking (OR=1.52, 95% CI=1.05-2.2), peer smoking (OR=1.86, 95% CI=1.22-2.83) and education. Respondents with college level education were less likely to smoke than those of primary school level (OR=0.55, 95% CI=0.39-0.79). More than half (58.5%) started smoking before 20 years of age, and 74.1% smoked more than 5 years. Twenty-five percent of smokers also used tobacco in other forms. Large numbers smoked at home (70.1%) and at public places (52.2%). It was found that smoking is prevalent among adult males, and most of them begin to smoke rather early in life and continue for many years which may lead to the development of various tobacco-related diseases. Health education and intensive anti-smoking campaigns through media are important to combat smoking and smoking-related health problems in the future.

INTRODUCTION

Tobacco is the single most important preventable cause of diseases and premature death in the world today. Tobacco causes about three and a half million death through the world and kills nearly 10,000 people worldwide everyday. Smoking causes a substantially increased risk of mortality due to lung cancer, upper gastro intestine tract and respiratory tract cancer, several other cancers, ischaemic heart diseases, stroke, chronic respiratory disease and a range of other diseases. Smoking also harms others, with definite health risks from passive smoking [1].

It is estimated that there are about 1100 million smokers worldwide, about one-third of global population aged 15 years and above. Smoking prevalence and cigarette consumption are decreasing in many developed countries. The reverse is unfortunately happening in developing countries, where a large proportion of adult men are dependent on some form of tobacco use. Nearly 73% of smokers live in developing countries where about 48% of males aged 15 years and above are smokers [2].

Unless immediate steps are taken to reduce the number of smokers, the number of deaths each year due to smoking will increase to 10 million within the next 30 years, of which 70% will occur in developing countries [3].

Observation on the difficulty of giving up smoking and the poor success rate of most
smoking cessation programs highlight the need for primary prevention. Knowledge about the distribution, associated factors and behavior of smokers is important because it could help to facilitate preventive actions and to formulate intervention strategies.

**Objectives**
- To determine the prevalence of smoking among adult males in Kyimyintine Township
- To find out the associated factors of smoking
- To identify the pattern of smoking and behavior of male smokers

**MATERIALS AND METHODS**

**Study area and population**
A cross-sectional community-based study was carried out in Kyimyintine Township in Yangon Division during March 2006. Two wards from this township were randomly selected and house-to-house survey was conducted. From each household, an adult male aged 15 years and above was chosen randomly. Information was obtained from male adults 15 years above, who gave consent to participate voluntarily. Of 510 houses, 486 completed the questionnaire.

**Data collection**
Trained interviewers administered a pre-tested, structured questionnaire. Informed verbal consent was obtained from each study participant. Respondents were assured of the confidentiality of the information, and every effort was made to ensure privacy. Socio-demographic information was obtained regarding age, marital status, educational level, occupational status and household monthly income. Regarding smoking status, respondents were asked: ‘Do you smoke?’ with possible responses being: ‘Yes – current smoker or past smoker’ and ‘Never smoker’. The current smokers were interviewed on their smoking behavior, like age of start smoking, length of time as a smoker, number of cigarettes smoked per day, use of tobacco in other forms, and smoking at home and in public places. Questions were also asked whether they ever attempted to quit or wanted to quit smoking and a paternal history of smoking.

Operational definitions were made for the study. Never-smokers were defined as those who had never smoked, past-smokers were those who had smoked in the past but had stopped for at least the previous six months. Current-smokers were those who are at present were smoking any amount of tobacco, either regularly or occasionally. Due to the small number of past-smokers, we merged this category into the category of never-smokers and termed them as non-smokers for the analysis.

**Statistical methods**
The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 10. The chi-square test and odds ratio with 95% confidence intervals were calculated using simple logistic regression to find out the factors associated with smoking.

**Ethical consideration**
This study was approved by the Medical Ethics Committee of the Department of Medical Research (Lower Myanmar).

**RESULTS**

**Background characteristics**
Mean age of the study population was $36.1 \pm 13.3$ years (range, 15-65 years). As shown in Table 1, 52.7 % were married and 14% had no schooling or primary level education only. The majority (67.3 %) had household income of less than 50,000 kyats per month. Regarding the occupation, 51.4% worked in office/business and 29.2 % were manual workers.

**Prevalence and associated factors of smoking**
Overall prevalence of current smokers in this study was 46.1%. Table 2 shows the factors associated with smoking. Apart from
Table 1. Background characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smokers n= 224</th>
<th>Non-smokers n= 262</th>
<th>Total n= 486</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;45</td>
<td>57(25.4)</td>
<td>81(30.9)</td>
<td>138(28.4)</td>
</tr>
<tr>
<td>30-44</td>
<td>82(36.6)</td>
<td>82(31.3)</td>
<td>164(33.7)</td>
</tr>
<tr>
<td>15-29</td>
<td>85(38.0)</td>
<td>99(37.8)</td>
<td>184(37.9)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>113(50.4)</td>
<td>143(54.6)</td>
<td>256(52.7)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>103(46.0)</td>
<td>109(41.6)</td>
<td>212(43.6)</td>
</tr>
<tr>
<td>Divorced / separated</td>
<td>8( 3.6)</td>
<td>10( 3.8)</td>
<td>18( 3.7)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school level</td>
<td>34(15.2)</td>
<td>34(13.0)</td>
<td>68(14.0)</td>
</tr>
<tr>
<td>Middle school level</td>
<td>72(32.1)</td>
<td>57(21.7)</td>
<td>129(26.5)</td>
</tr>
<tr>
<td>High school level</td>
<td>72(32.1)</td>
<td>88(33.6)</td>
<td>160(33.0)</td>
</tr>
<tr>
<td>Graduate</td>
<td>46(20.5)</td>
<td>83(31.7)</td>
<td>129(26.5)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Office</td>
<td>45(20.1)</td>
<td>55(21.0)</td>
<td>100(20.6)</td>
</tr>
<tr>
<td>Business</td>
<td>61(27.2)</td>
<td>89(34.0)</td>
<td>150(30.9)</td>
</tr>
<tr>
<td>Manual</td>
<td>72(32.1)</td>
<td>70(26.7)</td>
<td>142(29.2)</td>
</tr>
<tr>
<td>Student</td>
<td>13( 5.8)</td>
<td>18( 6.9)</td>
<td>31( 6.4)</td>
</tr>
<tr>
<td>Others</td>
<td>33(14.7)</td>
<td>30(11.4)</td>
<td>63(12.9)</td>
</tr>
<tr>
<td>Household income (kyats)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50,000</td>
<td>153(68.3)</td>
<td>174(66.4)</td>
<td>327(67.3)</td>
</tr>
<tr>
<td>50,000-100,000</td>
<td>45(20.1)</td>
<td>63(24.1)</td>
<td>108(22.2)</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>26(11.6)</td>
<td>25( 9.5)</td>
<td>51(10.5)</td>
</tr>
</tbody>
</table>

Figures in parenthesis denote percentages

demographic variables, paternal smoking and peer smoking were selected as associated factors of smoking and it was found that education was related with the smoking status. Respondents with university/college level education were less likely to smoke than those of no schooling or primary school level (OR = 0.55, 95% CI = 0.39-0.79). Paternal smoking (OR = 1.52, 95% CI = 1.05-2.2) and peer smoking (OR = 1.86, 95% CI = 1.22-2.83) were also associated with smoking. Other factors such as age, marital status, household income, and occupation were not found to have any significant association with smoking status (Table 2).

Smoker’s behaviors

Smoker’s behaviors are shown in Table 3. Mean age at start of smoking was 19.3 ± 5.9 years. The youngest age was 10 years and the oldest was 45 years. More than half (58.5%) started smoking before 20 years

Table 2. Associated factors of smoking (Univariate Analysis)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smoking prevalence (%)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;45</td>
<td>41.30</td>
<td>0.82</td>
<td>0.51-1.31</td>
<td>0.38</td>
</tr>
<tr>
<td>30-44</td>
<td>50.00</td>
<td>1.16</td>
<td>0.75-1.82</td>
<td>0.48</td>
</tr>
<tr>
<td>15-29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>44.10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>48.60</td>
<td>1.19</td>
<td>0.83-1.72</td>
<td>0.33</td>
</tr>
<tr>
<td>Divorced/ separated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>50.00</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school</td>
<td>55.80</td>
<td>1.26</td>
<td>0.69-2.28</td>
<td>0.44</td>
</tr>
<tr>
<td>High school</td>
<td>45.00</td>
<td>0.82</td>
<td>0.46-1.45</td>
<td>0.49</td>
</tr>
<tr>
<td>Graduate</td>
<td>35.70</td>
<td>0.55</td>
<td>0.39-0.79</td>
<td>0.04</td>
</tr>
<tr>
<td>Household income (kyats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50,000</td>
<td>46.80</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50,000-100,000</td>
<td>41.70</td>
<td>0.81</td>
<td>0.52-1.26</td>
<td>0.35</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>51.00</td>
<td>1.18</td>
<td>0.65-2.14</td>
<td>0.58</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Office</td>
<td>45.00</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>40.70</td>
<td>0.84</td>
<td>0.50-1.4</td>
<td>0.50</td>
</tr>
<tr>
<td>Manual</td>
<td>50.70</td>
<td>1.26</td>
<td>0.75-2.10</td>
<td>0.38</td>
</tr>
<tr>
<td>Student</td>
<td>41.90</td>
<td>0.88</td>
<td>0.39-2.00</td>
<td>0.77</td>
</tr>
<tr>
<td>Others</td>
<td>52.30</td>
<td>1.34</td>
<td>0.68-2.66</td>
<td>0.36</td>
</tr>
<tr>
<td>Paternal smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>40.00</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>50.30</td>
<td>1.52</td>
<td>1.05-2.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Peer smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35.10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>50.30</td>
<td>1.86</td>
<td>1.22-2.83</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 3. Smoking behavior among male smokers in Kyimyintine Township

<table>
<thead>
<tr>
<th>Smoker’s behaviors</th>
<th>Number</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of start smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>131</td>
<td>58.50</td>
</tr>
<tr>
<td>20-40 years</td>
<td>91</td>
<td>40.60</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>2</td>
<td>9.00</td>
</tr>
<tr>
<td>Mean ± SD 19.3 ± 5.9 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 years</td>
<td>58</td>
<td>25.90</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>166</td>
<td>74.10</td>
</tr>
<tr>
<td>Number of cigarettes smoked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 per day</td>
<td>140</td>
<td>62.50</td>
</tr>
<tr>
<td>5-10 per day</td>
<td>46</td>
<td>20.50</td>
</tr>
<tr>
<td>&gt;10 per day</td>
<td>38</td>
<td>17.00</td>
</tr>
<tr>
<td>Use of tobacco in other form</td>
<td>57</td>
<td>25.40</td>
</tr>
<tr>
<td>Smoked at home</td>
<td>157</td>
<td>70.10</td>
</tr>
<tr>
<td>Smoked at publics places</td>
<td>117</td>
<td>52.20</td>
</tr>
<tr>
<td>Want to quit smoking</td>
<td>142</td>
<td>63.40</td>
</tr>
<tr>
<td>Ever tried to quit smoking</td>
<td>136</td>
<td>60.70</td>
</tr>
</tbody>
</table>
and 75% smoked more than 5 years. Of all smokers, only 17% said they smoked more than 5 cigarettes/cheroots per day. About one-fourth (25.4%) of smokers also used tobacco in other forms. Large numbers smoked at home (70.1%) and at public places (52.2%) as well. Majority (63.4%) wanted to quit smoking and 60.7% had ever tried to quit. Sixty-four percent of smokers reported paternal smoking history.

**DISCUSSION**

The prevalence of smoking in this study was 46%. It was lower than 74% prevalence found in Cardiovascular Diseases Survey in 1993 [4]. But it was more or less comparable to 44.5% prevalence reported in Myanmar sentinel tobacco use prevalence study in 2001 [5] and 49% prevalence in World Health Survey 2003 [6]. Some participants in our study, particularly the young, might have hidden their smoking habit during the interview in the presence of other family members.

