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**Dot blot hybridization method for rapid detection of drug resistant
Mycobacterium leprae in Myanmar**

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Information of susceptibility for the *Mycobacterium leprae* isolates is beneficial for the proven multi-drug treatment and for verification of the efficacy of current leprosy control program. The simple genotypic method to detect mutations conferring resistance to dapsone (DDS), rifampin, and quinolone was exploited on the basis of hybridization with capture probe fixed to the glass slide. Mutations were discriminated by a series of oligonucleotide probes corresponding to each mutation in the *folP*, *rpoB*, and *gyrA* genes of *Mycobacterium leprae*. Based on these mutations, gene contained these mutation points (hot spots) was amplified by polymerase chain reaction (PCR) and followed by dot blot hybridization to detect mutations. This method is simple, rapid and not very expensive. The result can be obtained within 8 hours. A total of 100 mutibacillary leprosy cases attending the Central Special Skin Clinic, Yangon General Hospital were determined gene mutations. Ninety-three cases (93%) were susceptible to three drugs and seven cases (7%) were resistant. Among resistant cases, 3 were DDS resistance, 1 was rifampicin resistance, 2 were quinolone resistance and 1 was both DSS and rifampicin resistance (MDR).

INTRODUCTION

Current strategy of leprosy control relies mainly on multi-drug treatment (MDT). Although information of susceptibility for the isolates contributes to better treatment, susceptibility test is not done in almost all areas with high prevalence of leprosy. Comprehensive surveillance for the spreading of resistant cases offers useful information to evaluate efficacy of MDT and to prevent spreading of resistant strains, but a little is known about that. On the other hand, susceptibility test for anti leprosy drugs has been done by cumbersome and time-consuming mouse foot-pad method for over three decades, however, recent advances in molecular biology for drug resistance enabled to test susceptibility to dapsone,

rifampin, and quinolone by the detection of relevant mutations which confer resistance in each drug. Analysis of mutations is done by sequencing of target genes so far. Implementation of sequencing is not easy in some developing countries. In this context, a simple and rapid method to detect mutations was exploited and transferred to developing countries from Leprosy Research Center, National Institute of Infectious Diseases, Japan.

The data obtained by the method were compared with the results of sequencing to evaluate the reliability of the method. Prevalence of the drug resistant cases in a few Asian countries was also analyzed by the sequencing of the *folP*, *rpoB*, and *gyrA* genes.

MATERIALS AND METHODS

Sample collection

After getting informed consent, skin scrap samples were collected from leprosy patients attending the Central Special Skin Clinic (CSSC), Yangon General Hospital from 2005 to 2007 and 100 multibacillary (MB) patients with clinically and bacteriologically documented cases agreed to participate in the study. Leprosy patients were classified clinically and microscopically according to World Health Organization classification. The project was approved by Institutional Ethical Review Committee, Department of Medical Research (LM).

Specimen preparation

The collected skin scrapings were dipped in 1.5 ml tubes containing 0.5ml of 70% ethanol and stored at room temperature until test and prepared for DNA template by Klatser's method [1].

DNA template preparation (DNA extraction)

DNA was prepared from skin scraps according to the method of Klatser *et al.* Briefly, under sterile condition, the blade is scratched by wood prick and 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 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 inactivate proteinase K, which inhibits Taq polymerase during PCR.

Exploitation of dot blot hybridization method (DBH) for detecting mutation

A series of oligonucleotide probes corresponding to each mutation for the *folP*, *rpoB*,

and *gyrA* genes which revealed to confer drug resistance in previous studies [2, 3, 4 & 5) was designed and tested empirically for the use of capture probe. Capture probes consist of 8 kinds of oligonucleotide for the *folP* gene, 9 kinds of oligonucleotide for the *rpoB* gene, and 2 kinds of oligonucleotide for the *gyrA* gene respectively. Probes were absorbed on the glass slide. PCR to amplify simultaneously drug resistance determining regions (DMDRs) of the *folP*, *rpoB*, and *gyrA* genes was performed in a tube using primers (Table 1) where reverse primers are biotinylated. Two microlitre of PCR products was mixed with 38µl of hybridization buffer followed by the incubation for 80 min at 42°C. After discarding hybridization buffer, glass slide was washed for 60 min at 47°C. PCR products hybridized with probes, were detected by the color developing with oxidization of 3',3',5',5'-tetra methyl benzidine (TMB) for 80 min at room temperature and then read by computer scanner.

Table 1. Primers used for the amplification target region in the *folP*, *rpoB* and *gyrA* genes

Target gene	Forward primer	Reverse primer biotinylated
<i>folP</i>	5'- ATGGTCGCGGAAGGC GCGGC-3'	5'- CAAGTTCTTTTACGACA GG-3'
<i>rpoB</i>	5'- TCGCCGCTATCAAGG AATTC-3'	5'- TCACGCGACAAACCACC CGG-3'
<i>gyrA</i>	5'- GAGACTCCGGTTTCC GCCC-3'	5'- GCAGCGACCACGGCTG CGC-3'

RESULTS

Prevalence of drug resistance deduced by the dot blot hybridization is shown in Table 2. Many clinical samples showed negative PCR results because of inadequate sampling and preservation. Together with mutations revealed in this study, oligonucleotide capture probes for wild type and mutant type shown in Table 3 were spotted on the glass slide. Mutation detection using DBH method was successfully conducted at both places (Fig.1). Coincidence between results of

DBH and sequencing was 99% respectively. Discordant results were obtained when the intensity of color developing was not good in DBH.

Table 2. Results of DBH on 2005-2006 & 2006-2007 in Myanmar

Total tested	2005-2006	2006-2007	Total
	50	50	100
Susceptible to 3 drugs	46 (92%)	47 (94%)	93 (93%)
Total resistant cases	4 (8%)	3 (6%)	7 (7%)
DDS resistance	2 (4%)	1 (2%)	3 (3%)
Rifampin resistance	1 (2%)	0 (0%)	1 (1%)
Quinolone resistance	0 (0%)	2 (4%)	2 (2%)
*MDR (DDS & Rifam)	1 (2%)	0 (0%)	1 (1%)

*MDR = Multi-drug Resistance

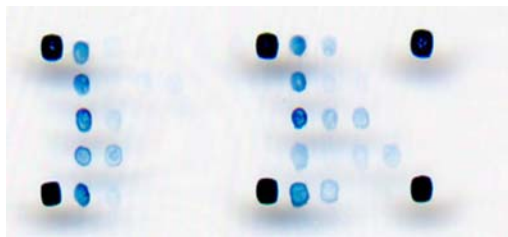


Fig. 1. DBH results for a susceptible case

Table 3. Sequence of capture probes

Gene	Position	Wild type	Resistant mutant
<i>folP</i>	53	GTGGCGAATCG	TGGCGAATCGGCCCGG
		ACCCGG	TGGCGAATCGGTCCGG
			TGGCGAATCGATCCGG
			GGCGAATCGAGGCGG
			TGGCGAATCGAGACGG
	55	CGGCCCGGTGC	GACCGGTCCGGTGCC
		CATTA	GACCGGCGCGGTGCC
			GACCGGCTCGGTGCC
<i>rpoB</i>	513	AGCTGTCGCAGT	AGCTGTCGGTGTTCAT
	516	TTCATGGATCAG	TTCATGAATCAGAACAA
		AA	TTCATGTATCAGAACAA
	526	CCTGACCCACAA	GCCTGACCTACAAGCGC
		GCGC	GCCTGACCGACAAGCGC
	531	CGCCGACTGTC	CCGACTGATGGCGC
		GGCGCTG	GCCGACTGTTGGCG
			GCCGACTGTTGCGG
	533	GCGCTGGGGCC	GGCGCCGGGGCCCGGG
		CGGGTG	TG
<i>gyrA</i>	89	ATCCGCACGGC	ATCCGCACTGCGACGCA
	91	CGGCGACGCAT	CGGCGACGTATCGATTT
		CGATTT	
Positive hybridization control in the <i>gyrA</i>		GGACCGTAGCC	
		ACGCTAA	
Negative hybridization control in the <i>gyrA</i>		GGACCGTCAT	
		CACGCTAA	

DISCUSSION

Detection of drug resistance in *M. leprae* is crucial for the efficient treatment of leprosy and the prevention of the spread of drug resistant strains. The elucidation of the genetic background of resistance by molecular methods has enabled the prediction of drug susceptibility of *M. leprae*. Drug resistance to dapsone, rifampicin and ofloxacin has evolved by mutation in drug resistance determining regions in the *folP*, *rpoB*, and *gyrA* genes respectively [6].

Although the direct sequencing of PCR products is definitive and allows rapid detection of resistant cases, it has the shortcomings of requiring expensive apparatus and high sequencing costs, so that it is not practical in many developing countries. The heteroduplex method (HAD) [7] and the PCR-single strand conformation polymorphism method (SSCP) [2] have been applied to the detection of mutants to overcome these disadvantages. The HAD method can identify mutations in the PCR amplified fragments by the electrophoretic mobility difference of heteroduplexes of wild-type products and test sample products, while the SSCP method analyzes that of single stranded products.

However, neither the HAD nor the SSCP method fully meets the required conditions in developing countries, since these methods demand complicated procedures and both methods detect silent mutations as resistant mutations. The recently developed Line Probe assay based on reverse hybridization can detect rifampicin resistant *M. leprae* simply and rapidly, but it cannot provide susceptibility information for other anti-leprosy drugs. The multiple-primer PCR amplification refractory mutation system is relatively simple but detects only nucleotide mutations and cannot distinguish silent mutations from missense mutations [8].

Our present study aimed to exploit a rapid, simple and simultaneous drug susceptibility test for three key anti-leprosy drugs to solve defects of each method previously reported,

based on DNA-DNA hybridization using a DNA microarray. The novel method, designated DBH, allows the simultaneous identification of mutations in three genes, responsible for resistance to dapsone, rifampicin and the quinolones. Easy accessibility and high reproducibility demonstrated by the studies with clinical materials in developing countries revealed the superior applicability of this method. The monitoring of drug resistant leprosy cases has been recommended in order to maintain the effectiveness of chemotherapy for leprosy [9, 10]. The DBH method developed in this study seems to be a simple and robust tool to assess the drug susceptibility of *M. leprae* in developing countries where susceptibility testing is rarely applied. Comprehensive data on the prevalence of resistant cases show that the level of drug resistance is low in some endemic countries [10].

It is, therefore, recommended to apply this method to samples from intractable cases and relapsed cases, to examine the susceptibility to anti-leprosy drugs and ensure effective treatment. Additionally, the capacity of the DBH method to identify the positions of mutations can be utilized for molecular epidemiological and geographical studies on the spread of drug resistant *M. leprae*.

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**Teaching learning intervention of motivation by close supervision
to low performance students of 2nd M.B.,B.S.(1/2005 batch) of
University of Medicine 2, Yangon**

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Thirty repeater-students from second year M.B., B.S. (1/2005 batch) were taken as low-performance students and they were divided into research and control groups of 15 students each. Fifteen students from research group were divided into three sub-groups of five students each. Each sub-group was undertaken in teaching learning intervention (remedial educational intervention), run by senior teachers from Anatomy, Physiology and Biochemistry, Department of University of Medicine 2, Yangon. The students from the research group were closely supervised on their academic difficulties, solved their extracurricular problems throughout the whole academic year. In the final examination, out of 15 research students, six students clearly passed the examination. Five students failed either in one or two subjects. They had the chance to sit the supplementary examination and they all passed the supplementary examination. Four students failed in all subjects. Therefore, out of fifteen research students, eleven students (73.3%) passed and four students (26.7%) failed in the final examination. In the control group, only two students clearly passed the examination. Seven students passed after the supplementary examination. Six students failed in all subjects. Therefore, in the control group, nine students (60%) passed and six students (40%) failed in the examination.

INTRODUCTION

There are many causes and problems which lead to failure in the medical education. Personal interest in medical subject is the personal factor which influences the medical student's characteristic. The social and financial problems are among the many problems which cause failure in final examination. The repeater students usually have difficulties or problems. They are also called slow learners, backward students, non-motivated students or low performance students [1]. Therefore, motivation to the repeater students may be very important and essential.

The stresses of medical education may be the information overload, long duration of

study, less time to interact with friends and family, repeated examinations/ tests, relocation and personal issues, weakness in English, poor academic performance, unexpected absence and irregular attendance, financial pressure, physical disturbances, alcohol and other substance abuse, withdrawal from outside activities, peers and family members, arguments, frights and behavioral excesses and mental disruptions [2]. The most prevalent source of academic stress was the test or examination [3]. Medical education can be a stressful experience. Competition seems to be more the style than cooperation among health professionals [4].

The causes of academic failure were widespread and ranged from deficient study

skills to financial, domestic and emotional problems. Provision of individually tailored remedial teaching is labourious, intensive and requires full faculty support [5]. Other motivations, such as social gains, financial benefits or family wish, were related to lower performance. Students motivated by the presence of chronic ill health in their families performed significantly lower. Social factors also play a role in medical students' performance [6]. Motives for choosing medicine were categorized into three indexes: 'people orientated', 'status/security orientated' and 'natural science orientated' motives. 'Person orientated' and 'natural science orientated' motives exerted the strongest influence on preferences and person orientated motives are most important for becoming a doctor [7]. The slow learner is the student formally diagnosed as "learning-disabled" by specialists in child psychology [8]. To motivate students, the teacher needs self-motivation. The teacher should also realize that motivation once established is never permanent and may require periodic 'booster' dose to build up the levels again to desirable plane.

The objective of this study was to improve the students' performance so as to pass final examination. At the end of the intervention, the students should attend the class regularly; should get passed marks in all the class tests and should significantly increase in confidence in studying Anatomy, Physiology and Biochemistry subjects.

MATERIALS AND METHODS

The students who took part in the study were altogether 30 repeater-students of second year M.B.,B.S. (1/2005 batch) students of University of Medicine 2, Yangon. Study period is the academic year 2005 - 2006.

The repeater-students were taken as low-performance students. The procedure of the research was explained to all the repeater-students. The control and research groups

were divided according to their informed consents. Fifteen repeater-students were assigned as research group and fifteen repeater-students were assigned as control group.

All these thirty students (both research & control group) had to attend the regular lecture classes, tutorial classes, practical classes and demonstration classes together with other regular second year students in all three subjects according to the respective teaching days. They also had to sit the completion class tests and final examination as other regular second year M.B.,B.S. students.

Fifteen students in research group were undertaken in remedial educational intervention run by senior teachers from three departments. The remedial educational intervention consisted of academic part and personal affair solving part. For academic part, fifteen students in research group had to attend the extra-tutorial classes, do extra assignments group discussion, MCQ exposure classes, MSQ and essay type questions discussion on respective teaching day of three subjects. All these attendance and individual attention were recorded for each student.

For personal affair solving part, fifteen students in research group were subdivided into three groups of 5 students each. Each five students were closely supervised by senior teachers from each department. Their personal histories were taken. They were interviewed with the semi-structured questionnaires (Table 1). Problems were listed (Table 2). The remedial programmed teachers were responsible for monitoring academic progress throughout the course and also closely supervised and solved personal affair problems and difficulties as feasible. Professors and Heads of the respective Departments were available for individual confidential pastoral support. Their class attendance and students performance were reported to the Rector and Pro-rector of the University.

Table 1. Semi-structured questionnaires for research group

No.	Questions	Yes	%	No	%
1	Do you attend lecture/tutorial /demonstration class regularly?	7	46.7	8	53.3
2	Do you have interest in Anatomy subject?	12	80	3	20
3	Do you have interest in Physiology subject?	11	73.3	4	26.7
4	Do you have interest in Biochemistry subject?	10	66.7	5	33.3
5	Do you enjoy this 2nd M.B.B.S course?	14	93.3	1	6.7
6	Do you understand the lectures?	5	33.3	10	66.7
7	Do you study alone?	13	86.7	2	13.3
8	Do you study with friends?	2	13.3	13	86.7
9	Do you have complete set of handouts/ practical manual, etc?	14	93.3	1	6.7
10	Do you have any problem attending the course?	0	0	15	100
11	Were the marks you got last year representative of your performance?	15	100	0	0
12	Do you have enough financial support?	14	93.7	1	6.7
13	Do you have any health problem?	15	100	0	0
14	Are you married or single?	1	6.7	14	93.3
15	How many members in your family?				
16	Who is the favorite one in your family?				
17	What is your hobby?				
18	Who support you to attend the medical course?				

Table 2. Problems list

No.	Problems	Number of students	%
1	Academic difficulties	10	66.7
2	Family problems	3	20
3	Financial problems	1	6.7
4	Interest in extra-curriculum such as sport (foot ball), TV games	3	20
5	Frequently going back to home at district	2	13.3
6	Marital problem	1	6.7

RESULTS

Regarding the class attendance, the repeater students were naturally uninterested in attending the lecture, tutorial and demonstration classes. However, as they were explained the benefit of the classes and were encouraged to attend the classes, so their

class attendance attained at least seventy-five percent. Total class-test marks carried 30% of the final examination marks as class work performance. Class work performance in research group had the average of 12.3 in Anatomy subject, 10.5 in Physiology subject and 11.6 in Biochemistry subject. Class work performance in control group had the average of 8.7 in Anatomy subject, 7.9 in Physiology subject and 8.9 in Biochemistry subject. There was significant difference between the control and research group in class work performance (Table 3).

Table 3. Class test performance of research and control group

No.	Anatomy		Physiology		Biochemistry	
	out of 30 marks		out of 30 marks		out of 30 marks	
	R	C	R	C	R	C
1	19	11	16	6	13	12
2	11	10	10	7	11	8
3	10	3	10	3	7	9
4	13	8	11	5	11	6
5	17	11	12	10	14	10
6	12	10	15	13	15	10
7	15	12	6	13	17	11
8	15	13	12	12	14	13
9	5	6	7	12	11	9
10	14	2	14	2	11	5
11	11	15	8	3	12	4
12	8	8	6	5	8	10
13	8	8	10	14	8	12
14	10	5	9	8	7	7
15	17	11	12	6	15	12

R = Research, C = Control

The whole second M.B.,B.S. students including repeater students were assessed by final examination. Out of thirty repeater students (both control and research group), altogether 8 students (2 control & 6 research) got clear passed result, 12 students (7 control & 5 research) failed and had to sit the supplementary examination after 6 weeks of intensive revision course. All these 12 students passed in the supplementary examination. Therefore, altogether 20 students had passed the examination. However, 6 students (3 from each) had failed in all three subjects. They had to repeat the course for another one year. Four students (3 controls & 1 research) had to be dismissed from the course due to third time failure in the examination.

After the remedial education intervention to repeater students, the final result obtained was as follows: out of 4 drop-out students, 3 students were from control group and one student was from research group. Out of 6 repeater students, 3 students each were from control and research group. Out of 12 supplementary students, 7 students were from control group and 5 students were from research group. These results showed that there were fewer drop-out students, less supplementary students from research group, and passed students were more in research group. The second-time failed students who become again repeater students were same from each group (Fig 1).

