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Association of *pvmdr1* Y976F mutation and *in vitro* chloroquine sensitivity of *Plasmodium vivax* in Kawthaung

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Plasmodium vivax is prevalent throughout tropics accounting for 25-40% of global malaria burden. Chloroquine (CQ) is the first-line treatment though chloroquine-resistant *Plasmodium vivax* had been reported since 1989. Vivax malaria has long been considered as benign but some recent reports on complicated and fatal forms of vivax malaria draw a great attention to monitor emergence of drug resistance in *Plasmodium vivax*. To find out *in vitro* CQ status and its association with polymorphism of *pvmdr1* gene of *P. vivax*, a total of 26 *P. vivax* isolates from Kawthaung District were investigated during transmission season of 2009. Polymerase chain reaction could detect *pvmdr1* Y976F mutation in 30.8% of isolates. Mean effective concentrations 90 (EC₉₀) of CQ was noted as 307.18 nM (95%CI, 162.46-580.84). Mean EC₉₀ of CQ among isolates carrying *pvmdr1* Y976F was higher than that of wild types (444.14 nM vs. 281.87 nM). Minimum inhibitory concentration (MIC) of CQ and number of isolates harboring *pvmdr1* Y976F mutation were found to be directly associated ($R^2=0.457$). The findings of the study could report applicability of molecular tool in relation to *in vitro* test for monitoring of CQ-resistant vivax malaria.

INTRODUCTION

Plasmodium vivax is widely distributed among tropics and affecting 40% of the world's population which accounts for 147-436 million clinical infections each year [1]. Chloroquine (CQ) is the first-line treatment since 1946 though chloroquine-resistant *Plasmodium vivax* had been reported starting from 1989. CQ-resistant *P. vivax* was first reported from Papua New Guinea [2]. Reports from Indonesia [3, 4], Myanmar [5, 6], and India [7] confirmed the emergence of CQ-resistant vivax malaria. Development of dormant hypnozoite stages of *P. vivax* in the liver resulting in relapse few weeks after the initial episode is the major obstacle for control measures.

In vitro susceptibility assays provide an alternative mean of assessing drug susceptibility of *Plasmodium* spp. without confounding effect of host immunity, relapse, and reinfection [8]. Lack of continuous culture

system for *P. vivax* is another obstacle to monitor CQ-resistant *P. vivax*. To better define the drug-susceptibility profile of *P. vivax*, a standardized *in vitro* assay was recently developed and validated [9, 10]. Using an *in vitro* susceptibility assay, a spectrum of chloroquine susceptibility in *P. vivax* can be defined. The findings of *in vitro* studies suggested *in vitro* test as useful tool in monitoring for the emergence of CQ-resistance [8].

The *in vivo* test fails to detect a reduction of drug sensitivity before the manifestation of overt resistance [11]. In 2005, the *mdr*-like gene in *P. vivax* was first identified and presence of two *pvmdr1* mutations at codon 976 and 1076 of the isolates from CQ-resistant *P. vivax* were found [12]. These two mutations were proposed as early molecular markers for CQ-resistant *P. vivax*. A study reported the association of *pvmdr1* Y976F mutation and 4-fold higher chloroquine IC₅₀, and *pvmdr1* Y976F mutation

was reported as a useful tool to monitor the emergence of chloroquine resistance [10]. Usefulness of molecular markers may vary in different geographical areas and their validity needs to be evaluated before applying them into drug resistance surveillance [13].

Diagnosis of CQ-resistant *P. vivax* requires a proof of adequate compliance to and absorption of therapy, given under supervision or, ideally, by determination of the levels of drug in blood [14]. A recurrent parasitaemia should not occur within 35 days of standard CQ therapy, if so, it is resistant to ordinary lethal exposure of CQ. Therefore, recurrent parasitemia, regardless of their origin, within 35 days of CQ therapy supports a provisional diagnosis of resistance [1]. Vivax malaria has long been considered as benign but some recent reports on complicated and fatal forms of vivax malaria draw a great attention to monitor emergence of drug resistance in *Plasmodium vivax*. In the studies conducted in Thailand and Papua New Guinea (PNG), 21-27% of patients with severe form of malaria were due to *Plasmodium vivax* mono-infection [15]. CQ-resistant vivax has faster growth rate and found phenotypically correlated with severe falciparum malaria [16].

Therefore, the study was conducted with the objective to determine the *in vitro* CQ sensitivity of *Plasmodium vivax* and to find out the prevalence of the molecular marker and its association to CQ sensitivity status, in Myanmar.

MATERIALS AND METHODS

Study site and study period

The study was conducted in Kawthaung Township during malaria transmission season of 2009.

Sample collection

Two milliliters of venous blood were collected aseptically from a total of 26 *Plasmodium vivax* infected patients who met

inclusion criteria after obtaining written informed consent.

One milliliter of collected sample was transferred onto 3M Whatman filter paper and then the filter paper was air dried and kept in self-sealed plastic bag individually. Collected filter paper samples were transported to Parasitology Research Division, Department of Medical Research (Lower Myanmar) and stored at -20°C till they were processed by molecular assay.

One milliliter of collected venous blood sample was transferred into sterile test tube containing 0.5 ml of Waymouth medium and 40 IU heparin, for *in vitro* chloroquine susceptibility test.

Inclusion criteria for sample collection

- Age over 6 months
- Mono-infection with *P. vivax* detected by microscopy
- Asexual parasite count >250/μl
- Axillary temperature $\geq 37^{\circ}\text{C}$ or history of fever during 48 hr before recruitment
- Patients with no history of hypersensitivity to antimalarial drug
- Patients with negative pregnancy test or not lactating

Exclusion criteria

- Patient with concomitant medical illness
- Patient with mixed malaria infection
- Patient who does not give consent to participate in the study

Ethical approval

The study was approved by The Institutional Ethical Committee for Research Involving Human Subjects in 2002.

In vitro susceptibility test

In vitro susceptibility test for CQ was conducted according to method of Congpuong [17], using WHO pre-dosed microtitre plates. Different concentrations of chloroquine predosed in the plate were 20, 40, 80, 160, 640, and 1280 nM, respectively. The procedure in brief was to add 0.1 ml of parasitized blood to 1.9 ml of medium containing RPMI medium plus Waymouth

medium at ratio of 1:1. Fifty microlitre aliquots were dispensed into each scheduled well of microtitre plate. Then, the microtitre plate was incubated at $37.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 36 hr applying candle jar method. After 36-hr incubation, thick smears were made from each well of plate. Microscopic differential counting of 200 asexual parasites of the smears made from different drug concentrations was performed. Effective concentrations were calculated using Probit calculus computer programme, provided by WHO. MIC (minimum inhibitory concentration) was defined as the minimum concentration of a drug at which more than 99% of parasites, relative to control, were inhibited from developing schizonts (parasites with six or more chromatin dots).

Molecular method

DNA was extracted from collected filter paper samples by applying Chelex method [18].

Polymorphism in *pvmdr1* gene was detected by polymerase chain reaction primer mismatch method described by Suwanarusk [10]. Primers used were *Pvmdr* 976F (5'-GGA TAG TCA TGC CCC AGG ATT G), *Pvmdr* 976R (5'-CAT CAA CTT CCC GGC GTA GC), and *pvmdr* Internal (5'-CGG CTG TAC TGA CCG GAA CGT A). A fifty microliters of PCR premix was prepared containing 10x PCR buffer, 2.5mM MgCl_2 , 0.20 mM of each dNTP, 1 μM each primer and 1.25 U of Taq DNA polymerase and 1 μl of genomic DNA. Mutant and wild controls were included in each PCR reaction. The reaction was performed at following cycling condition; 95°C for 10 min. and 40 cycle of at 94°C for 40 second, at 55°C for 1 min. and at 72°C for 2 min. PCR products then underwent agarose gel electrophoresis and examined under UV light by BioDot documentation system. Discrimination of wild *pvmdr1* Y976 and mutant *pvmdr1* F976 was made based on the band size. Mutant alleles show single band of 560 base pair and wild-type alleles show double bands having 560 base pair and 400 base pair.

Data analysis

Data analysis was made by applying SPSS version 12.0.

RESULTS

In vitro sensitivity status of *Plasmodium vivax* isolates

A total of 26 criteria-matched *P. vivax* isolates were investigated for their sensitivity status against chloroquine using WHO microtiter plates predosed with CQ. Mean effective CQ concentration 90 (EC 90) of 26 tested isolates was found to be 307.1884 nM (95% CI, 162.4631-580.8377).

Prevalence of *pvmdr1* 976 alleles

Polymerase chain reaction detected *pvmdr1* Y976 (wild type) in 69.2% (18 out of 26 isolates) and *pvmdr1* F976 (mutant type) in 30.8% (8 out of 26 isolates) (Plate 1).

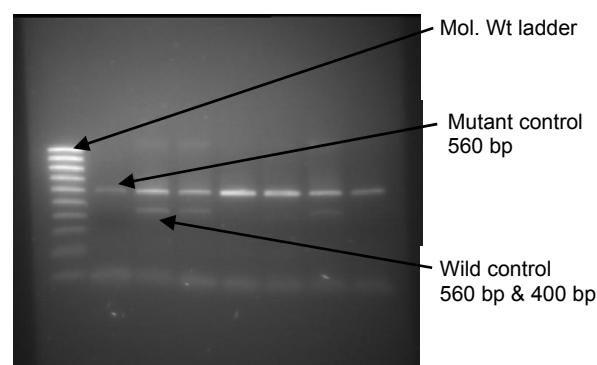


Plate 1. Photographic documentation of *pvmdr1* 976 alleles on agarose gel electrophoresis

Distribution of *pvmdr1* 976 alleles in MIC (minimum inhibitory concentration) status

More than 50% of all isolates showed growth inhibition at the MIC level of 320 nM. Most of the mutant isolates (63.33%; 5/8 mutant isolates) were distributed in the higher MIC level (>320 nM) (Table 1).

Mean EC90s (effective concentration 90) of chloroquine against *pvmdr1* 976 alleles

Mean EC90 of chloroquine against *P. vivax* isolates harboring *pvmdr1* F976 (mutant type) was noted to be 1.6 times higher than that of wild type isolates (Table 2).

Table 1. Distribution of *pvmdr1* 976 alleles in different chloroquine MIC status

<i>Pvmdr1</i> 976 allele	MIC in nM of chloroquine					Total
	80	160	320	640	≥1280	
Wild type (no of isolates)	4	4	3	2	5	18
Mutant type (no. of isolates)	1	0	2	2	3	8
Total (no of isolates)	5	4	5	4	8	26

Table 2. Effective concentration of chloroquine against *Plasmodium vivax* isolates harboring different *pvmdr1* 976 alleles

<i>Pvmdr1</i> 976 allele	Mean EC90 of chloroquine in nM	95% confidence Intervals	
		Lower	Higher
Wild type (n=8)	281.8717	138.8607	572.1681
Mutant type (n=18)	444.1423	90.8351	2171.6533

Association between numbers of *Plasmodium vivax* isolates harboring different *pvmdr1* 976 alleles and chloroquine MIC status

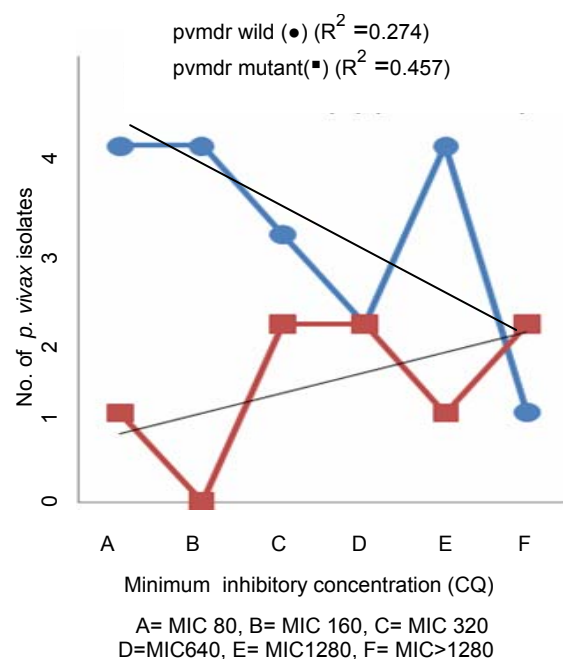


Fig. 1. Association between number of *P. vivax* isolates and chloroquine MIC

The number of *Plasmodium vivax* isolates harboring *pvmdr1* F976 mutations was found to have direct linear association with chloroquine MIC status ($R^2 = 0.457$) (Fig. 1).

DISCUSSION

Antimalarial sensitivity of *P. vivax* is significantly influenced by initial parasite stage and duration of incubation. Regarding parasite stage, the ratio of ring to trophozoite (RT ratio) is negatively correlated with inhibitory concentration of chloroquine because of relative insensitiveness of trophozoite to CQ. Duration of incubation is also negatively correlated with inhibitory concentration [9].

In this study, the average proportion of ring stage parasites was 40% and incubation time was 36 hours. Mean effective chloroquine concentration 90 (EC90) of overall tested isolates was noted as 307.1884 nM (95% CI, 162.4631 - 580.8377). A study tested on *P. vivax* isolates from northwestern Thailand in 1998-99, reported mean EC90 as 358 nM. [11]. Another study from Thailand conducted in the same region in 2001 presented mean EC90 of 511 nM [20].

In Myanmar, *in vitro* drug testing system for *P. vivax* using WHO pre-dosed microtitre plates was first established in laboratory setting in 2002 and field applicability was tested on 19 *P. vivax* fresh isolates in Butheedaung Township in 2004 and mean EC90 of chloroquine was noted to be 107 nM (Ye Htut, unpublished data). This is the first report of *in vitro* sensitivity status of chloroquine against *P. vivax*.

Monitoring of *P. vivax in vitro* sensitivity in Thailand showed an approximate 4-fold decrease in CQ sensitivity in Thailand within a couple of decades [21]. Although the sites of two studies in Myanmar were not the same, CQ sensitivity status on *P. vivax* was found to be decreasing in parallel with therapeutic efficacy (107 nM vs. 307 nM). As CQ-resistance has been evident since 1993 in Myanmar, regular monitoring of drug-resistant *P. vivax* is strongly recommended to track the spread of drug resistance. Among tested isolates, 30.8% (8 out of 26 isolates) harbored *pvmdr1* F976 (mutant type) while 69.2% (18 out of 26 isolates) harbored wild type.

Regarding prevalence rate, a wide range of rates has been reported by different studies.

The prevalence rate of mutant *pvmdr1* 976F was reported as 39% of isolates from PNG [19]. A Thailand study investigated a limited number of isolates and found mutant *pvmdr1* in 3 out of 5 Myanmar isolates and 1 out of 11 Thai isolates [22]. *Pvmdr 1* mutation was found in 93% of Papuan isolates and 25% of Thai isolates [10]. The discrepancy in mutation rates may be due to the geographical origin of isolates tested, having different CQ sensitivity status and different degrees of drug pressure.

Results of *in vitro* drug sensitivity test are usually presented with IC (inhibitory concentration) or EC (effective concentration). In this study, EC values calculated by Probit calculus computer programme were presented. Decreased sensitivity of CQ was observed among mutant *P. vivax* isolates (n=8) in this study. Mean EC90 (90% effective concentration) of the *P. vivax* isolates harboring mutant *pvmdr1* 976F was found 1.6 times higher than that of wild type (*pvmdr1* 976Y) carrying isolates. Similar finding on reduced CQ sensitivity of mutant isolates (*pvmdr1* 976Y) than wild type was reported by a Thailand study [23].

Chloroquine resistance can occur in *P. vivax* isolates harboring wild type (*pvmdr1* 976Y) and clinical cure can be achieved in mutant isolates [15]. Among mutant isolates, 3 out of 8 showed lowest MIC while 5 out of 18 wild isolates showed highest MIC in the study. This finding supported the above statement.

Molecular assay using genetic marker is an important tool for antimalarial resistance monitoring [8]. Usefulness of molecular markers may differ for different geographical areas and their validity needs to be evaluated before applying them into drug resistance surveillance [16]. Association of *pvmdr1* 976 allele and *in vitro* CQ susceptibility was first reported by Suwanarusk [10]. A total of 81 *P. vivax* isolates from Thailand and 45 isolates from Papua,

Indonesia were included in that study and *pvmdr1* 976F was proposed as useful tool for chloroquine-resistant *P. vivax*.

In this study, a moderate linear association was found between the number of *P. vivax* isolates harboring *pvmdr1* 976F mutations and chloroquine MIC status ($R^2=0.457$). The more the mutant isolates, the higher the MIC of chloroquine is. It states that as the number of mutant isolates increases, the higher concentration of chloroquine is required to kill the parasites.

Linking molecular tool with *in vitro* susceptibility tests would be of value to map out drug-resistant *Plasmodium vivax* particularly chloroquine resistance.

The report of the study is the first step towards monitoring and surveillance of drug-resistant *P. vivax* malaria emergence and spread.

Conclusion

This study is the first report on *in vitro* susceptibility status of *Plasmodium vivax* in Myanmar. It could provide the molecular evidence of chloroquine resistance in *P. vivax*, in terms of *pvmdr1* 976F mutation which has been proposed as marker of CQ-resistant *P. vivax*. The results also proved the direct association between the number of isolates bearing *pvmdr1* 976F mutation and minimum inhibitory concentration of chloroquine

Recommendation

1. Chloroquine sensitivity of *Plasmodium vivax* should be monitored regularly by means of both *in vivo* and *in vitro* tests.
2. Field as well as laboratory-based research works focusing on emergence and spread of CQ-resistant *vivax* malaria should be encouraged.

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**Phytochemical analysis of commonly known Banda trees
(*Terminalia catappa* Linn.) grown in Yangon Division**

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*Lwin Zar Maw, *Tin Ko Ko Oo, *May Thu Kyaw & *Yee Yee Sein

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The aim of the study is to identify and differentiate between two varieties of trees which are commonly known as Banda trees grown in Yangon Division. The morphological, phytochemical, and elemental studies of leaves, fruits and barks were done. Two different varieties of tree: *Terminalia catappa* Linn. variety 1 Catappa and *Terminalia catappa* Linn. variety 2 Pubescens, are almost similar in general appearance. The leaves are obovate to oblong, round or tapering at base and have yellowish spot-like glands on the lamina and base of midrib in variety 1 and red glands in variety 2. The mesocarp (fleshy part) of fruits are either pink or yellow in colour, pink are bitter and yellow are sweet. Both kinds of kernel contain catappa oil (25-40%). Barks are smooth or nearly rough, the bark of variety 1 provides more oil. The phytochemical tests have shown the presence of alkaloid, flavonoid, glycoside, tannin, phenolic compounds, carbohydrate, saponin, reducing sugar and absence of cyanogenic glycoside. But the content of flavonoid was higher in variety 2. In the elemental analysis, potassium, calcium and silicon were found in leaves, fruits and barks; iron, calcium and iodine were found in oil. Due to the different phytochemical and elemental contents of these two varieties of *Terminalia catappa* Linn. they may have different activities and these activities may be useful in the preparation of traditional medicine.

INTRODUCTION

India almond, commonly known in Myanmar as Banda or Jangli Banda (*Terminalia catappa* Linn.), is one of the valuable traditional medicine plants [1, 2]. It grows wild in open areas and is cultivated along the roadsides in Yangon Division. It is medium size tree growing up to 70 feet in height, deciduous in summer, forming upright and is often buttressed at the base. The branches are arranged in tiers giving them pagoda shape. The tree is a native of Malay having the lifespan of 60 to 100 years in suitable environment and can be cultivated on the land below 1300 feet in Myanmar. Genus-*Terminalia* is a member of the Family Combretaceae including 250 species and varieties. *Terminalia* means the

leaves and flowers which are born in cluster at the tips of the twigs and *catappa* stands for the name of Malaysian vernacular tree [3]. Flowering period is from April to May and fruiting is from June to July. The species have been useful for traditional medicine and in coastal communities, as a shelter from strong wind. The timber makes an excellent general purpose hard wood and is well suited for furniture and building. The leaves are traditionally used as food wrapping in Myanmar. The leaves, fruits and bark were reported to have anti-mutagenic, anti-cancer, anti-oxidant, hepatoprotective and anti-diabetic activity. The leaves are used for treating rheumatism in South India and ointment is prepared from the leaf extract for treating scabies, leprosy and other cutaneous diseases [4].

The aim of the study focused on identification of the correct subspecies of *Terminalia catappa* Linn. and phytochemical and elemental analysis of these varieties which are crucial in preparation of traditional medicines with different efficacy of the drugs.