The level of education has been associated with smoking in a large number of studies [7, 8]. In these studies, an inverse relationship was seen between level of education and the prevalence of smoking. Our study also showed significantly lower smoking prevalence among educated persons.

Paternal smoking was found to be associated with smoking in this study. Moreover, 64.3% of them gave history of parental smoking. Parents are supposed to be role models for their children. The children, whose parents smoke, are more likely to smoke when they become adults.

It was also found that those with friends who smoke were more likely to smoke. More than half (58.5%) started smoking before 20 years of age (mean 19.3 years) in our study. Peer pressure is one of the key reasons for start of smoking in young age.

In different parts of the world, the highest prevalence of smoking was found in younger age groups and fell steadily in older age [9, 10]. In contrast, age was not related with smoking status in this study. Those in the older age were still the current smokers despite the fact that majority (61%) of smoking subjects had ever tried to quit smoking.

There was a strong association between duration of smoking, the number smoked per day and the development of different diseases [11]. It was also reported that most smokers acquired their habit during their teenage years [12, 13]. In this study, more than half (58.5%) started smoking before 20 years of age, and 75% smoked more than 5 years which may lead to the development of various health problems. But only 17% of smokers said they smoked more than 5 cigarettes/cheroots per day. This figure seemed to be low because we took into account the number of cigarettes and cheroots smoked per day altogether in the analysis.

Use of tobacco in other forms such as chewing with betel (Smokeless Tobacco-ST) has been documented as hazardous and cause oral and esophageal cancer [14]. ST is also associated with ischaemic heart disease and stroke deaths [15]. In our study, twenty-five percent of smokers also used tobacco in other form as well.

There was ample evidence of the health hazards of passive smoking [16, 17]. It was alarming in this study that large numbers smoked at home (70.1%) and at public places (52.2%). This provides a potent risk factor for a large number of chronic and crippling diseases to their family members, workmates and colleagues.

In summary, the prevalence of smoking was high among adult males in the study area. Most of them began to smoke before 20 years and continued for many years which may lead to the development of various tobacco-related diseases. Anti-smoking campaigns need to be intensified to combat smoking and smoking-related health problems in the future. There is also a need to change the smoker’s behavior to avoid the
health hazards of passive smoking. Health education of the public through media is very important in this regard.

ACKNOWLEDGEMENTS

The authors would like to thank Director-General of the Department of Medical Research (Lower Myanmar), for his kind permission to conduct the study. We are also grateful to Township Medical Officer and basic health staff in Kyimyintine Township for their kind help during the study.

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Optimization of a multiplex polymerase chain reaction assay and its application for simultaneous detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in clinical specimens

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*Bacteriology Research Division  
**Blood Research Division  
Department of Medical Research (LM)  
***Department of Medical Microbiology  
University of Malaya, Malaysia

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections are common sexually transmitted infections with a similar pattern of clinical presentation and related sequelae such as urethritis, cervicitis, pelvic inflammatory disease, ectopic pregnancy and infertility. Chlamydial co-infections are also frequently found in patients with gonorrhoea. This study was conducted to optimize a multiplex polymerase chain reaction (M-PCR) assay which could be applied to detect *N. gonorrhoeae* and *C. trachomatis* simultaneously from genitourinary specimens. The procedure of a M-PCR assay, developed by Mahony et al. (1995), was modified by incorporating lambda phage-*Neisseria* hybrid DNA as an internal control for the detection of amplification inhibitors. Thermal cycling parameters were optimized by varying annealing temperatures and using different concentrations of magnesium chloride, dNTPs and primers. The optimized M-PCR assay had a detection limit of $2.885 \times 10^3$ genome copies for *C. trachomatis* and $8.995 \times 10^2$ genome copies for *N. gonorrhoeae*. It was used to detect *N. gonorrhoeae* and *C. trachomatis* in 90 endocervical swab specimens from women with vaginal discharge. The specimens were also tested for chlamydial antigen using Clear View test kit (Unipath, UK) and for *N. gonorrhoeae* by conventional culture. The sensitivity and specificity of the M-PCR assay were found to be superior to those of gonococcal culture and chlamydia antigen testing, making it a potentially useful test for the diagnosis of *N. gonorrhoeae* and *C. trachomatis* in lower genital tract infections in symptomatic women.

**INTRODUCTION**

*Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections are common bacterial causes of sexually transmitted infections. They have a similar pattern of clinical presentation and related sequelae such as urethritis, epididymitis, cervicitis, pelvic inflammatory disease, ectopic pregnancy and infertility. Both organisms colonize the mucosal epithelium of the genital tract with the cervix as the primary site in women and urethra in men. Concomitant gonococcal and chlamydial infections are frequently found in men and women [1].

Multiplex Polymerase Chain Reaction (M-PCR) assays permit simultaneous screening for multiple pathogens that might be causing a disease condition. In multiple-agent infections, all the pathogens causing
the disease can be detected in a single specimen subjected to a single reaction [2]. This makes M-PCR a useful tool for the determination of the prevalence of sexually transmitted infections in the community, thus enabling further interventions for the prevention and management of these infections [3].

Numerous studies have demonstrated that a small, but significant, proportion of clinical specimens contain substances that interfere with the nucleic acid amplification process. Unless inhibitory specimens are identified, negative amplification test results do not exclusively indicate the absence of infection. Inhibitory specimens can be identified by monitoring the amplification of a second target nucleic acid which serves as an internal control [4]. The inclusion of an internal control (IC) in a PCR assay may decrease the sensitivity of the test because of the competition between the IC and the target DNA. Hence, thermal cycling conditions have to be optimized to overcome this competition and ensure adequate test sensitivity.

**General objective**

- To optimize a multiplex PCR assay which can detect *N. gonorrhoeae* and *C. trachomatis* (Ng-Ct M-PCR) simultaneously from genitourinary specimens

**Specific objectives**

- To determine the optimal conditions for a Ng-Ct M-PCR assay which was modified by incorporating an internal control to identify inhibitory specimens
- To assess the applicability of the optimized Ng-Ct M-PCR assay in clinical specimens.

### MATERIALS AND METHODS

Laboratory tests were carried out from January 2005 to October 2006 at the Molecular Diagnostics and Research Laboratory, University of Malaya, Malaysia and Bacteriology Research Division, Department of Medical Research (LM).

**Optimizing the Ng-Ct M-PCR Assay**

**a. Construction of a hybrid internal control (IC) for Ng-Ct M-PCR assay**

A 650 base pair (bp) lambda phage DNA segment ( # SD 0011) was selected for use in a hybrid internal control, as its G+C content (51%) and melting temperature were similar to those of the *N. gonorrhoeae* ccpp gene.

The hybrid IC was created in a single PCR with GCIC 1 and GCIC 2 primers with the following DNA sequences:

- GCIC1 primer (sense)
  5’GCTACGCATACCCGCGTTGCTTCCGGTTTAAGGCGTTTGC 3’
- GCIC 2 (anti-sense)
  5’CGAAGACCTTCGAGCAGACATCATCCAGCGCGGCTGCTTT 3’

The resultant 730 bp hybrid IC DNA, consisting of the 650 bp lambda phage segment flanked by 2 short sequences complementary to HO 1 and HO 3 primers of *N. gonorrhoeae* [5], was purified with QIA Quick PCR Purification Kit (Qiagen).

A series of 10-fold dilutions of hybrid DNA was amplified with HO 1 and HO 3 primers and the dilution presenting the faintest positive band was selected as the optimal amount of hybrid internal control to minimize the competition between *N. gonorrhoeae* target DNA and hybrid IC DNA.

**b. Optimizing thermal cycling conditions for Ng-Ct M-PCR with hybrid IC incorporated**

Primers KL1 and KL2 were used to amplify 241 bp fragment of ORF 2 of a 7.5 kb genetically conserved plasmid in *C. trachomatis* as described in Mahoney JB et al., 1992[6]

- KL 1 5’ TCCGGAGCGAGTTACGAAGA 3’
- KL 2 5’ AATCAATGCCGGGTTAAGGCGTTTGC 3’

Primers HO1 and HO3 were used for the amplification of a 390 bp fragment of the ccpp gene of a 4.2 kb *N. gonorrhoeae* cryptic
plasmid as described in Ho BSW et al., 1992[5]:

- HO 1 5’GCTACGCAATCCGGTTC 3’
- HO 3 5’CGAAGACCTTCGAGCAGACA 3’

*N. gonorrhoeae* ATCC 49226 and *C. trachomatis* L2 strain were used as positive DNA controls.

With the incorporation of the hybrid IC into the PCR mixture, thermal cycling conditions were modified and optimized by varying the annealing temperature and using different concentrations of magnesium chloride, dNTPs and primers.

**Sensitivity of Ng-Ct M-PCR Assay**

*N. gonorrhoeae* DNA and *C. trachomatis* DNA were estimated using optical density measurements at 260 nm in a spectrophotometer (Eppendorf, Hamburg). The sensitivity of Ng-Ct M-PCR was assayed with 10-fold dilutions of *N. gonorrhoeae* DNA and *C. trachomatis* DNA.

**Specificity of Ng-Ct M-PCR Assay**

Ng-Ct M-PCR assays were performed with the DNA of non-target bacterial strains (*Escherichia coli*, *Klebsiella* spp, *Staphylococcus aureus*, *Gardnerella vaginalis*) which can be found in the female genital tract.

**Detection of *N. gonorrhoeae* and *C. trachomatis* in clinical specimens**

**DNA Extraction**

DNA was extracted from 90 endocervical swabs which were collected from women presenting with vaginal discharge. This method involved cell digestion by lysis buffer which contained Proteinase K, Nonidet and Tween-20 followed by DNAzol treatment [7].

**Ng-Ct M-PCR assay**

Ng-Ct M-PCR assay was carried out using a thermal cycler (Perkin Elmer 480, USA) and two sets of primers: HO1-HO3 and KL1-KL2. PCR products were analyzed by electrophoresis on a 1.5% agarose gel in 1x TBE buffer system.

**Detection of *C. trachomatis* antigens**

*C. trachomatis* was detected from endocervical swabs using Clear View test kits (Unipath, UK) according to the manufacturer’s instructions. This is a solid phase sandwich immunoassay using chromatography and monoclonal antibodies to a genus-specific LPS antigen.

**Detection of *N. gonorrhoeae* by culture**

*N. gonorrhoeae* was isolated from endocervical swabs by inoculating onto Thayer Martin and chocolate agar and incubating up to 48 hr in 5% CO₂. The isolates were confirmed by gram staining, oxidase test and Phadebact GC monoclonal antibody test.

**Analysis of the results obtained by Ng-Ct M-PCR, antigen detection test and culture**

The M-PCR results were compared with those of conventional culture and antigen detection methods. The specimens with discrepant results were retested with single PCR assays for *N. gonorrhoeae* and *C. trachomatis*, using HO1-HO3 and KL1-KL2 primers respectively.

**Ethical consideration**

Strict confidentiality is maintained with regard to the identity of the subjects.

**RESULTS**

**Optimization of the M-PCR**

10-fold dilutions of the hybrid IC generated with HO1 and HO3 primers were visualized in a gel electrophoresis with ethidium bromide staining. The faintest positive band was obtained for the 10⁻¹¹ dilution. Thus 1 µl of 10⁻¹¹ hybrid IC was used in the M-PCR assay. Following optimization, PCR components used in the reaction mixture for the Ng-Ct M-PCR are shown in Table 1.

