MYANMAR
HEALTH SCIENCES
RESEARCH JOURNAL

Volume 7, Number 3, December 1995

DEPARTMENT OF MEDICAL RESEARCH
No. 5, Ziwaka Road
Yangon 11191, Myanmar

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Printed at the Publications Division, Department of Medical Research

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The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Prescribing practices on contraception among general practitioners and basic health services staff in Taikkyi Township

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Sixty-eight health service providers including 14 Medical Doctors [7 Full-time General Practitioners (GPs) and 7 Government Medical Officers], 4 Health Assistants (HAs), 11 Public Health Supervisors (PHS I & II), 5 Lady Health Visitors (LHVs) and 34 Midwives (MWS) practising in Taikkyi Township, were interviewed using a self administered structured questionnaire, to assess their level of knowledge and prescribing practices for contraception. All of the service providers with the exception of some PHS have prescribed some form of contraception during their practice. Among the contraceptives stated to be most prescribed by them, oral contraceptives came first with 94%, followed by injections with 92%, rhythm method in 37%, intra-uterine device in 30%, female sterilization in 21%, and condoms were prescribed only by 16% of them. The real and perceived side effects experienced with various methods are also discussed. The findings suggest that health care providers need to be trained or given refresher courses on contraceptive technology and proper counselling and use of the available methods, appropriate choice of methods suitable for the individual, the real and perceived side effects from various methods and their management. Development of appropriate health education and counselling package for use in birth spacing programmes is also indicated.

INTRODUCTION

The National Health Committee of Myanmar has identified population policy formulation as a priority task and accepted the concept of birth spacing as an important measure for the improvement of the health of mothers and children. Thus the importance of systematic provision of contraceptive services by all health care providers is recognized, to obtain effective birth spacing, reduce fertility, abortion rate and abortion deaths.

The Department of Health had launched its first Birth Spacing Project in Zigone Township in 1991, and has now expanded to other townships as well. There is very little information on contraceptive use and prescription from the health providers perspective. Especially as the curriculum and job description of Basic Health Services staff had not included the provision of contraceptive services until lately, there is a clear need for research in this area. This paper focusses on the findings of interview of GPs and Basic Health Services staff in the area on their prescribing practices on contraception. It is an attempt to determine the prescribing practices of contraception among health care providers in Taikkyi Township.

MATERIALS AND METHODS

The study was designed as a cross-

sectional survey of all health service providers both government and private sector in Taikkyi Township. A self-administered semi-structured questionnaire was distributed and collected from all basic health services staff and GPs in the area.

RESULTS

The characteristics of the study population by category of health provider, revealed that there were 14 Medical Doctors, 4 Health Assistant (HAs), 11 Public Health Supervisors (PHS), 5 Lady Health Visitors (LHVs) and 34 Midwives (MWs). Among a total of 87 registered Health Providers 68 responded with a response rate of 78% (Table 1).

Table 1. Characteristics of study population

Category of health provider	No.	Percent
1. Medical Doctors (MOs)	14	20.6
2. Health Assistants (HAs)	4	5.9
3. Public Health Supervisors (PHS)	11	16.2
4. Lady Health Visitors (LHVs)	5	7.3
5. Midwives	34	50.0
Total	68	100.0

Overall response rate = 68/87 = 78%

The majority of providers (65%) had 10-29 years of service (Table 2).

Table 2. Study population by years of service

Service years	No.	Percent
<10 years	14	20.6
10-19 years	30	44.1
20-29 years	14	20.6
30+ years	10	14.7

Regarding contraceptive prescribing practices, it was found that almost all providers, 55% of Public Health Supervisors and 100% of other categories are prescribing contraception to some degree in their daily practice. Methods most commonly prescribed were oral contraceptive pills (OCP) (94%), injections (92%), safe period (rhythm) (37%), intrauterine device (IUD) (30%), female sterilization (21%) and condom (16%). Very few prescribed withdrawal (8%) and male sterilization methods (1.5%) (Figure 1).

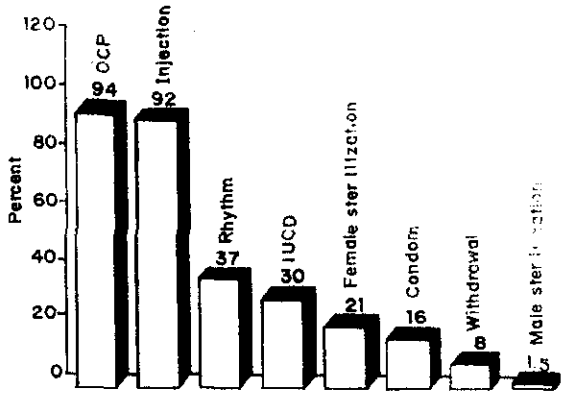

Fig. 1. Methods most commonly prescribed

The contraceptive methods best preferred by health providers were described in Table 3. Most doctors (21%) indicated OCP followed by injection. Lady Health visitors and midwives (46%) liked to prescribe injection

Table 3. Contraceptive methods best preferred by health providers

Medical Doctors	LHVs + MWs	HAs + PHS
1. OCP (21%)	Injection (46%)	OCP (50%)
2. IUCD (21%)	OCP (23%)	Rhythm (30%)
3. Steriliza tion (21%)	Rhythm (17%)	Injection (20%)
4. Injection (14%)	Sterilization (12%)	Condom (20%)
5. Condom (7%)	Condom (2%)	Steriliza-tion (10%)

whereas health assistants and Public Health Supervisors (50%) favored OCP.

Problems and complications experienced by providers were revealed by different methods (Table 4).

Table 4. Problems and complications experienced by providers prescribing contraceptive methods

1. **OCP**
   - Giddiness (53%)
   - Irregular pill taking (46%)
   - Menstrual problems (34%)
   - Palpitations (24%)
2. **Injections**
   - Menstrual problems (96%)
   - Giddiness & palpitations (41%)
   - Need injection (20%)
   - Changes in body weight (16%)
3. **IUCD**
   - Menorrhagia (25%)
   - pain in SPA (8%)
   - Headache (8%)
   - Lost loop (8%)
   - Expulsion (6%)
4. **Condom**
   - Not affordable (15%)
   - Not available (10%)
   - Ignorance of method use (5%)
   - Dissatisfaction of user (3%)
5. **Rhythm (Safe period)**
   - Non-compliance (25%)
   - Wrong/Unknown dates (14%)

It shows that main complication expressed for OCP was giddiness (53%), menstrual problem for injection (96%), menorrhagia for IUCD (25%), not affordable for condom (15%) and family non-compliance for safe period (25%).

Some 38% of providers have prescribed method switch. Reasons for method switch were mentioned as current method not satisfactory, complications, forgetting to take pills, long duration of use of current method and patient's request to switch method.

They also expressed that there were consumers' choice of method which mainly depended on: health reasons, socio-economic reasons, interest and satisfaction of client with particular method and self decision on suitable method.

DISCUSSIONS AND RECOMMENDATIONS

Even in townships without the government birth spacing programme for provision of contraceptive services, all health care personnel are found to be involved in prescribing contraception either at their private practice or other wise. This clearly shows a demand for contraceptive services from consumer's aspect as well. Another study by Le Le Win et al., in Sangyoung Township also revealed the same findings that all GPs practising in Sangyoung were involved to some extent in delivering family planning services. Despite the long lists of real and perceived problems experienced by their clients, hormonal methods are still the most commonly prescribed method by the health care providers, presumably because of their availability. The differences among the various health categories in their best preferred method could be due to the level of background knowledge on contraception by various categories; and also by locality of consumer population, GPs would most likely serve the urban population whereas the other basic health staff would be mostly involved with the rural population. A positive attitude of all health care providers as revealed by the percentage in favour of contraceptive use indicates that the Basic Health Services staff, even those not directly involved in MCH services (such as HAs and PHS) would be very receptive if trained to deliver counselling services to married couples, both men and women in appropriate birth spacing methods.