MATERIALS & METHODS

Sample collection

The leaves, fruits and barks were collected from Banda trees growing on roadsides in Yankin Township, Yangon Division. The leaves were collected from main branch before blooming season. Mature fruits were collected when the colour turned to brown (or) yellow. Barks were collected from stems above 4-5 feet ground level. All these materials were collected in the morning.

Inclusion criteria

Mature tropical almonds having no appearance of disease.

Exclusion criteria

The trees under 3 years of age

Identification

The plants were identified according to Hooker & Kurz (1877) [5-9].

Extraction of oil

By percolated method: Oils were extracted from two kinds of kernels by solvent extraction method [10]. Each 2 gm of kernels was soaked in 50 ml of petroleum ether (60-80°C) for two days. Two layers were seen. Then, the upper layer of oil was decanted and dried out at the room temperature. This process was easy, cost effective and provided 25-40% of oil from dry kernels.

The phytochemical analysis

The leaves, fruits and barks were dried at room temperature, were made into powder and the constituents were extracted. Detection of alkaloid was done by Mayer's

method, flavonoid by concentrated sulphuric acid method, glycoside by 10% lead acetate method, tannin by 1% ferric chloride test, phenol by 10% ferric chloride test, saponin by aqueous method and cyanogenic glycoside by sodium picrate method, respectively [11-14].

The elemental analysis

The analysis of mineral elements in leaves, barks and kernels of both types was done by using Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy at the University of Yangon Research Centre [15].

RESULTS AND DISCUSSION

Morphological and taxonomical characters of the plants were observed to agree with those of *Terminalia catappa* Linn. [5] standardized at the Department of Botany, Yangon University.

Morphological characters

Habit

Medium size, 20-25 meter long, bark smooth or nearly so, stem woody, cylindrical, girth 50-130 cm, trunk 1-1.5 feet in diameter, branch whorled, spreading or slightly ascending.

Leaves

Simple, alternate, 15-28 x 13-17 cm wide, obovate to oblong shape, round tapering at the cuneate base, gland present at each side of base of midrib and on the lateral veins, petiole short, thick, 0.7-1.5 cm long.

Inflorescences

Simple axillary spike, 13-15 cm long.

Flower

Apetalous, bisexual flowers at the base of inflorescence, 0.8-1 x 0.5-0.6 mm; male flowers towards the apex, 3-5 x 4-5 mm, actinomorphic, epigynous.

Calyx

Sepals 5, fused, cup-shaped, calyx lobes pointed teeth, glabrous.

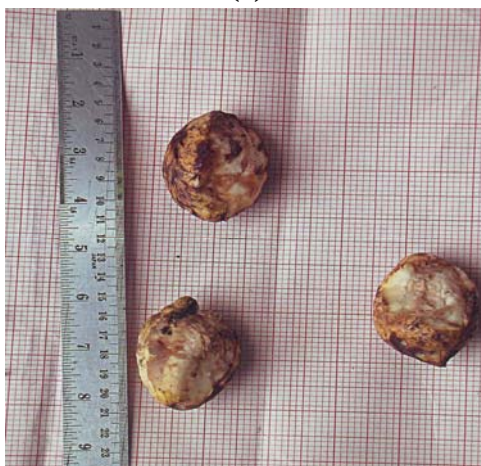
Variety 1



(a)



(c)



(e)

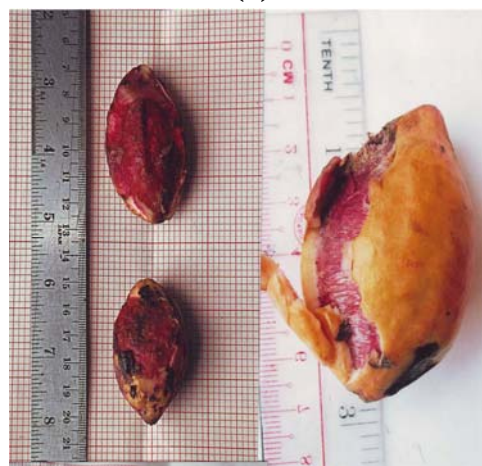
Variety 2



(b)



(d)



(f)

- a= Transverse section of leaf showing no pubescence beneath the lamina
 b= Pubescence present beneath the lamina
 c= Yellow spot-like glands on the lamina
 d= Red spot-like glands on the lamina
 e= Yellowish color of mesocarp
 f= Pink color of mesocarp

Fig. 1. The distinguishing characteristics of two varieties of *Terminalia catappa* Linn.

Corolla

Petal absent

Androecium

Stamen 5+5, biserate, flexed in bud, spreading when bloom, anther 3-5 mm, ditheous, dorsifixed, filament 1.5-2 mm long.

Gynoecium

Monocarpellary, unilocular, ovary ellipsoid, stigma 0.7-0.9 cm, filiform 3-5mm long, placentation pendulous, 2-3 ovules present.

Fruit

Drupes, oblong to oval, compressed, smooth with prominent edges, 2.5-4 cm long, seeds 1.5-2 cm long x 0.5-1 cm broad.

The distinguishing characters of *Terminalia catappa* Linn. variety 1 Catappa and *Terminalia catappa* Linn. variety 2 Pubescens are shown in Fig.1. There are no pubescence beneath the lamina in variety 1 but present in variety 2. In variety 1, the lamina has yellow spot-like glands but in variety 2, red spot-like glands are present on the lamina. The mesocarp of the fruit of variety 1 is whitish or yellowish in color and has sweet taste but that of variety 2 is pink and has bitter taste. These findings were certified by Department of Botany, Yangon University and they are also in accordance with the findings of Kurz which were mentioned in Forest Flora of British Burma in 1877.

Phytochemical analysis

Regarding phytochemical test using various solvents, both plants were found to contain alkaloids, flavonoids, glycosides, phenolic compound, tannins, saponins and carbohydrates whereas cyanogenic glycoside was not detected in both plants (Table 1). The only difference is the higher content of flavonoid in the leaves of variety 2.

Elemental analysis

The elemental analysis of *Terminalia catappa* in Table 2 shows that substantial contents of potassium (K), iron (Fe), calcium (Ca),

Table 1. Phytochemical screening of the extracts of variety 1 and variety 2

Test reagents	Solvents used	Observation	Leaves		Fruits		Barks	
			1	2	1	2	1	2
<i>Alkaloid</i>								
Mayer's	HCl (dil)	White ppt	+	+	+	+	+	+
<i>Flavonoid</i>								
Concentrated sulphuric acid	EthOH	Reddish pink	+	++	++	++	++	++
<i>Glycoside</i>								
10% lead acetate	H ₂ O	White ppt	+	+	+	+	+	+
<i>Tannin</i>								
1% ferric chloride	H ₂ O	Blue black	+	+	+	+	+	+
<i>Phenol</i>								
10% ferric chloride	H ₂ O	Blue or green	+	+	+	+	+	+
<i>Saponin</i>								
Water	H ₂ O	Frothing	+	+	+	+	+	+
<i>Carbohydrate</i>								
Benedict's	H ₂ O	Green or blue ppt	+	+	+	+	+	+
<i>Cyanogenic glycoside</i>								
Sodium picrate	H ₂ O	Yellow	-	-	-	-	-	-

Table 2. Elemental analysis of leaves, fruits, bark and kernel oils of two varieties of *Terminalia catappa* Linn.

Relative quantity (%)										
K	Fe	Ca	P	S	Si	Cu	I	Pb	Zn	
<i>Leaves</i>										
a.	23.4	3.3	34.7	25.9	11.4	-	1.1	-	-	-
b.	24.7	2.7	27.9	27.2	17.2	-	-	-	-	-
<i>Fruits</i>										
a.	19.1	5.5	5.2	-	-	68.9	0.5	-	-	0.51
b.	55	16	23	-	-	-	5.9	-	-	-
<i>Barks</i>										
a.	4.4	0.73	37.5*	-	3.4	53.5	-	-	-	-
b.	-	0.87	27.5	-	-	70.5	0.45	-	-	-
<i>Kernel oils (mg/L)</i>										
a.	-	149.3	89.3	-	-	-	50.6	288.8	157.3	45.0
b.	-	84.8	66.27	-	-	-	47.4	342.2	132.4	38.1

K=potassium, Fe= iron, Ca=calcium, P=phosphorus, S=sulphur, Si= silicon, Cu=copper, I=iodine, pb=lead, Zn=zinc

a= variety 1, b= variety 2

sulphur (S) and copper (Cu) were not much different in leaves of both varieties but the fruits of variety 2 contained more in

potassium, iron, calcium and copper than variety 1 except silicon and zinc which were found only in variety 1.

The bark of variety 1 was more rich in calcium but variety 2 contained more silicon. iron, calcium, copper, iodine, lead and zinc were found in the kernel oils of both varieties but the contents were higher in variety 1. Due to the different phytochemical and elemental contents of these two varieties of *Terminalia catappa* Linn., they may have different activities and these activities may be useful in the preparation of traditional medicine.

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**Clinical trial of blood glucose lowering effect of traditional medicine formula
No. 28 (TMF 28) on type 2 diabetes mellitus patients**

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In community, five hundred and twenty-two school teachers were screened for type 2 diabetes mellitus and among them, eighty-three were test positive. Thirty diabetic teachers who gave informed consent for participation were conducted phase 2 clinical trial before-after study according to objectives of determining hypoglycemic effect of TMF 28 (Thetyinnkalat Hsay) and its side effects. Before trial, fasting blood sugar, liver function tests, serum creatinine and ECG were done. All patients took seven tablets (300 mg/tablet) three times per day (6.3 g/day) with meal after getting diet control and regular physical exercise advice. Fasting and 2-hour post-prandial blood glucose were measured on day 7, 14, 21 and 28 with observation of any adverse effects during medication. After trial, all investigations were done on day 28. When trial was completed, patients were divided into three groups according to their blood glucose level - group 1 should continue with TMF 28 medication; group 2 was advised to switch to hypoglycemic medicine; group 3 did not need any medication apart from diet control, physical exercise and regular blood glucose examination. Fasting blood glucose levels reduced significantly from day 0 (8.91 mmol/l) to day 28 (7.49 mmol/l) ($p=0.000$). There was no side effect like severe hypoglycemia or hyperglycemic attack during study period. It was observed that there were no changes in ECG, serum bilirubin and creatinine. Elevations of SGOT and SGPT in two patients were found between before and after trial but these changes were within normal limit. The present study indicated that there was blood glucose lowering effect of TMF 28 on patients (63.3%) with type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic debilitating disease with severe complications including blindness, heart and renal diseases. Diabetes is given increased attention as the serious health problem that has increased steadily worldwide. World Health Organization (WHO) estimated that, by 2020 chronic diseases will account for almost three-quarters of all deaths worldwide, and that 75 percent of death due to stroke, 71 percent death due to ischemic heart disease and 70 percent death due to diabetes will occur in developing countries [1].

Diabetes can not be assumed to be a disease which predominates in developed countries. The number of people in developing world with diabetes will increase by more than 2.5-fold from 84 million in 1995 to 228 million in 2025 [2]. Diabetes causes about 5 percent of all deaths globally each year, contrary to common perceptions, almost 80 percent of diabetes deaths occur in low and middle-income countries. Almost half of diabetes deaths occur in people under the age of 70 years, and diabetes deaths will increase by more than 50 percent in next 10 years unless urgent action is taken [3]. For most of the developing world,

the main issue of public health is still the acute need for basic health care, which is sadly lacking even at the most elementary level. WHO indicates that more than half of the world's population does not have access to adequate healthcare service. This is due to the fact that poor people neither have access to nor could afford the present healthcare services. Therefore, innovative alternative approaches are needed to address this problem.

Medicinal plants offer alternative remedies with tremendous opportunities. They do not only provide access and affordable medicine to poor people but also generate income, employment and foreign exchange for developing countries. Many traditional healing herbs and plant parts have been shown to have medicinal value, especially in the rural areas and these can be used to prevent, alleviate or cure several human diseases. The WHO estimates that more than 80 percent of the world's population rely either solely or largely on traditional remedies for healthcare.

Plants, their extract or traditional formulas may also have a potential therapeutic role in controlling blood sugar level [4, 5]. TMF 28 has been used by Myanmar traditional medicine practitioners for a long time without adverse effect. The safety profile of TMF 28 has been tested and proved to be safe at the standard dosage regimen [6].

Therefore, this clinical trial was conducted to identify the blood glucose lowering effect of TMF 28, and to assess the side effect(s), if any, on type 2 diabetes patients.

MATERIALS AND METHODS

Materials used

- Test drug (300 mg tablets of TMF 28) developed by Department of Traditional Medicine
- Glucometers, lancet needles and test strips
- Blood pressure measurement cuff

Ingredients of TMF 28 (Thetyinnkalat Hsay)

Nine plant ingredients and one mineral/salt.

Scientific name	Myanmar name	Amount in g/100g
<i>Major plant</i>		
<i>Croton oblongifolius</i> (Root)	Thetyinngyee	50.0
<i>Other ingredients</i>		
<i>Capparis sepiaria</i> (Bark)	Hsoogauknet	5.6
<i>Crataeva religiosa</i>	Kadet	5.6
<i>Piper nigrum</i> (Fruit)	Ngayokkoun	5.6
<i>Osyris wightiana</i> (Fruit)	Zaunggyan	5.6
<i>Piper longum</i> (Fruit)	Peikchinn	5.6
<i>Sapium</i> sp.(Stem)	Awle	5.6
<i>Zingiber officinale</i> (Rizome)	Gyin	5.6
Unidentified (Root)	Hsaypale	5.6
<i>Mineral/salt ingredient</i>		
Roasted salt	Hsahlaw	5.6

Patient recruitment

Before clinical trial, patient recruitment was done from prevalence study of type 2 diabetes mellitus among school teachers of Mandalay City. In this study, screening was done by glucose-oxidase method in 522 teachers, among them, 83 teachers were test positive. Thirty out of eighty-three teachers who gave informed consent to participate in community-based phase 2 clinical trial were selected according to inclusion criteria. Twenty-eight days medication of TMF 28, 6.3 g/day (300 mg/tab, 7 tabs three times per day) was done together with diet control and regular physical exercise advice to control blood sugar level.

Inclusion criteria

Subjects were selected according to WHO definition

- Newly diagnosed type 2 DM, fasting blood sugar (FBS) ≥ 126 mg/dl (7.0 mmol/L), random blood sugar (RBS) > 200 mg/dl (11.1 mmol/L)
- Both sexes
- Age 40-60 years

Exclusion criteria

- Pregnant or lactating mothers
- Those receiving corticosteroid therapy
- Severe complications of DM
- Hypertension more than 160/95 mmHg
- History of bleeding tendency

Withdrawal criteria

- Subject's request
- Patient developing hypoglycemic shock or hyperglycemia
- Attending physician's recommendation because of side effects

Ethical consideration

Only those patients who gave voluntary written informed consent were included in this study. They were informed about the nature and purpose of the study and the right to withdraw at any stage without prejudice. The study was approved by the Institutional Ethical Review Committee of Department of Medical Research (Upper Myanmar).

Clinical trial procedures

Baseline examination

History taking, physical examination and appropriate laboratory investigations were carried out on each patient.

Pre-intervention and post-intervention tests

ECG, liver function tests (serum bilirubin, alkaline phosphatase, SGOT, SGPT) and serum creatinine test were done.

After recruitment of the patients, they were given TMF 28, 7 tablets (300 mg/tab) three time per day for 7 days and FBS and 2-hour post-prandial (2HPP) blood sugar (day 7) were measured by respective team at respective point. After reviewing the results, all subjects were provided with diet control and regular physical activity advice for the control of their blood sugar level and any adverse effects of drug were observed. The compliance of the subjects to the prescribed drug, diet and physical activities was assessed weekly by enquiring the subjects. This procedure was repeated during each of the three successive visits (on day 14, 21 and 28), respectively, when glucose levels were measured and any adverse effects were undertaken.

Signs and symptoms of hypoglycemia and hyperglycemia were observed. When hypoglycemic or hyperglycemic signs and symptoms appeared, necessary treatment

was given immediately. In case of emergency, assigned MO has to inform to Physician and to try (well-planned schedule prior to trial) to hospitalize as soon as possible after giving emergency care for hypoglycemia or hyperglycemia.

Statistical analyses

The data were presented as mean $\pm$ SD. Changes in FBS were analyzed by ANOVA of repeated measures; changes between weeks of medication and safety measures were analyzed using paired Student's t-test for pre and post-trial. The significance level was set at $p < 0.05$.

RESULTS

Blood glucose levels were measured in 30 school teachers with newly diagnosed type 2 diabetes mellitus, who received TMF 28, 7 tablets (300 mg/tab) three times daily for a period of 4 weeks. Within the first week of TMF consumption mean baseline blood glucose level was significantly reduced from 160.38 mg/dl (8.91 mmol/l) to 139.86 mg/dl (7.77 mmol/l) ($p = 0.006$). Mean FBS and mean 2 HPP of 30 patients at day 0, 7, 14, 21, and 28 are shown in Table 1.

Table 1. Mean FBS and 2 HPP of 30 patients enrolled in the study

	Day 0	Day 7	Day 14	Day 21	Day 28	P
Mean FBS mg/dl (mmol/l)	106.38 (8.91)	139.86 (7.77)	143.64 (7.98)	133.74 (7.43)	134.82 (7.49)	0:7 = 0.006 0:14=0.007 0:21=0.000 0:28=0.000
Mean 2HPP mg/dl (mmol/l)	Un- avai- lable	211.5 (11.75)	185.58 (10.31)	176.94 (9.83)	186.84 (10.38)	14:21=0.377 14:28=0.847 21:28=0.376

A significant interaction effect was observed between time and the group (repeated measure ANOVA) in the fasting blood glucose level of subjects. With repeated measure ANOVA pre-treatment blood glucose level (day 0) was significantly different with other groups (day 7, 14, 21, 28) as shown in Table 2a & b.

Table 2 a. Repeated ANOVA test measured between times in the mean fasting blood glucose level of subjects

Factor 1	Mean	Std. error	95% confidence interval	
			Lower bound	Upper bound
Day 0	8.91	.48	7.92	9.89
Day 7	7.77	.56	6.91	9.22
Day 14	7.98	.57	6.80	9.16
Day 21	7.43	.45	6.49	8.36
Day 28	7.49	.41	6.64	8.34

Table 2 b. Repeated ANOVA test measured between groups in the fasting blood glucose level of subjects

		Std. error	P	95%CI for difference
Day 0	Day 7	.28	.006	.84 (.2-1.4)
	Day 14	.32	.007	.92 (.2-1.5)
	Day 21	.25	.000	1.48 (.9-2)
	Day 28	.27	.000	1.41 (.8-1.9)
Day 7	Day 0	.28	.006	.84 (-1.4-.26)
	Day 14	.33	.805	.08 (-.6-.7)
	Day 21	.22	.009	.63 (.17-1.1)
	Day 28	.36	.130	.57 (-.18-1.3)
Day 14	Day 0	.32	.007	.92 (-1.5-.27)
	Day 7	.33	.805	.08 (-.76-.61)
	Day 21	.33	.105	.55 (-.12-1.2)
	Day 28	.36	.190	.49 (-.25-1.2)
Day 21	Day 0	.25	.000	.48 (-2.5-.9)
	Day 7	.22	.009	.63 (-1.1-.17)
	Day 14	.33	.105	.55 (-1.2-.12)
	Day 28	.28	.827	.06 (-.65-.52)
Day 28	Day 0	.27	.000	1.41 (-1.9-.85)
	Day 7	.36	.130	.57 (-1.3-.18)
	Day 14	.36	.190	.49 (-1.2-.25)
	Day 21	.28	.827	.06 (-.52-.65)

* The mean difference is significant at the 0.05 level.

Table 3. Reduction in the FBS of total patients, those with FBS above 180 mg/dl and those with FBS below 180 mg/dl

	Day 0	Day 7	Day 14	Day 21	Day 28
FBS of total patients (mg/dl) n=30	106.4	139.9	143.6	133.7	134.8
FBS of patients (>180 mg/dl) n=10	217.3	190.8	172.8	164.9	162.4
FBS of patients (<180 mg/dl) n=20	134.9	115.7	121.7	112.9	117.5

Each value shows mean $\pm$ SEM

At the baseline level, a subgroup of 20 subjects had glucose level below 10 mmol/l (180 mg/dl) and the other subgroups of 10 subjects had ≥ 10 mmol/l (≥ 180 mg/dl). Both subgroups were analyzed with repeated measure ANOVA. Clinically acceptable glucose levels were achieved during 4 weeks of therapy in the former subgroup. No side-effect was reported in all subjects (Table 3).

Safety parameters

Liver enzymes SGOT and SGPT were within normal range. No significant changes were seen in other parameters (alkaline phosphatase, bilirubin, creatinine and ECG) between pre-intervention and post-intervention.