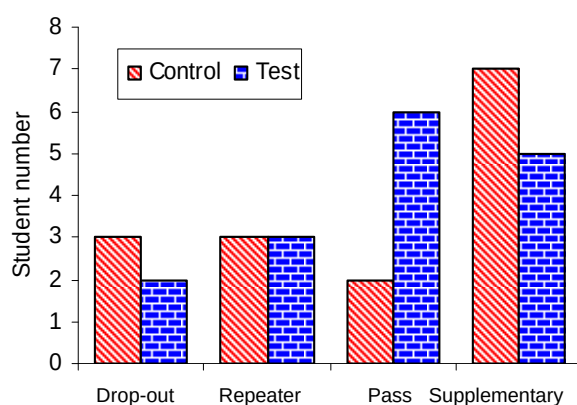


Fig.1. Comparison of final results between research and control group

DISCUSSION

By conducting the teaching learning intervention or remedial educational intervention on repeater students or low performance students, they became motivated, and the failure rate and drop-out rate reduced. They will be qualified as other ordinary students, their medical knowledge and skill will be properly trained to be an efficient health personals. Therefore, they can continue their medical course and become an efficient medical persons and competent basic undifferentiated doctors. The causes of academic failure are not only academic in origin. The students can get benefit from an individually tailored remedial programme

and get success in subsequent parts of the curriculum and examination.

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**Study of utilization of rapid diagnostic tests by community health workers
and acceptance of the served community of the diagnostic tests
in remote malaria-endemic areas**

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A descriptive study using quantitative and qualitative methods was conducted in seven remote malaria-endemic villages of Pyinmana Township, Central Myanmar from September 2006 through May 2007 with the main objective of studying the utilization of rapid diagnostic test (RDT) for malaria in remote endemic areas by community health workers (CHWs) and the community. The study revealed that RDT, 'HEXAGON MALARIA COMBI' has a good efficacy (sensitivity 88.64%, specificity 88.89% and accuracy 88.68%) emerged under an enthusiastic endeavour by seven CHWs among fifty-three study subjects of clinically suspected malaria. It was also noted that RDT was well-utilized by CHWs and well-accepted by the served community. It is concluded that RDT should be equipped to CHWs together with appropriate anti-malarials under the supervision of one of the basic health staff to ensure early diagnosis and effective treatment for the cases suffering from deadly malaria in remote endemic areas.

INTRODUCTION

Malaria causes one of the health problems in Myanmar resulting in about 600,000 reported cases and about 3,000 deaths annually [1]. The global malaria control strategy includes four main components with the first one - provision of early diagnosis and prompt treatment of the disease aiming at the elimination of mortality [2]. Diagnosis is very important in treating malaria and performed in three ways-by clinical methods, by microscopic examination of blood film and by a rapid diagnostic test (RDT) strip using a principle of immunochromatographic technology. People in remote rural areas have less access to proper health care, solely depending on basic health staff (BHS) and their community health workers (CHWs) there. Diagnostic facility is also very scarce and sometimes almost nil. Diagnosis based on signs and symptoms alone is found to be unreliable for treatment purpose. In such a condition RDT facilities are urgently needed

especially for those suffering from life-threatening falciparum malaria. Therefore, the present study is conducted with the objectives of: (i) to study the efficacy of RDT used by CHWs in remote malaria-endemic areas in Pyinmana Township, (ii) to study the utilization of RDT by CHWs to diagnose malaria and (iii) to study the acceptance of the community to have a diagnosis by RDT.

MATERIALS AND METHODS

It is a descriptive study using both quantitative and qualitative methods conducted in Gamontaung, Mepauk, Pinye, Myaymyo, Kyoedan, Gadoeseik and Inntha villages, Pyinmana Township in Central Myanmar (C 19° 43'N, 96° 11'E). The areas are characterized by extensive forests, mountains and hard-to-access. They are situated in remote hilly areas and about 40-45 miles away from downtown Pyinmana. The numbers of household in each village is estimated to be about 50-130 mainly living

on semisubsistence agriculture. In each village there is at least one CHW who had attended the basic health training on common diseases including malaria. Malaria occurring in these areas is featured with a generally low perennial transmission of *P. falciparum* and *P. vivax* with pockets of high transmission and marked seasonality. Study period is from September, 2006 through May, 2007. Study populations are local residents of above-mentioned villages. Sample size is estimated to be about fifty subjects (ie. $n = z^2 pq / d^2$; $p = 0.8$, proportion of patients with fever, chill and rigor seeing BHS during a transmission season: $n = 1.96^2 \times 0.8 \times 0.2 / 1.1^2 = 51$ subjects).

Data collection instruments and technique

A five-day training course, led by Principal Investigator (PI), for malaria diagnosis and treatment was held in Pinyinana Township Health Department and attended by seven CHWs, seven other BHS and ten research assistants (RAs) of Department of Medical Research (Central Myanmar) in August, 2006.

In phase I, during September, PI and RAs visited each village and performed field demonstrations which included diagnosis of malaria by signs and symptoms, RDT [HEXAGON MALARIA COMBI (HMC)] and blood film preparation. Then CHWs were let to perform the similar procedure by themselves on two malaria patients in front of RAs and BHS.

Then results of RDT (Illustration. 1) were read and blood films were examined by RAs and results were compared. Then CHWs were provided with RDT strips, anti-malarials [Tablet Mefloquine and Tablet Artesunate for Artemisinin-based Combination Therapy (ACT)], glass slides, lancets and Giemsa stain. HMC is an immunochromatographic rapid test for the detection of *Plasmodium* spp. in human whole blood. It is for the qualitative detection of histidine-rich protein 2 (HRP2) released from *P. falciparum* and aldolase, a pan-plasmodium antigen released from all *Plasmodium* species.

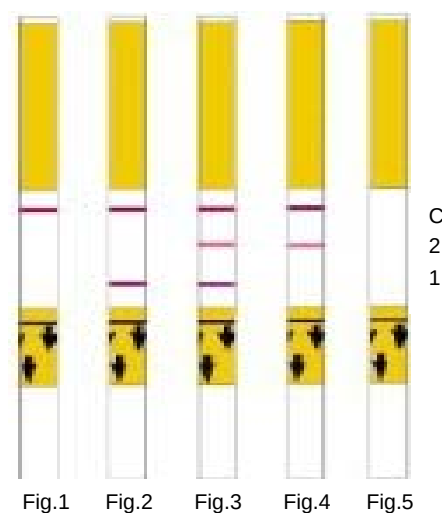


Illustration 1. Different results by HMC RDT:

Fig 1. Negative

Fig 2. Positive (*P. falciparum*)

Fig 3. Positive (*P. falciparum* or *P. falciparum* plus non-*P. falciparum*)

Fig 4. Positive (Non-*P. falciparum*, ie. *P. vivax*, *P. malariae*, *P. ovale* or mixed infection of them)

Fig 5. Invalid test

(1=line for HRP2, 2=line for aldolase, C=control line)

In phase II, from October onwards, CHWs treated malaria patients after asking about symptoms especially fever with chill and rigor in last 1-3 days and examining the signs in their villages using RDT and at the same time prepared blood films under supervision of public health supervisors or midwives till the end of May, 2007. If RDT showed positive result of *falciparum* malaria patient was administered ACT. If it was non-falciparum malaria mainly *P. vivax* Tablet Chloroquine followed by Tablet Primaquine were given as a radical cure [3]. If it showed negative result and fever still persisted Tablets Chloroquine were first given and patient was under watch. If he was not relieved and fever was still unresolved for another 12- 24 hours, treatment was changed to ACT paralleled with performing other investigatory methods for differential diagnosis at nearest hospital.

During Phase II CHWs had to report the cases including RDT results, and sent the prepared blood films weekly to DMR (CM) where they were examined microscopically by a well-experienced technician according to WHO criteria (100 thick film fields

before negativity declared). The results of blood films were sent back to CHWs. During the last week of May, PI and RAs visited the villages again and performed indepth interviews with nine key informants including three village heads there and seven CHWs using an interview guideline mainly on two diagnostic methods followed by treatment. Their opinion and ideas were recorded on tapes as their actual wordings and they were transcribed and translated.

Consent and data analysis

Ethical approval was obtained from Ethical Review Committee of DMR (CM). The study was explained to and consent was obtained from all participants. Data analysis was done by SPSS version 10.0. Efficacy of RDT was calculated against the microscopy as a gold standard method to produce (i) sensitivity (proportion of diseased people who are correctly identified as 'positive' by the test), (ii) specificity (proportion of non-diseased people who are correctly identified as 'negative' by the test) [4], (iii) positive predictive value (probability of the person having the disease when the test is 'positive'), (iv) negative predictive value (probability of the person not having the disease when the test is 'negative') [5] and (v) accuracy (the degree to which a measurement or an estimate based on measurements represents the true value of the attribute that is being measured) [6]. Indepth interview results were also analyzed and assessed.

RESULTS

Quantitative method

During study period, seven CHWs examined fifty-three clinically suspected malaria (CSM) patients, out of them forty-four (83%) were confirmed malaria cases (i.e. 42 *P. falciparum* cases and 2 *P. vivax*) finally diagnosed by microscopy. Out of confirmed cases 15 (34.1%) were contributed by children of 15 years and below and 27 (61.4%) by those of 16 - 60 years. Among total CSM patients 31 (58.5%) were males and 22 (41.5%) females. Their mean ages

$\pm$ SD were 23.13 ± 14.59 years and 23.14 ± 20.49 years respectively. The results of CSM patients diagnosed by CHWs with RDT and by microscopy as a gold standard method are shown in Table 1. Sensitivity of RDT is 88.64%, specificity 88.89%, positive predictive value (PPV) 97.5% and negative predictive value (NPV) 61.54%. Accuracy is 88.68% meaning that the degree to which detection of malaria parasites by two diagnostic tests - microscopy and RDT- represents true value (ie. presence and absence of the parasites among the CSM patients) is 88.68%.

Table 1. The results of clinically suspected malaria patients diagnosed by RDT and microscopy

Methods		Microscopy result		Total
		m.p. +	m.p. –	
RDT result	m.p. +	39	1	40
	m.p.–	5	8	13
Total		44	9	53

m.p.+ = malaria parasite positive,

m.p.- = malaria parasite negative

Sensitivity = $(39/44) \times 100 = 88.64\%$

Specificity = $(8/9) \times 100 = 88.89\%$

PPV = $(39/40) \times 100 = 97.5\%$

NPV = $(8/13) \times 100 = 61.54\%$

Accuracy = $[(39+8)/53] \times 100 = 88.68\%$

Qualitative method

To study the utilization of RDT by CHWs and acceptance of RDT by served community, indepth interviews were performed on seven CHWs and nine key informants of study villages. Their opinion and ideas were elicited and they did not fully represent for other areas. Findings by some interviewees are as follows:

The study areas are remote rural areas where locals mainly depend on their CHWs, some usually use RDT for malaria diagnosis there and some never using it also became to use after a training course.

"Our village is very far away ie about thirty-five miles and separated by several mountains from downtown Pyinmana. We solely depend on BHS and CHW for our health."

(A 45-year-old villager)

"On diagnosis of malaria I use 'paracheck Pf test'. Blood film preparation was not usually done because our village is very far from Pyinmana and it is very difficult to get the result."

(A 26-year-old CHW)

"I have never used the rapid test before. After a training course by the present malaria research team, I use it."

(A 45-year-old CHW)

Regarding feasibility and time, RDT is very feasible for CHWs and it is time-saving and results are obtained in minute. Therefore both CHWs and villagers were found to be satisfied with it.

"It is very easy and time-saving. It also gives immediate results. Generally I prefer RDT to blood film."

(A 45-year-old CHW)

"On detecting the disease malaria, I knew two methods – microscopy and RDT. I prefer RDT because disease causing germs and their types can be known within minutes ... say about fifteen minutes."

(A 55-year-old village head)

"According to the voluntary health worker in our village RDT could detect malaria germs nearly 99% correctly. I also know that the results can be read in 10 – 15 minutes. Compared to it, it takes longer to send a blood film to downtown where a microscopic facility exists. Therefore I like RDT more and accept it in treatment of malaria and do our villagers as well."

(A 40-year-old village head)

"Regarding treatment of malaria using RDT and blood film, I prefer the former to the latter as a result of the fact – a more rapid and easier method."

(A 45-year-old villager)

RDT's cost is reasonable and it is not expensive in terms of benefits obtained and so it is accepted by the user.

"If a blood film was used, the cost including the charges of transport and meals on the

way to Pyinmana was 6,000 Kyats. Out of two diagnostic methods – RDT and blood film, the former is better because of immediate delivery of the result and its (HMC) cheaper cost of 2,175 Kyats per strip."

(A 26-year-old CHW)

DISCUSSION

Malaria is by far the world's most important tropical parasitic disease killing more people than any other communicable diseases except tuberculosis. It is a public health problem in more than ninety countries inhabited by a total of some 2.4 billion people or 40% of the world's population with the prevalence of 300-500 million clinical cases and 1.5- 2.7 million deaths each year [7]. In Southeast Asia Region the disease is a major public health problem. Out of eleven countries all except Maldives have malaria accounting for 30% of global morbidity and 8% of mortality [8]. In Myanmar it is also the number one life-threatening disease. To fight against it WHO had laid down the global malaria control strategy comprising four components - disease management through early diagnosis and prompt treatment, planning and application of selective and sustainable preventive measures, early detection or prevention of epidemics and their containment, and regular assessment of malaria situation [2].

To carry out the first component effectively a health care provider who usually first contacts the patient at peripheral level should be a good health care provider. The provider is a CHW. CHW is defined as a community member who works almost exclusively in community settings and serves as a connector between health care consumers and providers to promote health among groups that have traditionally lacked access to care. If a CHW is trained to give treatment to those suffering from illnesses he will be able to do so. In one of the studies in Nigeria consumers preferred the CHW malaria treatment strategy over self-treatment at home [9]. In Myanmar, like

in other countries, CHWs are basic health care givers closest to the communities and thus in the present study they were conferred with the diagnostic aids and assigned to management of uncomplicated malaria cases in their villages situated very far away from urban settings. Within about eight months seven CHWs treated fifty-three CSM patients using RDT paralleled with preparation of blood films which were forwarded to and checked at DMR (CM).

CHWs had treated 15 children of 15 years and below – about one third of total confirmed malaria cases and 27 adults of productive age – about two thirds of total, so saving their lives in time using RDT. Regarding sex, males are more prone to get malaria due to their nature of occupation. *P. falciparum* species was found to be more prominent and there was no mixed infection. Falciparum vivax ratio was 21:1. So, life-threatening malaria dominated in the study areas.

The efficacy of RDT was found to be satisfactory showing sensitivity 88.64%, specificity 88.89% and accuracy 88.68% calculated on the sample size of 53 cases obtained in study period. Sensitivity is slightly lower than that prescribed [10] (ie. 90.2% - 99.2%) due to three factors - increased environmental temperature may alter the immune complex reaction on the strip, parasite count contained in blood drop tested may be less than 100/ μ l of whole blood [10], and mature gametocytes do not produce HRP2 while a very low trophozoite number may not provide sufficient levels of antigen to be detected by RDT strip [11, 12].

Regarding environmental temperature, in one of the studies [13], RDT could be safely used at rural health centre level without cold storage up to two months of store during which sensitivity and specificity did not vary significantly. Specificity was also lower than that prescribed [10] (ie. 100%) because of the fact that parasite could not be found when its density is low by micro-

scopy. Accuracy was nearly 90%. Anyway RDT is acceptable when compared to microscopy. When compared to other RDTs (14, 11), sensitivity and specificity were slightly lower than the respective values [ie. ParaSightTM-F, 90.9% and 97% (Shiff *et al.*) and ICT Malaria[®] Pf, 92.7% and 95.1% (Thepsamarn P *et al.*]. The only thing that HMC outweighs other RDTs is it can differentiate between *P. falciparum* and non- *P. falciparum* malaria.

In the present study, symptom-based diagnosis was not compared with RDT and microscopy because symptom fever is non-specific for malaria [15]. Regarding utilization of RDT by CHWs and acceptance of RDT by community indepth interview on CHWs and key informants expressed that almost all of them accepted the test and preferred to when compared to conventional blood film examination because of the facts that RDT provides the result within minutes thus immediate treatment can be followed, it is easy to perform by CHWs and it is not expensive. RDT was also found to be well-accepted by community in a Philippine study [15].

In future, if CHWs are empowered with RDT and ACT they may be satisfied in their villages where light microscopy is hardly possible or microscopy cannot be maintained at an appropriate standard. In connection with the cost it is quite justifiable in terms of benefits obtained. One thing to be considered is that ACT is the last resort treatment and if it was wrongly used the resistant parasites may develop. Thus, CHWs are to be kept under supervision of BHS.

In conclusion, RDT was found to have a good efficacy and be well-utilized by CHWs and well-accepted by the served community as well. Therefore such a rapid diagnostic test is recommended to be utilized by CHWs together with appropriate anti-malarials, under the supervision of one of the BHS, in remote malaria-endemic areas to ensure the early diagnosis and effective treatment for those suffering from life-threatening and economy losing disease malaria.

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**An exploratory study on willingness to change smoking practice
of urban males, Dagon Township, Yangon Division**

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Smoking is rising in developing world but falling in developed countries. In Myanmar, number of current smokers in urban is higher than those in rural areas. Since little is known about changes in future attitudes of smoking practice, the present study attempted to explore willingness to change smoking practice of urban males. A longitudinal cohort study was conducted in Dagon Township, Yangon Division. Thirty-nine male adult current smokers, who were literate involved voluntarily. Self-administered questionnaire was used 3 times to explore changing process of smoking; May, June and August 2007. To know their instantaneous perceptions of practice, second time was one month apart from the first time and to explore their gradual perceptions, the last time was made 2 months later. Out of 39 smokers, we could follow 30 and 22 smokers at the second and last times respectively. Their mean age was 29.4 years. The majority started smoking at the age between 16 to 20 years since their friends urged them to smoke for experience. At the last time, among 22 smokers, 13 were still smoking, 7 started quitting/reducing and 2 had stopped smoking. However, among 22 smokers, half of them had a plan to quit/reduce smoking in future. Factors relating to changes in smoking practice explored from this study like personal feeling, self-control and environment, will be useful in developing IEC materials for quitting smoking programme in our country.

INTRODUCTION

Smoking is on the rise in the developing world but falling in developed countries. In Myanmar, various smoking prevalence studies carried out between 1991 and 2005 showed overall community prevalence of smoking ranged from 38% to over 50% [1, 2]. Among adult population, prevalence of male smokers was higher than females, both in urban and rural areas (ranged from 58% to 74% for males and from 11% to 46% for females). Since little is known about changes in future attitudes towards smoking practices, the present study attempted to explore willingness to change smoking practice of urban males.

MATERIALS AND METHODS

A longitudinal cohort study was conducted in Dagon Township, Yangon Division. We aimed to study the cohort of respondents, who were current smokers, 18 years and above and literates for more than once. It was not possible to collect a list of current smokers who met with such criteria from the entire township due to time and budget limitations. Hence, through township medical officer and local authority person of the selected township we recruited study respondents for participating in the study voluntarily. Thirty-nine male adult current smokers, who were literate involved voluntarily. Pre-tested semi-structured self-administered

questionnaire was used 3 times to explore changing process of smoking; May, June and August 2007. To explore their instantaneous perceptions of practice, second time was one month apart from the first time and to know their gradual perceptions the last time was made 2 months later. First, we obtained verbal informed consent from study respondents and explained about study's nature and how to fill the semi-structured self-administered questionnaire. They were also asked to write down about family history of smoking, reasons for smoking, attempts to quit smoking or reduce the number of cigarettes/cigars, reasons for (not) quitting or reducing. We assisted them filling the questionnaire when necessary.

