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**Detection of *Mycobacterium leprae* by the polymerase chain reaction (PCR)
in nasal swabs of leprosy patients and their contacts**

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In the light of current leprosy control strategies, non-invasive samples such as nasal swabs may be more important than skin slit specimens as a source of material for epidemiological study. The objective of this research project was to investigate the use of a polymerase chain reaction (PCR) test to detect *M. leprae* in samples of nasal mucous from leprosy patients and asymptomatic household contacts of those patients. Nasal swabs from 15 paucibacillary (PB) and 55 multibacillary (MB) patients attending the Central Special Skin Center, Yangon General Hospital and 137 of their household contacts were tested for the presence of *M. leprae* by PCR and 33% of the samples of both patients and contacts were found to contain *M. leprae*. One of 32 (3.1%) swabs and 21 of 105 (20%) swabs were positive for *M. leprae* among contacts of PB and MB patients respectively ($p < 0.05$). Among the patients, PCR positivity for nasal swab was 3 out of 15 (20%) in PB patients and 45 out of 55 (81.82%) in MB patients ($p < 0.001$). Therefore, total 48 out of 70 (68.57%) in clinically diagnosed patients was PCR positive. PCR positivity of MB is significantly higher than PB in both patients and contacts. Although nasal carriage does not necessarily imply infection or excretion of bacilli, the finding of nasal carriage supports the theory of a disseminated occurrence of *M. leprae* in populations for which leprosy is endemic. This study is part of the molecular epidemiological study of leprosy in Myanmar.

INTRODUCTION

Following the introduction of the multidrug therapy (MDT) program in 1980, there has been a significant reduction in the estimated prevalence of leprosy worldwide, from around 12 million in early 1980, to approximately 1 million now. However, this decline in prevalence has not been mirrored by a concomitant fall in the observed incidence of the disease: over half a million new cases are still detected annually, a figure similar to that of 1985 [1]. This apparent discrepancy suggests that the widespread use of MDT is having little impact on the transmission of leprosy. Although the causative agent of leprosy, *Mycobacterium leprae*, has long been known, the exact mode of transmission of the disease remains to be fully elucidated

[2]. There appears to be few natural animal hosts of this bacterium in endemic areas: human-to-human contact is, therefore, thought to play a major role in providing a reservoir of infection. In addition, the inability to culture *Mycobacterium leprae* *in vitro* makes assessment of subclinical infection rates difficult.

It is thought that the nose is the usual site of primary infection with *M. leprae*, as a result of airborne infection [3]. The advent of polymerase chain reaction (PCR) technology has afforded the opportunity to specifically detect small amounts of DNA, and a procedure, which indicate the presence of DNA equivalent to as few as 20 *M. leprae* cells, has been developed by Hartskeerl, *et al.* [4]. Studies using this technique have detected *M. leprae* DNA on

swabs taken from nasal mucosa of clinically normal individuals in family contacts of leprosy patients. The significance of the presence of such DNA is as yet unresolved, but may represent a form of sub-clinical infection or transient carriage of *M. leprae*, which may in turn be important in the transmission of the disease.

The technique described here is simple, sensitive, and specific for use in large-scale epidemiological studies. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination programs in countries where the disease prevalence has been significantly reduced. This study is part of the molecular epidemiological study of leprosy in Myanmar.

MATERIALS AND METHODS

Sample collection

Patients and contacts: After taken informed consent, nasal swab samples were collected from leprosy patients attending the Central Special Skin Center (CSSC), Yangon General Hospital and their family contacts. Fifteen PB and fifty-five MB patients with clinically and bacteriologically documented disease, as well as 137 household contacts (HHC) agreed to participate in the study. HHC were defined as persons sleeping during the night under the same roof. Leprosy patients were classified clinically and microscopically according to WHO classification [5] which consists of two categories, paucibacillary (PB) and multi-bacillary (MB). PB leprosy is defined as five or fewer skin lesions with no bacilli in skin smears, and MB leprosy cases have six or more lesions and may be skin smear positive.

Nasal swabs: Nasal swabs were taken by introducing cotton tip swabs (sterilized JCB MENTIP, Japan) 2-3cm into each nostril successively, and rubbing gently on the lateral and median sides of each cavity.

Swabs were immediately chilled and transported to the Immunology Research Division, DMR (Lower Myanmar) and analyzed.

Specimen preparation

The collected nasal swab samples were dipped in 1.5 ml eppendorf tubes containing 1ml PBS with 0.05% Tween 20 to release the bacilli from the cotton swab by turning and squeezing, then subjected to high speed centrifugation 14,000 rpm for 10 minutes. The supernatant was discarded and the sediment was resuspended in 1 ml 70% ethanol and prepared for DNA extraction by Klatser's Method [6].

DNA template preparation (DNA extraction)

DNA was prepared from nasal swabs according to the method of Klatser *et al* [6]. Briefly, nasal swabs immersed in 70% ethanol were centrifuged at 14,000 rpm for 10 minutes. After discarding the supernatant, the precipitate was washed with PBS and centrifuged again at 14,000 rpm for 10 minutes to remove remaining alcohol. The washed precipitate was suspended in 50µl of lysis buffer containing proteinase K 10mg/ml in 1M Tris-HCL, pH 8.5 and 0.5% Tween 20 and incubated at 60°C for 18 hours. Five microlitre of mineral oil was over-layered microlitre to prevent evaporation of water from the mixture. After heating at 97°C for 10 minutes, the suspended solution was treated with freezing and thawing twice to extract DNA and also to inactivate proteinase K, which inhibits Taq polymerase during PCR.

The DNA polymerase chain reaction (PCR)

A set of primers (5'-AAA AAA TCT TTT TTA GAG ATA CTC GAG-3' and 5'-CAA GAC ATG CGC CTT GAA-3') was used for amplification of the specific region of *M. leprae* 16S rRNA gene. *M. leprae* chromosomal DNA was kindly supplied by Dr. M. Matsuoka, Leprosy Research Center, NIID, Japan. This DNA served as a positive control in all PCR experiments.

The 50 µl reaction mixture contained 10 µl of template solution, 0.2 µl of *Ex Taq* DNA polymerase (Takara Shuzo Co., Shiga, Japan), 1 µM of each primer, 5 µl of 10x DNA PCR buffer, 8 µl of dNTP solution and 25.8 µl of water. The reaction mixture was overlaid with mineral oil 5 µl. The reaction was performed with a Mastercycler personal Eppendorf AG, Hamburg, Germany. The reaction mixture was heated to 94°C for 1 min, rounds of amplification consisted of a 30 second denaturation step at 94°C, a 2 min annealing step at 44°C and a 3 min elongation step at 72°C for 45 cycles. The amplified DNA fragments were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels in Tris-Borate EDTA (TBE) buffer.

RESULTS

A total of 70 leprosy patients (55 MB and 15 PB) attending the Central Special Skin Center (CSSC), YGH and 137 of their house hold contacts (HHC) were studied to detect *M. leprae* from nasal mucosa by PCR using the 16S ribosomal gene amplification. In 55 MB patients including both Bacillary Index (BI) positive and negative cases, 45 patients (81.82%) were PCR positive. Out of the 15 PB patients (BI negative) 3 cases were found to be PCR positive, that is 20% (Table 1). Table 2 shows 21 cases (20%) out of 105 contacts of MB cases were PCR positive and 1 (3.12%) out of 32 contacts of PB patients were PCR positive.

Table 1. Detection of *M. leprae* by PCR from nasal swabs in different types of leprosy

Types of Patients	PCR Results		Total
	Positive	Negative	
PB	3 (20%)	12 (80%)	15 (100%)
MB	45 (81.82%)	10 (18.18%)	55 (100%)
Total	48 (68.57%)	22 (31.43%)	70 (100%)

PB = Paucibacillary MB = Multibacillary
 Pearson χ^2 (1) = 20.8988 Pr = 0.00001
 Odds ratio = 18.0 95% CI = 3.19-101.56

Table 2. Detection of *M. leprae* by PCR from nasal swabs in House Hold Contacts (HHC) of different types of leprosy

HHC in different types of patients	PCR Results		Total
	Positive	Negative	
HHC of PB	1 (3.12%)	31 (96.88%)	32 (100%)
HHC of MB	21 (20.00%)	84 (80.00%)	105 (100%)
Total	22 (16.06%)	115 (83.94%)	137 (100%)

HHC = House Hold Contacts, PB = Paucibacillary, MB = Multibacillary, Pearson χ^2 (1) = 5.1812
 Pr = 0.023, Odds ratio = 7.75, 95% CI = 0.95-63

DISCUSSION

For more than a century, the excretion of *M. leprae* through the nasal mucosa of MB patients has been documented, and it represents the most important portal of exit for the organism. Whether *M. leprae* invades the human body after deposition of aerosolized organisms on the nasal mucosa is unknown. In the past, many attempts were made to detect *M. leprae* in Ziehl-Neelsen-stained smears from nasal swabs, but this technique is always open to criticism, particularly because of its lack of specificity.

PCR targeting species-specific sites of *M. leprae* DNA offers promise, in terms of both specificity and sensitivity. We investigated the household contacts of a number of PB and MB leprosy patients. Sampling of the nasal mucosa through swabbing is not the optimal technique because it is impossible to standardize. However, under field conditions it is the only practical possibility; a nasal washing procedure as performed by Shepard [7] would be impossible.

M. leprae was detected by PCR in 1 (3.12%) out of 32 samples from contacts of PB patients and in 21 cases (20%) out of

105 samples from contacts of MB patients. The difference is significant ($p < 0.05$). Among the patients, PCR positivity for nasal swab was 3 out of 15 (20%) in PB patients and 45 out of 55 (81.82%) in MB patients. The difference is highly significant ($p < 0.0001$, odds ratio=18.0, 95% CI=3.19-101.56). Therefore, PCR positivity of MB is significantly higher than PB in both patients and contacts. This might be an indication that carriage of *M. leprae* in the nose is indeed related to exposure to type of leprosy.

The results of this study provide evidence that a majority of MB patients are carrying *M. leprae* in their noses and that carriage of *M. leprae* occurs among healthy people living in an area where leprosy is endemic. Nasal carriage by apparently healthy people might have an impact on leprosy control and thus be an important phenomenon from the public health point of view. New studies have to be undertaken to investigate whether and to what degree nasal carriage occurs in the general population of areas of endemicity and what might be the role of carriers in the maintenance of infection reservoirs and transmission of leprosy.

Our results differed from those published by Stefaan R, *et al* [8], who did not find a difference among contacts of PB and MB patients. The finding was 1 of 52 (1.9%) swabs and 13 of 164 (7.9%) swabs were positive for *M. leprae* among contacts of PB and MB patients. However, De Wit, *et al* [9] suggested that the percent positivity of the patients group was significantly higher than in both the group of occupational contacts and endemic controls. The amplification products were found in 55% of untreated patients, in 19% of occupational contacts, in 12% of endemic control, and in none of the non-endemic controls.

Although nasal carriage does not necessarily imply infection or excretion of bacilli, the finding of nasal carriage supports the theory of a disseminated occurrence of *M. leprae* in populations for which leprosy is endemic.

The technique described here is simple, sensitive, and specific for use in large-scale epidemiological studies. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination programs in countries where the disease's prevalence has been significantly reduced. This study is part of the molecular epidemiological study of leprosy in Myanmar.

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A comparative study of biological and biochemical properties of white, pale yellow and yellow Russell's viper (*Daboia russelii siamensis*) venoms

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Biological and biochemical properties of white, pale yellow and yellow venoms of Russell's viper (*Daboia russelii siamensis*) of same length (81-90cm) from Nyaungdon, Ayeyawady Division were studied by standard techniques. The results showed that biological and biochemical properties of these venoms were comparable except that the white venom possessed slightly potent defibrinogenating activity and lacked L amino acid oxidase activity compared to the yellow. The pale yellow venom had 1/3 and the white 1/2 of the arginine esterase activity possessed by the yellow. SDS-PAGE of the venoms showed that the white venom had fewer bands than the pale yellow and yellow venoms. Such variation in biological properties of the coloured venoms may be attributed to age (length) rather than colour variation of the Russell's viper venom.

INTRODUCTION

While studying geographical variation of Russell's viper (*Daboia russelii siamensis*) venom, we came across coloured venoms namely white, pale yellow and yellow. It has been observed that white venom contains less L amino acid oxidase than yellow venom [1]. Master and Kornalik [2] found that there are antigenic differences between yellow and white venom and the latter has weak necrotic activity compared to the former. Variation in colour of young and adult Russell's viper (*D.r.siamensis*) venom has been reported [3]. In the present study, we compared biochemical, biological and SDS-PAGE properties of white, pale yellow and yellow venoms of Russell's viper (*D. r. siamensis*) from our collected pools.

MATERIALS AND METHODS

418 Russell's vipers measuring 61-120 cm collected from 15 localities of 5 divisions of Myanmar were milked individually,

lyophilized, pooled according to their length, colour and locality and stored at +4°C. Venoms were pooled according to their length into two groups (<90 cm) (young) and (>90 cm) (adult).

Biological, biochemical and SDS-PAGE properties were determined on ten (white 3, pale yellow 3 and yellow 4) young viper (81-90cm) venoms of Nyaungdon, Ayeyawady Division.

Biological and biochemical properties of venom were also carried out on pooled 14 young (white) and 14 adult (yellow) Russell's vipers venom collected from Danubyu, Ayeyawady Division.

Methods

Biological properties of the venoms such as lethality, coagulant, haemorrhagic, necrotic, capillary permeability increasing and defibrinogenating activities were studied according to the WHO recommended techniques [4]. Biochemical properties: esterase activity was measured using substrate

Table 1. Biological properties of Russell's viper venoms from Nyaungdon, Ayeyawady Division

Length /color	No of snake	LD _{50 iv} µg/mouse*	MHD µg/rat	MND µg/rat	MDD µg/mouse	MCD µg/ml	MCPID µg/ml	Phospholipase activity** µg/mg/min	L amino acid oxidase activity** µg/mg/min	Arginine esterase activity** µg/mg/min
81-90 cm White	3	4.77 ± 1.8	24.6	26.9	2.4	0.07	0.0065	800 ± 5	ND	35.5 ± 0.3
Pale yellow	3	4.68 ± 1.0	24.0	29.2	4.2	0.09	0.0003	880 ± 2	6.6 ± 0.3	22.2 ± 1.2
Yellow	4	5.53 ± 2.5	24.3	31.6	3.3	0.06	0.0007	960 ± 1	6.6 ± 0.3	75.7 ± 0.3

Data are means of duplicate determination

** Mean ± SD (n=4)

*95% confidence limit

Table 2. Biological properties of Russell's viper venoms from Danuphyu, Ayeyawady Division

Length /color	No. of snake	LD _{50iv} µg/mouse *	MHD µg/rat	MND µg/rat	MDD µg/mouse	MCD µg/ml	MCPID µg/ml	PLA ₂ activity** µg/mg/min	L amino acid oxidase activity** µg/mg/min	Arginine esterase activity** µg/mg/min
71-90 white	14	4.42± 1.2	30.9	30.9	4.2	1.995	0.0004	1000± 5	ND	142± 1.5
91-120 yellow	14	6.38 ± 1.0	30.2	27.5	5.1	2.291	0.0018	1026 ± 3	13.6 ± 0.7	133± 0.6

Data are means of duplicate determination

** Mean ± SD (n=4)

*95% confidence limit

TAME [5], phospholipase activity using phosphatidyl choline [6], L-amino acid oxidase activity using L-leucine as substrate [7] and pyruvate formed was measured following development of colour with dinitro phenylhydrazine substrate. Enzyme activities were expressed in units/mg/min. SDS-PAGE of the venoms was carried out according to the method of Laemmli [8].

