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Enteric bacterial contamination and chemical composition of Oral Rehydration Salts packets in Yangon

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In acute diarrhoea except bacillary dysentery and cholera WHO recommends fluid therapy other than antibiotics to replace dehydration. There is no information on the enteric bacterial contamination and chemical composition of ORS (oral rehydration salts) packets which are available in Yangon and thus this study was carried out. It was conducted in Yangon from December 1996 to July 1997. The packets were bought from markets, drug shops and general practitioner clinics. Each packet was dissolved in water which was first boiled and then cooled. Faecal coliform count (FCC) was determined on those solution by multiple tube method at 0, 6 and 24 hour. Isolation of enteric bacterial pathogen was done by standard procedures. pH values, glucose, sodium and potassium concentrations were determined by standard methods. FCC was increased with longer duration of storage time. The enteric bacterial pathogens were not isolated from all types. pH value was acidic in solutions of "Royal D" and "Servidrat". Glucose concentration was higher and potassium concentration was lower in "Royal D" than ORS packets from Myanmar Pharmaceutical Factory.

INTRODUCTION

Oral rehydration with a solution of glucose and electrolytes has proved remarkably effective in the treatment of dehydration from acute diarrhoea. The rapidly absorbed WHO recommended oral rehydration solution was recognized as the standard solution. Although the WHO recommended solution is now widely used throughout the world, many children with diarrhoea are given other solutions, marketed by commercial manufacturers, which differ in composition from the standard solution. The unabsorbed sugars in these solutions could worsen diarrhoeal fluid losses [1].

Rapid growth of *E. coli*, an indicator of faecal contamination, would suggest that specific pathogens transmitted by this route, may also multiply in the presence of glucose and electro-

lytes. Black reported the rapid multiplication of *V. cholerae* and enterotoxigenic *E. coli* in oral rehydration salts (ORS) solution up to 48 hr of storage [2]. Significant bacterial growth was noted in ORS solution after 12 hr of storage [3].

These commercially available ORS packets must be in good quality and free from major diarrhoeal pathogens such as *E. coli*, *Salmonella*, *Shigella* and *Vibrio* spp. and thus bacterial contamination of commercially available ORS packets in Yangon was studied.

Since sodium bicarbonate, although stable in air, may slowly decompose in the moist air [4], it is necessary to investigate whether there is any decomposition of sodium bicarbonate in the presence of extra ingredients such as orange flavour or others. If

the decomposition takes place in the sugar salts mixture, then the sodium carbonate solution will be dangerous and unsuitable for therapeutic use.

The standard glucose-electrolyte solution (ORS) contains the sodium amount of 90 mmol/litre. This solution content is safe and useful in treating diarrhoea even in severely malnourished children [5]. Therefore the chemical composition of commercially available ORS packets in Yangon was also studied.

MATERIALS AND METHODS

Sample collection

The study was conducted in Yangon during December 1996 to August 1997. The oral rehydration salt (ORS) packets were bought from markets, drug shops and general practitioners' clinics. They were collected as packets. The condition of the outer surface of the packets was examined. The packets which had no holes and no stains were tested in this study. The ingredients must be easily moveable within the packets, that was the sugar-salts must not be solidified or hydrated. The ORS packets which were tested in this study were ORS (Myanmar Pharmaceutical Factory), ORS (Myanmar salt service), ORS (Central Pharmaceutical service), Electrobion (Merck), Royal D (Thailand), Diasalt, T.O.S. (To Chemical Ltd.), Servidrat (Servipharma) and Hydrite tablets. Twelve packets of each brand were tested and a total of 108 packets were tested for bacterial contamination in this study. Fifteen packets of each brand were tested and a total of 135 packets were tested for chemical composition.

Sugar salt mixture of oral rehydration salt packets was dissolved in water which was first boiled and cooled. They were dissolved in specified volume/packet or tablet which was

mentioned on the cover of the packets. The direction on 'Royal D' is written in 'Thai' language and thus it was translated. It was told to be written that to dissolve in one glass. Thus the sugar salt mixture in 'Royal D' was dissolved in 200 ml of water.

Determination of faecal coliform (FC) count

Faecal coliform count was determined on these solutions by multiple tube method at 0, 6 and 24 hours after dissolving in water. It was done by using the method described by International Commission on Microbiological Specifications of Food [6].

Isolation of enteric bacterial pathogens

The samples were plated onto MacConkey (MA), Salmonella-Shigella (SS) and Thiosulphate Citrate Bile Sucrose agar media and also inoculated into alkaline peptone water (APW) broth and incubated at 37°C overnight. They were also inoculated into Selenite-F (SF) broth and incubated at 44°C overnight. After 24 hours the specimen from APW and SF were inoculated onto TCBS and SS agar media respectively and incubated. The suspected colonies on these agar plates were tested biochemically by using triple sugar iron agar, lysine iron agar, urease agar and sulphide indole motility agar. The above procedure was done on 0, 6 and 24 hour after dissolving in water.

Determination of chemical composition

Chemical investigation of samples were conducted at 24 hours after preparation of salt solutions. pH values of the sample solutions were determined by pH meter (model/HM 208) using Beckman standard buffer calibrating solutions.

Atomic absorption spectrophotometer (PYE Unicam SP 9) was used for the electrolyte determinations using

reference hollow cathode tubes. Glucose concentration was carried out by titration method.

RESULTS

When pH values were determined, 'Royal D' and 'Servidrat' solutions were more acidic in pH as shown in Table 1. The other ORS solutions gave natural (or) slightly alkaline pH. When testing on glucose concentration, it was high in 'Royal D' solution and it was 4.5 gm%. On determination of sodium and potassium concentration, sodium was more or less similar but potassium was lower in 'Royal D' solution.

As shown in Table 2, FC count was 'O' up to 24 hours when tested on 'Servidrat', 'Hydrite' and 'T.O.S.' packets. However, it was increased with longer duration of storage time in the other brands of ORS tested. On isolation and identification of enteric bacterial pathogens on these solutions, E. coli, Salmonella, Shigella and Vibrio spp. were not isolated from all ORS solutions in this study.

DISCUSSION

In ORS packet from UNICEF the concentration of ingredients is: 20 gm of glucose, 3.5 gm of sodium chloride BP, 1.5 gm of potassium chloride BP and 2.5 gm of sodium bicarbonate BP. This concentration fulfills the rehydration of the patient caused by acute diarrhoea.

Rapid absorption of sodium and water to replace extracellular fluid deficits is essential in receiving the pathophysiological process in dehydration [7]. The relatively poor absorption of sodium and water appears to have resulted in part from osmotic effects of unabsorbed glucose [8]. In this study glucose concentration was increased in 'Royal D'. The unabsorbed glucose which was more than

the standard concentration may interfere the sodium and water absorption in dehydrated patients. Thus the dehydration may not be corrected.

The pH values of 'Royal D' and 'Servidrat' ORS solutions were acidic in this study. It may give rise gastritis to the users.

In a randomized double blind trial, infants with mild or moderate dehydration were rehydrated with simple solution containing table sugar and salt (without potassium or bicarbonate) or with complete glucose/electrolyte formula. Oral therapy with simple sugar/salt solution frequently developed hypokalaemia and greater volume of vomiting during treatment [9]. In 'Royal D' solution the potassium concentration was much lower than 'ORS (MPF)' solution. It can develop hypokalaemia and more vomiting to the diarrhoeal children during treatment.

The citrate-based oral rehydration solution was found to decrease the time before vomiting stopped, decrease period of dehydration, reduce period of diarrhoea and the quantity of stool output. The only disadvantage was found that the citrate-based solution was distasteful than bicarbonate-based solution [10]. Thus nowadays the citrate-based oral rehydration solution was effectively used in treatment of dehydration in acute diarrhoea.

A solution from tablet of ORS packet would have advantages of stability under environmental exposure. The increased dissolution lag time for the compact tablet is a disadvantage that can be overcome by instructions to crush the product immediately before use [11]. Thus 'Servidrat' and 'Hydrite' ORS tablet may have the longer shelf-life than others but it may be needed to crush the product immediately before use.

To formulate judicious recommenda-

Table 1. The chemical composition of oral rehydration salts (ORS) solution according to different types of ORS packets

Different types of ORS packets	Specified vol/packet or tablet	pH values				Glucose % (g/100 ml)	Sodium % (g/100 ml)	Potassium % (g/100 ml)
		0 hr	6 hr	12 hr	24 hr			
Hydrite (tablet)	100 ml	7.92	8.09	8.53	8.82	2.25	0.38	0.081
Servidrat (tablet)	120 ml	3.12	3.18	3.21	3.17	1.95	0.38	0.082
I.O.S.	150 ml	7.99	7.66	7.32	7.27	2.03	0.35	0.080
Diasalt	200 ml	8.09	7.66	7.38	7.19	1.99	0.37	0.089
Royal-D	200 ml	2.91	2.95	2.98	2.94	4.50	0.19	0.020
ORS (Myanmar salt service)	500 ml	9.06	9.13	9.32	9.25	2.10	0.25	0.079
ORS (Central Pharmaceutical Service)	500 ml	8.86	8.97	9.26	9.23	1.54	0.28	0.079
Electrobion	1000 ml	7.34	7.21	7.16	7.12	2.08	0.25	0.080
ORS (Myanmar Pharmaceutical Factory)	1000 ml	8.38	7.85	7.44	7.37	1.95	0.20	0.076

Mean of 15 packets results - 3 packets each were used for one assay

Table 2. The degree of contamination of oral rehydration salts (ORS) solution according to different types of ORS packets and different storage time

Different types of ORS packets	Specified vol/packet or tablet	FCC in different storage time (hrs)		
		0 hr	6 hr	24 hr
Hydrite (tablet)	100 ml	0	0	0
Servidrat (tablet)	120 ml	0	0	0
I.O.S.	150 ml	0	0	0
Diasalt	200 ml	0	0	4
Royal-D	200 ml	0	5	39
ORS (Myanmar salt service)	500 ml	0	21	39
ORS (Central Pharmaceutical Service)	500 ml	0	3	75
Electrobion	1000 ml	0	0	9
ORS (Myanmar Pharmaceutical Factory)	1000 ml	0	0	3

FCC = Faecal Coliform Count

Mean of 12 packets result, one packet each were used for one assay

tions for preparation, storage of such solution, the authors assessed the capability of recognized bacterial enteropathogens to survive and proliferate in solution made either with sterile water or river water collected in 2 developing countries. *V. cholerae* and ETEC pathogens which were

associated with transmission by food and water, reached concentration of 10^3 - 10^4 /ml by 12 hr and 10^4 - 10^6 by 24 hr after inoculation of solution made with river water and somewhat lower concentration in distilled water [12]. Significant bacterial growth was noted in oral rehydration solution

after 12 hr of storage. Faecal coliform (FC) count was also increased with longer storage of time in this study. Originally, all the types of ORS solutions were not contaminated because FC count was '0' at '0' hr. During preparation, pipetting and storage FC count was increased because enteric bacteria could survive and proliferate in these solutions in the presence of glucose and electrolytes. Thus health education is needed to give to prepare ORS solution with boiled water and use freshly. Usually the diarrhoeal children consumed less than one litre of solution in a day, and thus supply of half litre packets appears to be more practical.

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A study of HbA1c in diabetics of Mandalay General Hospital

Khin Ye Myint & Than Than Kyaing

Mandalay General Hospital

The Diabetes Control and Complications Trial (DCCT) demonstrated that in insulin dependent diabetes mellitus, the incidence of retinopathy, nephropathy and neuropathy could be reduced by intensive treatment and reduction of blood glucose concentration and glycosylated haemoglobin values to normal. The present methods of assessing diabetic control eg. urine glucose measurements or random blood glucose estimations tend to be unreliable. They only relate to blood glucose control over the preceding few hours, or minutes respectively. The newly available measurement of the degree of glycosylation of haemoglobin provides an index of integrated plasma glucose levels over a longer period of time, and a completely new tool to aid clinicians. Ninety-six diabetic cases from Medical Unit II and diabetic clinic, Mandalay General Hospital were studied over a 10-month study period from October 1994. Fasting blood sugar and HbA1c levels of patients with and without diabetic complications and risk factors were compared. There is a positive correlation between fasting blood sugar and HbA1c levels in this study. However, there is no significant difference in fasting blood sugar and HbA1c levels of diabetic patients with and without complications as patients were taking regular treatment and follow-up at the diabetic clinic. The mean duration of diabetes in diabetics with and without complications was 6.58 years and 2.69 years respectively. Patients with complications had a significantly longer duration of diabetes although their fasting blood sugar and HbA1c levels are level with those of patients without diabetic complications. This supports the evidence that long-term complications of diabetes are not solely dependent on glycaemic control and duration of diabetes plays an important role. Statistical analysis was done by correlation coefficient and Student's 't' test.

INTRODUCTION

Diabetes mellitus is a disease of metabolic dysregulation, most notably abnormal glucose metabolism, accompanied by characteristic long-term complications like retinopathy, nephropathy and neuropathy [1]. The Diabetes Control and Complications Trial (DCCT) demonstrated that in insulin dependent diabetes mellitus, the incidence of retinopathy, nephropathy and neuropathy could be reduced by intensive treatment and reduction of blood glucose concentration and glycosylated haemoglobin values to normal [2]. The present methods of assessing diabetic control (eg. urine glucose measurement or random blood glucose estimation) tend to be

inadequate and unreliable. They only relate to blood glucose control over the preceding few hours or minutes respectively. The newly available measurement of the degree of glycosylation of haemoglobin provides an index of integrated plasma glucose levels over a longer period of time, at least the preceding 6-8 weeks and a complete new tool to aid clinicians [1].

Haemoglobin A₁ is structurally identical to haemoglobin A₀ except for the addition of a glucose group to terminal amino acid of the B chain of the haemoglobin molecule. This is a post synthetic, nonenzymatic reaction and the rate of synthesis of HbA_{1c} accumulates throughout the

life span of red blood corpuscle and its concentration reflects the blood glucose level. Measurement of HbA1c is therefore used as a supplement to blood glucose estimation to monitor the overall degree of diabetic control achieved [3].

In advanced health centres glycosylated haemoglobin estimation test is a requisite for monitoring diabetic patients. Like advanced health centres, glycosylated haemoglobin estimation using the modified Phenol Sulfuric Acid (PSA) method estimated in Department of Biochemistry, Institute of Medicine, Mandalay was made available to us to assess the glycaemic status and diabetic control of our diabetic patients in MGH.

Aims and objectives

1. To detect the correlation between fasting blood sugar and haemoglobin A1c levels.
2. To determine the association between fasting blood sugar, haemoglobin A1c and diabetic complications and risk factors.

MATERIALS AND METHODS

Apparently normal healthy persons of both sexes between 35-55 years were sampled for establishment of reference value of glycosylated haemoglobin in control group. The donors were from Thatmadow Canning Factory, Mandalay. Diabetes mellitus cases from Diabetic Clinic and Medical Unit II were studied over a 10-month study period from October, 1994. Complete history and physical examination were performed and factors affecting HbA1c such as drugs (asprin, ascorbic acid and alcohol), pregnancy, haemoglobinopathy, hypertriglyceridaemia and renal failure were excluded in these patients. Blood samples for fasting blood sugar and HbA1c were taken. Other investigations for diabetes mellitus, its complications and risk factors were carried out according to a proforma.

The modified short duration Phenol-sulfuric Acid (PSA) method for glycosylated haemoglobin estimation [4] is one of the calorimetric methods. It is a relatively simple, inexpensive, cost-effective, accurate and sensitive method.

The Phenol-Sulfuric Acid (PSA) Method
The phenol sulfuric acid (PSA) method is one of calorimetric assay.

Results in the original PSA method

Normals = 2.32 ± 0.36 mg hexose/gm of Hb

Diabetic = 4.21 ± 0.66 mg hexose/gm of Hb

Glycosylated Hb = $\frac{\text{Fructose mg} \times 18.1}{\text{Total Hb}}$
as percentage of
total haemoglobin

By this conversion the glycosylated haemoglobin concentration for the control subject is $2.2 \pm 0.5\%$ and for the diabetic patient is $6.4 \pm 3.6\%$ [4] (Annex 1).

RESULTS

Ninety-six diabetic cases from Medical Unit II and Diabetic Clinic were studied.

Table 1 shows the frequency distribution of diabetic cases according to age groups. Most of the patients (90.36%) were between 31 to 70 years. Twenty were males and 76 were females. Male and female ratio is 1:3.8.

Figure 1 shows distribution of diabetic cases according to their complications. Out of 96 cases, ischaemic heart disease, retinopathy, nephropathy and neuropathy were detected in 52 cases (54.17%), 51 cases (53.13%), 43 cases (44.79%) and 32 cases (33.33%) respectively.

Figure 2 shows the frequency distribution of diabetic cases according to certain diabetic risk factors. Out of 96 cases, hypertension, hypercholesterolaemia and smoking were noted in

Table 1. Frequency distribution of diabetic cases according to age group

Age groups	No. of cases
11-20	1
21-30	4
31-40	9
41-50	22
51-60	34
61-70	22
71-80	3
81-90	1

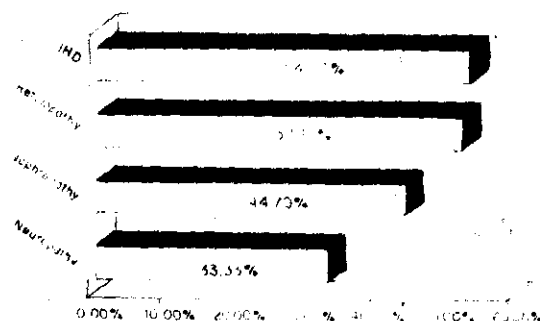


Fig. 1. Frequency distribution of diabetic cases according to complications

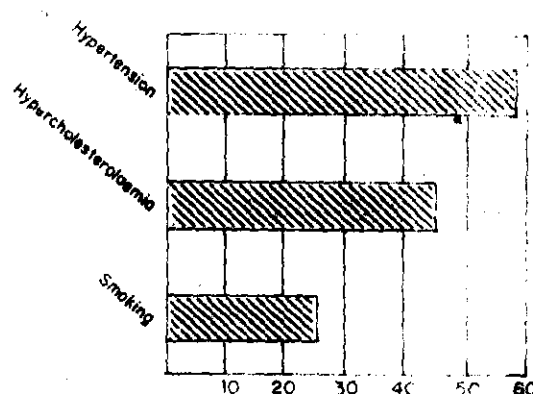


Fig. 2. Frequency distribution of diabetic cases according to certain risk factors

58 cases (60.42%), 45 cases (46.88%) and 26 cases (27.08%) respectively.