The thermal profile for the Ng-Ct M-PCR consists of 1 cycle of 95°C x 5 min followed by 40 cycles of 95°C x 1 min, 57°C x 1 min and 72°C x 2 min, and ending with a final extension at 72°C x 7 min.
Table 1. PCR components in the reaction mixture for Ng-Ct M-PCR

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile double distilled water</td>
<td>25.0</td>
</tr>
<tr>
<td>10 x PCR Buffer</td>
<td>5.0</td>
</tr>
<tr>
<td>dNTPs mix (10mM)</td>
<td>2.0</td>
</tr>
<tr>
<td>50 mM Magnesium chloride</td>
<td>4.0</td>
</tr>
<tr>
<td>5 U/µl Taq polymerase</td>
<td>0.4</td>
</tr>
<tr>
<td>HO 1 primer (5 pmole/µl)</td>
<td>0.5</td>
</tr>
<tr>
<td>HO 3</td>
<td>0.5</td>
</tr>
<tr>
<td>KL 1 primer (8 pmole/µl)</td>
<td>0.8</td>
</tr>
<tr>
<td>KL 2</td>
<td>0.8</td>
</tr>
<tr>
<td>DNA Template</td>
<td>10.0</td>
</tr>
<tr>
<td>Internal control (10⁻¹¹ dilution)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>TOTAL VOLUME</strong></td>
<td><strong>50.0</strong></td>
</tr>
</tbody>
</table>

The detection limit of M-PCR was calculated at the lowest dilution showing positive band for both Neisseria gonorrhoeae and Chlamydia trachomatis. Both N. gonorrhoeae and C. trachomatis were detected up to 10⁻⁴ dilution (Fig.1).

Sensitivity(Detection Limit) of Ng-Ct M-PCR

As a starting preparation of N. gonorrhoeae had 8.995 x 10⁶ genomes copies, the detection limit of it was 8.995 x 10² genome copies. Similarly, C. trachomatis which contained 2.885x10⁷ genomes copies in an initial preparation giving a detection limit of 2.885 x 10³ genomes.

Specificity of Ng-Ct M-PCR

None of the non-target bacterial strains were amplified, indicating that the primers used in Ng Ct M-PCR had a high specificity for N. gonorrhoeae and C. trachomatis.

Detection of N. gonorrhoeae and C. trachomatis in endocervical swabs by M-PCR

The M-PCR was positive for N. gonorrhoeae in 11 endocervical swabs and for C. trachomatis in 14, giving detection rates of 12.2% and 15.6% respectively. Specimens were classified as M-PCR negative only if the IC was amplified, indicating the absence of amplification inhibition (Fig. 2).

Analysis of results obtained by Ng-Ct M-PCR, antigen detection test and culture

The results of tests performed on the 90 endocervical swabs are summarized in Table 2. There were a total of 25 M-PCR
positive results, and 8 and 16 positives for Ng culture and Ct antigen testing respectively. Discrepant results were observed for 5 samples only, giving a concordance rate of 94.4% for paired M-PCR/Ng culture and M-PCR/Ct antigen tests. All specimens showing discrepant results between M-PCR and either Ng culture or Ct antigen detection was examined by corresponding monoplex PCR assays.

Table 2. Results of M-PCR compared to N. gonorrhoeae culture and antigen test for C. trachomatis

<table>
<thead>
<tr>
<th>Culture for Ng</th>
<th>Ag test for Ct</th>
<th>Total M-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>-</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>-</td>
<td>74</td>
<td>76</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>-</td>
<td>74</td>
<td>90</td>
</tr>
</tbody>
</table>

Of 11 M-PCR positives for Ng, 3 were culture negative. However, because the monoplex Ng PCR was also positive in these samples, they were classified as true positives. Similarly, in 2 M-PCR Ct negative but Clear View positive samples, the monoplex Ct PCR was negative, and these samples were classified as true negatives. Four specimens were positive for both Ct and Ng in the M-PCR. They were all positive for Ng by culture and in the CT antigen test.

Defining a true positive as a positive result in 2 different tests performed in a symptomatic patient, the clinical sensitivity and specificity of the M-PCR were found to be both 100%, while the corresponding sensitivities and specificities for Ng culture and Ct antigen test were 72.7, 100%, and 100%, 97.4% respectively.

DISCUSSION

Although conventional culture for N. gonorrhoeae and tissue culture for C. trachomatis remain as the gold standard methods for the detection of these organisms, there is an increasing use of nucleic acid amplification tests (NAAT) for the screening of sexually transmitted infections. In this study a Ng-Ct multiplex PCR was optimized with the inclusion of a suitable internal control for the detection of amplification inhibition. Its sensitivity and specificity were found to be adequate for the detection of both pathogens simultaneously from endocervical specimens of patients with vaginal discharge.

Many gonococcal and chlamydial infections are asymptomatic and therefore do not present at physicians’ clinics. For the screening of these cases, the use of non-invasive specimens like urine and low vaginal swabs is a preferred strategy. This strategy is made feasible with highly sensitive NAAT. The Ng-Ct M-PCR assay established in this study can be further optimized to increase its sensitivity for specimens with low number copies of N. gonorrhoeae and C. trachomatis so that it can also be used for the detection of asymptomatic infections using non-invasive specimens. This will make it an important tool not only for the diagnosis of overt infection but also for community-based prevalence studies of sexually transmitted infections.

ACKNOWLEDGEMENTS

We are grateful to Mr. Cheong Soon Fatt for helping with the designing of the Ng-lambda phage internal control used in the M-PCR. We also thank WHO HRP for the WHO Fellowship awarded to Wah Wah Aung that provided her the opportunity to conduct this study.

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Poisonous snakebites of Myanmar with special references to the bites and case fatality rate (1998-2005)

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Department of Medical Research (Lower Myanmar)
**Department of Health Planning
Ministry of Health, Naypyidaw

Retrospective study of data collected by the Department of Health Planning on bites and case fatality rate of poisonous snakes of the whole country from 1998 - 2005 were analyzed. The objective of the study was to determine the trend, number of bites and case fatality rate of poisonous snake bites of the states and divisions. The average poisonous snakebites (1998-2005) of the whole country were 8107 (6529-9600) with a case fatality rate of 7.43% (4.93-8.82%). The yearly trend of the snake bite is on increase and Mandalay, Magway, Sagaing and Bago (W) Divisions have the highest numbers of snakebite ranging from 1001 to 2000 per year and Chin, Kachin, Shan (East and North), Rakhine and Kayah States and Taninthayi Division, each has less than 50 per year. Townships with no report of snakebite are also highlighted. Ayeyawady Division has the highest case fatality rate 17.75%, followed by Rakhine State 10% and Magway Division 8.96%. The exceptionally high fatality rate (11-40%) of 24/26 townships of Ayeyawady Division needs to be investigated. The information obtained from the study will be useful for the policy makers and project managers concerned, in planning, distribution and in estimating the amount of antivenom required for the whole country.

INTRODUCTION

Snakebite is an occupational hazard of farmers and plantation workers. It is endemic in Myanmar. Epidemiology of snakebite of the country (1984-88) was presented at the National seminar on prevention and management of Russell’s viper bite in 1989 [1]. In the later years, few reports on epidemiological studies of snakebite have been published [2, 3]. Because of the increase demand of antivenom by the township hospitals, it is likely that the morbidity of snakebite is on increase. In order to know the situation of snakebites of the country, we carried out retrospective study of hospital data collected by the Department of Health Planning from all townships of the states and divisions of Myanmar from 1998 to 2005. The objectives of the study were to determine the morbidity and mortality and trends of snakebite occurring in townships and the states and divisions of the country in past 8 years and to identify townships with and without report of snakebites in order to estimate requirement and distribution of antivenom to township hospitals.

MATERIALS AND METHODS

Data related to poisonous snake bites and fatal cases (1998-2005) reported from 320 townships of the states and divisions of Myanmar collected by the Department of Health Planning, Ministry of Health were
studied. Coded data were entered and analyzed using SPSS version 11.5 software. Arbitral grading of the bites was used to stratify morbidity of the bites in the states and the divisions to cases ranging from 1001-2000/yr, 501-1000/yr, 101-500/yr, 51-100/yr and less than 50/yr and at township level to 101-200 bites/yr, 51-100/yr and less than 50/yr. Incidence of snakebite per 100000 was calculated based on population growth of 2% per year. Case fatality rate (CFR) was calculated by dividing number of fatal cases by the total number of bites and multiplied by 100.

RESULTS

Incidence of snakebite

The average poisonous snakebites per year of 8 years (1998-2005) of the whole country are 8107 (6529-9600). Mandalay, Magwe, Sagaing and Bago (west) Divisions have average poisonous snakebites per year ranged from 1001-2000, Ayeyawady, Bago (east) and Yangon (501-1000), Mon State (101-500) and Shan (south) and Kayin States (51-100). Majority of the states, Chin, Kachin, Shan (north and east), Rakhine and Kayah States and Taninthayi Division have average bite per year less than 50. Among them, Mandalay Division tops the chart (1828 bites/yr) followed by Magwe, Sagaing and Bago (west) Divisions. The snakebite morbidity per 100000 populations of the states and divisions are tabulated in Table 1 & 2. The incidences of snakebite per 100000 populations of all divisions except Taninthayi are higher than that of all states except Mon State. Bago (west) Division has the highest incidence of snakebite per 100000 populations throughout 8 years followed by Magwe, Mandalay, Bago (east) and Sagaing Divisions (Table 1). The yearly trend of CFR of snakebite of the country showed a rising trend (Fig. 2).

Trend of CFR

The yearly trend of CFR of the snakebite of the divisions (mean) and the states (mean) showed fluctuation between years with a rising trend in the former (data not shown) especially in Ayeyawady Division (Fig. 2). The yearly trend of CFR of snakebite of the country shows a similar rising trend.

Morbidity and mortality of snakebites of the states and divisions

Mandalay Division

All 31 townships of Mandalay Division reported incidence of snakebites during 8 years. Five townships (Myingyan, Kyaukpadaung, Taungtha, Meikhtila and Kyaukse) have incidence of snakebite (101-200/yr), 13 (51-100/yr), 7 less than 50/yr and 6 with no report of fatality
following the bites (data not shown). The CFR of the townships are less than 10% except in 3 townships (Kyaukpadaung, NyaungU and Sintkine) (11%).

Sagaing Division

Sagaing Division has the third highest snakebite in the country. Of 37 townships, Sagaing and Kantbalu townships have snakebite of 101-200/yr, 8 have 51-100/yr, 15 less than 50/yr and 7 with no report of fatality. Throughout 8 years, no report of snakebite was recorded in Nanyun, Mawlike, Layhae, Layshe and Indaw townships. Monywa, Pale and Yinmarbin townships have CFR 11-20%.

Bago (East and West) Division

Bago (E and W) Division has 14 townships each. Three townships from Bago (W) (Pyay, Thayawady & Paukkaung) and Nyaunghlaybin from Bago (E) have snakebites 101-200/yr. Nine townships from Bago (W) and 6 from Bago (E) have bites ranging from 51-100/yr and 2 from Bago (W) and 7 from Bago(E) have less than 5 bites/yr. Kawa, Thanatpin, Bago & Oaktwin townships from Bago (E)) have CFR of 11-25%.

Ayeyawady Division

Snakebite occurred in all 26 townships of Ayeyawady Division. Six have moderate bites (51-100/yr) and remaining 20 have less than 50/yr. Although snakebite morbidity was not so high, 92% (24/26) of the townships have CFR of 11-40%. This division has high yearly CFR throughout 8 years (Table 1). High CFR 31-40% was recorded in 4 townships (Zalun, Pyapone, Latbuta and Einme), 21-30% in 6 (Maubin, Pantanaw, Daedaye, Bogalay, Mawkyun & Kangyidaunk) and 11-20% in 14 townships.