RESULTS

Lyophilised venom of 418 Russell's vipers from collected pool showed that 64% (131/204) of young venom were white and 85% (182/214) of adult were pale yellow and yellow. Eighty to ninety percent of young viper venom from Danubyu (Ayeyawady Division), Indaing, Hmawbi and Kun-gyankone (Yangon Division) were white.

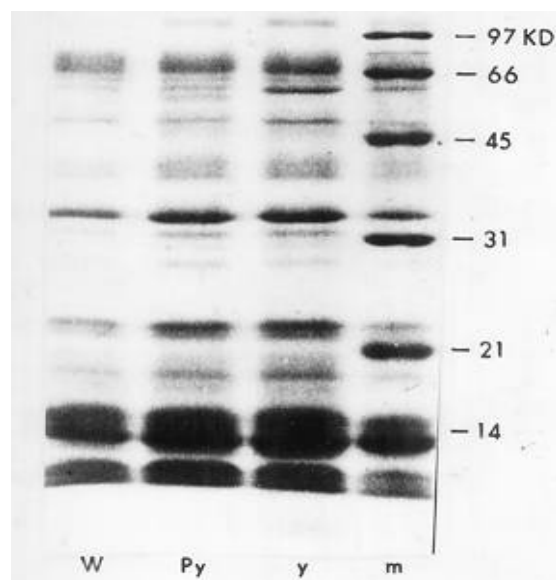


Fig. 1. SDS-PAGE patterns of white, pale yellow and yellow Russell's viper venoms of Nyaungdon (81-90cm). W=white, Py=pale yellow, Y= yellow venoms and m= molecular weight markers.

Biological and biochemical properties of white, pale yellow and yellow venoms of Russell's viper of the same length (81~90cm) from Nyaungdon (Table 1) were comparable except that the white venom possessed slightly potent defibrinogenating activity and lacked L amino acid oxidase activity compared to the yellow. The pale yellow venom had 1/3 and the white 1/2 of the arginine esterase activity possessed by the yellow venom. SDS-PAGE of the venoms showed the white venom had fewer bands than the pale yellow and the yellow venoms (Figure 1).

Comparative study of biological and biochemical properties of white (young) and yellow (adult) venoms of Danubyu (Table 2) shows that the former was more lethal and possessed more potent capillary permeability increasing activity than the latter. L-amino acid oxidase activity was not detected in the former. Phospholipase and protease activities (arginine esterase) were comparable.

DISCUSSION

The study highlighted that 64% of young Russell's viper (*D.r.siamensis*) (<90cm) venom were white and 85% of the adult (>90cm) were pale yellow to yellow. However, 80~90% of the young viper venom from Danubyu, Indaing, Hmawbi and Kungyankone were white.

Study of young Russell's viper venom of same length (81~90cm from Nyaungdon, Ayeyawady Division) suggested that biological properties of white, pale yellow and yellow venoms were comparable except that the white venom possessed slightly more potent defibrinogenating activity. However, on comparing white venom of young Russell's viper (*D.r.siamensis*) venom of Danubyu with that of adult yellow venom, the former was more lethal and possessed potent capillary permeability increasing activity than the latter. It was reported that the white venom of Indian

Russell's viper possesses weak necrotic activity compared to the yellow [2]. However, the other activities are comparable as observed in our Russell's viper (*D.r.siamensis*) venoms. It has also been documented that juvenile venom is more potent than that of adult [3] and such variation in biological properties of the coloured venoms may be attributed to age (length) rather than colour variation of the Russell's viper venom. Variation in protein bands of venom in SDS PAGE between young and adult [3] has been reported. A similar observation was made in our study.

Study on biochemical activity of the venom showed Indian Russell's viper (*Vipera russelli*) venom was found to have the same concentration of L amino acid oxidase and protease activities in both yellow and white venoms [2]. Our findings agree with observation made by Master and Kornalik [2] that proteolytic activity of *Vipera ammodytes* is comparable in both yellow and white venoms except the latter lacks L amino acid oxidase. In contrast, white venom of the Indian Russell's viper is found to have same concentration of L amino acid oxidase in both venoms. Age, species and geographical variation of venom composition [9-11] may account for the differences.

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Factors associated with the outcome of Dengue Haemorrhagic Fever/Dengue Shock Syndrome cases admitted to Yangon Children Hospital

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A prospective cohort study included the 303 mothers of Dengue Haemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS) cases admitted to Yangon Children Hospital (YCH). Case Fatality Rate (CFR) was highest in 1-5 year age group (8.53%), the patients ill for >5 days (10.95%), and more than 5 hours duration of shock at home (34.15%). CFR was low in the cases consulted with the medical personnel (4.18%), and the hospitalized patients referred by the health staff (4.46%). CFR was highest among the cases of illiterate mothers (16.13%) and vendors (9.47%), and lowest in those of mothers with high education (4.25%). Though the mothers gained high knowledge and attitude scores of 59% and 62.7% respectively, only 34.7% got high practice scores. The difference of knowledge, attitude, and practice scores was statistically significant to education of the mothers. CFR was high in the children of the mothers who had no action taken for preventive measures (8.1%). CFR was cent percent in Grade IV cases. Early and timely referral should be enhanced by modified health education processes.

INTRODUCTION

Dengue/dengue haemorrhagic fever (DHF) is now the most important and rapidly rising arbovirus infection in the world. Clinically, dengue fever has been recognized for more than 200 years and a disease similar to DHF was first described at the end of the past century. Currently, the incidence of DHF is greater by far in Asia than in the Americas. DHF continue to be a serious public health problem and a major cause of hospitalization and death among children in many Asian countries [1-2].

Aedes aegypti is a small black-and-white, highly domesticated mosquito that prefers to lay its eggs in artificial water-containers commonly found in urban areas of the tropics. Containers found in and around the home, such as those used for water storage,

flower vases, old automobile tyres, buckets and other junk items that collect rain water are examples. The adult mosquitoes are rarely noticed, preferring to rest indoors and to feed on humans during daylight hours in an unobtrusive and often undetected way [3].

DHF, a severe, often fatal, febrile disease caused by dengue viruses is characterized by acute febrile illness of 2-7 days duration, haemorrhagic tendency, and in severe cases, Dengue Shock Syndrome (DSS), a protein losing shock syndrome. It is currently thought to have an immunologic basis. At least four distinct types of dengue virus (type 1-4) have been isolated from patients with haemorrhagic fever. [4]

An increase in the number of reported cases was noted in Myanmar. DHF is now

occurring in many townships from 12 out of 14 States and Divisions in Myanmar. The recorded cases for YCH was 2242 with 31 deaths in 1997. Most of the reported cases are under 15 years of age. An increase in the number of reported dengue cases is noted in Myanmar. DHF is prevalent in Yangon throughout the year. Disease incidence rises during the rainy season. Highest number of cases occurs in July or August. The reported total cases in Yangon Division were 3943 and dead cases were 76 in 1967. DHF occurs annually in an endemo-epidemic form. Control of DHF is included in the VBDC program. Early diagnosis and prompt treatment, timely hospitalization, can prevent severe complication of DHF and it is cost effective.

DHF is prevalent in Yangon throughout the year. Disease incidence rises with the rainy season. Highest number of cases occurs in July or August. DHF/DSS is a disease mainly of children under 12 years. The most vulnerable age group is 3-8 years, which contribute 79.8% of DSS cases and 86.1% of fatal cases. Female preponderance was seen in DSS (male:female = 1:1.39). Most of the children with DSS developed shock on 3rd to 7th day of fever [5].

A previous infection makes people more susceptible to serious complications or to subsequent infections. The breeding habits of the main mosquito vector, *Aedes aegypti*, are closely related to poor sanitation or overcrowding and incorrect water storage [1]. Our interest is to find out the outcome of DHF influenced by maternal education and awareness. Early hospitalization based on awareness of the disease of the mother could favor the reduction of mortality, as DSS is a severe fatal outcome. The study will provide future intervention for reduction of Case Fatality Rate (CFR).

"HFA by the year 2000" targets to decrease the CFR in DHF/DSS to less than 1.8% in Myanmar health plan. Early recognition of the disease and early admission are the

prime importance to decrease CFR. This study is carried out to detect different presentations of DHF/DSS, which will give valuable information to decrease CFR.

Objectives

The general objective was to determine the factors associated with the outcome of DHF/DSS cases admitted to Yangon Children Hospital (YCH) from September to December 1998. The specific objectives were to determine the association of maternal education to knowledge, attitude, practice, and outcome of the disease, and to identify the risk factors associated with DSS.

METHODOLOGY AND DESIGN

A prospective cohort study was conducted at YCH from July to December 1998. The total sample was 303 mothers of DHF/DSS cases admitted to YCH. Systematic random sampling was done from the admission list. The hypothesis was that the CFR of the children of the educated mother is lower than that of the children of uneducated mother. Dependent variable was the outcome of DHF/DSS cases (recovery or death). Independent variables were maternal education, knowledge and practice, family income, days of fever at home, duration of shock at home, and stage of illness at the time of admission. Structured questionnaire, hospital registers, and charts were used as data collection tools. Data entry, and processing were performed using Epi-info 6.0.

Assumptions of the study were as follows:

1. The educated mothers are aware of DHF/DSS.
2. The children of the educated mothers consult at YCH regardless of hospital admission.

3. Maternal education and knowledge influence the outcome of DHF/DSS.

FINDINGS

Three hundred and three children of DHF/DSS cases admitted to YCH participated in the study.

Table 1. Characteristics of the children admitted to YCH with DHF/DSS

Variables	No.	Percent	CFR (%)
Age group			
<1	17	5.6	5.88
1-5	129	42.6	8.53
6-12	157	51.8	4.46
Mean age (SD)	3.6 ± 1.3		
Gender			
Female	148	48.84	7.43
Male	155	51.16	5.16
Day of fever at home			
1	12	4.0	0
2-4	218	71.9	5.05
>5	73	24.1	10.95
Mean day of fever (SD)	3.6 ± 1.3		
Duration of shock (hours) at home			
0	172	56.8	1.16
1-5	90	29.7	3.33
>5	41	13.5	34.15
Mean (SD)	2.63 ±		
Same illness among the child's associates			
Siblings	23	7.6	
Neighbors	4	1.3	
Classmates	44	14.5	
Consultation of the cases with medical personnel			
Yes	239	78.9	4.18
No	64	21.1	14.06
Advice for hospitalization of the cases			
Decision of the parents	104	34.3	3.85
Referred by health staff	157	51.8	4.46
Others	42	13.9	19.05
Accessible health facility of the cases			
Health center	40	13.2	10.0
Private clinic	254	83.8	4.7
None	9	3.0	3.3

Most of the cases fell in six to twelve year age group compared to under one year age

group (51.8% vs 5.6%). CFR was highest in 1-5 year age group. Males were 51.16% of the cases and CFR (5.16%) was lower than females (7.43%). The majority of the cases (71.9%) were febrile of the cases at home for 2-4 days. Mortality was highest among the patients ill for >5 days at home (i.e.10.96%). More than half of the cases did not suffer any shock at home. If duration of shock lasted for more than 5 hours, CFR was highest (34.15%). Some cases had history of same illness in the classmates (14.5%), and in the siblings (7.6%). The majority of the cases (78.9%) consulted with the medical personnel and CFR was lower than those without any consultation. The hospitalized patients referred by the health staff were more than 50% and CFR was 4.46%. Private clinics and health centres were the most accessible health facility (97%) (Table 1).

More than 30% of the mothers were high school and college level and only 10.2% were illiterate. CFR was highest among the cases of illiterate mothers and lowest in those of mothers with high education (Figure 1).

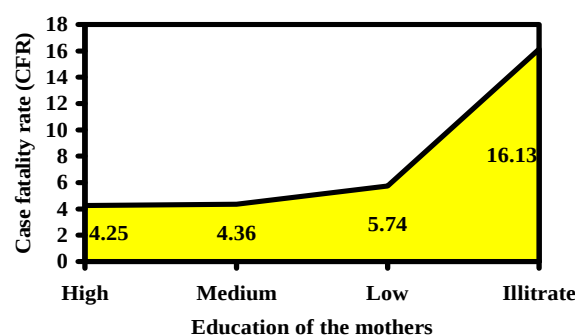


Fig. 1. Mother's education and outcome of the cases

Half of the mothers were dependent and 31.4% were vendors. CFR was highest in vendors (9.5%).

Mortality was high in the cases with associated diseases such as primary complex (10.6%), protein energy malnutrition (10%), malaria (10%), asthma (6.1%), etc. Though the mothers gained high knowledge and attitude scores of 59% and 62.7%

respectively, only 34.7% got high practice scores.

The difference of knowledge, attitude, and practice scores was statistically significant to education of the mothers (Table 2).

Thirty two percent of the mothers covered water container to prevent mosquito multiplication, and 23% exchanged water vases regularly. The mothers who had no action taken for preventive measures were 41% and CFR was highest in those cases.

Table 2. Maternal education and knowledge, attitude, and practice scores

	Education of the mothers				P value
	Illiterate	Low (primary school)	Medium (middle school)	High (high school & college)	
Knowledge scores					
- High	6	51	31	91	0.00*
- Low	25	71	25	3	
Attitude scores					
- High	5	23	16	69	0.00*
- Low	26	99	40	25	
Practice scores					
- High	2	17	15	71	0.00*
- Low	29	105	41	23	

* Statistically significant

Although Grade IV cases were very few (4.3%) at the time of admission, CFR was cent percent (Figure 2).

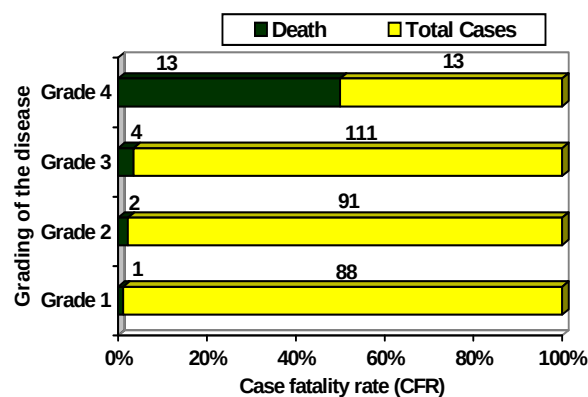


Fig. 2. Severity of admitted cases and outcome

DISCUSSION

As from the survey findings, DHF/DSS is common in 6-12 age group, school going age group, however, CFR is lower than other age groups. It should be taken into consideration that the disease might be transmitted in the school.

Although male preponderance was seen in the study, mortality is lower. No death was observed in the child consulted on the first day of fever. Most of the cases came to the hospital without shock at home and mortality rate was very low, indicating that early admission could lower the mortality.

CFR was highest in the children of illiterate mothers and vendors. Education of the mothers influenced knowledge, attitude, and practice on DHF preventive measures. Cases without associated diseases had very low fatality. Primary complex was the common disease among the studied cases.