Another finding, that there are those who do not favour contraception on

the grounds that it affects maternal health, also indicates the need for basic health services staff to be trained with refresher courses to know and realise the real and perceived side effects of each of the contraceptive methods available, so that they can counsel on the appropriate choice of method suitable for each individual. The findings that some 10% of respondents do not consider consumer's choice because of their ignorance of appropriate method, is consistent with the quotation of Dr. Firman Lubis of WHO/HRP that "Providers have a tendency to consider patients so ignorant that it is not helpful to provide information"; it is important that however ignorant the client may be, the provider should do his or her best to counsel and provide all necessary information and help the consumer make an informed choice.

Despite the efforts of AIDS and STD prevention programmes' attempt to promote the use of barrier methods such as condoms, the providers' prescription and preference for condoms is found to be surprisingly low in this study. This may be partly due to the consumers' sex, as it is the woman who usually seeks contraceptive service and not the male counterpart.

It is recommended that appropriate counselling and health education packages for health provider's use, be developed and training of health care providers or refresher courses be conducted on contraceptive technology, and use of available methods, the real and perceived side effects of each method, and appropriate choice of method suitable for the individual. The health providers in non-programme areas should also be trained as their involvement in birth spacing services is already high.

ACKNOWLEDGEMENT

The authors are indebted to Director-General & Deputy Director-General of Department of Medical Research for allowing us to undertake this study under DMR Research Grant, to all health care providers of Taikkyi Township for participating in the study, and to the staff of Epidemiology Research Division for their assistance in data analysis and report writing.

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Accepted for publication 12 Dec 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Development of enterotoxigenic Escherichia coli 'OK' and 'O' antisera for detection of E. coli in diarrhoea and dysentery cases

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Standard strains of enterotoxigenic Escherichia coli (ETEC) were used to raise 'OK' and 'O' antisera in rabbits. The antisera were obtained from O6K15, O8K40, O25K+, O27K+, O78K80, O148K+ and O159K+ strains. Random samples of 2730 isolates of E. coli from 305 cases of adults with diarrhoea or dysentery were tested with 'OK' antisera by slide agglutination and found that 96 cases showed agglutination. It was then serotyped using 'O' antisera by test tube serial dilution technique and found that 33 cases showed agglutination.

INTRODUCTION

Without question, diarrhoeal diseases constitute one of the greatest causes of morbidity and death on a global scale. Four classes of E. coli that cause diarrhoeal disease can be distinguished as enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC) and recently recognized enterohaemorrhagic (EHEC) strains. Each class manifests distinct features in pathogenesis, clinical syndrome and epidemiology and falls within a different set of serogroups. Enterotoxigenic strains are an important cause of diarrhoea in infants and travellers in less developed countries, causing three episodes per child in the first year of life in some villages. The disease ranges from minor discomfort to severe cholera-like purging of liters of fluid per day, leading to severe dehydration, particularly in children. The ETEC are acquired by the ingestion of contaminated food and water and adults in endemic areas are evidently usually immune. Ordinarily, large number of organisms (10^8) must be consumed to cause disease in a susceptible individual. The process requires intestinal colonization as well as the elaboration of one or more enterotoxins. Both factors are encoded on plasmids. This paper aims to produce antisera required for the diagnosis of E. coli isolates from diarrhoea and dysentery cases.

MATERIALS AND METHODS

E. coli 'OK' antisera are employed in the identification of the 'K' or envelope antigens in the slide agglutination technique. E. coli 'O' antisera are employed in the identification of the somatic antigens in the tube agglutination tests. Prior to the development of serological methods for identification of pathogenic E. coli, biochemical tests were used. Biochemical tests alone were inadequate because nonpathogenic and pathogenic E. coli with identical biochemical characteristics may possess different antigenic identities. The delineation of the enteropathogenic cultures from the 'nonpathogens' was facilitated by the work of Kauffmann [1] which results in a serological typing system for E. coli. Later work of Orskov [2] and studies of Ewing [3] contributed to the development of an antigenic scheme for these groups of organisms which

made possible the identification and characterization of non-pathogenic E. coli and those serotypes isolated from cases of diarrhoea.

Bacterial strains

The following ETEC standard strains obtained from Centre for Disease Control, Atlanta, USA were used for both 'OK' and 'O' antisera: 06K15 (Bi 7458/41), 08K40 (G 3404/41), 025K+ (E 47a), 027K+ (F 9884/41), 078K80 (E 38), 0148K+ (E 519/66) and 0159K+ (E 2476/72).

Rabbits for immunization

Japanese white rabbits were used for immunization. Female or male rabbits weighing between 2.5 to 3.5 kg were used. At least 2 rabbits were used for each antigen.

Preparation of 'OK' antigen

Each standard strains of E. coli was cultured in a tube containing 5 ml of Trypticase Soy Broth (TSB) and incubated at 37°C for 18 to 24 hours. A loopful of upper layer of the broth was then transferred to a new tube of TSB and again incubated at 37°C for 18 to 24 hours. It was proceeded to at least thrice and transferred onto Trypticase Soy Agar plates. Isolated 5 smooth colonies of E. coli were selected from the plates and then transferred again onto 5 tubes of Trypticase agar slants and then incubated at 37°C for 18 to 24 hours. The organisms were tested with standard 'OK' antisera by slide agglutination and normal rabbit sera was used as a control to check for roughness or smoothness. Slants of organisms which were smooth and showed a quick response to the tested homologous antisera were chosen for the preparation of antisera. The chosen slant was then transferred to 10 new TSA slants and to maintainence media for further use. Just before immunization, 3 ml of one per cent formal saline was added, suspended and transferred to a sterile centrifuge tube. It was spinned at 3000 rpm for 30 minutes and washed again with one per cent formal saline at least three times. It was then suspended in a sterilized saline to obtain 1 mg/ml concentration which was estimated by MacFarland Nephotometer tube No. 3.

Preparation of 'O' antigen

Plating and selection of smooth colonies was done as described in the 'OK' antigen. 5 smooth (s) colonies to be tested were checked by its respective antisera to yield a high titre of agglutination after growing on Blood Agar Base slants. Blood agar base media was used to enhance K antigen production. Normal rabbit sera and saline were used as controls. For agglutination it was done by putting 1 to 2 ml of normal saline in a slant, mixed gently and slide agglutination was done by using 'O' antisera. If agglutination did not occur the organisms were allowed to boil at 121°C for 1 hour and was checked again. Boiling was carried out two to three times to make sure that the capsule was uncovered.

A tube with smooth colony with high titre of agglutination was then taken to use in preparation of 'O' antigen. A few drops of culture was then transferred in 15 ml of Trypticase Soy Broth (TSB) in a conical flask and incubated at 37°C for 3 hours. Two ml broth was seeded on Blood Agar Base media supplemented with one percent Oxoid No. 1 agar, using stainless steel trays (200 ml/tray) or in large petridishes (150 mm diameter) and streaked onto the media's whole surface by hockey glass stick and incubated at 37°C overnight. The growth was removed from the plates by washing out with 0.5 Molar formal saline using a hockey stick into a

250 ml beaker.