Magwe Division

Twenty-four out of 25 townships of Magwe Division had reported incidence of snakebite during the study period except Gantgaw Township. Of 24 townships, Taungdwingyi and Aunglan have (101-200/yr) incidence of snakebite, 7 have 51-100/yr, 12 have less than 50/yr and 3 with no report of fatality following the bites. CFR of 11-20% were reported from 6 townships (Taungdwingyi, Natmauk, Myothit, Minbu, Pauk and Myaing).
Table 1. Snakebite morbidity and case fatality rate of divisions (1998-2005)

<table>
<thead>
<tr>
<th>Divisions</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
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<th>2003</th>
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<td>4.10</td>
<td>53.31</td>
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<td>6.00</td>
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<td>4.60</td>
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<td>3.60</td>
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Table 2. Snakebite morbidity and case fatality rate of states (1998-2005)

<table>
<thead>
<tr>
<th>States</th>
<th>1998</th>
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<tr>
<td>Mon</td>
<td>15.03</td>
<td>4.54</td>
<td>13.36</td>
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<td>5.49</td>
<td>7.85</td>
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<td>1.76</td>
<td>4.21</td>
<td>3.52</td>
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<td>6.45</td>
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<td>7.69</td>
<td>4.39</td>
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<td>2.68</td>
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<td>0.61</td>
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</table>
Majority of the states including Taninthayi Division have snakebites less than 500 in 8 years (<50/yr) except Mon, Southern Shan and Kayin States (50-200 /yr). No snakebite was reported in 12 townships (9.2%) (13/130) during 8 years and 57.7% (75/130) of townships have reports of snakebites with no fatality. The number of fatal cases reported in 8 years in the former (<50/yr) ranged from 1-8 and 1-18 in the latter (50-200/yr).

Township with the highest snakebites/ fatality in 8 years in each state and Taninthayi Division are, Nyaungshwe (159/7) (Southern Shan), Kutkhine (78/2) (Northern Shan), Kyaingtone (24/0) (Eastern Shan), Phyuso (38/0) (Kayar), Harkar (157/4) (Chin), Shwegu (66/1) (Kachin), Kawkayeik (180/13) (Kayin), Manaung (39/8) (Rakhine), Mudone (345/6) (Mon) and Palaw (41/1) (Taninthayi Division).

In summary, poisonous snakebites reported in 88.4% (383/320) of the townships of the country (7618 bites in 190 townships of the division and 489 in 130 townships of the states including one division) totaling to 8107 cases per year in 320 townships. Snakebite with no fatality was reported in 35.6% (101/383) of the townships (26 in the divisions and 75 in the states) amounts to 177 cases/yr (80 in the divisions and 97 in the states) (2% of the total bites). No report of snakebite was recorded in 11.5% (37/320) townships of the country (25 in the divisions and 12 in the states).

DISCUSSION

The trend of poisonous snakebite of the country of last 3 ½ decades (1970, 1980, 2000 and 2005) showed that there is much reduction in incidence of snakebite per 100000 populations in all states (1 to 8 times) and divisions (1.4 to 4.8 times) except in Bago Division (an increase from 54.5 to 73.63/100000), Mandalay Division (35.5 to 37.51/100000) and Shan State (6.1 to 7.48/100000) on comparing data at 1970 and 2005. However, the trend of snakebite morbidity of last 3½ decades indicated that there was a decrease in morbidity from 1970 to 1980, followed by an increase in the morbidity from 2000 to 2005. Fluctuation in the morbidity rate was observed between decades in Chin and Kayin States.

Later studies on epidemiology of snakebite of the country (1994-2002) indicated that the incidence of snakebite decreases from 1994 to 2000 and is on increase from 2001 to 2002 [2, 3]. The present observation on epidemiology of snakebite of last 8 years (1998-2005) is in agreement with the rising trend of snake bite and CFR.

The study showed that poisonous snakebite were reported in 88.4% of the townships of the country and its morbidity is high in the divisions (with reference to total numbers of the bites (7616/yr) as well as bites per 100000 populations) compared to the states (489 bites/yr). It is endemic in rice growing divisions namely Mandalay, Magwe, Sagaing, Bago (East and West), Ayeyawady and Yangon. Bago (W) Division has the highest incidence of snakebite per 100000 in the country with Bago (E) showing rising trend of snakebite morbidity. Most divisions are engaged in agricultural and plantation work and in some multiple cropping are in practice. Increase contact between man and snakes at work accounts for higher morbidity in the divisions compared to the states.

The average CFR of snakebite of the country (8 years) is less than 9%. However, high CFR (11-40%) recorded in townships with bites less than 50/yr in 4/7 townships in Bago (east) (11-25%), 7/11 townships in Yangon Division (11-40%) and Manaung (Rakhine State) (12.5-100% in 8 yrs) and in 24/26 townships in Ayeyawady Division (11-40%), raised a special concern in the current management of snakebite in the country. Failure of using protective wears at work, use of incorrect ineffective first aid
measures and delay in getting treatment at the health centres are important contributing factors for high CFR. It is high time to reevaluate the implementation of snakebite control programme of the country. Health education leading to change in attitude of farmers and plantation workers in using protective wears, correct first aid and avoid wasting valuable time should be achieved in order to bring down the morbidity and mortality of the snakebite. Further in-depth studies of the causes of high mortality in selected townships of the states and the divisions need to be carried out in order to find a remedy for it.

Community-based epidemiological study of snakebite carried out in Taungdwingyi Township (4) indicated that there was under reporting of morbidity of snakebite (17.5%) and CFR (38.7%) in data collected by the hospital. It is speculated that the morbidity and mortality rate of snakebite of the country could be much higher since not all snakebite cases sought medical treatment and usually terminal snakebite cases were discharged from the hospital on request.

Townships with reports of snakebite and no fatality
One hundred and one townships (35.6%) reported of poisonous snakebites with no fatality throughout 8 years, of which majority came from the states (75 townships). If the bites are due to genuine poisonous snakebites, then these bites could be attributed to green snake species, which inhabited elsewhere throughout the country and also could be result of “dry bites” of poisonous snakes. In clinical studies of snakebite cases, about 30% of the Russell’s viper (Daboia russellii siamensis) bites failed to inject venom into the victims or were “dry bites” [5]. Malayan pit viper (Calloselasma rhodostoma) bites could not be excluded in nonfatal bites occurring in Yale Township of Mon State and in Kayah State since the bites have been reported earlier in these places [6, 7]. It is suggested that inclusion of name of the species of snakes responsible for the bites in future study will be helpful in epidemiological survey of snakebites and selection of antivenom.

Townships with no report of poisonous snakebites
Throughout 8 years there were no reports of poisonous snakebites in 37 townships (11.5%) of the country including 19 townships in Yangon Division. Although green snake bites have been reported in Bahan Township in Yangon Division [8, 9] these were not on record of primary health centre of Bahan Township because the victims preferred to seek treatment at the Yangon General Hospital instead. Similar incidences might also occur in the other townships of the Yangon Division. It is likely that there were under reporting of the cases in primary health centres which needs to be taken into consideration in future compilation of the data. It is highlighted that antivenom could be spared in these townships with no report of snakebites.

Antivenom requirement and distribution
The study indicated the townships with different grades of morbidity, townships with no report of snakebite (n=37), townships with snakebite and no mortality (177 cases in 101 townships) and incidence of snakebites per 100000 population of the states and divisions of the whole country. Townships of the states and divisions of the country with high snakebite morbidity rate are also highlighted. It is hoped that the information provided from the study will be useful for health managers concern in quantifying antivenom required for the whole country and individual townships and distribution of antivenom to the target townships. Since 30% of the bites are “dry bites” [5], a substantial amount of antivenom could be saved. However, more useful data could be generated if species of poisonous snakes causing morbidity and mortality are known and it is suggested to be included in future hospital returns.
REFERENCES

Viability of the recombinant hepatitis B surface antigen expressed - *Hansenula polymorpha* yeast cells in Master Cell Bank

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For production of the recombinant hepatitis B (HB) vaccine, freeze-dried form of Master Cell Bank (MCB) containing the HBsAg expressed-*Hansenula polymorpha* yeast cells, stored at 4°C has been used as a starting material, followed by seed cultivation, fermentation and purification processes. In this study, freeze-dried form of MCB was reconstituted and viability of the HBsAg expressed-*Hansenula polymorpha* yeast cells was determined by observing cell morphology, growth pattern and presence of contamination during cultivation. It was found that there were actively growing viable yeast cells at different stages of cell divisions with specific characteristics. These cells also showed an increase in growth during cultivation with normal pattern of growth curve and were free from any contamination. Therefore, the recombinant hepatitis B surface antigen expressed-*Hansenula polymorpha* yeast cells in long-term storage at 4°C in MCB were found to be viable, stable and safe satisfactorily thus being suitable for further processing recombinant HB vaccine production.

**INTRODUCTION**

For production of vaccine using recombinant DNA technology, a gene that codes for a specific product can be isolated and propagated by insertion into a suitable vector with the aid of highly specific restriction endonuclease and ligase enzymes. The vector can then be introduced into host organisms, and individual clones that carry the desired genes can be selected and propagated in mass culture to obtain gene expression under controlled conditions with efficient synthesis of the encoded product [1]. At the CJ Pharmaceutical Corporation, Republic of Korea, the hepatitis B surface antigen (HBsAg) expressed-*Hansenula polymorpha* yeast cell was successfully developed using recombinant DNA technology in year 2000 for production of recombinant HB vaccine [2]. After cultivation of these cells with selective media, followed by mixing with skim milk solution, 0.5 ml of sterile cell media was aliquoted into individual containers. Then, lyophilization and sealing were done under sterile conditions. They were stored at 4°C and considered as Master Cell Bank (MCB). In this study, the viability of MCB which was stored at 4°C for about 4 years was determined with the aim to confirm the morphological stability and sterility of transformed yeast cells required for further production processes.

**MATERIALS AND METHODS**

The lyophilized form of MCB in a sterile glass ampoule, stored at 4°C for about
4 years, a product of CJ Corporation, Republic of Korea was reconstituted with 1 ml of autoclaved distilled water. The suspension was further used for the following tests.

**Growth pattern**

One-hundred microliter of MCB suspension was inoculated into 50 ml of 0.7% Yeast Nitrogen Base (YNB) with 2% glucose media broth in 250 ml conical culture flask, followed by incubation at 37°C in a shaking incubator (200 rpm) for 24 hours. The samples were taken from the above culture media every 4 hours for 24 hours and the optical density (i.e. cell growth) of each sample was measured in a spectrophotometer at 600 nm. The cell growth curve was determined by plotting the different OD against different times.

**Morphological stability**

The morphology and activity of the yeast cells in the culture samples were observed using an optical microscope at 1000 times magnification.

**Contamination test**

The presence of other microorganisms (bacteria or fungi) except the HBsAg-expressed yeast strain was checked in MCB strain as follows:

Culture plates for growth of different microorganisms such as Brain Heart Infusion agar, Lactose agar, Nutrient agar, Sabouraud Dextrose agar and Tryptic Soy agar were prepared. Then, 100 µl of MCB suspension in different dilutions i.e. $10^{-2}$, $10^{-4}$, $10^{-6}$, and $10^{-8}$ were inoculated to the above mentioned culture plates. These plates were kept at respective temperatures in an incubator for 72 hours and were checked every day for colony formation. After 3 days, the number, size and shape of colonies were observed. Among them, 10 colonies from each plate were randomly picked up and checked under an optical microscope for confirmation of shape of cells and colonies, and microbial species.

**RESULTS**

The growth pattern of MCB in culture broth is illustrated in Fig. 1. The yeast cells started to grow at 8 hours and growth rapidly increased at 16 hours and reached the peak level at 24 hours of cultivation.

![Fig. 1. Growth pattern of the HBsAg expressed (MCB) during cultivation](image)

The morphology and activity of these cells under the microscope are shown in Fig. 2. The specific appearance of *H. polymorpha* yeast cells was confirmed and actively growing cells with different stages of cell division were observed.

![Fig. 2. Morphological observation of MCB by optical microscope](image)

On daily checking of culture plates, there was no isolation of other microorganisms except *Hansenula polymorpha* cells which
showed their specific size and shape. In morphological observation under microscope, they showed specific appearance of *Hansenula polymorpha* cells only. It confirmed the identification of viable *Hansenula polymorpha* and free of contamination of other organisms of MCB strain during the growth period of 3 days culture (Table 1).