RESULTS

At the first time

Most of the respondents were aged between 15 and 25 years with the mean age of 29.4 years (Table 1). The majority was unmarried (61.5%) and had passed at least middle school level (92.3%). Most were employees of non health-related job (51.3%).

Table 1. Socio-demographic characteristics of study respondents (n = 39)

Characteristics of the study respondents							
Age gr Yrs	%	Marital status	%	Education	%	Occupation	%
15-25	48.7	Married	35.9	Lower level	7.7	Employee from non health-related job	51.3
26-35	28.2	Un-married	61.5	Middle level	20.5	Employee of health-related job	15.4
36-45	7.7	Un-known	2.6	Higher level	51.3	Dependent/ student	15.4
46-55	15.4			College/ graduate Unknown	15.4	Others	18.0
					5.1		

Most had family history of smoking (64.1%), especially grand parents (40%) and/or their fathers (68%). The majority of their friends were smokers (94.9%), of whom; their close friends were the largest (72.9%). The majority of their colleagues at work places were also smokers (93.9%). The

majority (69.2%) started smoking at the age between 16 to 20 years and one respondent started smoking at the age of six. Most were cigarette smokers: 41% smoked cigarettes only and 54% smoked both cigarettes and cheroots. The majority (70.3%) smoked 1 to 5 cigarettes (range 1-20) and 65.2% smoked 1 to 3 cheroots (range 1-6) a day respectively. The main reasons of start smoking were persuasion of their friends (59%) that they used to sit at coffee shops where cigarettes were easily available and wanting new experience or fun (12.8%).

Between first and second time

Among 39 smokers, 30 respondents were able to follow at the second time. Between first and second times, the majority (73%) had attempted to quit smoking and reduce the numbers of cigarette/cheroot rolls and about 27% did not try to quit and reduce (Table 2). At Time 2, the majority was still smoking (63.3%), some started quitting/reducing (20%) and a few said they stopped smoking (16.7%). Because of health problems and bad consequences of smoking, they tried to stop and start quitting/ reducing.

Table 2. Attempts of smoking practice between Time 1 & 2 by smoking status at Time 2

Attempted to quit/ reduce between Time 1 & Time 2	Smoking status at Time 2			Total no. (%)
	Still smoking no. (%)	Start quitting/ reducing no. (%)	Stop smoking no. (%)	
Either quitted or reduced	11 (57.9)	6 (100)	5 (100)	22 (73.3)
Neither quitted nor reduced	8 (42.1)	0	0	8 (26.7)
Total	19 (63.3)	6 (16.7)	5 (20)	30

Between second and third time

Out of 30 respondents, 22 smokers were able to contact at the last time. Among them, while the majority had tried to quit/ reduce during second and third time (86.4%), only a few had neither tried to quit nor reduce (13.6%) (Table 3). At the last time, 13 respondents (59.1%) were still smoking with reasons of meeting with friends at coffee shops, unable to avoid smoking and depression. Two respondents (9.1%) said

they had stopped smoking because of health problems. Seven respondents (31.8%) had started to quit/ reduce and their reasons were health problems, bad consequences of smoking and to stop smoking completely. During the period of quitting and reducing, they got help from their parents, friends and their senior colleagues. A few said they tried by themselves.

Table 3. Attempt of smoking practice between Time 2 & 3 by smoking status at Time 3

Attempted to quit/ reduce between Time 2 & Time 3	Smoking status at Time 3			
	Still smoking no. (%)	Start quitting/ reducing no. (%)	Stop smoking no. (%)	Total no. (%)
Either quit or reduced	10 (76.9)	7 (100)	2 (100)	19 (86.4)
Neither quit nor reduced	3 (23.1)	0	0	3 (13.6)
Total	13 (59.1)	7 (31.8)	2 (9.1)	22

After third time

When 22 respondents were asked their smoking plan in future (i.e., after the study was completed), 31.8% had intention to quit or reduce in future because of health problem, 2.9% had already stopped and 27.3% had at the process of stop smoking. One third of respondents did not have a plan to quit or reduce after the study (Table 4).

Table 4. Smoking plan after Time 3 by smoking condition at Time 3

Smoking plan after Time 3	Smoking condition at Time 3			
	Still smoking n = 13 (%)	Start quitting /reducing n = 7 (%)	Stop smoking n = 2 (%)	Total n = 22 (%)
Plan to either quit or reduce	6 (46.2)	1 (14.3)	0	7 (31.8)
Plan to neither quit nor reduce	7 (53.8)	0	0	7 (31.8)
Already stopped smoking at Time 3	0	0	2 (100)	2 (9.1)
Process of stop smoking at Time 3	0	6 (85.7)	0	6 (27.3)

Statements expressed in the questionnaire showed their different attitudes toward changes in smoking practices as follows:

When I felt unhappy, cigarette is my only good companion. People are not trustable. One by one, they left me. But cigarettes never left me and it is the truth. I will neither quit nor reduce in future. I will smoke until I die or the price of cigarette will increase, say 2000 Kyats per roll of cigarette.

25 years old, 9th grade, single,
employee of private business

Previously, I tried once to quit and three times to reduce because I play football. I could not play well and felt tired easily due to smoking. With my own decision I tried as much as possible to avoid sitting at coffee shop with my friends who are smokers. At first, I thought I would be able to quit by reducing the number of rolls gradually. However, after about one week, I could not resist smoking, especially after lunch. I substituted sweets and gum for the cigarette but felt sour in mouth, so I smoked again. But I am very fond of playing football, thus I have determined to quit smoking.

19 years old, 10th grade, single,
employee of private business

Quitting was not an easy task. I could stay without cigarette for only three days. During which, I felt sour in mouth and my parents told me to chew gums and betel. However, I remembered cigarette after lunch, while sleeping at night and while toileting. I thus smoked again. Now, I began to stop smoking with a lot of efforts to overcome my desire to smoke.

29 years old, 10th grade, single,
supervisor of private business

DISCUSSION

This exploratory study indicated that, although the sample size was not large, the study respondents had positive intention to some extent in changing their smoking practice. Health problems and bad

consequences of smoking were the main reasons to stop and start quitting/reducing. Similarly, health reasons and cost were among the causes to quit smoking of the Malay and Korean smokers [3, 4]. We found that those who had tried to quit, reduce and stop smoking were higher after one month of study began. It gradually decreased after one month. Most could not continue their changed practice - quitting and/or reducing the rolls of cigarettes. It reflected the human nature of wanting to try new message or experience within a short period when it was introduced [5].

Though they could not maintain the changed practice, they received support and advice from their family members, friends and senior colleagues during their quitting/reducing attempts. Other studies done in Korea, East Java and Taiwan also reported that parents and peers were the main sources for protecting teenaged smokers from smoking [4, 6, 7].

However, during the study period, majority of the respondents had not only started to quit/reduce and stopped smoking, but also were willing to quit, reduce and give up smoking in future. Findings revealed that whether they wanted to quit, reduce or smoke, this did not depend on their socio-demographic characteristics, but on their health problems, personal feeling, self-control, environment and easy accessibility to cigarettes.

In conclusion, the study highlights the need of expansion of health dissemination of school-based smoking programme, particularly on quitting methods for the public and to publicise the message widely.

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Hypoglycemic activities of ethanolic *Gynura procumbens* leaves extracts in rats

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Traditional medicinal plants including those with anti-hyperglycemic effect have been widely used in Myanmar. Indeed, hundreds of plants were used traditionally for management and/or control of both insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus worldwide. There was no recorded evidence of *Gynura procumbens* leave for its anti-hyperglycemic effect in Myanmar. The present study aimed to evaluate the anti-hyperglycemic potential of *G. procumbens* leaves on adrenaline-induced hyperglycemic rats. It was a preclinical animal model, conducted during May, 2007 in DMR (Upper Myanmar). The extracts (different doses), glibenclamide and water were administered orally to different groups of adrenaline-induced hyperglycemic albino rats of both sexes (7 rats in each group). Oral administration of *G. procumbens* ethanol extracts (150 and 300 mg/kg) showed hypoglycemic effect in adrenaline-induced hyperglycemic rats. The efficacy was comparable with glibenclamide (0.5mg/kg). *G. procumbens* ethanol extract (300 mg/kg orally) significantly inhibited hyperglycemia induced by adrenaline (0.2 ml/kg). *G. procumbens* leaves extract with the dosage of 300 mg/kg was more potent than glibenclamide at the first 2 hours (1 hour and 2 hour) (P value = 0.0484, 0.0035), respectively. However, in 3 hour and 4 hour, there was no significant differences in tested extract and glibenclamide (P value = 0.4864, 0.4256), respectively. The results indicated that the ethanol extract of *G. procumbens* leaves possess anti-hyperglycemic activity.

INTRODUCTION

The World Health Organization's expert committee on diabetes mellitus estimated that about 150 million people worldwide suffered from diabetes mellitus in 1995; and predicted that this number will rise to an alarming figure of 300 million by the year 2025 [1]. Life expectancy can be halved by this disabling disease, particularly in developing countries of the world where its prevalence is increasing steadily, and adequate treatment regimens are often unavailable or economically unaffordable. In fact, phytotherapy has been widely used by the Myanmar population since time immemorial. There is a growing global interest in herbal and other forms of traditional medicine.

Herb has long been an important source of effective drugs. Among WHO recommendations is the need for the investigation of local medicinal plants for their potential therapeutic efficacy [2]. This practice continues to be prevalent in the urban as well as in the rural environment because of the low cost and the easy availability of medicinal plants in the market all over the country. However, the efficacy study on antidiabetic plants used in Myanmar since 1991 has begun to evolve in the last few decades. Moreover, there is no extensive documentation of data on antidiabetic activities of this plant extract. *Gynura procumbens* Merr. belongs to the family Compositae. It is known in Myanmar as "Pyar Mee Swae". The leaves are edible.

Gynura procumbens is found in various parts of Southeast Asia. It has been used for the treatment of eruptive fevers, rash and kidney disease [3]. Medicinal uses of the species including treatment for kidney trouble, fever and rash have been reported [4]. It was used traditionally in Thailand as tropical anti-inflammatory, anti-allergic agents and recently it was reported to be effective against the herpes simplex virus [5]. Leaves extract of *G. procumbens* has also been reported to have hypotensive [6] and anti-inflammatory effects [7]. In Myanmar, by reviewing the current literature, there was no previous research on the pharmacological properties of this plant. Therefore, experiments were carried out to test the antidiabetic activities of this plant. The present study was undertaken to evaluate the possible hypoglycemic activity of the ethanolic extracts of *G. procumbens* leaves (which have been reported in Ayurveda to be useful in diabetes) in the adrenaline-induced hyperglycemic rats.

MATERIALS AND METHODS

Plant material and extract preparation

The leaves (*Gynura procumbens*) were collected from the herbal garden and identified by U Kyaw Nyan Linn (Research Officer), keeper of herbarium, DMR (Upper Myanmar). Fresh leaves were cut into small pieces (about 1 cm). The air dried *G. procumbens* leaves (100 g) were pulverized and then exhaustively extracted with ethanol. The extract was evaporated in vacuo and the yield was 5.37% (5.37 gm of ethanolic extracts were achieved by extraction of 100 grams of dry leaves).

Animals

Albino rats (180-250 gm) of both sexes were obtained from the Laboratory Animal Services Division, DMR (Upper Myanmar). These rats were randomly divided into 5 groups of 7 rats each.

Induction of hyperglycemia in rats

Screening of adrenaline-induced hyperglycemia in experimental animals was done

one week before starting experiment. Diabetic rats (n=28) were induced by single intraperitoneal injection of freshly prepared adrenaline (MPF, Myanmar) (200 mcg/kg body weight) to overnight (18 hours) fasted rats. Negative controls (n = 7) were injected with sterile distilled water alone. Before induction, fasting blood glucose concentration was determined by Bayer's glucometer Elite. The ethanolic extracts of *G. procumbens* leaves in aqueous solution were administered orally at a concentration of 150 and 300 mg/ml body weight / rat.

Preparation of test solution

The aqueous solution of *G. procumbens* was prepared by mixing 5 grams of *G. procumbens* extracts with 25 ml of distilled water and was osterized until in a completely liquefied state. Resulting solution contained 200 mg of *G. procumbens* per 1ml aqueous solution.

Treatment schedules

The experimental rats were divided into 5 groups of 7 rats each. Group I and II were treated with 150 and 300 mg/kg body weight of extracts of *G. procumbens* leaves. In group III, the rats were treated with glibenclamide (0.5 mg/kg) (Braan Laboratories Ltd, Batch-42184 E, Expiry date–January 2008, India) and in group IV, the rats were treated with 2ml/kg body weight of distilled water. These groups III and IV were taken as positive controls. The rats in negative control group (group V) were given only distilled water.

Collection of blood samples

Blood samples (0.02ml) were collected from the tail vein at 0 hour, 1 hour, 2 hour, 3 hour and 4 hour post administration.

Statistical analysis

Data were checked and entered using Epi-info 3.1 software. All values were expressed as the mean $\pm$ SD values obtained from a number of experiments using one way ANOVA. The results were expressed in Table.

RESULTS

The effects of oral administration of different doses of the plant extract and the positive controls serum glucose levels of adrenaline-induced hyperglycemic rats are presented in Table 1.

Table 1. Effects of *G. procumbens* leaves on rats

Time of blood collection (hour)	Blood glucose level (mg/dl)				
	G-1	G-2	G-3	G-4	G-5
	<i>G. pro-cumbens</i> 150 mg/kg	<i>G. pro-cumbens</i> 300 mg/kg	Glibenclamide standard drug	Pc Dw only	Nc Dw
0	4.9	4.1	4.9	4.08	4.0
1	5.8	4.7	6.5	9.47	4.1
2	4.5	4.4	6.0	8.4	4.3
3	4.5	4.0	4.4	7.0	3.5
4	4.2	4.0	3.7	5.5	3.5

G=Group, Pc=positive control, Nc=Negative control
Dw= Distilled water

P value = 0.0484 at 1 hour, 0.0035 at 2 hour, 0.4864 at 3 hour, 0.4256 at 4 hour

n = 35 (7 rats per group for 5 groups)

Test = single ANOVA test

At 1 hour post-administration, serum glucose level of adrenaline-induced animals reached a peak but plant extracts treated groups and glibenclamide treated control group did not reach up to peak level. The high dose of extracts (300 mg/kg) produced significant decrease in serum glucose levels compared to glibenclamide treated group. This dose also produced a significant decrease in serum glucose at (1 & 2 hour) ($p=0.0484$, $p=0.0035$, respectively) compared to reference western drug (glibenclamide). Low dose extract (150 mg/dl) had significantly lowered the serum glucose level at 1st and 2nd hours. But 3 hours and 4 hours glibenclamide inhibition effects were more prominent than this low dose extract.

DISCUSSION

Although the morbidity and mortality associated with diabetes mellitus is primarily due to its complications, these longterm complications depend on a long-standing history of poor glycemic control. In the adult population worldwide, diabetes is the leading cause of end-stage renal

disease, blindness, kidney failure, neuropathy, gangrene and amputation not resulting directly from accident or trauma. Although insulin still remains the main therapeutic agent for the management and control of type 1, juvenile-onset, IDDM, the major classes of synthetic oral hypoglycemic agents currently available for the management and/or adult-onset type 2 NIDDM include the sulphonylureas, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, and so on. However, the use of these synthetic products is usually accompanied by some serious adverse effects. Therefore, there is an urgent need to find safe, cheap and effective pharmacological interventions for diabetes mellitus.

The hypoglycemic effect of ethanolic extract of *G. procumbens* was determined by evaluating its effects in adrenaline-induced hyperglycemic rats. As shown in Table 1, the glucose levels of *G. procumbens* extract treated rats showed significant reduction in glucose level at 1st and 2nd hours compared to glibenclamide. *G. procumbens* leaves extract with the dosage of 300 mg/kg had more reduction level of glucose than glibenclamide at the first 2 hours (at 1 hour, $p=0.0484$) and (2 hour, $p=0.0035$). However, in 3 hour and 4 hour, there was no significant differences in tested extract and glibenclamide ($P=0.4864$, 0.4256 , respectively). Zhang *et al.* [8] demonstrated that the ethanolic extracts of *G. procumbens* at 3 doses levels orally, significantly suppressed the glucose levels in diabetic rats. Rasadah MA [7] stated that methanol extract of *G. procumbens* leaves at the dose of 1 gm/kg presented a significant decrease in blood glucose level which was observed only at 60 minutes after administration of glucose as compared to vehicle (5% gum acacia).

In Myanmar, many plants have been reported to possess hypoglycemic activities. Indeed, hundreds of plants are now being used traditionally for the management and/or control of both insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus worldwide. To date, however, only

a few of these plants have been subjected to scientific scrutiny either in laboratory animals or human subjects. In the present study, the hypoglycemic activity of the ethanol extract of *G. procumbens* was evaluated in the adrenaline-induced diabetic rats. Under the same conditions, glibenclamide produced a significant glucose reduction in adrenaline-induced diabetic rats. One study stated that the ethanolic extract of *G. procumbens* leaves had antihyperglycemic and antihyperlipidaemic activities in streptozotocin-induced rats [8]. It is well known that glibenclamide which is a derivative of sulfonylurea causes hypoglycemia by stimulating pancreatic β cells to release more insulin, and inhibiting glucagon secretion. As these effects require a functional pancreas, it can lower blood sugar levels in non-diabetic subjects [9]. The results showed that the ethanol extract of *G. procumbens* was assumed to stimulate insulin release in pancreatic β -cell lines. Therefore, extract of *G. procumbens* can represent a source of potential new hypoglycemic agent.

Conclusion

This study has confirmed the traditional uses of *G. procumbens* for treating diabetes. This plant possesses hypoglycemic activity in adrenaline-induced rats and also contains substances, which are able to stimulate insulin secretion, thus contributing to the anti-diabetic property of the plants. Therefore, *G. procumbens* has the potential to become a dietary adjunct for the treatment of diabetes and potential source for the discovery of new orally active agents for diabetes therapy.