DISCUSSION

Diabetes mellitus patients were treated with combination of hypoglycemic drugs in conventional medicine. Formulae contain minerals and metals in addition to multiple plants like a study drug TMF 28. In our country, human trials of traditional medicine were done mainly for single herb e.g *Momordica charantia* (bitter melon), *Orthosiphon aristatus*, *Fenugreek* etc. Ayurvedic practitioners assert that traditional medicine formula is more potent than the constituents taken individually and, further minerals and metal components boost the formula's potency. In Myanmar, TMF 28 has been used for a long time by senior traditional medicine practitioners.

Most trials examined supplements as an adjunct to conventional treatment with diet and/or medication and in these controlled trials, statistically significant treatment effects were reported in 88% of those examining single herbs, 60% of those examining combination herbs, and 67% of those examining vitamin and mineral supplements [7, 8, 9]. The literature mainly consisted of studies upon single or compound herbal plants; however, in clinical Ayurvedic practice, herbs are usually given

in combination [10]. In 29 trials that evaluated herbal medicines combined with hypoglycemic drugs, 15 different herbal preparations showed additional better effects than hypoglycemic drugs monotherapy.

Considerable number of alternative medicine experiments concerning diabetes mellitus are done all over the world. The results of clinical trials with human as well as animal studies, have confirmed the anti-diabetic effect of herbal medicines. A total of 108 human trials of herbs and vitamin/ mineral supplements for glycemic control were done by the American Diabetes Association. In these trials, the most common outcome parameters encountered in other trials included the following parameters of glycemic control: fasting and postprandial plasma glucose, response to glucose tolerance tests (GTTs), insulin and C-peptide levels, protein glycosylation (long-lived intracellular glycated hemoglobin and shorter-lived plasma fructosemine), and clinical insulin requirements. A significant positive change in at least one of these important parameters was required to categorize the trial as positive.

In present study, main outcome measure was mean fasting blood glucose level. Results of present trial showed a significant decrease in mean fasting glucose level. The average fasting blood sugar reduced from 161.44 mg/dl to 131.98 mg/dl ($p < 0.001$), and post-prandial glucose decreased from 211.50 mg/dl (day 7) to 186.84 mg/dl (day 28). There was a reduction of fasting blood sugar level as early as after the first week of treatment. We considered this reduction was due not only to drug but also to awareness of disease which caused diet restriction and life style change in patients.

It was found that reduction of fasting blood glucose level started after 1st week of treatment and it maintained up to four weeks. The hypoglycemic activity continued as long as the drug was given, therefore, we suggested that TMF 28 had blood glucose lowering effect on type 2 diabetic patients.

Then, subjects were separated into two groups; group 1 with FBS above 180 mg/dl (10 participants) and group 2 with FBS below 180 mg/dl (20 patients). Both groups received same dose and duration of TMF 28. There was a decrease in the mean fasting blood sugar from initial 217.26 mg/dl to 162.36 mg/dl (group 1) and from 134.86 mg/dl to 117.51 mg/dl (group 2) after the treatment period. Other studies showed hypoglycemic effect of drug according to different dosage, different duration and combined with other hypoglycemic drugs [11].

For safety of TMF 28, we measured liver enzymes (SGOT, SGPT and ALP), serum bilirubin, serum creatinine and ECG at pre-intervention and post-intervention periods. It was observed that there were no changes in ALP, serum bilirubin, serum creatinine and ECG. Safety measures showed post-intervention increase in SGOT in one patient and post-intervention SGPT change in another patient but their changes were within normal limit.

Therefore, TMF 28 was found to be a safe drug during study period, which needs to be further evaluated for its long-term safety and efficacy as anti-diabetic agent. Some herbal medicines show hypoglycemic effects in type 2 diabetes. However, these findings should be carefully interpreted due to the low methodological quality, small sample size and limited number of trials. In the light of some positive findings, some herbal medicines deserve further examination in high-quality trials.

In summary, this preliminary evidence suggests that TMF 28 had glucose lowering effect on type 2 diabetes mellitus. There is still insufficient evidence to draw definitive conclusion about the efficacy of TMF 28 for diabetes mellitus. The nature of multiple constituent and botanical products has made standardization a challenging task. The available data suggest that it may need further study with adequate designed randomized controlled trial and longer study period.

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Prevalence and risk factors of diabetes mellitus among middle-aged school teachers in Mandalay City

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This cross-sectional, descriptive study was explored prevalence of diabetes mellitus (DM), latent DM and risk factors among 40-60 years old of school teachers in Mandalay City for further strengthening of prevention strategy. Collecting data by interview using structured questionnaires, anthropometry measurement and laboratory investigation of fasting blood glucose level from respective respondents were performed. A total of 522 subjects including 455 women (87.2%) and 67 men (12.8%), gave informed consent to participate in this study. Average age was 49.5 ± 5.1 years. Prevalence of total cases was 15.9% (83/522). Among them, 4.2% (22/522) cases were identified as known cases. Prevalence of newly diagnosed case was 12.2% (61/500). To identify significant risk factors, bivariate and multivariate logistic regression analysis was done using R statistic software. Age, sex, marital status, history of delivery of big baby, family history of DM, smoking, drinking, exercise and BMI were included in modeling of logistic regressions. Age group over 45 (OR=2.69, $p=0.01$), family history in siblings (OR=2.39, $p=0.018$) and BMI: pre-obese (OR=2.08, $p=0.015$) and obese (OR=3.35, $p=0.033$) were identified as major risk factors for DM. Therefore, people with these factors should be treated as a target group for intervention, prevention or early detection of DM.

INTRODUCTION

Diabetes mellitus (DM) is a challenging health problem because of its chronicity and high demands for daily monitoring and care. The highest prevalence of DM in the Southeast Asia Region is between 45 and 65 years of age, the more economically productive age group [1]. Risk factors associated with type 2 diabetes are older age, obesity, family history of the disease, hypertension, dyslipidemia, physical inactivity, and belonging to certain ethnic groups. The greater the number of risk factors a person has, the greater the chance of developing the disease [2].

In 2001, a study on antidiabetes traditional medicine was conducted in Mandalay City among 522 school teachers of both sexes,

aged 30-60 years. It showed that the proportions of DM among male school teachers were 3.2% and 4% at age 40-50 and 51-60 years, respectively. The rates of DM among female school teachers were 2.3%, 3.2% and 7% at the age groups of 30-40, 41-50, 51-60 years, respectively. Present study aimed to identify the prevalence rate and the associated risk factors of DM among middle-aged school teachers in Mandalay City. The outcome of the study would be utilized as baseline data for prevention and control of DM.

MATERIALS AND METHODS

Study population

The study was conducted in five townships of Mandalay City in the middle-aged (40-60 years) school teachers. Sample size was

calculated by using Epi-info 6 software for descriptive study using simple random sampling with the population size of 1834 teachers. The prevalence of diabetes was assumed to be about 7%. It is estimated that for the prevalence of DM with a precision level of $\pm 2.4\%$, the required sample size is 446 in number.

The sampling frame was constructed by making a list of teachers ranging from 40 to 60 years of age from the register of high school teachers in Mandalay City under the Department of Basic Education No. 2. Subjects were chosen by simple random sampling from the sampling frame. Randomizing was done by using random number generated from Microsoft Excel version 2003. If a chosen subject did not give consent or had to be excluded by the criteria, the subject was replaced by the second nearest person of the same sex working in the same school.

Data collection was done from 17 November 2008 to 30 January 2009. It was a community and laboratory-based, cross-sectional, descriptive study. The questionnaire consisting of 13 questions were explored. Those with a known history of DM and hypertension were included. Two millilitres of blood were withdrawn from each subject after obtaining their informed consent, and glucose analysis was done. A total of 522 blood samples were analyzed for the study.

The study was accepted by the Ethical Committee on Medical Research (Upper Myanmar) on 12th September, 2008.

Measurement

Height (centimeters) and weight (kilograms) were measured and BMI was calculated. Blood samples were collected in sodium fluoride tubes after at least 8 hours of fasting and glucose analysis was done on the same day at the Biochemistry Research Division, Department of Medical Research (Upper Myanmar). Plasma glucose levels were determined by the glucose-oxidase method as recommended by WHO using

Spectrophotometer UV-1601 PC Shimadzu, Japan [3].

Data entry and analysis

Data entry was done using Microsoft Excel version 2003. The Microsoft Excel database was transferred into STATA format by using Stat-transfer version 8. After cleaning and checking the consistency of the database, the data were analyzed by using R Statistical software version 2.9.2 and Epicalc package 2.9.1.2. Risk factors were identified using logistic regression modeling.

RESULTS

A total of 522 teachers (455 women and 67 men) participated in the study. Average age $\pm$ SD was 49.5 ± 5.1 years with a range of 40-60 years. The mean glucose level of participants ($n=522$) was 113.7 ± 38.2 mg/dl with a range of 70-390 mg/dl. Among 522 participants, 83 were shown to be positive for DM. Therefore, the prevalence of DM was estimated to be 15.9% among school teachers in Mandalay City. Of the total subjects, 4.2% (22 cases) already known that they were diabetics while 11.7% (61 cases) did not know they had the disease. Proportion of newly identified diabetics was 2.8 times higher than that of previously diagnosed subjects.

Bivariate analysis of the association between blood glucose and age, sex, hypertension, family history of father, family history of mother, family history of siblings, father's relatives, mother's relatives, smoking, alcohol, exercise and BMI are shown in Table 1.

In this study, significantly higher prevalence of DM was found in the subjects with the age of 45 years and above. Number of female school teachers was more than that of male school teachers in the study population. Among 455 female school teachers, 69 (15.2%) were diabetics, compared with 14 out of 67 male school teachers (20.9%).

Significantly higher prevalence of DM was also found among married persons, those

Table 1. Prevalence of diabetes among subjects

Variables	Total n=522	No. of diabetes (%)	OR (95%CI)	p
Age (years)				0.004
<=45	127	10 (7.9)	1	
>45	395	73 (18.5)	2.65 (1.30-5.95)	
Sex				0.231
Male	67	14 (20.9)	1	
Female	455	69 (15.2)	0.68 (0.35-1.40)	
Marital status				0.024
Single	209	24 (11.5)	1	
Ever married	313	59 (18.8)	1.79 (1.05-3.12)	
History of delivering big baby				0.112
No	212	33 (15.6)	1	
Yes	50	13 (26.0)	1.9 (0.84 - 4.15)	
Other (male, un-married women)	260	37 (14.2)	0.9 (0.52 - 1.55)	
Hypertension				0.031
No	372	51 (13.7)	1	
Yes	150	32 (21.3)	1.7 (1.01-2.85)	
Family history in father				0.009
No	469	68(14.5)	1	
Yes	53	15(28.3)	2.32 (1.12-4.61)	
Family history in mother				0.231
No	466	71(15.2)	1	
Yes	56	12(21.4)	1.52 (0.69-3.1)	
Family history in siblings				<0.001
No	451	59(13.1)	1	
Yes	71	24(33.8)	3.38 (1.84-6.13)	
Family history in paternal side				0.018
No	478	70(14.6)	1	
Yes	44	13(29.5)	2.44 (1.11-5.08)	
Family history in maternal side				0.058
No	475	71(14.9)	1	
Yes	47	12(25.5)	1.95 (0.88-4.07)	
Smoking				0.989
No	497	79(15.9)	1	
Yes	25	4 (16)	1.01 (0.24-3.1)	
Alcohol				0.773
No	488	77 (15.8)	1	
Yes	34	6 (17.6)	1.14 (0.37-2.94)	
Exercise				0.405
No	372	56(15.1)	1	
Yes	150	27(18.0)	1.24 (0.72-2.1)	
BMI				0.013
Normal	335	42(12.5)	1	
Pre-obese	162	34 (21.0)	1.85 (1.09-3.13)	
Obese	25	7 (28)	2.7 (0.9-7.3)	

who have the history of hypertension, those with the diabetic father, sibling or paternal relative and those who were obese.

No association was found between smoking, alcohol or exercise and diabetes mellitus in our study. As 22 subjects were already known to be DM, only 500 subjects were

analyzed for the determination of risk factors in multivariate analysis.

The abovementioned variables, together with results of logistic regression model in association between diabetes and selected factors are shown in Table 2.

Table 2. The association between diabetes and selected factors as calculated by logistic regression method

Factors	Adj. OR (95%CI)	p (Wald's test)	p (LR test)
Age			
Above 45 vs. 40-45	2.69 (1.17, 6.15)	0.019	0.010
Gender marital: ref.= Male single			0.034
Female single	1.16 (0.14, 9.6)	0.888	
Male ever-married	4.39 (0.51, 37.96)	0.179	
Female ever-married	1.36 (0.17, 11.11)	0.774	
without history of big baby			
Female ever-married	2.56 (0.29, 22.86)	0.399	
with history of big baby			
Family history in siblings	2.39 (1.16, 4.91)	0.018	0.024
ref.=Yes vs. No			
BMI ref =Normal			0.017
Pre-obese	2.08 (1.15, 3.77)	0.015	
Obese	3.35 (1.1, 10.18)	0.033	

History of hypertension, history of having DM in mother and her relatives, some risk factors such as smoking, alcohol drinking and doing exercise have been related to DM in many other studies; however, these variables were not identified as risk factors in our population.

The significant relationship between getting DM and family history in mother, smoking, alcohol and exercise in univariate analysis were lost, when adjusted for the other variables in the multivariate model. Age, family history and BMI were found as significant predictors.

Although composite interaction-term variable of gender, marital status and history of delivering big baby was significant at LR test, all these three factors were not likely to increase ORs for DM individually since Wald's test p values were larger than 0.05.

DISCUSSION

In our study, prevalence of diabetes was explored among school teachers population in Mandalay City. The prevalence of diabetes was 15.9% in this particular study. The prevalence of known diabetes was 4.2% and that of previously undiagnosed diabetes was 11.7%, so that the proportion of undiagnosed diabetes was 2.8 times greater than that of previously known cases. This is probably due to teachers' lack of knowledge, possibly thinking the symptoms (i.e., thirst, diuresis, less sensation in lower limbs) were due to their work load and not realizing that these were symptoms of the disease. Similar findings on adult Moroccan Sahraoui women were reported [4]. In univariate analysis, the higher prevalence in subjects above the age of 45 found in our study is similar to the findings of a study from Turkey [5], indicating the higher prevalence among males.

Although we found a relationship between reported history of hypertension and DM in univariate analysis which was similar to the study in Turkey, the evaluation of blood pressure cannot be considered solely as a risk factor. The gradual downgrading in renal function in diabetic patients may lead to secondary hypertension according to natural history of disease. Hypertension was, therefore, not included as a potential risk factor for diabetes in the multivariate analysis. DM is known to have a hereditary component.

We did not find any association between smoking, alcohol or exercise and DM among school teachers despite these variables having being reported in other studies to be related with DM. The low proportion of teachers who smoke or drink alcohol may have limited the power to detect any association.

It was found that DM is associated with age (above 45), BMI (>25), and family history in siblings. Although gender and reproductive

life were associated with DM in logistic regression model, we could not find any statistical significance between DM and individual factors (such as gender, marital status and history of delivering a big baby). The high proportion of obese among our school teachers is of concern. This may be related to the sedentary nature of life style. Reducing BMI may be an important component of any strategy in reducing the risk of DM among this population.

Conclusion

The risk factors of DM among teachers are multi-factorial. Among them, BMI is potentially modifiable risk factor and appropriate body weight should, therefore, be maintained to help reduce the risk of DM. The rest, non-modifiable risk factors such as age (above 45), family history in siblings, are also significantly associated with DM in our study. Therefore, people with these factors should be treated as a target group for intervention for prevention or early detection of DM.

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Adiponectin in relation to lipid profile in type 2 diabetes mellitus patients

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Adiponectin (ADPN) has demonstrated insulin-sensitizing, anti-atherogenic, and anti-inflammatory properties, and may be an important risk factor for coronary heart disease and diabetes. Plasma adiponectin (PADPN) level is reduced in middle-aged obesity and in patients with type 2 diabetes mellitus and coronary artery disease. The purpose of this study was to investigate the association of PADPN level to lipid profile in T2DM patients. Twenty-eight apparently healthy volunteers and 30 T2DM patients participated in the present study. The determination of PADPN, lipid profile (serum total cholesterol, serum low density lipoprotein cholesterol, serum triglyceride and serum high density lipoprotein cholesterol), baseline characteristics, anthropometric and clinical examination was done in both groups. The mean PADPN was significantly higher in women (19.46 ± 4.29 $\mu\text{g/ml}$) than in men (11.8 ± 1.36 $\mu\text{g/ml}$) in apparently healthy subjects ($p < 0.001$). The mean PADPN value of T2DM males was significantly lower than T2DM females (males, 4.52 ± 1.1 $\mu\text{g/ml}$, and females, 7.19 ± 0.99 $\mu\text{g/ml}$, $p < 0.001$). The mean PADPN value of male T2DM patients (4.52 ± 1.1 $\mu\text{g/ml}$) was significantly ($p < 0.001$) lower than that of control males (11.8 ± 1.36 $\mu\text{g/ml}$). Mean PADPN value of T2DM female patients (7.19 ± 0.99 $\mu\text{g/ml}$) was also significantly lower than that of control females (19.46 ± 4.29 $\mu\text{g/ml}$; $p < 0.001$). In a multivariable analysis, serum HDL concentration was positively correlated with PADPN ($r = 0.55$, $p < 0.001$) and BMI ($r = -0.432$, $p < 0.05$) and serum TG concentration were negatively correlated with PADPN ($r = -0.429$, $p < 0.05$) in T2DM patients. When analyzed according to two categories of PADPN concentration, T2DM patients with hypoadiponectinemia (PADPN level < 6 $\mu\text{g/ml}$) were significantly higher in body weight ($p < 0.05$) and in serum TG ($p < 0.05$), but significantly lower in serum HDL ($p < 0.01$) than T2DM patients with PADPN level ≥ 6 $\mu\text{g/ml}$. These results showed that increased ADPN levels might be associated with better lipid profile in normal and in diabetic subjects and hypoadiponectinemia (PADPN level < 6 $\mu\text{g/ml}$) is significantly associated with cardiovascular risk factors such as atherogenic lipid profiles in T2DM patients.

INTRODUCTION

Dyslipidemia is a major cardiovascular risk factor in type 2 diabetes mellitus (T2DM) with coronary heart disease (CHD) which is the most common cause of death. According to the WHO, the prevalence of T2DM with CHD increases worldwide each year due to profound changes in diet and lifestyle of the population. In Myanmar, the prevalence of diabetes mellitus increases yearly. There was 6.8% of total population in 1992 and it increased up to 6.9% by the year 2002 and the

overall mortality being 2% of total patients [1]. The coexistence of obesity, glucose intolerance, dyslipidemia, and hypertension, is termed as insulin resistance syndrome (metabolic syndrome). The association of hyperinsulinemia with hypertriglyceridemia and low levels of serum high density lipoprotein cholesterol (HDL-C) was reported [2]. A study in Myanmar pointed out that high level of total cholesterol (TC), high level of total triglyceride (TG) and low level of HDL-C were associated with the high risk of diabetes mellitus [3].

Adiponectin (ADPN) is a protein specifically and abundantly expressed by adipocytes, which is important in metabolic and vascular diseases, has been reported to exhibit protective effects against atherosclerosis as well as an insulin-sensitizing effect. One study stated that this adipocyte protein seems to play a protective role in experimental models of vascular injury, perhaps because it suppresses the attachment of monocytes to endothelial cells [4]. Hypoadiponectinemia is caused by interactions of genetic factors such as small nuclear proteins in the ADPN gene and environmental factors causing obesity. The ADPN receptors, adipoR1 and adipoR2, which mediate the anti-diabetic metabolic actions of ADPN have been cloned and are downregulated in obesity-linked insulin resistance. Upregulation of ADPN might be a mechanism of the insulin-sensitizing and anti-diabetic actions of thiazolidinediones [5]. Furthermore, adiponectin concentrations are found to be lower in diabetics than in non-diabetics.

ADPN is found abundantly in the circulation accounting for approximately 0.01% of total plasma protein [6]. Plasma adiponectin (PADPN) concentrations seem to be gender-dependent, being higher among women than men [7]. Exercise and proper diets help to increase level of ADPN [8]. Serum ADPN is also important to maintain energy homeostasis in energy shortage conditions, such as during female pubertal development. Although various studies in different populations have demonstrated the association of PADPN to plasma lipid profile, it is still unknown in Myanmar population. This study investigated the plasma ADPN in apparently healthy Myanmar subjects to define the baseline reference values. The study was also extended to have further information on the effect of plasma ADPN level in some clinical situation such as T2DM.