After being subjected to heat at 121°C for 2 hr (Roschka antigen) [4], it was checked by slide agglutination using its respective antisera, normal rabbit sera and normal saline. If there was slow reaction in agglutination, the antigen was discarded and repeated again from the beginning. Only after satisfactory with agglutination it was spinned at 10,000 rpm for 30 mins. The sediment was washed again with 200 ml of formal saline and spinned again. To the sediment, 100 ml of 95 percent ethanol was added and mixed thoroughly by using a glass rod and kept overnight. It was spinned again and the sediment was transferred directly into absolute ethyl alcohol. The bacteria was emulsified and suspended as much as possible in the alcohol. This suspension was placed in the incubator at 37°C for 2 hours and spinned again. The sediment was then resuspended in absolute alcohol and placed in the incubator for additional 2 hours. It was centrifuged and the bacteria were allowed to dry and suspended in 50 ml of acetone in a motor and ground with a pestle. It was placed in a fume hood overnight until the acetone was evaporated. The dried bacteria was ground until powder form was obtained and was stored in an air-tight moisture-proff container. When animals were to be injected, a small amount of powder density of the suspension which was approximate to that of 24 hour broth culture was used. Although this alcohol treatment does not completely inactivate the flagellar antigens of the bacteria, the production of 'H' agglutinin is minimized, especially when poorly motile strains are selected.

Immunization schedule

All injections were made in the marginal ear vein. For 'OK' antisera 0.5 ml, 1 ml, 2 ml, 3 ml and 4 ml of antigen were immunized in rabbits on day 1 to day 17, with an interval of 4 days (Table 1). On 24th day some of the blood were withdrawn from the artery and tested for the titre of antisera. If the titre was more than 320 the sera was collected from the rabbit. If the titre was lower than 320 booster injection with 4 ml of antigen was done and wait for another one week to 10 days. The antisera were preserved in 0.1 percent sodium azide.

For 'O' antisera it was also made in the marginal ear vein. The first dose given was 0.5 ml, the second 1.0 ml, the third 2.0 ml and the fourth, fifth and sixth 3.0 ml each. Time interval for immunization ranged between 4 to 5 days (Table 1).

If the titre was not greater than 1:1280 another booster dose of 3 ml antigen was immunized and after 7 days the animal was bled. After the immunization schedule, the animals were bled into separate containers. The sera were not pooled until after the tests were completed. The antisera were preserved by addition of an equal volume of glycerol.

Absorption of antisera

The bacteria were cultured in 15 ml trypticase soy broth for 3 to 4 hr to reach MacFarland No. 3 nephotometer tube. It was then seeded onto large tray or large petridishes and incubated at 37°C overnight. The cells were then scrapped with formal saline and heated at 121°C for 2 hours. It was then spinned at 10,000 rpm for 20 minutes. The supernatant was discarded and the remaining supernatant was removed by soaking on a filter paper and the amount of cells were checked according to weight. Then one millilitre of antisera was added to approximately 1 gram of packed cells and suspended with a glass rod

Table 1. Immunization schedule

Sr. No.	'OK'		'O'	
	Time (day)	amount of antigen injected (ml)	Time (day)	amount of antigen injected (ml)
1	1	0.5	1	0.5
2	5	1.0	5	1.0
3	9	2.0	9	2.0
4	13	3.0	13	3.0
5	17	4.0	17	3.0
6	24	bled	24	bled
	>1:320		If>1:1280	
7	If<1:320	4.0 (boost)	If<1:1280	3.0 (boost)
8	day 31	bled	day 31	bled

and kept at 48°C waterbath for 2 hours. It was then spinned at 15,000 rpm for 45 minutes and the antisera was kept in a sterile vial. The absorbed antisera was then checked with the unrequired reactive antigen and if still showed some cross reaction, the antisera was then reabsorbed again as described above. It is well known that cross reaction occurred in some serogroups such as 086 with 0127, 0111 with 0119, 0127 with 086, etc. as described in Ewing [5].

Serotyping of E. coli

2730 isolates of E. coli from diarrhoea and dysentery cases of 305 adults attending the Infectious Diseases Hospital and Military Hospital (2) were tested for serotyping firstly by 'OK' sera by slide agglutination. If agglutination occurred 'O' serogrouping was also done by test tube serial dilution technique as described by Ewing [5].

RESULTS

Approximately 40 to 80 ml of antisera was obtained from each rabbit. For 'OK' antisera the lowest titre was 1:20 and for 'O' antisera the lowest titre was 1:320 (Table 2). Absorption was also needed in some cases such as 086, 0127 etc.

For every E. coli serotyping, OK polyvalent antisera was used by slide agglutination test and further confirmed by monovalent antisera. Later, the E. coli was tested with specific 'O' antisera by tube agglutination test [5] and this confirms the presence of specific somatic antigen of the E. coli.

Out of 2730 isolates of E. coli from diarrhoea and dysentery cases 96 isolates showed agglutination. When tested by tube agglutination it was observed that 52 isolates from 33 cases showed agglutination by either one of 'O' serotypes. The serotypes obtained were 06, 08, 025, 027, 078, 0148 and 0159 (Table 3). The titre of O antigen of E. coli isolated from diarrhoea and dysentery cases is shown in Table 4. It was found that each of the four isolates showed low titres of 1:40 and 1:80. Eight, seventeen and seven isolates showed at the titre of 1:160, 1:320 and 1:640 respectively and twelve isolates showed high titre of more than 1:640.

Table 2

Antisera Antigen	Code No.	Homologous titre of 'OK' antisera using standard strains by slide agglutination							Homologous titre of 'O' antisera using standard strains by test tube dilution method							
		06 K51	08 K40	025 K+	027 K+	078 K80	0148 K+	0159 K+	'O'	06	08	025	027	078	0148	0159
06K15	Bi7458/41	80							06	1280						
08K40	G3404/41		80						08		640					
025K+	E47a			80					025			640				
027K+	F9884/41				160				027				640			
078K80	E38					20			078					320		
0148K+	E519/66						160		0148						320	
0159K+	E2476/72							80	0159							320

DISCUSSION

The serotyping scheme for E. coli is based on that described by Kauffmann [1] and depends on the identification of the heat stable lipopolysaccharide somatic or 'O' antigens, the flagellar 'H' and the surface or capsular 'K' antigens. The term 'K' antigen was originally used for surface antigen that cause 'O' inagglutinability and the phenomenon in which the agglutination of these organisms in an antiserum prepared with a heated vaccine is inhibited or blocked by 'K' antigen.

Table 3. Number of isolates and cases agglutinated by 'O' serotypes

Serotype	No. of isolates	No. of cases
06	10	6
08	8	3
025	14	8
027	6	5
078	4	2
0148	5	4
0159	5	5
Total	52	33

Table 4. Titre of 'O' antigen of E. coli isolated from diarrhoea and dysentery cases

'O'	Isolates	1:40	1:80	1:160	1:320	1:640	>1:640
06	10		1	1	3	2	3
08	8	1			2		5
025	14		3	2	7		2
027	6	1			2	2	1
078	4			1	1	2	
0148	5	1		2	1		1
0159	5	1		2	1	1	
Total	52	4	4	8	17	7	12

It is apparent from published literature that there are hundreds of serotypes of Escherichia [5]. It was also reported by many investigators that serotypes that belong to many 'O' antigen groups occur commonly in the colon of normal human and lower animals. These serotypes cause no apparent ill effects as long as they are confined to that area. The E.

coli flora of the normal human is not static. It changes constantly, as indicated by repeatedly (usually weekly) examination of faecal specimens by continuous culture method from healthy individuals for a year or more. Some of the serogroups of E. coli that constitute the 'normal' flora (what is normal in one person may differ considerably in another) are more invasive than others, and cause extraintestinal disease when they gain entrance to other anatomic sites such as in bacteriemias, urinary tract infection, meningitis.