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Assessment of community's behaviors focusing on knowledge and attitude towards cancer in Hlinethaya Township, Yangon Division in 2006

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This cross-sectional study was carried out in Hlinethaya Township, Yangon Division in 2006 to assess community's knowledge, opinion, and attitude towards cancer. A total of 400 randomly selected subjects were interviewed. The mean age of participants was 39 ± 11.33 years. Median income was 50,000 Kyats per month (10000- 300000). Forty-four percent finished primary level of education and 71% were females. All of respondents were found to have common knowledge regarding cancer. One-third of respondents were aware that the canned food also causes cancer. Thirteen percent of them had cancer patients in their families and relatives. The health personnel (33.1%) and the neighbors (32.3%) were found to be common knowledge sources regarding cancer. The breast, cervix, and stomach were most common sites for cancer which was answered by 41.4% of the respondents. Breast self examination (86.2%) and mammography (86.7%) were found to be methods of diagnosis for cancer responded by interviewees. Regarding treatment, surgery (33.6%) and radiotherapy (95.5%) were commonly cited methods. More than 90% knew that smoking and alcohol drinking behaviors were related to cancers. According to their opinions, early diagnosis (87.4%) of cancer is important and can be hereditary (66.4%) in nature. The knowledge of cancer was not related to age, sex, and income but it was strongly related to education. So it is recommended to introduce cancer information in upper, middle and high school curriculum to develop favorable attitude that leads to promote better preventive practice.

INTRODUCTION

Of all the maladies that human beings have to suffer, cancer imposes as a most fearful and burdensome disease. Worldwide, approximately 10 million people are diagnosed with cancer annually and more than 6 million died of the disease every year. The burden of cancer is distributed unequally between the developed and the developing world. The total cancer burden is highest on affluent societies, mainly due to high incidence of tumors associated with smoking and western life style. In developed countries the overall mortality due to cancer is

more than twice as high as in developing countries. But now in some western countries, cancer mortality rates have recently started to decline due to reduction in smoking, improvement in early case detection and advance in cancer therapy [1].

Formerly, populations in developing countries are vulnerable to cancer in which infectious agents play a significant role. Now, it is due to adoption of western life style, such as consumption of tobacco, alcohol abuse, sedentary habits without adequate physical exercise and over-indulgence that lead to double burden for cancer in these communities. On the other

side, due to increased life span and better diagnostic facilities, more cases of cancer are detected in general population. But in most cases, the patients present themselves to a medical facility when the disease is far advanced and is not amenable to treatment [1]. Therefore, it is necessary to inform people thoroughly about cancer. Before giving information about cancer to community, the behavior of community about cancer has to be assessed first. So, the findings of this study will hopefully provide information to develop appropriate Information Education Communication (IEC) material for cancer prevention.

General objective

- To assess the knowledge, opinion, and attitude regarding cancer among the community in Hlinethaya Township.

Specific objectives

- To document socio-demographic characteristics of study subjects.
- To identify source of information regarding cancer among community members.
- To assess knowledge of signs and symptoms, sites of cancer, diagnostic method, treatment, and risk about cancer.
- To assess opinion and attitude of community regarding cancer.
- To find out association between the knowledge of cancer and attitude, opinion and demographic characteristics

MATERIALS AND METHODS

Study area and population

A cross-sectional community-based study design was used. Both urban and rural setting of Hlinethaya Township, Yangon Division was selected because the reported cases of cancer in hematology unit of Yangon General Hospital were increasing. Five out of 20 wards from urban and 3 out of 15 villages from rural were randomly selected. Eligible house-holds were selected consecutively. Age between 18-60 years and

both genders were eligible for the study if they were willing to give consent. If there were more than one eligible subject in the household, each individual per household was randomly selected.

Sample size determination

The probability of people who never heard & never come across cancer cases (q) was 0.5, the desired precision was set at 5%. The calculated sample size was about 384 at 95% confidence level. In this study, a total of 400 subjects were recruited.

Data collection and analysis

Before the data collection, informed consent was obtained from each participant. Respondents were interviewed (face to face) individually by trained interviewers using pre-tested structured questionnaires. Before the data entry, data were checked daily for error, incompleteness and inconsistency. Data entry was done by Epi Data 2.1b and range and consistency check was carried out before analysis. Data analysis was performed with computer software Epi info 6.04d. Univariate analysis was used to show the frequency distributions for categorical data and mean $\pm$ SD for continuous data. The chi-square test was used to examine the significance of association between categorical variables. P value at <0.05 was considered as the association was statistically significant. Logistic regression analysis was done to find out the association between knowledge, attitude, opinion and demographic variables.

Ethical consideration

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

RESULTS

Characteristics of study samples

Total number of respondents was 399, of which 71% were females. Mean age of participants was 40.35 ± 12.43 years in men and 38.66 ± 10.83 years in women. Median

income of their families was 50,000 Kyats per month (ranged from 10,000-300,000). More than half of them were dependents and about 45% completed primary school education. Seventy-eight percent were married. Wooden houses (49.6%) and that of bamboo (35.6%) were commonest types of housing. The median year of living in the study area was 6 and it ranged from 1 to 43 years. Nearly 14% had family history of cancer in their families.

Ninety-four out of 399 (23.6%) respondents were smokers. They started to smoke at the age of 18.5 years (median) and it ranged from 5 to 48 years. Thirty percent of respondents had betel chewing practice. Among male respondents, 8.8% were drinking alcohol. Median time of consuming vegetable was 8 times (1-24), fruit was 4 times (0-15), and that of meat and fish were 10 times (0-21) per week. The reported consumption of green vegetables and fruits more than 7 times a week were 47.7% and 17.8% respectively. Among the respondents, 18.3% consumed meat between 4-6 times per week. Foods (28%) and behaviors (8%) were found to be causes of cancer reported by participants. Eleven out of 399 (2.8%) respondents identified cancer could be due to genetic. But majority (52.7%) of participants did not know the potential causes of cancer.

Early signs and symptoms of cancer reported by respondents

Breast swelling, swelling or thickening of any part of body and difficulty in swallowing were the most frequently responded early symptoms of cancer (91%, 89.9%, and 76% respectively). Forty percent of respondents knew that constipation more than 2 weeks was early symptom of cancer (Table 1).

Source of knowledge of cancer

The health centers and neighbors were common sources for cancer information cited by 33% and 32% of respondents respectively. Eighteen percent of study

subjects received cancer information from mass media.

Table 1. Distribution of early signs and symptoms of cancer reported by respondents

Signs and symptoms (N=399)	Freq * (%)
Non-healing ulcer	223 (57.0)
Abnormal bleeding or secretions	237 (60.6)
Gradual hair loss	236 (60.4)
Swelling or thickening of any part of the body	351 (89.8)
Chronic cough	202 (51.7)
Bloody cough	201 (51.4)
Sudden chest pain	188 (48.1)
Breast swelling	356 (91.0)
Difficulty in swallowing	297 (76.0)
Constipation > 2 weeks	153 (39.1)
Frequent bone pain	217 (55.5)

*Multiple responses allowed

Knowledge of cancer

The most commonly stated sites of cancer were breast, cervix and stomach (41.4%) and it was followed by lung (18.3%) and liver (8.0%). Nearly 12% could not describe where the cancers developed from. Smoking (92.3%), alcohol drinking (93.6%), betel chewing (86.0%), and tobacco chewing (82.4%) were most commonly mentioned risks of cancer by the respondents. But only half of study sample knew occupational exposure could be possible cause of cancer.

Knowledge of diagnosis and that of treatment methods

Regular medical check-up was reported as a method of diagnosis by 95.4% of respondents. The other known methods were breast self examination (87.5%), mammography (88%), and Pap smear (78.1%) examination. Concerning treatment methods, 96.2% of participants knew radiation and 92.4% recognized surgery as methods of cancer treatment. Few (23.7%) identified hormonal method of cancer treatment (Table 2).

Attitude and opinion towards cancer

Nearly all (93.5%) of participants viewed cancer is a serious problem and 85.5% thought some cancer can be detected early. Few (14.5%) believed that cancer is

contagious. Almost all (98.7%) of participants agreed that cancer had to be detected early to save the lives. Sixty-seven percent showed the disagreement to accept traditional mean for treatment of cancer. Of respondents, 57.1% concerned about what they eat (Table 3).

Table 2. Distribution of knowledge of diagnosis and treatment methods

Knowledge variables	Freq* (%)
<i>Diagnosis methods (N=397)</i>	
Regular annual medical check-up	375 (95.4)
Breast self examination	344 (87.5)
Mammography	346 (88.0)
Stool examination for blood	265 (67.4)
Pap smear	307 (78.1)
<i>Treatment methods (N=396)</i>	
Surgery	366 (92.4)
Radiation	381 (96.2)
Chemotherapy	337 (85.1)
Hormone	94 (23.7)
Combination treatment	244 (61.6)

*Allowed multiple responses

Table 3. Opinion and attitudes towards cancer

Opinions & Attitudes	Freq (%)
<i>Opinions</i>	
Cancer can be cured if detected early	
Yes	347 (87.4)
Cancer is a serious problem	
Yes	371 (93.5)
Cancer is a punishment for sin	
Yes	228 (57.4)
Only smoker will get cancer	
Yes	133 (33.5)
Some cancers can be detected early	
Yes	339 (85.4)
Cancer is a rare disease that affects the unlucky one	
Yes	148 (37.3)
Some cancers are hereditary	
Yes	265 (66.8)
Cancer is contagious	
Yes	59 (14.9)
<i>Attitudes</i>	
I like cancer to be detected early to save my life	
Agree	394 (98.7)
All individuals are at risk of getting cancer	
Agree	293 (73.4)
I prefer traditional treatment for breast cancer	
Disagree	264 (66.3)
I will not see a doctor even if I have early signs of cancer	
Disagree	368 (92.2)
I don't like to sit next to a smoker	
Agree	350 (87.7)
I am not concerned about what I eat	
Disagree	228 (57.1)

Association between knowledge and demographic characteristics, attitude, and opinion among respondents

The knowledge score was developed from combination of knowledge of symptoms, diagnosis, methods and risks. Among respondents 61% belonged to high knowledge group and the rest was in low level knowledge group. The age, sex, income, residence, and family history of cancer were not significantly related to knowledge of cancer. But the education, attitude, opinion, and years of living in the study area were related to knowledge of cancer in bi-variable analysis (Table 4).

Table 4. Association between knowledge and demographic characteristics, attitude, and opinion among respondents

Variables	Total knowledge		χ^2	p value
	High	Low		
Age (N= 394)				
Young (≤ 38 yrs)	119	87	1.51	0.219
Old (> 38 yrs)	120	68		
Sex				
Male	67	49	0.58	0.446
Female	172	106		
Education				
$\leq$ middle school	199	144	9.77	0.008
High school	11	6		
$\geq$ Graduate	29	5		
Income (n=391)				
Low	122	75	0.41	0.522
High	114	80		
Residence				
Urban	193	121	0.42	0.517
Rural	46	34		
Family history of cancer				
Present	37	16	2.15	0.143
Absent	202	139		
Years living in the study area (n=391)				
≤ 5 years	105	87	5.55	0.018
> 5 years	132	67		
Attitude (n=391)				
Favorable	67	85	28.5	0.000
Unfavorable	170	69		
Opinion (n=389)				
Correct	97	84	7.65	0.006
Wrong	140	68		

Note: The total knowledge score was 31, if the respondent could answer all the questions correctly. The minimal score of knowledge was 1 and maximal was 31. The mean score 22 was used as cutpoint for determining low and high knowledge score.

Logistic regression analyses examining association of selected characteristics and high knowledge

In logistic regression analysis, high school level of education ($p=0.029$) was strongly related to high level of knowledge after controlling for other variables. Living in urban was marginally related with high knowledge ($p=0.061$) (Table 5).

Table 5. Odds ratio (and 95%CI) from logistic regression analyses examining the association of selected characteristics and high level of knowledge

Characteristics	Odds ratio	95% CI of odd		p value
		Lower	Upper	
1. Residence				
Rural (reference)	1.000			
Urban	1.935	0.970	3.857	0.061
2. Education				
=< Middle school (reference)	1.000			
High school	1.353	0.425	4.311	0.609
=> Graduate	0.298	0.101	0.882	0.029

Note: Age, sex, income, family history of cancer, attitude and opinion were also included in analysis. But these were not fit to model thus they were omitted.

DISCUSSION

Hlinethaya is the satellite township with the total population of 273,879. The number of people residing in urban dwelling is 220,387 so, that of rural is only 53,492 [2]. Most of men were working far from the houses as breadwinners and thus majority of respondents in the present study were women. Regarding previous exposure to cancer risk, the proportion of being smokers in the current study was lower than that of the study by Nyo Nyo Kyaing *et al* (23.6% vs. 31.1%) [3]. This is probably due to large number of females who participated in the current study. But for the betel chewing, the proportions were similar in both studies (30% vs 33%). It highlighted that significant number of females were also engaged in betel chewing practice which is one of the risks of cancer.

Although the finding of consumption of green vegetables in meal was higher in this

study (47.7% vs. 31.9%), the consumption of fruits (17.8% vs 17%), and meat (18.3% vs 21.5%) in a week were more or less the same as in Omar Hasan Kasule Sr *et al* study [4]. Knowledge of food in relation to cancer was low in this study (28% vs. 94%) as compared to that in Egypt because the study population in Egypt was students [5] but the study sample in present study was community members. This showed the importance of education in determining level of knowledge. Despite most of study sample were women with an older age, breast swelling (91%), swelling and thickening of any part of body (89.9%) and difficulty in swallowing (76%) were frequently recognized as early symptoms of cancer in our study, this was different view from study among students in Malaysia in which gradual hair loss (75.4%), bloody cough (72.8%), chronic cough (66.7%) were identified as main symptoms. Despite the priority setting in knowledge of cancer sites and that of diagnosis methods (regular medical check up 95.4% vs. 83.2%, mammography 88% vs. 68.2%, self breast examination 87.5% vs. 66.2%) and that of treatment options (surgery 92.4% vs. 85.1%, radiotherapy 96.2% vs. 49.4%, and chemotherapy 85.1% vs. 33.6%) were the same in present study and previous study. But the proportion with correct responses was high in ours. Due to an older age of our participants, they had ever come across cancer patients in their neighbors than students. Although the knowledge of most cancer risk factors between studies were similar, knowledge of occupational exposure (53.6% vs. 45.5%) as cancer risk was lower in both study participants. Because they were not workers, they probably did not aware occupation as a risk.

The respondents get cancer information from diverse range of sources with neighbors and health centers playing the major role. According to culture, Myanmar people are very friendly with their neighbors and willing to help each other. This could be explained why people get information from neighbors even though they are not trust-

worthy. Not only government health sector but also local NGO and international NGO that give curative and preventive services in the study area may be responsible for source of cancer information. Some opinions such as, 'cancer is a punishment for sin', 'only smoker will get cancer', 'cancer is a rare disease that affects the unlucky one', and 'cancer is contagious' have to change in order to accomplish for better preventive practice. Similarly, a few attitudes like preference of traditional treatment for breast cancer, unwillingness to see a doctor even if they had early sign of cancer, and no concern about what they ate were needed to change in order to get proper prevention, early and effective management of cancer. These findings pointed out proper health education message are necessary to improve their existing knowledge that also guided in changing or modifying more favorable opinion and attitude.

As in other studies the knowledge of cancer is strongly related to level of education in present study. This is also consistent with the study on medical students' KAP regarding cancer prevention in which KAP variables showed better scores for continuing than new students [6]. It is well known fact that the education will leave a lifelong impact on students because they will become responsible adults in a few years. This is the important fact that our study findings revealed that the difference in knowledge could be seen only after high school education. Actually the ages between upper, middle and high school children are very receptive to new ideas, while their opinions and attitudes are still amenable to change and modification. These emphasize

the significance of incorporating health education section regarding cancer in school curriculum.

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Immunohistopathology of liver in autopsy cases of dengue shock syndrome

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Dengue hemorrhagic fever (DHF) is a severe febrile disease, characterized by abnormalities in hemostasis and increased vascular permeability, which in some cases results in hypovolemic shock and in dengue shock syndrome. The clinical features of DHF include plasma leakage, bleeding tendency and liver involvement such as enlargement of the liver. During the peak period of outbreak from July through October, of 2005 and 2006, a total of 13 fatal cases of dengue hemorrhagic fever (DHF)/ dengue shock syndrome (DSS) were collected from Intensive Care Unit, Yangon Children Hospital to study their immunohistopathology of DHF. The liver seems to be a target for dengue virus, so postmortem examinations were performed to investigate histopathological changes and whether the virus and viral antigens were present in target cells of the liver. We detected Kupffer cell hyperplasia in 100%, hemorrhage in 92%, mixed midzonal and centrilobular necrosis in 85%, and hemophagocytosis in 77% by using hematoxylin and eosin stain of routine histopathological standard method. Dengue virus antigens of the structural (envelop) and non-structural (NS1) proteins were detected in 54% and 77% respectively in both hepatocytes and Kupffer cells, using immunohistochemistry. There was no recruitment of polymorphonuclear cells; however, minimal degree of portal infiltration by lymphocytes was detected in the liver lesions of patients who died from DHF. Thus, our findings conclude that the hepatocytes and Kupffer cells may be target cells supporting virus localization and replication to provide more information for understanding the pathogenesis of liver involvement in DHF.

INTRODUCTION

Only a few histopathologic studies of liver has been published that describe the cellular and organ damage done by dengue viruses and the accompanying host responses that characterize dengue hemorrhagic fever/ dengue shock syndrome (DHF/DSS) [1-5]. No general histopathologic studies on fatal dengue infections have been published within the past twenty-five years. Conventional studies on dengue pathology have been supplemented by efforts to isolate dengue virus or localize dengue viral

antigens in peripheral blood leukocytes, biopsies or organs at autopsy using fluorescent antibody or in situ hybridization [6-11]. French workers have demonstrated dengue 1 antigen in hepatocytes from a patient dying during a dengue infection; these cells also showed apoptosis [12]. Recently, studies of autopsy tissues have been complemented by efforts to grow dengue viruses in human tissue explants, primary cells and human cell lines. Several studies have shown the inability of dengue 1 virus to replicate in mature Kupffer cells *in vitro*, while hepatocytes and cell lines do become infected but

then promptly die as the result of apoptosis [13-16]. These studies raise questions concerning the sites of infection during human dengue infection. An understanding of the mechanisms that produce symptoms of disease and the fatal vascular permeability, hepatic damage and metabolic acidosis rests ultimately with gaining definitive understanding of dengue virus-host cell interactions during infections. To achieve this goal, a comprehensive study of liver obtained at autopsy is essential. The liver seems to be a target for dengue virus, so autopsy is essential for immunohistopathological studies of DHF/DSS.

MATERIALS AND METHODS

Thirteen autopsies of DHF/DSS cases were collected following a designated protocol, at Yangon Children's Hospital (YCH) during 2005 and 2006. Ethical clearance had been taken from Ethical Review Committee, DMR (LM). After taking written informed consent from parents/guardian, autopsies were conducted as soon as possible after death in order to minimize autolysis and post-mortem artifacts using a proforma protocol. Subjects were placed in the mortuary cold room of YCH, the internal temperature of which is kept at 4-8°C constant. Age, sex, date of onset of fever, date of hospitalization and date of death were recorded for each study subject. During autopsy, gross observations of liver were also recorded. The liver tissue specimens were not allowed to dry out during the preparation phase and were placed into fixatives 10% neutral buffered formalin and Optimal Cutting Temperature (OCT) compound.