Figure 3 shows the correlation between fasting blood sugar and HbA1c

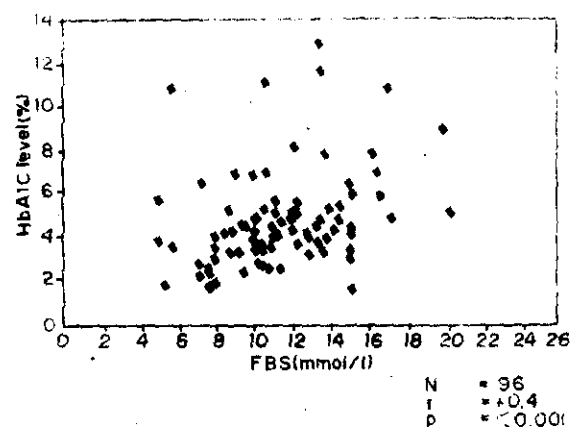


Fig. 3. Correlation between fasting blood glucose and HbA1c levels in diabetes mellitus cases

levels. There is a positive correlation between fasting blood sugar and HbA1c levels ($r = +0.4$, $n = 96$, $a = 2.3$, $b = 0.18$, $y = 2.3 \pm 0.18 x$).

Table 2 compares the mean $\pm$ SD HbA1c levels of diabetics with and without diabetic complications and risk factors. The mean $\pm$ SD HbA1c levels were $4.36 \pm 1.32\%$, $4.43 \pm 1.64\%$, $4.63 \pm 1.6\%$, $4.32 \pm 1.52\%$, $4.5 \pm 1.62\%$, $4.41 \pm 1.5\%$, $4.38 \pm 1.55\%$ for those with neuropathy, retinopathy, nephropathy, ischaemic heart disease, hypercholesterolaemia, hypertension and smoking respectively. The mean $\pm$ SD HbA1c levels were $4.37 \pm 1.55\%$, $4.34 \pm 1.2\%$, $4.25 \pm 1.31\%$, $4.45 \pm 1.44\%$, $4.39 \pm 1.26\%$, $4.4 \pm 1.38\%$, $4.08 \pm 1.42\%$ for those without neuropathy, retinopathy, nephropathy, ischaemic heart disease, hypercholesterolaemia, hypertension and smoking respectively. These results show that there was no significant difference between HbA1c levels of diabetic patients with and without risk factors and complications.

Table 3 compares the mean $\pm$ SD fasting blood sugar levels between diabetic patients with and without risks and complications. The mean $\pm$ SD F.B.S. levels were 11.94 ± 2.99 , 11.44 ± 3.25 , 11.94 ± 2.84 , 11.13 ± 3.37 , 11.69 ± 3.41 , 10.9 ± 3.31 , 10.22 ± 2.82 for those patients with neuropathy, retinopathy, nephropathy, ischaemic heart

Table 2. Mean $\pm$ SD HbA1c level of diabetic patients with and without diabetic complications and risk factors

Diabetic complications and risk factors	Mean $\pm$ SD HbA1c levels of patients with complication and risk factors %	Mean $\pm$ SD HbA1c levels of patients without complication and risk factors %
Neuropathy	4.36 $\pm$ 1.32	4.37 $\pm$ 1.55
Retinopathy	4.43 $\pm$ 1.64	4.34 $\pm$ 1.20
Nephropathy	4.63 $\pm$ 1.60	4.25 $\pm$ 1.31
IHD	4.32 $\pm$ 1.52	4.45 $\pm$ 1.44
Hypercholestromlaemia	4.50 $\pm$ 1.62	4.39 $\pm$ 1.26
Hypertension	4.41 $\pm$ 1.50	4.40 $\pm$ 1.38
Smoking	4.38 $\pm$ 1.55	4.08 $\pm$ 1.42

Statistically not significant, using Student's 't' test

Table 3. Mean $\pm$ SD FBS levels of diabetic patients with and without complications and risk factors

Diabetic complications and risk factors	Mean $\pm$ SD FBS levels of patients with complication and risk factors (m-mol/l)	Mean $\pm$ SD FBS levels of patients without complication and risk factors (m-mol/l)
Neuropathy	11.94 $\pm$ 2.99	10.10 $\pm$ 3.22
Retinopathy	11.44 $\pm$ 3.25	11.23 $\pm$ 3.05
Nephropathy	11.94 $\pm$ 2.84	10.86 $\pm$ 3.28
IHD	11.13 $\pm$ 3.37	11.45 $\pm$ 2.97
Hypercholestromlaemia	11.69 $\pm$ 3.41	11.03 $\pm$ 2.93
Hypertension	10.90 $\pm$ 3.31	11.87 $\pm$ 2.90
Smoking	10.22 $\pm$ 2.82	11.78 $\pm$ 3.19

Statistically not significant, using Student's 't' test

Table 4. Duration of diabetes mellitus

	Mean $\pm$ duration diabetes mellitus (in years)
With complications	6.85 $\pm$ 5.76
Without complications	2.69 $\pm$ 1.92

(p = <0.001 (Statistically significant, using Student's 't' test)

disease, hypercholestromlaemia, hypertension and smoking respectively. The mean $\pm$ SD FBS levels of diabetic without complications and risks were 10.10 $\pm$ 3.22, 11.23 $\pm$ 3.05, 10.86 $\pm$ 3.28, 11.45 $\pm$ 2.97, 11.03 $\pm$ 2.93, 11.87 $\pm$ 2.9, 11.78 $\pm$ 3.19 for patients without neuro-

pathy, retinopathy nephropathy, ischaemic heart disease, hypercholestromlaemia, hypertension and smoking. There was no significant difference in fasting blood sugar levels between diabetic patients with and without complications and risks.

Table 4. shows the duration of diabetes in patients with and without complications and risk factors. The mean $\pm$ SD duration of diabetes with and without complications were 6.85 $\pm$ 5.76 years and 2.69 $\pm$ 1.92 years respectively. The duration of diabetes was significantly longer in those with complications.

DISCUSSION

In this study out of 96 cases, 94.8% were NIDDM and only 5.2% were IDDM. Male and female ratio was 1:3.8. Majority of the patients (90.63%) were between 31 years and 70 years.

In this study there was a positive correlation between FBS and HbA1c levels ($r=+0.4$, $n=96$, $p<0.001$). Similarly in a study by Beisswenger, there was a significant correlation between mean blood glucose and HbA1c levels ($r=0.66$, $p=0.001$) [5]. Koenig studied the increased levels of haemoglobins A1a + A1b and A1c in diabetic patients and determined that changes in diabetic control caused parallel changes in the levels of these haemoglobins [6]. Gonem noted the positive correlation of FBS and HbA1c ($r=0.74$, $p<0.001$, $n=55$) in maturity onset diabetic patients [7]. These studies supported that the measurement of nonenzymatically glycosylated haemoglobin has been widely used in clinical practice to assess glycaemic control in diabetes mellitus and offers significant advantages over traditional methods of clinical assessment of glycaemic control.

In this study there was no significant difference between mean HbA1c levels and FBS levels between diabetic patients with and without complications and risk factors. Both patients with and without diabetic complications and risk factors had mean HbA1c $<4.5\%$. This may be attributed to the fact that these patients with complications were taking regular treatment and follow-up at our diabetic clinic. The DCCT conducted a 9-year study on IDDM patients and showed that if blood glucose levels were maintained at nearly normal levels by intensive therapy, there was an approximate 60% reduction in the risk of retinopathy, nephropathy and neuropathy [8]. Reichard reported that in IDDM smoking habits were correlated with total number of

complications (retinopathy, neuropathy, nephropathy) deteriorating as was HbA1c during the study ($p<0.001$) [9]. Klein stated that there is a positive relationship between incidence and progression of retinopathy and HbA1c and this suggests a strong and consistent relationship between hyperglycaemia and incidence and progression of retinopathy [10]. The Wisconsin epidemiologic study on diabetics noted that risks for retinopathy were hypertension, hypercholesterolaemia, smoking and age [11]. Ford reported that age, sex, current smoking and hypertension were associated with coronary artery disease mortality among diabetics [12].

In this study the mean duration of diabetes in diabetics with and without complications was 6.85 years and 2.69 years respectively. Patients with and without complications had a significantly longer duration of diabetes although their fasting blood sugar and HbA1c levels are level with those of patients without diabetic complications. In the UK hospital clinic population, a cross-sectional multicentric study involving 6487 diabetic patients examined in 188 clinics, neuropathy increased with both age and duration of diabetes mellitus until it was present in $>50\%$ of NIDDM subjects aged over 60 years [9]. In Orchard's study, background retinopathy was virtually universal after 20 years of diabetes mellitus. He reported proliferative retinopathy affects 70% of IDDM after 30 years duration [13]. Duration of diabetes was the most important risk supported by Wisconsin epidemiologic study of diabetic retinopathy [11]. Chen reported that diabetic retinopathy was correlated with duration of diabetes, age at onset of diabetes, type of diabetes treatment, in higher serum creatinine levels and lower serum cholesterol levels [14]. A cross-sectional study in Norway reported that retinopathy was found in 32.8% of diabetes. Patients with

retinopathy had a significantly higher mean age reflecting a longer mean duration of diabetes and had a higher mean HbA1c [9]. According to Donhorst there is a positive correlation between levels of glycaemic and retinopathy, the prevalence increasing with increasing duration of the disease. They reported approximately 80% of diabetic subjects will have detectable background retinopathy by 15 years, with a smaller percentage having maculopathy or proliferative retinopathy [15]. Said reported that the most important risk for neuropathy was longer duration of diabetes mellitus [16]. Krowleski reported that retinopathy depends on duration of disease [17]. Monske reported that there is association of both higher HbA1c levels and number of years of diabetes mellitus in patients with nephropathy [18]. These studies support our findings that long-term complications of diabetes are not solely dependent on glycemic control and duration of diabetes plays an important role.

CONCLUSION

It can be concluded that HbA1c is an effective method of measuring the degree of blood glucose control over a longer period of time in diabetics. In our study we have found that long-term complications of diabetes mellitus are not solely dependent on glycaemic control but also on the duration of diabetes mellitus.

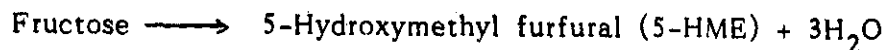
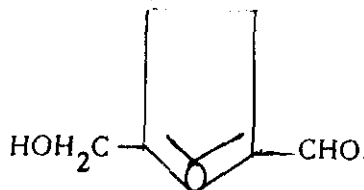
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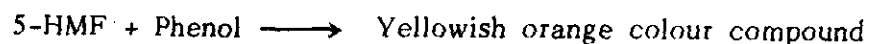
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ANNEX 1

The phenol-sulphuric acid (PSA) method



The aldehyde group of 5-HMF is condensed with phenol, and the colour produced is measured with a photoelectric colorimeter or a spectrophotometer at 480 nm.



Cost analysis of hospitalized malaria patients in Taikkyi Township

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A hospital-based study was conducted in Taikkyi Township to elicit the various types of cost incurred and factors influencing these costs. Data were obtained from malaria cases of different severity admitted to the hospital from October, 1995. All study subjects were interviewed by using a pretested standardized questionnaire. The total costs of illness per patient for one episode of malaria were estimated as kyats 2582 for uncomplicated malaria case, kyats 4056 for cerebral malaria case, kyats 4568 for other severe and complicated malaria case and kyats 4758 for malaria with other disease case. In the cost before and during hospitalization, direct cost was more than indirect cost. Cost for income lost and drug cost were the highest. Multivariate analysis revealed that days of illness and days of absence from work before hospitalization, malaria parasite density status, income lost of patient and total attendant's cost before hospitalization, family income, distance between home and hospital and days of actual illness were important determinants for various types of cost incurred for hospitalized malaria patients. This study will focus on the need for large-scale similar studies in the country in future.

INTRODUCTION

Malaria has been considered as the first national priority disease in Myanmar [1,2]. It affects the socio-economic condition of the country. For instance, reduction in labour force and re-allocation of household income to health expenses can lead to poor economy of the people [3].

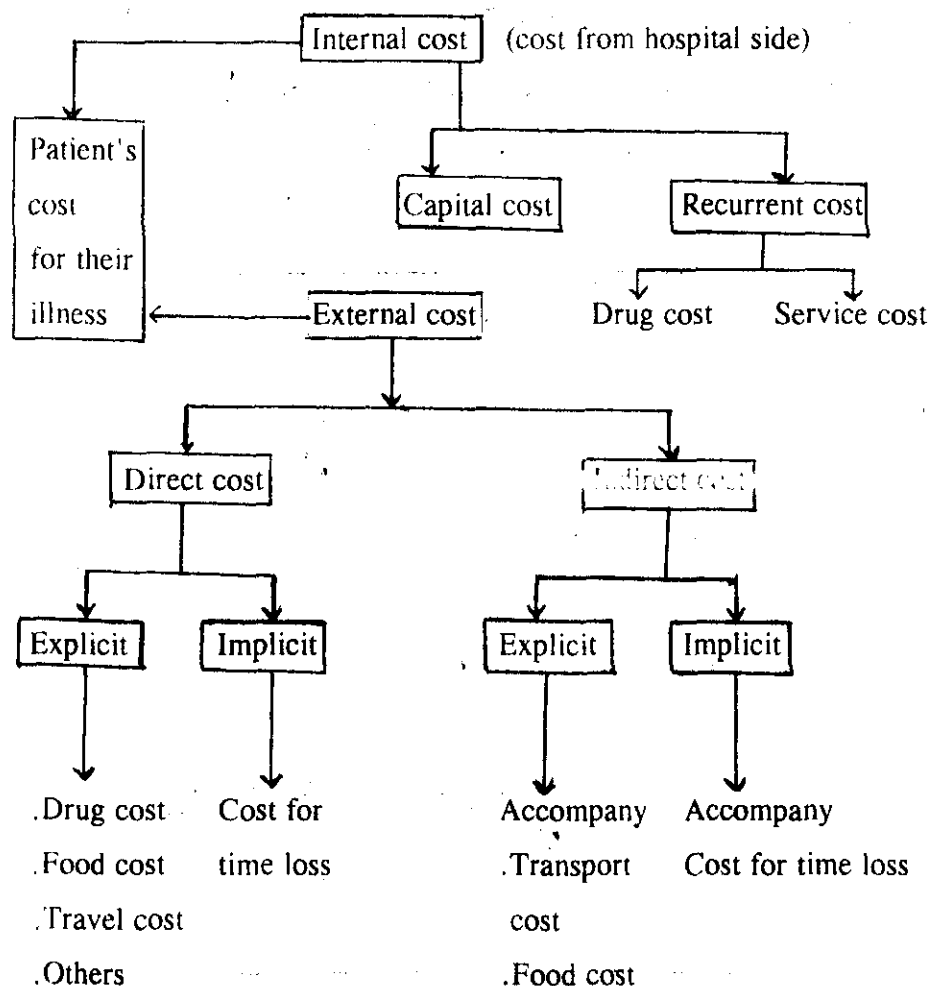
There were many studies regarding the economic costs of malaria in other countries. In a study done by Ettling, 1991, comprehensive estimate of direct and indirect economic cost of malaria were estimated. Representative household surveys were carried out. Cost per case averaged and cost per capita were estimated by patients as well as by providers [4,5].

This study was a hospital-based

study and the characteristics of in-patient malaria cases and the various types of cost incurred by these patients during their illness were described as external and internal cost. The conceptual framework for these costs [6,7] are shown in figure 1.

There were many factors determining the costs of malaria patients during their illness. Personal, socioeconomic, disease and cost variables were important factors. Personal factors included age, sex, behaviour of patients before and during hospitalization. Socio-economic factors included marital status, education, occupation, number of family members, family income and personal income [8]. Disease factors included different severity of disease, duration of illness and days of absence from their work for their illness and duration of hospitalization.

Figure (1): Conceptual model for malaria cost incurred by patients during their illness



The aim of the study was to determine the various types of cost for malaria patients and the factors influencing the cost incurred by malaria patients in Taikkyi Township.

MATERIALS AND METHOD

This study was carried out in Taikkyi Township, a malaria endemic area in Yangon Division. It is about 49 miles north of Yangon along the Yangon-Pyay Highway. The land area is 669 square miles and the total population was 188,000 with urban rural population ratio of 1 to

2.7 in 1994. Most of them (70%) were farmers. In Taikkyi Township, there are one 50-bed township hospital, three 16-bed station hospitals, three rural health centers, one school health team, three maternal and child health centers, three disease control units, one traditional medicine clinic, two co-operative clinics, one meditrade clinic and 22 private clinics. During 1994, out of a total of 3733 in-patients, 726 patients (16.2%) were hospitalized due to malaria and case fatality rate was 5%. Slide positivity rate for malaria patients was 31.3%.

Study subjects

The study subjects consisted of malaria in-patients admitted to Taikkyi Township Hospital and Okkan Station Hospital from July to October 1995, representing more severe forms of malaria cases. These in-patients were cases with clinically suspected malaria, cerebral malaria, other severe and complicated malaria and malaria with other diseases. The clinical diagnoses [9] were made not only by the responsible medical officer in-charge of township hospital but also by the investigator and were confirmed again by laboratory examination for malaria parasites (MP). The patients with fever due to other diseases and pyrexia of unknown origin and who were MP negative and not treated with anti malarial drugs were excluded from the study.