Table.1. Contamination test for microorganisms in different cultures

<table>
<thead>
<tr>
<th>Types of culture media</th>
<th>Condition (Temp)</th>
<th>Microorganisms allowed</th>
<th>Microorganisms detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Heart Infusion agar</td>
<td>30 ºC</td>
<td>fastidious microorganisms fungi, yeasts</td>
<td>H. polymorpha Other microbes</td>
</tr>
<tr>
<td>Lactose agar</td>
<td>37 ºC</td>
<td>coliform bacteria</td>
<td>+ –</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>37ºC</td>
<td>majority of the less fastidious microorganisms</td>
<td>+ –</td>
</tr>
<tr>
<td>Sabouraud Dextrose agar</td>
<td>30ºC</td>
<td>Yeasts, molds, acidic micro-organisms</td>
<td>+ –</td>
</tr>
<tr>
<td>Tryptic Soy agar</td>
<td>37ºC</td>
<td>Fastidious &amp; nonfastidious microorganisms</td>
<td>+ –</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Hepatitis B viral infection is a major health problem worldwide and caused by the HB virus. There is no cure for chronic HB infection, that is why prevention is so important. The HB vaccine is the best protection against HB infection [3]. In Myanmar, since the beginning of year 2004, the Hepatitis B Vaccine Plant, Department of Medical Research (LM), Ministry of Health has been producing recombinant HB vaccine and plasma derived HB vaccine as test run under the supervision of CJ / Samsung Corporation through the EDCF loan agreement from the government of the Republic of Korea. According to the contract, the plant will have a capacity of producing 5-million and 2-million pediatric doses of recombinant and plasma derived HB vaccines respectively after complete transfer of technology. For production of recombinant HB vaccine, the CJ corporation has provided a starting material i.e MCB containing the HBsAg expressed- *Hansenula polymorpha* yeast cells which have to further undergo fermentation and purification processes to produce the desired HBsAg protein for production of recombinant HB vaccine.

In this study, freeze-dried form of MCB stored at 4ºC for about 4 years was found to be viable and stable with normal growth pattern on cultivation. Specific morphological appearance and identification of yeast cells were also confirmed. Moreover, no growth of other microorganisms and absence of contamination were confirmed. Therefore, stored MCB containing the HBsAg expressed- *Hansenula polymorpha* cells was still viable with no notable difference from that in the pre-lyophilization stage. It could be concluded that both lyophilization procedure and long term storage of freeze-dried form of MCB at 4ºC do not affect the viability and sterility of the HBsAg expressed- *Hansenula polymorpha* cells. Therefore, the lyophilized form of MCB strain can be used effectively and safely in further processes of recombinant HB vaccine production in the HB Vaccine Plant.

**REFERENCES**

Antihypertensive effect of *Plantago major* Linn. whole plant (Ahkyawpaung-tahtaung) on mild to moderate hypertensive patients

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***Pharmaceutical Toxicology Research Division
****Chemical Toxicology Research Division
Department of Medical Research (LM)
*****Traditional Medicine Hospital

A clinical trial to determine the antihypertensive effect of medicinal plant, *Plantago major* Linn. (Ahkyawpaung-tahtaung) (whole plant) crude powder tablet was carried out on 10 mild to moderate hypertensive patients attending the out patient department (OPD) of Thingungyun Sanpya Hospital, Yangon and Traditional Medicine Hospital, Yangon. After washout period of 3 days, patients were treated orally with *Plantago major* Linn. whole plant crude powder tablet 3 g three times daily for 12 weeks. Blood pressure was monitored at 0 hr, 0.5hr, 1hr, 2 hr, 3 hr after first dose of trial drug. Monitoring of blood pressure and vital signs were done on day 1, day 2, day 3 and weekly up to 12 weeks. Laboratory investigations such as blood for complete picture, platelet count, random blood sugar level, liver function test, renal function test and ECG were done before and after the study. The results showed that reduction of blood pressure from baseline level was found at (0.5 hr-1 hr) after the first dose of the trial drug and was maintained up to 3 hr post dose. After 12 weeks of treatment with this trial drug, it was observed that significant reduction of mean blood pressure was from 150 ± 2.58/98 ± 1.33 mmHg (baseline blood pressure) to 129 ± 2.77/86 ± 1.63 mmHg (P<0.001). This trial drug decreased the mean systolic blood pressure and diastolic blood pressure from baseline level by 21 mmHg (p<0.001) and 12 mmHg (p<0.001) respectively. No side effects were observed. Therefore, it can be concluded that *Plantago major* Linn. showed significant antihypertensive effect on mild to moderate hypertensive patients with no side effects.

**INTRODUCTION**

Globally, hypertension is a common health problem found in both developed and developing countries [1]. There are many complications of hypertension principally involved the central nervous system, the retina, the heart, the vessels and the kidneys. Hypertension is a common cause of death in cardiovascular disease [2]. Today, the management of hypertension is a challenge to the medical profession. There has been a continuous search for a remedy which produces the least side effects and cost effectiveness. In Myanmar traditional medicine and Ayurvedic medicine of India, there are many medicinal plants which are known to have antihypertensive activity. People have used many antihypertensive plants even though they have not been investigated scientifically. *Plantago major* Linn. (Family - Plantaginaceae) (Fig.1) commonly known as (Ahkyawpaung-tahtaung) in Myanmar is a perennial herb with erect stout root stock which grows wild along streams, river banks and moist places.
in Shan State, Maymyo, and the Kachin State [3]. This medicinal plant has been widely used in traditional medicines of Myanmar and India for many years. The leaves have been known to be useful in fever, cuts and wounds and arthritis. Whole plant decoction has been used as diuretic [4, 5].

Fig. 1. Plantago major Linn. (Ahkyawpaung-tahtaung)

It was found that a soluble pectin polysaccharide, isolated from leaves of this plant had antibacterial effect [6]. It was also reported that pectin substance, plantaglucide isolated from the leaves has been effectively used in the treatment of peptic ulcer [7].

In the review article of Samuelsen (2000) [8] it was reported that a range of biological activities has been found from this plant extracts including wound healing activity, antiinflammatory activity, antioxidant activity, immunomodulatory activity and antisucregenic activity.

Myanmar people traditionally use this plant in the belief that it produces a fall in blood pressure [3]. Khin Kyi Kyi et al. (1971) [9] and Aye Than et al. (1977) [10] reported that this medicinal plant, Plantago major Linn. whole plant extract had shown hypotensive effect in dog model. It was also reported that this plant showed no acute toxic effect on mice. Thaung Hla et al. (2000) [11] stated that this plant had no sub-acute toxic effect on rats.

It has also been characterized physico-chemically and botanically [12]. Tin May Nyunt and Ohnmar May Tin Hlaing et al. (2003) [13] reported that in the clinical trial of short-term antihypertensive efficacy of crude powder of this whole plant in mild to moderate hypertensive patients, this plant showed significant antihypertensive effect with no side effects. In view of the above findings, this study was performed to determine the long-term antihypertensive effect of this medicinal plant on mild to moderate hypertensive patients.

**Objectives**

(1) To investigate the long-term antihypertensive effect of medicinal plant, Plantago major Linn. (whole plant) on mild to moderate hypertensive patients.

(2) To determine the side effects of this trial drug.

**MATERIALS AND METHODS**

*Plant collection and preparation of Plantago major Linn. tablet*

The mature fresh Plantago major Linn. whole plants collected from Shan State (Taunggyi) were carefully washed with tap water to remove dust and foreign material and dried under shade at room temperature. The air dried whole plants were powdered with electric grinder and made tablets with 500 mg each.
Patients selection

After ethical clearance from Department of Medical Research (LM), eligible patients from out patient department of Thimgungyun Sanpya Hospital and Traditional Medicine Hospital were selected according to the criteria. Only those patients who gave the voluntary informed consents were allowed to participate in this study and they had the right to withdraw at any stage.

1. Inclusion criteria

(i) Established essential hypertensive patients who had supine systolic blood pressure (SBP) ranged (140-170mmHg) and supine diastolic blood pressure (DBP) ranged (90-110 mmHg) were selected.
(ii) Both sexes of age between 30-70 years

2. Exclusion criteria

(i) Subjects who were not included in above inclusion criteria
(ii) Subjects with severe hypertension and hypertension with target organ damages
(iii) Patients taking regular treatment with long acting antihypertensive drugs (e.g. Amlodipine, Enalapril, Lisinopril etc)
(iv) Patients with other diseases (such as infectious diseases, central nervous system disease, heart disease, lung disease, liver disease, renal disease and endocrine organ diseases)
(v) Pregnancy, lactating mothers and children
(vi) Patients with regular consumption of alcohol

3. Withdrawal criteria

(i) Subject's request
(ii) Patients developing uncontrolled blood pressure
(iii) Attending physician's recommendation because of side effects of the trial drug
(iv) Patients with no response to the trial drug

Study design

The study design was an open typed (single arm) clinical trial. Ten mild to moderate hypertensive patients of both sexes were selected from out patient department of Traditional Medicine Hospital, Yangon and Thimgungyun Sanpya Hospital, Yangon.

Trial procedure

After getting the informed consent from each patient, history taking, physical examination and laboratory investigations such as blood for complete picture, platelet count, ECG, LFT (Liver Function Test), renal function test (ie. urine RE, blood urea and serum creatinine) and random blood sugar level were done before the trial drug study.

Ten established hypertensive patients with supine blood pressure ranged from 140/90 mmHg to 160/100 mmHg of both sexes took part in this study. Any previous anti-hypertensive therapies were stopped and wash out period for 3 days was done before entry into the trial. The patients were also advised not to take alcohol, smoking and salty diet during the study period.

As a preliminary study, 7 mild hypertensive patients with the blood pressure ranged from 140/90 mmHg to 150/100 mmHg were treated orally with trial drug 1.5 g (3 tablets) and blood pressures were measured before giving the trial drug and at 30 min, 1hr, 2hr, 3hr after taking the first dose. Then, the trial drug 1.5 g per dose for 3 times per day daily was administered to the patients. Follow up was done on day 2 and day 3. Blood pressure and vital signs such as heart rate, pulse rate and respiratory rate were monitored at each follow up. It was found that there were no changes in blood pressure up to 3 days. So, on the 4th day, the dose was increased to 3 g (6 tablets) per dose and the patients were treated with the trial drug 3 g three times daily. Then, blood pressure measurement and monitoring of vital signs were done as described above. Follow up was done on 2nd day and 3rd day of this dose. If there were reductions in blood pressure with 3 g three times daily dose, the patients were treated...
daily with this dose up to 12 weeks. Then, follow up was done weekly up to 12 weeks. Blood pressure and vital signs such as heart rate, pulse rate and respiratory rate were monitored at each follow up.

The remaining subjects with moderate hypertension were also treated with the trial drug 3 g three times daily for 12 weeks. Follow up and monitoring of blood pressure, heart rate, pulse rate and respiratory rate at each visit were also done as described above up to 12 weeks. Dosage adjustment was done according to the patient’s response. Side effects were recorded at each visit.

Blood pressure was measured on the right forearm by a standardized mercury sphygmomanometer and stethoscope by the same observer. Supine BP and standing BP were measured in this study. Supine BP was measured after the patient was in recumbent position for 10 minutes and standing BP was measured after patient had been standing for 2 minutes. Blood pressure was monitored in duplicate. Under each condition, the average of two measurements was taken [14]. Laboratory investigations such as blood for complete picture, platelet count, ECG, LFT (Liver Function Test), renal function test (ie. urine RE, blood urea and serum creatinine) and random blood sugar level were done again at the end of the study.

Data analyses

The results were shown in mean ± standard error and compared statistically with baseline levels applying student paired ‘t’ test.

RESULTS

Ten mild to moderate hypertensive patients of mean age 51.3 years (ranged from 40-70 years) of both sexes with mean blood pressure (X±SE), 150±2.58/98 ± 1.33mmHg (ranged from 140/90 to 160/100mmHg) took part in this study. The results of reduction of mean systolic and diastolic blood pressure from baseline levels after the first dose on the first day of the trial drug and day 2, day 3 and at each week are shown in Table 1.