Staining methods

The following methods were applied to serial section: hematoxylin and eosin (H&E) and immunohistochemical staining using EnVision™ kit (Dako). This kit is a peroxidase-conjugated polymer backbone, which, in addition, also carries secondary antibody molecules directed against mouse immunoglobulins. Endogenous biotin will not affect

staining results. The primary monoclonal antibody against NS1 and envelope protein and positive control of dengue virus injected suckling mouse brain were produced and provided by Medical Molecular Biology Unit, Siriraj Hospital. They were applied for 30 minutes at room temperature for primary antibody and 30 minutes for ready to use EnVision™ reagent. Counter stain was done by Mayer's hematoxylin.

RESULTS

The ages of the patients varied from 3 years to 9 years. Seven cases were aged 3 to 3 and half years, 2 cases were 4 to 5 years, 2 cases were 7 years and 2 cases were 9 years old. Five cases were males and 8 cases were females. All the patients went through two clinical stages, febrile and shock, before succumbing to the disease. Hospital stay varied from 1 to 8 days. The children who died within 24 hours were 2 cases, within 48 hours were 4 cases, 3 days and 4 days were 3 cases each and 8 days were 1 patient. All cases have hepatomegaly on clinical examination.

Gross autopsy findings

Most of the subjects appeared well developed and well nourished. Hemorrhage appeared as petechial rashes or purpura, especially around needle punctures sites. The rashes were particularly striking over the lower limbs; frank hemorrhage appeared in patches in subcutaneous tissue. Hemorrhage presented in the mucosa of the nose, gums and gastrointestinal tracts, as well as in liver. The livers were enlarged and subcapsular hemorrhages were noted in most fatal cases. The cut surface of the liver revealed yellowish colour suggesting fatty metamorphosis.

Microscopic findings

Microscopic examination of the liver was made in 13 patients. Necrosis of the liver cells was present in 11 cases, some of which showed only a few necrotic liver cells in a single focus, while in others a major part of

the hepatic lobules was involved. Where necrotic foci were small they occurred focally or in the paracentral part of the lobules, usually on one side of the central veins separated by a few rows of intact liver cells. The liver cells in the necrotic foci were swollen, their cytoplasm was acidophilic and vacuolated. The nuclei showed pyknosis, karyorrhexis and karyolysis, and occasionally had disappeared. The foci of necrotic liver cells were usually surrounded by histiocytic mononuclear cells or altered Kupffer cells. Polymorphonuclear leucocytes were rarely present. Centrilobular necrosis, hemorrhage, Kupffer cell hyperplasia and hemophagocytosis in necrotic areas were graded as in Table 1.

Table 1. Grading of histological changes in liver of DHF/DSS

<i>Centrilobular necrosis</i>	
Grade I	Necrosis area extend 1/3 from central vein to portal triad
Grade II	Necrosis area extend 2/3 from central vein to portal triad
Grade III	Necrosis area extend from central vein to portal triad
<i>Hemorrhage</i>	
Grade I	Hemorrhagic area extend 1/3 from central vein to portal triad
Grade II	Hemorrhagic area extend 2/3 from central vein to portal triad
Grade III	Hemorrhagic area extend from central vein to portal triad
<i>Kupffer cell hyperplasia</i>	
Grade I	With effort
Grade II	Occasional
Grade III	Easy to find
<i>Hemophagocytosis in necrotic areas</i>	
Grade I	With effort
Grade II	Occasional
Grade III	Easy to find

Histological changes in liver were observed by two pathologists at the same time who are investigators and this grading classification is based on their working definition.

In two cases, the necrotic foci were small and were seen in only a few lobules. Five cases showed moderate-sized foci, while in four cases the necrosis was fairly extensive (Table 2). In one case, no necrosis of the liver cells or haemorrhage was observed. In all cases the Kupffer cells showed hyperplasia and acidophilia of the cytoplasm. Councilman bodies were noted in one case

of liver. Fatty metamorphosis of varying degree was noted; where it was not extensive the central and paracentral zones were mostly involved. Typical features of hepatitis were not seen in all fatal cases.

Table 2. Grading of histological changes in liver

	GI (%)	GII (%)	GIII (%)	No changes	Total number
<i>Liver</i>					
Centrilobular necrosis	2 (15%)	5 (38%)	4 (31%)	2 (15%)	13
Hemorrhage	3 (23%)	5 (38%)	4 (31%)	1 (8%)	13
Kupffer cell hyperplasia	0 (0%)	6 (46%)	7 (54%)	0 (0%)	13
Hemophagocytosis	7 (54%)	2 (15%)	1 (8%)	3 (23%)	13

G= Grade

Immunohistochemical results

A total of 13 liver tissues, 11 cases (85%) were NS1 positive and 9 cases (69%) were envelop protein positive.

DISCUSSION

Despite the mixed midzonal and centrilobular necrosis of the liver, minimal degree of portal infiltration by lymphocytes was noted in all cases of DHF autopsy. But Kupffer cell hyperplasia was observed in Grade II and III of these cases. In this series of 13 autopsy cases, only one case showed Councilman bodies, frequently described in the literature for common pathologic change in the liver of dengue infection at autopsy. The causes of midzonal necrosis and centrilobular necrosis of the liver should be different. Regarding the vascular supply of the hepatic lobules, the centrilobular necrosis seems to be secondary to hypoxia. The midzonal necrosis seems to be immunologically induced, but it is difficult to explain why zone 1 and 3 are spared. The observation of the dilatation of subcapsular lymphatic spaces (Fig. 1) without any evidence of inflammation needs correlation with the existence of right pleural effusion and ascites from the clinical and gross autopsy profile in each case.

In the histopathological analysis of this fatal case of DENV-3 infection, in Rio de Janeiro,

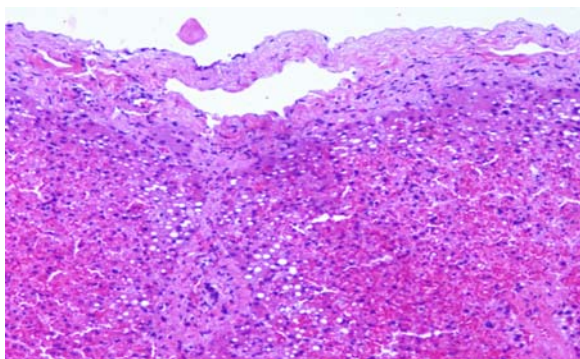


Fig.1. Histological changes of capsular lymphatic dilatation in liver (H & E)

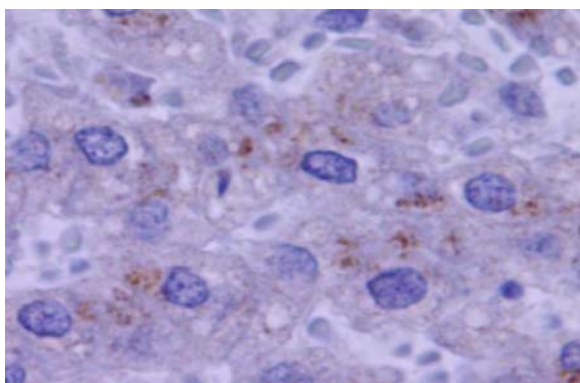


Fig. 2A. Dengue viral antigens (NS1) detected in hepatocytes and Kupffer cells (Magnification x 40)

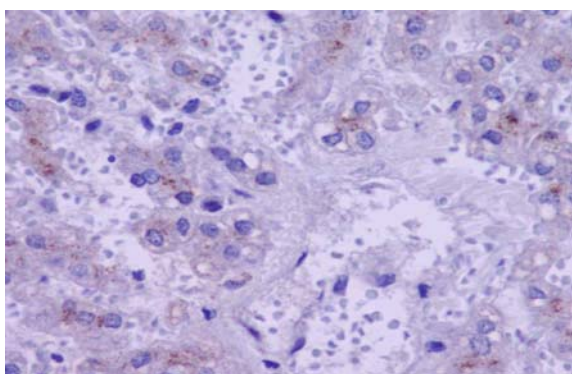


Fig. 2 B. Dengue viral antigens (E protein) detected in cytoplasm of hepatocytes and Kupffer cells (Magnification x 10)

Brazil found severe damage to the vascular system, hepatic injury, and mononuclear cell infiltration in many target organs. These data corroborate the conception that the liver is an important site of dengue virus replication. Intense injury to many organs was demonstrated, such as focal vacuolization of hepatocytes, and increasing concentrations of inflammatory cells inside sinusoid capillaries. This suggested the participation

of cytokines and inflammatory mediators, causing alteration of vascular permeability [17]. In our patient, viral antigen was found in hepatocytes and Kupffer cells. Similarly, dengue virus has previously been identified within liver tissue, and dengue virus antigen was identified within Kupffer cells of infected individuals [18]. That expression of the NS1 and envelope protein antigens of dengue in the hepatocytes and Kupffer cells in the paraffin-embedded tissue is not quite distinct as that observed in the paraffin-embedded tissue of suckling mouse brain that was inoculated by heavy dosage of dengue viruses used as a positive control. Elevation of serum aminotransferase activity is almost universal in individuals with DHF [19]. The histopathology showed marked macrovesicular fatty changes, mid-zonal hepatocellular necrosis and focal lymphocytic and polymorphonuclear leucocyte infiltration in a case of dengue hemorrhagic fever with fulminant hepatic failure [20]. In our patient, hepatocellular injury was also observed, and focal microvesicular steatosis was found around the centrallobular vein. The hepatic alteration consisted of diffuse necrosis of sinusoid capillaries, suggesting release of cytokines and activation of the complement system, as well as endothelial dysfunction. In conclusion, the patient in our study, infected with dengue virus died with considerable hepatic injury.

The observation that the immunohistochemical assay was as sensitive in detecting the number of infected cells may have several explanations. First, the fatal form of the disease is clearly associated with massive viral proliferation in the liver with a concomitant large amount of viral protein being synthesized, resulting in ample amounts of target. Second, the multistep method employed allows a greater sensitivity in general for immunohistochemical detection of a variety of proteins. The immunohistochemical assay showed that the viral protein localized to the cytoplasm in a punctate pattern (Fig 2A & 2B). In this study, NS1 protein antigen was 85% positive in all liver tissue of patients,

envelop protein antigen as positive in 6% of these cases. Thus, the NS1 protein was found predominantly associated with the liver, where hepatocytes appeared to represent major target cells.

Dengue fever is a common disease that, in most cases, is self-limited. Presumably, this represents an active and successful immunologic response to the virus. In the rare and often fatal forms of dengue fever, such as hemorrhagic dengue fever, the virus is able to apparently escape immunologic surveillance and rapidly and massively proliferate at certain sites, such as the liver as documented in this study. The marked hepatic dysfunction associated with the viral infection certainly could explain, in part, the hemorrhagic tendencies characteristic of the disease. A better understanding of pathogenesis and viral proliferation in dengue infection need to study changes in multi-organs by using IHC using viral markers and other markers as macrophages, dendritic cells, endothelial cells, B and T lymphocytes, compliments, cytokines and molecular techniques such as in situ hybridization and RT-PCR.

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Syndromic versus laboratory-based diagnosis for lower genital tract infections among married women in selected sub-urban communities, Yangon

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The syndromic diagnosis is the most realistic and cost-effective strategy for controlling sexually transmitted infections (STI) in the developing countries. This approach relies on symptom recognition by an infected person who as a consequence seeks medical care. A cross-sectional study was carried out in January and April 2007 at Indaing and Hmawbi areas to determine the prevalence of certain reproductive tract infections (gonorrhoea, trichomoniasis, candidiasis and bacterial vaginosis) and to evaluate the accuracy of the syndromic diagnosis by comparing with laboratory diagnosis. Of 212 symptomatic and asymptomatic married women (93 from Indaing and 119 from Hmawbi), the prevalence of gonorrhoea was 3.8%, trichomoniasis 17.5%, candidiasis 15.6 % and of bacterial vaginosis 13.2%. Syndromic diagnosis for lower genital tract infections (LGTI) was made on the basis of presence of clinical symptom of vaginal discharge and signs such as creamy white or greenish-yellow vaginal discharge and/or vaginitis and/or cervicitis on speculum examination. Among 80 laboratory-confirmed LGTI (trichomoniasis, candidiasis and bacterial vaginosis), syndromic diagnosis can detect 47.5% (38/80) of these infections. There is unsatisfactory agreement between laboratory diagnosis and syndromic diagnosis (Kappa coefficient= 0.04). The present study found out the occurrence of asymptomatic reproductive tract infection in the community and clinical judgement based on symptoms and signs seemed to be less indicative for LGTI. The lack of association between symptoms and laboratory-confirmed infections underscores the challenge of diagnosing LGTI when laboratory testing is not available.

INTRODUCTION

Reproductive tract infections (RTIs) continue to cause significant morbidity and mortality throughout the world. Chlamydia, gonorrhoea, candidiasis, trichomoniasis and bacterial vaginosis are the common curable RTIs and they are mainly transmitted by sexually. The World Health Organization (WHO) estimates that 340 million new cases of curable RTIs occurred throughout the world in 1999 in men and women aged 15-49 years [1].

The RTIs are important health priority not only because of their complication but also because of their association with Human

Immunodeficiency Virus (HIV) and AIDS. The presence of RTIs has been found to facilitate the acquisition and transmission of HIV infection. Therefore prevention and control of RTIs have gained greater importance with the emergence of HIV/AIDS.

In Myanmar, RTI is one of the priority health problems among 39 priority diseases in the National Health Plan from 2006-2011. A recent study carried out on 426 symptomatic women attending various clinics in Mandalay, showed high prevalence of bacterial vaginosis (40.1%) in 299 symptomatic women attending general clinics, with candidiasis (23.7%) being the second most common RTI. In this study, chlamydia,

gonorrhoea, syphilis and trichomoniasis were 4, 2.7, 2.0 and 2.7% respectively in general clinics and 14.2, 14.4, 13.4 and 3.1% respectively in sexually transmitted diseases (STD) clinics [2]. The other study on 90 symptomatic women at the Central Women's Hospital, Yangon, found a prevalence of 27.5% for bacterial vaginosis, 18% for chlamydiosis, 2.2% for gonorrhoea and 3.3% for trichomoniasis [3].

There are limited prevalence data available from community-based studies concerning RTIs in Myanmar. The recent community-based study on RTIs among currently married women in Yangon reported the prevalence of 1.11% (chlamydiosis), 2.23% (candidiasis), 7.06% (trichomoniasis) and 0.74% (gonorrhoea) in urban area and 1.89% (chlamydiosis), 1.68% (candidiasis), 7.55% (trichomoniasis) and 2.1% (gonorrhoea) in semi-urban area [4]. The most realistic and cost-effective strategy for control of RTIs in developing countries is syndromic management approach as recommended by the World Health Organization (WHO). The syndromic approach has the potential advantage of giving immediate treatment at the first contact with health care system without waiting for laboratory results [5]. However, the algorithms proposed by WHO should be periodically evaluated under field conditions. Failure to diagnose and treat the curable RTIs can have serious complications such as infertility, pelvic inflammatory diseases, miscarriage, prematurity and neonatal infections in men, women and newborns. [6].

This study was focussed upon gonococcal, trichomonas, candida and bacterial vaginosis infections which usually cause vaginal discharge as a symptom in infected women. However, these infections can either have no symptoms or symptoms as mild as to pass unnoticed. Thus, our study was carried out to reveal the magnitude of RTIs among married women in semi-urban area as they are an important group for transmission of RTIs. Then we evaluated the accuracy of a WHO syndromic algo-

rithm for women with symptoms and signs of vaginal discharge to diagnose lower genital tract infections (trichomoniasis, candidiasis and bacterial vaginosis) in comparison with laboratory test results.

Objectives

- To detect gonococcal, and trichomonas, candidiasis and bacterial vaginosis infections among married women in semi-urban area (Indaing and Hmawbi military communities)
- To evaluate the accuracy of the syndromic diagnosis for women with vaginal discharge by comparing with laboratory diagnosis

MATERIALS AND METHODS

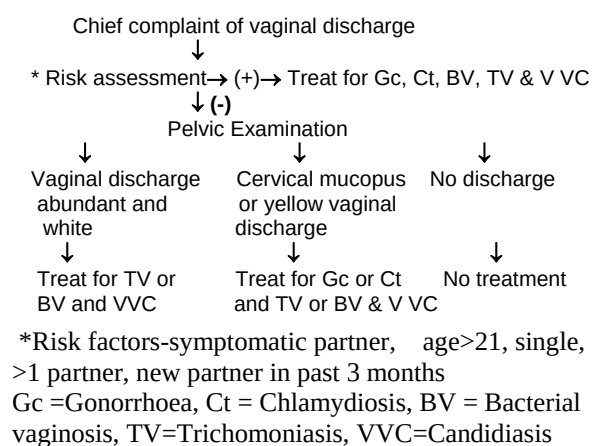
A cross-sectional community-based study was carried out among currently married women in Indaing, 50 Km from Yangon, and Hmawbi, 40 Km from Yangon in January and April 2007. The study population comprised 93 women from Indaing and 119 women from Hmawbi.

The participants were invited to come to a health centre/clinic at the study area. The informed consent was obtained before the enrolment. The clinicians recorded data on socio-demographic characteristics, contraceptive usage, obstetric, gynaecological history and STD history and presence of a complaint of vaginal discharge. Participants were categorized as symptomatic when she was presenting with vaginal discharge at the current visit or one month before this visit. All participants were undergone a standardized pelvic examination (including speculum and bimanual examination) with special attention given to inflammation of vulva, vagina and cervix; the characteristics of the cervical and vaginal discharge; and abnormal cervical, uterine and adnexal tenderness. The endocervical swab and high vaginal swab specimens were collected to perform laboratory procedure.

The clinicians diagnosed the lower genital tract infections (LGTI) including tricho-

moniasis, candidiasis and bacterial vaginosis according to a WHO syndromic algorithm for diagnosis of women with vaginal discharge (shown below). Syndromic diagnosis was made on the basis of presence of clinical symptom of vaginal discharge and signs such as creamy white or greenish-yellow vaginal discharge and/or vaginitis and/or cervicitis on speculum examination. Participants who were diagnosed as having LGTI were treated with metronidazole 200 milligram for three times a day for 7 days and intravaginal cotrimazole tablet 500 mg single dose at the health centre/clinic at the study area. If the laboratory results revealed an infection which had not been treated, the study team informed the laboratory results to the medical officer in charge of the respective areas to give the treatment to the participant.

A WHO Algorithm for clinical presentation with vaginal discharge



Specimen collection for laboratory procedures

One endocervical swab was taken for detection of *N. gonorrhoeae*. This swab was inoculated onto Amies Transport medium. Two high vaginal swabs were taken for detection of bacterial vaginosis, candidiasis and trichomonas infections. One swab was put into an eppendorf tube containing 0.9% normal saline and the other swab was inoculated onto Diamond's trichomonas medium. The specimens were kept in an ice box and carried to Bacteriology Research Division, DMR (LM). Laboratory procedures

were carried out according to the method described by Van Dyck *et al.*, 1999 in WHO manual [7].

Detection of Trichomonas vaginalis

Both microscopy and culture method were used to detect trichomoniasis. Wet mount microscopic examination was carried out to detect motile *T. vaginalis*. The trichomonas medium was incubated at 35° C up to 7 days and positive growth was checked by microscopy on wet films and Giemsa stained smears.