For calculation of the sample size, the formula for estimation population values [10] was used.

$$n = \frac{Z^2 \alpha^2}{\delta + \frac{Z^2 \alpha^2}{N}}$$

n = Sample size

Z = Value from the standard normal distribution for alpha error at the 5% level
= 1.96

α^2 = Variance of total cost for illness of malaria patients. The value was obtained from the result of a pilot study of 30 malaria in-patients and is equal to kyats 1599.6

δ^2 = Proportion of acceptable error, set at the 10% level (10% of mean costs)
= (361.6)²

N = Total number of malaria in-patients admitted to the hospital within one year
= 726 patients. This figure was the average of the five-year data of in-patient malaria cases in Taikkyi Township.

In order to fill up cost data in the formula, the pilot study as mentioned

above was conducted on 30 in-patients selected by consecutive sampling method using the same pretested standardized interview-administered questionnaire for the main study. Total sample size was thus estimated to be about 100 cases. Therefore, the remaining 70 patients were further interviewed to obtain the required data.

Collection of data

The following data were collected.

(1) Socio-demographic-economic characteristics of patients

This consisted of age, sex, marital status, education, occupation, number of family members, patients' and family monthly income.

(2) Behaviour of patients before hospitalization

It consisted of history of visiting to malaria endemic areas within 3 months, previous history of malaria, number of malaria episodes within 1 year, days of illness before hospitalization, days of absence from work before hospitalization, methods of treatment taken and days of absence from work for attendance.

(3) Behaviour of patients during hospitalization

This included distance between home and hospital, means of travel, travel time, days of hospitalization, number of accompanying persons to hospital, number of attendance during hospitalization, days of absence from work for attendance, malaria parasite status, type of malaria parasite, diagnosis and outcome.

(4) Various types of cost of illness for one episode of malaria

These were costs incurred by patients before hospitalization as explicit and implicit portion of direct and indirect cost. Costs incurred for patients during hospitalization was examined as internal and external costs. The internal cost is the cost incurred for malaria patients from hospital as drug cost and service cost. The

external cost is the cost incurred by patient and determined as explicit and implicit portion of direct and indirect cost. The aggregate cost is the cost incurred by patients before and during hospitalization.

(a) Direct cost (for patient)

Explicit cost: Cost incurred by patient for treatment, travel cost and food cost.

Implicit cost: Cost incurred for sickness leave, travel time and waiting time of the patients.

(b) Indirect cost (for accompanying person)

Explicit cost: Cost incurred for the accompanying persons as travel cost and food cost.

Implicit cost: Cost incurred for travel time and waiting time for accompanying persons.

Analysis of data

The data from the questionnaire were analyzed by using the EPI-INFO, SPSS software and Time Series Processor (TSP) Micro-software. Drugs cost and other treatment cost incurred by malaria patients were calculated according to standard price lists obtained from the hospital Community Cost Sharing (CCS) scheme, Central Medical Store Depot (CMSD) price list for drugs and sale price of a retail drugs seller in Taikkyi Township. Mean cost, standard deviation, maximum and minimum costs incurred by malaria patients were calculated. The differences in costs by severity of illness were tested by Kruskal-Wallis 1-way ANOVA test from SPSS SOFTWARE. Mean costs for different categories of malaria patients were ranked and summed. Then, differences were tested after correcting for ties. Correlation between total cost of

illness and its cost components was done by using Spearman Rank correlation test. The determinants of cost for malaria patients were elicited by applying the multiple regression model in TSP micro-software. The dependent variables were various types of cost and independent variables were characteristics of patients. Therefore, the strength of relationship between dependent and independent variables were found out by looking at the value of regression coefficients and set the priority of determining variables.

RESULTS

Characteristics of study subjects

Out of the total 100 respondents, 36% were uncomplicated malaria cases, 20% cerebral malaria cases, 25% other severe and complicated malaria cases and 19% malaria with other diseases. Mean age of the malaria cases was 31 years and showed male preponderance. Most of the patients were in low educational status and were farmers. Mean number of family members for malaria patients was 5. Mean personal and family income were kyats 2039 and kyats 3594 respectively (Table 1).

More than half of the patients gave past history of malaria and mean previous number of episodes for each patient was 3. Mean number of days of illness before hospitalization was 6 and mean number of days of absence from work before hospitalization was also 6. Nearly half of the patients received the treatment from general practitioners before hospitalization and one-third went to quacks.

Mean distance between home and hospital was 6 miles and mean travel time to come to hospital was one and a half hour. Mean number of accompanying persons to hospital was 4 and mean number of days of absence from work for attendance during

Table 1. Mean $\pm$ standard deviation ($X \pm SD$) of socio-demographic, economic and behavioural variables of malaria patients

Variables	Non-complicated malaria n = 36	Cerebral malaria n = 20	Severe & complicated malaria n = 19	Malaria with other disease n = 19	Combined n = 100
Age	33 $\pm$ 16	27 $\pm$ 16	26 $\pm$ 16	35 $\pm$ 19	31 $\pm$ 17
Family members	5 $\pm$ 2	5 $\pm$ 2	6 $\pm$ 2	5 $\pm$ 2	5 $\pm$ 2
Personal income (kyats)	1724 $\pm$ 2539	2262 $\pm$ 2636	2731 $\pm$ 1627	1558 $\pm$ 703	2039 $\pm$ 1750
Family income (kyats)	2983 $\pm$ 2175	3955 $\pm$ 2451	4380 $\pm$ 2451	3337 $\pm$ 1614	3594 $\pm$ 2255
Previous history of malaria	2 $\pm$ 1	3 $\pm$ 1	2 $\pm$ 1	3 $\pm$ 1	3 $\pm$ 1
Days of illness before hospitalization	6 $\pm$ 5	6 $\pm$ 5	6 $\pm$ 5	7 $\pm$ 9	6 $\pm$ 6
Days of absence from work	5 $\pm$ 5	6 $\pm$ 4	7 $\pm$ 4	7 $\pm$ 8	6 $\pm$ 5
Distance between home & hospital (miles)	6 $\pm$ 4	7 $\pm$ 4	6 $\pm$ 3	8 $\pm$ 6	6 $\pm$ 4
Travel time (minutes)	73 $\pm$ 60	89 $\pm$ 50	102 $\pm$ 77	97 $\pm$ 78	87 $\pm$ 67
Days of hospitalization	5 $\pm$ 2	6 $\pm$ 2	7 $\pm$ 4	8 $\pm$ 4	6 $\pm$ 3
Number of accompany person	4 $\pm$ 2	5 $\pm$ 3	4 $\pm$ 2	5 $\pm$ 2	4 $\pm$ 2
Days of absence from work for attendance	3 $\pm$ 3	8 $\pm$ 4	10 $\pm$ 12	10 $\pm$ 5	8 $\pm$ 7
Days of actual illness	10 $\pm$ 5	11 $\pm$ 5	13 $\pm$ 7	14 $\pm$ 9	12 $\pm$ 3

hospitalization of the patient was 8. Mean duration of hospitalization was 6 days and duration of actual illness for one episode of hospitalized malaria case was 12 days. Blood slides examination showed 99% with falciparum malaria and more than half of the patients were in hyper-parasitaemic stage (Table 1).

Among the 100 malaria cases, 3 died, 4 were referred and 93 recovered without sequelae.

Cost incurred for malaria patients

Cost before hospitalization

The mean cost before hospitalization

was kyats 930. Sixty-four per cent of patients incurred less than kyats 1000. Of the cost, 83% was due to direct cost. Again, of the direct cost, 27% was drug cost, 7% travel cost, 20% food cost and 35% income lost for patients due to absence from work (Table 2).

Cost during hospitalization

The mean aggregate cost during hospitalization was kyats 2845. Of these cost, 98% (kyats 2784) was direct and indirect cost incurred by patients and 2% (kyats 61) was incurred from hospital as internal cost (Table 3).

Table 2. Mean cost $\pm$ standard deviation ($\bar{X} \pm SD$) of in-patients before hospitalization by type of cost and severity of malaria

Type of cost	Non-complicated malaria n = 36	Cerebral malaria n = 20	Severe & complicated malaria n = 19	Malaria with other disease n = 19	Combined n = 100
Direct cost					
Explicit					
Drug cost	153 $\pm$ 165	308 $\pm$ 277	249 $\pm$ 217	320 $\pm$ 469	238 $\pm$ 281
Travel cost	44 $\pm$ 23	35 $\pm$ 18	61 $\pm$ 55	183 $\pm$ 280	67 $\pm$ 110
Food cost	148 $\pm$ 101	181 $\pm$ 123	221 $\pm$ 134	235 $\pm$ 257	189 $\pm$ 156
Other	529 $\pm$ 558	150 $\pm$ 55	400 $\pm$ 82	424 $\pm$ 618	445 $\pm$ 471
Sub total	397 $\pm$ 340	462 $\pm$ 388	533 $\pm$ 417	674 $\pm$ 1120	497 $\pm$ 592
Implicit					
Income lost	263 $\pm$ 223	500 $\pm$ 1082	342 $\pm$ 201	278 $\pm$ 233	328 $\pm$ 495
Total direct cost	652 $\pm$ 454	862 $\pm$ 1145	834 $\pm$ 504	908 $\pm$ 1322	788 $\pm$ 845
Indirect cost					
Total indirect cost	97 $\pm$ 125	89 $\pm$ 114	277 $\pm$ 317	171 $\pm$ 358	271 $\pm$ 282
Grand total cost	710 $\pm$ 476	947 $\pm$ 1158	1125 $\pm$ 722	1075 $\pm$ 1161	930 $\pm$ 998

Table 3. Mean cost $\pm$ standard deviation ($\bar{X} \pm SD$) of in-patients during hospitalization by type of cost and severity of malaria

Type of cost	Non-complicated malaria ^{a5} n = 36	Cerebral malaria ^{b6} n = 20	Severe & complicated malaria ^{c7} n = 19	Malaria with other disease ^{d8} n = 19	Combined n = 100
Direct cost					
Explicit					
Drug cost	281 $\pm$ 206	755 $\pm$ 403	896 $\pm$ 629	966 $\pm$ 912	671 $\pm$ 616
Travel cost	199 $\pm$ 213	398 $\pm$ 472	263 $\pm$ 285	363 $\pm$ 451	292 $\pm$ 351
Food cost	348 $\pm$ 171	420 $\pm$ 221	458 $\pm$ 301	518 $\pm$ 270	435 $\pm$ 236
Other	168 $\pm$ 331	127 $\pm$ 139	147 $\pm$ 211	187 $\pm$ 314	159 $\pm$ 268
Sub total	965 $\pm$ 437	1708 $\pm$ 756	1737 $\pm$ 1049	2017 $\pm$ 1230	1506 $\pm$ 947
Implicit					
Income lost	265 $\pm$ 151	280 $\pm$ 252	387 $\pm$ 316	339 $\pm$ 336	339 $\pm$ 264
Total direct cost	1229 $\pm$ 546	1988 $\pm$ 813	2124 $\pm$ 1336	2356 $\pm$ 1439	1819 $\pm$ 1119
Indirect cost					
Total indirect cost	600 $\pm$ 336	1063 $\pm$ 569	1247 $\pm$ 1503	1261 $\pm$ 660	987 $\pm$ 909
Grand total	1796 $\pm$ 787	3052 $\pm$ 1132	3370 $\pm$ 2684	3617 $\pm$ 1989	2784 $\pm$ 1876
Internal cost	39 $\pm$ 20	54 $\pm$ 36	86 $\pm$ 55	62 $\pm$ 37	61 $\pm$ 38
Aggregate cost	1835 $\pm$ 782	3106 $\pm$ 1140	3457 $\pm$ 2690	3680 $\pm$ 2011	2845 $\pm$ 1889
Total cost of illness	2582 $\pm$ 1057	4056 $\pm$ 1873	4568 $\pm$ 3082	4758 $\pm$ 2765	3787 $\pm$ 2374

a = Two cases referred to DSGH on third day of admission because they are army persons and requested to refer to DSGH

b = Two death cases, one died at first day of admission due to pulmonary oedema and another at third day of admission

c = One case died at third day of admission due to hepato-renal failure

d = Two cases referred to YGH, one at third day of admission due to haematemeses and another case at fourth day due to chronic diarrhoea not responded to treatment

Table 4. Mean ranks of various types of cost for different severity of malaria cases

Types of cost	Non-complicated malaria n = 36	Cerebral malaria n = 20	Severe and complicated malaria n = 19	Malaria with other disease n = 19
<u>Total cost before hospitalization</u>				
. Mean ranks	49.93	48.10	61.78	46.84
χ^2 value =	5.1114	χ^2 value =	5.1125(Corrected)	
p value =	0.1638	p value =	0.1637(Corrected)	
<u>Aggregate cost</u>				
. Mean ranks	31.94	60.55	59.28	63.53
χ^2 value =	23.2472	χ^2 value =	23.2491(Corrected)	
p value =	0.0000	p value =	0.0000(Corrected)	
<u>Total cost of illness</u>				
. Mean ranks	33.17	56.59	62.16	61.21
χ^2 value =	20.4672	χ^2 value =	20.4689(Corrected)	
p value =	0.0000	p value =	0.0000(Corrected)	

Table 5. Factors determining cost before hospitalization for malaria patients

Independent variables	Coefficient value, β	Standard error of β	P - value
<u>Total cost before hospitalization^a</u>			
Constant	414.916	168.772	0.016
Days of illness before hospitalization	30.677	9.945	0.003
Severity of malaria parasite status	-88.850	42.245	0.039
Income lost of patients before hospitalization	1.065	0.103	0.000
Total attendance cost before hospitalization	2.205	0.214	0.000
<u>Total cost of illness during hospitalization^b</u>			
Constant	-1694.009	836.680	0.046
Family monthly income of patients	0.235	0.067	0.001
Days of illness before hospitalization	144.579	65.219	0.030
Days of absence from work before hospitalization	-541.243	94.288	0.000
Distance between home and hospital	100.205	42.458	0.021
Days of actual illness	487.600	46.365	0.000

^a The multiple regression prediction model for total cost before hospitalization

Cost before hospitalization = 414.916 + 30.677 (Days of illness before hospitalization) - 88.85 (Malaria parasite status) + 1.065 (Income lost before hospitalization) + 2.205 (Total attendants' cost before hospitalization)

^b The multiple regression prediction model for total cost of illness during hospitalization

Total cost of illness = -1649.01 + 0.235 (Family income) + 144.579 (Days of illness

before hospitalization) - 541.243 (Days of absence from work
before hospitalization + 100.205 (Distance between home and
hospital) + 487.6 (Days of actual illness)

Total cost for illness

Mean total cost of illness for non-complicated malaria cases was kyats 2582, for cerebral malaria cases was kyats 4056, for severe and complicated malaria cases was kyats 4568 and for malaria with other disease was kyats 4578. Mean total cost for illness of in-patient malaria cases was kyats 3787. From that total cost, 25% (kyats 930) was incurred before hospitalization, 73% (kyats 2784) during hospitalization by patient and only 2% (kyats 61) from the hospital (Table 3). Out of 100 patients, 60% incurred more than kyats 3000.

Test of significance

Using Kruskal-Wallis 1-way ANOVA test, there were significant differences in mean costs during hospitalization and total cost of illness among different categories of malaria ($p < 0.005$). However, mean costs by severity of malaria incurred by patients before hospitalization were not significantly different ($p = 0.164$) (Table 4).

The Spearman Rank Correlation between total cost of illness and other costs for one episode of malaria showed that if the direct cost during hospitalization, total cost during hospitalization or aggregate cost is large, the total cost of illness will also be large. The correlations between total cost of illness and each of the direct cost during hospitalization, total cost during hospitalization and aggregate cost are highly significant, the correlation coefficient γ -values were all about +0.92 ($p < 0.001$). However, total cost of illness and cost before hospitalization are moderately correlated, γ -value being +0.58 ($p < 0.05$).

Factors influencing the costs

The dependent variables were cost before hospitalization and total cost of illness of malaria patients. The factors that significantly influenced the cost before hospitalization were days of illness before hospitalization, severity of malaria parasite status, income lost of patients before hospitalization, total attendance cost before hospitalization and those that had significant influences upon total cost of illness were family monthly income of patients, days of illness before hospitalization, days of absence from work before hospitalization, distance between home and hospital and days of actual illness (Table 5). The prediction models for various types of cost were described by applying the coefficient values of independent variables.

DISCUSSION

Although malaria is the important public health problem in Myanmar, there were few research on its cost studies. In this study, we tried to assess the cost and the factors determining the cost incurred for hospitalized malaria cases, non-complicated and complicated malaria patients such as cerebral malaria, other severe and complicated malaria, malaria with its sequelae and combined with other diseases due to effect of malaria. Cost of illness for complicated malaria cases was more than that of non-complicated cases. We found that the complicated cases took long time before getting admission and during which ineffective treatment led to late referral, threatening the life of patients and had to spend more money outside and during hospitalization. Moreover, we also found

that the average distance between home and hospital for complicated malaria cases was more than that of non-complicated cases. This fact can hinder taking of early diagnosis and prompt treatment facilities resulting in prolonged illness and more severe disease leading to more costly illness.

Therefore, we recommend that early diagnosis, prompt and effective treatment are necessary for reduction in socio-economic loss due to malaria. To obtain these facilities, not only the health education given to the community but also upgrading and expanding the diagnosis facilities to the rural communities are required. Besides, treatment facilities by appropriate means and affordable method from both the community and the government such as mobile clinics should be provided especially in the endemic areas having difficult communication.

During hospitalization, nearly half of the total cost of illness was incurred as direct cost especially for drugs cost. Patients and their family have to buy some drugs from the market. So, the authority concerned should arrange for buying drugs from sources of insured quality drugs with affordable cost.

Malaria is one of the important health problems for urban and rural poor community. One episode can waste a large amount of money [11]. We found that the mean personal income for malaria patients was kyats 70 per day. So, one episode of malaria which was severe enough to hospitalize can waste about 54 days' income of the patients [12].