Table 1. The effect of Plantago major Linn. on mean lying systolic and diastolic blood pressure in mild to moderate hypertensive patients (n=10)

<table>
<thead>
<tr>
<th>Time</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Mean difference from baseline</th>
<th>P value</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Mean difference from baseline</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>150 (2.58)</td>
<td>98 (1.33)</td>
<td>-</td>
<td>6.5 &lt;0.01</td>
<td>80 (2.58)</td>
<td>-</td>
</tr>
<tr>
<td>30min</td>
<td>140.5 (3.69)</td>
<td>9.5 &lt;0.001</td>
<td>91.5 (2.36)</td>
<td>6.5 &lt;0.01</td>
<td>81 (2.33)</td>
<td>7 &lt;0.01</td>
</tr>
<tr>
<td>1 hr</td>
<td>140.5 (3.37)</td>
<td>9.5 &lt;0.001</td>
<td>90.5 (2.41)</td>
<td>7.5 &lt;0.01</td>
<td>82 (2.41)</td>
<td>7.5 &lt;0.01</td>
</tr>
<tr>
<td>2 hr</td>
<td>139 (3.79)</td>
<td>11 &lt;0.001</td>
<td>87.5 (2.01)</td>
<td>10.5 &lt;0.001</td>
<td>89 (2.01)</td>
<td>10.5 &lt;0.001</td>
</tr>
<tr>
<td>3 hr</td>
<td>135.5 (3.2)</td>
<td>14.5 &lt;0.001</td>
<td>85.5 (2.01)</td>
<td>10.5 &lt;0.001</td>
<td>88 (2.01)</td>
<td>10.5 &lt;0.001</td>
</tr>
<tr>
<td>Day 2</td>
<td>140 (3.33)</td>
<td>10 &lt;0.001</td>
<td>90 (2.58)</td>
<td>8 &lt;0.001</td>
<td>92 (2.58)</td>
<td>8 &lt;0.001</td>
</tr>
<tr>
<td>Day 3</td>
<td>138.5 (2.99)</td>
<td>11.5 &lt;0.001</td>
<td>90 (2.58)</td>
<td>8 &lt;0.001</td>
<td>92 (2.58)</td>
<td>8 &lt;0.001</td>
</tr>
<tr>
<td>1st wk</td>
<td>137.5 (2.71)</td>
<td>12.5 &lt;0.001</td>
<td>88 (2)</td>
<td>10 &lt;0.01</td>
<td>92 (2)</td>
<td>10 &lt;0.01</td>
</tr>
<tr>
<td>2nd wk</td>
<td>134.5 (3.37)</td>
<td>15.5 &lt;0.001</td>
<td>88 (2.5)</td>
<td>10 &lt;0.001</td>
<td>92 (2.5)</td>
<td>10 &lt;0.001</td>
</tr>
<tr>
<td>3rd wk</td>
<td>136 (3.06)</td>
<td>14 &lt;0.001</td>
<td>89 (1.8)</td>
<td>9 &lt;0.001</td>
<td>92 (1.8)</td>
<td>9 &lt;0.001</td>
</tr>
<tr>
<td>4th wk</td>
<td>134 (3.06)</td>
<td>16 &lt;0.001</td>
<td>89 (2.33)</td>
<td>9 &lt;0.001</td>
<td>92 (2.33)</td>
<td>9 &lt;0.001</td>
</tr>
<tr>
<td>5th wk</td>
<td>134 (3.06)</td>
<td>16 &lt;0.001</td>
<td>85.5 (2.63)</td>
<td>12.5 &lt;0.01</td>
<td>88.5 (2.63)</td>
<td>12.5 &lt;0.01</td>
</tr>
<tr>
<td>6th wk</td>
<td>133.5 (2.12)</td>
<td>16.5 &lt;0.001</td>
<td>85.5 (1.5)</td>
<td>11.5 &lt;0.001</td>
<td>88.5 (1.5)</td>
<td>11.5 &lt;0.001</td>
</tr>
<tr>
<td>7th wk</td>
<td>131.5 (2.36)</td>
<td>18.5 &lt;0.001</td>
<td>84 (1.63)</td>
<td>14 &lt;0.001</td>
<td>86 (1.63)</td>
<td>14 &lt;0.001</td>
</tr>
<tr>
<td>8th wk</td>
<td>132 (3.35)</td>
<td>18 &lt;0.001</td>
<td>86 (2.67)</td>
<td>12 &lt;0.001</td>
<td>88 (2.67)</td>
<td>12 &lt;0.001</td>
</tr>
<tr>
<td>9th wk</td>
<td>131 (3.15)</td>
<td>19 &lt;0.001</td>
<td>85 (2.11)</td>
<td>13 &lt;0.001</td>
<td>87 (2.11)</td>
<td>13 &lt;0.001</td>
</tr>
<tr>
<td>10th wk</td>
<td>129 (2.33)</td>
<td>21 &lt;0.001</td>
<td>84.5 (1.57)</td>
<td>13.5 &lt;0.001</td>
<td>86.5 (1.57)</td>
<td>13.5 &lt;0.001</td>
</tr>
<tr>
<td>11th wk</td>
<td>131 (2.33)</td>
<td>19 &lt;0.001</td>
<td>86.5 (1.5)</td>
<td>11.5 &lt;0.001</td>
<td>88.5 (1.5)</td>
<td>11.5 &lt;0.001</td>
</tr>
<tr>
<td>12th wk</td>
<td>129 (2.77)</td>
<td>21 &lt;0.001</td>
<td>86 (1.63)</td>
<td>12 &lt;0.001</td>
<td>88 (1.63)</td>
<td>12 &lt;0.001</td>
</tr>
</tbody>
</table>

wk = week          hr = hour

Statistical comparisons were made between mean baseline blood pressure and mean blood pressure at different times, days and weeks.

The results of mean hourly systolic and diastolic blood pressure changes after treatment with the first dose of the trial drug on the first day of the study and weekly blood pressure changes up to 12 weeks
treatment with the trial drug are shown in Fig 2 & 3.

Mean pulse rates (X ± SE) were 81 ± 2.3 pulses /min at baseline and 75.2 ± 1.72 pulses/min at the end of 12 weeks treatment. Mean heart rates (X ± SE) were 81.2 ± 2.29 beats/min at the baseline and 76.2 ± 2.1 beats/min at the end of 12 weeks treatment. Mean respiratory rates (X ± SE) were 23 ± 2.11 times/min at the baseline and 20.8 ± 2.26 times/min at the end of 12 weeks treatment. It was found that at the end of the 12 weeks treatment with the trial drug, there were no significant changes in mean pulse rate, heart rate and respiratory rate from baseline levels (p >0.1).

![Fig 2](image)

**Fig 2.** Effect of *Plantago major* Linn. (whole plant crude powder tablet) on mean systolic and diastolic blood pressure (hourly changes) after the first dose of trial drug (X± S.E) *p<0.05, *p<0.01, ***p<0.001.

**DISCUSSION**

Khin Kyi Kyi *et al.* (1971)[9] reported that hypotensive screening tests of various extracts of this plant on dog model showed hypotensive activity in some extracts. Aye Than *et al.* (1977) [10] reported that the various extracts of this plant had been screened for their hypotensive activity on dog model. PM-9 fraction possessed the most effective hypotensive activity. No evidence of the hypotensive action was detected directly at the vascular smooth muscle, at the adrenergic neurone and at the sympathetic ganglion. The fact that PM-9 caused hypotension by acting centrally was confirmed in experiment with dogs.

Tin May Nyunt and Ohnmar May Tin Hlaing *et al.* (2003) [13] carried out the clinical trial of antihypertensive effect of *Plantago major* Linn. whole plant (crude powder tablet) in 10 mild to moderate hypertensive patients for one month. They reported that this plant showed significant antihypertensive effect on mild to moderate hypertensive patients and side effects were not detected.

This study was the 3 months clinical trial of antihypertensive effect of *Plantago major* Linn. whole plant (crude powder tablet) on 10 mild to moderate hypertensive patients.

In this study, the results showed that the significant reduction of blood pressure from baseline level was found at 0.5 hr–1hr after first dose and was maintained up to 3 hr post dose. Weekly blood pressure was maintained at less than 140/90 mmHg. After the 12 weeks treatment with this trial drug, it was observed that significant reduction of mean lying blood pressure was from 150 ± 2.58/98 ± 1.33 mmHg (baseline
blood pressure) to 129 ± 2.77/86 ± 1.63 mmHg (p<0.001). This trial drug decreased the mean lying systolic blood pressure and diastolic blood pressure from baseline level by 21 mmHg (p<0.001) and 12 mmHg (p<0.001) respectively. It was observed that initial blood pressure between 140/90 mmHg – 150/100 mmHg could be controlled with the trial drug dose of 3 g three times daily and for control of initial blood pressure 160/100 mmHg, 3.5 g three times daily was needed. Throughout the study period, no significant changes in mean pulse rate, heart rate and respiratory rate from baseline levels and no side effects were found. At the end of 12 weeks, it was found that there were no changes in laboratory investigations described above.

In conclusion, Plantago major Linn. (whole plant) showed significant antihypertensive effect on mild to moderate hypertensive patients with cost effectiveness and no side effects. In future, this herbal drug may be useful in the treatment of mild to moderate hypertensive patients.

ACKNOWLEDGEMENT

We would like to thank Professor Dr Paing Soe, Deputy Minister (Ministry of Health) for his valuable advice to perform this study. We also wish to thank Director-General and Deputy Director-General (Department of Medical Research, Lower Myanmar) for their encouragement and supporting funding to this study. We are also grateful to Daw Khin Taryar Myint, Research Officer (Pharmacology Research Division, Department of Medical Research, Lower Myanmar) for her help in buying the plants from Taunggyi.

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Detection of glucose-6-phosphate dehydrogenase G6PD enzyme deficiency in the field for treatment of malaria

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**Department of Health

Malaria is the first priority health problem in Myanmar and early diagnosis with prompt and effective treatment is essential for reduction of morbidity and mortality due to the disease. Primaquine is the only effective drug to prevent relapses of liver form of *Plasmodium vivax* and *Plasmodium ovale* and can also be used to kill gametocyte form of *Plasmodium falciparum* and *Plasmodium malariae*. Primaquine can cause haemolysis in glucose-6-phosphate dehydrogenase G6PD deficient individual and prevalence of G6PD deficiency varies among different ethnic races. Malaria survey was done during 2001-2003 in Mon and Shan States and prevalence of G6PD deficiency was investigated by rapid screening method of Hirano using DEAE (Di-Ethyl Amine-Ethylene) and Sephadex mixture. In normal person the test shows orange ring due to the presence of G6PD enzyme which is absent in G6PD deficient person. Among 1079 samples tested, 47 (4.5%) was found to have severe type of G6PD deficiency by the test. In relation to the ethnic region, G6PD deficiency rate was 5.5% (29/338) among Burmese, 3.2% (6/191) among Chinese, 3.4% (5/146) among Indians, 3.3% (3/92) among Mons, 5.1% (3/59) among Shans and 6.7% (1/15) among Kayin races. This rapid test can detect severe G6PD deficiency in the field, thus primaquine can be prescribed safely to malaria patients.

INTRODUCTION

Malaria ranks as the top priority health problem in Myanmar and nearly 600,000 outpatients and 120,000 in-patients are recorded annually in public health facilities. Early diagnosis and prompt, effective treatment is essential for reduction of morbidity and mortality due to the disease. Among currently using various antimalarials, primaquine is the only effective drug to prevent relapses of the liver forms of *Plasmodium vivax* and *Plasmodium ovale* and can also be used to kill gametocytes of *Plasmodium falciparum* and *Plasmodium malariae*. However, primaquine can cause severe haemolysis in persons with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency, whose prevalence varies among different races in malaria endemic countries. Routinely used methods for screening of G6PD deficiency [1, 2, 3] are costly or time-consuming, thus inconvenient for use in the field. Hirano et al. [4] have developed a rapid, single-step screening method for G6PD testing which requires only 5µl of blood, without expensive equipment and gives a test result within 40 minutes. This method was used to detect G6PD deficiency status among different races in Shan and Mon States together with malaria field surveys during 2001 to 2003. Some G6PD deficient blood samples were collected to identify the genetic pattern of deficiency to compare with those of other countries.