Detection of Neisseria gonorrhoeae by culture

An endocervical swab in Amies Transport media was inoculated onto Modified Thayer Martin agar and chocolate agar and incubated at 35°C up to three days in the moist candle jar. Positive cultures from chocolate and Thayer Martin agar were identified by colony morphology, Gram staining, positive oxidase reaction, and the phabedact agglutination.

Detection of candidiasis

By KOH wet prepration and culture on Sabouraud's agar.

Detection of bacterial vaginosis (BV)

By testing of vaginal pH (ranged 4.0-7.0), Gram-stained smear and wet mount examination for clue cells. Women were defined as having a specific RTI if any of the laboratory tests for *T. vaginalis*, *N. gonorrhoeae*, *C. albicans* and bacterial vaginosis were positive.

Data analysis & ethical consideration

Only women in whom all laboratory results were available were considered in the analysis. The data were checked and analysed by using SPSS for Windows software version 12.0. This study was approved by the ethical committee, Defence Services Medical Academy.

RESULTS

Socio-demographic data

The demographic data and relevant history of 212 married women (93 from Indaing,

and 119 from Hmawbi) are shown in Table 1. Ninety-eight (46.2%) women had symptoms of vaginal discharge at the current visit and 114 (53.8%) were asymptomatic.

Table 1. The demographic data and relevant history of 212 married women

Demographic data	Number	Percentage
Age		
19-56 years		
Mean 35.7 SD $\pm$ 7.68		
Occupation		
Dependent	184	86.80
Own business	20	9.40
Sedentary	8	3.80
Education		
Primary school	91	42.90
Middle school	51	24.10
High school	63	29.70
University	7	3.30
Parity		
Parity 0 to 1	41	19.30
Parity 2 to 4	146	68.90
Parity 5 to 7	25	11.80
No. of marriage		
Single marriage	207	97.60
Married for >1 time	5	2.40
Presenting symptoms		
Vaginal discharge	98	46.20
Characteristics of vaginal discharge		
Creamy colour	39	18.40
Greenish yellow color	18	8.50
Associated with pruritus	37	17.50
Associated with offensive odour	31	14.60
Profuse amount	27	12.70
Moderate amount	39	18.40
Scanty amount	32	15.10
Type of current contraceptive usage		
Oral contraceptive pills	97	45.80
Contraceptive injection	91	42.90
Barrier method	5	2.40
Intra uterine device	21	9.90
Condom	14	6.70
History		
Past history of abortion present	53	25.00
Previous STD history present	2	0.90

Prevalence of RTIs

Table 2 represents the prevalence of RTIs in the study population. Of 212 married women, 83 (39.2%) had at least one of the tested infections. Double infections (*N. gonorrhoeae* + *T. vaginalis* or *C. albicans*/ *T. vaginalis* + *C. albicans* or bacterial vaginosis/ *C. albicans* + bacterial vaginosis) were found in 16 (7.5%) and triple infections (*N. gonorrhoeae* + *T. vaginalis* + *C. albicans* or *T. vaginalis* + *C. albicans* + bacterial vaginosis) were found in 3 (1.4%) of the study population.

Table 2. Prevalence of RTIs among 212 married women (93 from Indaing and 119 from Hmawbi)

Infection	Area	No.	Total No. (%)
<i>N. gonorrhoeae</i>	Indaing	7	8
	Hmawbi	1	(3.8 %)
<i>T. vaginalis</i>	Indaing	24	37
	Hmawbi	13	(17.5 %)
<i>C. albicans</i>	Indaing	23	33
	Hmawbi	10	(15.6 %)
Bacterial vaginosis	Indaing	18	28
	Hmawbi	10	(13.2%)
Double infections	Indaing	15	16
	Hmawbi	1	(7.5 %)
Triple infections	Indaing	2	3
	Hmawbi	1	(1.4 %)
Overall infections	Indaing	53	83
	Hmawbi	30	(39.2%)

RTIs among symptomatic and asymptomatic women

The proportion of gonorrhoea, trichomoniasis, candidiasis and bacterial vaginosis is shown among women with presence of symptoms of vaginal discharge at current visit and those without the symptoms. Higher proportion (44.7%) of overall RTIs was found among the asymptomatic women (Table 3).

Table 3. Percentage of RTIs among symptomatic and asymptomatic married women

Infections	Symptomatic (n=98)	Asymptomatic (n=114)
<i>N. gonorrhoeae</i>	4 (4.1%)	4 (3.5%)
<i>T. vaginalis</i>	15 (15.3%)	22 (19.3%)
<i>C. albicans</i>	13 (13.3%)	20 (17.5%)
Bacterial vaginosis	9 (9.2%)	19 (9.0%)
Overall infections	32 (32.7%)	51 (44.7%)

Comparison of syndromic diagnosis and laboratory-based diagnosis for lower genital tract infections (trichomoniasis, candidiasis and bacterial vaginosis)

Syndromic diagnosis for lower genital tract infections (LGTI) was made on the basis of presence of clinical symptom of vaginal discharge and signs such as creamy white or greenish-yellow vaginal discharge and/ or vaginitis and/or cervicitis on speculum examination. Ninety-five women were diagnosed to have LGTI. Among 80 laboratory-confirmed LGTI (trichomoniasis, candi-

diasis and bacterial vaginosis), syndromic diagnosis can detect 47.5% (38/80) of these infections (Table 4).

Table 4. Lower genital tract infections (trichomoniasis, candidiasis and bacterial vaginosis) diagnosed by syndromic diagnosis and laboratory-based diagnosis

Syndromically diagnosed LGTI	Laboratory diagnosed LGTI		Total
	Present no.	Absent no.	
Present no.	38	57	95
Absent no.	42	75	117
Total	80	132	212

Kappa coefficient= 0.04,

Unsatisfactory agreement between laboratory and syndromic diagnosis

DISCUSSION

The present study provides prevalence data of four important reproductive tract infections in married women in sub-urban community in Myanmar. These include the prevalence of gonorrhoea 3.8%, trichomoniasis 17.5%, candidiasis 15.6% and of bacterial vaginosis 13.2%. The prevalence detected in our study was lower than that of a recent study on symptomatic women attending various clinics in Mandalay, which showed high prevalence of bacterial vaginosis (40.1%) candidiasis (23.7%) and gonorrhoea (14.4%) [2]. However the prevalence of all tested RTIs in our setting is higher than a recent community-based study on RTIs among married women in semi-urban area in Yangon during 2002 which reported the prevalence of 1.68% (candidiasis), 7.55% (trichomoniasis) and 2.1% (gonorrhoea) [4]. It showed that occurrence of asymptomatic infections in our study population accounts for higher rate of infection than Nyein *et al.*'s study.

The mean age of the participants was 35.7 years and 79.3% were in the age group of 25-45 years. Sixty-seven (80.7%) of tested RTIs were found in this reproductive age group which is an important group for transmission of STIs. Some 86.8% of women were dependants and 67% had primary and middle school education. High rate of 98.7% used oral and injection form contraceptives

and low rate of 6.7% used condoms. Condom used within marriage was found to be only 2.4%. These findings could provide the necessary information to the National AIDS/STD Control Programme for implementation of the prevention, control and health education programs targeted on married women and urgent need for condom promotion in sub-urban area.

Overall prevalence of tested RTIs in Indaing area (53/93, 56.9%) was higher than that of Hmawbi area (30/119, 25.2%), however, socio-demographic background characteristics and hygienic factors such as source of water supply and sanitary habit data collected in our study of two areas were not much different, so that the association of higher prevalence with these factors can not be documented. However, spouses of married women from Indaing area are highly mobile. Mobility is the strong risk factor for RTIs.

Higher proportion (44.7%) of overall RTI was found among the asymptomatic women and only 32.7% were detected among symptomatic women in the study population. The syndromic approach relies on symptom recognition by an infected person who as a consequence seeks medical care. Reliance to reported symptoms may miss asymptomatic infections. Individuals may also fail to recognize symptoms as manifestation of disease. To achieve STD control in this and similar population, the present findings support the fact that public health programmes should target asymptomatic infections. Presence of double infection (7.5%) and triple infection (1.4%) among the study population also highlighted the importance of case finding and treatment of RTIs in the community.

Syndromic diagnosis for lower genital tract infections (LGTI) was made on the basis of presence of clinical symptom of vaginal discharge and signs such as creamy white or greenish-yellow vaginal discharge and/or vaginitis and/or cervicitis on speculum examination. There is unsatisfactory agreement between syndromic diagnosis and

laboratory diagnosis (Kappa coefficient =0.04). Among 80 laboratory-confirmed trichomoniasis, candidiasis and bacterial vaginosis, syndromic diagnosis can detect 47.5% (38/80) of these infections. Similar findings were seen in a study on RTIs in Mandalay clinics by DOH & Population Council, Thailand, 2002 [2].

Thus, clinical judgement based on symptoms and signs seemed to be less indicative for LGTI. Lack of association between syndromic diagnosed cases and laboratory-confirmed infections underscores the challenge of diagnosing LGTI when laboratory testing is not available. The present study recommended the need of large scale multicentre studies to evaluate the syndromic management for RTIs, to cultivate protective condom culture in high-risk communities and to encourage annual screening for RTIs in high-risk communities.

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**Hypoglycemic effect of *Curcuma comosa* Roxb. (Nanwin-ga)
rhizomes on rabbits model**

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This study was carried out to determine the phytochemical constituents, acute toxicity and hypoglycemic effect of crude powder and 80% ethanolic extract of dried rhizomes of *Curcuma comosa* Roxb. (Nanwin-ga). Adrenaline-induced hyperglycemic rabbits were used to study the hypoglycemic effect. Oral route of administration was used in this study. The phytochemical studies of the crude powder and 80% ethanolic extract of this plant showed that both contained alkaloids, flavonoids, glycosides, steroids, saponins, tannins and amino acid. In acute toxicity study in mice, it was observed that the crude powder of the rhizomes was not toxic up to the maximal feasible dose of 5g/kg. But, 80% ethanolic extract of the dried rhizomes showed mild acute toxic effect and median lethal dose (LD₅₀) was determined to be 5.2 g/kg and its confident limit was 4 g/kg - 6.76 g/kg. The results showed that the 80% ethanolic extract of the rhizomes at the dose level of 1.5 g/kg had significant hypoglycemic effect at 2 hr, 3hr and 4 hr ($p < 0.01$ - $p < 0.05$) after oral administration when compared with those of the controls. But, the crude powder of the rhizomes at the dose level of 3 g/kg showed no significant hypoglycemic effect. It was observed that the hypoglycemic effect of 80% ethanolic extract was inferior to that of the standard drug glibenclamide. Therefore, it can be concluded that the 80% ethanolic extract of the dried rhizomes of *Curcuma comosa* Roxb. (Nanwin-ga) possessed significant hypoglycemic effect on adrenaline-induced hyperglycemic rabbits model and the effect was inferior to that of glibenclamide.

INTRODUCTION

Diabetes mellitus is a common clinical syndrome and complications of diabetes mostly effect on the eyes, the kidney, nervous system and vascular system [1]. Diabetes mellitus is worldwide in distribution and the estimated worldwide prevalence of diabetes in 1997 derived from WHO data was 124 million people and the number of people with diabetes is expected to increase to 221 millions in 2010 [2]. The present day drugs including insulin and oral hypoglycemic agents are used to control the blood sugar level in diabetes mellitus. But these drugs have considerable side effects and toxicities.

Even today a large number of herbal drugs are being used for the treatment of diabetes mellitus in different regions of the world. Mya Bwin and Sein Gwan [3] mentioned a large numbers of herbal drugs which are reputed for the treatment of diabetes mellitus.

Curcuma comosa Roxb. (Nanwin-ga) (Family-Zingiberaceae) grows widely in Myanmar and Thailand. In Myanmar traditional medicine, this plant is known to be useful in the treatment of diabetes mellitus, hypertension, fever, hyperlipidemia and stress [4]. It also contains in some Myanmar traditional medicine formulations for hypertension and diabetes

mellitus. But, there is no scientific report of hypoglycemic effect of *Curcuma comosa* Roxb. in experimental animal model and human in Myanmar. Therefore, this study was performed to investigate the hypoglycemic effect of the rhizomes of *Curcuma comosa* Roxb. on rabbits model.

MATERIALS AND METHODS

Type of study and study design

Type of the study was experimental animal study. Parallel study design for acute toxicity study in mice and cross over study design for hypoglycemic activity study in rabbit model were used. Site of the study was Pharmacology Research Division, Department of Medical Research (LM).

Ethanollic extraction of the rhizomes of Curcuma comosa Roxb.

The rhizomes of *Curcuma comosa* Roxb. collected from Yangon were air dried in the shade and then powdered by electric grinder. One-hundred grams of the air-dried rhizomes powder were put into conical flask of 2 litres capacity and 80% ethanol was added up to 500 ml capacity. Then, the conical flask was put onto the water bath and heated at 70°C for 12 hours. After 12 hours of extraction, the resultant extract was cooled down to room temperature and the supernatant solution was decanted. The supernatant solution was filtered through the cheese cloth. The filtrate was placed in the petridish and evaporated to dryness on a boiling water bath [5].

Phytochemical studies and physicochemical tests

Phytochemical studies of both crude powder and 80% ethanolic extracts of the rhizomes of *Curcuma comosa* Roxb. were performed qualitatively for the presence of alkaloids, flavonoids, glycosides, steroids, saponins, tannins, resin, amino acid, phenol and cyanogenic glycoside using the method of Physicochemical standards of Unani formulations [6]. The physicochemical tests

of the crude powder of the rhizomes of this plant were done by the method of WHO, 1998 [7].

Acute toxicity studies of Curcuma comosa Roxb. rhizomes on albino mice

The acute toxicity test was done to determine the symptomatology consequent, degree of toxicity to administration of the drug and to find out the median lethal dose (LD₅₀) by the method of Litchfield & Wilcoxon, 1949 [8].

Fifty albino mice of both sexes, weighing 25-35 gm were used in this study. The mice were separated into 5 groups and each group contained 10 mice. Food was withheld for the period of 18 hours before the experiment but they were allowed free access to water. One group served as the control and only drug vehicle (i.e 10% tween 80) was administered orally. 80% ethanolic extract of *Curcuma comosa* Roxb. was dissolved in 10% tween 80 and the required doses were administered orally with intragastric needle to every mice. Each group of mice received each dose level of the 80% ethanolic extract (i.e 2g/kg, 4g/kg, 6g/kg and 8g/kg body weight). Then, the mice were observed for behavior, neurologic and autonomic signs 1 hourly up to 6 hours after administration of the extracts and daily up to two weeks. The crude powder of the dried rhizomes of *Curcuma comosa* Roxb. was suspended in 1% methyl cellulose and the same procedure described above was applied for acute toxicity study on albino mice at the dose levels of 1g/kg, 2g/kg, 4g/kg and 5g/kg body weight.

The effect of the crude powder of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

Six adult healthy rabbits (JW strain) of both sexes weighing 2.73±0.07 Kg were used in this study. The rabbits were kept without food for 18 hours with free access to water before the experiment.

In the control group, only drug vehicle (1% methyl cellulose 10ml/kg) was administered orally to each rabbit by using the intragastric tube connected to a plastic syringe containing the calculated volume of 1% methyl cellulose. Before the drug vehicle administration, 0.1ml of blood was collected from marginal ear vein as the baseline blood sample. Then, 1% methyl cellulose was administered orally and just after that, the rabbit was made hyperglycemic by injecting the adrenaline (0.2 mg/kg) (MPF) subcutaneously [9]. Similar sample of 0.1ml of blood was collected at 1 hr, 2 hr, 3 hr and 4 hr intervals after subcutaneous injection of adrenaline. Blood glucose levels were determined at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr by using the glucometer (Elite, Bayer Company limited, USA). After drug free interval of one week, the same 6 rabbits were used again and the same procedures were performed as above for determination of the blood glucose levels of the rabbits after oral administration of the crude powder of *Curcuma comosa* Roxb. rhizomes (suspended in 1% methyl cellulose) at the dose of 3g/kg.

The effect of 80% ethanolic extract of Curcuma comosa Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

Six adult healthy rabbits (JW strain) of both sexes weighing 2.16 ± 0.329 kg were used in this study. The rabbits were kept without food for 18 hours with free access to water before the experiment. In the control group, only drug vehicle (i.e 10% tween 80 10ml/kg) was administered orally to each rabbit by using the intragastric tube. The same procedures were performed as above for induction of hyperglycemia with adrenaline, collection of blood and determination of blood glucose levels. After drug free interval of one week, the same 6 rabbits were used again and the same procedure was repeated before the experiment. Then, the 80% ethanolic extract of this plant was dissolved in 10% tween 80 and the same procedures were performed as above for

determination of blood glucose levels of the rabbits after oral administration of the extract at the dose levels of 0.375g/kg and also for 0.75g/kg and 1.5g/kg.

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

After drug free interval of one week, the same six rabbits were used again and the same procedure was repeated. Then, the standard drug glibenclamide 4mg/kg (Hovid Company Ltd, Malaysia) dissolved in distilled water was administered to the rabbit orally. Then, the same procedure was performed as above for determination of blood glucose levels.

Data analysis

The results were shown in (mean $\pm$ standard error). Student 't' tests were used for calculation of statistical significance between control and treated groups.

RESULT

Extraction of the rhizomes of Curcuma comosa Roxb.

The 80% ethanolic extraction of dried rhizomes powder of *Curcuma comosa* Roxb. yielded 11.9 g ethanolic extract per 100g of dried rhizomes powder (i.e 11.9 %).

Phytochemical studies and physicochemical tests

The results are shown in Table 1.

Table 1. Phytochemical studies of the crude powder and 80% ethanolic extract of the rhizome of *Curcuma comosa* Roxb.

Pythochemical constituents	Crude powder	80% ethanolic extract
Alkaloids	(+)	(+)
Flavonoids	(+)	(+)
Glycosides	(+)	(+)
Steroids	(+)	(+)
Saponin	(+)	(+)
Tannin	(+)	(+)
Amino acid	(+)	(+)
Phenol	(-)	(-)
Resin	(-)	(-)
Cyanogenic glycoside	(-)	(-)
+ present - absent		

Physicochemical tests of the crude powder of *Curcuma comosa* Roxb. rhizomes showed moisture content (11.8%), swelling index (0.6 cm), foaming index (<100%), watery extract (18.7%), ethanolic extract (7.3%), chloroform extract (8.8%) and petroleum ether extract (2.4%).

Acute toxicity study of the rhizomes of *Curcuma comosa* Roxb.

In the present study, it was found that the crude powder of the rhizomes of this plant showed no toxic effect and lethality up to the maximal feasible dose of 5g/kg body weight. In the acute toxicity study of 80% ethanolic extract of this plant's rhizomes, it was observed that the mice died within 7 days after single dose oral administration of the extract at the dose level of 4g/kg, 6g/kg and 8g/kg. It was observed that there were decrease in motor activity, sedation, reduction of screen grip, ataxia, piloerection and finally, they died at these dose levels. On the autopsy examination, the internal organs such as brain, heart, lungs, liver, stomach, intestine, kidneys, spleen and pancreas were found to be grossly normal. In this study, LD₅₀ of 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes was found to be 5.2 g/kg and its confidence limit was 4-6.76 g/kg.