In our country, Malaria Control Programme covers the whole nation by Primary Health Care approach [1]. The unit cost for early diagnosis and prompt treatment in the field, selective DDT spray and epidemic control

activities of the Malaria Control Programme were estimated and the sum of the unit costs for these malaria prevention and control activities was found to be about kyats 140. The preventive cost was 18 to 34 times less costly than the hospitalized curative cost [12]. Therefore, refresher training course for prevention and treatment of malaria for all health staff and general practitioners should be given periodically in malaria highly endemic areas.

In the study, for calculating the drug cost incurred by patients during hospitalization, exact information could not be obtained in some cases and the standard price list for Community Cost Sharing drugs [13,14] and market price for relevant drugs stated at one day during the study period had to be used. The cost of time loss for some patients and attendants was calculated by using the minimum daily wages and average daily wages set by group consensus of health care providers from Taikkyi Township. During calculation of the internal cost, the cost for capital items was excluded. Therefore, there was some weakness in calculating the costs incurred for the patients. However, the conduct of a pilot study for the development of a pre-tested questionnaire and the interview of all the study subjects by the principle investigator will reduce the bias with assurance of confidentiality. Moreover, this study can identify the various components of total cost for illness and the cost structure of malaria patients.

This study was based on one township hospital only, and the study period was quite brief which cover only the four rainy months. The selected illness was also based on single episode of the malaria attack only, although a single patient may have had repeated attack of malaria episodes within a year. Therefore, further study on other townships, if

possible, a nation-wide representative study should be carried out to get more comparative and general information in future.

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Coagulation profile in common malignancies

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The study of coagulation abnormalities in 55 cases of biopsy proven malignancies (21 cases of carcinoma cervix, 20 cases of carcinoma breast, and 14 cases of carcinoma lung) was undertaken at cancer ward of Yangon General Hospital. Tests included whole blood coagulation time, Quick's prothrombin time, activated partial thromboplastin time, thrombin time, estimation of fibrinogen and FDP level and platelet count. Ninety-six per cent of these patients had one or more coagulation abnormalities. The commonest abnormalities were elevated fibrin degradation products and abnormal prothrombin time. Compared to thrombocytopenia the coagulation abnormalities were more commonly found in this study. The data illustrated that subclinical coagulopathy is relatively frequent in patients with malignancy. These coagulation disorders were not related to liver metastasis nor cancer therapy. There was no significant difference of abnormal coagulation tests results between different malignancies that have been studied. In relation to DIC, these patients were considered to be in a compensated state. Platelet count, thrombin time test, estimation of fibrinogen and FDP level are the most important coagulation tests for evaluating DIC. These tests are suggested to be done in all cancer patients for early detection of subclinical coagulopathy and for prevention of undesirable consequences of bleeding.

INTRODUCTION

Malignancy is one of the major causes of death in the world. Eventhough many of the cancer can be cured, it presents a major clinical problem. In developing country like Myanmar, the number of cancer patients has been increasing. During the year 1988-1990 a total 11915 cases of cancer were diagnosed in which the most common cancers are carcinoma lung in male and carcinoma cervix and carcinoma breast in female [1].

There were many correlations between the malignancy and coagulation system [2-5]. Haemorrhage or thrombosis is the final clinical event in many of these patients [6,7]. Malignancy of various types can also lead to serious DIC [8,9]. It is important to assess

the alteration of haemostasis so that the frequency of complications arising from haemostatic abnormalities could be easily reduced by preventive therapy as to achieve a better prognosis of the diseases.

The aim and objectives of this study were to study the coagulation status and common solid malignancies using available facilities, to correlate the coagulation defects with haemostatic clinical manifestations, to compare the coagulation changes in malignancies of different organs and to assess the value of coagulation parameters in the management of a patient with malignancy.

PATIENTS, MATERIALS AND METHODS

A total of 55 cases of biopsy proven

malignancies from cancer ward of Y.G.H. were studied. These were 21 cases of carcinoma cervix, 20 cases of carcinoma breast and 14 cases of carcinoma lung. Out of the total 55 cases, USG was done in 21 cases. The duration of the study was approximately one year from May 1994 to April 1995.

History taking and clinical examination was done and the findings were recorded. Screening tests for coagulation such as (1) whole blood clotting time by capillary tube method (2) prothrombin time test by Quick's one stage method (3) activated partial thromboplastin time with kaolin (4) thrombin time (5) plasma fibrinogen estimation by dry weight method (6) detection of FDP in serum by staphylococcal clumping test and (7) platelet count by visual method using improved Neubauer counting chamber were carried out [10].

RESULTS

Out of 55 cases, 4 (27%) were found to have bleeding manifestations (Table 1). Thirty-six cases (65%) were shown to have abnormal whole blood clotting time (Table 2) in which 30 cases were prolonged and 6 cases were shortened.

In total 55 cases, the abnormal prothrombin time was seen in 39 cases (71%) and the abnormal partial thromboplastin time was seen in 36 cases (65%). The abnormal thrombin time was found in 20 cases (36%) of cancer patients in which 15 cases (27%) were prolonged and 5 cases (9%) were shortened. Abnormal plasma fibrinogen level was found in 31 cases (56%) of cancer patients out of which 9 cases were reduced level and 22 cases were increased. The increased FDP level was found in 40 cases (73%). Thrombocytopenia was found in only 8 cases (15%) (Table 2).

Table 1. Clinical bleeding manifestations

Bleeding manifestations	No. of cases	% of all cases
Without bleeding	51	93
With bleeding	4	7

Table 2. Screening tests for coagulation

No. Screening tests	Total cases	Normal	Abnormal
1 Whole blood clotting time	55	19(35%)	36(65%)
2 Prothrombin time	55	16(29%)	39(71%)
3 P.T.T.K.	55	19(35%)	36(65%)
4 Thrombin time	55	35(64%)	20(36%)
5 Plasma fibrinogen level	55	24(24%)	31(56%)
6 FDP	55	15(27%)	40(73%)
7 Platelet count	55	47(85%)	8(15%)

Table 3. Abnormal coagulation tests in malignancy

Total 55 cases	No. of cases	% of cases
Normal coagulation tests	2	4
Abnormal coagulation tests (singly or in combination)	53	96

The overview of this study showed out of 55 cases of cancer patients the abnormal coagulation was found in 53 cases (96%) (Table 3).

The correlation between abnormal coagulation tests results in malignancies of different organs showed no significant differences (Table 4).

Total 21 cases of USG proven malignancies, 2 cases (10%) presented with normal coagulation tests with no liver metastasis and 19 cases (90%) presented with abnormal coagulation tests in which 4 cases (19%) were associated with liver metastasis

Table 4. Correlation between abnormal coagulation tests, in malignancies of different organs

Abnormal lab. tests	Total no. of cases	Ca Cx (21 cases)		Ca breast (20 cases)		Ca lung (14 cases)	
		No. of cases	% of all	No. of cases	% of all	No. of cases	% of all
Coagulation time	36	13	62	14	70	9	64
Thrombocytopenia	8	4	19	3	15	1	7
Prothrombin time	39	12	57	14	70	13	71
P.T.I.K.	36	14	67	12	60	10	71
Thrombin time	20	8	38	4	20	8	57
Fibrinogen level	31	11	52	12	60	8	57
FDP	40	16	76	12	60	12	86

Table 5. Association of platelet count with FDP

Platelet count	Total	Normal FDP	Increased FDP
Normal	47(85%)	15(27%)	32(58%)
Abnormal (Thrombocytopenia)	8(15%)	-	8(15%)

and 15 cases (71%) were not associated with liver metastasis.

Table 5 shows association of platelet count and FDP. Of 55 cases, 47 cases (85%) had normal platelet count in which 15 cases (27%) were associated with normal FDP level and 32 cases (58%) were associated with increased FDP level. All 8 cases (15%) with thrombocytopenia were found to have increased FDP level.

DISCUSSION

Clinical finding related to bleeding manifestations

Out of 55 cases of biopsy proven malignancies, only 4 (7%) presented with bleeding manifestations. Although clinical bleeding manifestations are uncommon in cancer patients, subclinical coagulation abnormalities may be more expected in these

cases [5].

The screening tests for coagulation

The abnormal results obtained from the screening tests indicate that there are defects in coagulation mechanism. Tumor cells have (1) procoagulant activity-factor X activator activity and thromboplastin like activity (2) fibrinolytic activity [2,4, 11].

The prolonged prothrombin time and activated partial thromboplastin time are probably due to increased consumption of coagulation factors in the formation of intravascular clotting process stimulated by procoagulant activity of tumor cells. The shortened prothrombin time and activated partial thromboplastin time may be due to the increased synthesis of coagulation factors in the compensated state of DIC [4,12].

The abnormal thrombin time was significantly observed in cancer patients. The prolonged thrombin time may result either from the presence of FDP or hypofibrinogenemia. The shortened thrombin time was not usually detected in cases of other coagulation disorders but in cases of malignancies its presence may be due to hyperfibrinogenemia during compensated state of DIC [9].

The abnormal fibrinogen level was definitely present in these patients. Hyperfibrinogenemia is probably due to hypercoagulable or compensated state and hypofibrinogenemia is probably due to consumption of fibrinogen in fibrinolytic process [7,9].

Estimation of FDP is the most sensitive test for evaluating DIC. The abnormal amount of FDP in serum indicated excessive fibrinolysis. The increased FDP was found in 73% of cancer patients. It may be due to fibrinolytic activity of cancer cells or secondary fibrinolysis following intravascular fibrin deposition. Thus the abnormalities in fibrinolysis is definitely present in these patients who did not show any clinical bleeding. They may be in the state of subclinical disorders associated with compensated DIC [3,4,7,9].

Platelet count is one of the indicators of assessing DIC. Only 15% of cancer patients presented with thrombocytopenia which may be due either to the effect of cancer therapy or association with DIC. In association with FDP level, all patients with thrombocytopenia have increased FDP level. Thus thrombocytopenic patients are considered to be in the state of DIC [11,13,14].

Comparison between the abnormal coagulation tests in malignancy of different organs

All malignancies presented with abnormal coagulation tests but there were no significant difference between each types. This may be due to the small sample size of each group of malignancies.

Association between abnormal coagulation and liver metastasis in 21 cases (USG proved)

The abnormal coagulation tests results are found in cancer patients whether they are associated with liver metastasis or not. It indicates that the

tumor cells themselves produce coagulation abnormalities and it is not due to liver involvement.

Role of DIC in malignancy

Subacute or chronic DIC are probably more common than dramatic acute DIC in malignancies [3,4]. According to the study of Sun [7] based on the 'Owen & Bowies' hypothesis, FDP and platelet count were used as indicators to separate the patients into 3 groups: the patients with no DIC (normal FDP), those with compensated DIC (elevated FDP but normal platelet count) and those with decompensated DIC (elevated FDP and decreased platelet count). In this study, 27% of the cancer patients were found to have no DIC, 15% of these patients have shown laboratory evidence of acute DIC and 58% of these patients were found to be in the state of compensated DIC [9,14].

CONCLUSION

The screening tests for coagulation were performed in 55 cases of biopsy proven malignancies (21 cases of carcinoma cervix, 20 cases of carcinoma breast and 14 cases of carcinoma lung) from cancer ward of Y.G.H.

The coagulation abnormalities (singly or in combination) were present in 96% of these cases even though the incidence of bleeding manifestation was low.

Sixty-five per cent of total cases presented with abnormal whole blood coagulation time, 71% presented with abnormal prothrombin time and 64% presented with prolonged PTTK results.

Abnormal thrombin time results were found in 36% of cases and abnormal fibrinogen level and elevated FDP level were detected in 56% and 73% of total cases respectively. Thrombocytopenia was found in only 15% of

these patients. Compared to thrombocytopenia, coagulation abnormalities were more commonly found in this study. The commonest abnormalities of coagulation time tests are abnormal prothrombin time and elevated FDP level.

Further investigation such as individual clotting assays would have given more specific information [6]. Although these specific assays could not be done, the abnormal screening tests results obtained in this study provide a good clinical guide as they detect the presence of haemostatic abnormalities before clinical detection is possible.

Comparison of the abnormal coagulation tests results between 3 different malignancies (carcinoma cervix, breast and lung) showed no statistically significant difference. In relation to DIC, these cancer patients were considered to be in the compensated state but it is possible that they can progress to decompensated state at any time after being provoked by various stimuli such as infection, radiotherapy and chemotherapy [7,12]. By this study we have to conclude that the abnormal coagulations were significantly found in 96% of the cancer patients even though they presented no overt clinical bleeding. Only the screening tests for coagulation can detect the subclinical coagulopathy. Thus these tests were worthwhile to be done in all cancer patients for early detection of subclinical coagulopathy before the overt bleeding manifests and such early detection can prevent the undesirable consequences of coagulation disorders or DIC.

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**Cryptosporidiosis among children with diarrhoea and dysentery from
Yangon Children's Hospital**

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Cryptosporidiosis among children who attended Yangon Children's Hospital from March to October 1996 was studied. A total of 396 stool samples were collected after admission to the hospital. Simultaneously, a set of questions was filled to ascertain the duration and motion of diarrhoea of children. Stool characteristics were also recorded. Cryptosporidium oocysts were detected by staining with Kinyoun's acid modified method by Haley and Standard 1973. Random samples of 238 male and 158 female children of ages ranging from one month to ten years old were included in this study. It was found that the duration of diarrhoea ranged from one to 150 days. Number of motions also ranged from one to 40 times per day. Diarrhoea with either blood or mucous (dysentery) was found in 61 cases (15.4%). Cryptosporidium oocysts were found in 5 cases (1.3%) of children; three were from watery diarrhoea cases and two from dysentery cases. From the cases detected, the age range was from 10 months to five years and the duration of illness was from 3 to 30 days. Four cases were with moderate degree of dehydration and one case was associated with HIV infection.

INTRODUCTION

The protozoa that parasitize the intestinal and urogenital systems of humans belong to four groups: amebae, flagellates, ciliates and coccidia. The species of intestinal protozoa vary in prevalence and in pathogenicity. Some species are rarely encountered in patients of developed countries but may be found in travellers who travel to areas in which the organisms are endemic and in persons from those areas who visit or emigrate to developed countries. In addition to the protozoan species generally considered human parasites some species parasitic in animals may also infect humans. Cryptosporidiosis due to a coccidian protozoan parasite was first recognized to cause disease

in humans in 1976 [1]. Worldwide prevalence of Cryptosporidiosis has been documented and established that Cryptosporidium causes acute gastroenteritis. It was found more frequently in the stool of children with diarrhoeal disease than in controls [2]. Cryptosporidium has also been identified as the causative agent in the outbreaks in day-care centers [3] and in waterborne transmissions [4].

Cryptosporidium species infect the brush border of intestinal epithelial cells and causes villus atrophy. They may occasionally infect the cells of other organs in immunocompromised hosts. Clinically apparent infections with Cryptosporidium species are separable into two categories. Patients

with intact immune function develop a profuse, watery diarrhoea accompanied by mild epigastric cramping pain, nausea and anorexia which is generally self-limited and patients such as those having AIDS or receiving immunosuppressive therapy develop a more severe, long-lasting infection. Symptoms are as noted above, but the disease is prolonged, with profuse, watery diarrhea persisting from several weeks to months or years. Cryptosporidiosis was found to occur in Myanmar patients with acute diarrhoea and Cryptosporidium oocysts were found in three to four per cent of infants between two and eleven months of age [5]. Thus, it is the aim of this study to detect the occurrence of Cryptosporidium oocysts among children with ages ranging from one month to ten years, at the Yangon Children's Hospital.

MATERIALS AND METHODS

Study site

This study was conducted at Yangon Children's Hospital (YCH). This is a 300 bedded hospital situated in Dagon Township and serves as treatment centre for children from various areas. The stools were collected from four places (wards M1, M2, M3) and from Out Patient Department (OPD), from 10th March to 7th October, 1996. A random selection of 396 children, 335 cases with watery diarrhoea and 61 cases with bloody diarrhoea were included in this study. It comprised of 195 males and 140 females in 335 cases with watery diarrhoea, 43 males and 18 females in 61 cases with bloody diarrhoea. Their ages ranged from one month to 10 years. A set of questionnaires for mothers was filled to ascertain the duration and number of motions per day before coming to the hospital for treatment.

Preparation of stool samples

Fresh stool samples, collected directly

in clean bottles were characterised and examined within 4 hours. Faecal matter admixed with mucous was selected by using a sterile applicator stick and spread evenly over 2x3 cm area of a cleaned flamed glass slide and allowed to air dry and stained by using the method of Haley and Standard [6]. Under oil immersion objective at x 100, the Cryptosporidium oocysts were detected as distinct deep-pink round or oval bodies approximately about 4-5 micrometer in diameter. Most of the oocysts were surrounded by a clear halo embedded in the blue background of mucus or fecal matter. Some oocysts exhibit either a clear center and darker periphery or dark center and dark periphery. Nuclei were observed in some of the oocysts.

RESULTS

Sex and age distribution among children with diarrhoea and dysentery

Sex and age distribution among children with watery diarrhoea and dysentery are shown in Table 1. Among 396 cases, comprising of 238 males (60.1%) and 158 females (39.9%), the study children included 195 males (58.2%) and 140 females (41.8%) from diarrhoea cases; 43 males (70.5%) and 18 females (29.5%) from dysentery cases. Age distribution is also shown in this Table with 99 cases (25%) in children less than 6 months old. It was noted that 142 cases (35.9%) were from the 7-12 month range, 94 cases (23.7%) from 13-24 month range, 33 cases (8.4%) from 25-36 month range, seven cases (1.8%) from 37-48 month range, ten cases (2.5%) from 49-60 month range and eleven cases (2.8%) from more than 60 month age group. This study included one case who was ten years old, three cases of nine year olds, four cases of eight year olds, three cases of six year olds and the rest were less than five years of age.