Regarding the knowledge scores of the mothers, the majority had a high level of knowledge about the DHF/DSS features, transmission and prevention. Even some mothers with low education level had high knowledge, attitude, and practice scores; the reason was that most of the mothers were aware of DHF which is fatal. Low education level with low practice score was the majority group. Practices scores were not satisfactory although the education level was high.

Fatality of the cases consulted with medical personnel was very low. Doctor's advice was the major role for hospitalization of the cases. Some cases came to the hospital by the parents' own decision. It had double reasons: (1) Mothers are alarmed for DHF/DSS. (2) Financial deficit to consult at the outside clinic.

Many patients were accessible to general practitioner's clinic. CFR was too high in the patients without accessible health because

these cases were from the rural area where transportation was difficult.

The majority of the mothers took no action to prevent the process of transmission mosquito multiplication and mortality was the highest. Some mothers covered water containers. Only few mothers knew to remove the breeding places for mosquito multiplication. Some mothers didn't know that DHF/DSS was transmitted by the mosquito bite.

At the time of admission, most of the cases were stage 3. Stage 4 at the time of admission was very few, due to the fact that most of the mothers were aware of DHF and they came to the hospital in the early stage and critical cases expired at home were under-reported.

Recommendations

DHF/DSS is a principal epidemic disease in Myanmar. Consideration for effective prevention and control measures is essential. DHF frequently occurs in school going age. Some cases are likely to be transmitted in the school. Health education for DHF/DSS features, transmission and preventive measures, and early sign should be strengthened not only for the mothers but also for the teachers and students.

Awareness of the mothers plays a major role in the incidence and outcome of the disease. Promotion of information system relevant to prevention DHF/DSS is essential through different media.

Motivation of the NGOs and community to participate for source reduction and early referral should be emphasized. Effective intervention programme for the reduction of CFR of DHF is a need for the vulnerable group. Early and timely referral should be

enhanced by modified health education processes.

Limitations of the study

- Though our target population includes all children, we have restricted the study population to children with diagnosis of DHF/DSS admitted to YCH because of the anticipated difficulty in identifying the cases without reporting.
- Due to constraints of time and finance, our study is limited to a selected institution. However, we do not anticipate much variation among the community.
- The accuracy of the response of mother was difficult to ensure.

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The efficacy of *Artemisia annua* crude extracts on *Anopheles dirus* in the laboratory and whole plants on other mosquitoes in the field

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Evaluation of the efficacy of an aromatic plant *Artemisia annua* against *Anopheles dirus* mosquito (a major vector of forest malaria in Myanmar) was conducted in the laboratory and in the field situation. Repellent effect on adult mosquito was observed at the concentration as low as 0.5% *Artemisia* extract solution. At the concentration of 6.0 %, the repellency was markedly increased and the protection provided was found to be 94.74 % during an exposure period of six hours ($r = 0.95$, $df = 6$, $p = 0.001$). The larvicidal effect on 3rd and 4th instar larvae of *An. dirus* showed that the LC_{50} and LC_{90} were 0.04 % and 0.14 % respectively based on the dose-effect probit analysis ($\chi^2 = 3.05$, $df = 3$, $p < 0.05$). The residual effect persisted for at least 6 days at 0.25% and 0.50% concentrations. Regarding the ovicidal effect, a series of concentrations starting from 0.025% were tested and at 0.40% concentration, the relative reduction in egg hatching rate was 93.85% compared to that of control ($r = 0.92$, $df = 4$, $p = 0.026$). However, protection from mosquito bites was not observed when *Artemisia annua* plants were placed around baits under field situation ($\chi^2 = 1.69$, $df = 1$, $p > 0.05$). The prospects for the use of indigenous plants and its extracts for personal protective measures in prevention and control of vector borne diseases are also discussed.

INTRODUCTION

Anopheles dirus is one of the major vectors of malaria in mainland Southeast Asia, including Myanmar, Cambodia, Bangladesh and Thailand [1]. Despite the importance of this species as a vector of malaria, there is no published record of its response to repellent, larvicidal and ovicidal actions of *Artemisia annua* plant and plant extracts. The use of repellents and other personal protective measures to minimize contact with malaria vectors in Southeast Asia is especially important for two reasons.

Firstly, the region is the center for the occurrence of *Plasmodium falciparum* drug resistance causing ineffective chemotherapy in many areas [2]. Secondly, *Anopheles dirus* is largely exophilic and therefore unaffected by interior spraying of houses with residual insecticides [3]. Synthetic insecticides that have been developed in agriculture, forestry and public health against plant pests and vectors of human diseases, do not fulfill the requirements for integrated pest management [4]. For these reasons as well as the increasing problems

of insecticide resistance, interest in insecticidal properties of plants has grown rapidly during recent years.

During the last decade, extracts and compounds of pyrethroid have attracted the special interest of entomologists and phytochemists resulting in the wide use of bed nets treated with pyrethrum insecticide as personal protective measures in malaria control. *Artemisia annua* plantations have been encouraged due to the parasitocidal effect of its derivatives such as artesunate and artemether. In parallel to this, it seems appropriate and worthwhile to conduct efficacy studies of *Artemisia annua* and its extract on mosquitoes for personal protection and other properties.

Thus, the general objective is to explore the repellent effect and other insecticidal properties of indigenous plant *Artemisia annua* against malaria vector mosquitoes with special reference to the primary malaria vector *An. dirus* for the development of future alternative vector control measures. The specific objectives are: (i) to determine the repellent action of *Artemisia annua* plant extract on *Anopheles dirus* adult mosquitoes under laboratory conditions; (ii) to assess the larvicidal and ovicidal effects of *Artemisia annua* extract on *An. dirus* under laboratory condition and (iii) to investigate the repellent action of *Artemisia annua* on *Anopheles* mosquitoes under field condition.

MATERIALS AND METHODS

Study area

The laboratory studies were carried out in the Medical Entomology Research Division, Department of Medical Research (DMR) (Lower Myanmar) and the field study was undertaken at Do-gwin plantation site, Myaingyi village, Pyin-Oo-Lwin Township, Mandalay Division.

Study design

One arm of this study consisted of experimental laboratory studies namely: (i) repellency (ii) larvicidal (including persistency test) and (iii) ovicidal tests on *An. dirus*. This is the major component of the present study. Another arm of this study involved cross-sectional field comparative study comparing the total number of mosquitoes caught on cattle-baits with or without *A. annua* plants regardless of whether *An. dirus* are present or not.

Sample size

This study was not based on human population but on mosquitoes and therefore minimum sample size determination was not applicable for this situation. Nevertheless, enough mosquitoes had been utilized for each experiment.

1. Laboratory studies

The required plants were obtained from the Agricultural Department in Yangon. The *An. dirus* colonies reared in the insectary were utilized. *Artemisia annua* crude extract was obtained with the assistance provided by the Pharmacology Research Division (DMR-LM). A 10 percent emulsified concentration (EC₁₀) stock solution of *Artemisia annua* (Methanol extract) was prepared by dissolving it in acetone. It was tested in the laboratory for repellent, larvicidal and ovicidal properties using fifteen concentrations ranging from 0.01 percent to 6.00 percent. Detailed testing was according to the W.H.O larval susceptibility standard method [5], Frances [6], Abdulcada and Sasamananabu [7] and Swaroop [8].

1.1 Repellent testing

Tests were carried out on two human volunteers based on a method described by Schreck [9]. Laboratory reared 6 to 7-day-old nulliparous female *An. dirus* mosquitoes were used. For each test, 50 mosquitoes were placed overnight in a screen wire cage

measuring 30cm x 30cm x 30cm and 10% sugar solution was used for feeding. Mosquitoes were starved for 12 hours before testing. Three replicates were carried out.

Tests were conducted by exposing untreated and repellent-treated human forearms to the mosquitoes according to the method of Frances [6]. Two millilitres of the solution was applied because this volume evenly covered the surface area. The treated arm was then exposed in the test cage for 15 minutes and subsequently exposed at 30-minute intervals. A number of bites for untreated and treated arms in each trial were recorded and percent protections were calculated using standard formula [10] and [11].

1.2 Larvicidal action testing

Larvicidal effect of *Artemisia annua* plant crude extract on *Anopheles dirus* was conducted in the laboratory at $27 \pm 1^\circ\text{C}$, 75-80% Relative Humidity (RH). A series of five *Artemisia annua* plant crude extract concentrations were used. In each replicate ten late-third and fourth instar larvae were exposed to 30 ml of each concentrations for 24 hours and for control purpose, the same number of larvae was exposed using 100 ml polythene beakers. A total of 250 larvae was utilised.

Five replicates were carried out and mortalities were recorded and checked daily. The lethal concentrations of LC_{50} and LC_{90} values were determined using dose-effect probit analysis.

1.3 Ovicidal action testing

Beakers (100 ml) containing a series of *Artemisia annua* plant crude extract concentrations (30 ml each) were used. One hundred eggs were exposed to each concentration for 48 hours to 72 hours and for control purpose, the same number of eggs were exposed using distilled water. After 48 hours and 72 hours, the number of hatched eggs were counted and recorded. Six replicates were carried out using a total

of 3,600 eggs.

2. Field studies

The plants were collected from Pyin-Oo-Lwin Botanical and Agricultural Garden and the field study site was conducted in Pyin-Oo-Lwin Township where *Artemisia annua* plants were grown by Agricultural Department. Mosquitoes caught were identified according to Rattarithikul [12].

There were two catching stations. In one catching station, there were two big mosquito-net traps. One net had ten *Artemisia annua* plants around the inner periphery of the net and a cattle-bait was tied at the centre. Another net had no *Artemisia annua* plants but only cattle-bait. Mosquitoes that came into the net were caught hourly from dusk to dawn. To exclude bias in site, the positions of the plants were reversed in the next catching station. Cattle-baits were used because of the pronounced attraction to mosquitoes in comparison to human baits. The total number of mosquitoes caught for 8-collection nights was recorded separately according to the respective catching station.

Data analysis

Data entry and processing were made using data base software and data analysis was carried out using Epi-info version 6 software. Probit analysis (dose effect analysis) in determining effect of insecticide concentration on mortality was drawn manually and calculated according to Swaroop [8].

RESULTS

Repellent action

Artemisia annua extract in the concentrations of 0.5%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0% and 6.0% provided 15.78%, 36.36%, 56.52%, 75.0%, 80.0%, 86.6% and 94.74% protection respectively during the exposure period of six hours for the principal malaria vector *An. dirus*. There was a strong

correlation between *Artemisia annua* methanolic extract concentration and percentage protection from *An. dirus* bites ($r = 0.95$, $df = 6$, $p = 0.001$) (Fig 1).

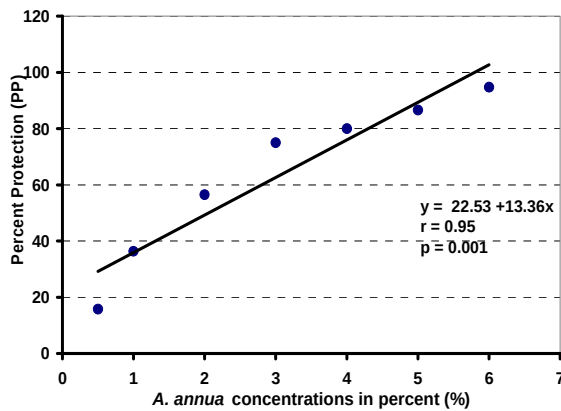


Fig. 1. Regression analysis of *Artemisia annua* concentrations against percent protection (PP) from *An. dirus* adult mosquito bites

Table 1. Larvicidal effect of *Artemisia annua* extract on the immature stages of *An. dirus* mosquitoes

<i>A. annua</i> concentration (%)	Dead/ Tested	Observed mortality (%)	Expected mortality (%)	Con-tribution (O-E)	tribution to χ^2
0.01 %	4/50	8%	4.75	3.25	0.023
0.025 %	12/50	24%	28	4	0.007
0.05 %	27/50	54%	58	4	0.006
0.10%	45/50	91%	86	5	0.021
0.25%	50/50	100%	99.1	0.64	0.004
Total				0.061	

Larvicidal action

The summary of dose-effect mortality and testing the goodness of fit for *Artemisia annua* methanolic extract concentrations against *An. dirus* larvae are shown in Table1& Fig. 2. The LC_{50} value and the LC_{90} value were found to be 0.04% and 0.14% concentrations respectively based on the dose-effect probit analysis ($\chi^2 = 3.05$, $df = 3$, $p < 0.05$). There was a strong correlation between *Artemisia annua* methanolic extract

annua extract concentrations on the immature 3rd and 4th stage of *An. dirus* larvae is shown in Table 2. The effects of *Artemisia annua* extract 0.25 percent and above persisted for 6 days.

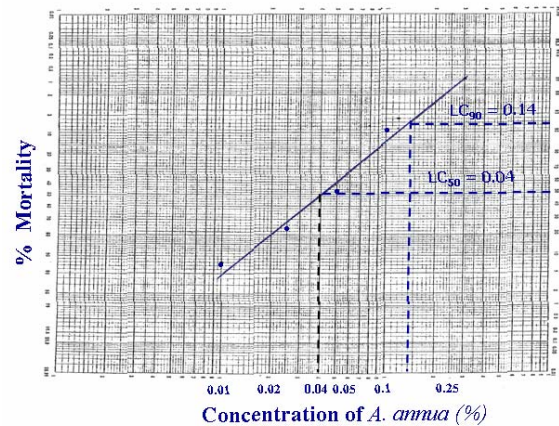


Fig. 2. Testing the goodness of fit for dose - effect analysis of *A. annua* concentration and *An. dirus* mortality

Ovicidal action

Artemisia annua extract in the concentrations of 0.025%, 0.05%, 0.10%, 0.20% and 0.40% reduced *An. dirus* egg hatching by 10.07%, 37.59%, 50.12%, 76.16% and 93.85% respectively. There was a strong correlation between *Artemisia annua* methanolic extract concentrations and percent reductions in eggs hatching ($r = 0.92$, $df = 4$, $p = 0.026$) (Fig. 3).

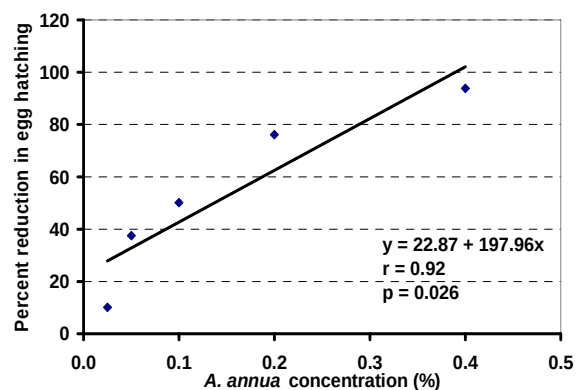


Fig. 3. Regression analysis of *Artemisia annua* concentration(%) against *An. dirus* percent reduction in eggs hatching

concentrations and *An. dirus* larval mortality. The persistent effect of *Artemisia*

Table 2. The effective persistancy of *A. annua* extract EC₁₀ application on immature stages of *An. dirus* mosquitoes

<i>A. annua</i> concentr- ation (%)	Effect of <i>A. annua</i> methanolic extract EC ₁₀ on <i>An. dirus</i> immature stages (% mortality in days)												Remarks
	Day (1)		Day (2)		Day (4)		Day (6)		Day (8)		Day (10)		<i>A. annua</i> methanolic extract 0.25% persists up to 6 days. Percent mortality was still 50% on Day 6.
	T *	C **	T	C	T	C	T	C	T	C	T	C	
0.05	63	0.0	40	0.0	23.3	0.0	13.3	0.0	6.6	0.0	3.3	0.0	
0.075	76.6	0.0	66.6	0.0	46.6	0.0	26.6	0.0	13.3	0.0	6.6	0.0	
0.10	93	0.0	70	0.0	56.6	0.0	30	0.0	16.6	0.0	10	0.0	
0.25	100	0.0	93.3	0.0	70	0.0	50	0.0	26.6	0.0	13.3	0.0	
0.50	100	0.0	96.6	0.0	80	0.0	73.3	0.0	46.6	0.0	23.3	0.0	

* T = Test ** C = Control

Field studies

Species and number of adults biting females mosquitoes collected from cattle baits were during field study were found in catching station (1). A total of 1011 mosquitoes were collected. Out of these 501 mosquitoes were caught where the plants were present along with cattle baits and 510 mosquitoes were caught where the plants were absent. Similarly, in catching station 2 out of 2290 mosquitoes collected, 1191 mosquitoes were caught where *Artemisia annua* plants were present and 1099 mosquitoes were caught where the plants were absent.