MATERIALS AND METHODS

Study area

Malaria endemic villages of Phar Auk and Kyaik Pun of Mudon Township, Mon State and Ho Peik and Mae Han of Lashio Township, Northern Shan State.
Study period

2001-2003

Method

Reaction mixture was prepared in a 1.5ml microcentrifuge tube as reported by Hirano et al. [4]. It contained 200µl each of DEAE (Di Ethyl Amine Ethylene) and Sephadex A 50Gel (Sigma Co. USA) equilibrated with 0.1Tris-HCl buffer pH 6.4 with 10mol. MgCl substrate mixture of 5 nM glucose-6-phosphateG6P), Boehringer, Germany, 0.4nM NADP (Nicotinamide Adenine Dinucleotide Phosphate) and 0.2% saponine.

Blood was collected from finger tip of malaria patients through a sterile single prick and 5µl of blood was mixed with 200µl of the above mixture in a microcentrifuge tube. The tube was kept in a dark plastic bag for 10 minutes and reading was done for a development of blue to purple ring (The ring was absent in G6PD deficient blood) (Fig 1 & 2). Heparinized blood of healthy volunteer with normal GPD activity and 0.9% saline were prepared for positive and negative controls respectively.

Inclusion criteria

Clinically suspected malaria patients of all ages and both sexes were included in the study for malaria microscopy and rapid G6PD testing.

RESULTS

The study showed that among 1079 samples tested for G6PD deficiency in Myanmar, 47 samples (4.4%) were found to have severe deficiency. Regarding to the respective races, Bamar (5.3%), Shan (5.1%) and Kayin (6.7%) were above the national value of 4.4%, while Chinese (3.2%), Indian (3.2%) and Mon (3.3%) were below the national value. It showed that severe G6PD deficiency varies widely among the different races of Myanmar (Table 1).

<table>
<thead>
<tr>
<th>SN</th>
<th>Races</th>
<th>Number of blood sample tested</th>
<th>Severe G6PD deficient samples</th>
<th>G6PD deficiency status (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bamar</td>
<td>550</td>
<td>29</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>Chinese</td>
<td>191</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Indian</td>
<td>146</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>Mon</td>
<td>39</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>Shan</td>
<td>59</td>
<td>3</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>Kayin</td>
<td>15</td>
<td>1</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>Others</td>
<td>26</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1079</td>
<td>47</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Glucose - 6 - phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy, affecting over 400 million people around the world. Vulliamy et al. [5] with the highest prevalence in the tropical Africa, the Middle East, tropical and subtropical Asia, parts of the Mediterranean and in Papua New Guinea. It was estimated as G6PD deficiency rate of 0.1% in Japan and Northern Europe, 25-30% in Africa and Asia [6]. In Myanmar, it was reported as 4-14% among various ethnic groups [7] and 15-17% among populations residing in the malaria endemic areas [8].
The gene encoding the G6PD enzyme displays X linked inheritance, implying that hemizygous males and homozygous females are the most likely to show clinical manifestations of the disease. G6PD deficient persons are prone to acute haemolytic anaemia, usually triggered by exposure to a variety of oxidants including antimalarials such as primaquine or quinine (or) by certain foods (fava beans) or most commonly by infections.

Primaquine is currently the single available drug, effective for the elimination of liver hypnozoites of Plasmodium vivax and P. ovale and also for killing of gametocyte forms of Plasmodium falciparum and P. malariae. Administration of primaquine to severe G6PD deficient person can give rise to acute severe haemolysis which is undesirable for malaria patients. Having G6PD deficiency status of malaria patient in the field is quite essential for radical treatment and successive implementation of malaria control strategies.

Routinely used G6PD tests are quite expensive and inconvenient to be used in the field. Rapid screening for G6PD test, as introduced by Hirono et al. [4] is quite suitable for malaria endemic countries especially in the field, where other G6PD tests are not suitable.

ACKNOWLEDGEMENTS

Reagents required for the study were kindly supplied by Professor Fumihiko Kawamoto of Oita University, Japan. The investigators are grateful to basic health staff and VBDC personnel whose active participation in the field is essential for smooth implementation of the study in the fields.

REFERENCES

Prevalence of malaria in Shwe Zar and Myothagyi villages of Maungdaw Township, Rakhine State, Myanmar

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*Parasitology Research Division  
**Immunology Research Division  
***Medical Entomology Research Division  
****Clinical Research Division  
***** Department of Medical Research (Lower Myanmar)

Shwe Zar and Myothagyi villages of Maungdaw Township, Rakhine State are in the border area of Myanmar and Bangladesh. These villages are in the coastal area of the Bay of Bengal. In May 2004, we studied the prevalence of malaria in these villages. Two hundred and fifty one villagers were recruited from Shwe Zar village and 400 villagers were recruited from Myothagyi village. Diagnosis of malaria was done by thick and thin blood films with direct microscopy. The prevalence of malaria in Shwe Zar and Myothagyi villages were found to be 12.75% and 8.5% respectively. In Shwe Zar and Myothagyi villages, P. falciparum parasite positivity rates in total malaria positive patients were 81.25% and 79.41%. P. vivax were 18.75% and 17.65%, gametocyte positive rates were 0.39% and 0.25% and Parasite Density Index (PDI) were 1.9 and 1.79 respectively. Only one mixed (P.f+P.v) infection was found in Myothagyi village. Further detailed study of parasitological, entomological, immunological and sociological studies need to be conducted for epidemiological perspective.

INTRODUCTION

Malaria is one of the most widespread diseases and is a major public health problem of tropical and subtropical countries. In Myanmar, malaria has been identified as one of the most important public health problems in every national health plan since 1978. It is still the main public health problem in Myanmar. It has been accorded first priority disease in third People's Health Program (PHP) (1986-1990). Malaria is caused by a protozoan parasite of the genus Plasmodium. Plasmodium falciparum, Plasmodium vivax, and Plasmodium malariae are commonly known to infect people living in Myanmar. Although P. vivax and P. malariae infections are not generally life threatening, sometimes they can cause severe acute illness. Plasmodium falciparum is one of the most dangerous species compared to others; it causes complications, such as cerebral malaria and sometimes death. Plasmodium falciparum is the dominant malaria parasite species in Myanmar, causing 85% of the infected cases [1, 2, 3, 4] but incidence of P. vivax is gradually rising in some parts of the country [5]. However, reliable determination of the site of transmission is problematic for Myanmar. Anti-malaria programmes are being implemented in many areas of the country where villages are located close to the suitable habitats for vectors, such as forests, forested foothills, rubber plantations and rice fields etc. Myanmar is one of the malaria endemic areas in Southeast Asia region because of its climate and geographic situation, which provide favorable ground for breeding of
mosquitoes. The most troublesome areas in the country are located mainly in the borders with forested mountain regions where mosquitoes can breed well. Border areas in Myanmar are mostly forest-fringe foothills and swamp areas where temperature and rainfall would most likely allow the Anophelean mosquitoes to survive and multiply. An. dirus and An. minimus are primary vectors in Myanmar and they are widely distributed in forests, forested foothills, rubber plantations and rice fields [6, 7]. Tun Lin et al., [3] had already reported on An. dirus occurrence in water wells within a coastal village where all shade comes from occasional fruit trees. Incidence of malaria is also high in cold-dry season in coastal areas [8, 9, 4]. An. dirus is widely distributed in Bangladesh and Thailand [10, 11, 12, 13, 14]. The present study was planned to investigate the prevalence of parasite, risk area and malaria infection in human population from the border area of Maungdaw Township, Rakhine State.

MATERIALS AND METHODS

Study area and population

The study was conducted in Shwe Zar and Myothagyi villages of Maungdaw Township, Rakhine State which are border areas of Myanmar and Bangladesh. The area is situated in the North-west part of the Rakhine State; Shwe Zar is four miles away from Myothagyi and near a rocky hill area. Paddy fields, creeks, water pools, streams, wells and ponds are also present in these areas. The total population of Shwe Zar and Myothagyi are about 5000 and 6000 respectively. Ninety percent of the population are farmers. The climate of these areas comprises of heavy rainfall and heavy wind in the rainy season. Some local people fish in streams, coastal area, sea and deep sea water and some are doing prawn culture.

Most of the villagers go into the hilly area to cut bamboo and wood for general use. The houses are made of wood and bamboo and some houses are constructed of brick. About 90% of the villagers are Muslims. Maungdaw area is a border area of Myanmar and Bangladesh.

Blood collection

For prevalence of malaria in the village population, 251 villagers from Shwe Zar and 400 villagers from Myothagyi villages were recruited for the malaria study. Finger-tip blood specimens were collected on grease-free clean glass slides. Thick and thin blood films were made on the glass slides. Thick film was used for the detection of malaria parasite and thin blood film was used for species identification.

Staining and examination of malaria parasite

Thin blood films were fixed with 100% alcohol then dried in room temperature, after that thick and thin blood slides were stained with 10% Giemsa's stain for 10 minutes. After staining, slides were washed with buffer water and dried in room temperature. Thick and thin films were examined under high power oil immersion lens (x 100), Olympus. Malaria parasites were counted against 300WBC according to WHO method.

Data analysis

Microsoft excel was used for malarriometric calculations.

RESULTS

Microscopic examination of malaria parasite

The result of malaria prevalence study in Shwe Zar and Myothagyi villages of Maungdaw Township, Rakhine State is shown in Table 1 & Fig. 1. Of the recruited population from Shwe Zar, 12.75% were found to have malaria, out of which 81.25% were P. falciparum and the rest 18.75% were P. vivax positive. In Myothagyi village, 8.5% of the population were malaria positive. Among these cases, 79.41% were due to P. falciparum and 17.65% were
caused by *P. vivax* and 2.94% were mixed infection in Myothagyi village.

Table 1. Parasite positive rate by microscopic examination in villages of Maungdaw Township, Rakhine State

<table>
<thead>
<tr>
<th>Study location examined</th>
<th>Total examined</th>
<th>Total positive</th>
<th>Malaria parasite species</th>
<th>Gametocyte positive</th>
<th>Parasite density index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shwe Zar</td>
<td>251</td>
<td>32</td>
<td>12.8%</td>
<td>81.3%</td>
<td>(0.39) 1.9</td>
</tr>
<tr>
<td>village</td>
<td></td>
<td>26</td>
<td>6%</td>
<td>88.1%</td>
<td></td>
</tr>
<tr>
<td>Myothagyi</td>
<td>400</td>
<td>34</td>
<td>8.5%</td>
<td>79.4%</td>
<td>(0.25) 1.79</td>
</tr>
<tr>
<td>village</td>
<td></td>
<td>27</td>
<td>6%</td>
<td>94.1%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>651</td>
<td>66</td>
<td>10.4%</td>
<td>80.3%</td>
<td>(0.31) 1.81</td>
</tr>
<tr>
<td>population</td>
<td></td>
<td>53</td>
<td>12%</td>
<td>58.8%</td>
<td></td>
</tr>
</tbody>
</table>

*P. f = P. falciparum*  
*P. v = P. vivax*  
*mixed = P. falcip + vivax*

Gametocyte rate

Gametocyte positivity rate of Shwe Zar and Myothagyi villages were 0.39% and 0.25% respectively (Table 1, Fig. 2).

Parasite Density Index (PDI)

Parasite Density Index of Shwe Zar and Myothagyi villages were 1.9 and 1.79 respectively (Table 1, Fig. 3).