Table 1. Sex and age distribution among children with watery diarrhoea and bloody diarrhoea (dysentery)

Age groups	Diarrhoea & dysentery			Diarrhoea			Dysentery		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
<6	59	40	99(25.0)	50	40	90(26.9)	9	-	9(14.8)
7-12	85	57	142(35.9)	82	54	136(40.6)	3	3	6(9.8)
13-24	62	32	94(23.7)	46	26	72(21.5)	16	6	22(36.1)
25-36	12	21	33(8.4)	7	16	23(6.9)	5	5	10(16.4)
37-48	4	3	7(1.8)	4	2	6(1.8)		1	1(1.6)
49-60	8	2	10(2.5)	4	1	5(1.5)	4	1	5(8.2)
60>	8	3	11(2.8)	2	1	3(0.9)	6	2	8(13.1)
Total	238 (60.1)	158 (39.9)	396	195 (58)	140	335	43 (29.5)	18	61

Figures in parentheses denote percentages

Characteristics of stool among diarrhoea and dysentery cases of children

Characteristics of stool are shown in Figure 1. It was observed that 56.7% was watery type of stool, 1.8% with mucous, 30.8% with watery form with mucous in diarrhoea cases. Among the dysentery cases, 9.8% produced mucous, 19.7% was with blood and 29.5% was of watery form with blood and mucous.

Duration of diarrhoea and dysentery among children

Duration of the illness in days prior to hospital admission was recorded. The longest duration was five months in a child with persistent diarrhoea who was a one year old female from Thingangyun area. One 18-month-old child had a duration of three months, a male from Ywathagyi Township. Another child had a duration of two months, a three-month-old male from Thakayta. It is shown in Fig. 2 that 226 cases (67.5%) of children had a duration of 5 days; 58 cases (17.3%) had a duration between 6 to 10 days; 20 cases (6.0%) had a duration of 14 to 18 days; seven cases (2.1%) had a duration of 19 to 25 days; 14 cases (4.2%) had duration of one to five months and ten cases (2.9%) were not sure. In dysentery

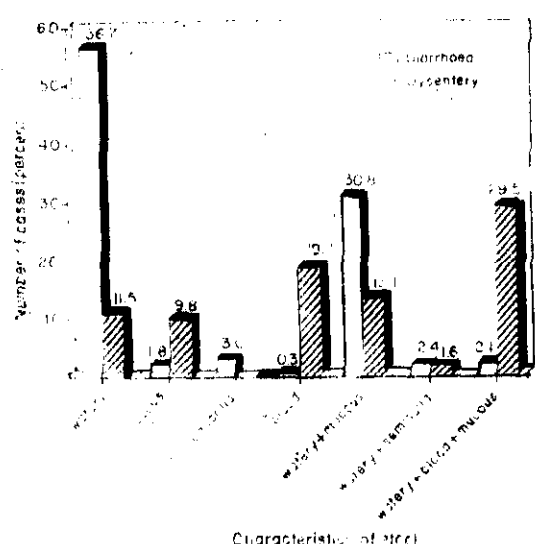


Fig. 1. Characteristics of stool among diarrhoea and dysentery cases

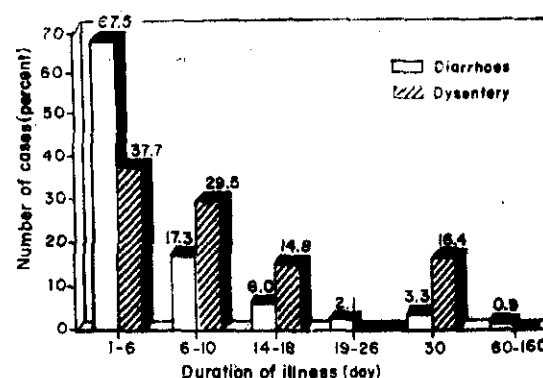


Fig. 2. Duration of illness in diarrhoea and dysentery cases

cases, 23 cases (37.7%) had a duration of one to five days; 18 cases (29.5%) had a duration of six to ten days; nine cases (14.8%) had a duration of 14 to 18 days and ten cases (16.4%) had a duration of one month.

Number of motions per day among cases of children with diarrhoea and dysentery

The maximum number of motions per day was 40 times and this occurred in three cases; one from Dala was a two-month-old male; the second was a male one year old from Hlinethaya and the third, a 38-month-old male from Thingangyun area from among the diarrhoea cases. It is shown in Fig. 3 that 2.7% and 1.6% had a motion of 30 times per day among diarrhoea and dysentery cases respectively.

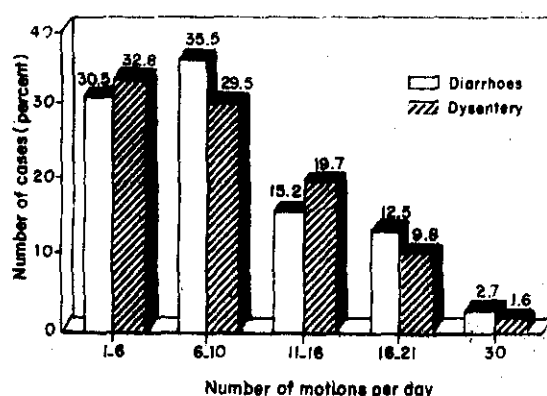


Fig. 3. Number of motions per day among diarrhoea and dysentery cases

Occurrence of Cryptosporidiosis among children

Cryptosporidium oocysts are very small (4 to 5 micrometer) and can easily be missed in fecal preparations. Both immature and mature oocysts may be present, although usually those present are mature and contain four naked sporozoites; sporocysts are not present. Also, oocysts have a low specific gravity and are usually found in the upper levels of the mount, just below the cover slip. Oocysts do not stain with iodine (unless they are exposed to it for

long periods), but yeast cells do stain, thus helping to distinguish oocysts from yeast cells. Oocysts stain intensively acid-fast, whereas yeast cells and fecal material do not. In many mature oocysts, sporozoites can be seen.

In this study *cryptosporidium* oocysts were identified from five cases (1.3%) of children; three from among the diarrhoea cases and two from among the dysentery cases. Findings of these five patients are shown in Table 2. One case of diarrhoea had

Table 2. Findings of *Cryptosporidium*-associated diarrhoea cases

No. of cases studied	396 cases who attended the Yangon Children's Hospital
Study period	10.3.96 to 7.10.96
No. of cases detected	5 (1.3%) cases
Age range	10 months - 5 years
	Mean 21 months
Sex	Male 4, Female 1
Duration of diarrhoea	Range 3-30 days
Degree of dehydration	4 cases with moderate degree of dehydration
Other clinical finding	1 case with HIV infection

HIV infection as well. It was found that the minimum age with infection was a female ten months old with HIV infection who expired after hospitalization. She passed motion 20 times/day with watery diarrhoea and mucous and had moderate dehydration. She suffered from diarrhoea very frequently since birth. The other two cases of diarrhoea were two males who were eight and ten months old respectively. They had a past history of diarrhoea with nine to ten motions per day with moderate dehydration. Their duration of diarrhoea before coming to the hospital was ten days. Two dysentery cases who were three and five years old respectively were both from outskirts of Yangon with a past history of diarrhoea and had a duration of one

month with a motion of 7 times per day. They also had moderate dehydration. The five year old boy had no past history of diarrhoea within one month prior to hospitalization and had a motion of 2-25 times per day for eight days.

DISCUSSION

Cryptosporidiosis infection is caused by a coccidian parasite originally described a century ago and until recently, not considered as a human pathogen. Life cycle is complex with sexual and asexual reproduction, an autoinfectious cycle and the ability to complete its development within a single host. After excretion from the stool, oocysts are environmentally resistant, and can exist for long periods of time in the environment. Primary hosts are domestic livestock and human infection is usually zoonotic. Water supplies are often contaminated with oocysts which are resistant to chlorination. Transmission via ingestion of fecally contaminated swimming pool water, food, fomites, and sexual activities facilitating fecal-oral inoculation have been demonstrated [7].

The major target of Cryptosporidium in the host is the intestinal epithelial cells, resulting in diarrhoea, sometimes profuse and persistent, although it may also infect other organs such as the gall bladder and lungs. Pathogenesis involves attachment, probably with Cryptosporidium parvum. Tanya-lesel reported that oocystic form of Cryptosporidium spp. were found in 18 (17%) of 106 samples with neoplasia and diarrhoea patients [8]. Dupont reported the infectivity of Cryptosporidium parvum in healthy volunteers by infecting with a dose of 300 or more oocysts to 16 subjects and found that five subjects (20%) become infected, whereas at a dose of 1000 or more oocysts to seven volunteers all seven became infected and concluded that with no serologic evidence

of past infection with Cryptosporidium, a low dose of Cryptosporidium oocyst is sufficient to cause infection [9]. From other studies, outbreak of waterborne cryptosporidiosis was associated with public water. In the United Kingdom, all 135 cases involved with cryptosporidiosis lived in a part of the city which received drinking water supply from a single water treatment source [4]. Another study conducted in Milwaukee, showed that water contaminated with Cryptosporidium spp. caused a widespread outbreak of that disease [10].

Morgan also reported waterborne cryptosporidiosis associated with a borehole supply and 64 cases of cryptosporidiosis were diagnosed within one district health authority [11]. Cryptosporidium oocysts were detected among pigs, sheep [12]; ostrich chicks [13] and beef cattle [14] which are the sources of contamination of the environment. It was reported that cryptosporidiosis in Myanmar infants with acute diarrhoea occurred in 3.4% infants between two to 11 months who were found passing Cryptosporidium oocysts [5]. In our findings 1.3% of children (five children) out of 396 cases tested were passing Cryptosporidium oocysts. Out of five patients one case was an infant suffering from HIV infection and the other four cases had persistent and longer duration of diarrhoea. Three cases were less than ten months old, one case was three years old and the remaining case was five years old. Out of 396 cases most of the children suffering from diarrhoea were from one to 36 months old. Only 28 cases with diarrhoea were in the range of 36 months to ten years. This study clearly shows that diarrhoea occurs commonly among children less than three years old and is still a major health problem in Myanmar, especially during the age of seven to 12 months. ie after the weaning period. It was significantly shown that mothers should be aware that

cleanliness is important in feeding their infants after the weaning period. As food from outside is mostly contaminated with bacterial, viral and parasitic pathogens it is better to serve the children with food prepared in a hygienic way to prevent diseases transmitted by the faecal-oral route. It is an established fact that *Cryptosporidium* spp. causes intractable diarrhoea in immunocompromised patients especially those with HIV infection. It can occur as an opportunistic infection in adults and, as this study shows, also in very young children less than 10 months old and also in older children. The occurrence in children especially in developing countries is due to faecal contamination of food and water. Malnourished children and children with HIV infection will be particularly susceptible.

It is therefore of utmost importance to prevent this still exotic parasitic infection from gaining a foothold in our country by prevention case detection and treatment before it becomes widespread.

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Serum calcium in normal and preeclamptic pregnant Myanmar women

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To study the changes during normal pregnancy and in preeclampsia, serum calcium concentration was measured in 26 apparently healthy pregnant Myanmar women and in 15 preeclamptic patients. They were 20-40 years of age. In healthy pregnant women, serum total calcium levels measured at 24th week, 28th week, 32nd week and 36th week of gestation were 2.45 ± 0.30 mmol/l, 2.47 ± 0.29 mmol/l, 2.41 ± 0.29 mmol/l and 2.46 ± 0.29 mmol/l respectively and ionized calcium levels at these periods were 1.24 ± 0.15 mmol/l, 1.23 ± 0.14 mmol/l, 1.21 ± 0.17 mmol/l and 1.20 ± 0.16 mmol/l respectively. In preeclamptic patients, the mean serum total calcium level (2.26 ± 0.24 mmol/l) was significantly lower than that (2.52 ± 0.23 mmol/l) of control group. However, the mean serum albumin adjusted calcium levels (2.42 ± 0.27 mmol/l) and ionized calcium level (1.23 ± 0.14 mmol/l) of preeclamptic patients were not significantly different from those (2.43 ± 0.24 mmol/l and 1.24 ± 0.13 mmol/l respectively) of control.

INTRODUCTION

Preeclampsia is a common disorder of pregnancy and a major cause of maternal and neonatal mortality and morbidity [1]. Thus its prevention would have a significant impact on maternal and perinatal outcome. Prevention not only requires knowledge of pathophysiologic mechanism of disease but also availability of method of early detection and means of intervention and correction of pathophysiologic changes.

Many theories on the pathophysiologic basis for pregnancy-induced hypertension (PIH) and preeclampsia (PE) have been postulated. One hypothesis is that decreased serum ionized calcium level could be expected to favour the development of PIH and PE as a result of deranged production of vascular endothelium derived vasodilator (nitric oxide) [2]. If this hypothesis is true, PIH and PE could be easily preventable by calcium

supplementation during early pregnancy. This hypothesis is in agreement with the fact that significant reduction in serum ionized calcium level was found in hypertensive pregnancy [3]. A reduction in PE rate was reported in calcium supplemented pregnant women [4].

The normal blood calcium level of Myanmar males and non-pregnant females were reported [5,7]. Information concerning the serum calcium in pregnant Myanmar women and PE is not yet available. Therefore, the present investigation is aimed to determine the total and ionized calcium concentrations of pregnant Myanmar women and preeclamptic patients.

MATERIALS AND METHODS

Subjects

The subjects who volunteered to participate in this study were recruited

ted from the Maternity Unit, North Okkalapa General Hospital. They were selected by stratified sampling method according to age, period of gestation, presence or absence of disease and treatment.

Normal pregnancy

The study was conducted on 72 apparently healthy pregnant Myanmar women of 24th weeks of gestation. Age (20-40 years) and gestational period matched control subjects were studied. Their serum calcium levels were measured at 4 weekly interval until 36th weeks of gestation. Only 26 women completed the study.

Preeclamptic patients

The subjects were selected according to the criteria for preeclampsia laid down by Haynes [8]. Fifteen preeclamptic patients were selected. Blood and urine samples were taken on admission before any treatment. Those taken treatment with Nifedipine were excluded from the study. All the preeclamptic patients included in the study recovered completely from hypertension and proteinuria after delivery. Those having persistent proteinuria and hypertension were excluded from the study.

Non-pregnant women

Age matched 25 apparently healthy non-pregnant women from North Okkalapa were studied.

Blood samples

In order to minimize biological variability, blood samples for biochemical analysis were collected with minimum stasis between 09:00 am and 11:00 am with the subject in sitting position. Sera were separated as soon as possible and stored at -20°C until analyzed.

Methods

1. Blood pressure measurement was done with a mercury sphygmomanometer by using method described by World Health Organization [9].

2. Serum total calcium concentration was measured by Atomic Absorption Spectrophotometry method.
3. Serum total protein was measured by Biuret method [10].
4. Serum albumin concentration was determined by Bromo Cresol Green dye binding method [11].
5. Serum ionized calcium concentration was calculated by using formula described by Pottgen & Davis [12].

$$\text{Ionized calcium (mg/dl)} = \frac{[6\text{Ca} - (K/3)]}{(K + 6)}$$

$$K = (0.19 - P) A$$

$$P = \text{Total protein (g/dl)}$$

$$A = \text{Albumin (g/dl)}$$

5. Serum total calcium was adjusted for albumin by using formula described by Paynes et al., [13].

$$\text{Albumin adjusted calcium} = \text{(mg/dl)}$$

$$\text{Total calcium} - \text{Albumin} + 4$$

$$\text{(mg/dl)} \quad \text{(g/dl)}$$

Statistical analysis

Data were expressed as mean $\pm$ SD. Comparisons were made using Student's 't' test for unpaired samples and for paired samples respectively. Differences were considered significant if $p < 0.05$.

RESULTS

Table 1 shows comparison of serum total calcium, albumin adjusted calcium and ionized calcium levels in non-pregnant and pregnant women at various gestational periods. Mean serum total calcium level of pregnant women was found to be lower than that of non-pregnant women and significant difference was found at 32nd week of gestation. However, in the case of mean albumin adjusted calcium level, pregnant women has higher level than non-pregnant women although the difference was not statistically significant. No significant difference was found for mean serum ionized calcium level between pregnant

Table 1. Comparison of serum total calcium, albumin adjusted calcium and ionized calcium levels between non-pregnant and pregnant (at various gestation periods) women (mean $\pm$ SD)

	Non-pregnant	Pregnant women			
		24th week	28th week	32nd week	36th week
Total calcium (mmol/l)	2.57 $\pm$ 0.20	2.45 $\pm$ 0.30	2.47 $\pm$ 0.29	2.41 $\pm$ 0.29	2.46 $\pm$ 0.29
Albumin adjusted calcium (mmol/l)	2.42 $\pm$ 0.24	2.45 $\pm$ 0.30	2.43 $\pm$ 0.27	2.47 $\pm$ 0.24	2.45 $\pm$ 0.27
Ionized calcium (mmol/l)	1.21 $\pm$ 0.12	1.24 $\pm$ 0.15	1.23 $\pm$ 0.14	1.21 $\pm$ 0.17	1.20 $\pm$ 0.16

Table 2. General characteristics of preeclamptic and control groups

Characteristics	Control group	Preeclampsia
Age (yrs.)	20-40	20-40
No. of subjects		
Primary gravida	14	7
Multi-gravida	12	8
Mean gestation periods (weeks)	36.62 $\pm$ 2.03	37.4 $\pm$ 2.60
Blood pressure (mmHg)		
Systolic	103.80 $\pm$ 8.98 (90-120)	158.00 $\pm$ 15.68 (140-190)
Diastolic	65.38 $\pm$ 5.82 (60-80)	98.67 $\pm$ 13.02 (90-140)
Edema	-	+
Proteinuria	-	trace to solid

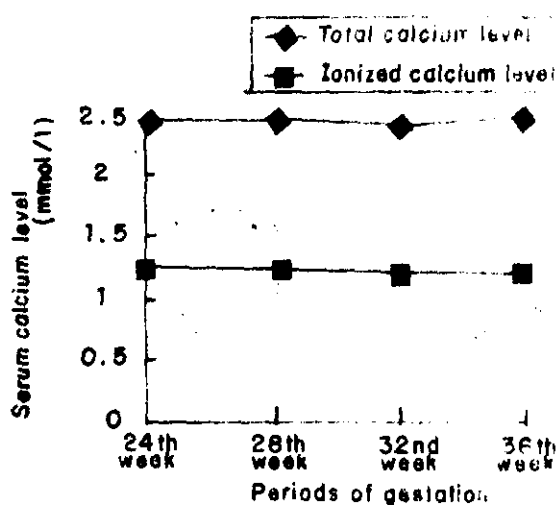


Fig. 1. Mean serum total calcium and ionized calcium levels in different periods of gestation

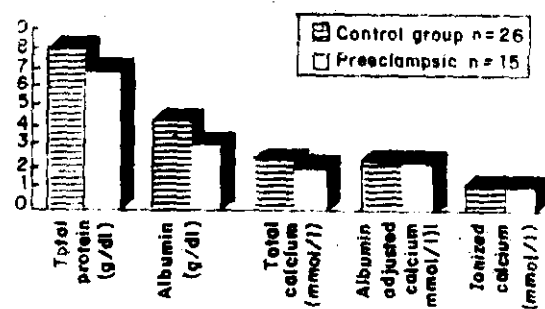


Fig. 2. Comparison of serum total protein, albumin, total calcium, albumin adjusted calcium and ionized calcium levels between control group and preeclamptic patients

and non-pregnant women. Mean serum ionized calcium level declined with advancement of gestation.