General objective:

- To find out the relationship between plasma adiponectin and lipid profile in patients with type 2 diabetes mellitus.

Specific objectives

- To determine the PADPN level in apparently healthy control adults and patients with T2DM of different sexes.
- To determine lipid profile, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride concentration of apparently healthy control adults and T2DM patients.
- To compare the plasma ADPN level between T2DM patients and a group of the control adults in same age and gender.
- To find out the association between plasma ADPN level and lipid profile in both control and T2DM patients.

MATERIALS AND METHODS

Study design

A cross-sectional, hospital-based, analytical study.

Study population and study area

This study was carried out on 28 healthy volunteers (14 males and 14 females) attending the North Okkalapa General Hospital (NOGH) and out-patient department of Thingangyun Sanpya Hospital (TSPH) and 30 T2DM patients comprising 15 males and 15 females from out-patient and in-patient Medical Ward in NOGH and TSPH in Yangon. The informed consent was obtained from healthy volunteers and T2DM patients.

Inclusion criteria

Healthy volunteers and T2DM patients

- Both sexes
- Ages between 30 and 60 years
- Screening fasting blood sugar was <126 mg/dl or 7 mmol/L for healthy volunteers and >126 mg/dl or 7 mmol/L for T2DM patients

Exclusion criteria

Healthy volunteers

- One who has significant renal or hepatic disease, cardiovascular disease,

uncontrolled endocrine disease and diabetes mellitus

- Taking treatment for hypertension receiving anti-coagulants, hypolipidemic drug, oral contraceptives
- Chronic alcoholism
- Pregnant and lactating women
- Subject with any history of mental illness

T2DM patients

- Taking treatment with thiazolidione
- Patients with significant renal or hepatic diseases, infectious diseases and other uncontrolled endocrine diseases

Procedures

Subject selection

Subjects were selected according to inclusion and exclusion criteria. They were recruited between February-April, 2006 at the Medical Ward of NOGH/TSPH.

Personal history and clinical measurements

Subjects were assessed for questionnaire history taking, clinical measurements and collection of blood for laboratory measurements.

History taking and physical examination were done according to the proforma of the study. Height, weight and BMI were measured and data were recorded.

Blood sample collection and laboratory assays

An overnight fasting venous blood sample (total 10 ml) was collected with a sterile disposable vacutainer for determination of biochemical parameters. First of all, 3 ml of venous blood sample were withdrawn into a tube containing EDTA disodium anti-coagulant (for determination of plasma ADPN) and then, 4 ml of venous blood were put into sterile test tube to analyze fasting serum lipid profile. Finally, 3 ml of blood were put into the test tube containing sodium fluoride anticoagulant for determination of fasting glucose. After the collection, the blood samples in the ice packed container were transported to the Common Research Laboratory of the

University of Medicine 2. In the laboratory, they were centrifuged at 2000 rpm at 4°C for 10 minutes. The plasma was separated within 3 hours of collection. Plasma obtained from EDTA blood for the analysis of ADPN was stored at -80°C. The serum obtained from 4 ml of blood was separated and stored at -20°C for the measurement of serum lipid profile. The fasting glucose and serum lipid profile were determined immediately after the separation of plasma and serum on the day of collection. The plasma ADPN level was determined by enzyme-linked immunosorbent assay (ELISA) kit developed by B-Bridge International Inc. (USA) with a co-efficient of variation of 6.4% [9] after the three-month period when the sample collection was completed.

Data analysis

Data were itemized and coded manually. All calculations were performed by using computer-based statistical package of statistical product and service solution (SPSS) software version 13. Descriptive statistics of numerical variables were expressed as mean and standard deviation. Description of categorical variables was expressed as percentage. Difference between each group was examined by the unpaired Student's 't' test. Pearson correlation coefficients were calculated to evaluate a relationship between plasma ADPN levels and study variables such as age, BMI, lipid profiles and fasting glucose. Level of significance was set at $p < 0.05$.

Ethical consideration

The study was approved by the Research and Ethical Committee, University of Medicine 2, Yangon.

RESULTS

Table 1 shows the baseline characteristics of controls and T2DM patients studied were analyzed for comparison. Regarding race and religion, all were Myanmar and Buddhists. Mean BMI of the control females was significantly higher than that of

male control ($p<0.001$). The mean BMIs of male and female T2DM patients were statistically not significant. Smoking habit and family history of diabetes were found to be more in T2DM patients than in control subjects. Hypertension and ischaemic heart disease were also found only in T2DM patients.

Table 1. Baseline characteristics of control subjects and T2DM patients

Demographic characteristics	Control subjects		T2DM patients	
	Male n=15	Female n=15	Male n=15	Female n=15
Age in years	44.43 ± 11.20	45.5 ± 9.52	44.67 ± 2.61	45.0 ± 2.41
Race	Myanmar	Myanmar	Myanmar	Myanmar
Religion	Buddhist	Buddhist	Buddhist	Buddhist
<i>Personal characteristics</i>				
Weight (kg)	52.36 ± 6.33	51.79 ± 1.32	58.00 ± 6.80	53.47 ± 5.30
Height (meter)	1.63 ± 0.04	1.54 ± 0.06	1.60 ± 0.05	1.54 ± 0.06
BMI	19.69 ± 2.10	21.76 $\pm 1.99^*$	22.80 ± 2.65	22.62 ± 2.03
Current smoking	2 (14.3%)	2 (14.3%)	6 (42.9%)	4 (28.6%)
Family history of diabetes	4 (28.6%)	2 (4.3%)	7 (50%)	10 (71.4%)
Other medical history	0	0	2.60 ± 1.40	1.80 ± 1.70
Duration of diabetes in years			11 (78.6%)	8 (57.1%)
Hypertension			4 (28.6%)	2 (14.3%)
Ischaemic heart disease			2 (14.3%)	1 (7.1%)
<i>Oral hypoglycaemic drugs</i>				
Sulphonyl ureas and biguanides			0	2 (14.3%)

Results are shown as mean $\pm$ SD and percentage

When comparing between control male and female subject, the respective parameters showed no significant difference between them except PADPN value which was significantly higher in female ($p<0.001$) than in male subjects. The same results were seen between T2DM male and female patients. The mean serum TC, serum LDL, serum TG and FBG value of T2DM male patients were significantly higher than those of control males ($p<0.001$). There was the same finding in comparison of mean values of serum TC, serum LDL, serum TG and FBG and PADPN level between control females and T2DM females (Table 2).

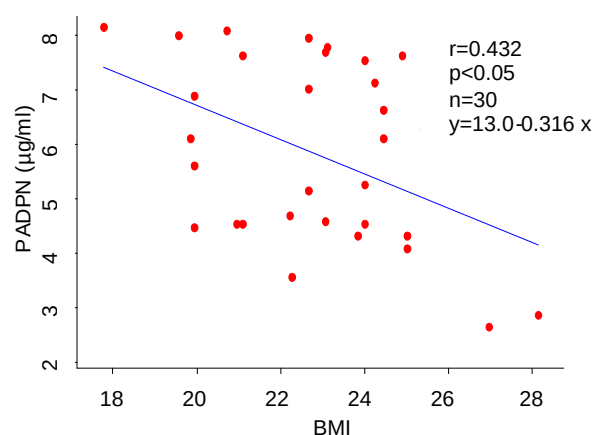


Fig 1. Correlation between PADPN and BMI in T2DM patients

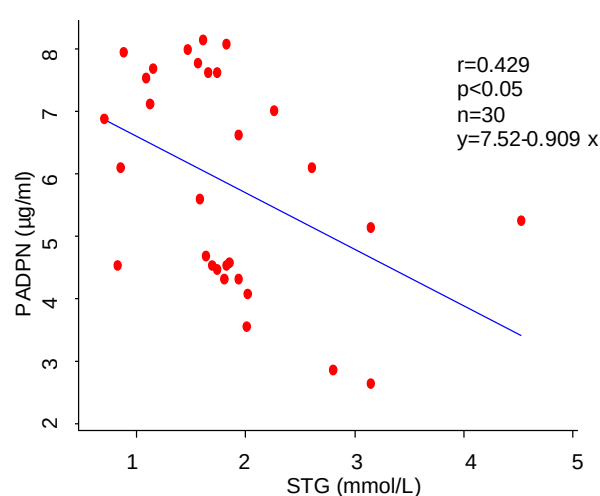


Fig 2. Correlation between PADPN and serum TG in T2DM patients

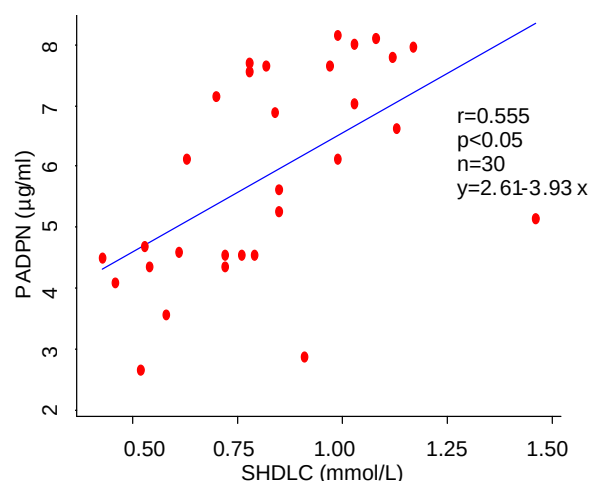


Fig 3. Correlation between PADPN and serum HDL in T2DM patients

Correlation between PADPN levels and various parameters of control subjects are shown in Fig. 1, Fig. 2 and Fig. 3.

Table 2. Comparison of FBG, serum lipid profile and plasma ADPN level between control male and female subjects, between T2DM male and female patients, between control male and T2DM male and between control female and T2DM female

	Control subjects		P ₁	T2DM patients		P ₂	P ₃	P ₄
	Males n=15	Females n=15		Males n=15	Females n=15			
Serum TC (mmol/L)	4.23±0.84	4.24±0.97	0.977	6.78±1.03	7.21±1.25	0.315	<0.001*	<0.001*
Serum HDL (mmol/L)	1.09±0.37	1.37±0.59	0.145	0.66±0.15	0.99±0.19	0.000	<0.001*	0.026*
Serum LDL (mmol/L)	2.69±0.94	2.42±1.30	0.535	5.32±1.21	5.35±1.16	0.952	<0.001*	<0.001*
Serum TG (mmol/L)	0.99±0.47	0.99±0.33	1.000	1.76±0.66	1.90±0.94	0.630	0.001*	0.002*
FBG (mmol/L)	4.06±0.52	4.11±0.63	0.812	7.94±2.02	8.33±2.57	0.650	<0.001*	<0.001*
PADPN (µg/ml)	11.80±1.36	19.46±4.29	<0.001*	4.52±1.10	7.19±0.99	<0.001*	<0.001*	<0.001*

Results are shown as mean±SD , *=Statistically significant

P₁= The probability level in comparison between control male and female mean values

P₂ = The probability level in comparison between T2DM male and female mean values

P₃ = The probability level in comparison between control male and T2DM male mean values

P₄ = The probability level in comparison between control female and T2DM female mean values

There was negative correlation between PADPN and BMI ($r=-0.432$, $p<0.05$) in T2DM patients (Fig.1) and between PADPN and serum TG ($r=-0.429$, $p<0.05$) in T2DM patients (Fig. 2). The positive correlation was found between PADPN and serum HDL ($r=0.55$, $p<0.001$) in T2DM patients (Fig. 3). Table 3 shows the association of physical and biochemical characteristics by PADPN level in T2DM patients. T2DM patients with hypoadiponectinemia (PADPN level <6 µg/ ml) were significantly more in weight ($p<0.05$) and in serum TG ($p<0.05$), but significantly lower in the serum HDL than T2DM patients with PADPN level >6 µg/ml ($p<0.01$)

Table 3. Association of biochemical characteristics by PADPN level in T2DM patients

	PADPN level		P
	<6 µg/ml (n=15)	>6 µg/ml (n=15)	
	mean±SD	mean±SD	
Wt (kg)	58.47±6.52	53±5.18	0.017*
Ht (m)	1.59±0.05	1.55±0.06	0.051
BMI	23.26±2.39	22.16±2.2	0.198
Serum TC (mmol/L)	6.96±1.22	7.03±1.1	0.875
Serum HDL(mmol/L)	0.72±0.26	0.94±0.17	0.009*
Serum LDL(mmol/L)	5.26±1.11	5.41±1.25	0.73
Serum TG (mmol/L)	2.17±0.9	1.50±0.54	0.019*
FBG (mmol/L)	8.40± 2.25	7.87±2.36	0.539

Results are shown as mean±SD

* =Statistically significant

DISCUSSION

ADPN, synthesized in the adipose tissue, appears to play an important role in hyperglycemia and dyslipidemia, as well as in inflammatory mechanisms, which lead to a markedly increased atherosclerotic risk in diabetic subjects. In the present study, most of all in the study groups were Myanmar and Buddhists, following the usual race and religion distribution of the country. There were some reports of racial difference of type 2 diabetes in other countries, mostly common in American Indians [10].

Smoking habit was seen only in 4 out of 28 control subjects and 10 out of 30 T2DM patients and it was less than that of Western and Asian studies [11]. This may probably be due to the smoking habit of Myanmar which is less than that of other Westerners and Asians. The mean duration of diabetes in the study group was short ranging from 0 to 6 years. This short duration may be due to the fact that most of the patients (19 out of 28) in the study group were newly diagnosed patients. Regarding complications of T2DM patients in this study, 19 patients got hypertension and 4 had IHD. The increased number of hypertensive patients is not surprising because hypertension is the first and most common macrovascular complication for the diabetic patients.

In the present study, the mean BMI of the T2DM patients was higher than that of control subjects in both sexes. However, the significant difference was found only in males between control and patient groups. The BMIs of T2DM were less than 25, indicating that most of the Myanmar T2DM patients were not obese. Mean BMIs of T2DM patients and control subjects in both sexes were less than those of international studies. Level of BMI in Myanmar T2DM is lower than Western and other Asian data. This may be probably due to the genetic factor and environmental factors such as racial difference, dietary pattern and life style.

Higher BMI seen in T2DM of the study group could be explained by the consequences of increased insulin resistance [12]. Abnormalities in lipid metabolism are thought to contribute to atherosclerotic vascular damage in diabetes. Diabetic subjects of both sexes in present study were found to have a higher level of STC and SLDL cholesterol than the control subjects ($p < 0.05$). Some surveys on patients with type 2 diabetes mellitus have demonstrated the contradictory results that show a little difference in STC and serum LDL cholesterol level as compared with those of non-diabetic patients [13].

The higher or normal level of LDL in T2DM patients had impaired LDL catabolism which could be due to a reduction of the number of LDL receptors or decreased affinity for their receptors and secondary to apoB glycation leading to the formation of small dense LDL particles and foam cells and the increased number of plasma oxidized LDL particles [14].

Oxidized LDLs stimulate the formation by macrophages of cytokines, such as tumour necrosis factor α (TNF α) or factor interleukin 1 (IL1), which amplify the inflammatory atherosclerotic process. The results of serum TC and LDL obtained from the present study agreed that the above explanation of the significant kinetic abnormalities in LDL particles can be a high risk factor for the T2DM patients.

In the present study, the HDL level of T2DM patients of both sexes was significantly lower than that of the normal control subjects. One of the main reasons for increased catabolism of HDL particles in insulin-resistant states and type 2 diabetes is the increased pool of triglyceride-rich lipoproteins (mainly VLDL) which drives, through CETP, leading to the formation of triglyceride-rich HDL particles. A statistically significant low level of serum HDL seen in T2DM patients of the present study could be attributed to the above facts.

In the present study, mean serum triglyceride level of T2DM patients was significantly higher than that of control (both males and females). Hypertriglyceridemia predominates due to the lack of insulin, reduced lipoprotein lipase activity and thereby decreased clearance of circulating triglycerides and low HDL levels [15].

Adiponectin, the adipose-specific proteins, is known to correlate negatively with insulin resistance in patients with obesity and type 2 diabetes. In the present study, the mean PADPN level in T2DM patients was significantly lower than that of normal control subjects of same age and sex ($p < 0.001$). The PADPN levels of healthy control females and T2DM female patients were significantly higher than those of males of both study groups. PADPN levels were predominantly determined by visceral fat tissue instead of subcutaneous fat, therefore, these sex differences of PADPN levels are explained by the body fat distributions in which women tend to have less visceral fat than subcutaneous fat. Sexual dimorphism in PADPN is also explained by the inhibitory effect of male sex hormone on ADPN gene expression [16].

In this study, one of the drawbacks is limitation of the number of subjects which may not represent the whole population. For this reason, although there was a sex difference of PADPN level in control and

patient groups, association and correlation analysis of PADPN and lipid profile between control and T2DM was done only on total number of study groups. In the present study, the PADPN of T2DM patients was positively correlated with serum HDL level which is in accordance with a previous report [17]. These facts suggest that adiponectin plays an important role not only in insulin resistance but also in atherosclerotic cardiovascular disease.

Hypoadiponectinemia may be directly linked to early atherosclerotic vascular damage and subsequent endothelial dysfunction [18]. In this study, among the cut-out points (3 µg/ml, 4 µg/ml, 5 µg/ml or 6 µg/ml) postulated for hypoadiponectinemia that significantly associated with dyslipidemia was found only with 6 µg/ml. With this cut-out point, the significant results on association of biochemical characteristics by PADPN could be obtained only with both sexes combined (total number of patients) although there was a sex dimorphism of PADPN in the present study. The apparent results which were related with gender difference could not be obtained because of the few number of male patients with PADPN >6 µg/ml and the few number of female patients with PADPN <6 µg/ml.

In the present study, type 2 diabetes mellitus patients with hypoadiponectinemia (PADPN level <6 µg/l) were significantly more in weight and serum TG, but significantly lower in serum HDL levels than T2DM patients with PADPN level >6 µg/ml. The serum FBG level was more (but not significantly different) in T2DM patients with PADPN level <6 µg/ml.

In conclusion these findings suggest that lower plasma ADPN levels are significantly associated with cardiovascular risk factors such as insulin resistance (high FBG level) and atherogenic lipid profiles (increased serum TG and decreased serum HDL concentration) in T2DM patients and increased adiponectin levels might be valuable target for decreasing the atherosclerotic risk present in diabetes.

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Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency in Chin State

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The oxidant drug primaquine is used to get complete cure from *Plasmodium vivax* infection. Primaquine can cause haemolysis in severely deficient G6PD enzyme activity. The laboratory facility to detect the G6PD enzyme activity is limited. The aim of this research was to find out the prevalence of G6PD enzyme deficiency in Chin race. A total of 408 healthy adults (147 male subjects and 261 female subjects) from Hakha Township, Chin State were recruited in June 2009. These subjects were not related to each other. Blood samples of the male subjects were tested by methaemoglobin reduction test (Brewer's test) followed by agarose gel electrophoresis. G6PD enzyme deficiency is the X-linked recessive gene disorder, which is hidden in female heterozygous carrier. The cytochemical staining method can detect the female heterozygous carrier. In male subjects, 4.76% (n=7) had enzyme deficiency and among them, 0.68% (n=1) had severe deficiency. In female subjects, 2.68% (n=7) had enzyme deficiency and among them, 0.38% (n=1) had severe enzyme deficiency.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is relatively common in populations exposed to malaria. In Myanmar, 15-17% of people (both male and female) living in malaria endemic areas have G6PD enzyme deficiency [1]. This deficiency appears to provide some protection from malaria infection, but it can also cause haemolysis after administration of some antimalarial drugs. The risk of drug-induced G6PD deficiency-related haemolysis depends on a number of factors including the G6PD variants, the drug and drug dosage schedule, patient status and disease factors. [2]

In Myanmar, about 80% of the population are residing in malaria transmission areas [3] and the incidence of *Plasmodium vivax* is 21.55% in malaria confirmed cases in 5 years average 2001-2005 [4]. In chemotherapy of *Plasmodium vivax* infection, the

oxidant drug primaquine is required for the elimination of this infection. The risk of relapse of *Plasmodium vivax* malaria without primaquine therapy ranged from 5% to 80% or more depending largely upon geographic location [5]. Haemolytic anaemia after administration of the antimalarial drug primaquine (also known as plasmoguin and plasmochin) was reported as early as 1926 [6]. However, it was not until the 1950s that a series of investigations by United States Army researchers identified G6PD enzyme deficiency was the cause of haemolysis after the administration of the related antimalarial primaquine [7]. Different variants of G6PD enzyme deficiency are found in high frequencies in African, Mediterranean and Asiatic populations. WHO classified the G6PD enzyme activity in five groups [8]. Class I includes severely deficient variants that are associated with chronic non-spherocytic haemolytic anaemia. Class II variants have less than 10% of residual

enzyme activity, but are not associated with chronic spherocytic haemolytic anaemia. Class III are moderately deficient (10-60% of enzyme activity), Class IV has normal enzyme activity and in Class V, the enzyme activity is increased.