This field study revealed that the primary vector *An. minimus* and other secondary vectors *An. annularis* and *An. culicifacies* were present in the study area. There were no significant differences in collected mosquitoes species between catching stations and presence or absence of *A. annua* plants ($\chi^2 = 1.69$, df = 1, $p > 0.05$).

DISCUSSION

The primary objective of the present study is to gain more knowledge on mosquito control with insecticides extracted from plant ingredients which are locally available. A notable species was found to be

Artemisia annua plant [13]. *Artemisia annua* species (composite *Anthemideae*) are a group of aromatic plants with worldwide distribution.

Essential oils like Lemon grass, Thyme, Citronella etc. have mosquito repellent action. The fractionation of these essential oils has been carried out and the repellent effect of these fractions has been studied. Often, many unrelated plants have similar repellent constituents. Eucalyptus, alpine and camphor are the most commonly used natural oils as mosquito repellents before the artificial compounds are not available [14]. The problem with the natural oils is that they provide repellent action only for a short duration often not exceeding a few hours.

Moreover, a number of synthetic repellents have been developed. Some promising ones are DEET (N, N-diethyl-meta-toluamide) and DMP (dimethyl phthalate). These are most commonly used throughout the world to protect human against biting insects. The discovery of DEET in 1954 has ushered a new era in repelling mosquitoes.

The present study indicated that methanolic extract of *A. annua* could be considered as a potential repellent. It possessed repellency property and at 6.0 percent concentration, 94.74% protection was achieved during 6 hours period. This period is long enough if the

repellent is applied during the peak biting period. However, its repellency could not be compared with chemicals such as DEET in both efficacy and residual duration since these chemical compounds provide better and longer protection against many biting insect (ED₅₀ and ED₉₅ level = 0.37-25.37 µg/cm², 3-8 hours) [15-17].

The previous report of the repellent tolerance of *Ae. aegypti* was higher than the present finding which showed that the repellent action concentration was much lower with *An. dirus* [18]. As mentioned earlier, *Artemisia annua* methanolic extract 6.0 percent provided the protection up to 6 hours. Thus, it was found that *Ae. aegypti* was less susceptible to *Artemisia annua* than *An. dirus* in the laboratory test.

The results of the larvicidal effect of *Artemisia annua* extract on *An.dirus* larvae showed that LC₅₀ value was 0.040% and LC₉₀ value was 0.14%. The LC values were within statistically insignificant range. The regression lines were linear and the range of concentration to get a kill of 50% to 90% mortality is nearly 14 times of the initial concentration. Using the statistical analysis adopted by Swaroop's chi square test, it was found that the test results were highly significant. The present study differed with the finding of Htay Aung [18] where *Artemisia annua* extract on *Ae. aegypti* larvae (LC₉₀) was found to be 0.75% compared to only 0.14% in our studies on *An. dirus*. This could be that *An. dirus* is more susceptible than *Ae. aegypti* under laboratory condition.

Studies on the persistence effect of *Artemisia annua* on *An. dirus* larvae showed *Artemisia annua* methanolic extract 0.25 percent and above persisted up to 6 days. These findings also differed from the finding of Htay Aung [18] who reported that persistence effect of *Artemisia annua* on *Ae. aegypti* larvae lasted for 30 days at 1 percent to 2 percent concentration. It must be noted that the concentration in our study was 40 times less. The lesser concentration

is better in the sense that scarcity of the extract could be an operational problem.

Regarding ovicidal effect, observations at lower concentration showed that *Artemisia annua* 0.025 percent concentration provided 10.07 percent reduction in egg hatching, whereas in the higher concentration 0.40 percent concentration provided 93.85 percent reduction in egg hatching. The lack of data for comparison is due to the fact that this is the first time ovicidal effect has been studied.

Contrary to the findings under laboratory condition, our field results indicated that the repellent action of *Artemisia annua* plants on the field mosquito species was not promising. Although *An. dirus* were not caught, *An. minimus* (also primary vector of malaria) were caught. It showed that there were no significant differences in collected mosquito species between 2 catching stations with and without *A. annua* plants. These findings are similar to the finding of Htay Aung [18]. One possible explanation could be that "whole plants" are used in the field but the "pure extracts" are used in the laboratory. Further studies are needed to clarify these findings.

Employing *Artemisia* (EC₁₀) solution is safe to use as a larvicide, though with different characteristics and mode of action unlike other existing insecticides. *Artemisia* is not a conventional pesticides. It does not produce the non-discriminatory rapid and directly non-toxic effects that are associated with traditional medicine. The use of simple and cheap local indigenous *Artemisia* products such as crushed *Artemisia* leaves (plants), seems promising for treatment of temporary water pools in towns and villages in developing countries especially during the post-monsoon season for prevention of mosquitoes breeding.

The overall laboratory results indicated that *Artemisia* is an effective repellent against *An. dirus*. It can be useful and cost-effective in areas where insecticides can not be sprayed due to administrative and financial

reasons and also in areas where there is no effective method for protection against mosquito vectors. Night biting activity of *An. dirus* and other potential *anopheline* mosquitoes in Myanmar is during the 1st and 2nd quarters of the night. The application of *Artemisia* (6 percent) during this period is recommended as prophylaxis against bites of *An. dirus* mosquitoes. Thus, in the present context of malaria situation, application of *Artemisia annua*, an indigenous plant, as mosquito repellent, larvicides or ovicides could be cost-effective for the communities.

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Prevalence of hypertension in Pardagyi village, Kyauktan Township

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A cross-sectional community survey was carried out in Pardagyi village, Kyauktan Township in October 1998 to determine the prevalence of hypertension in adults aged 25 to 59 years and to identify the potential risk factors for hypertension in that area. A total of 394 subjects participated in the study. Overall prevalence of hypertension was 15.48% (diastolic greater than or equal to 90 mmHg, or systolic greater than or equal to 140 mmHg, or currently taking antihypertensive medication). The distribution of different categories of hypertension showed mild hypertension (diastolic blood pressure 90-104 mm Hg) to be the most frequent form (83.6%). Among the hypertensives, 75.4% were aware of their diagnosis and 51.5% were taking treatment. Hypertension awareness and treatment were found to be significantly higher in women ($p < 0.01$). Regarding the risk factors, older age (45 to 59 years), being a current smoker, Body Mass Index equal to or more than 25 and self-reported diabetes mellitus were associated with increased odds of being hypertensive in this study.

INTRODUCTION

The improved control of infectious diseases during recent decades has dramatically changed the health profile of many economically developing countries [1]. This is reflected in a prolongation of life expectancy and emergence of cardiovascular disease as a leading cause of morbidity and mortality in such countries [2]. Evidence from epidemiological studies indicates that a strong correlation between the arterial blood pressure and cardiovascular mortality and morbidity. The higher the blood pressure, the greater the risk for an individual to experience stroke, myocardial infarction, angina pectoris, heart failure and renal failure[3].

The prevalence of hypertension has been also increasing in Myanmar during the last decade. In previous cardiovascular disease survey, prevalence of hypertension was 12.5% and 14.6% for rural and urban areas of Myanmar respectively [4].

The prevalence and potential risk factors for hypertension in rural areas of Kyauktan Township were previously unknown. So this study was conducted with the following objectives.

1. To determine the prevalence of hypertension in adults aged 25 to 59 years living in Pardagyi village, Kyauktan Township.
2. To identify the potential risk factors for hypertension in the study population.

MATERIALS AND METHODS

Study area and population

Community survey was carried out in Pardagyi village, Kyauktan Township in October 1998. A cross-sectional study design was employed. A total of 394 persons aged 25 to 59 years participated in this study.

Methods of data collection

Before the actual data collection commenced, the study team was trained for

the use of research instruments and methods of data collection. To minimize the inter and intra-observer variation, method of blood pressure (BP) measurement was instructed and validity assessment was done on five medical officers included in the survey team. Consistency assessment was done on standard mercurial sphygmomanometers and weighing scales were also calibrated.

During the actual data collection, the surveyors created a friendly atmosphere, interviewed the subjects, measured their blood pressure and made anthropometric assessments.

Data on demographic, socioeconomic and dietary patterns were included in the interview. Blood pressure in lying down position was taken twice (5 minutes apart) only after the interview which usually lasted about 10 minutes, so as to eliminate the influence of activity on blood pressure. Systolic blood pressure (SBP) corresponded to Korotkoff phase I and diastolic blood pressure (DBP) to phase V. SBP and DBP for each individual were determined by averaging of the two readings.

Working definitions

For the purpose of prevalence of hypertension, persons were considered hypertensive if they met one of the following conditions set by the 1988 Report of the Joint National Commission on Detection, Evaluation and Treatment of High Blood Pressure (JNC 1988):

- (1) a diastolic blood pressure (DBP) of greater than or equal to 90 mm Hg;
- (2) a systolic blood pressure (SBP) greater than or equal to 140 mm Hg;
- (3) reporting currently using antihypertensive medication (regardless of blood pressure measurements) [5]. Prevalence estimates for the specific categories also followed JNC 1988 guidelines.

Statistical methods

The number of hypertensive subjects was

divided by the number of respondents to yield the prevalence of hypertension in the study area. Bivariate analysis was done for the identification of possible risk factors. The association between independent and dependent (outcome) variable was expressed as an odds ratio (OR) with its 95% confidence interval (CI). $P < 0.05$ was used as the definition of statistical significance.

Ethical consideration

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

RESULTS

Background characteristics

A total of 394 persons participated in this survey. Among them, 136 (34.5%) were males and 258 (55.5%) were females. Their mean age was 38.4 years (range, 25 to 59 years). Approximately half of the participants (48.1%) were manual labourers and 30.3% were dependents. Of the remainder, 17.6% were engaged in own business and 3.4% in office work. Median family income per month was 6000 Kyats (range, 550 to 90000 Kyats). Regarding the educational status, 60.8% were primary school level. Illiteracy rate was 7.9% in this village.

Prevalence of hypertension

Out of 394 participants, 61 subjects were found to have hypertension ($SBP \geq 140$ and/or $DBP \geq 90$). Consequently the prevalence of hypertension in the study area was estimated to be 15.48% (95% CI=12.05-19.43).

Further analysis of prevalence of hypertension in relation to age groups revealed a steadily increasing trend towards older age groups (Table 1). There was no sex difference among hypertensives where female and male hypertensives were 16.28% and 13.97% respectively ($p = 0.54$).

Table 1. Prevalence of hypertension in different age groups

Age groups (years)	Study subjects	Hypertensive	Prevalence	95%CI
≥25-34	165	11	6.60%	3.37-11.62
≥35-44	114	15	13.15%	7.56-20.77
≥45-54	78	19	24.35%	15.35-35.4
≥55-59	37	16	43.24%	27.1-60.51

Chi-square = 36.76 p = <0.001

The proportions of different types of hypertension such as borderline isolated systolic hypertension (BISH), isolated systolic hypertension (ISH), mild, moderate and severe hypertension were calculated and these are summarized in Table 2. Mild hypertensives were the most common (83.6%) followed by moderate hypertension (8.2%) and severe hypertension (6.5%).

Table 2. Distribution of different categories of hypertension*

Types	Criteria	Number (percent)
Borderline isolated systolic hypertension (BISH)	SBP 140-159 mmHg DBP <90mmHg	1 (1.64%)
Isolated systolic hypertension (ISH)	SBP≥160mmHg DBP<90mmHg	Nil
Mild hypertension	DBP 90-104 mmHg	51(83.6%)
Moderate hypertension	DBP 105-114 mmHg	5 (8.2%)
Severe hypertension	DBP≥115mmHg	4 (6.56%)

* JNC 1988 criteria

Among the cases, 46 (75.4%) were aware of their diagnosis but only 35 (51.5%) were taking antihypertensive treatment. Hypertension awareness and treatment were found to be significantly higher in hypertensive women (Table 3).

Risk factors analyses

Potential risk factors for hypertension in the study area were presented in Table 4. Older age group (45 to 59 years), being a current

smoker, Body Mass Index (BMI) equal to or more than 25 and self-reported diabetes mellitus were associated with increased odds of being hypertensive.

Table 3. Hypertension awareness and treatment among hypertensive individuals by gender

Group	All hypertensives	
	Number (%) aware	Number (%) treated
Total (n=61)	46 (75.4%)	35 (51.5%)
Sex		
Men (n=19)	10 (52.6%)	6 (31.5%)
Women (n=42)	36 (85.7%)	29 (69.1%)
p-value	0.005	0.006

Table 4. Potential risk factors for hypertension in the study area

Variables	Odds Ratio	95%CI	p-value
Age (RC=≥25-34 years)			
≥35-44 years	1.96	0.85-4.48	0.11
≥45-54 years	5.16	2.34-11.4	<0.001
≥55-59 years	9.54	3.89-23.4	<0.001
Sex (RC=male)			
Female	1.58	0.86-2.92	0.14
Education(RC=illiterate)			
Less than high school	2.87	0.67-12.43	0.16
High school and above	2.42	0.45-12.88	0.32
Marital status(RC=single)			
Married/widow	2.23	0.85-5.82	0.12
Family history (RC=no)			
Yes	1.39	0.87-1.71	0.1
Smoking (RC=non-smoker)			
Ex-smoker	0.95	0.21-4.38	0.98
Current drinker	1.79	1.02-3.16	0.04
Alcohol drinking (RC=never drinker)			
Ex-drinker	2.24	0.87-5.71	0.09
Current drinker	0.91	0.42-1.97	0.82
Obesity (RC=BMI<25)			
BM ≥ 25 BM(I)	1.95	1.12-3.39	0.03
Physical activity (RC=sedentary)			
Mild	0.76	0.49-1.28	0.11
Moderate	0.58	0.19-1.24	0.07
Salt consumption (RC=<30 ticals per day)			
≥30 ticals per day	1.67	0.96-2.92	0.08
Self-reported diabetes mellitus (RC=no)			
Yes	11.25	1.01-126.1	0.04

RC = Reference Category

DISCUSSION

Overall prevalence of hypertension in the present study was 15.48%. According to the nationwide cardiovascular diseases survey, that of hypertension in rural area of Myanmar was reported to be 12.5% [4]. Other surveys carried out in different areas of Myanmar have given wide range of results. It was found that the highest prevalence was 38.4% at Thonegwa [6] and the lowest was 2.1% at Laydaunggan [7]. Regional variation in prevalence of hypertension could be explained in part by ethnicity, dietary constituents, different cutoff BP values in defining hypertension and different age range of the study populations.