DISCUSSION

Malaria is considered the first national priority disease in Myanmar [15] since it also affects the socioeconomic condition of the country. Anti-malaria programme has successfully decreased the incidence in many areas of the country where villages are located close to the suitable habitats for vectors. However malaria is gradually rising in some parts of country, such as forests, forested foothills, rubber plantations and rice fields. Previous studies [3,6] from Thabye-wa village, Oktwin Township, Bago Division and a coastal area of Yepyu Township, Thanintharyi Division [4] found that, the parasite positivity rates were 46.8%, 30.1% and 54% respectively. Those are higher than our findings (12.75% in Shwe Zar and 8.5% in Myothagyi). A study at Ann Township of Rakhine State [17] revealed that the parasite positive rate was 29.64%. It is higher when compared with our present study. Malaria parasite positive rate detected by microscopy in Tachileik Township was 10.76% [18] and it is similar to our present study. Another study in the same place showed 14.77% in pregnant women, 17.33% in delivery cases, 22.66% in placenta and 2.66% in neonatal blood respectively [19]. Several researchers reported that *P. falciparum* is the dominant
species (>80% in different parts of Myanmar [16, 20] and the present study also showed 81.25% and 79.41% P. falciparum prevalence in villagers from Shwe Zar and Myothagyi. The present study was conducted in April and May and the parasite positive rate was found to be lower, but it may be higher in transmission season. Gametocyte positive rates were 0.39% and 0.25% in Shwe Zar and Myothagyi villages. Parasite Density Index (PDI) of Shwe Zar and Myothagyi villages were 1.9 and 1.79 respectively. The PDI was lower than that of Tha-by-e-wa village i.e. 3.23 in the study of Tun Lin et al., (1995). An. dirus and An. minimus are primary malaria vectors of Myanmar. An. dirus is widely distributed in forested and forest foothill areas [20, 21, 16,3] and also distributed in domestic wells at Mon State and Tanintharyi Division [8,4]. Khin Maung Kyi et al., [6] epidemiologically studied that area and assumed that An. annularis could be a vector in Sittway. During the cyclone disaster in Rakhine State in August-September 1968, a severe malaria epidemic occurred and during that time An. annularis was found to breed in profusion around villages and also found resting in human habitations. Another study found that An. barbiostrias, An. hyrecanus, An. vagus, An. maculatus and An. culicifacies were the most dominant mosquito species in Ann area of Rakhine State whereas An. annularis is the primary vector [17]. Based on the topography of the areas, possible vectors may be An. minimus and An. annularis. Both species were collected near the study areas during the survey conducted by MERD, DMR (LM) in 2006 (unpublished record). The changes in ecology influence the mosquito population, vector prevalence and human behavior. During the study period of April and early May 2004, parasitological studies indicated that gametocyte carriers are present in the villages and >10% were positive for malaria parasite. It is assumed that, seasonal changes in post monsoon situation may aggravate the malaria prevalence in these areas and therefore, proper surveillance is required. Seasonal parasitological, entomological, immunological and social studies are required to have a complete epidemiological picture of these border areas.

ACKNOWLEDGEMENTS

The authors thank our Director-General for his support and the staff of the Medical Entomology Research Division for assistance rendered in conducting this research. We are grateful to the authorities concerned, the Township Medical Officers and staff of Maungdaw Township and the residents of Shwe Zar and Myothagyi villages for their help and cooperation.

REFERENCES


SHORT REPORT

Profile of immunoglobulin deposits detected by immunoperoxidase method in patients with idiopathic glomerulonephritis

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Department of Medical Research (Lower Myanmar)
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Department of Health

Glomerulonephritis (GN) refers to inflammation of glomeruli. Glomerulonephritis can be considered as primary when the major problem appears to start in the glomerulus, and secondary when involvement of glomeruli is part of a systemic disease [1]. The prognosis of various glomerulonephritis varies widely. Identification of the histology of GN enables an initial diagnosis to be made and a prognosis to be given.

Based on the histopathology of idiopathic glomerular disease, a modified classification by WHO is as follows: Minimal change nephropathy, focal proliferative GN, focal and segmental glomerulosclerosis, membranous glomerulopathy, diffuse mesangial proliferative GN (Ig A and Ig M), diffuse endocapillary GN, mesangiocapillary GN (type I and II) (also known as membrano-proliferative GN), and crescentic GN [2].

The correct and appropriate treatment for the many diverse types of renal diseases depends on the accurate diagnosis which will rest mainly on laboratory findings. This will complement the clinical diagnosis which, on its own, will not suffice since clinical manifestations and features are non-specific.

Immunohistochemical methods are usually performed in various tissues to identify immunoglobulins, complement factors and surface antigens. Immunoperoxidase (ImPx) method has been already preferred among others in immunohistochemistry using light microscopy and has become a standard staining technique in most of the diagnostic pathology laboratories worldwide [3,4,5].

ImPx, either direct or indirect, method is employed to renal specimens as a routine procedure at Department of Medical Research (Lower Myanmar) (DMR-LM). Renal biopsy specimens of patients with glomerulonephritis are sent routinely to the Pathology Research Division from the Renal Units of Yangon General Hospital and Thingangyun General Hospital. Report of Haematoxylin and Eosin staining, performed at respective hospitals, also accompanies each of the paraffin-embedded specimen.

At the Pathology Research Division, each paraffin-embedded specimen was cut into six serial sections, each section 3 micron thick. Immunostaining was done using rabbit antisera to human IgA, IgG, IgM, C3c Complement by direct method and C4c and C1q Complement by indirect method. The reagent activity of the antisera was always checked with known positive and negative controls of biopsy tissues. The immunostained biopsy tissue slides were examined and viewed under ordinary light microscope. Brownish yellow stains or staining deposits were taken as positive. Only glomerular positive stains were taken as significant particularly in mesangium, capillaries, membranes and matrix of
glomeruli. Tubular stains are neither important nor significant.

During the period from 1 January 2004 to 31 December 2006, 371 renal biopsies had been received. Among them, membranoproliferative glomerulonephritis, diffuse mesangial proliferative glomerulonephritis, focal and segmental glomerulonephritis, crescentic glomerulonephritis and minimal change nephropathy constituted 148 (39.9%), 115 (31.1%), 35 (9.5%), 33 (8.9%) and 12 (3.2%) respectively. The profile of immunoglobulin and complement deposits detected by ImPx method for each disease category is shown in Table 1.

**Table 1.** Summary of immunoglobulin deposits detected by immunoperoxidase method in patients with idiopathic glomerulonephritis (2004-2006)

<table>
<thead>
<tr>
<th>Disease Category</th>
<th>Immunoglobulin deposits detected in each disease category (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranoproliferative GN (MPGN)</td>
<td>IgA: 67.3, IgG: 59.2, IgM: 34.7, C3c: 27.9, C4c: 73.4, C1q: 66.6</td>
</tr>
<tr>
<td>Diffuse mesangial proliferative GN (DMPGN)</td>
<td>IgA: 60.9, IgG: 58.3, IgM: 33.1, C3c: 27.8, C4c: 72.2, C1q: 51.3</td>
</tr>
<tr>
<td>Focal and segmental glomerulosclerosis</td>
<td>IgA: 82.9, IgG: 48.6, IgM: 42.9, C3c: 91.5, C4c: 91.5</td>
</tr>
<tr>
<td>Membranous glomerulonephritis (membrano-</td>
<td>IgA: 66.7, IgG: 42.4, IgM: 21.2, C3c: 75.8, C4c: 60.6</td>
</tr>
<tr>
<td>proliferative GN)</td>
<td></td>
</tr>
<tr>
<td>Crescentic GN</td>
<td>IgA: 74.9, IgG: 49.9, IgM: 41.7, C3c: 58.3, C4c: 83.3</td>
</tr>
<tr>
<td>Minimal change nephropathy</td>
<td>IgA: 100, IgG: 100, IgM: 100, C3c: 60.6</td>
</tr>
<tr>
<td>SLE nephropathy</td>
<td>IgA: 66.6, IgG: 49.9, IgM: 49.9, C3c: 66.6, C4c: 49.9</td>
</tr>
<tr>
<td>Others</td>
<td>IgA: 59.7, IgG: 35.5, IgM: 63.9, C3c: 63.9</td>
</tr>
</tbody>
</table>

n = No. of cases

According to the data for the reporting period, there were 148 cases of membranoproliferative glomerulonephritis and was found to be the commonest disease category constituting 39.9% of the total. In this category, C4c was detected in 73.4%, IgA in 67.3%, C1q in 66.6%, IgG in 59.2%, IgM in 34.7%, and C3c in 27.9% of cases.

There were 115 cases of diffuse mesangial proliferative GN. This category constituted 31.1% of the total and was the second commonest disease category. In this category, C4c was found in 72.2%, IgA in 60.9%, IgG in 58.3%, C1q in 51.3%, IgM in 33.1%, and C3c in 27.8% of cases.

As the report was based on the data from routine procedures of DMR-LM, the main drawback in this short report is that we are unable to make a comparison of the findings between ImPx and other methods like Immunofluorescence Microscopy and Electron Microscopy. We also lack to correlate clinical manifestations and glomerular pathology.

**REFERENCES**

SHORT REPORT

ABO blood groups distribution among Russell's viper (Vipera russelli) bite patients with systemic envenomation

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The ABO-blood group distribution is not uncommonly associated with many diseases [1, 2, 3]. It is also well documented that Russell's viper bite envenomation, one of the major health problems in the countries of Southeast Asia region including Myanmar, induces haematological and coagulation changes in victims [4, 5]. However, ABO blood group distribution among the victims of Russell's viper bite envenomation has not been studied yet. Therefore, it is necessary to determine whether ABO blood group distribution is related to systemic envenomation and various complications in the victims following Russell’s viper bite. This was a hospital-based, non intervention, descriptive study, carried out on Russell's viper bite patients admitted to the Tharyarwady Township Hospital, Bago Division. Cases of Russell's viper bite were confirmed by identifying the dead snake brought with the patient to the hospital and/or detection of the development of signs and symptoms of Russell’s viper bite envenomation. Finger stick blood specimens were taken from these victims at the time of admission. ABO-blood group typing was performed by conventional technique using commercial reagents; known anti-A and anti-B. These patients were thoroughly examined and kept under close observation for a minimum of 5 days in the hospital. Anti-snake venom therapy was indicated according to the guidelines recommended by National Seminar on Snake Bite held in Myanmar in 1989 and complications following Russell’s viper envenomation were also recorded. It was found that the percentage distribution of A,B,AB and O blood groups in 112 Russell’s viper bite victims were found to be 25.9 %, 31.3 %, 8.9 % and 33.9 % of total cases respectively. The various complications following Russell's viper bite envenomation such as disseminated intravascular coagulation, clinical proteinuria, oliguric acute renal failure, hypotension, systemic bleeding and death among these victims were observed in 42.9 %, 41.1 %, 20.5 %, 12.5 %, 10.7 % and 8% of total cases respectively. Table 1 summarizes the detailed distribution of Russell's viper bite patients with different ABO blood groups among various complications following systemic envenomation in this study.

It is apparent from our findings that there was no significant relationship between frequency distribution of ABO blood groups and various types of complications following envenomation in Russell’s viper bite victims. Numbers of Russell's viper bite victims with different ABO blood groups were found to be equally distributed in all types of complications. In other words, there was an equal distribution of ABO blood groups in all types of complications following systemic envenomation encountered in Russell's viper bite victims. There was no possibility that ABO blood
Table 1. Distribution of ABO blood groups in Russell’s viper bite patients with systemic envenomation and various complications.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Total cases studied</th>
<th>No of patients with types of complications (Percentage)</th>
<th>DIC</th>
<th>Proteinuria</th>
<th>Oliguric</th>
<th>ARF</th>
<th>Hypotension</th>
<th>Systemic bleeding</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29</td>
<td>13 (44.8) 12 (41.4) 6 (20.7) 4 (13.8) 3 (10.3) 2 (6.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>35</td>
<td>15 (42.9) 15 (42.9) 8 (22.9) 4 (11.4) 3 (11.4) 2 (8.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>10</td>
<td>4 (40) 4 (40) 2 (20) 1 (10) 1 (10) 1 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>38</td>
<td>16 (42.1) 15 (39.5) 7 (18.4) 5 (13.2) 4 (10.5) 3 (7.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>48 (42.9) 46 (41.1) 23 (20.5) 14 (12.5) 12 (10.7) 9 (8)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

\[X^2\] 0.09 0.09 0.22 0.15 0.03 0.27

p value 0.99 0.99 0.97 0.98 0.99 0.44

Significance NS NS NS NS NS NS

Percentage of cases studied are shown in parentheses. Significance p values were calculated by using the Chi-square test. NS means not significant at the 5% probability level.

group distribution is related with Russell's viper bite envenomation and development of various complications.

REFERENCES