Among distinct O serogroups, it is now becoming increasingly recognized that some clinical EPEC strain produce enterotoxin such as ST or LT. Scotland [6] demonstrated that 0114 and 0128 produce ST or LT. 044 also produce ST. Thus, some of serogroups are grouped as Enterotoxigenic E. coli (ETEC) and their common serogroups are 06, 08, 015, 025, 027, 063, 078, 0115, 0148, 0153, 0159 and 0167. In this study, 06, 08, 025, 027, 078, 0148 and 0159 strains were used to raise as ETEC antisera. It has been shown that certain strains of E. coli produce enterotoxins detectable and these ETEC tend to belong to particular serogroups but differ from those associated with EPEC. Serotyping is always worthwhile in outbreaks of diarrhoea.

Further study is necessary to correlate between ETEC and serotypes and to know the virulency of these serotypes.

ACKNOWLEDGEMENTS

We would like to thank Miss L. Britt and Dr. I.K. Wachsmuth of CDC, Atlanta for providing us the standard E. coli strains and transferring the technology for 'O' antisera and to Professor H. Hayashi and Dr. T. Tsukamoto for the strains and technology for 'OK' antisera. Thanks are also due to Director-General Prof. Myint Lwin and Director Dr. Myint Lwin for their guidance in research activities, to staff of animal house for providing rabbits and to USAID and JICA for providing the materials and media for use in this research.

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Accepted for publication 26 Sep 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Case-control study of risk factors for cholera in Yangon

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A case-control study was conducted on 100 culture-confirmed cholera cases who were admitted to Yangon Infectious Diseases Hospital during 12 July to 11 October 1993 and their age-matched controls from their neighbourhood to determine the risk factors for cholera. V. cholerae 01, El Tor Ogawa, was isolated from the drinking water sample of one case-patient. A number of factors, including poultry farming, consumption of food and iced drinks from street vendors, drinking unboiled water and lack of hygenic habits were found to be significantly associated with cholera transmission.

INTRODUCTION

Vibrio cholerae 01, the aetiological agent of cholera, causes severe or mild watery diarrhoea, muscle cramps, vomiting, dehydration and death if treatment is delayed or inadequate. Yangon is an endemic area where sporadic cases of cholera occur throughout the year. In 1993, 647 cholera cases from various townships of Yangon Division were reported to Yangon Infectious Diseases Hospital. In August 1993, small outbreaks of cholera also occurred in Thazi and Setkalay villages of Twantay Township, 5 miles away from Yangon City and situated on the banks of Twantay Canal. Seventeen people had to be hospitalized and all the patients interviewed gave a history of eating Ohn-no-khauk-swe, noodles served in coconut milk gravy, at a meals-offering ceremony held in a nearby village within the 3-day period before they became ill.

The Ministry of Health implemented control measures by disinfecting case households with calcium oxide, improving community sanitation and intensifying health education activities. The Government also banned the roadside food stalls when the incidence was high. This study was carried out to determine the risk factors and possible vehicles and modes of transmission of cholera in Yangon Division.

MATERIALS AND METHODS

From medical records it was observed that 647 culture-confirmed cases of Vibrio Cholerae 01 were admitted to the Infectious Diseases Hospital during January to December 1993. The cases selected for the case-control study were the patients who were admitted to the hospital during the period 12 July to 11 October. After culture-confirmation they were asked by use of a questionnaire about their consumption of food (rice and curries, vegetables, sea food, canned food, snacks and fruits), water (municipal water, shallow-well

water, tube-well water, pond water and rain water) and ice preparations (ice lollies, iced drinks, ice creams) in the three days before the onset of illness. Number of persons and income per household, occupation and standard of education of adult subjects or mothers of children subjects, daily consumption of water, poultry farming, consumption of food from street vendors, storage of water, habit of drinking boiled water, hand immersion into drinking water, filtering and filter washing practices, type of latrine, defaecation practices, hand washing practices before preparation and taking food were also investigated. For infants and children, parents were questioned. For each index case an age-matched (± 5 years) control was selected from neighbourhood who had no diarrhoea in the previous month. Controls were asked the same questions as the cases about their consumption of food and drinks during the three days before the onset of illness in the patient.

Rectal swabs were collected from the controls and plated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. After incubation at 37°C for 24 hours the presence of V. cholerae 01 was examined and controls whose rectal swabs yielded V. cholerae 01 were excluded. Samples of water used by case and control families were taken from household drinking water storage containers, transported in ice-coolers to the laboratory and tested for the presence of V. cholerae 01 by membrane filtration method [1]. Each sample was then enriched in alkaline peptone broth for 6 hours and streaked on TCBS agar. After overnight incubation at 37°C, suspicious colonies were subcultured and tested for agglutination with V. cholerae 01 polyvalent and monovalent antisera. The isolates were also biochemically indentified. Statistical analyses of 95% confidence intervals (CI) on odds ratios (OR) were used to identify risk factors for matched samples [2].

RESULTS

Out of 647 culture-confirmed cases of V. cholerae 01 admitted to the Infectious Diseases Hospital in 1993, 349 (53.9%) were males and 298 (46.1%) were females; 146 (22.6%) were children under 12 years old including 91 (14.1%) males and 55 (8.5%) females. The incidence was high in the months of April, May (hot season) and July to October (rainy season) (Fig. 1 & 2).

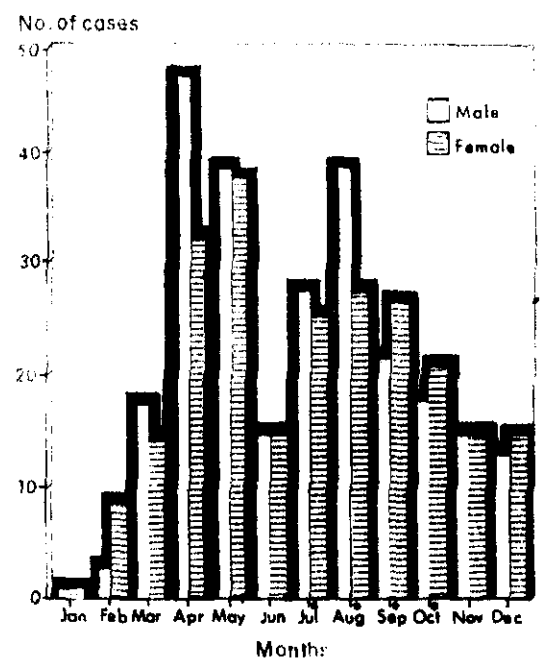

Fig. 1. Bacteriologically confirmed cholera cases (12 years and over) admitted to Infectious Diseases Hospital, Yangon, 1993
*Study period

Case-control study was conducted between July and October and 100 patients and 100 controls were inter-

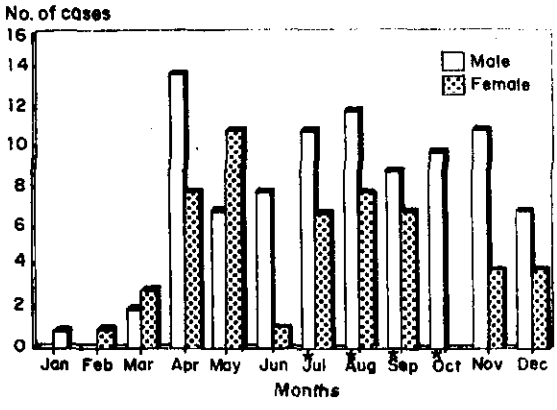

Fig. 2. Bacteriologically confirmed cholera cases (under 12 years) admitted to Infectious Diseases Hospital, Yangon, 1993
*Study period

viewed. Cases occurred sporadically throughout Yangon Division comprising 19 townships. Case patients ranged in age from 1.3 to 85 years (mean 29.7 years), 76 were adults (12 and more than 12 years) including 37 males and 39 females. Twenty four were less than 12 years of age including 15 males and 9 females.