Certain findings in our study replicated the patterns observed in other BP surveys. For instance, the general pattern of an increasing prevalence of hypertension with age has been well recognized in other studies abroad [3]. Likewise, the better awareness and treatment of hypertension in women has been consistently documented [8, 9, 10] but the reasons are not entirely clear. Differences in health seeking behaviors could contribute to this gender-related difference.

The distribution of different categories of hypertension showed mild hypertension to be the most frequent form (83.6%). It was consistent with previous hypertension survey in Thonegwa Township where mild hypertensives formed the majority (78.1%) [6]. It was also in line with the position elsewhere in other countries, for example in Spain (66%) and in United States (80%) [11, 12].

Older age, being a current smoker, high BMI and self-reported diabetes mellitus turned out to be significant risk factors for hypertension in our study. The association of hypertension with BMI and diabetes mellitus has been reported previously on numerous occasions [13, 14, 15]. The effect of smoking on cardiovascular system and its

association with hypertension was also well-known.

It was reported in Thonegwa BP survey that family history, consumption of salt and soda rich snacks were strongly associated with hypertension. Contrary to this and other studies [6, 16, 17] physical inactivity, family history and salt intake were not found to be significant correlates of hypertension. Lack of association with these well-known risk factors in the present study may be due to its small sample size.

There was a regional variation in hypertension prevalence and risk factors in our country. To what extent well-establish risk factors such as age, weight, salt intake and physical inactivity, accounted for observed regional variation in prevalence is an important issue worthy of additional investigation. The role of these and other possible risk factors such as ethnicity, dietary constituents, socioeconomic status and education level should be explored further in other areas of Myanmar.

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Sea snake bites in Letkokekone: a situation analysis

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Although sea snakebite occurs among fishermen in Myanmar, the incidence has not been documented. In order to determine incidence, case fatality and treatment seeking behaviour of the victims, a community-based study was conducted in fishing community of Letkokekone, Yangon Division. A house-to-house visit was conducted and structured questionnaires were asked to the victims. The cumulative incidence of sea snakebite for 4 years is 318/100000 with male preponderance (96%). The mean age of the victim was 39 yrs and 92% were fishermen. Fifty-five percent were bitten after dark. Sixty percent of them did not apply first-aid and none of them used prophylactic measure. Sixty-six percent sought treatment from traditional healers consisting of wound incision and suction, 23% home remedy (taking coconut flesh and jaggery) and 11% at hospital. Twenty-one percent of them gave a history of previous sea snakebite and sting from jellyfish. Activities like setting up, drawing and unloading stake net expose leg (77.5%) and hand (22.5%) to risk. Since only 11% sought medical treatment, the incidence based on hospital statistics is grossly underestimated. Health education on use of correct first-aid and prophylaxis at work should be promoted. Practice of harmful treatment of traditional healers and unscientific home remedy should be discouraged.

INTRODUCTION

Sea snakebite is an important occupational hazard of fishermen. Accidental bite occurs while unloading fishing net, sorting fish especially under insufficient light [1] and catching fish using net in shallow seashore. According to Reid [2] less than 20% of the sea snake bite victims are envenomed, of which 40% or more are severely envenomed. Severe envenomed cases succumbed with neuromuscular paralysis and renal insufficiency secondary to myoglobulinuria. Specific antivenom is not available in Myanmar. Only conservative treatment could be given. The incidence of sea snake bite based on hospital statistics is 0.4% [3]. The traditional practice in fishing community is referring sea snakebite cases with neurotoxic features to hospital. The

fact that the true incidence of sea snakebite may far exceed the hospital data is supported by community-based study carried out elsewhere [4-8]. However, epidemiological studies on sea snakebite cases have not been carried out in Myanmar. The objective of the present study is to determine incidence and treatment-seeking behaviour of sea snake bite victims of Letkokekone, Yangon Division.

MATERIALS AND METHODS

A community-based study was conducted in four villages of Letkokekone Township namely Letkokekone, Kanyinkone, Wetkite, Kanlyal shae and Dayeboo. A house-to-house visit was undertaken by assigned midwives. Set proforma including size of the household, age and sex of sea snakebite victims within last 4 years was asked to the

head of household and the victim was identified.

Structured questionnaires designed to cover circumstances of the bite, fatality, treatment-seeking behaviour, use of first-aid and prophylaxis were asked to the victim or next of kin if the victim is dead. For children, guardians or parents were asked. Validity of the data was checked by the researcher TP and AAM. In-depth interview was carried out with two key informants from Letkokekone who have been engaged in fishing for over 10 to 18 years. In in-depth interview, incidence, circumstances of the bite, use of first-aid and prophylaxis, traditional belief in fishing community and treatment-seeking behaviour were asked. Collected data were coded and analysed using epi info 6 version (6.04d) software.

RESULTS

A total of 14777 populations residing in the 4 villages of Letkokekone were included in this study. Of them, 47 (45 males and 2 females) were bitten by sea snake in 4 years (1999-2002). Mortality and morbidity of sea snakebite by year is shown in Table 1 and demographic characteristics of the snakebite victims in Table 2. The mean age of the victims is 39 yrs (youngest 16 and the oldest 87 yr) with male preponderance (96%). Most victims (92%) were bitten while engaged in fishing. Fifty-five percent of the bites occurred after dark (7pm-6am). The bite occurred throughout the year with its peak in March and May.

Table 1. Morbidity and mortality of sea snakebite in Letkokekone by year

Year	Total populations	No. of snakebite victim	No. of death	Incidence x100000	Case fatality rate %
1999	13908*	22	4	158.1	18
2000	14191*	11	-	77.5	
2001	14481*	10	-	69	
2002	14777	4	-	27	
Total	57357	47	4	331.6	8.5

*Growth rate calculated on 2%

Table 2. Demographic characteristics of the victims

Age	Mean 39 yr (16- 87yrs)
10-20yr	8.9%
21-30yr	15.6%
31-40yr	31.1%
41-50yr	24.4%
51-60yr	17.8%
61 ++	2.2%
Sex	Male 95.6%
	Female 4.4%
The bite	Throughout the year (peak - March and May).
Time bite	55% after dark (7pm-6am). 45% day time (6am-7pm)
Sites of bite	81% (38) Legs 19% (9) Hands 0% Others
Occupation	93.4% - Fishing business 2.2% Farmer 2.2% Carpenter 2.2% Hawker
Activity	82.3% Fishing 11.1% Walking seashore 4.4% Sitting at seashore 2.2% Sorting fish

In-depth interview of the key informants revealed that estimated yearly incidence of the bite accounted for 10-20 bites. Recent decrease in the incidence to 10 per year were attributed to introduction of modified fish catching technique and advancement of sandbank at seashore making unfavourable condition for catching fish in shallow water. Case fatality rate also dropped from 2-3 per year to none in recent year. Accidental bite occurred while sorting fish and drawing/unloading content of fishing net especially after dark with poor illumination. Most bites occurred in October-November and majorities were bitten on legs while setting up stake net under sea and drawing/unloading content of the net.

Site of bite

Majority (81%) of the victims was bitten on legs (right leg 44.7% + left leg 36.2%) and 19% on hands (right hand 14.99% + left hand 4.2%). Fang marks were detected in 82% and local pain in 91%. Majority 85% (40/46) were bitten during fishing and 15% (7/46) on seashore (walking 7 + sitting 1).

One 87 yr old man was bitten while walking on seashore. There were four fatal cases (8.5%) (three treated at station hospitals and the other by a traditional healer).

First-aid and prophylaxis

Majority (60%) of the victims did not apply first aid. Wound treatments carried out by the victims include wound incision 19% (9), herbal extract to wound 11% (5), tourniquet 6% (3) and coagulation 4% (2). No prophylactic measures were taken against sea snake bites (100%).

In-depth interview of the key informants revealed that use of prophylaxis against snakebite and first-aid were not aware of and were not used in fishing community. Wound incision and suction was widely practiced until last year before the death of the reputed local healer whose specialty was wound incision and suction. Now application of herbal extract or meditated oil to the wound is often practiced.

Treatment seeking behaviour

Treatment seeking behaviour of the victims is shown in Table 3. Treatments provided by local healers are wound incision and suction (100%). Home remedy consists of advising to take coconut flesh with jaggery.

Table 3. Treatment seeking behaviour of the victims

Treatment	Cases	%	Treatment given
Traditional healer	31/47	66	Wound incision and suction
Home remedy	11/47	23	Coconut flesh and jaggery
Hospital	5/47	11	Conservative

All of the victims (9/9) from Chaungwa and 73.5% (22/30) from Letkokekone sought treatment from local healers. In Gadegone village, 6/8 victims were treated with home remedy since thus was no traditional healer around. In Letkokekone and Gadegone, 13.3% (4/30) and 25% (2/8) of the cases

seek treatment at station hospitals respectively.

In-depth interview of the key informants revealed that sea snakebite occurs at or near seashore and takes 1-3hr to get ashore and treated by local healer advising to take coconut juice and jaggery. The victim is kept awake by constant arousing or talking to him. Since majority of the victims recovered with local treatment, they are reluctant to seek hospital treatment unless the victim is presented with severe neurotoxic features.

Symptoms following the bites

Time taken for development of symptoms following the bites ranges from 15 minute to 4hr. Fifty-eight percent of the cases developed symptoms within ½ to 1hr after the bite and 24% in 15 minutes. The symptoms recalled by the victims are shown in Table 4. Four out of 47 victims were fatal.

Table 4. Symptoms of the sea snakebite victims

Symptoms	Cases	%
Drowsiness	46	97.8
Limb muscle pain	30	64
Limb muscle tenderness	14	30
Heavy upper eyelids	8	17
Passing dark coloured urine*	6	13

* (Myoglobulinuria)- feature of systemic envenoming

History of previous sea snake bites and stings

History of sea snake bite in the last 5 years was recalled in 21.2% (10/47) and sting from jellyfish in 21% (10/47) of the victims.

Perception

Majority (91%) of the victims knew species of sea snake (77% three species) and believed (91%) that all were poisonous. Majority (95%) of the victims saw the snake after the bite. Knowledge of practice of prophylaxis against sea snakebite was nil.

Circumstances of the bites

Majority of the fishermen were shallow water fish/prawn catchers, who used stake net and bag net (Figure 1) for catching fish/prawns. Majority of them 77.5% (31/40) were bitten on leg and 22.5% (9/40) on hand while setting up/unloading stake net and catching prawn using a bag net. Seven victims recalled being bitten by sea snakes (6 walking and one sitting on shore).



Fig. 1. Fishing with net

In Chaungwa and Letkokekone, most were bitten while setting up stake net (57.5%), while catching fish with bag net (10%) and in a few cases while walking /sitting on shore (15%). In Gadegone village, most were bitten during drawing and unloading the fishing net (32.5%).

In-depth interview of the key informants revealed that shallow water fish catchers used stake net and bag net in catching fish. Majority of the victims were bitten on legs while setting up stake net under sea, drawing/unloading fishing net, catching fish using a bag net. Recent practice of modified fish catching technique results in decline in prevalence of sea snake bite cases.

DISCUSSION

Incidence

The study highlights that sea snake bite is an occupational hazard for fishermen. Although yearly incidence of the bite is decreasing, they are still high. Recent decrease in the

incidence of the bite is attributed to use of modified fish catching technique and advancement of sand bank in seashore. Since only 11% of the victims sought medical treatment, incidence of sea snake bite based on hospital statistics is grossly underestimated. Emphasis on seeking early medical treatment irrespective of the severity of the bite should be made in imparting health education to the fishing community.

Prophylaxis/first-aid

The study highlights that prawn catchers of shallow water were mainly bitten on legs and some on hands while setting up stake net and catching fish using a bag net. Health education on taking precaution/wear of protective gloves while drawing/unloading the net and sorting fish should be given and promoted. Since majority of the bites occur after dark, provision of good illumination at work is essential. Two sea snakebite cases admitted to Yangon General Hospital were bitten after dark while sorting fish under insufficient light [1]. Protective boots should be worn [9] when walking along seashore. Since majority of the victims did not apply first aid, use of correct first-aid (pressure-immobilisation technique using crepe bandage) [10] recommended for neurotoxic envenoming should be promoted.

Clinical features

Usually sea snake bite is painless [2]. However, local pain present in all victims probably reflects misinterpretation of pain inflicted by local wound treatment. Clinical features are similar to those reported in sea snakebite cases [1-2]. However, if no myoglobulinuria (dark coloured urine) develops in 4 or more hours, it is unlikely that the victim will develop systemic envenoming [11]. Passing dark coloured urine was observed in fatal cases (13%). According to Reid [2], majority of sea snakebite cases are not envenomed and less than 20% are envenomed, of which 40% or more are severely envenomed.

Treatment seeking behaviour

Sea snake bite fails to envenom in 80% of the victims [2]. Most victims treated by local healers belong to this category and majority will recover without treatment. This favourable outcome has created a good impression among the community not knowing the real clinical outcome of the disease. The community should be discouraged on use of unscientific home remedy and harmful treatment of local healers. Early referral to better-equipped hospitals with capability of performing renal dialysis and assisted ventilatory support to treat renal failure and neurotoxic respiratory paralysis in severe envenomed cases should be practiced.

CONCLUSION

The study highlights that incidence based on hospital statistics is underestimated since a small proportion of the victims sought medical treatment. It is suggested that victims should be encouraged to seek medical treatment by giving health education or the disease should be made a notifiable one. Moreover the fishing community needs to be educated on clinical course, outcome of sea snake bite, use of prophylaxis and correct first aid in order to reduce the incidence and fatality following the bite.

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**Epidemiological study of sea snakebite victims of
Kyaikkami Township (Mon State)**

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An earlier community-based epidemiological study of sea snakebites highlights that its prevalence based on hospital statistics is grossly underestimated and possible influence of fishing technique on its prevalence. In order to clarify the statements, a community-based study of sea snake bite victims was carried out in Kyaikkami, Mon State where its fishing technique differs from the earlier study. A house to house survey was conducted and structured questionnaires designed to cover circumstance of the bite, treatment-seeking behavior, fatality, use of first-aid and prophylaxis were asked to the victims. The total number of the bite for 4yrs is 46. Yearly incidence for 4 yrs (1999-2002) is 27.7, 11.9, 15 and 22.8/100000 respectively. Majority (67%) of them were bitten on hands and 33% on legs while drawing/unloading fish net. Majority of the cases are still using no longer recommended first aid-treatment. No prophylactic measures were taken against the bite (97.8%). Majority (57%) sought treatment from traditional healers consisting of either application of herbal medicine to wound or wound incision and suction. Nine (19.6%) sought hospital treatment, 8 (17.4%) at local clinic and 3 (6.5%) sought home remedy (advising to take coconut juice and rub wound with lime). Since only 19.6% sought hospital treatment, the incidence of sea snakebite based on hospital statistics is grossly underestimated. Health education on use of correct first-aid and taking precautions/prophylactic measures at work should be given to fishing community and practice of harmful treatment of traditional healers and unscientific home remedy should be discouraged.