The effect of the crude powder of *Curcuma comosa* Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

In the control group, the significant increases in blood glucose levels were observed at 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate ($P < 0.001$) (Fig.1).

The mean blood glucose levels of the rabbits treated with the crude powder of *Curcuma comosa* Roxb. rhizomes 3g/kg at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate were $78.5 \pm 4.53\text{mg/dl}$, $252.25 \pm 24.13\text{mg/dl}$, $321.67 \pm 12.52\text{mg/dl}$, $386.33 \pm 16.39\text{mg/dl}$ and $366.5 \pm 21.38\text{mg/dl}$ respectively. It was observed that there was no significant decrease in

blood glucose levels when compared with those of the controls. The results are shown in Fig. 1.

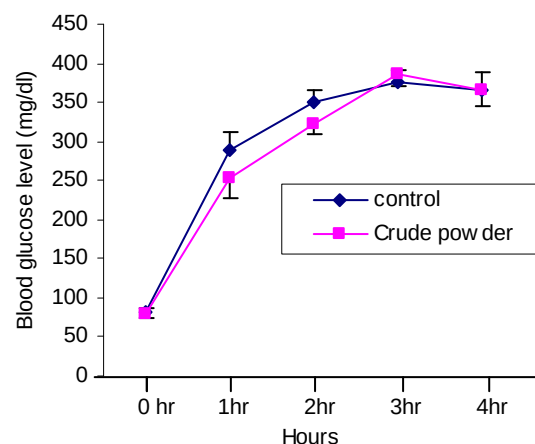


Fig. 1. Hypoglycemic effect of the crude powder of *Curcuma comosa* Roxb. rhizomes on adrenaline-induced hyperglycemic rabbits (n=6). The results in mean $\pm$ SE.

The effect of 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes on blood glucose level in adrenaline-induced hyperglycemic rabbits

In the control group, the significant increases in blood glucose levels were observed at 1hr, 2hr, 3hr, and 4hr after subcutaneous injection of adrenaline tartrate ($P < 0.001$) (Fig. 2).

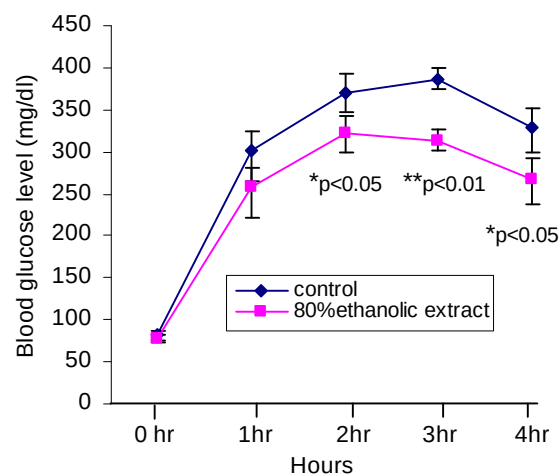


Fig 2. Hypoglycemic effect of 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) on adrenaline-induced hyperglycemic rabbits (n=6). The results in mean $\pm$ SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (0.375g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline tartrate were 77 ± 3.54 mg/dl, 279.83 ± 34.61 mg/dl, 334.83 ± 37.38 mg/dl, 327.33 ± 40.64 mg/dl and 310.83 ± 54.91 mg/dl, respectively. It was found that there was no significant decrease in blood glucose levels when compared with those of the controls. The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of the rhizomes (0.75mg/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline tartrate were 71.5 ± 6.07 mg/dl, 263.33 ± 35.29 mg/dl, 343.17 ± 25.62 mg/dl, 333.67 ± 25.24 mg/dl and 306.83 ± 40.78 mg/dl, respectively. It was found that there was no significant decrease in blood glucose levels when compared with those of the controls.

The mean blood glucose levels of the rabbits treated with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline tartrate were 77.33 ± 5.33 mg/dl, 258 ± 35.96 mg/dl, 321.17 ± 26.65 mg/dl, 313.83 ± 12.01 mg/dl and 268.33 ± 36.54 mg/dl, respectively. Significant decreases in blood glucose levels were found at 2hr ($P < 0.05$), 3hr ($P < 0.01$) and 4hr ($P < 0.05$) when compared with those of the controls (Fig. 2).

The effect of standard drug (glibenclamide) on blood glucose level in adrenaline-induced hyperglycemic rabbits

The mean blood glucose levels of the rabbits treated with standard drug, glibenclamide (4mg/kg) at 0hr, 1hr, 2hr, 3hr and 4 hr after subcutaneous injection of adrenaline tartrate were 75 ± 2.46 mg/dl, 245.5 ± 39.73 mg/dl, 271.33 ± 19.35 mg/dl, 241.5 ± 26.74 mg/dl and 216 ± 33.76 mg/dl, respectively. Significant decreases in blood glucose levels were found at 1 hr ($P < 0.05$), 2 hr ($P < 0.001$), 3 hr ($P < 0.01$) and 4 hr ($P < 0.05$) when compared with those of the controls. The mean percent reductions of

blood glucose levels with glibenclamide were 25.53 ± 9.93 %, 32.14 ± 3.69 %, 44.61 ± 9.35 % and 44.29 ± 12.45 % for 1 hr, 2 hr, 3 hr and 4 hr, respectively. The mean percent reductions of blood glucose levels with ethanolic extract of *Curcuma comosa* Roxb. rhizomes were 15.12 ± 4.74 %, 22.05 ± 3.39 %, and 25.32 ± 9.97 % at 2 hr, 3 hr and 4 hr, respectively. The results are shown in Fig. 3.

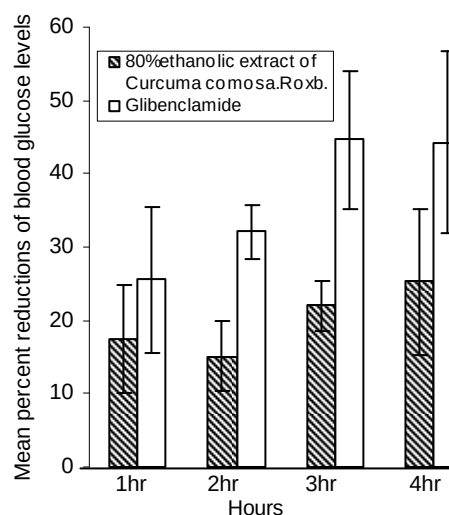


Fig 3. Mean percent reductions of blood glucose levels with 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) and glibenclamide (4mg/kg) in adrenaline-induced hyperglycemic rabbits

DISCUSSION

In the phytochemical studies of the crude powder and 80% ethanolic extract of the rhizomes of this plant, both did not contain toxic plant compound like cyanogenic glycoside. In the acute toxicity studies of the crude powder and 80% ethanolic extract of the rhizomes of *Curcuma comosa* Roxb. oral route of administration was used because this route of administration is the intended route for use in man. In this study, it was found that the crude powder of the rhizomes of the plant showed no toxic effect and lethality up to the maximal feasible dose of 5g/kg. So, the LD₅₀ of the crude powder was more than 5g/kg.

In the acute toxicity study of 80% ethanolic extract of rhizomes of the plant, it was

found that median lethal dose (LD₅₀) was 5.2 g/kg and its confidence limit was 4 g/kg -6.76 g/kg. So, 80% ethanolic extract of the dried rhizomes of *Curcuma comosa* Roxb. showed mild acute toxic effect on mice. In this study, adrenaline-induced hyperglycemic rabbit model was used as the transient hyperglycemic model because adrenaline can produce hyperglycemia by three mechanisms. They are by inhibiting the release of insulin from pancreas, by reducing the peripheral utilization of glucose and by causing glycogenolysis in liver [10]. This adrenaline-induced hyperglycemic rabbits model is also the commonly used model.

In the study of hypoglycemic effect of the crude powder, at the maximal feasible dose of 3g/kg, it was found that there was no significant hypoglycemic effect. So, the higher dose of the crude powder was needed to show the significant hypoglycemic effect. In this study, it was observed that 80% ethanolic extract of *Curcuma comosa* Roxb. rhizomes (1.5g/kg) showed the significant hypoglycemic effect at 2 hr (P<0.05), 3 hr (P<0.01) and 4 hr (P<0.05) after oral administration of the extract when compared with those of the controls. Mean percent reduction of blood glucose level with 80% ethanolic extract ranged from 15.12% to 25.32%. But there were no significant hypoglycemic effects at the lower dose levels of 0.375 g/kg and 0.75 g/kg of the extracts. In the present study, the standard drug, glibenclamide 4mg/kg showed significant hypoglycemic effect from 1hr up to 4hr (P<0.05-P<0.001) after oral administration of the drug. Mean percent reduction of blood glucose level with glibenclamide ranged from 25.53% to 44.61%. In the comparison between hypoglycemic effect of 80 % ethanolic extract of *Curcuma comosa* Roxb. rhizomes with that of standard drug, glibenclamide, it was found that the effect of 80% ethanolic extract was less than (ie. 56.85%) that of glibenclamide (P<0.05). Oliver Bever & Zahnd [11] reported that the chemical

constitutions of the hypoglycemic principles from the plants are flavonoids, tannin, alkaloids, steroids and glycosides. Therefore, in this study, hypoglycemic action of 80% ethanolic extract of the rhizomes of *Curcuma comosa* Roxb. may be due to one or more compounds (i.e alkaloids, flavonoids, glycosides, tannin, steroids) obtained in this extract. It can be concluded that 80% ethanolic extract of rhizomes of *Curcuma comosa* Roxb. could produce significant hypoglycemic effect on adrenaline-induced hyperglycemic rabbit model and the effect was inferior to that of glibenclamide.

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Seroprevalence of anti-HEV(IgG) in apparently healthy adults in Yangon

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In Myanmar, hepatitis A, B, C and E are endemic, with the disease occurring subclinically or clinically in sporadic or epidemic forms. Hepatitis E is a small, non-enveloped RNA virus that is leading cause of non-enterically-transmitted viral hepatitis worldwide. The prevalence of antibody to HEV in suspected or documented endemic region has been lower than expected (3% to 26%) and this antibody in nonendemic region has been much higher than anticipated (1% to 3%). The aim of this study was to evaluate the anti-HEV IgG seropositivity in adult study population in Yangon. A total of 84 serum samples of adults (15-60 years) comprising of 43 males and 41 females were tested for anti-HEV IgG using the recombinant baculovirus expressed Viral Like Protein (VLP) of HEV antigen ORF2 by the ELISA (Enzyme Linked Immunosorbent Assay) test. Twenty-four subjects (28.57%) comprising of 16 males and 8 females were found to be positive for anti-HEV IgG. The prevalence of HEV was variable according to age group. The highest prevalence was between 20-30 years and 30-40 years age groups. Anti-HEV positivity rate was 37.2% in male and 19.52% in female subjects. In other studies, the prevalence of anti-HEV remained constant at 10-40% in young adults with no difference between males and females. The presence of anti-HEV IgG is the evidence of past infection. The use of baculovirus expressed ORF2 apparently had a sensitive and specific rate of detection to anti-HEV. Recombinant protein test detected more than 90% of the undiluted known positive sera. Anti-HEV was associated with partial protection from hepatitis E. Anti-HEV has been found to persist as little as few months to 14 years. Recombinant protein assays can detect anti-HEV in human serum obtained 13 years after infection.

INTRODUCTION

In Myanmar, hepatitis A, B, C and E are endemic, with the disease occurring subclinically or clinically in sporadic or epidemic forms. Hepatitis E is a small, non-enveloped RNA virus that is leading cause of non-enterically-transmitted viral hepatitis worldwide [1]. It is transmitted mainly by the faecal-oral route and large epidemics due to this virus are often associated with

contaminated water [2]. It is a positive strand RNA virus (7.5 Kb) and grouped with Caliciviridae, a family of small round-structure virus. There are two strains of hepatitis E known as old world isolate (Myanmar) and new world isolate (Mexico). These strains are 90.5% identical in genetic sequence [3]. All HEV strains have same serotype [4]. It was first recognized in 1980 and 32-34 nm viral like particles were visualized by immunoelectron microscopy

(IEM) in 1983 [5]. Its genome consists of 3 Open Reading Frames (ORFs) such as ORF1-5079 nucleotides (nonstructural protein), ORF2-1980 nucleotides (structural protein) and ORF3-369 nucleotides (highly immunogenic protein of unknown function). Antigens derived from ORF2 and expressed in *Escherichia coli* or insect cells have also been used for detection of anti-HEV by Western blot and the entire ORF2 has been expressed in insect cells and a part of the expressed antigen spontaneously forms virus like particles used in ELISA test for anti-HEV. Recombinant protein-based tests have higher sensitivity than the prior assays, detecting 90-95% of anti-HEV in acute hepatitis cases in endemic area [6]. The common age group was highest in young adults (15-40 years) and attack rates were higher for males than for females [4]. The rising titre of anti-HEV IgG is also diagnostic and exists for a long time. Anti-HEV IgG peak starts between 2-4 weeks after onset of hepatitis and 100% of young adults are still positive 20 months after onset of hepatitis and some adults are still positive for 14 years after this infection. The prevalence of anti-HEV in suspected or documented endemic region has been lower than expected 3% - 26%. This antibody in non-endemic region has been much higher than anticipated 1%-3% [4]. In Myanmar, epidemic HEV infection occurred in Mandalay (1976-1977), Moulmein (1978) and Yangon (1982) which was spread by the fecal-oral route and with sporadic cases occurring all over Myanmar [7]. Hepatitis E infection is commonly transmitted from infected mothers to their babies with significant perinatal morbidity and mortality. All infants had anti-HEV IgG in their birth serum samples and five infants had HEV RNA in their serum at birth by polymerase chain reaction [8].

The objective of the present study is to determine the anti-HEV IgG positivity in apparently healthy adults aged 15-60 years who are living in Yangon and to evaluate the seroprevalence of anti-HEV IgG in different age groups and gender.

MATERIALS AND METHODS

Anti-HEV IgG was detected in human serum samples by Enzyme Linked Immunosorbent Assay (ELISA). Hepatitis E viral like particles (HEV-VLP) the structural protein, with its terminus truncated (amino acid residues 112 to 660 of ORF2) was expressed in insect Tn5 cells by a baculovirus. The efficient expression of N-terminally truncated HEV capsid protein was derived from a Myanmar strain isolated in 1986 [9].

Construction of recombinant transfer vectors

In 1986, acute-phase stool specimens from patients with Non A, Non B hepatitis in Myanmar were pooled and injected into Rhesus monkeys (*Macaca mulatta*) via the intravenous route. Bile which contained large number of HEV virions were collected from these monkeys and the total RNA was extracted by using the RNAzol (Biotex Laboratories) which contained large numbers of HEV virions. The poly (A)-containing RNA was purified with Oligotex-dT 30 (Super-Roche Diagnostic System Tokyo, Japan).

Amplification of the entire ORF2 by polymerase chain reaction (PCR) was carried out with specific primers HEV D2 (5'-CAACAGAAAGAAGGGGGGCACAA-3') and HEV U2 (5'-CAACAGAAAGAAG-GG GGGCAC AA-3'). The product of PCR were cloned into a TA cloning plasmid vector PCR II (Invitrogen) to generate pHEV 5134/7161 which was digested with restriction enzymes *NruI* & *XbaI* and resultant 2-kb fragment was ligated with a transfer vector, pVL1393 (Pharmingen) which was digested with *SmaI* & *XbaI* to produce plasmid vector pVL 5147/7126. DNA fragments with deletion of the N-terminal 111 amino acid encoded by ORF2 were amplified by PCR with HEV D13 (5'-AAGGATCCATGGCGGTCGCTCCAGCCCATGACACCCCGCCAGT-3') and HEV U14(5'-GGTCTAGACTATAACTCCCGAGTTTACCCACCTTCATCTT-3') primers

ORF1 5105 123aa 5473

28 1693aa 5106 5147 660aa 7126

ORF2

▽_N _____ X

pHEV5134/7161 △

▽_N _____ X

pVL5147/7126 △

▽_B _____ X

pVL5480/7126 △

5' B N N H E 7194 3'

HEV RNA

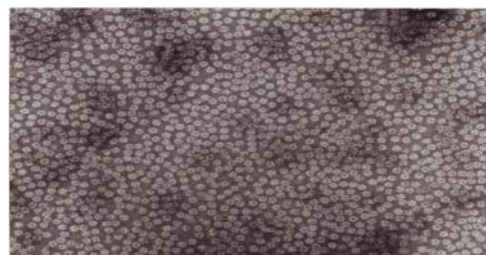
▽ initiation codon ▽ newly introduced initiation codon

△ termination codon N - Nru I H - Hind

 E - EcoRI X - XbaI

Purification of Viral Like Proteins (VLPs) of HEV antigen

CSCl₂ equilibrium density gradient centrifugation. These VLPs, 23.7 nm, small round empty, viron-like particles were observed under electron microscope (EM) and used as coating antigens for antibody of HEV-ELISA assay test for antibody (Fig. 2). The protein concentration was measured with a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.) using Bovine Serum Albumin (BSA) as standard. The VLPs (50KDa) protein was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method. The purified VLPs protein (1 µg/ml) was used to detect anti-HEV IgG and IgM by Enzyme Linked Immunosorbent Assay (ELISA) method. A total of 84 serum samples of apparently healthy adults with 15-60 years age group, comprising of 43 males and 41 females, were tested for anti-HEV IgG using the recombinant baculovirus expressed VLP (Viral Like Protein) of HEV antigen ORF2 by ELISA test.



Detection of anti-HEV IgG by ELISA test

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and distilled water (negative control) were used as control. After washing the plate with PBS-T for 3 times, 2µl of horseradish peroxidase (HRP) conjugated goat anti-human IgG (1:5000 dilution in 1% skim milk with PBS-T) was added into each well and incubated at 37°C for one hour. After washing the plate with PBS-T for 6 times, 100 µl of substrate (5 mg of OPD, 12.5 ml of phosphate citrate buffer, 5µl of hydrogen peroxide) was added into each well and incubated at room temperature for 30 min and then 50 µl of 4N H₂SO₄ was added for stopping the reaction. The optical density (O.D) was read by ELISA reader at 492 nm wave length. The cut-off value was determined as 0.5 and positive control showed two times higher than the cut-off value (>1.0) and negative control showed lower than the cut-off (0.5).

RESULTS

Twenty-four subjects (28.57%) comprising of 16 males and 8 females were found to be positive for anti-HEV IgG. The highest prevalence was between 30-40 years age group of male healthy subjects. In female subjects, 50-60 years age group had the highest prevalence of anti-HEV. The highest prevalence of age group was 20-29 years in other studies. In this study, the anti-HEV IgG positivity rate was 37.2% in males and 19.52% in females in normal healthy subject. Anti-HEV IgG was not detected in 60 cases. Anti-HEV IgG was absent in 50-60 years age group among males and under 20 years and 40-50 years groups among females in this study (Table 1).