According to the results (Table) drinking unboiled water was found to be a significant risk factor: 87% of case-patients drank unboiled water vs 61% of controls (odds ratio [OR] = 4.3, 95% confidence interval [CI] = 0.53-1.47). Consumption of food from street vendors or road-side food stalls was more common among case-patients than among controls (OR = 3.8, CI = 0.55-1.45). Hand immersion into drinking water was found to be associated with significant risk (OR = 3.7, CI = -0.15-2.15). Lack filtering drinking water and washing water filters were also found to be risk factors (OR = 1.9, CI = -0.32-2.32 and OR = 3.2, CI = 0.46-1.54 respectively). Lack of proper hand washing with soap before preparation and eating of food and after defaecation were also associated with risk (OR = 1.9, CI = -0.07-2.07; OR = 1.7, CI = -0.22-2.22 & OR = 2.8, CI = 0.34-1.66 respectively). Poultry farming (OR = 2.3, CI = -0.17-2.17) and consumption of soft drinks and ice preparations (OR = 2.1, CI = 0.23-1.77) were more common in case-patients than in controls.

Table. Risk factors for cholera among patients and controls

Risk factors	Proportion exposed		Odds Ratios
	Patients (%)	Controls (%)	
Drinking unboiled water	87	61	4.3
Street vendors	78	48	3.8
Hand immersion into drinking water	98	93	3.7
No washing of water filters	78	52	3.2
Poultry farming	13	6	2.3
Soft drinks	53	35	2.1
No filtration of drinking water	16	9	1.9
Handwashing without soap after defaecation	80	59	2.8
Handwashing without soap before preparation of food	85	75	1.9
Handwashing without soap before eating food	82	72	1.7

The occupations of adult patients were 11 bazaar sellers, 4 traders and brokers, 3 trishaw peddlers, 3 pensioners, 11 housewives and 32 in miscellaneous jobs. The majority of them had low incomes. Concerning the education standard of mothers of 24 cases of children, 13 had primary education (5 years of schooling) and

only 8 reached middle school level (5th to 8th standard). Of adult cases 23 (30.3%) reached primary level, 16 (21.1%) middle level and 17 (22.4%) high school level (9th and 10th standard). Only 2 (2.6%) had University education and 18 (23.7%) were able to read and write.

Ohn-no-khauk-swe, mohinga (rice noodles with fish gravy) and lethoke (a kind of Myanmar salad prepared with condiments, vegetables, noodles, etc.), the popular foods of Myanmar, were probably vehicles of infection during the cholera season. Thirty six percent of patients had eaten mohinga, 26% had eaten lethoke and 16% had eaten ohn-no-khauk-swe within 3 days before their illnesses. Other foods consumed by patients included fried bamboo shoots (8%), potato curry (6%), djenkol bean pickle (4%) and egg-plant curry (4%). Only 2 patients had eaten sea foods.

No significant differences were observed between cases and controls in some of the variables as type of water used, daily consumption of water, number of persons per household and toilet facilities. Majority of cases and their controls used household water from municipal pipes or tube wells. In rainy season rain water was also used. The common types of latrines were pour-flush and pit latrines. V. cholerae, El Tor Ogawa, was isolated from drinking water from the home of a patient.

DISCUSSION

Seafood has been believed to be among the important vehicles of cholera transmission [3,4,5,6] but in Myanmar most of the people eat fresh water fish only after well cooking and seafood are not popular food items. Only two patients ate sea fish and crab before they became ill.

The food consumption histories of patients from Thazi and Setkalay villages suggest that Ohn-no-khauk-swe was the most likely vehicle of transmission. It is a mixture of noodles and coconut milk gravy and one of the popular food items in Myanmar. The soup is usually boiled and V. cholerae cannot survive but the noodles, in salty condition can serve as a medium for the organisms to survive and even multiply if contaminated.

Isolation of V. cholerae from drinking water of one patient suggests that drinking contaminated water also played a role in the transmission of the disease. Most of the patients and controls used water from municipal pipes or tube wells. Although municipal water had been chlorinated, tube-well water was usually unchlorinated and household water can also become contaminated during collection, home storage and use [7]. Reduction in cholera attack rates was observed in households in which narrow-necked drinking-water containers were used and hence preventing hands to dip into the water and contaminate [8]. In this study 98% of the case-patients stored drinking water in containers in which hands had been introduced into the water. Drinking unboiled water was also more common in these people than in controls. Because of the high cost of fuel in Myanmar, it is not feasible to drink boiled water for people of low socio-economic level, hence cheaper but effective method of decontaminating household water by putting alum had been described [9].

Ice preparations and soft drinks were found to be more commonly consumed by cases than by controls. Consumption of foods or beverages from street vendors and roadside food stalls was also found to be associated with illness. Street vendors had

been shown to be carriers of pathogens [10] and were suspected sources of cholera [11]. The foods sold by them were also frequently contaminated with enteric bacterial pathogens [12]. Accordingly they were banned when the cholera incidence was high.

Contaminated hands play an important role in diarrhoeal diseases transmission, either directly or indirectly through food, water and other objects. The people in Myanmar usually use water after defaecation and hands can readily become contaminated but thorough hand washing with soap and water was found to be effective in decontaminating them [13]. In this study the habit of washing hands with soap after defaecation as well as before preparation and eating of food was more common among controls than among case-patients.

The introduction of cholera into a country is impossible to prevent but its transmission can be contained by appropriate control measures. In addition to providing safe water and good sanitation, our observations suggest the importance of consuming safe water and safe food, thorough hand washing with soap after defecating and before preparing or eating food to reduce the risk of cholera. Street vendors will need to be taught about the safe food-handling practices.

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Accepted for publication 10 Oct 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Breath methane analysis in the diagnosis of rice carbohydrate malabsorption

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Rice carbohydrate malabsorption is common in Myanmar village children and adults. The diagnosis is dependent on the use of a rice breath hydrogen test which has potential limitations. As methane production has been identified in almost 20% of Myanmar children under age 5, it was possible that an increased carbohydrate load in the colon consequent upon rice malabsorption may provide increased substrate for methanogenic bacteria in the left colon. A rise in methane production might be reflected in fasting breath methane concentration and therefore simplify the diagnosis of rice malabsorption. 79 children had a rice breath hydrogen test with measurement of hydrogen over a four hour period allied with a breath methane measurement and anthropometric measurements. There was no correlation between rice malabsorption and methane production and the measurement of breath methane does not, therefore, correlate with the rice absorption status.

INTRODUCTION

It has previously been demonstrated that rice carbohydrate malabsorption in Myanmar village children is common, being present in approximately two-thirds of the subjects, with a similarly high prevalence of rice malabsorption in adults [1]. This malabsorption is associated with significantly diminished linear growth [2,3]. The diagnosis is dependent on the use of a rice breath hydrogen test [1] which has potential difficulties in that it requires fasting, with frequent breath samples over a three hour period, allied with the problem that ten per cent of subjects have no detectable hydrogen present in the breath. In addition, false positive tests may occur in the presence of small bowel bacterial overgrowth, together with elevated breath H_2 levels in subjects not strictly fasting.