INTRODUCTION

Sea snakebite is encountered among fishermen working in shallow and deep sea. Some are bitten while sorting out fish at sea especially under insufficient light [1] and others while drawing/ unloading content of fishing net. When bitten at deep sea, it takes several hours to get to shore depending on the level of tide. However, 60-70% of the accidental sea snakebites fail to envenom victims [2]. Specific antivenom is not

available in Myanmar. The incidence of sea snake bite based on hospital data is 0.4% [3]. Community-based studies of snakebite cases elsewhere highlight that incidence of snakebite based on hospital statistics is grossly underestimated [4-8]. Recent community-based study of sea snakebite cases of fishing community of Letkokekone, Yangon Division [9] supports the observation and highlights the possibility of influence of fishing technique on its incidence. The present study aims to verify the above assumptions.

MATERIALS AND METHODS

Community-based epidemiological survey of sea snake bites was carried out in Kyaikkami Township (Kyaikkami, Setse, Karupi and Pa-nga) (Figure 1) with the help of basic health staff of the township. A house to house survey was conducted and structured questionnaire designed to cover circumstances of the bite, mortality, treatment-seeking behavior, first-aid and prophylaxis were asked to the sea snakebite victims or next of kin if the victim was dead. For children, guardian or parents were asked. An interview on key informants and a follow-up study were also carried out. Coded data were entered and analysed using Epi info version (6.04d) software.

Map of survey area of kyaikkami

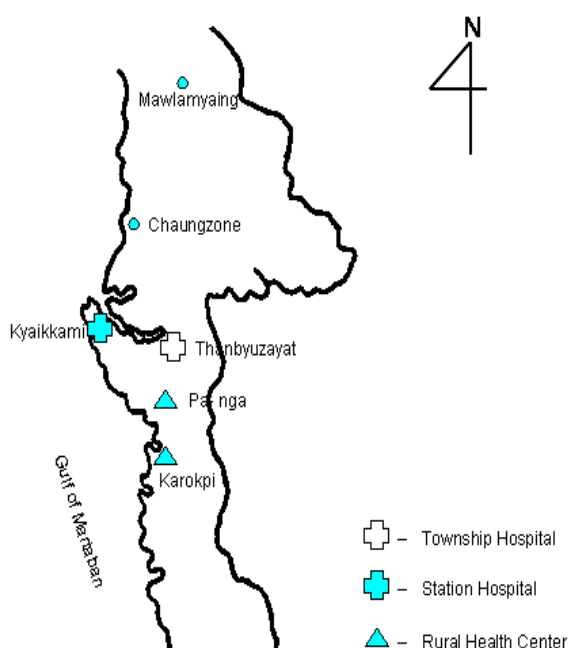


Figure 1. Map of study area of Kyaikkami

RESULTS

Prevalence

The total population of the study areas is 61209. The total number of sea snake bite

covering a period of 4 yrs (1999-2002) is 46 and yearly incidence for 4yr is 27.7, 11.9, 15 and 22.8/100000 respectively. Case fatality rate for 2002 is 7%. The mean age of the victim is 32 yrs (youngest 12 and oldest 65yr) with male preponderance (98%). Majority (98%) were fishermen and most bites (63%) occurred after dark (7pm-6am). The bites occurred throughout the year with peak incidence in October, December and February. Majority of the victims were bitten on hands (70%) and 30% on legs.

First-aid and prophylaxis

Majority (96%) of the victims carried out wound treatment namely tourniquet 26% (12/46), application of herbal extract to wound 23.9% (11/46), wound incision plus application of herbal extract to wound 21.7% (10/46), incision 17.4% (8/46), coagulation 6.5% (3/46) and none 4.3% (2/46). None of them carried out prophylaxis against sea snakebite.

Treatment seeking behavior

Majority 56.5% (26/46) sought treatment with local traditional healer, 19.6% (9/46) hospital, 17.4% (8/46) local clinic and 6.5% (3/46) home treatment.

Treatments from local healers were either application of herbal extract to wound (77%) and wound incision plus suction. Home remedy recommends drinking coconut juice and rubbing the wound with lime.

Pattern of treatment seeking behaviour

Majority 100% (25/25) of the victims from Setse and 67% (2/3) of Karupi seek treatment from traditional healers, 55.5% (10/18) at hospital and clinic. Home treatment was sought in 16% (3/18) of the victims in Kyaikkami.

Symptoms following the bites

The symptoms recalled by the victims were drowsiness (87%), heavy upper eye- lids

(84%), stiffness of limb muscle (80%), tenderness, ache and pain of limb muscle (74%) and passing dark coloured urine (71%).

Circumstances of the bites

Majority 70% (32/46) of the victims were bitten on hands (24 while drawing/unloading conical net + 5 sorting fish + one catching fish using a bag net + one while carrying fish basket). Thirty percent (14/46) of them were bitten on legs while drawing/unloading conical net (10/14), catching fish using a bag net (2/14) and while carrying fish baskets (2/14). History of sea snakebite bite was recalled by 24% of the victims.

Perception

Majority (80%) of the victims were aware of sea snake and 94% had seen the snakes and 89% were familiar with two species of sea snake. Majority (98%) of the victims believed that all snakes are poisonous.

DISCUSSION

According to the survey it is evident that sea snakebite is an occupational hazard of our fishermen. The yearly prevalence of sea snake bite for 4 yrs (1999-2002) is less than that of Letkokekone, another fishing community in Yangon Division (158.1, 77.5, 69, and 27/100000 respectively) [9]. It suggests that fishermen from Letkokekone are more prone to sea snakebite because of their technique of catching fish in shallow water seashore using stake net compared to deep-sea fish catchers of Kyaikkami who use conical net (Figure 2). It appears that shallow water fish catchers also carry 10 times more risk to get sting from jelly fish during summer and 2 times more risk to sea snake bite.

Majority of shallow water fish catchers are bitten on legs while setting up/drawing the fishing net under sea [9]. In contrast, deep sea fish catchers are bitten on hands (63%) while drawing/ unloading fishing net and

sorting fish, suggesting that the site of bite is related to technique of catching fish. According to the survey, some fishermen are using rubber gloves as a protective measure against injury sustained from drawing the net. Health education on taking special precautions/ measures at work should be further emphasized and promoted. Majority of the victims were bitten after dark. It has been reported that two sea snake bite cases admitted to Yangon General Hospital were bitten on hands while sorting fish at sea after dark under insufficient light [1]. Need for provision of good illumination at work is essential and should be emphasized.



Figure 2. Picture of a conical net

Since majority are still practicing no longer recommended first-aid treatment as in the previous study [9], the community should be discouraged on taking harmful wound treatments and educated on use of correct first-aid (compression immobilizations first-aid using crepe bandage) [9].

The treatment seeking behavior of two fishing community (Letkokekone and Kyaikkami) appears to be similar [8]. Majority of them seek treatment from local traditional healers [9] whose harmful treatment should be discouraged. More numbers of victims from Kyaikkami sought medical treatment compared to that of Letkokekone. One of the reasons for seeking early medical treatment by the victims is their wish to return to work as soon as possible. The favorable outcome of the cases treated by traditional healers

attracts higher number of victims seeking treatment with them. It was found that all 25 victims from Setse village sought treatment from traditional healers. Practice of unscientific home-remedy should be discouraged since it gives a false sense of security to the victim.

Since only 9/46 victims sought medical treatment, the incidence of the bite based on hospital data is grossly underestimated. In order to get true incidence, sea snakebite should be made a notifiable disease and victims should be encouraged and educated to seek medical treatment in order to receive proper medical care.

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Bacteriological analysis of drinking water in selected schools

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Bacteriological analysis was undertaken on 90 samples of water from primary, secondary and high schools in 10 townships of Yangon area. Most of the schools (8.81 % of primary; 55.56% secondary & 70% high schools) have artesian wells. It was also found that 18.18%, 33.33% and 30% of primary, secondary and high schools respectively received water supply from Yangon City Development Committee (YCDC). In this study, only one secondary school used water from hand dug well; one high school had stainless steel tank; some schools had aluminium tanks and some had concrete brick storage containers. Purified water bottles with different brand were used for drinking water in most of the schools. From drinking water sources, coliforms were identified from purified water (66.67%), YCDC water (100%), artesian water (82.35%). Although faecal coliforms were not identified from purified water, they were isolated from YCDC water sources (83.33%), and artesian water (64.71%) which were used as drinking water. For domestic water source, coliforms and faecal coliforms were identified from YCDC water (100% & 87.50%) and artesian water (70% & 45%) respectively. From water used as sanitary purposes, coliforms and faecal coliforms were identified from YCDC water (100% & 62.50%) and artesian water (95% & 70%) respectively.

INTRODUCTION

The major causes of disease and death from waterborne illness in the history of humanity have been microbiological factor, and only rarely other components. Waterborne infection occurs through excreta contaminating supply of water or by contaminated through persons. The coliforms bacilli are thus, the most reliable indicators of faecal pollution [1]. Illness in a community, associated with an episode of water contamination with sewage was reported in a rural Irish town [2]. Outbreaks of gastrointestinal illness associated with contamination of main water supply were shown by various investigators [3-17]. Drinking water should contain no impurity that offends the sense of sight, taste or smell. Although water is abundant on the

earth, it is becoming more and more difficult problem to provide fresh water, on account of growth of the population and the rise in the standard of living and also due to the development of modern industrial technology. Thus, availability and adequate use of safe water supply round the year would decrease the incidence of a number of specific indicator conditions which in turn would improve the health status of the community. The study was conducted with the following objectives:

General objective:

To determine the bacteriological quality of water in schools.

Specific objective

To determine the sanitary quality of water by determination of coliforms, faecal coliforms, total counts and specific pathogens (*Salmonellae*, *Shigellae*, *Escherichia coli* and *Vibrios*).

MATERIALS AND METHODS

Primary, middle and high schools from 10 townships of Yangon area were selected randomly as study areas from January to March, 2003. Drinking water samples from 24 artesian wells, from one campus in January, 2002 (cool dry) and May 2003 (hot wet) were tested for comparison. Approximately one litre each was collected aseptically from drinking water sources, domestic use water and from the sources of water used for sanitary purposes. All samples were carried under cold chain transportation to reach the laboratory within 3 hours after collection [1].

Bacteriological examination

All procedures were carried out as in the standard manual described by WHO [1]. Examination includes determination of coliforms and faecal coliforms by multiple tube and membrane filtration methods; and isolation, identification, characterization and serotyping of the isolated bacterial pathogens [2].

RESULTS

Types of water sources from Yangon schools

Three different types of water sources were observed from selected schools; 18.18% primary schools received water from YCDC and 81.81% have artesian wells. Among secondary schools, 55.56% have artesian wells, 33.33% have YCDC supply and only one school has hand dug well. It was also observed that 70% high schools also have artesian wells and 30% have YCDC supply (Fig. 1).

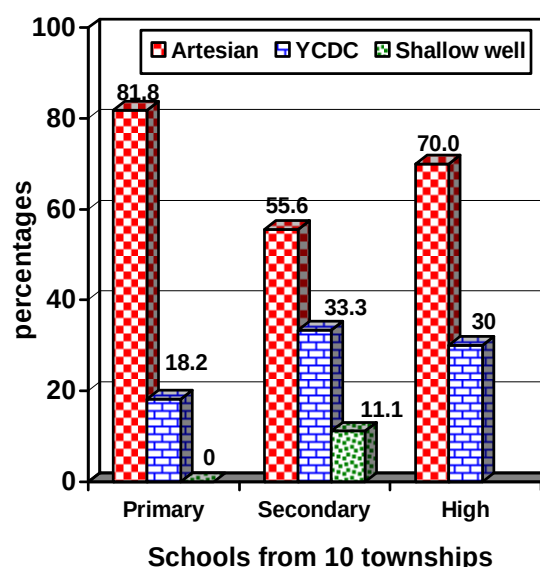


Fig. 1. Types of water source from primary, secondary and high schools from 10 townships of Yangon

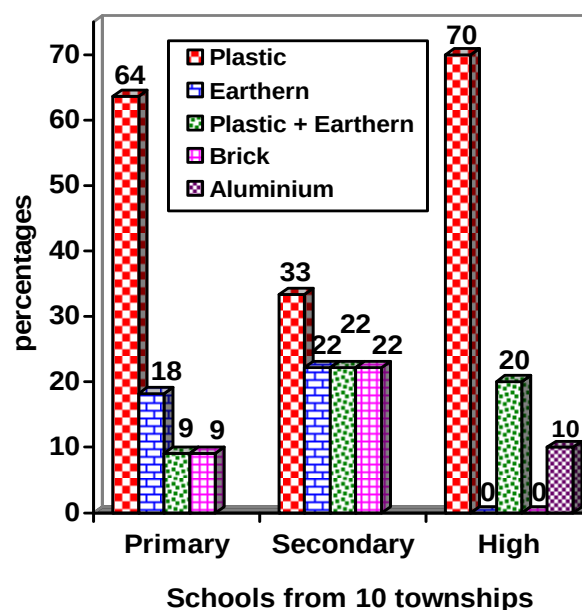


Fig. 2. Types of storage containers used for drinking water in schools

Types of storage containers used for drinking water

Most of the schools provided drinking water in good containers, either in big plastic bottles (20 litres) with cap; or in earthen wares, or they used both type of containers. One of the schools used an aluminium tank provided with taps. Some were made of

bricks and connected with taps. Those types of storage containers used in primary, secondary and high schools are shown in Fig. 2. It was observed that 63.64% of primary schools used plastic bottles, 18.18% used earthen wares, and 9.09% used both types and 9.09% used brick type. From secondary schools, 33.33% used plastic bottles, 22.22% each used earthen types, plastic and earthen types or brick types respectively. In high schools, 70% used plastic bottles, 20% used plastic and earthen pots and 10% used aluminium tank. The most prominent type they usually used was of polyethylene with polypropylene stopcocks.

Determination of coliforms and faecal coliforms by multiple tube method and by membrane filtration techniques

The presence of coliforms and faecal coliforms from three different sources from each schools is shown. From drinking water sources; faecal coliforms were not identified but coliforms were identified from 66.67% of purified water (with different brands) used in those schools. From YCDC water, all tested samples were contaminated with coliforms and 83.33% with faecal coliforms., Some 82.35% of coliforms and 64.71% of faecal coliforms were isolated from artesian water source (Fig.3).

All samples of domestic water from YCDC source were contaminated with coliforms and 87.50% with faecal coliforms. Artesian water source was contaminated with 70% of coliforms and 45% of faecal coliforms (Fig. 3).

Water for sanitary purposes from YCDC also had coliforms in all the tested samples and 62.50% had faecal coliforms. Artesian water source was with shows 95% of them with coliforms and 70% with faecal coliforms (Fig. 3).

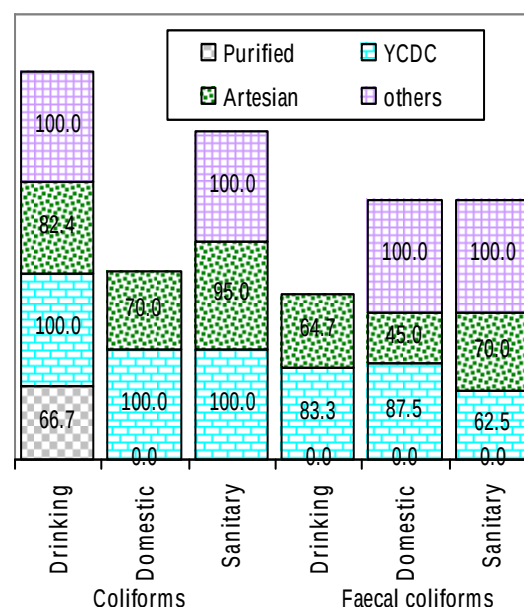


Fig. 3. Contamination of coliforms and faecal coliforms from water sources

When comparing the presence of coliforms and faecal coliforms in cool dry and hot wet seasons in one campus area, the isolation of coliforms 71.43% in cool dry gave rise to 85.71% in hot wet season. Similarly, the faecal coliforms isolated from 14.29% in cool dry increased up to 66.67% in hot wet season (Fig. 4).