Table 1. Anti-HEV IgG positive cases in apparently healthy adults in Yangon

Age groups (yrs)	Male			Female		
	Total no. of cases	No. of positive cases	%	Total no. of cases	No. of positive cases	%
<20	11	6	54.54	2	0	0
20-30	21	5	23.80	23	4	17.39
30-40	7	4	57.14	12	3	25.00
40-50	3	1	33.33	1	0	0
50-60	1	0	0	3	1	33.33
	43	16	37.20	41	8	19.52

Table 2. Anti-HEV IgG negative cases in apparently healthy adults in Yangon

Age groups (yrs)	Male			Female		
	Total no. of cases	No. of negative cases	%	Total no. of cases	No. of negative cases	%
<20	11	5	45.45	2	2	100
20-30	21	16	76.19	23	19	82.6
30-40	7	3	42.85	12	9	75
40-50	3	2	66.66	1	1	100
50-60	1	1	100.00	3	2	66.66
	43	27	62.79	41	33	80.48

DISCUSSION

The prevalence of hepatitis E virus infection in general population differs widely in many countries (1.5% in UK, 1.8% in Netherlands, 7.4% in Chile and 16.5% in Hong Kong) and varied according to geographical regions of the world [10]. In endemic countries, anti-HEV IgG was detected in 20% of population [11]. In this study, the seroprevalence of anti-HEV was found to be 28.57% in all apparently healthy subjects although the sample size was not enough for statistical evaluation for age and gender preponderance. The prevalence in males was nearly twice as high as females. Findings from other studies showed the prevalence of anti-HEV has remained constant at 10-40% in young adults with no difference between males and females [2].

In this study, the highest prevalence of anti HEV was seen in 30-40 years age group and the prevalence was not different between male and female subjects. The use of baculovirus expressed ORF2 antigen apparently had a very sensitive and specific rate of detection to anti-HEV. IgG antibodies to ORF3 do not persist as long as antibodies to ORF2, because of different response of the immune system to the different HEV proteins [12]. Recombinant protein-based (Immunodominant epitopes of ORFs 2&3) test has demonstrated increased sensitivity compared to Western blot and Enzyme immunoassays (EIAs) and detecting anti-HEV(IgG) in 90-95% of HEV infective

cases in endemic region [6]. IgG response begins to develop shortly after the IgM response and its titres increased throughout the acute into convalescent phase, remaining high for 1-4.5 years after the acute phase of disease. The presence of anti-HEV IgG is evident of past infection of hepatitis E virus [13]. Recombinant protein assays could detect anti-HEV IgG in human serum obtained after 13 years of infection [6]. The prevalence of anti-HEV IgG was associated with partial protection from hepatitis E. The cleaved protein from ORF2 spontaneously forms Viral Like Protein (VLP) and appears to be more immunogenic than the full length of ORF2 product and it is also basis for a highly immunogenic candidate vaccine. Naturally acquired anti-HEV IgG correlates with the protection in humans in epidemics of hepatitis E infection [3].

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**Hypocholesterolaemic potential of Russell's viper
(*Daboia russelii siamensis*) venom in experimental mice**

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With an aim to determine the hypocholesterolaemic potential of Russell's viper venom (RVV), changes in serum cholesterol levels of experimental mice, envenomed with different dosages of RVV were studied at various time intervals after envenomation, and were compared with changes in serum total protein and albumin. This *in vivo* study demonstrated that the serum cholesterol levels in these mice significantly reduced at half an hour after administration of RVV, obtaining its lowest level at one hour after envenomation. Although the degree of hypocholesterolaemia did not directly associate with respective dosage of RVV administered, the rates of fall in serum cholesterol level within first 24 hours were found to be apparently correlated with the relapsing time intervals after envenomation. Besides, RVV was found to have a direct lowering action on serum cholesterol in *in vitro* test. It could be concluded that the development of hypocholesterolaemia in experimental mice at early hours after administration of RVV indicates the sign of envenomation, and RVV also seems to be promising in cholesterol lowering action which could be used as a diagnostic indicator of envenomation.

INTRODUCTION

Being a national health problem in Myanmar, much research has been conducted on Russell's viper (*Daboia russelii siamensis*) venom with special inference to purification, characterization and biological activities of the different components for many decades [1]. However, little is known about the effects of RVV on serum lipid profiles in Russell's viper bite victims. In recent years, Pattnaik *et al.* reported that phospholipase A₂ (PLA₂) of *Crotalus adamanteus* snake venom was found to have an action on human serum high density lipoprotein (HDL) [2]. Sun *et al.* also showed that extract of snake venom of *Agkistrodon hylas* has little effect on the regression of atherosclerosis but it prolongs blood clotting and lowers serum cholesterol in experimental Japanese quail [3]. Winkler *et al.*

reported that venom of *Vipera palaestinae* induced decrease in serum cholesterol level in human victims and determination of total serum cholesterol may serve as an indicator of the severity of clinical syndrome [4]. These findings prompt us to conduct this study with an aim to investigate the effects of RVV of Myanmar on serum total cholesterol levels of experimental mice being administered with different dosages of RVV at various time intervals after envenomation.

MATERIALS AND METHODS

Desiccated crude RVV was purchased from Myanmar Pharmaceutical Factory (MPF), Yangon. All chemicals used in this experiment were of analytical grade from Sigma Chemical Co. Ltd., U.S.A. Adult albino mice

of both sexes, Institute of Cancer Research (ICR) strain, weighing 20 ± 2 gm were obtained from Laboratory Animals Services Division, Department of Medical Research (Lower Myanmar), Yangon. After determining the LD₅₀ of RVV on mice, *in vivo* effect of RVV on serum cholesterol, protein and albumin level at various time intervals after envenomation and in different dosages on mice were carried out. Finally, *in vitro* direct effect of RVV on these parameters was carried out. The detailed procedures were as follows:

Determination of intramuscular (IM) median lethal dose (LD₅₀) of RVV on mice

Six groups of mice each consisting of six animals were envenomed with different concentrations of RVV ranging from 5 to 160 ug/0.1ml. Death of mice within 24 hours was noted. The I.M LD₅₀ of RVV on mice was calculated by Spearman-Kärber method, 1981 [5].

Effect of RVV on mice at various time intervals after envenomation (in vivo test 1)

Six groups of mice each consisting of five animals were injected intramuscularly into the inner aspect of thigh with calculated LD₅₀ dose of RVV. They were then sacrificed at 0.5, 1, 2, 4, 6 and 24 hours post envenomation. The mice of the control group received the same amount of normal saline only. Their blood samples were taken out through the opened cardiac puncture.

Effect of RVV in different dosages on mice (in vivo test 2)

A total of 25 mice, equally divided into five groups were tested. They were administered with I.M RVV in different dosages i.e. 2.5, 5, 10, 15 (i.e. LD₅₀) and 20 ug/0.1 ml into the inner aspect of the thigh. They were sacrificed at one-hour post envenomation and their blood samples were taken out through the opened cardiac puncture. The control mice were injected with the same volume of normal saline only.

Direct effect of RVV on serum of mice (in vitro test)

In this experiment, blood samples of unenvenomed mice were taken transcardially after being anesthetized and sera were separated out and collected. Then 0.1 ml of serum was mixed with 0.1 ml of RVV in concentration of LD₅₀ dose. Another 0.1ml of serum was also mixed with same volume of normal saline. These two types of mixture were incubated in a water bath at 37°C for half an hour with gentle shaking. Determinations of cholesterol, total protein and albumin in these sera and mixtures obtained above were carried out by using the standard assay procedures [6, 7, 8].

RESULTS

The I.M LD₅₀ of RVV on mice was calculated to be 15ug/0.1ml (i.e 0.75ug/gm or 15ug /20gm mouse) (Table 1).

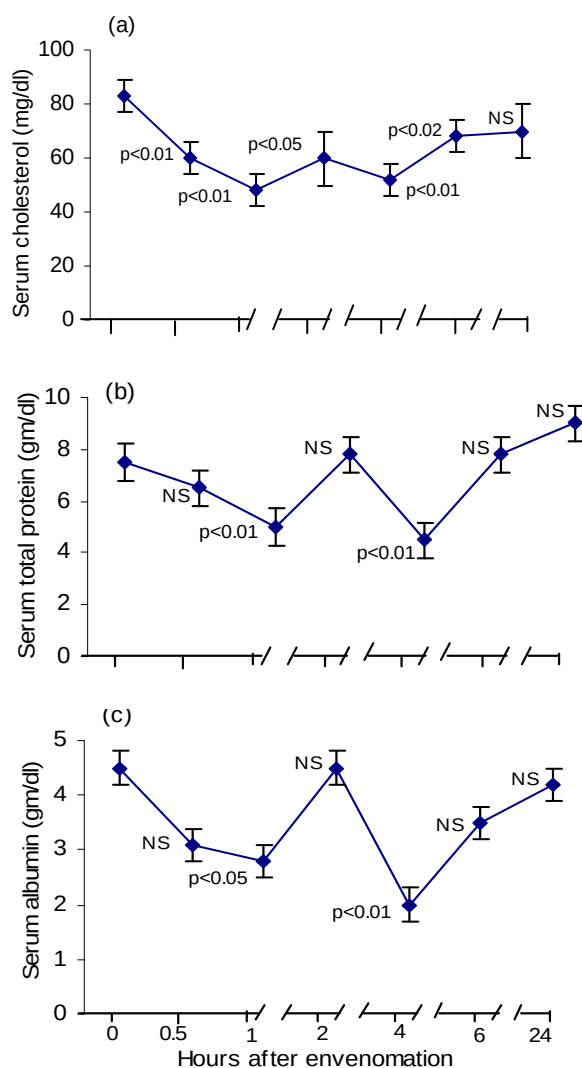
Table 1. Determination of intramuscular median lethal dose (LD₅₀) of RVV on mice

Concentrations of RVV	Deaths of mice within 24 hr
160 ug/0.1ml	6/6
80 ug/0.1ml	6/6
40 ug/0.1ml	6/6
20 ug/0.1ml	5/6
10 ug/0.1ml	0/6
5 ug/0.1ml	0/6
Normal saline (control)	0/6

Numerator indicates number of mice died and denominator indicates numbers of mice used.

Calculated LD₅₀=15 ug/0.1ml (i.e 15 ug/ 20gm mouse or 0.75 ug/ gm body weight)

Fig. 1a shows the effect of RVV on serum cholesterol levels of mice at different time intervals after being administered with LD₅₀ dose of RVV. It was found that serum cholesterol level started to decline significantly at half an hour after envenomation and reached its lowest level at one hour. The persistence of an apparent reduction in serum cholesterol level was observed till four hours. Then, it started to rise again at six hours and retain its initial level at 24 hours after envenomation. Changes in serum total

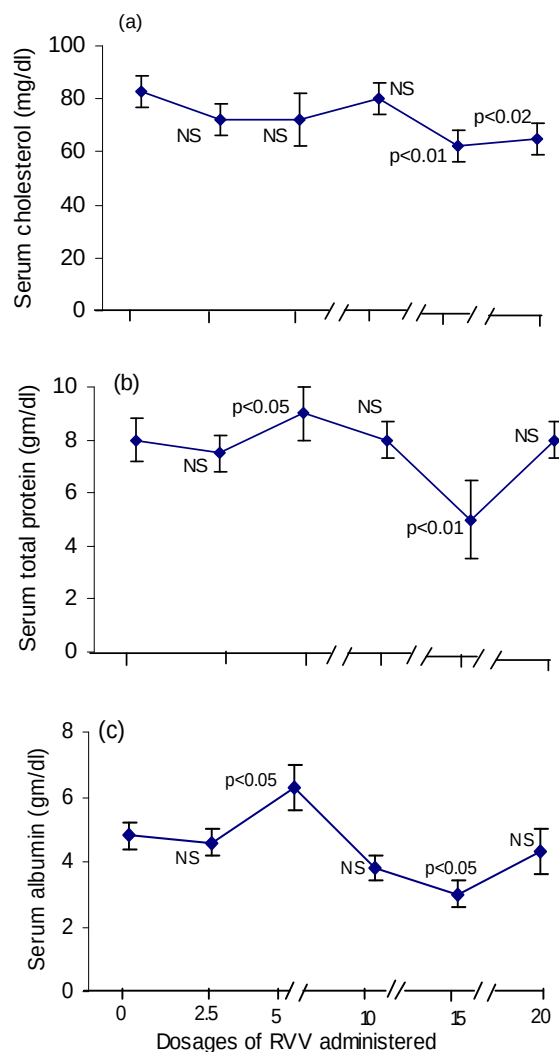


Each value represents Mean $\pm$ SEM (n=5).
 'p' value was calculated compared to the respective initial level, using Student's unpaired 't' test.
 'NS' means not significant at the 5% probability level.

Fig 1. Changes in serum cholesterol, total protein and albumin levels of mice at various time intervals after administration with RVV in LD₅₀ dose

protein levels of envenomed mice at various time intervals after envenomation were clearly illustrated in Fig.1b. Its levels showed a slight reduction at 0.5 hour, and significant reduction at one hour and four hours after envenomation. Total protein concentration retained its nearly initial level at two hours and six hours, and reached beyond the initial level at 24 hours after envenomation. Similarly, changes in serum albumin levels of mice at various time intervals after envenomation were clearly

visualized in Fig.1c. The significant decrease in levels was observed only at one hour and four hours. Serum albumin level regained its initial concentration at two hours and 24 hours after envenomation.



Each value represents Mean $\pm$ SEM (n=5)
 'p' value was calculated compared to the respective initial level, using Student's unpaired 't' test.
 'NS' means not significant at the 5% probability level.

Fig 2. Changes in serum cholesterol, total protein and albumin levels of mice at one hour after administration with RVV in different doses

Fig. 2a demonstrates the changes in serum cholesterol levels of mice at one hour after administration of RVV in different dosages. The apparent reduction in serum cholesterol levels was found after envenomation with each and every dose. The significant decrease

in levels was observed at the RVV in dosage of 15 ug/0.1ml (i.e. LD₅₀ dose). Changes in serum total protein and albumin levels of mice after being envenomed with different dosages of RVV can be observed in Fig. 2b & 2c respectively. Although the obvious reduction was seen at some dosages of envenomation, the significant rise in total protein and albumin levels were observed at RVV in dosages of 5 ug/0.1ml unexpectedly.

Table 2 summarizes the direct effect of RVV on serum concentration of cholesterol, total protein and albumin. These parameters were found to be significantly reduced after incubation with RVV, thus indicating that RVV had a direct lowering (i.e. destructive) effect on cholesterol, protein and albumin in the sera.

Table 2. Determination of the direct effects of RVV on serum cholesterol, total protein and albumin (*in vitro* test 1)

Parameters	Serum concentrations (mg/dl)		Significance 'p' value
	without RVV	with RVV	
Cholesterol	70.4 ± 6.6	48.0 ± 5.1	<0.05
Total protein	8.4 ± 0.67	4.8 ± 0.85	<0.05
Albumin	3.9 ± 0.51	2.5 ± 0.12	<0.05

Each value represents Mean ± SEm (n=5)

Amount of RVV used = 15 ug/0.1ml (i.e. LD₅₀)

NS means not significant at the 5% probability level

'p' value was calculated using Student's unpaired 't' test

DISCUSSION

In our clinical practice in the management of Russell's viper bite victims, presence and/or development of RVV evenomation is an indication for administration of anti-snake venom therapy and is manifested only by appearance of incoagulable (non-clotted) blood and clinical proteinuria in Russell's viper bite victim. These parameters are detected before the appearance of clinical features by using 20-minute whole blood clotting test and bed side boiling test respectively [9, 10]. These parameters usually show the abnormalities about 2 to 6 hours after bite. Therefore, it is necessary to detect

the presence of envenomation in Russell's viper bite victims at the early hours by using a more rapid and simple procedure or parameter which could be available in district and township hospitals where most of the Russell's viper bite cases are admitted at the early hours after bite.

From our findings, it is strongly evident that RVV induced the significant lowering of blood cholesterol level within 24 hours after evenomation and presence of evenomation in animal victims could be detected as earliest as half an hour after evenomation at which a significant degree of hypocholesterolaemia was apparently detected (Fig.1a.). In addition, different degrees of hypocholesterolaemia in accordance with RVV in different dosages were also observed in animal victims after being administered with RVV (Fig. 2a).

Although an apparent reduction of serum total protein and albumin were observed only at one hour after en-venomation and their degrees of reduction were not consistently and directly related to the time elapsed. The fluctuation in serum total protein and albumin levels were observed in first 24 hours after evenomation (Fig.1b & 1c). It may be due to the prompt and compensatory synthesis of protein and albumin by liver. Besides, the degree of hypoproteinaemia and hypoalbuminuria did not totally reflect the amount of RVV administration since both total protein and albumin levels unexpectedly increase after administration of RVV in some doses (Fig. 2b & 2c).

The mechanism of the apparent lowering effect of RVV on serum cholesterol, total protein and albumin may be through the direct effect (Table 2). Since the presence of phospholipase A₂ (PLA₂) enzyme in RVV, it seemed to induce a direct destruction of cholesterol resulting in a significant hypocholesterolaemia. The RVV also contains many proteolytic enzymes that are supposed to directly destroy the serum protein and albumin, inducing a significant lowering of

their concentrations in sera. Another possibility for hypocholesterolaemic action of RVV may be the indirect mechanism, in which several toxic components of RVV such as proteolytic enzymes, hyaluronidase, phospholipase, phosphodiesterase cause capillary endothelial damage in victims, resulting in extravasation of plasma proteins.

The decrease in cholesterol levels may be due to lipoprotein leakage through the damaged endothelium. However, since the dimensions of lipoprotein particles are considerably greater than that of the albumin molecule, capillary leakage of lipoprotein alone would not be sufficient explanation for hypocholesterolaemia that was encountered earlier than hypoproteinaemia and hypoalbuminemia in our study.

Besides, changes in cholesterol levels in envenomed mice were not apparently correlated with changes in total protein and albumin levels in both various time intervals and different dosages of RVV. Therefore the hypocholesterolaemia encountered in envenomed mice is probably due to the direct destructive action of PLA₂ on serum cholesterol rather than an indirect action through the endothelial leakage. With recovery from envenomation, the cholesterol pool can be released into the blood stream by the liver in the form of newly synthesized very low-density lipoprotein (VLDL). Therefore, hypocholesterolaemic effect of RVV was transient and it restored to normal level at 24 hours after envenomation.

Our study showed a totally time-dependent and a partially dose-dependent hypocholesterolaemia in animal victims envenomed with RVV. Therefore, prompt and simple measurement of serum cholesterol in RVV bite victims at early hours of clinical course may indicate the presence of envenomation. This may facilitate the decision on early administration of anti-snake venom (ASV) to the victims before the development of the abnormalities of other parameters for envenomation and severe clinical features. It

may also reduce the untoward side effects of unnecessarily administering ASV (i.e. horse antiserum) such as pyrogenic reaction and anaphylactic reaction which occurred about 100% and 13% respectively of victims receiving ASV [11].

Based on our findings, it could be concluded that RVV has a definite lowering effect on blood cholesterol levels in experimental mice and development of hypocholesterolaemia in victims after Russell's viper bite can be considered as a simple indicator for early detection of envenomation. The hypocholesterolaemic potential of RVV on serum cholesterol level in human victims needs to be studied in the near future for possible use in clinical practice.

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