A simpler method of diagnosis of rice carbohydrate malabsorption would therefore have considerable potential. The measurement of breath methane is attractive in that it does not require fasting. Breath methane production varies in different ethnic groups [4], being common in a Black African population in contrast to a white and Indian population [5]. Overall there are methane producers in approximately fifty per cent of the adult populations.

Both Flourie et al. [6] and Pitt and her colleagues [4] have demonstrated an increase in methane production following a high starch diet and lactulose, respectively. Tomlin and his colleagues [7] have demonstrated the abolition of methane production on a fibre-free diet.

As methane is produced in the left colon by reduction of hydrogen and carbon dioxide, it seemed possible that an increased carbohydrate load,

such as that consequent upon rice malabsorption in a rice eating population, may provide increased substrate, initially for hydrogen and carbon dioxide production with a subsequent increased substrate for methanogenic bacteria in the left colon. A rise in methane production might then be reflected in breath methane concentration, and if present in children, suggest rice malabsorption.

SUBJECTS AND METHODS

Subjects and breath tests

A total of 72 children under the age of 12 years (32 boys and 47 girls) with a mean (SD) age of 4.98 (2.86) years were recruited into the study. After obtaining informed consent of the parents/guardians, the children were requested to visit the field center early morning before breakfast.

For all subjects, age, sex and a short clinical history were recorded. Those with a history of acute or chronic gastrointestinal diseases were excluded from the study. Anthropometric measurements (weight and supine length for children under 3 years, weight and standing height for those over 3 years) were also carried out. Weights were measured on beam balances for infants up to sixteen kilograms and for heavier children on bathroom scales sensitive to ±0.25 kilograms. Supine lengths were measured to the nearest 0.1 centimetre by use of an infantometer made of teak for field use.

All subjects were fed a graded dose of 1 gram carbohydrate (3 gram cooked rice) per kilogram of weight. The rice meal consisted of polished white rice cooked in 1.5 times its volume of water until all the water had been absorbed. The total carbohydrate content (including fibre) of this type of cooked rice was 34.2 gms of carbohydrate per 100 gms of cooked rice. To stimulate gastric emptying, 100 mls of diluted vegetable soup (without solid matter) was given to all subjects. Breath hydrogen samples were obtained immediately before the test meal was administered with additional samples being collected at 20 minute intervals for the first hour and then at 30 minute intervals thereafter for a total of 4 hours. The detailed methodology for breath sample collection had been described elsewhere [1]. Additional samples for determination of breath methane were taken at 3 hours and 4 hours after the test meal. End-expiratory breath samples were obtained by making use of T-valve breath sampling plastic bags and the samples were immdeidately transferred to 30 ml air-tight plastic syringes [8] and transported to the Department of Medical Research for breath hydrogen and methane measurements within 24 hours.

Analysis of hydrogen, oxygen and methane in breath samples

A calibrated gas chromatograph (model 12 Microlyzer, Quintron Instruments Co., Inc., Milwaukee, WI 53215) and a portable oxygen analyser (Oxywarn 100 I, Draeger Instrument Division, Blyth, Northumberland, Australia) were used for determinations of breath hydrogen and oxygen respectively. Detailed methodology had been reported previously [1].

Methane was measured with a Model 16 Microlyzer equipped with a molecular sieve chromatographic column (Quintron Instrument Co., Inc., Milwaukee, WI 53215). Dry air was used as the carrier gas at a flow rate of 35 ml per minute. The chromatograph was calibrated with a methane-referenced mixture in compressed air (Quingas, Quintron Instrument Co.). The smallest detectable concentration of methane was 2 microlitres per litre, with a linear

accuracy response range of 2 to 200 microlitres per litre.

Working definitions

Rice malabsorption was defined as a peak excess hydrogen > 10 parts per million (ppm) above baseline values in the 90-, 120-, 150-, 180-, 210-, or 240- minute samples after a rice test meal [1]. Peak excess was calculated as the difference between the highest normalized breath-hydrogen value in any one of the breath samples collected 90-180 minutes after a rice meal, and the lowest normalized breath-hydrogen value in any one of the breath samples collected at 0, 20, 40, or 60 minute. Any individual who excrete at least 2 parts per million (ppm) of methane was considered to be methane producer.

Assessment of nutrition status

Three types of indices were used. First, standard deviation scores (SD scores) were calculated for weight-for-age, length-for-age and weight-for-length of each child, by subtracting the median value of the National Centre for Health Statistics (NCHS) reference from the child's value, then dividing by the SD value of the NCHS reference [9].

For the definition of malnutrition, the cut-off point used was weight-for-age or length-for-age < -3 SD of the corresponding NCHS median or weight-for-length < -2 SD of the NCHS median [10]. The Ehrenberg Index of weight-for-length [11] and the Dugdale index of weight-for-length [12] were also calculated.

Statistical analysis

Data analysis was performed with CRISP (Crunch Interactive Statistical Package Ver 3.05; Crunch Software Corporation, San Francisco, CA 94116, U.S.A.) on an IBM PC XT computer. Student's 't' test was used for comparison of nutritional status indices and methane excretion concentrations among rice absorbers and rice malabsorbers, and methane producers and non methane producers. Chi-square test was used for comparing proportions. Where frequencies in various cells of a contingency table were small, Fisher's exact test was used instead. Differences were considered to be significant if $p<0.05$.

Ethical considerations

The study was approved by the Local Ward Law and Order Restoration Committee and the Medical Ethics Committee of the Department of Medical Research, Yangon, Myanmar.

RESULTS

Rice absorption status

Of the 79 children (32 males and 47 females), 70% were categorised as rice malabsorbers and 30% were rice absorbers. Age related prevalence of rice malabsorption ranges from 100% under one year of age to 56% in 3-4 year age group (Table 1). Gender did not influence rice malabsorption, 78.1% of the boys and 66% of the girls were rice malabsorbers. Rice absorption status also did not correlate with methane production. Of methane producers, 69% were rice malabsorbers, but 70% of the non methane producers were also found to be rice malabsorbers. In addition, no significant difference in breath methane concentration was observed among rice absorbers and rice malabsorbers (Table 2).

Nutritional status

Although rice malabsorbers were lighter and shorter than rice absorbers, statistical significant difference was noted only for height ($t = 2.136$, $df = 77$, $p<0.05$) (Table 3). There was no significant difference in weight-for

Table 1. Age specific prevalence of rice absorbers and rice malabsorbers amongst Myanmar children

Age (months)	No. of children	Rice absorber N (%)	Rice malabsorber N (%)
0-11	2	0 (0%)	2 (100%)
12-23	6	1 (17%)	5 (83%)
24-35	11	1 (9%)	10 (91%)
36-47	9	4 (44%)	5 (56%)
48-59	14	2 (14%)	12 (86%)
> 60	37	15 (40%)	22 (60%)
Total	79	24 (30%)	55 (60%)

Table 2. Rice absorption and methane production status

Characteristics	Rice absorber N (%)	Rice malabsorber N (%)
Total (N=79)	23 (29.1%)	56 (70.9%)
Sex*		
Males (N=32; 40.5%)	7 (21.9%)	25 (78.1%)
Females (N=47; 59.5%)	16 (34.0%)	31 (66.0%)
Methane production status**		
Yes (N=29; 36.7%)	9 (31.0%)	20 (69.0%)
No (N=50; 63.3%)	14 (28.0%)	36 (72.0%)
Mean ± SD methane excretion concentration in ppm***	1.56±3.37	0.79±0.97

*Not significant between males and females using chi-square test
**Not significant between methane production status using chi-square test
***Not significant between rice absorbers and rice malabsorbers using t-test

age, length-for-age or weight-for-length between rice malabsorbers and rice absorbers. In Table 4 the nutritional indices of methane producers and non methane producers are compared. There was no statistically significant difference in degree of malnutrition between methane producers and non-producers.