Figure 1 shows serum total calcium and ionized calcium levels changes

with period of gestation. Total calcium level was found to be lowest at 32nd week of gestation and raised thereafter. Ionized calcium level was found to be decreased with the advancement of gestation. However, the decrease is statistically not significant.

The characteristics of the control group and preeclamptic patients are shown in Table 2.

Figure 2 shows comparison of mean serum total protein, albumin, total calcium, albumin adjusted calcium and ionized calcium levels between control and preeclamptic patients. Serum total protein (7.01 ± 0.85 g/dl), albumin (3.34 ± 0.56 g/dl) and total calcium (2.26 ± 0.24 mmol/l) of preeclamptic patients were found to be significantly lower than those (8.18 ± 0.51 g/dl, 4.37 ± 0.48 g/dl, 2.52 ± 0.23 mmol/l respectively) of control group. There were no significant differences in albumin adjusted calcium (2.42 ± 0.27 mmol/l vs 2.43 ± 0.24 mmol/l) and ionized calcium levels (1.23 ± 0.14 mmol/l vs 1.24 ± 0.13 mmol/l).

DISCUSSION

Mean serum total calcium concentration during pregnancy was found to be lower than that of non-pregnant women, lowest value at 32nd week of gestation and rising slightly thereafter. Similar findings have been reported by Olatunbasur *et al.* [14] and Pitkin *et al.* [15].

Paynes, Little and Wilkins suggested that preadjustment of serum total calcium level with serum albumin should be made before deciding increased or decreased level [16]. When serum total calcium level was adjusted for albumin, it was found to be greater during pregnancy than that of the non-pregnant women. However the adjusted serum total calcium level did not vary with the period of gestation. These findings were in agreement with that of

Paynes *et al.* who have suggested that striking changes in maternal calcium metabolism take place during pregnancy. It started with increase in 1,25 DHCC synthesis that stimulates intestinal absorption of calcium leading to hypercalcaemia [17].

Mean serum total calcium level of preeclamptic patients was found to be significantly lower than that of control group. Richards *et al.* described that there was no significant difference in serum total calcium level between preeclamptic patients and control group. However, even in their study, serum total calcium level of severe preeclamptic patients (2.2 ± 0.2 mmol/l) was found to be lower than that of control group (2.3 ± 0.1 mmol/l) [18].

Regarding serum ionized calcium concentration, Lopez-Jaramillo *et al.* recorded decrease in serum ionized calcium concentration during pregnancy in Ecuadorian Andean whose dietary calcium intake was low [19]. They hypothesized that any decrease in serum ionized calcium concentration necessary for activation of nitric oxide synthase enzyme could be expected to favour development of PIH and PE. In this population, preeclampsia rate was reduced by calcium supplementation during pregnancy. Normal dietary intake of Myanmar is also observed to be low [7] which is similar to that of Ecuadorian Andean. However, the serum ionized calcium level did not decrease in normal pregnant group and preeclamptic patients. Since the present finding does not agree with that of Lopez-Jaramillo and co-workers, calcium supplementation may not definitely help in reducing PE rate in Myanmar.

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Effect of adenosine triphosphate (ATP)-induced macrophage cell death (apoptosis) on mycobacterium viability and production of nitric oxide (NO)

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The effect of ATP-induced macrophage cell death (apoptosis) on mycobacterium viability and production of nitric oxide was studied. Murine monocytic cell line (J774) and human monocyte - derived macrophages were stimulated to die by apoptosis following treatment with ATP in different concentrations. The morphological feature of cell death was examined by fluorescence light microscopy to determine if macrophage apoptosis or necrosis had occurred. It was then confirmed by DNA-gel analysis. The viability of mycobacteria (BCG) was determined by ³(H)-uridine incorporation and the release of nitric oxide (NO) was quantified by Greiss reagent assay. The murine cell line (J774) and human monocyte-derived macrophages were shown to die rapidly within one hour by apoptosis following treatment with ATP (3mM), resulting in a 40-60% reduction in bacterial viability. A high level of NO release was associated with the death of murine cell line although it was not in human macrophages. The results suggest that the release of nitric oxide is not associated with the triggering of macrophage cell death by apoptosis. Thus, there might be two different pathways in control of intracellular mycobacterial infection by macrophages.

INTRODUCTION

Studies were undertaken to examine the effects of the induction of macrophage cell death on the survival of intracellular mycobacteria and its possible association with nitric oxide release. Recent published data by Molloy et al. in 1994 has suggested that human macrophages can be induced into apoptosis following treatment with purine nucleotide adenosine triphosphate (ATP) [1]. Furthermore, the induction of cell death by ATP was shown to be accelerated by activation of the macrophages with IFN- γ . Such ATP-induced apoptosis was found to significantly reduce intracellular BCG viability within human macrophages in 2-6 hours. Previous reports in the literature, have also suggested that activation-induced cell death (apoptosis), by various agents in murine macrophages, results from the production of nitric oxide [2-4]. Cumulatively, these

findings suggest that apoptosis may be an important process whereby macrophages may control an intracellular infection in-vivo.

This study was performed to answer the following questions.

- (1) Does ATP induce apoptotic cell death within in-vitro cultured murine monocytic cell lines and in human monocyte-macrophages?
- (2) Does the induction of cell death by ATP correlate with nitric oxide production by the cells?
- (3) Does the induction of cell death in BCG - infected macrophages by ATP result in a reduction in intracellular bacterial viability? and
- (4) Can this antimycobacterial effect be correlated with nitric oxide production?

MATERIALS AND METHODS

In this study, human monocyte-derived macrophages and murine cell lines

were cultured *in-vitro* and treated with various concentrations of ATP for different times. The cells were then assessed for:

- (1) Apoptosis, morphologically by fluorescence microscopy with Acridine Orange Staining and DNA-gel analysis.
- (2) Nitrite production by Greiss reagent.
- (3) Intracellular bacterial (BCG) viability by ^3H -uridine incorporation.
- (4) Cell viability, by ^{51}Cr -release assay.

RESULTS

Apoptotic cell death occurred rapidly within 1 hour resulting in a 40-60% reduction in intracellular bacterial viability (Figure 1).

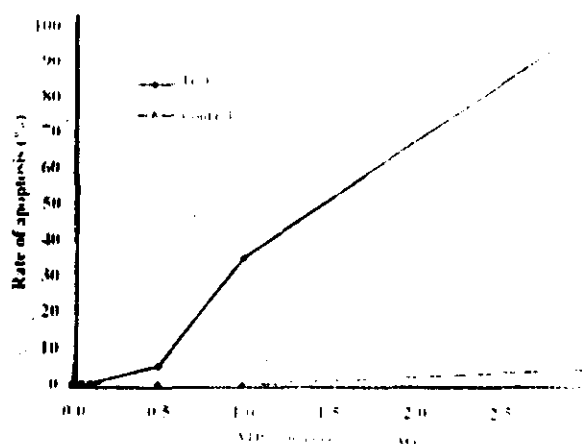


Fig. 1. Rate of opoptosis of human macrophages after incubation with different concentration of ATP for one hour at 37°C

A characteristic step ladder patten of DNA fragmentation was noted in macrophages treated with ATP (Figure not shown).

A high level of NO release was associated with the death of murine cell lines, whereas human macrophages did not produce a detectable level of NO after ATP induction.

BCG infected ATP treated cells

showed a progressive decrease in ^3H -uridine incorporation (Figure 2).

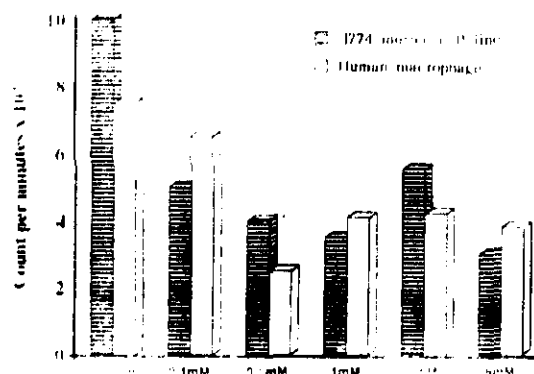


Fig. 2. ^3H -uridine incorporation in two types of BCG infected cells treated with various concentrations of ATP

Rate of ^{51}Cr -release in BCG infected ATP treated cells was lower than that of non-infected cells.

All these results were obtained from a single experiment. Repetition of experiment could not be done because of time limitation.

DISCUSSION

Two mechanisms of cell death were usually observed in a granuloma; (1) apoptosis and (2) necrosis. Apoptosis is a programmed cell death, whereby a rapid and profound changes in nuclear organization occurred and there is DNA-fragmentation. This can be induced by ATP and various cytokines.

In contrast, necrosis is an accidental cell death, where there is irreversible plasma membrane damage leading to osmotic dysregulation.

Molloy had mentioned that apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular *Bacillus Calmette-Guerin* and that this could be induced by ATP [1]. The results in this study agreed with that of Molloy.

Denis and Chan had reported that

apoptosis in murine macrophages was associated with NO production [2-5]. The results in this study also agreed with it. However a detectable level of NO release was not noted within human cells following ATP treatment.

The fact that percentage ^{51}Cr -release in BCG infected ATP treated cells was lower than that of non-infected cells indicated that BCG infection makes such cells more resistant to the cytolytic effects of ATP. Even then there still was a certain extent of cell death.

BCG infected ATP treated cells showed a progressive decrease in ^3H -uridine incorporation suggesting subsequent loss of intracellular bacterial viability thus resulting in limited bacterial growth.

Overall findings suggested that ATP treatment results in loss of intracellular bacterial viability and that the triggering of macrophage cell death by apoptosis was not associated with the release of NO in human macrophages.

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A preliminary study of CD4+ T-lymphocyte count in tuberculosis patients

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We evaluated the CD4+ T cell count in tuberculosis patients, whether it may be a supportive indicator reflecting the immune status of the patient. A total of 55 patients and 50 controls were included. Patients were within the age range of 19-65 years with a mean of 42.836 ± 16.24 SD. These included 43 male and 12 female subjects with pulmonary and extrapulmonary tuberculosis. Age and sex matched subjects were included as controls. CD4+ T-lymphocyte count was determined manually with Coulter^R Manual CD4 Count Kit. A significant reduction in mean CD4 count against control subjects was observed ($p=0.004$, d.f. 103). A correlation coefficient of 0.546 was observed between CD4 count and percentage of differential lymphocyte count from the peripheral blood. The results indicate that measurement of CD4 count using above method is worthwhile for tuberculosis patients as an adjunct to clinical parameters for the assessment of the immune status of the patient.

INTRODUCTION

The recent re-emergence of tuberculosis (TB) as a global health problem highlights the importance of the cellular immune responses particularly in cases complicated with HIV infection. Acquired cellular immunity to Mycobacterium tuberculosis infection is characterised by the emergence of protective helper/inducer CD4+ T-lymphocytes [1]. The kinetics of this protective immunity, which leads to the control and containment of the infection, and the onset of bacterial clearance are closely associated with the kinetics of emergence and loss of CD4+ T-lymphocytes that secrete large amount of the cytokine interferon- γ (IFN- γ) [2-5]. Thus, changes in the number of CD4+ lymphocyte count may be of critical importance in determining the overall response of the infection to treatment.

It may be of additional value in the assessment of the prognosis of disease. This study was performed to evaluate the CD4+ T-cell count in tuberculosis patients.

MATERIALS AND METHODS

A total of 55 patients diagnosed to have tuberculosis from clinical signs and symptoms, radiography and sputum smears were studied from December 1996 to February 1997. Both pulmonary and extrapulmonary TB cases were studied for absolute CD4+ T-lymphocyte count. The mean CD4+ T-lymphocyte count of 50 volunteers (blood donors) was used as control. Patients were within the age range of 19-65 years with a mean of 42.836 ± 16.24 SD. These included 43 male subjects and 12 female subjects. Both cases and controls were screened

for HIV infection and HBsAg. Initial screening of HIV with SimpliRed^R test kit (SimpliRed^R HIV-1/HIV-2 antibody Test Kit; a rapid whole blood agglutination test for the detection of antibodies to HIV-1/HIV-2) was followed by ELISA assay. Hepatitis B virus surface antigen (HBsAg) was tested by ELISA assay. All samples were collected in the morning to minimise circadian variation. Samples were taken within two weeks of the beginning of anti-tuberculous therapy. Detail clinical history, including past medical history and social history were noted. Hematological, bacteriological and immunological studies were performed. Skin tuberculin test was done simultaneously with blood collection and read on the third day.

Method in detail

Venous blood was collected in K₃EDTA bottles. Hemoglobin concentration, total and differential white cell count, and erythrocyte sedimentation rate (ESR) were evaluated by standard procedures. The detection of acidfast bacilli (AFB) in sputum was carried out for three consecutive days. A simple manual method of CD4⁺ T-cell count was performed using monoclonal antibody coated tiny latex beads (cytospheres) manufactured by Coulter Corporation (Coulter Manual CD4⁺ Count Kit). Cell counting was done under ordinary light microscope. In brief, 100ul of whole blood (collected in K₃EDTA anticoagulant) was gently mixed for 2 minutes with Reagent A containing MY4 cytospheres monocyte blocking reagent (MY4 monoclonal antibody-labeled spheres in PBS, 0.2% BSA & 0.1% NaN₃), to block the unnecessary CD4 receptor containing monocytes. Again, 10ul of Reagent B containing CD4 cytospheres reagent ie. 1% suspension of Coulter clone T4 monoclonal antibody-labeled spheres in PBS, 0.2% BSA and 0.1% NaN₃ was added and mixed gently for 2 minutes. Then

10ul of the blood-latex spheres mixture was put into the tube containing 100ul of Reagent C containing lysing reagent (2% acetic acid in distilled water and 0.025% crystal violet stain) for lysis of unnecessary background red cells. It was gently mixed for 15 minutes. The final solution was loaded in a Modified Neubauer Counting Chamber on both side and stand for 2-3 minutes to allow the cells to settle. Under the microscope, CD4⁺ cells were those with 3 or more large latex spheres attached to the cell surface.

Statistical analysis

Statistical analysis was done by using Epi-info programme. Descriptive analysis of the data was performed also in Microsoft Excel version 5 and presented in histograms. Unpaired 't' test was performed and significance expressed in 'p' values. Kruskal-Wallis one way analysis of variance and Mann-Whitney U test were applied as appropriate.

RESULTS

There was no significant correlation between CD4⁺ T-lymphocyte count and clinical parameters; like sex difference, history of smoking, alcohol drinking habit, marital status, family size, underlying medical diseases, history of TB contact, previous TB treatment, tuberculin positivity and sputum AFB positivity.

Hematological and immunological profile are presented in Table 1. Also no significant correlation could be drawn with hemoglobin concentration, total WBC count and ESR. However, a correlation coefficient of 0.546 was observed between CD4 count and percentage of lymphocyte count (Fig. 1).

Figure 2 shows means and SDs of CD4 count between TB patients and control group. A significant reduction in mean CD4 count from control

Table 1. Hematological and immunological profiles of 55 tuberculosis patients, Yangon, Dec. 1996 - Feb. 1997

	Hb conc. (g/dl)	TWBC (x10 ⁹ /L)	Lymphocytes (%)	ESR (mm/1st hr)	CD4+ lymphocyte (per ul)
Mean	11.036	10.036	22.073	63.527	629.400
SD	2.457	5.26	11.986	37.404	407.817

Hb conc. = Hemoglobin concentration
 TWBC = Total White Blood Cell Count
 ESR = Erythrocyte Sedimentation Rate

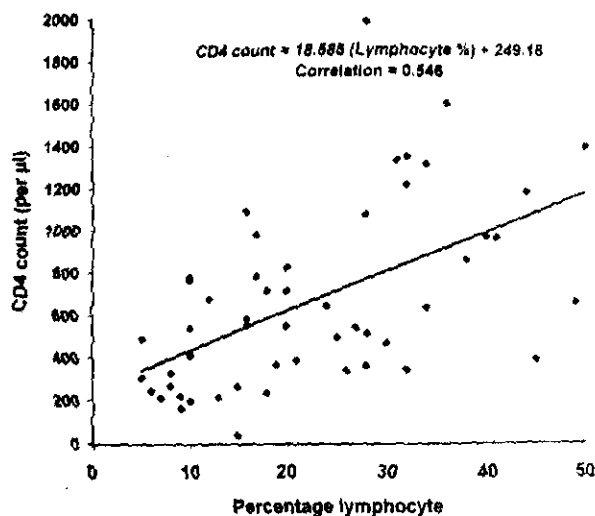


Fig. 1. Correlation between CD4 count and percentage of lymphocyte count

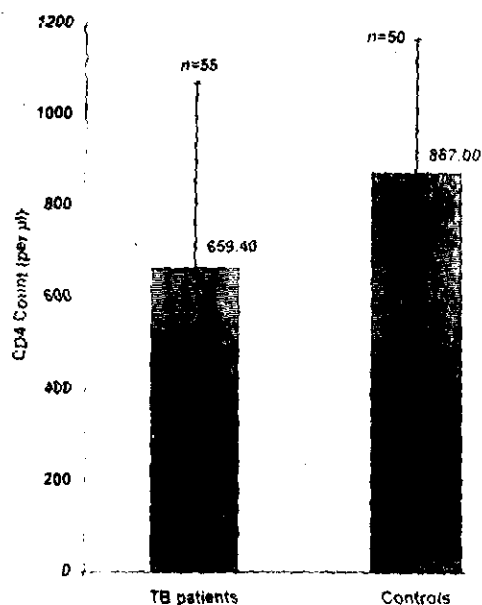


Fig. 2. Mean (+) SD of CD4 count between TB patients and controls

subjects was observed ($p=0.004$, d.f.=103).