In G6PD classification of Myanmar, there are 3 types of G6PD variants. Gd^{B+} has normal enzyme activity. Gd^{B-} has 10-60% enzyme activity and $Gd^{Myanmar}$ has less than 5% of enzyme activity [9]. In Myanmar, many researchers and scientists have carried out the research on the prevalence of G6PD enzyme deficiency. Its prevalence in male was 9% in Bamar, 4% in Mon, 14% in Kayin, 8% in Kachin and overall incidence was 9% [10]. In another study, its prevalence rate was 5.5% in Bamar, 3.3% in Mon, 5.1% in Shan, 6.7% in Kayin, 3.2% in Indian and 3.4% in Chinese. [11]. The prevalence of G6PD enzyme deficiency in Kayar, Chin and Rakhine are yet to be studied. Hence, in order to find out the prevalence of G6PD enzyme deficiency in Chin national race, a cross-sectional descriptive study was conducted on the unselected, unrelated healthy 18 years old and above males and females who were born from Chin race.

Objectives

- To find out the G6PD enzyme deficiency in healthy male subjects in Chin State
- To determine the G6PD enzyme activity of these subjects according to the WHO classification
- To differentiate heterozygote and homozygote in G6PD enzyme deficient female subjects in Chin State

MATERIALS AND METHODS

This study was done in Hakha Township of Chin State in June 2009. Sample size determination in male subjects was calculated by basing upon the assumption of G6PD deficiency occurrence in nearly 10% of the population in Myanmar, the required

sample size was 138 at 95% confidence level and 5% precision.

G6PD enzyme deficiency is the X-linked recessive genes disorder, hidden in female heterozygous carrier. The prevalence of hemizygous male (q) is nearly 10% and according to Hardy-Weinberg Equilibrium, the prevalence of female carrier is $2q=20\%$ [12]. Based upon the assumption of G6PD deficiency occurrence in nearly 20% of the female population in Myanmar, the required sample size was 246 at 95% confidence level and 5% precision. According to that sample size calculation, 147 male subjects and 261 female subjects were recruited.

Biochemical tests

For male subjects

- 1) Methaemoglobin reduction test (Brewer's Test) [13] was performed to detect whether the person was deficient or not.
- 2) Agarose gel electrophoresis, a semi-quantitative test [9] was carried out for the detection of G6PD enzyme activity according to WHO classification. That agarose gel electrophoresis revealed three genotypes in the subjects namely Gd^{B+} , Gd^{B-} and $Gd^{Myanmar}$. After staining with enzyme substrate solution, Gd^{B+} gave a deep purplish blue colour (normal enzyme activity) and Gd^{B-} type gave a faint colouration (mild deficient type, 10-60% enzyme activity) while $Gd^{Myanmar}$ showed no band (severely deficient type, less than 5% of enzyme activity).

For female subjects

The differentiation of homozygote and heterozygote in female was carried out by sensitive cytochemical staining method for G6PD activity in individual erythrocytes [14]. In that method, considerable formazan (yellowish brown) production occurs in most erythrocytes of a healthy person and only a small percentage of the cells appeared to be negative. Two cells populations of one completely negative and other with a variable amount of formazan per cell in almost equal size would be discerned in

heterozygote for G6PD deficiency. Homozygous deficiency leads to a population of negative cells with a few positive cells after staining.

The ethical clearance was obtained from the Institutional Ethical Review Committee, Department of Medical Research (LM) in 2008.

RESULTS

A total of 408 healthy adults (147 male subjects and 261 female subjects) were recruited for the screening of G6PD enzyme deficiency and the enzyme activity. In male subjects, 7 had deficient enzyme activity and among these subjects, 6 subjects had mild deficiency and one had severely deficient enzyme activity according to WHO classification (1989) (Table 1).

Table 1. Results of methaemoglobin reduction test (Brewer's test) and agarose gel electrophoresis in seven G6PD deficient male subjects

Code no.	Methaemoglobin reduction test	Agarose gel electrophoresis (Band density)	G6PD variants	WHO classification of G6PD enzyme activity (%)
CM 38	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
CM 39	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
CM 44	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
CM 65	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
CM 90	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)
CM 109	Deficient	Severe (no band)	Gd ^{Myanmar}	Class II (<10)
CM 143	Deficient	Mild (faint blue)	Gd ^{B-}	Class III (10-60)

In 261 female subjects, 6 had mild deficiency and one had severely deficient enzyme activity according to the Van Noorden and Vogels method (1985) (Table 2).

Table 2. Results of cytochemical staining method in seven G6PD deficient female subjects

Code no.	Stained erythrocytes (%)	Mild/severe	G6PD variants	WHO classification of G6PD enzyme activity (%)
CF 2	59.2	Mild	Gd ^{B-}	Class III (10-60)
CF27	58.4	Mild	Gd ^{B-}	Class III (10-60)
CF53	55.0	Mild	Gd ^{B-}	Class III (10-60)
CF72	28.2	Mild	Gd ^{B-}	Class III (10-60)
CF99	40.6	Mild	Gd ^{B-}	Class III (10-60)
CF102	4.8	Severe	Gd ^{Myanmar}	Class II (<10)
CF197	58.6	Mild	Gd ^{B-}	Class III (10-60)

Table 3. Sex distribution of G6PD enzyme activity in healthy adult subjects in Chin State

Sex	Total	Normal enzyme activity Gd ^{B+} (%)	Mild enzyme deficiency Gd ^{B-} (%)	Severe enzyme deficiency Gd ^{Myanmar} (%)
Male	147	140 (95.2)	6 (4.08)	1(0.68)
Female	261	254 (97.3)	6 (2.3)	1(0.38)
Total	408			

DISCUSSION

Deficiency of G6PD is the most common known enzyme deficiency and it is estimated to affect 400 million people worldwide, mainly in tropical regions [15, 16]. As the gene encoding G6PD is linked to the X-chromosome, common clinical manifestations such as neonatal jaundice and acute haemolytic anaemia are usually more severe in males. Acute haemolytic anaemia is precipitated by a number of oxidative drugs such as primaquine, by infection or foods, but most of G6PD deficient individuals are clinically asymptomatic and have never had a haemolytic episode. Primaquine is widely used for the radical treatment of *Plasmodium vivax* infection. However, primaquine-induced haemolytic crises in G6PD deficient individuals have caused serious problems and restrictions in chemotherapeutic malaria control activities. Thus, it is important to screen out G6PD deficient individuals before the start of malaria control programme [17].

So far, various screening methods for detection of G6PD deficiency have been reported. WHO recommended that screening procedures are brilliant cresyl blue (BCB) dye test, methaemoglobin reduction test and MTT linked spot test. In this study, the screening of enzyme activity in male subjects was done by methaemoglobin reduction test and followed by semi-quantitative test of agarose gel electrophoresis. In female subjects to detect the heterozygous carrier "A sensitive cytochemical staining method for glucose-6-phosphate dehydrogenase activity in individual erythrocytes" by Van Noorden and Vogels, 1985 was used.

G6PD enzyme deficiency is the X-linked recessive genetic condition. In male subjects, they have only one X chromosome and affect on this only one gene of this chromosome. So, in male subjects, if the X chromosome is not affected, he has the normal genotype and phenotype of normal red blood cell. If the X chromosome of male is affected, he is the hemizygous genotype of G6PD deficiency. The phenotype of G6PD enzyme activity was classified by agarose gel electrophoresis method. Of the 147 male subjects, phenotype of 140 (95.2%) had normal enzyme activity, six (4.08%) were mild deficient and one (0.68%) was severely deficient.

In female subjects, due to their 2X chromosomes, the G6PD enzyme deficiency may be affected on one or both genes. If one chromosome is affected, genotype is heterozygote and if both chromosomes are affected, it is homozygote. Therefore, the genotype of female in G6PD enzyme deficiency may be heterozygote or homozygote. In female subjects, we used the cytochemical staining method and in this method, analysis of enzyme content in red blood cell as normal, heterozygous and homozygous categories were easily distinguished. In our study, out of 261 female subjects, 254 subjects had normal enzyme activity (ie., more than 90% of the red blood cells were stained). Six subjects had from

28.2 to 59.2% enzyme activity and they were heterozygote. Only one had 4.8% enzyme activity and she was a homozygous enzyme deficiency.

In malaria infection, as the oxidant drug primaquine is used as a gametocycidal drug, it can inhibit the malaria transmission in *Plasmodium falciparum* and in treatment of hypnozoite stage (latent stage) of *Plasmodium vivax* infection. The drawback of primaquine usage is massive haemolysis in G6PD deficient persons. The laboratory facilities to detect the G6PD enzyme deficiency are limited in many parts of the country. Investigation of baseline G6PD enzyme activity in each national race is helpful for the management of *Plasmodium falciparum* and *Plasmodium vivax* infections.

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Changes in blood arsenic level and haematological parameters of male subjects living in Kyaungkone Township

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This study was carried out to determine blood arsenic level and its effect on haematological parameters in people who drink arsenic contaminated groundwater in Kyaungkone Township, Ayeyawady Division, Myanmar. Seventy volunteered male subjects of age 18-50 years who have been drinking arsenic-contaminated groundwater for more than 5 years were included in this study. Blood arsenic levels of 41 subjects (58.57% of total study population) were $>0.025 \mu\text{g/ml}$ and thus, they were regarded as high blood arsenic group and those of the remaining 29 subjects (41.43% of total study population) were $\leq 0.025 \mu\text{g/ml}$ and they were regarded as low blood arsenic group. The differential neutrophil count ($p=0.021$) and differential eosinophil count ($p=0.007$) of the high blood arsenic group had significant difference when compared with the low blood arsenic group. There were a significant negative correlation between blood arsenic level and differential neutrophil count and a significant positive correlation between blood arsenic level and differential eosinophil count. RBC count was less than 4.5 million cells/ μl in 25.71% of the total study population, but there was no significant correlation between blood arsenic level and total RBC count. The results of this study suggested that arsenic had toxic effect on haematopoietic system and the effect was seen more so in some blood cells like neutrophils and eosinophils than others. Variability in arsenic content in the drinking water, individual body defense and antioxidant status may also contribute to variation in the manifestations of arsenic toxicity.

INTRODUCTION

Arsenic (As) is widely distributed in the natural environment as a pure element and in combination with sulfur, nickel, iron, copper, lead and other metals in geologic deposits [1]. Groundwater can become contaminated with arsenic through natural geological processes and through mining and smelting of copper [2]. In acute arsenic poisoning, haemoglobinuria, bone marrow depression, severe pancytopenia and basophilic stippling are found [3].

Arsenic contamination of groundwater in Myanmar first came to attention in the year 2000 [4]. Since then, a cross-sectional

survey was conducted to assess the arsenic content of groundwater in three divisions (Ayeyawady, Bago and Mandalay) and three states (Kachin, Shan and Rakhine) [5]. A survey of 25 villages in the Ayeyawady Division during 2002 found high levels of arsenic in 67% of the wells examined [6].

According to data of Occupational Health Division in Myanmar, arsenic content of groundwater in Kyaungkone Township, Ayeyawady Division was higher than the maximum permissible limit of arsenic recommended by WHO (0.01 mg/l) [7]. Since groundwater is the primary source of drinking water in this area, many people in this area drink arsenic-contaminated water.

Amounts of arsenic in groundwater are much too small to pose an immediate health threat. However, if consumed over a long period of time, it can cause adverse health effects.

There may be some associations between blood arsenic level and changes in haematological parameters in people who drink arsenic-contaminated groundwater. The purpose of this study was to find out the relationship between blood arsenic level and changes in haematological parameters in people who drink arsenic-contaminated groundwater in this area. The knowledge of relationship between arsenic exposure and adverse health effects will help in estimation of health risk and prevention of arsenic poisoning in the future among the residents in areas where wells are contaminated with high levels of naturally occurring arsenic.

MATERIALS AND METHODS

Subjects

Seventy apparently healthy male volunteers aged between 18-50 years who have been drinking arsenic-contaminated groundwater for more than 5 years in Kyaungkone Township, Ayeyawady Division, Myanmar were chosen by history taking and clinical examination.

Determination of haematological parameters

Haematological parameters including haemoglobin level (Hb), haematocrit (Hct), total red cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total and differential white blood cell count, and platelet count were determined. These parameters were measured by automatic coulter counter (ABX Micros OT, Serial Number 001-OT 6718). Determination of haematological parameters was done within 6 hours after blood collection [8] at Common Research Laboratory of University of Medicine 2.

Blood film preparation

Blood film preparation for differential WBC count (neutrophil, eosinophil, lymphocyte and monocyte count) was done according to the method described by Dacie and Lewis [9].

Determination of blood arsenic level

Blood arsenic level was determined by Spectrophotometric method [10] at Laboratory of Occupational Health Division, Department of Health.

Procedure

Written informed consent was obtained before starting the study. On the first visit, the subjects were instructed not to eat sea foods within 3 days before collection of samples to avoid overestimation of exposure to inorganic arsenic because some fish varieties contain several mg/kg of arsenic as arsenobetain that is nontoxic and mainly excreted in urine. On the second visit, 10 ml of blood of previously chosen subject were collected. It was done at rural health centre of Bryke Village, Kyaungkone Township. Determination of haematological parameters was done at Common Research Laboratory, University of Medicine 2. Determination of blood arsenic level was done within one month of sample collection at the Laboratory of Occupational Health Division, Department of Health, Yangon.

Collection of blood

Before collection of blood specimen, the antecubital area of each subject was cleaned with 70 percent ethanol and allowed to dry. Ten milliliters of venous blood sample were with-drawn. Five milliliters of blood were delivered to EDTA treated bottle (Complete Picture bottle) for the determination of haematological parameters [9]. The remaining 5 ml of blood were used for determination of blood arsenic level. The blood samples were collected and carried in cold chains (4°C). Blood for arsenic determination was stored at -20°C until analysis [11]. The stability of blood arsenic was maintained up to 2 months if kept refrigerated or frozen.

Statistical analysis

Data were analyzed by using the computer-based statistical package of statistical product and service solution (SPSS) version 11.5. Results were reported as mean±SD. Correlation between blood arsenic levels and changes in haematological parameter in total study population were done by Pearson correlation. Haematological parameters between high blood arsenic group and low blood arsenic group were compared by Student 't' test.

RESULTS

The blood samples of 29 subjects (41.43%) contained arsenic level ≤ 0.025 $\mu\text{g/ml}$. Forty-one subjects (58.57%) had blood arsenic level >0.025 $\mu\text{g/ml}$ (reference value: 0.001-0.025 $\mu\text{g/ml}$) (Table 1).

Table 1. Blood arsenic level in study population

Blood arsenic level ($\mu\text{g/ml}$)	Subjects (no.)	Percentage (%)
≤ 0.025 (low blood arsenic group)	29	41.43
>0.025 (high blood arsenic group)	41	58.57
Total	70	100

In about 79% of the study population, duration of arsenic exposure was at least 10 years (Table 2).

Table 2. Duration of arsenic exposure of the study population

Duration (years)	Subjects (no.)	Percentage (%)
5-10	15	21.43
10-20	40	57.14
>20	15	21.43
Total	70	100

Comparison of haematological parameters between high blood arsenic group and low blood arsenic group and independent 't' test results are shown in Table 3. The values are described in mean±standard deviation for all data.

A significant negative correlation between blood arsenic level and differential neutrophil count ($p=0.028$) (Fig. 1a) and a signifi-

Table 3. Comparison of hematological parameters between high and low blood arsenic groups

Hematological parameters	Study group		p
	High blood arsenic group (n=41) Mean±SD	Low blood arsenic group (n=29) Mean±SD	
WBC count (10^3 cells/ μl)	10.05±2.05	9.49±1.94	>0.05
RBC count (million cells/ μl)	4.99±0.55	4.86±0.56	>0.05
Hb (g/dl)	14.9±1.32	15.21±1.47	>0.05
Hct (%)	41.59±3.69	42.12±4.36	>0.05
Platelet count (10^3 cells/ μl)	277.02±69.47	248.79±49.88	>0.05
Neutrophil count (%)	48.68±9.12	53.12±6.14	$<0.05^*$
Eosinophil count (%)	10.42±4.85	7.49±3.44	$<0.01^*$
Lymphocyte count (%)	34.16±8.12	33.01±5.97	>0.05
Monocyte count (%)	5.76±2.35	6.58±2.71	>0.05
MCV (fl)	85.00±6.80	87.10±6.67	>0.05
MCH (pg)	30.79±2.92	31.40±3.02	>0.05
MCHC (g/dl)	36.16±0.98	35.96±0.84	>0.05

*indicates significant difference between two groups

ficant positive correlation between blood arsenic level and differential eosinophil count ($p=0.004$) were seen in total study population (Fig.1b). The mean differential neutrophil count of the high blood arsenic group was $48.68\pm 9.12\%$, and that of low blood arsenic group was $53.12\pm 6.14\%$. Therefore, the differential neutrophil count of the high blood arsenic group was signifi-

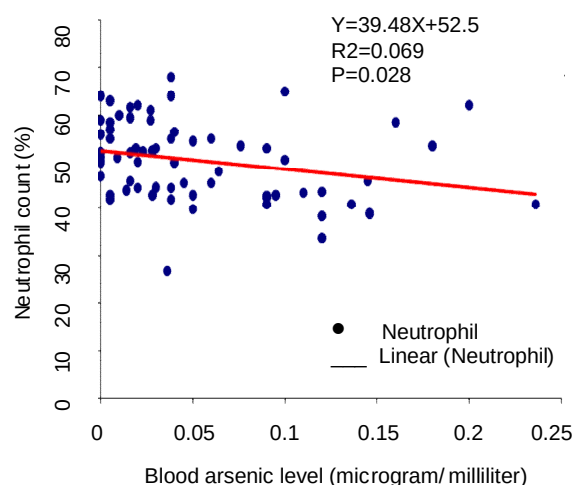


Fig.1a. Relationship between blood arsenic level and differential neutrophil count in people living in Kyaungkone Township

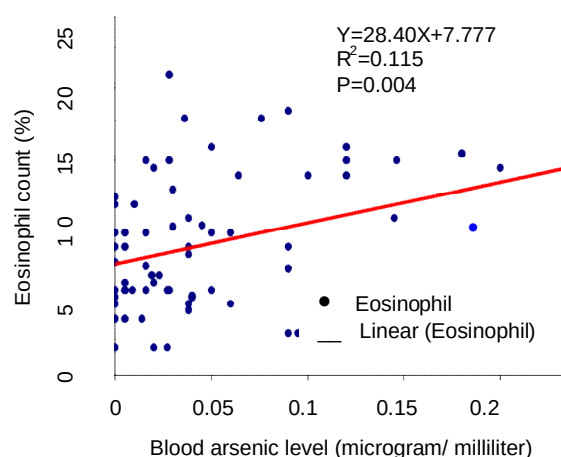


Fig.1b. Relationship between blood arsenic level and differential eosinophil count in people living in Kyaungkone Township

cantly lower than that of the low blood arsenic group ($p=0.021$). The mean differential eosinophil count of the high blood arsenic group was $10.42 \pm 4.85\%$ and that of the low blood arsenic group was $7.49 \pm 3.44\%$. Therefore, the differential eosinophil count of the high blood arsenic group was significantly higher than that of the low blood arsenic group ($p=0.007$).

DISCUSSION

In the present study, the mean blood arsenic level of the high blood arsenic group was $0.080 \pm 0.008 \mu\text{g}/\text{ml}$ and that of the low blood arsenic group was $0.008 \pm 0.002 \mu\text{g}/\text{ml}$. In one study conducted in Mongolia, the mean blood arsenic level of cases was $0.0421 \mu\text{g}/\text{ml}$ and that of controls was $0.0073 \mu\text{g}/\text{ml}$. The concentration of arsenic in their drinking water was $0.41\text{mg}/\text{L}$ and duration of exposure was 18 years [12].

In 2004, a study was done on 25 arsenical exposed patients with melanosis and keratosis. They drank arsenic-contaminated water of $0.029\text{--}0.13 \text{mg}/\text{L}$ for 3–10 years, but stopped drinking arsenic-contaminated water for about 2 years before the time of blood collection. The mean blood arsenic concentration of these individuals was $0.0263 \mu\text{g}/\text{ml}$. Although all of these patients stopped consumption of arsenic-contamina-

ted water, blood samples still contained arsenic. It was concluded that arsenic in blood could come from food that contained arsenobetaine [13]. Recently, it was reported that arsenobetaine was detected in rice and some commonly used spices [14].