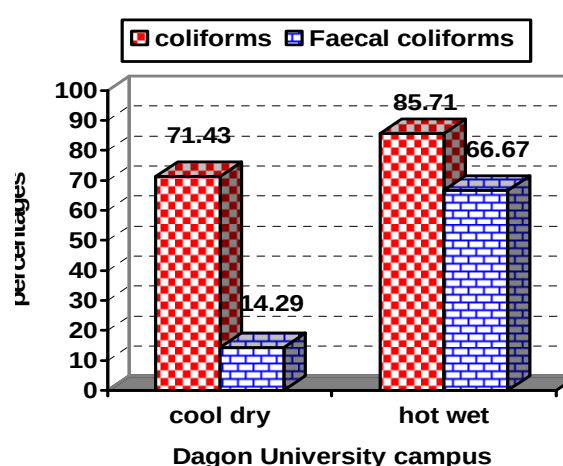


Fig. 4. Changing pattern of coliforms and faecal coliforms in two seasons from drinking water sources from Dagon University campus

Most probable number of bacteria from different water sources

Each sample from different sources varied in bacterial contamination. It was found that all samples from YCDC used for drinking water purposes were contaminated with heaviest growth of coliforms (>1800MPN/100ml).

Distribution of coliforms and faecal coliforms from school water in different townships

From 30 schools, in 10 townships, school water from Thaketa township seems to be contaminated more than in other townships. Faecal coliforms were not isolated from water from all three schools of Insein and South Okkalapa township where they used artesian wells. It indicated that the original source (>200 feet) was found to be free from faecal coliforms which reflects that natural water is pure. It also indirectly indicated that contamination occurs through carriage of water by some means.

DISCUSSION

There are interesting trends in the common causes of gastroenteritis with the aspect of new and re-emerging infectious organisms through water sources. Drinking water was identified as a probable source of infection by enterotoxigenic *Escherichia coli* (ETEC) in homes of children with diarrhoea in Thailand [19] and in Bangladesh [20]. Occurrence of drug-resistant bacteria in water [21, 22] and long term starvation-survival of bacteria [23] were reported. Environmental research with special emphasis on water was reported and the survival of bacteria in water was described in this locality [24]. In this study, the distribution of coliforms and faecal coliforms was high. It was assumed that these were contaminated more after handling and carrying processes after storage of water. It was suggested that after

obtaining of good quality water, a systematic pipeline system should be developed especially for drinking water purpose. More health education measures should be undertaken.

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Bacterial and fungal contamination of chillie powder available in local markets

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Contamination of bacteria and fungi in three kinds of chillie powder (ayaung-tin; akyan & ahlaw; n=70) available in local markets were tested by standard microbiological methods from May 2001 to April 2002. From chillie powder (ayaung-tin), coliforms (88%), faecal coliforms (48%) and fungi (68%) were isolated from the tested 25 samples. From chillie powder (akyan), coliforms (85.7%), faecal coliforms (42.9%) and fungi (57.14%) were isolated from the tested 21 specimens. Moreover, from baked chillie powder (a-hlaw), coliforms (79.1%), faecal coliforms (50.0%) and fungi (54.16%) were isolated out of 24 samples. Bacterial species identified were *Escherichia coli*, *Enterobacter* species, *Klebsiella* species, *Serratia* species, *Citrobacter* species and *Alcaligenes* species.

INTRODUCTION

One of the major health problems is the large number of gastrointestinal cases and outbreaks throughout the year contributing highly to the overall morbidity rate in many parts of the world. Typhoid and paratyphoid fever; bacillary dysentery and bacterial food poisoning still play major causes of morbidity as reported by South East Asian Medical Information Centre, 2001 [1-4]. The major reasons are: malnutrition, parasitic infestations and presumably *Salmonella* spp., *Shigella* spp., enteropathogenic *Escherichia coli* (EPEC) and *Vibrio cholerae* are the major potential bacterial infections. In Myanmar, chillie is one of the major items used in various preparations of food and is usually eaten raw in many cases. The role of bacteria in chillie needs to be explored and this study aimed to determine the presence of bacteria in different chillie powder available from local markets.

MATERIALS AND METHODS

Sample collection

A total of 70 samples of chillie powder were

chosen in 3 forms (a) akyan (b) ayaungtin and (c) ahlaw and collected from different local markets from North Dagon, Tamwe, Pabedan, Dagon and Latha townships.

Determination of coliforms and faecal coliforms

It was processed according to the method of WHO [6].

Determination of bacterial pathogens

Bacterial isolation was done by the standard method as described by WHO, 1981 [6]. Isolation of Enterobacteriaceae was done by direct inoculation onto MacConkey agar, (MA), Salmonella-Shigella (SS) agar and followed by enrichment media Selenite F broth; Micrococcaceae by Mannitol salt agar and Nutrient agar; Pseudomonadaceae by Ashdown agar. *Salmonella*, *Shigella*, *Escherichia coli* and other Enterobacteriaceae were isolated by the method of Ewing, 1986 [7]. Fungal isolation was done on Sabouraud Dextrose Agar and *Candida albicans* was confirmed by germ tube test. Biochemical tests and serotyping were done whenever necessary.

RESULTS

Determination of coliforms and faecal coliforms

Distribution of coliforms and faecal coliforms from different kinds of chillie are shown in Fig. 1. Almost all chillie types were contaminated with coliforms; 88% in ayaungtin, 85.7% in akyan, 79.1% in ahlaw powder. Also faecal coliforms were found in 52% of ayaungtin, 42.9% of akyan and 50% in ahlaw powder.

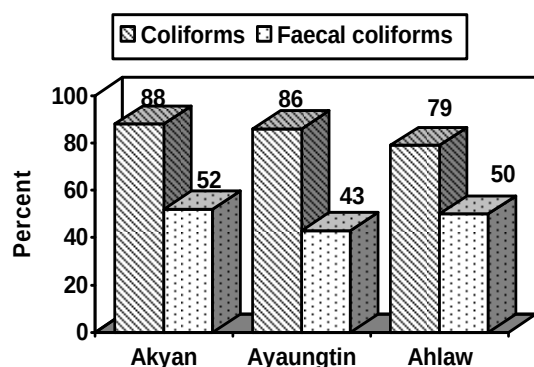


Fig. 1. Distribution of coliforms and faecal coliforms in different kinds of chillie powder

Bacterial count in different chillie powders

The bacterial count of coliforms ranged from 240 to >1800 MPN/100gm in ayaungtin, 12 to >1800 MPN/100gm in akyan, 7 to >1800 MPN/100 gm in ahlaw powder. Faecal coliform counts were found in 4 to >1800 MPN/180gm of ayaungtin, 4-240 MPN/100 gm of akyan and 7 to >1800 MPN/100 gm in ahlaw powder (Table 1).

Bacterial species isolated from different chillie powders

As shown in Table 2, bacterial species isolated were *Escherichia coli* from 52% of ayaungtin, 42.9% of akyan and 50% of ahlaw powder; and *Enterobacter* species from 24% of ayaungtin, 28.6% of akyan, 12.5% in ahlaw powder and *Klebsiella* spp. from 8% of ayaungtin, 4.8% of akyan, 8.3% of ahlaw powder. Other species identified included *Serratia* spp., and *Citrobacter* species.

Fungi isolated from chillie powder

As shown in Table 2, fungal species were isolated from 68% of ayaungtin, 57.1% of akyan, 54.1% of ahlaw powder respectively.

DISCUSSION

Food poisoning is a major public health concern worldwide. Mass food production, catering and wide distribution of food would undoubtedly increase the incidence of food poisoning especially if there is improper or unhygienic food handling or preparation. Awareness of emerging infections is also important [8-10]. The food itself provides sufficient nutrients and with adequate moisture, warmth and time lapse between preparation and consumption of food, multiplication of the pathogen will inevitably take place. If chillie is contaminated with bacteria or fungi, food will be a potential media for propagation of pathogens.

Many of the coliforms are inhabitants of the human and animal intestine. Coliform can survive and grow in an environment associated with food processing in which enteric pathogens die or are overgrown. Coliforms other than *Escherichia coli* are good indicators of unsatisfactory processing or sanitation and the presence of large numbers of coliforms in processed foods indicate that the opportunity for proliferation have occurred. Coliforms can persist and grow in improperly cleaned equipment and utensils. They can establish themselves as part of the resident flora of food processing establishments where they are difficult to eliminate. Coliforms may also enter the food product after processing from the hands or garments of food-handlers, or from dirty utensils or surfaces or from unsafe water used.

Coliforms do not necessarily indicate contamination from faecal source. However, it does indicate inadequate processing or post processing contamination, most probably from workers, or from dirty

equipment or from the raw food before processing. The presence of a large number of coliforms does not necessarily indicate an immediate health hazard, but it indicates lack of good sanitary practice. Presence of faecal coliforms indicate faecal-oral contamination. *Escherichia coli* does not usually persist for long in the environment other than the intestine. Thus, the presence of *Esch. coli* in foods generally indicates recent pollution of faecal origin. Recent pollution of faecal origin may be contamination directly by faeces or indirectly by faecally contaminated materials.

One aspect of food-borne infections can be attributed especially to the use of unsafe water in the preparation of foods and washing of the utensils. Other factors that are known to contribute to the risk of a foodborne disease include contamination from infected or colonized food handlers, inadequate boiling temperatures, improper holding temperatures, contaminated equipments and utensils. Thus, food preparation and handling process play a key role in causing food poisoning outbreaks.

Table 1. Bacterial count of coliforms and faecal coliforms in MPN/100g of chillie powder

Chillie powder	Coliforms (MPN/100g)	Faecal coliforms (MPN/100g)
Ayaungtin	240 to >1800	4 to >1800
Akyan	12 to >1800	4 to 240
Ahlaw	7 to >1800	7 to >1800

In this study, isolation of coliforms and faecal coliforms did not markedly decrease when compared with the the raw products. It reflects the situation that the storage might play a key role in contamination (Fig.1). Low isolation of faecal coliforms in chillie (akyan) demonstrated that the bacteria could not easily propagate in that akyan due to the high salt concentration in it (Table 1).

Similar species of bacterial isolates do occur in all three specimens with similar distribution. This showed that these environmental bacteria could easily contaminate the foods (Table 2).

Table 2. Isolation of bacterial and fungi from different kinds of chillie powder showing in percentages

Species	Ayaungtin (n=25)	Akyan (n=21)	Ahlaw (n=24)
<i>Escherichia coli</i>	52.0	42.9	50.0
<i>Enterobacter aerogenes</i>	24.0	28.6	12.5
<i>Klebsiella aerogenes</i>	8.0	4.8	8.3
<i>Citrobacter freundii</i>	4.0	4.8	8.3
Fungi	68.0	57.1	54.1

The general requirements for prevention of food poisoning from microorganisms could be applied to *Bacillus cereus* and *Esch. coli* to be used in well established hygienic principles in the manufacture, preparation, storage and serving food, training of food service personnel; health education, licensing of food service establishments depending on recognized qualifications in food hygiene for their managers and supervisors and compliance with the National Food Regulation.

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**Cost of in-patient care at 16-50 bedded township hospitals
in Yangon Division (2003-2004)**

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Economists define cost as the value of resources used to produce something which includes a specific health service or a set of services. By analyzing and understanding the cost of in-patient care at township hospitals, it could provide one piece of information for policy formulation on cost recovery schemes at the township hospitals. The objective of the study is to examine the cost of in-patient care in 16-50 bedded hospitals so as to provide inputs for policy formulation on cost recovery schemes at the township hospitals. Two 50-bedded, four 25-bedded and two 16-bedded township hospitals from Yangon Division were included in the study. Cost per in-patient day was calculated for each of these hospitals for one fiscal year from 1 April 2003 to 31 March 2004. The general approach that was used in the cost calculation involved calculating: capital cost and recurrent cost incurred by health care providers for each hospital for the fiscal year 2003-2004; patient-days for each hospital for the same fiscal year; and cost per patient-day. A comparison was also made between the unit costs of the hospitals included in the study. The study identified that cost per patient day ranged from Kyat 439.52 to Kyat 5269.41, with an average of Kyat 1931.60. It was revealed in the study that there were 3 hospitals which could be considered lagging behind in efficiency *relative to* other hospitals in the study. This quick and simple methodology of calculating in-patient care cost per patient day can be a useful managerial tool to understand performance of 16-50 bedded hospitals in Myanmar where general care services are being provided mainly for acute patients.

INTRODUCTION

Economists define cost as the value of resources used to produce something which includes a specific health service or a set of services [1]. Costs are generally categorized as capital (or developmental) and recurrent (or operating), the distinction between the two types being based on life expectancy [2]. Resources having a life expectancy of one year or more usually are called capital costs and resources that are purchased and used (or replaced) within one year's time are referred to as recurrent costs. Costs can

then be further classified as direct or indirect. Direct costs are directly attributable to the service. Indirect costs are the costs of supporting the services. These basic classifications of cost form the frame in cost analysis of our study.

Cost information is useful in meeting requirements for accountability and judging efficiency as well as achieving other goals. Health service delivery unit is more efficient when it provides more beneficial effects from the use of a given set of resources. If we can calculate cost for each resource input in

terms of an absolute value, we can calculate average (unit) costs, for example, cost per in-patient. Generally, cost per in-patient is expressed as cost per patient day [3].

By analyzing and understanding the cost of in-patient care at township hospitals, it could provide one piece of information for policy formulation on cost recovery schemes at the township hospitals.

Objective

The objective of the study is to examine the cost of in-patient care in 16-50 bedded township hospitals so as to provide inputs for policy formulation on cost recovery schemes at the township hospitals.

STUDY METHODS

Scope of the study

The scope of the study for cost analysis was on providers' inputs incurred for in-patients admitted to 16-50 bedded township hospitals in Yangon Division during one fiscal year from 1 April 2003 to 31 March 2004.

Sample size

Township hospitals were the sampling units. There are ten 16-50 bedded township hospitals in Yangon Division, four in the northern part and six in the southern part. Initially, five hospitals were chosen randomly so that the sample included two hospitals from the north and three hospitals from the south. However, due to availability of funding for travel expenses to include additional hospitals in the study, four hospitals were selected randomly and included in the study. All these four hospitals were from the southern part. In describing and discussing the study data in this paper, those data from one of the 50-bedded hospitals from the northern part (Hmawbi) was excluded because it was the pre-test site and some changes were made in

data collection approach basing on the experiences of the pre-test.

Two 50-bedded township hospitals --- Taik-kyi (TKI) and Thon-gwa (TGW), four 25-bedded hospitals --- Kun-chan-kon (KCK), Kyauk-tan (KTN), Kayan (KYN) and Twante (TTE), and two 16-bedded hospitals --- Htantabin (HTB), Kaw-hmoo (KHM) --- were included in the study. All these hospitals are acute hospitals although some chronic patients with emergency situations may be hospitalized for certain periods.