Table 3. Nutritional status of 79 children in relation to rice absorption status (values are mean ± S.D.)

Characteristic	Rice absorber N=23	Rice malabsorber N=56
Age in years*	4.66± 2.90	5.74± 2.66
Weight in kg*	15.78± 5.83	13.79± 4.12
Length in cm**	105.71±17.71	97.12±15.56
Weight for age Z scores*	-1.79± 1.23	-1.83± 0.94
Length for age Z scores*	-1.35± 1.64	-1.50± 1.53
Length for weight Z scores*	-0.93± 2.55	-0.71± 2.29

*Not significant between rice absorbers and rice malabsorbers using Student's 't' test
**Significant difference ($p < 0.05$) between rice absorbers and rice malabsorbers using Student's 't' test

Table 4. Nutritional status of children in relation to methane production status (values are mean ± S.D.)

Characteristic	Methane producer N=29	Methane non-producer N=50
Age in years*	5.45± 2.69	4.70± 2.94
Weight in kg*	15.24± 4.56	13.86± 4.81
Length in cm*	102.52±15.69	97.94±16.98
Weight for age Z scores*	-1.56± 1.45	-1.39± 1.62
Length for age Z scores*	-1.73± 1.13	-1.87± 0.96
Length for weight Z scores*	-0.65± 2.3	-0.84± 2.41

*Differences not significant between methane producers and methane non-producers using Student's 't' test

Distribution of nutritional status in relation to breath methane production was studied (Table 5). With regard to weight-for-age SD scores, 10.4%

Table 5. Distribution of nutrition status in relation to breath methane production

Nutrition status using SD scores	Methane producer N=29	Methane non-producer N=50
	N (%)	N (%)
Weight for age		
< -3 SD	3 (10.4%)	3 (6%)
< -2 SD	9 (31%)	19 (38%)
< -1 SD	9 (31%)	22 (44%)
At median	5 (17.2%)	4 (8%)
+1 SD	3 (10.3%)	1 (2%)
+2 SD	0 (0%)	1 (2%)
Length for age		
< -3 SD	4 (13.9%)	4 (8%)
< -2 SD	7 (24.1%)	15 (30%)
< -1 SD	8 (27.6%)	14 (28%)
At median	7 (24.1%)	10 (20%)
+1 SD	2 (6.9%)	4 (8%)
+2 SD	1 (3.4%)	3 (6%)
Weight for length		
< -2 SD	5 (17.2%)	11 (22%)
< -1 SD	8 (27.6%)	20 (40%)
At median	8 (27.6%)	13 (26%)
+1 SD	6 (20.6%)	4 (8%)
+2 SD	2 (7%)	2 (4%)

of methane producers in comparison to 6% of methane non-producers were malnourished. With length-for-age SD scores 13.9% of methane producers and 8% of non-producers were malnourished. For weight-for-height SD scores 17.2% of methane producers and 22% of methane non-producers were malnourished.

The Ehrenberg Index of weight-for length for methane producers and non-producers were 0.37 and 0.36 respectively. The Dugdale Index of weight-for-length for methane producers (92.4) and non-methane producers (90.5) were also similar.

DISCUSSION

The measurement of breath methane does not correlate with rice absorption status and, therefore, does not provide an alternative, simpler field test for the diagnosis of rice malabsorption. The study therefore, does not support the hypothesis of an increased carbohydrate load in the right colon providing an increased substrate for hydrogen and subsequent methane production.

In contrast to other population studies on methane production, it demonstrates a much greater incidence of methane production in children from a developing country than it has previously been disclosed in other studies from Africa [5] and Hong Kong [11] where the finding of methane production in children under the age of five years is unusual.

While there is some impact of rice malabsorption demonstrated in reduced mean weight and mean length, there is no significant difference between rice absorbers and malabsorbers, whether or not they are methane producers, in terms of more accurate nutritional markers of length-for-age, weight-for-age and weight-for-length. To overcome racial differences in weight and height between Myanmar children and the NCHS reference American children, the indices of Ehrenberg and Dugdale, which are independent of age [13] and race [14,15] were used to evaluate the nutrition status of these children. While the indices for the Myanmar children are lower than those described for the developed countries, there were no significant differences between the children with or without rice carbohydrate malabsorption.

The concept that increased methane production might reflect carbohydrate malabsorption is not supported by this study. The finding remains unexplained in that no association has been found with occupation, education, income, water source,

laterine type, diarrhoea, antibiotic usage or socioeconomic status [16].

ACKNOWLEDGEMENT

We thank Professor May May Yi and Professor Myint Lwin for their enthusiasm and encouragements; Dr. Than Swe and Dr. Tin Nu Swe for organizing logistics support; the staff of Clinical Research Division, Department of Medical Research, Ministry of Health, Union of Myanmar for their enthusiatic participation in both field and laboratory work.

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Accepted for publication 14 Nov 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Knowledge, perception and AIDS related behaviours among currently married couples in Yangon

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A community based study was conducted in 1991 in Dagon and Thakayta Townships to assess the existing level of knowledge and perception of AIDS among 594 currently married couples and to find out the type of educational needs on AIDS for married couples. Only a few study subjects believed that AIDS was a curable disease and the majority did not know some important preventive measures. Many of the studied couples could not identify those who could get AIDS within the society and the high risk group among community members. Knowledge of the source of AIDS transmission and on prevention of AIDS were also found to be low among them. Condom utilization by male having sex with spouse was also found to be low. Health education of AIDS dissemination through leaflets and health talks was found to be insufficient.

INTRODUCTION

AIDS, an incurable but preventable disease, was first reported in Myanmar in 1988 [1]. Up to the end of December 1993, 189 AIDS cases were reported from Yangon, Mandalay, Taninthayi Divisions and Shan State. It showed that male-female ratio was 8:1 and 96.8% of the cases were 20-49 year age group. These figures implied that the reproductive age group of women and the similar age group of men were the majority who had exposed to AIDS/HIV [2].

From these statistics, we have hypothesized that, married couples in these population are lack of knowledge on AIDS, and are unaware of risk behaviour relating to AIDS. Till now, studies on knowledge and perception on AIDS among married couples were not found. This study attempts to find out the gap between the present situation and the desired level which will be useful for remedy of the suitable health education programme for AIDS prevention and control. The study aims to assess the existing level of knowledge and perception of AIDS among currently married couples and to find out the type of educational needs on AIDS for married couples.

MATERIALS AND METHODS

Study subjects

A community-based cross-sectional study design was used. The study

was conducted in Dagon and Thakayta Townships in the City of Yangon. The study sample consists of 594 couples, 254 couples from Dagon Township and 340 couples from Thakayta Township, and the response rate was 79.2%.

Sampling

Multistage sampling was carried out to choose one township each randomly from downtown and peri-urban. This procedure was done after excluding port township, cantonment area, and new settlement area. If there were more than a couple in one household, only one couple was chosen randomly. The study was carried out during 1991.