A study showed that the mean blood arsenic level of cases was $0.08 \pm 0.06 \mu\text{g}/\text{ml}$ and that of controls was $0.02 \pm 0.02 \mu\text{g}/\text{ml}$. The arsenic concentration of drinking water was more than $0.1\text{mg}/\text{L}$ and duration of exposure was more than 5 years [15].

Variations of blood arsenic levels in various studies may be due to the difference in the methods of blood arsenic determination, the difference in the concentration of arsenic in drinking water, amount of water intake and duration of exposure. Moreover, arsenic exposure from various sources other than drinking water may also differ from one individual to another. Environmental arsenic concentrations affect exposures that do not come from drinking water, and those concentrations vary among locations because of different natural background levels and local anthropogenic sources [16]. Arsenic content in foodstuff which in turn depends on the arsenic concentration in the soil, in the water used for washing, cooking, and irrigation, in pesticides and in different cooking process also affect individual's arsenic exposure [17].

In the present study, blood arsenic levels of 41 subjects were $>0.025 \mu\text{g}/\text{ml}$ and it was regarded as high blood arsenic group and those of the remaining 29 subjects were $\leq 0.025 \mu\text{g}/\text{ml}$ and, low blood arsenic group. The mean blood arsenic level of high blood arsenic group was $0.08 \pm 0.008 \mu\text{g}/\text{ml}$ and that of low blood arsenic group was $0.008 \pm 0.002 \mu\text{g}/\text{ml}$. The differential neutrophil count of the high blood arsenic group was significantly lower than that of low blood arsenic group ($p=0.021$). Neutropenia was seen in 46.3% of high blood arsenic group. But, in low blood arsenic groups, it was seen only in 20.7% of the subjects. The possible reason for neutro-

penia in chronic arsenic poisoning may be probably due to the effect of arsenic on bone marrow [18] or arsenic-induced neutrophil apoptosis [19] or the inactivation of JAK-STAT pathway by arsenic [20].

The differential eosinophil count of the high blood arsenic group was significantly higher than that of low blood arsenic group ($p=0.007$). Eosinophilia was seen in 70.7% of the subjects in high blood arsenic group and 55.2% of the subjects in low blood arsenic group. The proposed mechanism for eosinophilia was the allergic factor which may be related in the arsenic effect on haematopoietic organs [21].

Neutropenia and eosinophilia were seen not only in arsenic poisoning but also in many other conditions. Because of limitations of the present study, possible causes other than arsenic exposure for neutropenia and eosinophilia could be excluded by history taking and clinical examination alone. Any laboratory investigation or invasive technique could not be done to exclude these causes. Therefore, these causes may also be considered as the possible factors for neutropenia and eosinophilia in the present study.

But, in the present study, neutropenia and eosinophilia were significantly found in the subjects with high blood arsenic level. Moreover, there was a significant negative correlation between blood arsenic level and differential neutrophil count and a significant positive correlation between blood arsenic level and differential eosinophil count. Thus, findings of neutropenia and eosinophilia could be regarded as toxic effect of arsenic on haematological system. Other haematological parameters (haemoglobin level (Hb), haematocrit (Hct), total red cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell count, and platelet count) showed no statistically significant difference between the high blood arsenic group and low blood arsenic group. The results of this study suggested

that arsenic may not significantly affect other haematological variables though it may cause disorders of differential white blood cell count in the study population.

The variability in toxicity of arsenic may be due to the dose, duration of exposure, variability in arsenic metabolism, genetic polymorphisms related to arsenic metabolism, arsenic tolerance mechanisms and protective responses such as nutrition and antioxidants. The arsenic problem and its impact on the health of the population are relatively new to Myanmar and the full magnitude of the problem is still unknown.

Considering the knowledge about arsenic in Myanmar, it is recommended that further and thorough evaluation study should be done on the extent of the arsenic contamination of groundwater and its effects on various systems of the body in people who drink arsenic-contaminated drinking water. Studies are also required to link toxic manifestation with possible genetic polymorphism, age, gender, nutritional status and the protective role of vitamins, minerals and antioxidants. It is hoped that the present study will serve as a baseline for further studies of arsenic in Myanmar population.

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Relationship between blood arsenic level, blood pressure and creatinine clearance in subjects living in Kyaungone Township, Ayeyawady Division

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Chronic human arsenic exposure via drinking water containing high inorganic arsenic has been associated with hypertension, ischaemic heart disease, renal tubular necrosis and skin diseases in arsenic exposed population. The aim of the study was to find out relationship between the blood arsenic level, blood pressure and 2-hour creatinine clearance in arsenic exposed population living in Hmyawthey Village, Kyaungone Township, Ayeyawady Division. Apparently healthy 70 male subjects aged between 18-50 years with history of continuous arsenic exposure (>5 years) were divided into two groups according to blood arsenic level (normal limit-0.025 µg/ml) high blood arsenic group, (n=40) and low blood arsenic group (n=30). Blood pressure was measured by indirect sphygmomanometry. Serum and urine creatinine were measured and 2-hour creatinine clearance was calculated. Blood arsenic levels of two groups were 0.085 ± 0.06 and $0.008 \pm 0.01 \mu\text{g/ml}$, respectively ($p < 0.001$). The percentage of hypertension was 24.29% among arsenic exposed population. High blood arsenic group had significantly higher systolic and diastolic blood pressures ($p < 0.05$) and lower 2-hour creatinine clearance ($p < 0.05$) than low blood arsenic group. There were significant positive correlations between blood arsenic level and arterial blood pressures (SBP, $r = 0.274$; DBP, $r = 0.298$; $p < 0.05$) and a negative correlation between blood arsenic level and 2-hour creatinine clearance ($r = -0.245$, $p < 0.05$). It was concluded that long-term arsenic exposure was found to be associated with increased blood pressure and renal insufficiency.

INTRODUCTION

Though arsenic is found everywhere in the environment, arsenic concentration in groundwater is the highest because of both very high dissolving power in water and a strong interaction of arsenic with rock and soil under physical and geochemical conditions which favour for arsenic dissolution and accumulation [1]. According to WHO guideline (1993), acceptable level of arsenic in drinking water is 10 µg/l [2]. It was noted that the arsenic concentration in groundwater has a very wide range from <0.5 to 5000 µg/l in Bangladesh [3].

Accumulating body of evidence suggests that arsenic exposed populations in the

affected areas have many health problems. Arsenic toxicity may result from a single large dose (acute poisoning) or from repeated small doses (chronic poisoning). There were a large number of studies in human and animals on the toxic effects of ingested arsenic. Most human arsenic exposure occurs from consumption of drinking water containing high amount of inorganic arsenic. Chronic effects of arsenic exposure via drinking water include skin lesions, neurological effect, hypertension, peripheral vascular disease, cardiovascular disease, respiratory disease, diabetes mellitus, and malignancies including skin cancer [4]. Association between long-term arsenic exposure and cardiovascular diseases such as hypertension, ischemic

heart disease and heart failure [5] and hypertension [6] has also been reported. Moreover, arsenic toxicity has become a health challenge since individual susceptibility to arsenic poisoning varies widely - most of the victims do not have any complaint or symptom until they are detected through a screening survey and some persons have been known to develop a tolerance to doses that would kill others [7].

According to the data issued from Occupational Health Division of Myanmar, arsenic content of drinking water is high in some regions in Myanmar including Thaboung, Kyonpyaw, Zalun and Kyaungone townships in Ayeyawady Division [8].

Arsenic concentration of water samples taken from all tube wells (shallow as well as deep tube wells) in Hmyawthey Village, Kyaungone Township was also analysed. It was found that 46% of all tube well samples were in the range of 100-200 µg/l, 44% between 201-300 µg/l and 10% more than 301 µg/l. Yet, there is no study of health impact of arsenic in this area. Therefore, this study was aimed to find out the relationships between blood arsenic level and arterial blood pressure, and between blood arsenic level and creatinine clearance in people living in Hmyawthey Village, Kyaungone Township, Ayeyawady Division, Myanmar.

MATERIALS AND METHODS

A total of 70 apparently healthy male subjects aged between 18-50 years with more than 5 years of continuous arsenic exposure in Hmawthey Village, Kyaungone Township, Ayeyawady Division, Myanmar were recruited and their blood arsenic levels were determined. Personal data, the present and past medical history, and current drug history were taken using proforma. General clinical examination was done to exclude acute illness, cardiovascular diseases and renal diseases. A written informed consent was obtained individually. Systolic blood pressure and diastolic blood pressure were

measured with indirect method using mercury sphygmomanometer (ALPK 2, Japan) and stethoscope. Serum and urinary creatinine concentrations were measured by Jaffe'-reaction using Humalyzer and creatinine clearance was determined. Blood arsenic level was determined by spectrophotometric method using spectrophotometer [9].

On the day of experiment, each subject took a rest for 30 minutes in sitting position. Then, systolic blood pressure and diastolic blood pressures were measured three times. After that, they were instructed to void urine three times with one to two minute intervals to ensure the completeness of bladder emptying. After drinking the water (20 ml/kg body weight) within 5 minutes, urine samples were collected for 2 hours at 15-minute intervals and a venous blood sample (15 ml) was taken at mid-point of the urine clearance period (i.e. at one hour) under aseptic condition. The subject was remained in sitting position throughout the experiment except during voiding.

Urine volume was measured with volumetric flask. About 10 ml of urine were collected in a capped bottle. Blood samples (15 ml) were collected and carried in cold chains (4°C). (1) Five millilitres of blood sample were used for determination of creatinine at Common Research Laboratory, University of Medicine 2 and (2) Ten millilitres of blood sample were stored at -20°C until arsenic determination was done at laboratory of Occupational Health Division, Yangon. Based on the blood arsenic level, the subjects were divided into two groups: high blood arsenic group (n=40), whose blood arsenic level was above the reference value (i.e. >0.025 µg/ml) and low blood arsenic group (n=30), whose blood arsenic level within the reference value (i.e. 0.001 to 0.025 µg/ml). The data of two study groups were compared by Student's unpaired "t" test. Pearson's correlation coefficient was used to assess relationship and Chi-square test was used to assess association between blood arsenic level and other parameters collected in this study.

RESULTS

The reference range of serum arsenic level was 0.001 to 0.025 µg/ml. Mean serum arsenic level of the high and low arsenic groups were 0.085 ± 0.06 µg/ml and 0.008 ± 0.01 µg/ml, respectively. It was also noted that there was a significant positive correlation ($r=0.316$, $n=70$, $p<0.05$) between blood arsenic level and duration of arsenic exposure in all subjects. Systolic blood pressure, diastolic blood pressure and mean arterial blood pressure were significantly higher ($p<0.05$) and 2-hour creatinine clearance was significantly lower in the high blood arsenic group than in the low blood arsenic group ($p<0.05$) (Table 1).

Table1. Comparison between blood arsenic level, blood pressures and creatinine clearance of high and low blood arsenic groups in arsenic exposed population

Parameters	High blood arsenic group (n = 40)	Low blood arsenic group (n = 30)	P
	Mean±SD	Mean±SD	
Systolic blood pressure (mmHg)	127.3±17.54	116.3±12.45	0.005*
Diastolic blood pressure (mmHg)	84.75±10.12	78.67± 7.76	0.005*
Mean arterial pressure(mmHg)	98.91±12.22	91.22±8.23	0.004*
Creatinine clearance(ml/min)	112.4±61.64	148±73.51	0.031*

*indicates significant difference between two groups.

In the high blood arsenic group, 14 out of 40 subjects (35%) had blood pressures (both SBP and DBP) higher than normal values (130/90 mmHg) and in the low arsenic group, 3 out of 30 subjects (10%) had blood pressures (SBP and DBP) higher than normal values. Thus, altogether 17 subjects (24.3%) of the arsenic exposed population had hypertension. It was also observed that blood arsenic level was significantly associated ($\chi^2=5.827$; $p=0.016$) with the percentage of hypertension in the arsenic exposed population.

Regarding 2-hour creatinine clearance, in the high blood arsenic group, 24 out of 40 subjects (60%) had lower 2-hour creatinine

clearance lower than normal value (97 ml/min) and in the low blood arsenic group, only 8 out of 30 subjects (26.67%) had lower creatinine clearance lower than normal value. Therefore, 32 subjects (45.71%) had a decrease in glomerular function which indicated as lower 2-hour creatinine clearance. It was also observed that blood arsenic level was significantly associated ($\chi^2=7.675$; $p=0.006$) with the percentage of decreased 2-hour creatinine clearance in the arsenic exposed population.

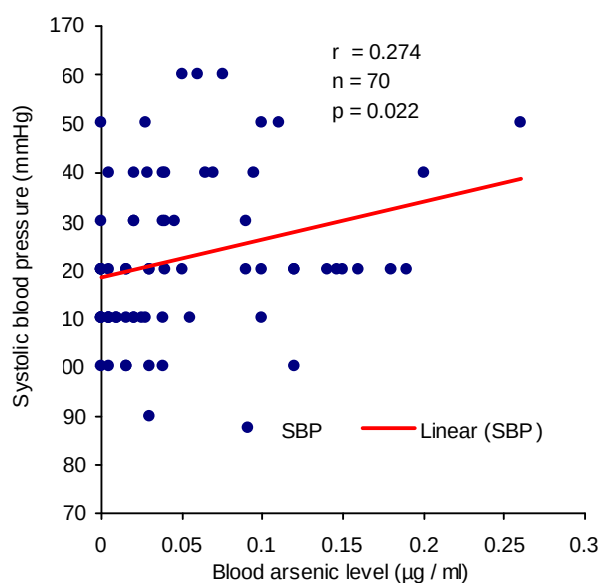


Fig. 1. Correlation between blood arsenic level and systolic blood pressure

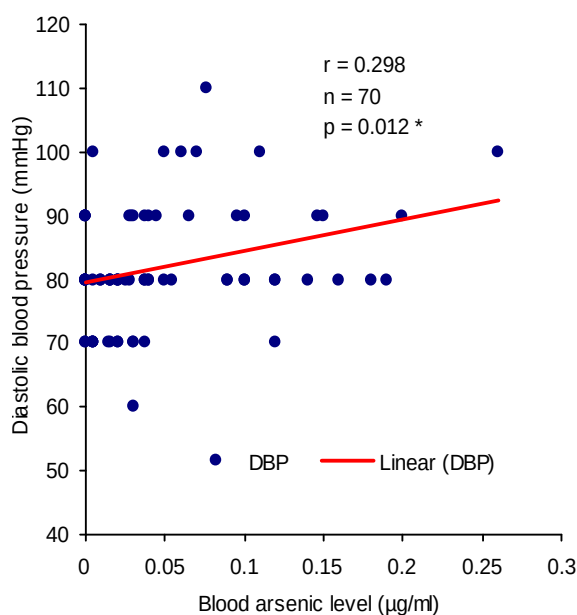


Fig. 2. Correlation between blood arsenic level and diastolic blood pressure

Figure 1 and 2 show significant positive correlations ($p < 0.05$) between blood arsenic level and blood pressure parameters (systolic blood pressure and diastolic blood pressure) among total subjects participated in this study ($n=70$).

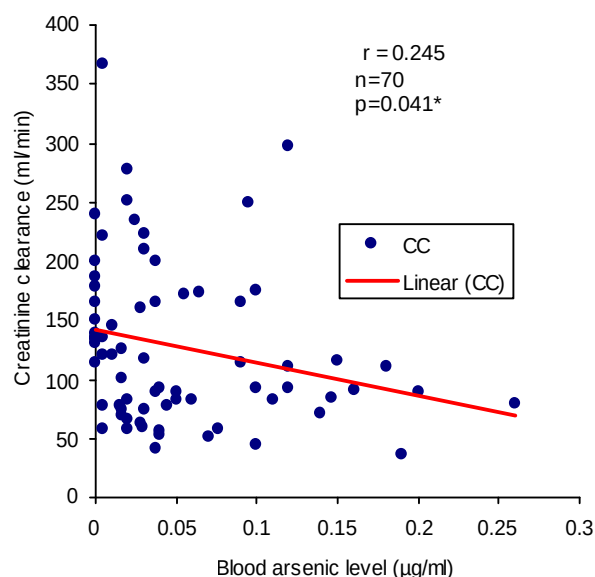


Fig 3. Correlation between blood arsenic level and 2-hour creatinine clearance

Fig. 3 shows a significant negative correlation between blood arsenic level and 2-hour creatinine clearance among total subjects in arsenic exposed population.

DISCUSSION

Mean blood arsenic level of the high blood arsenic group ($n=40$) was 0.085 ± 0.06 µg/ml and that of the low blood arsenic group ($n = 30$) was 0.008 ± 0.01 µg/ml. According to the data recorded by Rural Health Centre in Kyaungone Township (2007), 46% of the study population ($n=70$) used drinking water containing arsenic concentration of 100-200 µg/l, 44% used 201-300 µg/l and 10% used >301 µg/l. Out of 30 subjects participated in the low blood arsenic group, 12 subjects (40%) used drinking water containing 201-300 µg/l and 3 subjects (10%) used drinking water containing arsenic concentration >301 µg/l. Surprisingly, their blood arsenic levels were found to be lower than normal limit (i.e. 0.025 µg/ml)

though they used drinking water with high arsenic level (>200 µg/l). Thus, we can not definitely conclude that arsenic in drinking water was related to arsenic in the blood.

The present finding did not agree with those of another two studies carried out in Inner Mongolia, China and in West Bengal, India. They demonstrated that case subjects who drank well water containing arsenic level of 400 µg/l had blood arsenic level which is six times higher than that of control subjects who were exposed to arsenic concentration of 200 µg/l (0.0421 µg/ml vs. 0.0073 µg/ml) [10]. In one study, blood arsenic concentration of case subjects ($n=25$) was 0.0263 ± 13.8 µg/ml and control ($n=16$) was 0.0022 ± 6.68 µg/ml. Out of 41 subjects, 16 subjects drank water containing arsenic concentration of 55 µg/l; 12 subjects drank water containing arsenic 130 µg/l and 13 subjects drank water containing 163 µg/l [11]. Moreover, it has been stated that blood arsenic levels of subjects not known to have been exposed to arsenic are in the range of only a few micrograms per litre, while persons who are exposed to water containing high levels of blood arsenic levels exceed 50 µg/l [2].

Though it was unable to estimate the amount of water consumed by the subjects during their lifetime, the duration of arsenic exposure might also reflect the extent of arsenic exposure. Our finding, i.e. significant positive correlation between blood arsenic level and duration of arsenic exposure, indicates that the longer the duration of exposure, the higher the blood arsenic level. Among the factors which determine blood arsenic level, it seems that the duration of arsenic exposure might be more important than the arsenic content of drinking water from contaminated wells. It could be explained by the difference of individual sensitivity to toxic effect of arsenic.

Generally, majority of these subjects in the present study had similar occupations, socioeconomic status, lifestyle and dietary habits and all drank arsenic-contaminated

drinking water, but their blood arsenic levels quite varied. A study suggested that difference in sensitivity of human population to toxic effect of arsenic in large part related to different nutritional status [12]. One limitation of the present study was the difficulty in analysis of food intake for these subjects because of their consumption of various dietary constituents in the meal. Thus, it was unable to calculate the individual intake of nutrients quantitatively such as carbohydrate, protein, saturated fat, sodium and potassium etc.

In addition, nutrition has a substantial role in the etiology of arsenic-related diseases [13]. Poor nutritional status might decrease the ability of an individual to methylate arsenic, resulting in increased arsenic concentrations in tissues and the development of toxic effects [14]. High protein diet helps in the clearance of inorganic arsenic by increased methylation and protects against toxic effect of arsenic. The antioxidants vitamins A, C and E play an important role. Protein-rich diet and antioxidants can detoxify arsenic poison. Antioxidants such as vitamin C and α -tocopherol showed cytoprotective activity against arsenic trioxide [15]. Therefore, the discrepancies in individual sensitivity of the subjects to toxic effect might probably be due to differences in their dietary content.

In a cross-sectional analysis of 10910 participants consuming elevated ground-water arsenic concentration in Bangladesh, average systolic, diastolic and mean arterial blood pressures for male were 115.7 mmHg, 73.7 mmHg and 87.7 mmHg, respectively [16]. In the present study, mean values of systolic blood pressure and diastolic blood pressure and mean arterial blood pressure of the high arsenic group were 127.25 mmHg, 84.75 mmHg and 98.91 mmHg, respectively. They were significantly higher in the high arsenic group than in the low arsenic group ($p < 0.05$). Similar finding was also reported in a study that analyzed 1185 respondents who drank arsenic-contaminated well water for 20 years, in

which people drank well water with arsenic concentration of $>10 \mu\text{g/l}$ had significantly higher systolic blood pressure and diastolic blood pressure than people with $<2 \mu\text{g/l}$ [17].