Figure 3 shows means and SDs of CD4 count in TB patients by age groups. The reduction in CD4 count below mean of normal was observed mostly in under 20-year age group

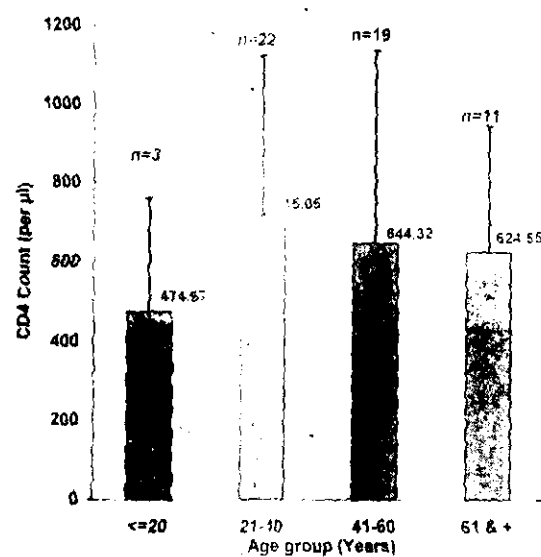


Fig. 3. Mean (+) SD of CD4 count in TB patients by age groups

followed by those above 60 and between 41-60. Those age groups between 21-40 were found to have higher CD4 count compared with others. However, the differences between the CD4 count in each age group were not statistically significant ($p>0.05$).

Figure 4 shows means and SDs of CD4 count by clinical presentation, which is categorised according to chest radiographic report. They include 28 patients with extensive pulmonary tuberculosis (ept), 5 with ept complicated by pleural effusion

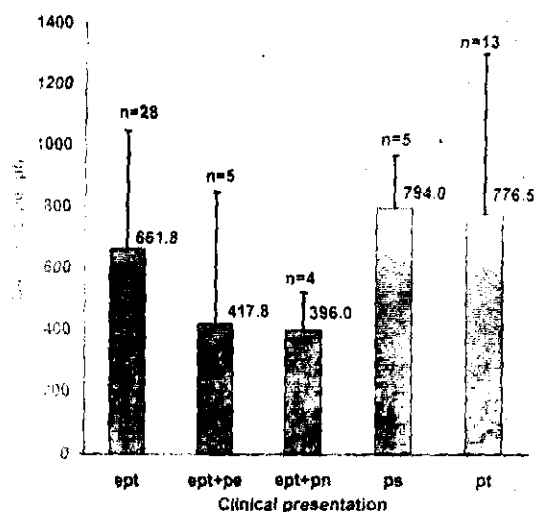


Fig. 4. Mean (+) SD of CD4 count by clinical presentation

ept = extensive pulmonary TB
 pe = pleural effusion
 pn = pneumothorax
 ps = Pott's spine
 pt = pulmonary TB

(pe), 4 with ept and pneumothorax (pn), 5 with TB of bone and joints (ps) and 13 with localised apical pulmonary TB (pt). Reduction of CD4 count below mean of normal was more evident in ept with complications like pe and pn. Those presented with TB of bone and joints or other extrapulmonary TB have a higher CD4 count compared with the pulmonary TB. A significant reduction in CD4 count was observed in extensive pulmonary TB with pleural effusion compared to simple localised pulmonary TB ($p=0.049$). Also a significant difference was found in extensive pulmonary TB with pneumothorax and TB of bone and joints ($p=0.014$).

DISCUSSION

In this study we observed a significant reduction in mean CD4 count of TB patients against healthy controls. A similar CD4 lymphopenia and a reduction of CD4/CD8 ratio were observed in patients with reactive tuberculosis by Ashtekar [6] and Laurence [7].

Although the reference range (normal values) given by the manufacturer of the kit is from 450ul to 1870ul (mean 1060ul), the range of CD4 count obtained from our local control subjects is between 457-1941ul (mean 867 + S.D 295) [8]. It was mentioned by Laurence in 1993 that many factors could influence these cell populations; like age, sex, ethnic origin, circadian rhythm, physical and psychological stresses, drugs, antilymphocytic antibodies and splenectomy [7]. Thus, in this study we took mean CD4 count as 867 as a cut-off value for our subjects. A study done on 680 subjects by Reicherts had shown a variation in mean CD4% and CD4:CD8 ratio with age and sex [9]. In our study the reduction of mean CD4 count for TB patients below normal was more marked in extreme age groups. i.e. under 20-year group and above 60, as shown in Fig. 3. However, the differences between the CD4 count in each group were not statistically significant. We concluded this as a small sample size in under 20 group ($n=3$) and above 60 ($n=11$) compared with middle age group ($n=41$). A better correlation could be drawn if the study could be proceeded with a larger sample size and equal distribution of samples between different age ranges. A study by Ladel et al. in 1995 had mentioned that CD4+ T cells are highly effective in containing *Mycobacterium bovis* BCG within distinct granulomatous lesions, but fail to eradicate their intracellular pathogen and it appears most likely that CD8 T cells are also required to achieve this goal [10]. We need to continue our study also with simultaneous CD8 cell count rather than counting CD4 alone. Bose et al. in 1995 had mentioned that a long standing bacillary load in drug resistant pulmonary tuberculosis patients probably results in persistent dysregulation of homeostasis of blood T-lymphocytes and this in turn delays their clinical and immunological recovery, even when

therapy is adequate [11]. Also Khomenko [12] and Singhal [13] have also shown that during the course of pulmonary tuberculosis, patients who show clinical and bacteriological improvement also showed changes in their immunological parameters. In our study a significant correlation was found between CD4 count and clinical severity of the disease as described in Fig. 4 and we hope that a better results and information could be obtained if we could proceed our study in the entire course of treatment. Furthermore clinical improvement in correlation with CD4 count could be obtained which could be a useful prognostic indicator. Finally, we concluded that even with a very limited facilities we obtained a certain understanding regarding the reduction in CD4 count in TB patients which in turn reflects an impairment in acquired cellular immunity in these patients. A further study could be proceeded on TB patients during the course of chemotherapy and their correlation with the immune status [11-13].

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Etiologic agents, clinical and laboratory characteristics of acute versus persistent diarrhoea in children who attended the Yangon Children's Hospital

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To identify the etiologic agents, clinical and laboratory characteristics of acute and persistent diarrhoea in children less than 12 years of age, a hospital-based prospective study was carried out for 18 months in Yangon Children's Hospital. A total of 487 children, 327 with acute and 160 with persistent diarrhoea participated in the study. Intestinal pathogens including bacterial agent 31%, viral agent 6.3% and protozoa 33% were detected in 71% of persistent diarrhoea cases, whereas in acute diarrhoea cases intestinal pathogens were identified in 64%, among which bacterial, viral and protozoal agents were 28%, 11.5% and 25% respectively. More than one enteric pathogen was detected in 13.7% and 16.5% of persistent and acute diarrhoea cases respectively. Male children who suffered from diarrhoea were more than females and peak incidence of acute and persistent diarrhoea occurred in the 2-11 month age group. Fever and vomiting were found more frequent in persistent than acute diarrhoea during second week of illness and differences were statistically significant. *Shigella* species, ETEC and *E. histolytica* were equally isolated from both acute and persistent diarrhoea whereas rota virus was found more often in acute than persistent diarrhoea. The presence of leucocytes and reducing substances in the stool was equally frequent.

INTRODUCTION

Diarrhoeal diseases have been well recognized as a major cause of morbidity and mortality among children in the developing countries including Myanmar. Although acute diarrhoea caused by the well-known classic pathogens produces severe and well-known symptoms and are reasonably easy to diagnose, persistent diarrhoea has not been adequately studied. A number of studies have been tried to

find out if particular pathogens are associated with persistent diarrhoea. Knowledge of the clinical characteristics of the early phase of illnesses that culminate in persistent diarrhoea could assist in the initial management of such episodes to shorten duration and reduce their adverse effects.

The relative distribution of major enteric pathogens responsible is crucial in planning and implementing control and treatment strategies.

The aim of the study was to determine the etiologic agents, clinical and laboratory characteristics of acute versus persistent diarrhoea in hospitalised children with diarrhoeal illness.

MATERIALS AND METHODS

The study was conducted at Yangon Children's Hospital for 18 months starting from March 1996. A total of 487 under-twelve-year old children admitted to medical wards for diarrhoea episodes were recruited for the study, only after informed consent of the parents/guardians had been obtained.

A questionnaire was completed for each patient to ascertain the details of clinical history, pre-illness feeding practice and demographic information. Physical examination and anthropometric measurements were done and stool samples/rectal swabs were taken within 24 hours of admission. Rectal swabs were taken on admission, if stool could not readily be obtained. Specimens were also collected on day 7 and on day 14 from the subjects with persistent diarrhoea episodes. Daily assessment of the patients was done till they were discharged from the hospital.

Stool specimens were brought, to Department of Medical Research (DMR) laboratory and divided for immediate bacteriological culture and examination and for virological study.

Working definitions

Diarrhoea was defined as three or more loose, liquid or watery stools over a 24-hour period or one or more loose stools containing blood in the same period and one diarrhoea episode was separated from another episode by at least two diarrhoea-free days.

Acute diarrhoea was defined as an episode which lasted less than 14 days and persistent diarrhoea as an

episode which lasted 14 days or longer.

Bacteriological examination

Rectal swabs collected in Cary Blair's transport media were plated onto MacConkey agar (MA), Salmonella-Shigella (SS) agar and Thiosulphate-Citrate-Bile salt (TCBS) media and incubated at 37°C for 24 hours. *E. coli*, Salmonella, Shigella and *Vibrio* species were identified by standard method as described by the WHO manual [1].

Campylobacter was isolated from 5% sheep blood Brucella agar with vancomycin (10 mg/L) and cephalothin (15 mg/L) after 48 hours of anaerobic culture using gas generation kit (Oxoid, BR-56).

Parasitic examination

Fresh stool samples were also examined microscopically for trophozoites of *Entamoeba histolytica*, *Giardia lamblia* and for helminth ova by standard methods [2].

Virological examination

Rota virus was detected by Dakopatts Rota virus enzyme-linked immunosorbent assay (ELISA). The coating antibody was rabbit antibody to rota virus and the detector system was peroxidase conjugated rabbit antibody to rota virus [3].

The presence of reducing substances in the stool was determined using Benedict's solution. Faecal leucocytes were sought by direct microscopy from a faecal smear stained with methylene blue [4].

RESULTS

A total of 487 children, 327 with acute diarrhoea and 160 with persistent diarrhoea were enrolled in the study. The mean age of acute diarrhoea cases was 16±1.2 months and of persistent diarrhoea was 21±1.3 months. The patterns of age distribu-

tion among cases were similar in both types of diarrhoea, the proportion being greatest in 0 to 11 month age group and then declined steadily thereafter (Figure 1). Anthropometric

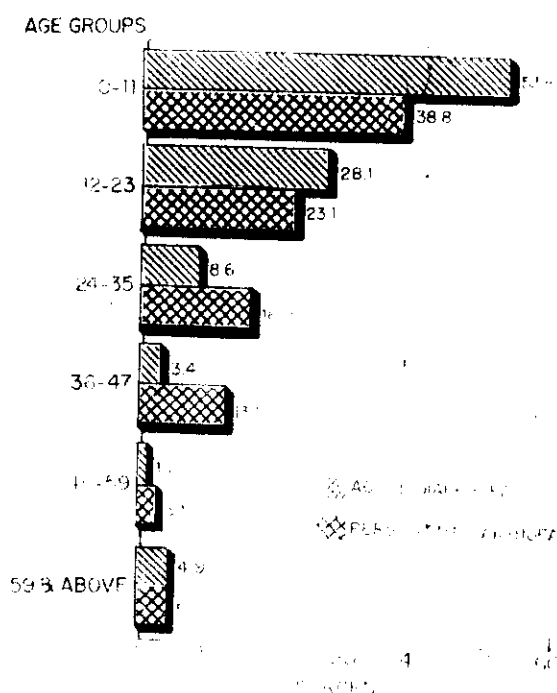


Fig. 1. Percent distribution of children with acute and persistent diarrhoea by age

measurements, monthly family income and family size were similar in both groups (data not shown in the table). Approximately 60% of the cases were boys in both types of diarrhoea. Maternal illiteracy rate and proportion with surface latrine used were slightly higher in persistent diarrhoea cases although the differences were statistically not significant. Total number of patients expired during the study period were 29 with case fatality rates of 2.4% (8/327) and 13.1% (21/160) for acute and persistent diarrhoea respectively (Table 1).

Among those expired cases, single pathogen was isolated from 8 cases, two from eight patients and three from one and no pathogen was isolated from 12 patients. *E. histolytica* and *Shigella* species combination were found to be the commonest, followed

Table 1. Socio-demographic and outcome by diarrhoeal episodes

Characteristics	Acute diarr n = 327	Persistent diarr n = 160
1. Mean age $\pm$ S.D (months)	16.02 $\pm$ 1.17	21.08 $\pm$ 1.28 ^a
2. Sex:		
Male	192 (58.7%)	95 (59.4%)
Female	135 (41.3%)	65 (40.6%)
3. Maternal education		
Illiteracy	29 (9.3%)	22 (14%)
4. Type of latrine used		
Surface	43 (13.2%)	27 (17%)
Direct pit	24 (7.4%)	15 (9.4%)
5. Outcome		
Expired	8 (2.4%)	21 (13.1%)

by *E. histolytica* with EPEC combination among these expired cases infected with mixed organisms.

Total enteropathogens detected were 64.5% in acute diarrhoea cases including 27.5% with bacterial agents, 11.3% viral and 25.4% with protozoal infection; whereas in persistent diarrhoea cases it was 71% including 31%, 6.3% and 33.7% with bacteria, viral and protozoal agents respectively. Helminth infections were detected equally in both groups. Mixed infection with two or more enteric pathogens were detected slightly higher in acute than persistent diarrhoea (16.5% vs 13.7%) but the differences were statistically not significant.

Rota virus was detected more frequently in acute than persistent diarrhoea, whereas *E. histolytica* was found to be more common in persistent than acute diarrhoea although the differences did not reach statistical significance. Other pathogens were equally detected in both groups (Table 2).

Watery stools at onset were similarly common in both types, whereas, mucoid stool was more common in persistent diarrhoea. Presence of leucocytes and reducing substance in

Table 2. Number and percentage distribution of enteropathogens isolated from stool samples of children with acute and persistent diarrhoea

Microorganisms	Acute diarr n = 160		Persistent diarr n = 327	
	No.	%	No.	%
1. <i>E. coli</i>				
*EPEC	24	7.3	15	9.4
*ETEC	16	4.9	7	4.4
*ETEC	1	0.3	1	0.6
2. <i>Shigella</i> species	30	9.2	17	10.6
3. Cholera	12	3.7	5	3.1
4. <i>Klebsiella</i>	8	2.4	5	3.1
5. Rotavirus	37	11.3	10	6.3
6. <i>E. histolytica</i>	78	23.8	49	30.6
7. <i>Giardia lamblia</i>	2	0.6	2	1.2
8. <i>Cryptosporidium</i>	3	0.9	3	1.8

Table 3. Number, percent distribution and characteristics of stool samples from acute and persistent diarrhoeal episodes

Characteristics	Acute diarr n = 327		Persistent diarr n = 160	
	No.	%	No.	%
1. Stool consistency				
*Watery	112	34.0	37	30.0
*Mucus	25	7.6	21	13.1
*Blood	2	0.6	3	1.9
*Blood and mucus	35	10.7	15	9.4
2. Stool leucocytes (+ve)	103	31.5	50	31.3
3. Stool reducing substance (+ve)	80	24.5	44	27.5

stool was found to be similar in both groups (Table 3).

Fever (56.2% vs 33%) and vomiting (29% vs 15%) during second week of illness were more common in persistent than acute diarrhoea and the differences were statistically significant ($P < 0.02$).

DISCUSSION

This study gives a considerably complete picture of bacteria, parasites and viruses associated with acute and persistent diarrhoea in hospitalised children. Black *et al.* had demonstrated that the aetiology of the initial infection is known to be a factor in determining the duration of diarrhoea; diarrhoea due to enterotoxigenic *E. coli* or shigella infection lasts longer than that due to Rota virus [5]. Maiya and colleague [6] showed that shigella was associated with more protracted diarrhoea. Mata suggested that shigella and giardia are likely to be agents of persistent diarrhoea in Guatemala because they tend to persist in the intestine [7]. The present study supported the fact that shigella was associated with more protracted diarrhoea and it was likely to be one of the agents of persistent diarrhoea. Intestinal amoebiasis and giardiasis are endemic in the tropics.