Cost calculation and analysis

The following **general approach** was used.

- (1) Capital cost and recurrent cost incurred by health care providers for each hospital under study were calculated for the fiscal year 2003-2004.
- (2) Patient-days (for hospitalized patients) were calculated for each hospital for the same fiscal year.
- (3) Unit cost per in-patient day was calculated.
- (4) A comparison was made between the unit costs of the nine township hospitals included in the study.

The basic calculation of a unit cost (often called average cost) is:

$$\text{Unit cost} = (\text{total cost})/\text{quantity}.$$

In our study, unit cost per in-patient day = (total cost)/total patient-days for the study period.

The capital and recurrent cost categories (for the fiscal year 2003-2004 and all reported in 2003-2004 prices in Kyat) were included. They were either used as direct or as indirect health service costs. Only those costs to the Township Health Department (Department of Health, Ministry of Health) were measured and valued. Time, travel or other private costs incurred by patients (and their families) when using health services were not assessed. The following is the description of the methodology of calculating the costs in our study.

For the calculation of **capital cost**, the cost categories were categorized broadly into three: (1) capital cost contributions from existing hospital buildings; (2) capital cost contributions from medical and surgical instruments that were directly related to in-patient care; and (3) cost contributions from capital items, other than those from the previous two categories, that were indirectly related to in-patient care (for example, an electricity generator, a vehicle, a hospital bed, a cabinet, etc).

Capital cost contribution from existing hospital buildings was calculated as follows.

- In our study, the original costs of most of the hospital buildings were not available. So, we used the price of the buildings for the year under study. This could be estimated by estimating the Per Area Estimate (PAE), with the price for the fiscal year 2003-2004, for all the existing buildings in the hospital compound. By proceeding the calculation from this point means the previous life span of the building had already been taken into account [2]. The PAE estimation was done with the assistance of engineers from Township Construction Department. In this approach, the life expectancy for each hospital building is the remaining life span of the building.
- The research team made an assumption that the life expectancy of all these structures was 10 years. The current rate of interest is taken as 10%. Then a reference was made to an “annualization table”[2]. The table gave the annualization factor value of 0.1627, for an asset with 10 years life expectancy and an interest rate of 10%.
- Another assumption was made that proportion of building space utilized for in-patient care was 30% and accordingly a multiplication factor 0.3 was used for to calculating cost contribution from existing space for in-patient care.
- Then, capital cost contribution from

existing hospital buildings was calculated as: PAE of township hospital A in Kyat (for the fiscal year 2003-2004) x 0.1627 x 0.3

Calculation of the capital cost contributions from other two categories followed the same steps described above, except for the first step. Instead of estimating PAE, market price estimations were made for the capital items (for the fiscal year 2003-2004) with the assistance of merchants engaged in selling and purchasing similar items (old and new).

For the calculation of **recurrent cost**, the cost categories were categorized broadly into three: (1) salaries of staff directly and indirectly involved in in-patient care; (2) cost contributions from recurrent items that were related directly to in-patient care (tablets, injections, syringes, needles, plaster, bandages, cotton wool, methylated spirit, etc.); and (3) cost contributions from recurrent items related indirectly to in-patient care (electricity, water supply, etc.).

Before calculating recurrent cost contributions from salaries of staff, interviews were made at study hospitals with staff directly and indirectly involved in in-patient care as regards their opinions on the average proportion of time spent by each category of staff in in-patient care. These proportions were taken as multiplication factors for calculating recurrent costs contributed by salaries of staff for in-patient care. After multiplying the annual total salary of each staff with respective multiplication factor, the results were added up. This gave the recurrent cost contributed by the hospital staff salary for in-patient care.

For calculation of recurrent cost contributed by recurrent items directly related to in-patient care, lists of these items used for in-patient care during the study period were prepared from sub-stock books available at the study hospitals. The price lists for these items for the study period were collected

from drug sellers. The total number of each item used was next multiplied by the price for each item. Adding up the results gives the recurrent cost contributions from items directly related to in-patient care. Because of lack of proper patient recordings, the amount of recurrent items used, and related cost, for each disease category of patient could not be calculated.

For the cost contributions from recurrent items related indirectly to in-patient care, information was obtained from budget sheets of each hospital. Because of difficulties in estimating the portions of each of the items utilized for in-patient care, the total figures for the study period were taken, since most of them had been utilized for in-patient care one way or either.

Patient-days for each hospital was calculated by adding the lengths of hospital stay in days for all patients admitted during the one-year study period [3].

STUDY RESULTS

Cost of in-patient care per patient day, average length of hospital stay in days and range of hospital stay in days for each study hospital for the year under study are shown in Table 1. The results indicate that cost of in-patient care per patient-day at 16-50 bedded township hospitals during the fiscal year 2003-2004, from providers' perspectives, ranged from Kyat 439.52 to Kyat 5269.41, with an average of Kyat 1931.60.

A matrix was prepared where the cost of care per patient-day was plotted against the average length of hospital stay (in days) for the eight hospitals as shown in Fig. 1. In this matrix, the cut-off point for a long average length of stay was taken as 6.4 days which is the national average length of stay for 16-50 bedded hospitals in Myanmar [4]. The cut-off point for cost of care per patient-day

is taken from the average of costs of care of the 8 hospitals included in the study. This figure was rounded as Kyat 1900.

Table 1. Cost of in-patient care in Kyat per patient day, average length of hospital stay in days and range of hospital stay in days for each study hospital (2003-2004)

Sr. No.	Hospital (bedded)	Cost of in-patient care in Kyat per patient day	Average length of hospital stay & (range of hospital stay in days)
1	TKI (50)	1123.49	4.7 (1-43)
2	TGW (50)	1142.99	7.2 (1-39)
3	KCK (25)	1039.15	4.8 (1-29)
4	KTN (25)	643.05	5.2 (1-29)
5	KYN (25)	439.52	5.7 (1-29)
6	TTE (25)	2671.12	5.1 (1-34)
7	HTB (16)	3124.07	3.9 (1-26)
8	KHM (16)	5269.41	6.8 (1-30)

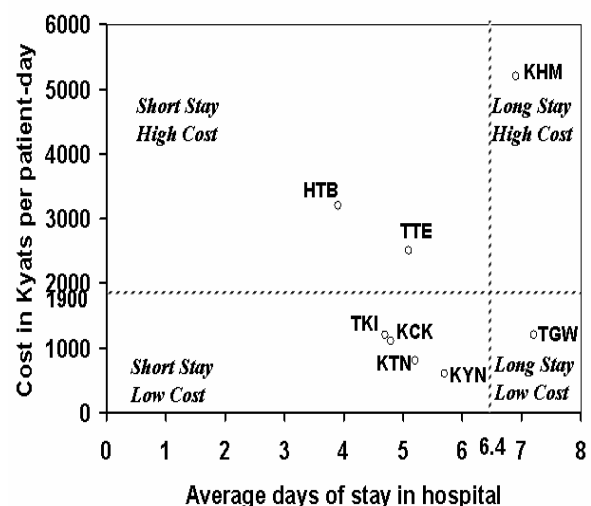


Fig. 1. Graph showing the relationship between cost of in-patient care per patient-day and average length of hospital stay for the eight hospitals (2003-2004)

The graph indicates that two hospitals (HTB and TTE) fell into “short stay/high cost” category, and that one hospital (TGW) fell into “long stay/low cost” category.

DISCUSSION

For hospitals of comparable sophistication and quality, a low cost per patient-day is an indication of good efficiency, while a high cost per patient-day may suggest poor efficiency [5]. We attempted to compare the *relative* efficiency of the eight hospitals in our study after plotting a graph shown in Figure 1.

Roughly, 3 hospitals with high cost of in-patient care per patient-day could be considered lagging behind in efficiency *relative to* other hospitals in the study. Those hospitals that fell either into the “short stay/high cost” category or into the “long stay/low cost” category needed further analysis. “Short stay/high cost” could arise out of underutilization of in-patient care services, or due to certain disease conditions requiring high cost therapies. The most possible explanation for the “long stay/low cost” category was that most of the expenses for care at the hospital being incurred by the patients themselves.

In our study, due to small sample size, there is a mix of small numbers of 16-bedded and 50-bedded hospitals. Although these are acute hospitals and there do not exist specialist care services, bed strengths and staff strengths differ between these hospitals. This is a limitation in our study in attempting to prepare a graph like the one shown in Figure 1 for observing relative efficiency between different hospitals included in our study. We should have prepared separate plots for different bedded hospitals if we have more sample sizes for each of these categories.

CONCLUSION

In this study, we measured only use of health service resources through duration of in-patient stay. In this endeavour, we could not list diagnoses of in-patients by codes in the International Classification of Diseases, due to incompleteness of information in

diagnostic and therapeutic recordings in many of the in-patient record sheets. Thus it was not possible for us to calculate cost by disease category or by using a diagnosis related groups (DRG) system as used in other studies [6-7].

Even though the costs estimated in our study could be considered gross estimations, the study did provide information on the cost of care being borne by the government in 16-50 bedded hospitals in Yangon Division. The data, when compared between the hospitals under study, also gave some information on relative efficiency of performance by these hospitals.

This quick and simple methodology of calculating in-patient care cost per patient day can be a useful managerial tool to understand performance of 16-50 bedded hospitals where general care services are being provided mainly for acute patients.

We propose that the methodology should again be applied in other 16-50 bedded township hospitals and basing on the experiences encountered it should be further refined to make it user-friendly for health services managers at central and State/Division levels. The methodology can be applied as a managerial tool to monitor trends in in-patient care costs and efficiency of performance of 16-50 bedded hospitals.

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SHORT REPORT

In-house ELISA for the diagnosis of childhood tuberculosis

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There are no conventional methods for diagnosis of childhood tuberculosis. Children can not cough out sputa and thus sputum for smear and culture methods are not useful for them. Tuberculin skin tests may give false positive results in children who were immunized with BCG. Chest X-ray may give mimic results with other lung disorders. A rapid and reliable test for diagnosis of childhood tuberculosis would have great applicability as a clinical tool. Therefore, use of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house test) as a serodiagnostic test for childhood tuberculosis was studied.

This study was carried out from May 2002 to April 2003. It was a cross-sectional, laboratory and hospital-based study. The coating antigen was prepared in the Tuberculosis Culture Laboratory and Immunology Research Division, Department of Medical Research (Lower Myanmar). It was prepared from *Mycobacterium tuberculosis* cultured on 3% Ogawa media. Whole cell soluble *My. tuberculosis* antigen was prepared using the method of Nicholl's (1975)[1]. Phenolized non-viable tubercle bacilli were collected, treated with N sodium hydroxide and heated at 80°C for 30 minutes. After washing and centrifuging, antigen soluble in double distilled water was used as the coating antigen in the ELISA test.

This study was done on 50 children admitted to the North Okkalapa General Hospital and Thingangyun Sanpya Hospital. The consents were asked from their parents or guardians. After getting informed consent chest X-rays and tuberculin skin tests were carried out on all cases. The parents and guardians of all subjects were interviewed on the BCG vaccination status of the subject. Two milliliters of blood were taken and whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house ELISA) was done on their sera.

Sera samples were assayed to detect the IgM antibodies, whole cell soluble *My. Tb.* antigen was used as the coating antigen, horse-radish peroxidase conjugated goat anti human IgM (Dako, Denmark) was used as the conjugate and orthophenylene diamine (OPD) (Wako, Osaka, Japan) was used as the substrate. Mean optical density (OD) values were obtained from the samples tested in duplicate. Sera samples from normal apparently healthy children who had visited the Vaccine and Diagnostic Clinic of Department of Medical Research (Lower Myanmar) were used as negative controls. The cut-off value of controls was OD-0.264.

There was no significant association between ELISA and BCG status ($X^2=0.08$, $p=0.786$). The sensitivity and specificity of

the in-house ELISA test was 72% and 80% respectively for IgM in childhood tuberculosis. Our findings point to a reasonable specificity (80%) and sensitivity (72%) of the test when IgM antibody titers are considered for the diagnosis of childhood tuberculosis.

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SHORT REPORT

Evaluation of microscopy for detection of acid-fast bacilli from sputum after overnight treatment with sodium hypochloride

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Microscopy of direct sputum smears for acid-fast bacilli (AFB) remains the gold standard for diagnosis of tuberculosis (TB) in most laboratories of developing countries. The specificity of positive acid-fast smear is high [99.3% to 99.9%] but the sensitivity is low ranging from 22% to 78% [1]. Previous studies showed that the sensitivity of AFB smear is improved by digestion of sputum with 5% sodium hypochlorite (NaOCl) or household bleach and concentration of bacteria by centrifugation [2,3,4].

However, this method needs access to a centrifuge and it may cause major inconvenience for microscopic centers in remote areas with no electricity and limited laboratory facilities. Thus, this study was carried out to detect whether sedimentation of NaOCl treated sputum by overnight standing at room temperature could replace the step of centrifugation.

Three hundred and seventy eight sputum samples were collected from tuberculosis patients attending TB Centre, Yangon. Slides for direct smears were prepared from purulent part of sputum and stained by Ziehl-Neelsen method for direct microscopy.

The remaining sputum was divided into two parts and each was transferred to 10ml screw capped tube and mixed with equal volume of 5% NaOCl. The mixture was incubated at room temperature for 10

minutes. Then 6-8 mililitre of distilled water were added to each sample and one tube was centrifuged at 3000g for 15-20 minutes. After discarding the supernatant, the sediments were stained by Ziehl-Neelsen method for microscopic examination.

The other tube was allowed to stand at room temperature overnight after adding distilled water. Then the next morning the supernatant was discarded and the sediments were stained by Ziehl-Neelsen staining and examined under ordinary light microscope.

Out of 378 sputum samples, 110 samples were positive by direct smear microscopy, 122 samples were positive by NaOCl-centrifugation and 109 samples were positive by NaOCl-overnight standing. It was found that there is no improvement in sputum smear microscopy of AFB by NaOCl-overnight standing method and the smear results can be obtained only on the next day. Almost all direct smear positive samples were also positive in NaOCl-overnight standing (only one slide showed negative with NaOCl-overnight standing method but that sputum sample had insufficient amount) and all NaOCl treated sputum samples showed no putrefaction on the next day at room temperature.

As NaOCl is a disinfectant, it can be used safely in condition where the sputum samples have to be kept for some times at

room temperature with lower risk of laboratory infection without interfering the positive rate of direct microscopy. But it cannot be applied to samples intended for culture because NaOCl kills mycobacteria. In our method, we add 6-8 millilitre of distilled water to the sputum after treating with equal volume of 5% NaOCl for 10 minutes. Therefore, disintegration of bacilli by acting too long with NaOCl did not have much effect in our method, as the morphology and average number of AFB seen per microscopic field were not quite different from direct smear microscopy.

Finally, it was concluded that sedimentation of NaOCl treated sputum by overnight standing could not replace the step for centrifugation for improvement of smear sensitivity. However, it could be used in remote microscopic center with no access of centrifuge and refrigerator when sputum specimens have to be collected late in the evening and must be kept as such at room temperature until tomorrow morning for direct microscopic examination. Moreover, this method reduces the risk of laboratory

infection and putrefaction of the sputum specimens.

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