Moreover, there were positive correlations between systolic blood pressure and blood arsenic level ($r=0.274$, $p < 0.05$) and diastolic blood pressure and blood arsenic level ($r=0.298$, $p < 0.05$) in subjects in arsenic exposed population ($n=70$), respectively. Therefore, these findings suggest that increased blood arsenic level might correlate with an increase of blood pressures (systolic blood pressure and diastolic blood pressure) in arsenic exposed population. The positive relationship between arsenic exposure and high systolic blood pressure was also shown in a study [16].

Previous prevalence studies had reported that age and sex adjusted hypertension prevalence of residents (382 men and 516 women) in arsenic hyper-endemic area in Taiwan was 23.2% (95% CI, ranged 19.3-27.1%) [18] and the prevalence of hypertension among 1595 subjects with and without arsenic exposure in Bangladesh was 17% (95% CI, ranged 0.8-3.3) [6]. In the present study, the percentage of hypertension among arsenic exposed population was 24.29% (95% CI, ranged 14.83-36.01%) and the high arsenic group had the greater percentage of hypertension than the low arsenic group (35% vs. 10%). It was consistent with the finding of a report which also found a significantly higher prevalence of hypertension among residents in arsenic endemic area when compared with those in non-endemic areas [18]. In fact, the sample size of our study was too small to express like the prevalence study. Therefore, a large-scale study is recommended to find out the real prevalence rate of hypertension among arsenic exposed population in Myanmar.

In the present study, mean 2-hour creatinine clearance of the high and low blood arsenic groups were $112.38 \pm 61.64 \text{ ml/min}$ and $148.03 \pm 73.51 \text{ ml/min}$, respectively. Among

them, it was noted that 60% of the high blood arsenic group had low 2-hour creatinine clearance whereas in the low blood arsenic group, it was only 26.67%. Moreover, 2-hour creatinine clearance was significantly decreased in the subjects of the high blood arsenic group when compared with the low arsenic group ($p < 0.05$). There were no studies on 2-hour creatinine clearance in arsenic exposed population with high arsenic content in drinking water.

One study showed that mean urinary arsenic level of patient with IV melarsoprol, an arsenic compound was $426 \pm 59 \mu\text{g/l}$, creatinine clearance was $72.9 \pm 1.2 \text{ ml/min}$ and estimated creatinine clearance was $99.1 \pm 29.4 \text{ ml/min}$ [19]. The values of creatinine clearance were found to be lower than our data. It might be attributable to the difference in analyzing methods. In that study, 2-hour creatinine clearance was significantly decreased in case group, but there was no correlation between estimated creatinine clearance and urine arsenic value. However, our study observed a negative correlation between 2-hour creatinine clearance and blood arsenic level in total subjects in arsenic exposed population ($r = -0.238$, $p < 0.05$). Therefore, it is concluded that chronic exposure to arsenic-contaminated drinking water may affect the renal glomerular function.

Conclusion

Our findings suggest that long-term arsenic exposure is associated with increased blood arsenic level, increased risk of hypertension and renal insufficiency. Moreover, arsenic-related hypertension observed in this study might probably be the result of renal defects induced by arsenic.

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The effect of Vipassana meditation towards behavioural and social changes among inmates of Central Jail, Insein, Yangon

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A study was carried out to assess the social and behavioural effects of Vipassana meditation sessions among inmates of Insein Prison, Yangon. In-depth interviews were done with 17 inmates, those who were older than 18 years, who had attended meditation training and who had the same criminal background. Key informant interviews were done with five prison staff. All the respondents stated that meditation had changed them in various aspects, mentally, socially and physically that resulted in having clear and constructive future perspective. They realized that meditation affected more on their outlook of mind than on social and physical changes. They all agreed that “mind” is the key phenomenon for the changes. They also noticed that attitudes and behaviour of the staff toward them had changed to a positive way. In addition, they also noticed about the changes in their physical appearance and health conditions. Every respondent said their ways of thinking and living will be changed when they were free from prison. They had intended to continue meditation practices throughout their life. Similarly, from the staff’s aspect, the supervision and dealing with the inmates would be much better, if the staff themselves had meditation practices. All of the respondents suggested the necessity to arrange for regular and frequent meditation sessions for the long period for both inmates and the staff. They all had similar thoughts that meditation is the best for the inmates for changing their attitudes and behaviours in this present life and also for the next existences.

INTRODUCTION

‘Vipassana’, which means ‘insight’ or ‘to see things as they really are’ in Sanskrit, is the essence of Buddha’s teachings. On the contrary, crime is essentially a social problem, which disrupts the harmony and peace of a society. Until now, the commonest way of dealing with criminals has been to imprison them, and found the criminals were repeatedly returning to the prison many times having committed worse crimes than the ones before. Realizing that this method of punishment was proving unsuccessful, various reform measures have been adopted to rehabilitate prisoners and equip them with the inner ability to transform themselves.

Vipassana meditation, as taught by SN. Goenka is being used as a prison reform measure with the ultimate objective of reducing recidivism, and reintegration of prisoners back into the society once they are released. The very first Vipassana course in a prison took place in Jaipur, India, in 1975. However, it was only after almost 20 years that Vipassana established itself as a tool for social and prison reform in the 1990s. The tremendous impact and change that it brought about in the prisoners was the turning point and very soon more and more prisons began organizing these meditation courses for the prisoners. Today, the meditation practice is not just confined to India but its non-sectarian and universal application is being recognized in prison

facilities in many other parts of the world, especially North America, where Vipassana has had the same positive effects on the prison inmates [1, 4].

In Myanmar, Vipassana meditation practice has been widespread for many years. But the meditation training in prison was initiated in 2008. Thus, it is an attempt to find out the effect of Vipassana meditation towards behavioural and social changes among inmates of Insein Prison, Yangon and findings of this study will be applicable to rehabilitation program for the inmates in Myanmar.

Objective

To assess the social and behavioural effects of Vipassana meditation among inmates of Insein Prison, Yangon.

MATERIALS AND METHODS

A cross-sectional study design with a qualitative method was used. The study was conducted at Insein Prison, Yangon Division during August 2009. Seventeen inmates who were older than 18 years, who had attended the meditation training and who had the same criminal background were recruited for in-depth interviews (IDI). Following dimensions were used for the recruitment of IDI.

- Sex: male and female
- Prison term: short period (i.e., 10 years or less) and long period (i.e., more than 10 years)
- Frequency of meditation sessions attended in the prison: 1 to 2 times and more than 2 times

To triangulate the findings, key informant interview (KII) with five prison staff, who had attended meditation session in the prison at least once, was also conducted. Ethical approval was obtained from Ethical Review Committee, Department of Medical Research (Lower Myanmar). With the interviewee's permission, the interviews were taperecorded and the findings were analysed manually.

RESULTS

Seventeen inmates who met the inclusion criteria involved in the study as follows.

Table 1. Number of inmates involved in study by selection dimension

Attending meditation sessions	Male		Female		Total
	Prison term		Prison term		
	Short term	Long term	Short term	Long term	
1 – 2	1	2	3	4	10
> 2	1	4	1	1	7
Total	2	6	4	5	17

The characteristics of the inmates and the prison staff are shown in Table 2 and Table 3, respectively.

Table 2. Characteristic of the inmates

Characteristic	Male	Female	Total
<i>Age (in years)</i>			
20 – 35	5	2	7
36 – 50	2	5	7
51 – 65	1	2	3
<i>Marital status</i>			
Single	4	1	5
Married	4	5	9
Divorced	0	2	2
Widowed	0	1	1
<i>Education</i>			
Primary level	1	1	2
Middle level	3	1	4
Higher level	2	4	6
Graduate	2	3	5
<i>Previous occupation</i>			
Own business	4	3	7
Seller	2	2	4
Dependant	0	4	4
Odd-jobs	2	0	2
<i>Years of prison term</i>			
1 – 10	4	4	8
11 – 40	0	4	4
No limitation/Death sentence	4	1	5
<i>Attending meditation sessions</i>			
1-2	4	7	11
> 2	4	2	6
Total	8	9	17

All the respondents, both inmates and the staff, stated that meditation had changed them in various aspects, mentally, socially and physically that resulted in having the clear and constructive future perceptive. They realized that meditation affected more on their outlook of mind than on social and physical changes. They all agreed that "mind" is the key phenomenon for the changes.

Table 3. Characteristic of the prison staff

Characteristic	Male	Female	Total
<i>Age (in years)</i>			
24	0	1	1
50 – 57	3	1	4
<i>Education</i>			
Middle level	1	0	1
Higher level	2	0	2
Graduate	0	2	2
<i>Rank</i>			
Officer	0	1	1
Other rank	3	1	4
<i>Service at present post (in years)</i>			
1 – 5	2	1	3
6 – 10	1	1	2
<i>Total service (in years)</i>			
1 – 5	0	1	1
6 – 10	0	0	0
11 – 15	1	0	1
16 – 20	0	0	0
21 – 25	1	0	1
26 – 30	1	1	2
<i>Attending meditation sessions</i>			
1	3	1	4
3	0	1	1
Total	3	2	5

Intention to enter meditation sessions

Among the study respondents, some had experiences on meditation before jailing, but not the Goenka's method. All of them said they had a strong desire to enrol in the meditation session when they were aware of opening the session in the prison. One-third of them had joined more than twice after being in the prison. However, whenever they heard about opening of the session, they were very interested in signing it up again. So did for those who had never experienced with the meditation.

One male respondent, who had joined more than once, had a different opinion on the session and he explained as follow:

I had never practised meditation. For the first time, I signed up at third meditation session. After that, I felt I could not concentrate on it. So, I thought I might have sinful thinking if I enrolled again. I had a feeling of revulsion then. Thus, I just meditated in my room till I could control my

mind totally. Then, I enrolled for the second time at the eleventh meditation session.

(A male respondent, 30 years, married, 8th standard, five-year punishment)

Changes in outlook

The majority admitted their nature as ruthless and short-tempered, particularly those who were sentenced for a long period, either life sentence or death sentence. They were so sensitive and always ready to fight against each other even for the small matters such as letting other inmates used a place for sleeping, being touched by others or using one's soap by other.

During the period of practising, gradually but varied between the individuals, they said they had developed optimistic views. The following quotations expressed how they had changed.

Previously I considered endlessly of being jailed. But now, I console myself that I won't be here for always. I will be free one day.

Here, we feel inconvenient with everything. After practising the meditation, I wanted to share my food to those who are living in appalling conditions. I realised that obsession with self had decreased.

I could understand my family now if they did not come regularly. Previously, I was furious when they did not visit me or they did not bring things and food that I had asked for.

My view toward the staff had changed. Earlier, I misunderstood with them. I didn't have any problems with them any more.

I think, for a person, mind is the main object. It is only "mind" that commands a person to do right things or the wrong things. If we could live with the u. pe' kha (ignorance) throughout the life, then there will be no mistakes and wars. And all of us will be in peace. This is the way I'm now practising.

Not only their outlooks had changed, but they also noticed that attitudes and behaviour of the staff toward them had also changed to a positive way. For instance,

they were greeted *min gāla pa* (say hello in a polite way) and were called as *yo: gi* (one who practises religious meditation) by the staff. They appreciated this very much as they were recognized like this because of meditation.

After understanding the essence of meditation, they began to accept their current situation. They regretted of committing a crime as nearly all of them said if they had practised meditation years before, they would not be here. On the other hand, some considered the punishment as the betterment that they would not know the meditation if they were not in the prison.

Social changes

They all have the same opinion that after understanding the insights of meditation, their attitudes had changed. They started talking with caution and did not argue back. They tried to understand why other people talked or behaved unmannerly toward them. They gave priority to other people to make themselves less angry, which could avoid fighting. Some said they could ignore the events when these were not concerned to them.

They also admitted that because of their greed, anger and ignorance they had committed the crimes, and they realized that cultivating the desire to revenge made them suffer. Once they tried to forgive others, within a moment, they said that they could feel peace in mind. All the changes lead to the peaceful state although their underlying causes were different. The following narrations illustrated their experiences.

I did not believe myself how I had changed totally after arriving here. I became so rude and wild. It was not my nature. Since I was blamed for other people's fault, I thought of revenging to the person who accused me. But now I understand that this is worthless and everyone has his own way.

(A female respondent, 43 years, divorced, graduated, three-year sentence)

They were able to live with other people in peace: they shared their meditation

experiences to other inmates, encouraged them to practise the session. One female respondent recalled how she helped her fellow cellmate.

During last month, another girl arrived to my cell with death sentence. She is only 26 years. She was so frightened and could not sleep at nights. At that time, I had entered the session once, so I told her about a na. pa na. (in and out breathing) as much as I knew and gave the religious books to read. I also urged her to sign up the meditation session.

(A female respondent, 29 years, married, graduated, death sentence)

Physical and health changes

Apart from the improvement in their perspectives, mentally and socially, they had also noticed about the changes in their appearance and health conditions. Some said they gained weight and had good looks. A few said though they went to bed late they had woken up feeling fresh. Further, while some said they did not feel any pain, or their pain had reduced to some extent, some said they did not need to take medicine for heart problems, hypertension, headache, pain and stress. They related the improvement of their health condition with meditation as they believed that meditation could lead to calm them, which in turn, reduce tension that caused them illness.

Future perspectives

Every respondent said their ways of thinking and living will be changed when they were free from prison. They had intended to continue meditation practices throughout their life. Majority said they wanted to be *dā ma* worker (a person who helped the *yo: gi* with the meditation) and a few wanted to be a *dā ma* preacher. Those who were sentenced for life said while serving in the prison, meditation was the only hope for them. Besides, the attitude toward the religious belief, although the individual's situation differed from one another, they all had decided to help their family and their environments. Here are the different forethoughts of the respondents.

I have never been felt so peaceful before. This may be due to meditation. I want to share my experience to my family when I am free.

(A female respondent, 43 years, divorced, graduated, three-year sentence)

In the past, I did not take any responsibility for my family. I drank alcohol and used marijuana. I am the only child in my family; all my uncles and aunties are unmarried. My mother and my wife thus had to look after the family for earning and other things. Now I had decided to take my family's responsibility when my term is completed. I will teach my children how to behave, I will stay in compatibility with the society and I will encourage my family, friends and relatives to practise the meditation.

(A male respondent, 30 years, married, 8th standard, five-year punishment)

I now believed in kan (one's deed, word or thought which predetermines one's future) and its effect. I am a type of person relying on the practical experience. I vow not to do any misdeeds in future not because of terrifying the law and prison, but because I'm really afraid of kan and misdeeds and their effects.

(A female respondent, 29 years, married, graduated, death sentence)

Suggestions for improvement

All of the respondents suggested arranging for regular and frequent meditation sessions for the long period for both inmates and the staff. They all had similar thoughts that meditation is the best for the inmates for changing their attitudes and behaviours in this present life and for *lo: kou' tara* (the way to escape from worldly desires and attachments) till attaining the *nirvana*. They explained that this concept should be accepted and adopted the changes for the whole life because practical experiences were more effective than teaching or explaining. Another suggestion was to share the experiences by the inmates to other people when they were free. Then there would be fewer crimes and less lawless

persons. For the administrative purpose, both types of respondents expected the better dealing between them, which could only have if the staff also practised the meditation. They suggested that everybody should practise at young age to differentiate between merits and misdeeds.

DISCUSSION

'Vipassana' is a straightforward way to achieve peace of mind and to live a happy and useful life. It equips one with the inner strength to face the vicissitudes of life in a calm, balanced manner, and gain mastery over one's mind. It is a practical and experiential way of understanding the mind-matter phenomenon and purifying one's mind of underlying negativities. This truth was evident among the inmates and their administrators of Insein Prison. Though we could not explain in numbers since the findings were based on the qualitative method, to a larger extent, the meditation sessions had converted them as optimistic persons. Beliefs, ways of thinking and behaving by all the study respondents had changed toward the positive direction, mentally, socially and physically.

The inmates became to accept and adapt their present situation and they were able to communicate with other inmates and the staff friendly. The inmates also had brighter images for their future than on the past. They all had desire to keep meditating once they are free. Not only for their present life, but also for the next existences, they all believed Vipassana as the only mean to have peace and calm life for themselves and their loved ones. The staff also changed their style of managing the matters of the inmates and they supervised them with positive attitudes. All study respondents suggested about expansion of meditation sessions, course duration and frequency of sessions, for the benefit of reducing criminals. Similar situations were observed among the prisoners of America and India, where 10 day meditation courses were held in the respective jails with the purpose of

investigating the effect of Vipassana meditation. These studies reported that there was a decrease in recidivism, psychopathological symptoms, and the level of propensity among the inmates. Additionally, after attending the sessions, the inmates had strength of mind to self-correct themselves, and they were also willing to cooperate with prison authorities [5, 7].

The present study and the reviewed studies pointed out that Vipassana meditation had not only improved the prison environment but also the would-be-released and the released inmates.

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Fatty acid profiles of commonly consumed fish in Yangon

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To determine the fatty acids concentration of commonly consumed fish in Yangon, the items were freely listed by interviewing with fifty housewives from five different occupational groups. A total of 16 items of fish (8 fresh water fish, 5 marine water fish and 3 brackish water fish) were identified as commonly consumed ones and these food samples were purchased for analysis from different markets of Yangon City. The total oil from edible portions were extracted by petroleum ether and methylated to methyl esters by methanolic boron trifluoride. The fatty acids were analyzed by gas chromatography with Optima (FFAP) column, flame ionization detector and maximum 220°C temperature program. Among studied items of fish, the highest amount of ω -3 fatty acids (3190 ± 20.6 mg/100g of oil) was found in featherback (*Notopterus notopterus*) (ig;Z ,). The highest level of ω -6 fatty acids (1430.9 ± 101.8 mg/100 g of oil) was found in snapper (*Lutjanus johnii*) (ig;y;e). Sardines (*Clupeichthys aesarnensis*) (ig;aOWm;ig;) are the rich source of saturated fatty acids as well as ω -3 fatty acids (337.7 ± 30.5 mg and 2831.8 ± 40.9 mg of oil, respectively). Therefore, not only marine water fish but also the freshwater fish (featherback) (ig;Z ,) is the rich source of essential fatty acids among their respective items. In conclusion, the persons who are not used to consume marine water fish should eat more fresh water fish with rich in essential fatty acids, like featherback (ig;Z ,).

INTRODUCTION

The humans can synthesize some fatty acids, however, two fatty acids cannot be synthesized in the body by any known chemical pathways due to lack of the desaturase enzymes required for their production. Therefore, they must be obtained from the diet and they are known as essential fatty acids (EFA). There are two families of essential fatty acids: ω -3 (omega-3 or n-3 or α -linolenic acid) (C18:3) and ω -6 (omega-6 or n-6 or linoleic acid) (C18:2) [1]. In the body, essential fatty acids serve multiple functions. In each of these, the balance between dietary ω -3 and ω -6 strongly affects function. They are modified to make the classic eicosanoids (affecting inflammation and many other

cellular functions), the endocannabinoids (affecting mood, behavior and inflammation), the lipoxins and resolvins (in the presence of aspirins, and downregulating inflammation), the isofurans, neurofurans, isoprostanes, hepoxilins, epoxyeicosatrienoic acids and neuroprotectin D. They act on DNA (activating or inhibiting transcription factors as NF κ B, which is linked to pro-inflammatory cytokine production [2]. They form lipid rafts (affecting cellular signaling) [3].

Almost all the polyunsaturated fats in the human diet are from EFA. Some of the food sources of ω -3 and ω -6 fatty acids are fish and shellfish (mackerel, herring, tuna, sardines and salmon), legumes (mung beans, kidney beans, pinto or lima beans, soybean, and split peas), flaxseed (linseed),

hemp oil, soybean oil, canola (rapeseed) oil, pumpkin seeds, sunflower seeds, walnuts and leafy vegetables (lettuce, broccoli, kale, and spinach) [4, 5].

The increasing demand for protein for human and animal consumption, and the high price and habit of meat avoidance have resulted in increased demand of fish and beans as a source of dietary protein. Fish are a rich source of ω -3 and ω -6 fatty acids and its lipids are well known to be rich in long chain ω -3 fatty acids, especially, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6]. Fatty acid composition of the fish differs due to climatic condition, diet, age, maturity and types of species [7].

Consumable oils are different from countries to countries according to their availabilities. Peanut oil, soybean oil, olive oil, and palm oil are used commonly as cooking oils. Most of the fat in soybeans is polyunsaturated (linoleic acid and linolenic acid). Primary fatty acids of olive oil are oleic acid (55%-85%), monounsaturated fatty acid although 50% of fatty acids in palm oil are saturated [8].