Intestinal amoebiasis and giardiasis have been reported in children under 2 years of age [8,9]. Nandinisheety *et al.* had reported that a high prevalence of amoebiasis was seen in the 0-6 month (12%) and 7-12 month (20.3%) age groups, while giardiasis was uncommon in children under 6 months old but occurred in 8-10% of all other age groups in paediatric diarrhoea cases in South Indian population [10]. Our study supported that *E. histolytica* accounts for the major identifiable cause of acute as well as persistent diarrhoea suggesting that our children are frequently exposed to this pathogen. However, isolation of *Giardia lamblia* is found

to be low.

A prospective study from India [11] reported a strong association between isolation of entero-aggregative *E. coli* and persistent diarrhoea; this type of *E. coli*, however, was not investigated in our study. In this study, predominant bacterial agents detected were similar in both types of diarrhoea; rota virus was commonly found in acute diarrhoea and *E. histolytica* was more frequently observed in persistent than acute diarrhoea. This investigation also demonstrated that children with persistent diarrhoea were infected with the same enteric pathogens or putative enteric pathogens as children with diarrhoea of shorter duration. Furthermore, children with persistent diarrhoea did not excrete the same organism for extended period of time, but rather had infection with different organisms during the time they were hospitalised.

In a study done in Brazil [12], it was shown that children with persistent diarrhoea have an increased frequency of having multiple organisms isolated from a stool culture, suggesting that those children were more susceptible to infection with more than one organism, or that mixed infections more often lead to persistent diarrhoea. In this study mixed infection was noted more frequently in acute diarrhoea but the difference was statistically not significant. Mixed infection was observed more in the patients excreting *E. histolytica*, shigella species, EPEC and ETEC serogroups. Salmonella was not isolated in our study and did not appear to play a significant causal role in childhood diarrhoea, as has also been reported from Zaire [13].

In India and Peru [14], the peak incidence of persistent diarrhoea occurred in infants less than 1 year of age. In Brazil [12], it was in children aged less than 6-24 months. Late age peaks have been observed in other countries, but in all studies

most episodes occurred in first three years of life. Our study also demonstrated that peak incidence being under one year of age and most episodes occurred in the first three years of life.

Episodes of persistent diarrhoea although fewer in number than those of acute diarrhoea are more likely to have severe consequences. Our findings highlighted that a substantial proportion of the diarrhoea-associated deaths was seen in persistent than in acute diarrhoeal cases in hospitalised children. This results is in agreement with the study done by Chowdhury and Bhan [15,16] who found that diarrhoea associated deaths in young children are from persistent diarrhoea. Although many aspects of the development of persistent diarrhoea are unclear, its high incidence of fatality rates deserve much more attention for control of disease and death due to diarrhoea.

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**Clinical features and response to antivenom of Russell's viper bite cases
of Myinmu, Sagaing Division**

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A total of 43 Russell's viper bite cases were studied during 1995-96. The median age of the victim is 27 years (7-71 yr) and the time interval between the bite and admission to the hospital is 1.50 hours (20 min-8.15 h). Sixty per cent of the bites occurred in lower limb and 86% were bitten while at work in the field. Length of the dead snakes brought varied from 23 to 50 cm (median 29 cm). Eighty-six per cent applied tourniquets and 67% carried out immobilisation. Fourteen per cent of the bites developed systemic, 72 per cent local and 14% no features of envenoming. Out of 6 systemic cases, only one presented with a spectrum of spontaneous systemic bleeding. One to 4 ampoules of antivenom was given to all cases irrespective of the state of envenoming. Six antigen negative cases were also given antivenom. Antivenom reactions were present in 21% of the cases. Venom levels of the cases with local and systemic manifestations were 10-45 ng/ml and 50-70 ng/ml respectively. There was no fatality in this study. Vomiting (32%) was observed in non systemic cases as well.

INTRODUCTION

Russell's viper (Daboia russelii siamensis) bite is an occupational hazard of our farmers. Recent reports on variation of biological properties of Russell's viper venom [1-6] and clinical features of Russell's viper bite cases from different localities [7-8] prompted us to study clinical features of Russell's viper bite cases from different localities of endemic divisions. This study concerns with Russell's viper bite cases of Myinmu, Sagaing Division.

MATERIALS AND METHODS

All snakebite cases admitted to the Myimu hospital, Sagaing Division during 1995-1996 were studied. Clinical details of the patients were recorded in standard proforma. Twenty minute clotting test [9] was used to determine the degree of envenoming. A total of 4 ampoules

of monospecific antivenom were given to all snake bite cases at admission. Clotting test was carried out at 2 hourly intervals on cases presenting with clottable blood and 6 hourly after giving antivenom on systemic cases until normal clot restoration occurred. Two millilitres of blood were taken for clotting test. Sera were collected onto filter paper strips, air dried, stored in a sealed polythene bag and later transported to the Department of Medical Research once a month. Venom antigen levels of the samples were determined by enzyme immunoassay technique [10] at the Immunology Research Division. Liquid monospecific antivenoms were given in the study.

RESULTS

A total of 43 Russell's viper bite cases were available for the study. Clinical details of the RV bite cases

Table 1. Clinical details of 43 Russell's viper bite cases

Total (43)	6 systemic + 31 local + 6 non envenoming	%
Sex: Male : Female	31:12	72:28
Time bite: Day (6 am - 6 pm) : night (6 pm - 6 am)	37:6	86:14
Age (yr) (median)	27 (7-71)	
Activity when bitten: working : walking : playing	37:4:2	86:9:56
Site of bite: lower limb : upper limb	26:17	60:40
Distance travelled (miles) (median)	8 (0.25-20)	
Time interval between the bite and admission to hospital (h) (median)	1.50 (0.20-8.15)	
Antivenom pretreatment at village	1	2
Bite and antivenom therapy (h) (median)	2 (0.30-820)	
First aid: tourniquet	37	86
immobilisation	29	67
Number of dead snake brought	39	90
Length of the snake (cm) (median)	29 (23-50)	

are shown in Table 1.

Site of bite on upper limb (17/43, 40%) were 13 fingers (30%), 3 dorsum of hand (7%), 1 forearm (2%) and that of lower limb (26/43, 60%) were 9 toes (21%), 7 malleolus (16%), 9 dorsum of the foot (21%) and 1 leg (2%).

Local blackening 14% (5) and bruising 5% (2) were observed in envenomed cases (n=37).

Response to antivenom

Of 43 bites, 14% (6/43) had systemic, 72% (31/43) local and 14% (6/43) no features of envenoming. All cases received 1-4 ampoules of antivenom irrespective of the state of envenoming. Six antigen negative cases received antivenom. Antivenom reactions were present in 21% (9/43) of the cases. Twenty-eight per cent received antivenom within 1 h, 44% in 2 h, 70% in 3 h and 79% in 4 h. Clot restoration times of 6 systemic cases were 6 h in 2 (33.3%), 12 h in 2 (33.3%) and 18 h in 2 (33.3%).

Venom antigen levels

Venom levels of local envenoming cases range from 10-45 ng/ml and that of systemic 50-70 ng/ml.

Clinical features of systemic cases

Out of 6 systemic cases, only one presented with a spectrum of spontaneous systemic bleeding such as epistaxis, haematuria, malena, haematemesis, subconjunctival haemorrhage, oedema and albuminuria who was bitten by a 30-cm-long snake and was admitted to the hospital 4.20 h after the bite. There was no fatality in this study. Vomiting (32%, 12/38) was observed in both (33%, 2/6) of systemic and 42%, 13/31) of local cases.

Length of the snake and degree of envenoming

A total of 37 juvenile snakes (230-500 mm) brought by the victims were studied. Seventy per cent (26/37) of the bites resulted in local envenoming (mean venom level 17 ng/ml, range 10-45 ng/ml), 16% (6/37) systemic (mean venom level 62 ng/ml, range 50-70 ng/ml) and blank bites in 14% (5/37).

Effect of immobilisation on admission venom levels

The mean length of the snakes of immobilised group (n=18) is 317 mm and mean admission venom level is 20.6 ng/ml and that of unimmobilised

group (n=12) are 267 mm and 29.6 ng/ml respectively. There is a significance difference in length of the snake and admission venom level between two groups ($p < 0.05$).

DISCUSSION

The study shows that majority of the Russell's viper bite victims are young adult which are the working force of our country. Unlike in other localities, the snakes responsible for the bites are all juvenile (23-50 cm in length) which are two times more effective in injecting venom into the victims (70% of the bites resulted in local and 16% systemic compared to that of juveniles from Taungdwingyi [7] which led to 32% local and 16% systemic envenoming).

Early high dose routine administration of antivenom combined with the practice of immobilisation of the limb may have contributed to less number of local envenoming cases turning into systemic and hence to low incidence of systemic cases and mortality rate in our study. The fact that effective bite of juvenile snakes could lead to severe envenoming was observed in the present and previous study [8].

If antivenom had been routinely given to all snakebite cases (38% were blank bites). However, in the present study only 14% blank bites were given antivenom because of the practice which could have been saved if guidelines for antivenom administration were followed. It is suggested that 1 to 2 ampoules of antivenom is sufficient to neutralise incoming venom in local envenomed cases and 4 ampoules in systemic [11].

Since all cases received antivenom, vomiting observed in local cases was likely due to antivenom reaction which was present in 21% of the cases.

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The use of Moringa oleifera (dan-da-lun) seed for the sedimentation and decontamination of household water

Part II: Community-based study

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The seeds of Moringa oleifera were tested as clearing and sedimentation agents in household water in Thaung Gyi Lay village with 110 households. Questionnaires were completed for each household and follow-up visits were carried out to ascertain the hypothetical acceptability (attitude), initial acceptability (behaviour) and experimental acceptability. It was observed that 78.9 per cent of the people accepted to use Moringa oleifera seeds if these were easily available. For continuous use of Moringa oleifera seeds, 47.3 per cent wanted to use, 44.7 per cent could not decide and only three households (2.7 per cent) did not want to use these. It was observed that the taste and pH of water did not change after treatment with Moringa oleifera seeds. There was no complaint about the treated water. This study will highlight the acceptance to use Moringa oleifera seeds for the sedimentation of turbid water.

INTRODUCTION

Moringa oleifera is a small or median sized tree, about 10 meters high, found growing wild in the subhimalayan tract. These are also cultivated throughout Myanmar and the fruits and leaves are used in soups. Fruits are obtained after 18 months and could survive up to 50 years. About 10,000 seeds could be obtained from each plant within a year. If the plants are grown about two meters apart on two and a half acres of land, it is estimated that the seeds will clear 250 cubic meters of water daily. There is an abundance of Moringa oleifera trees growing in many parts of Myanmar and some fruits remain unused on trees. Also plant bears approximately 50 to 300 fruits in each flowering season which falls at least three times a year. A

fruit contains about 5-12 seeds and average seed weighs approximately 0.2 gram. It is useful as an ornamental tree and well-known for its nutritive value. This study aimed to determine the effectiveness of Moringa oleifera seeds in decontamination of household waters. It is also aimed to determine whether the community practise using the seeds of Moringa oleifera as decontaminant and sedimentation agent.

MATERIALS AND METHODS

A community-based study: Decontamination effect of Moringa oleifera seeds on water

Thaung Gyi Lay village from Hlinethaya Township where the community used uncleaned turbid pond water was selected for community-based

study area. The village is situated about 8 kilometers from Hlinethaya and is 30 kilometers north of central Yangon. There were about 200 households residing in that place and their occupations are factory workers, daily-wage earners and farmers. Most of them live in traditional style houses built of thatches, bamboos and woods. Households using the pond water throughout the whole season during the whole year were selected. The study was conducted during the cool dry (pre-monsoon) and hot wet (monsoon) seasons.

A total of 110 households from that area were selected for the hypothetical acceptability (attitude), initial acceptability (behaviour) and experimental acceptability tests.

Data collection procedure

One house-wife or an influential person from each household was interviewed using a pretested questionnaire before and after treatment of domestic water with Moringa oleifera. Selected household characteristics as well as the hypothetical and initial acceptability of Moringa oleifera as a clearing agent for drinking and domestic water were assessed on the first visit. Experimental acceptability was ascertained, three months after treatment. The same person was interviewed in each household during the followup visit. Test retest reliability was carried out in five per cent of study population.

Treatment procedure

Moringa oleifera seeds were stocked during May, 1996. The seeds were grinded to powder, packed into 100 mg packets and were given to each household to mix with water in a ratio of 100 mg to one litre of water (equivalent to one average size seed to one gallon of water).

RESULTS

A community based study

Decontamination of household water by Moringa oleifera seed powder

Throughout the whole season the villagers consume water from the pond as a source for drinking and cooking. However, during rainy season most of them use rain water. In the field study 110 households were interviewed and followup visits were carried out. Prior to the treatment of Moringa oleifera seed powder in their household water, the acceptability test was ascertained (Table 1).

Table 1. Acceptability test prior to the treatment of water with Moringa oleifera seeds

Accepted	78.9 per cent
Not known	21.0 per cent
Not accepted	2.4 per cent

It was found that 78.9 per cent of the households accepted to use the seed powder if it was easily available, 47.3 per cent wanted to use continuously, 44.7 per cent could not decide and only 2.7 per cent (3 households) did not want to use (Table 2). It

Table 2. For continuous utilization of Moringa oleifera seeds for sedimentation of household water

Wanted to use	47.3 per cent
Could not decide	44.7 per cent
Did not want to use	2.7 per cent

was also noted that the taste and pH of water did not change after treatment with Moringa oleifera. No complaint was observed about water after treatment with Moringa oleifera seeds powder.

DISCUSSION

In Myanmar, the main sources of water supply for both drinking and domestic purposes are dugwells (used by 43.9 and 43.5 per cent of total households) followed by tubewells

(18.6 and 19.6 per cent of total households), ponds (15.0 and 9.1 per cent of total households), river water (8.5 and 9.8 per cent of total households) and gravity flow water systems (7.9 and 7.6 per cent of total households) [1]. According to the report on the health impact study of the dryzone tube well water supply project [2], almost all the samples collected from main water sources for drinking and general use revealed the presence of total coliform in all the study villages. They also stated the presence of relatively higher proportion of faecal coliforms in that area.

There are many ways for elimination of contaminated pathogens from water, including physical and chemical treatment. The effectiveness of chemical agents and alum on contaminated water was reported, but, these chemicals also altered the original pH [3,4]. In some African countries, plant materials from the family Papilionaceae, such as seeds from broad beans, white beans or ground nuts were already observed being in use from the beginning of the last century [5]. However, they are weak, slow acting coagulants leaving the water still milky opaque several hours and failing to clarify waters with mainly colloidal turbidity. In some parts of Myanmar, Ka-baung-ye-kyi was used for water purification although the water remains still opaque after some time.

In more recent times Sudan Arab women discovered that clarification of muddy Nile water to "tap water quality" could be achieved in one to two hours by adding the pounded seeds from a "clarifier tree" which was wrapped in a clean cloth and turned for 20-30 minutes within water [6]. This tree is now known as Moringa oleifera. It was reported that one litre of turbid water could be cleared by using 100 mg (0.1 gram) of the seed which is equivalent to one ywe only. For one bucket of water (10 litres) one gram of seed

is necessary. Moreover, the advantage of using Moringa oleifera seeds is that the pH value of water has not changed even up to three days of storage at 25°C. The plant itself could grow easily in most parts of Myanmar. The seed could be turned into powder form very easily by using kitchen utensils. These are non toxic and easily accepted by many people. The procedure to treat water is also very simple and easy. During this study, surprisingly, two persons from the village also explained that after consuming the treated water with Moringa oleifera seeds they stopped suffering from diarrhoea.

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SHORT REPORT

Heat stability of factor X activator of Russell's viper venom

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Russell's viper envenomation causes disseminated intravascular coagulation, particularly in renal glomerular capillary leading to acute renal failure [1]. Macfarlane [2] showed that Russell's viper venom reacts with Factor X in serum to form a product which, with phospholipid and Factor V, causes rapid prothrombin conversion to thrombin. Furie et al. [3] demonstrated activation of bovine Factor X by the venom coagulant protein of Vipera russelli. In 1976, Kisiel et al. [4] isolated Factor X activating enzyme from Russell's viper venom.

In this paper, Factor X activating enzyme of Russell's viper (Daboia russelli siamensis) venom obtained from Myanmar Pharmaceutical Factory was purified. Coagulant protein of Russell's viper venom was purified by two steps namely DEAE-cellulose ion exchange chromatography followed by Sephadex G-200 gel filtration chromatography [3]. The effect of the purified coagulant protein on Factor X in blood coagulation was studied using Factor X-deficient plasma and bovine Factor X. Recalcification time of normal plasma was measured by one-stage assay method [5]. It was found that the purified fraction possessed Factor X activating property.

The heat stability of the purified coagulant protein was tested in duplicate for 10 minutes at 50°, 60°, 70°, 80° and 90°C. The clotting time of normal citrated human plasma was 2.0 minutes, and when the unheated purified fraction was added, it was found to be 30 seconds. There-

fore, the shortened clotting time, 1.5 minutes, was regarded as the initial clotting activity (100%) of unheated coagulant protein. Its activity was found to be stable when heated up to 60°C for 10 minutes (100% of initial activity). It retained 70% of its initial activity when heated at 80°C for 10 minutes, and 50% at 90°C for 10 minutes.

Heat stability of other enzymes was studied by Mori et al. [6]. They made a comparative study of two arginine ester hydrolase, E-I and E-II from the venom of Crotalus ruber ruber (red rattlesnake). E-I and E-II were found to have high heat stability. Both enzymes were stable up to 60°C for 10 minutes, and retained considerable activity, 65% at 80°C.

According to Iwanaga and Suzuki [7], Factor X activator was a heat labile glycoprotein. This may probably be due to the geographic variation in the composition of Russell's viper venom itself [8]. The coagulant protein possessing Factor X activating property may be composed of a component other than glycoprotein, which is heat stable up to 60°C.

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