Consumption of fish varies from region to region in Myanmar. People residing in coastal region are used to consume more marine fish than fresh water fish. Marine fish are well known to have particularly high levels of EPA and DHA [9]; however, fatty acids composition of fresh water or farmed fish which are consumed more by people of inland region have not been studied yet. Actually, fish themselves can not produce ω -3 fatty acids, but the fatty acids composition of fish depend on their dietary sources, i.e., micro-algae. Fish could fill in some extent of protein and fat requirement for the persons who cannot afford expensive meat in their diet.

Therefore, the general objective of this study was to investigate the commonly consumed fish with rich source of essential fatty acids and the specific objectives were to determine the fatty acids compositions in fresh water fish, marine water fish and

brackish water fish which are commonly consumed by people residing in Yangon.

MATERIALS AND METHODS

Commonly consumed items of fish were explored after listing freely of 50 volunteers with five different groups of occupations living in Yangon. Yangon was purposely selected because being the city, people with heterogeneous occupations are residing, and various kinds of fish are available from markets and wholesale centers. A total of 16 items of fish (8 fresh water fish, 5 marine water fish and 3 brackish water fish) were identified as commonly consumed ones and these food samples were purchased from different markets of Yangon, packed in plastic bags and transported to the laboratory in ice box. The edible portions (skinless fillets) of collected fish samples (composite samples) were cut into small portions and frozen on the day of sample collection. Then, the moisture of frozen samples was removed under -10°C for 10 hours to obtain dried sample with constant weight. They were stored at -80°C until fat extraction.

Extraction of crude oil from fish tissue

Duplication of each item of fish was done for crude oil extraction, i.e., 32 extractions for total 16 items of food samples. The crude oils were extracted in petroleum ether by using Soxhlet apparatus [10]. The extracted oils were then stored at -18°C until fatty acid analysis.

Derivatisation of fatty acids

Fatty acid methyl esters (FAME) were prepared according to the AOCS method Ce 1b-89 [11]. Approximately 100 mg of extracted fat were placed in a screw-cap tube containing 0.5 mg of tricosanoic acid (23:0) methyl ester internal standard, 1.0 ml of 0.5N alcoholic potassium hydroxide added and the solution was blanketed with nitrogen before heating at 100°C for 5 min. After cooling, 400 μl of BF_3 /methanol were added and the solution was heated at 100°C for 15 min. After cooling to 30 - 40°C , 2 ml

of distilled water were added, the solution was vortexed for 30 sec and 3 ml of petroleum ether were immediately added. After vigorous agitation, the tubes were left to stand until clear petroleum ether was separated. The upper layer was collected and an additional extraction of the bottom layer was done using another 3 ml of petroleum ether. The combined petroleum ether extractions were concentrated under a nitrogen stream to purify fatty acid methyl ester and then redissolved methyl esters into 1 ml of chloroform. The fatty acid methyl esters samples were then stored at -18°C until analyzed by gas chromatography. The FAME (fatty acids methyl ester) standards were dissolved into chloroform directly.

Gas chromatography

Analysis of the FAME samples was carried out using a Shimadzu GC-17A equipped with a split/splitless injector, FID detector and the column with 25 m long, 0.25 mm ID and 0.25 µm phase thickness (df). The operation parameters for the GC separation were set according to AOCS method Ce 1b-89. The column temperature was ramped up from 50°C to 220°C according to the temperature program of the method. The temperatures in the injector port and the detector were 250°C and 260°C, respectively. The column flow rate was 1.5 ml/min with a split ratio of 1:20. Ultra high purity nitrogen (99.999%) was used as carrier gas. The FAME samples (0.5 µl) as well as fatty acids methyl standards were manually injected into the GC.

Data processing and analysis

The FAME samples were injected three times into the GC and the concentration of each fatty acid was calculated from each the peak area of fatty acids of FAME standards. The results were recorded and processed using the programmed software in GC 17A. The percent of each fatty acid was computed from the total peak area. The absolute concentrations of each fatty acid were calculated and converted into mg per 100 g of oil.

RESULTS

The crude oil contents (g/100g of the food) are shown in mean values in Table 1. The highest contents of oil are found in Paradise threadfin (ig;yPm;) (8.38±0.06 g/100g), Striped catfish (ig;we) (10.98±0.24 g/ 100 g), and Spanish mackerel (ig;ui;&y) (38.46 ±0.28 g/100g) among studied items of brackish water fish, fresh water fish and marine water fish, respectively.

Table 1. Crude oil (g/100g of food) contents of commonly consumed fish

Name of food	Scientific name	Myanmar name	Crude oil (g/100g food)
<i>Brackish water fish</i>			
Hilsa shad	<i>Tenulosa ilisha</i>	ig;oa v m u l	0.81
Paradise threadfin	<i>Polynemus paradiseus</i>	ig;yP m ;	8.38
Seabass	<i>Lates calcarier</i>	u u w p l	0.42
<i>Fresh water fish</i>			
Striped catfish	<i>Pangasius hypophthalmus</i>	ig;we l	10.98
Rohu	<i>Labeo rohita</i>	ig;jr p c i l ;	2.86
Globe-fish	<i>Diodom hystrix</i>	ig;yw o i l	0.75
Dwarf catfish	<i>Mystus pulcher</i>	ig;p i l i ;	2.28
Featherback	<i>Notopterus notopterus</i>	ig;z , l	0.42
Walking catfish	<i>Clarias batrachus</i>	ig;c l	1.9
Mrigal	<i>Cirrhinus mrigala</i>	ig;l u i ;	10.79
Striped snakehead	<i>Channa striata</i>	ig;& l	2.1
Tilapia	<i>Oreochromis sp.</i>	w l v m ; A ; , m ;	0.86
<i>Marine water fish</i>			
Sardine	<i>Clupeichthys aesarnensis</i>	ig;a o w m i g ;	0.32
Mackerel	<i>Scomberomorus guttatus</i>	ig;u i l i ; & y l	38.46
Croaker	<i>Lutjanus johnii</i>	ig;y g ; e l	0.07
Bombay Duck	<i>Harpadon nehereus</i>	ig;E y l	0.86

The fatty acids compositions of the brackish water fish are shown in mean values in Table 2. In 100 gm of oil, Seabass (u u w p) contains 131.5±0.21 mg of total saturated fatty acids, 73.09±0.14 mg of total monounsaturated fatty acids and 18.6±0.6 mg of linoleic acid (C18:2). Seabass is rich in fatty acids among brackish water fish. However, 2494.2±12.59 mg and 2308.9 ±80.5 mg of C22:6 (DHA) were found in 100 gm oil of Hilsa shad (ig;O a v m u) and

Table 2. Fatty acid composition (mg/100g of oil) in studied brackish water fish

Fatty acids	Hilsa shad (<i>H. ilisha</i>)	Paradise threadfin (<i>P. m.</i>)	Seabass (<i>S. u.</i>)
<i>Saturated fatty acids</i>			
C12:0	-	-	3.3
C14:0	14.36	22.69	18.1
C16:0	30.14	40.72	51.18
C18:0	17.86	34.07	49.93
C20:0	0.95	1.971	3.52
C22:0	3.97	5.897	-
C24:0	3.63	5.008	5.46
Total	70.91	110.35	131.5
<i>Monounsaturated fatty acids</i>			
C14:1	-	0.01	-
C16:1	9.06	-	26.85
C18:1	19.9	22.53	43.33
C20:1	0.9	1.56	2.92
C22:1	0.91	-	-
C24:1	0.02	-	-
Total	30.78	24.09	73.09
<i>ω-3 fatty acids</i>			
C18:3	-	-	-
C22:6	2494.2	2308.89	1840.6
Total	2494.2	2308.9	1840.6
<i>ω-6 fatty acids</i>			
C18:2	9.86	1.2	18.6
C20:4	-	-	-
Total	9.86	1.2	18.6

Paradise threadfin (*P. m.*), respectively. Therefore, they are the rich sources of ω-3 fatty acids. α-linolenic acid (C18:3) and arachidonic acid (C20:4) were not detected in all samples of brackish water fish.

The fatty acids compositions of the marine water fish are shown in mean values in Table 3. Among four marine water fish, sardine (*S. m.*) is the most abundant in all forms of fatty acids, i.e., 337.7±15.9 mg of saturated fatty acid, 82.69±2.7 mg of monounsaturated fatty acids, 2831.8±65.32 mg of ω-3 fatty acids (C18:3 and C22:6), and 811.6±36.45 mg of ω-6 fatty acids (C18:2 and C20:4) in 100g of oil. Moreover, C18:3 (linolenic acid) was found only in sardine. Croaker (*C. y.*) contains the highest amount of ω-6 fatty acids (C18:2 and C20:4), i.e., 1430.9±45.45 mg/100 gm of oil, but Bombay Duck (*B. y.*) contains

Table 3. Fatty acid composition (mg/100 gm of oil) in studied marine water fish

Fatty acids	Sardine (<i>S. m.</i>)	Mackerel (<i>M. u.</i>)	Croaker (<i>C. y.</i>)	Bombay Duck (<i>B. y.</i>)
<i>Saturated fatty acids</i>				
C12:0	5.19	-	-	2.55
C14:0	72.95	19.54	11.02	9.39
C16:0	87.18	36.86	512.20	16.32
C18:0	117.76	35.65	70.16	10.28
C20:0	4.86	0.8	-	-
C22:0	49.73	-	13.44	3.47
C24:0	-	3.16	-	-
Total	337.7	96.19	145.8	42.01
<i>Monounsaturated fatty acids</i>				
C14:1	-	-	-	-
C16:1	66.84	26.96	25.43	13.25
C18:1	15.85	12.56	16.36	5.8
C20:1	-	0.6	-	-
C22:1	-	-	-	-
C24:1	-	-	-	-
Total	82.69	40.13	41.79	19.05
<i>ω-3 Fatty acids</i>				
C18:3	98.57	-	-	-
C22:6	2733.2	1090.6	325.3	571.9
Total	2831.8	1090.6	1325.3	571.9
<i>ω-6 Fatty acids</i>				
C18:2	13.71	1.43	11.3	1.37
C20:4	797.89	-	419.6	-
Total	811.6	1.43	1430.9	1.37

the lowest amount.

The fatty acids compositions of the fresh water fish are shown in mean values in Table 4. Among nine fresh water fish, Featherback (*F. z.*) was the most abundant in all forms of fatty acids, i.e., 190.4±5.9 mg of saturated fatty acids, 102.51±10 mg of monounsaturated fatty acids, 3190.6±62.32 mg of C22:6 (DHA), and 21.58±6.45 mg of C18:2 (linoleic acid) /100g of oil. ω-3 Fatty acids were not found in Striped catfish (*S. w.*) and ω-6 fatty acids were not found in Rohu (*R. p.*) and Mrigal (*M. i.*).

Featherback (*F. z.*) was abundant in monounsaturated fatty acids and ω-3 fatty acids among studied fresh water fish whereas Croaker (*C. y.*) was one of the rich sources of ω-6 fatty acids among studied marine water fish.

Table 4. Fatty acid composition (mg/100 g of oil) in studied fresh water fish

Fatty acids	Striped catfish	Rohu	Globe-fish	Dwarf catfish	Feather-back	Walking catfish	Mrigal	Striped snakehead	Tilapia
<i>Saturated fatty acids</i>									
C12:0	-	-	2.72	4.99	-	-	0.9	-	-
C14:0	10.8	2.23	13.97	13.2	35.09	2.9	2.75	3.44	1.01
C16:0	29.6	22.7	38.32	33.7	82.99	10.9	20.9	24.8	3.41
C18:0	13.7	13.5	33.39	35.5	62.98	10.1	11.1	27.7	2.96
C20:0	-	1.77	-	2.04	-	-	4.45	-	-
C22:0	-	1.93	1.45	1.85	-	3.17	-	-	-
C24:0	-	0.81	2.67	3.86	9.34	2.33	-	-	-
Total	54.1	42.9	92.51	95.1	190.4	29.5	40.1	56	7.38
<i>Monounsaturated fatty acids</i>									
C14:1	-	-	-	-	-	-	-	-	-
C16:1	2.39	4.82	23.2	20.8	55.24	9.85	-	7.5	0.84
C18:1	30.2	27	11.99	16.2	39.74	8.93	30.66	29.7	3.32
C20:1	-	1.9	0.76	1.2	7.53	0.72	-	-	-
C22:1	-	-	-	-	-	-	-	-	-
C24:1	-	2.45	-	-	-	-	-	-	-
Total	32.6	36.1	35.95	38.2	102.5	19.5	30.66	37.2	4.16
<i>ω-3 fatty acids</i>									
C18:3	-	-	10.91	-	-	-	-	-	-
C22:6	-	438	1342	1828	3191	693	65.53	2135	162.5
Total	-	438	1353	1828	3191	693	65.53	2135	162.5
<i>ω-6 fatty acids</i>									
C18:2	1.32	-	-	2.73	21.58	1.46	-	7.18	9.43
C20:4	-	-	25.3	19.9	-	-	-	-	-
Total	1.32	-	25.3	22.6	21.58	1.46	-	7.18	9.43

DISCUSSION

In comparison between crude oil contents of Striped catfish (*Pangasius hypophthalmus*) and Seabass of present study and those of respective species of Mekong Delta, Vietnam, crude fat content was higher in catfish but lower in Seabass than those of Vietnam's fish [11]. The method of oil extraction was different between present study and that of Tawfik's study in Egypt [12] so that the significant difference between oil contents of Spanish mackerel in Saudi Arabia and that of our study (4.1 % vs. 38.5%) was found.

In the present study, the major saturated fatty acids identified in all fish were C14:0 (myristic), C16:0 (palmitic) and C18:0 (stearic). However, the major unsaturated fatty acids were C18:1 (oleic acid), C16:1 (palmitoleic) and C22:6 (docosahexaenoic). These findings were consistent with those of

Tawfik's study in Egypt [12]. In general, fish are relatively low in SFA (<30%), except for certain species [13]. The total SFA contents were <10%, even in sardine, therefore the remaining fatty acids of all species of fish were mono- and polyunsaturated fatty acids in this study.

Among ω-3 series, C22:6 (docosahexaenoic acid) was commoner than C18:3 (α-linoleic acid) in the studied fish. However, C20:5 (eicosapentaenoic acid) could not be detected in all studied fish although it is one of the rich sources of marine fish oil. It may be due to short length of gas chromatographic column. It was also found that not all fish products contain appreciable quantities of EPA [14]. Among ω-6 series, C18:2 (linoleic acid) was commoner than C20:4 (arachidonic acid) in all species of fish. α-linolenic acid and arachidonic acid were not found in the studied brackish water fish. These fish are

migratory in nature and their fatty acids composition may depend on climatic condition, diet, and age of fish.

In human nutrition, the important ω -3 fatty acids are α -linoleic acid (C18:3), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6). The human body cannot synthesize ω -3 fatty acids de novo, but it can form C20:5 and C22:6 from C18:3. The conversion occurs competitively with ω -6 fatty acids which are essential closely related chemical analogues that are derived from linoleic acid (C18:2). Both the ω -3 α -linolenic acid and ω -6 linoleic acid are essential nutrients which must be obtained from food. Thus accumulation of long-chain ω -3 fatty acids in tissues is more effective when they are obtained directly from food or when competing amounts of ω -6 analogs do not greatly exceed the amounts of ω -3. Competitive interactions with the ω -3 fatty acids affect the relative storage, mobilization, conversion and action of the ω -3 and ω -6 eicosanoid precursors. Linoleic acid (C18:2), the shortest-chained ω -6 fatty acid, is an essential fatty acid [15].

Arachidonic acid (C20:4) is a physiologically significant ω -6 fatty acid and is the precursor for prostaglandins and other physiologically active molecules. The biological effects of the ω -6 fatty acids are largely mediated by their conversion to ω -6 eicosanoids that bind to diverse receptors found in every tissue of the body. Dietary sources of ω -6 fatty acids are poultry, eggs, nuts, oils of soy bean, sunflower seeds, pumpkin seeds, corn and rapeseeds [16].

In the present study, all commonly consumed fish were not oily and crude oils were extracted from tissues of fish. Fish oil supplement is oil derived from the tissues of oily fish. Fish do not actually produce ω -3 fatty acids, but instead accumulate them from either consuming micro algae that produce these fatty acids, or as is the case with fatty predatory fish, by eating prey fish that have accumulated ω -3 fatty acids from micro algae. Therefore, ω -6 fatty acids

content of the studied fish may derive from their dietary sources because they are not rich in fish oil. The differences in fatty acids profile of farmed and wild types of fish may be attributed primarily to the dietary intake of the fish. The study could not show the differences between fatty acid profile of farmed and wild-caught fish because food samples were collected from the markets. In addition, regional and seasonal variations in the lipid and fatty acid patterns of different body parts, such as the head, dorsal muscle, ventral muscle and caudal muscle, need to be analyzed for better understanding of the fatty acids profile of different species.

Based on the results of our study, we concluded that not only marine water fish but also the freshwater fish (featherback) (*i;Z*,) is the rich source of ω -3 fatty acids among their respective items. Due to the general trend towards increased standards of living worldwide, people are becoming more and more health conscious, preferring to consume nutritious food with some added health benefits. Fish oils are rich in poly-unsaturated fatty acids, therefore, the persons who are not used to consume marine water fish can eat more fresh water fish with rich in essential fatty acids, like featherback (*i;Z*,).

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SHORT REPORT

Compliance with chemotherapy among female breast cancer patients in Medical Oncology Unit, Yangon General Hospital

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Worldwide, approximately 10 million people are diagnosed with cancer annually and more than 6 million died of the disease every year. Among female cancers, breast is the most common site for malignancy. In Myanmar, according to Yangon cancer registry, the incidence of breast cancer stands third for nearly 30 years (1974-2000), however, it has become the most common type of cancer from 2000 onwards [1].

The burden of breast cancer in developing countries is enormous [2]. The patients commonly present late, with advanced disease and are frequently lost to follow-up early in management with poor overall survival rate in different centers across Africa [3]. The major role of chemotherapy in management of breast cancer and its efficacy in prolonging survival has been well documented [4]. Among various strategies for managing cancer, patient's compliance with medical advice and procedures is crucial to successful treatment. Compliance with treatment regimens has been shown to be a significant problem in many areas of medicine. The beneficial effects of chemotherapeutic agents can only be realized if patients comply with their use. Compared with the developed countries, patients' compliance with chemotherapy in developing countries has been shown to be poorer [5]. Compliance (also adherence or concordance) describes the degree to which a patient correctly follows medical advice. For this reason, the present study

tried to explore magnitude of and reasons for poor compliance of chemotherapy among breast cancer patients.

A total of 174 female breast cancer patients who received chemotherapy were recruited in Medical Oncology Unit, Yangon General Hospital during 2008 and 2009, using consecutive sampling. They were interviewed with pre-tested questionnaire and their medical records were reviewed. The ages ranged from 30 to 76 years with a mean of 48.9 ± 9.5 years, of which, 56.3% were under 50 years. Most were at least high school level, married and dependents. Monthly median family income was 70,000 kyats (range: 6,000-800,000) and 80% did not meet their income.

The commonest co-morbidity was hypertension (27.6%), among them, 60% have been taking maximum two types of drug regularly. Majority (88%) of the patients had completed six cycles of chemotherapy and had good compliance. Most patients (62%) received CMF combination regimen and 2 of them switched to hormonal therapy; one was due to financial problem after receiving 4 cycles of chemotherapy and another one was due to drug side effects (such as fever, loss of appetite and/or weight and/or hair, palpitation and depression) after 1 cycle of chemotherapy.

Majority of breast cancer patients (98%) had been counseled by the doctor about disease, treatment option, side effect, treatment

benefits and follow-up. Majority of them (95%) also accepted that follow-up visit is important for breast cancer patients. However, non-adherence in 12% was seen across all age groups and disease stages of II and III. Of them, only 5 received radiotherapy as multimodality therapy. Among the defaulted patients, only 2 patients came back within a month of defaulting and completed 6 cycles of chemotherapy. The main reason for non-compliance was financial constraints (62%), followed by unbearable drug side effects (28.6%) and fallen CD4 count (4.7%). Among the non-compliant patients, majority were aged between 37-58 years, married and all of them had adjuvant chemotherapy (one-third received AC combination chemo regimen and half received CMF combination regimen) and had received only one or two cycles of chemotherapy. Only a few of them had either stage II (9 patients) or stage III (4 patients).

In this study, compliance with chemotherapy among breast cancer patients is good (88%) and main cause of non-adherence to chemotherapy is financial

problem (62%). It highlights the fact that not only financial and social supports, counseling about the requirement of continuation treatment are also important for compliance with chemotherapy among breast cancer patients.

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