Data collection

The structured questionnaire was used after pretesting and refinement. A couple was interviewed separately at the same time by the trained interviewers; female interviewer for wife and male interviewer for husband.

RESULTS

Respondents

Among the couples, 26-40 year age group was found to be the highest at 55.2%. Most (53%) of the couples had passed primary school level, followed by middle school level (19.5%) and graduate (10%). Among the husbands, 36.4% were skilled, 34.3% were unskilled labourers and 13.8% professionals while 65% of wives were housewives, 19.4% unskilled and 6.7% were skilled labourers.

Knowledge on AIDS

When the couples were asked about the mode of transmission, about 50% of the couples knew that it can be transmitted from mother to fetus and 30% from prostitutes (Table 1).

Table 1. Knowledge on mode of transmission

Modes of transmission	Husband n=594	Wife n=594	Total n=1188
Mother to fetus	50.9	55.4	52.9
From prostitutes	33.2	26.9	30.1
Homosexual relationship	33.1	13.1	23.3
Unsafe sex	10.9	7.2	9.1
Vaginal fluid	2.2	2.5	2.4

Among those who knew the mode of transmission, only 12 males and 10 females believed AIDS was a curable disease.

Out of 550 couples, who had knowledge on sources of AIDS transmission, 37.6% of them identified the brothels as the major place of transmission, followed by hospitals (20.5%), private clinics (6%) and dispensaries (0.7%).

Five hundred and one (42.2%) wives or husbands of the total respondents could identify at least one preventive measure. Among them, only a few knew about use of sterilized needle (3.4%), use of condom in sexual relationship (2.4%) and stopping use of heroin intravenously (2.4%) (Table 2).

The majority (47.5%) of husbands believed that intravenous drug users (IVDU) could get AIDS, followed by female prostitutes (28.7%) and gays (19.8%). The reverse was found among the wives: most (47%) of them stated that AIDS could get from female prostitutes, followed by IVDU (38.9%), and gays (8.9%). Among those who were able to identify the risk group, 68.3% of the couples identified the female prostitutes as the most risky group, and IVDU as the second group (23%) (Table 3).

Perception

Among the total respondents (both

Table 2. Knowledge on each preventive measure among wives or husbands who knew the measures

Preventive measures	Respondents (n=501)
Avoid sexual contact with female prostitutes	63.9
Faithful to spouse	26.5
Use sterilised needles	3.4
Stop use drug intravenously	2.4
Use condom while having sex	2.4
Avoid homosex	1.4
	100.0

Table 3. Knowledge on high risk groups for AIDS

High risk groups	Husband n=256	Wife n=249	Total n=505
Female prostitutes	60.9	75.9	68.3
Intravenous drug users	26.6	19.3	23.0
Bi-sexuals	7.5	2.8	5.1
Multiple blood/blood product recipients	2.3	1.6	2.0
Frequent travellers	2.7	0.4	1.6

wives and husbands), 100 (8.4%), 77 (6.5%) and 31 (2.6%) thought that hospitals, private clinics and dispensaries could be sources of AIDS transmission respectively.

Over 60% of the couples perceived that AIDS could not be transmitted by avoiding extra marital sex, 13% perceived that AIDS is a curable disease and 6.1% stated that AIDS patients should not eat and sit together with others (Table 4).

Condom utilization

Regarding utilization of condom, only 18 (3.03%) out of 594 husbands said they had ever used it. Almost all of them (11 out of 18 husbands, ie., 61.1%) had experience in using condoms when having sex with spouse.

Table 4. Perception on AIDS

Perception	Husband n=594	Wife n=594	Total n=1188
Could not transmit if faithful to spouse	66.5	69.2	67.8
AIDS is curable	15.3	10.6	13.0
Could get through anal sex	7.2	3.7	6.3
Should not sit and eat together with AIDS patients	6.1	6.1	6.1

Media for AIDS

Most husbands stated newspapers as the major source of media for AIDS and the wives stated the AIDS posters. Newspapers were identified by wives or husbands as the main source of information on AIDS transmission (38.3%) and on factors that caused AIDS (41.3%). The other sources for the above two groups were identified as posters (21.2%, 19%), Radio (18.5%, 15.9%), TV (10.2%, 11.3%) and health talks (0.5%, 0.2%) respectively (Table 5).

Table 5. Source of information on AIDS knowledge known by wives or husbands

Source of information	AIDS transmission (n=519)	Factors that caused AIDS (n=426)
Newspapers	38.3	41.3
AIDS posters	21.2	19.0
Radio	18.5	15.9
TV	10.2	11.3
Periodicals	8.1	9.2
Leaflets	3.2	3.1
Health talks	0.5	0.2

DISCUSSION

Although health messages on AIDS were disseminated throughout the country by various methods, it was found that in the study areas of Yangon, only a few study subjects believed that AIDS was a curable disease. This is also found in eastern Shan State [3]. The majority did not know it can be prevented by using condom while having sex, using sterilized needles and not using heroin through intravenous route. Many of the studied couples could not identify those who were likely to get AIDS within the society and the high risk group among community members. Knowledge on source of AIDS transmission and prevention of AIDS were also found to be low among them.

In view of their perception towards AIDS, the majority of the female respondents revealed that they would not have such a disease if there were no extra-marital sex. It may be influenced by cultural factors, which made housewives or married women more confided in their husbands.

Condom utilization at extra-marital sex by male was also found to be low. Moreover, experience of using condom while having sex with spouse was found to be much lower. The similar findings were also observed in Eastern Stan State [3].

Disseminating of health messages of AIDS through leaflets and health talks was likely to be insufficient.

It would seem that the present system and approaches of disseminating AIDS knowledge and the use of various media channels for AIDS did not seem to reach the target population.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the Director-General of the Department of Medical Research for the opportunity to conduct this study as well as for funding it. We also thank Township Medical Officers and their staff of Dagon and Thakayta Townships and the responsible personnel from the Ward Law and Order Restoration Council for making necessary arrangements in their respective areas for undertaking this study. Lastly, we are much obliged to all the respondents of the townships for giving consent to be interviewed in the study.

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Accepted for publication 27 Oct 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Morbidity and mortality pattern of hospitalized elderly patients in YGH (1985-1987)

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A record analysis of all elderly patients admitted to YGH during 1985 to 1987 was done to determine the morbidity pattern of hospitalized elderly patients, aged 55 years and above. There was an average admission rate of 7000 patients per year constituting approximately 18% of all admissions. The mean duration of stay in hospital for each hospitalized person was approximately 19 to 21 days. The most common morbidity condition for admission to hospital according to International Classification of Diseases (ICD) grouping is neoplasms in 18.9% of admissions, followed by diseases of the circulatory system in 18.7% and injuries or poisonings in 16.9%, digestive system diseases 12.8%, and other ill defined causes led to be 21.8%. The case fatality for hospitalized elderly is 12.8% for the three years. Implications of the study in planning for geriatric services are discussed.

INTRODUCTION

In Myanmar, health care for the elderly has been recognized as one of the priority health care programmes in its National Health Plan 1991-92. Although programmes for the elderly are becoming urgent, available information on health issues specific to older people are scanty. Thus it is an attempt to identify some problems of hospitalized elderly in YGH.

MATERIALS AND METHODS

A total of 21,020 medical records of elderly patients admitted to YGH (1985-1987) were collected from Medical Record Session. Relevant information were retrieved from the medical records to determine the morbidity and mortality pattern of hospitalized elderly patients, aged 55 years and above. WHO International Classification of Diseases (ICD), 1975 revision, vol. 2, was used for disease classification and grouping. Data analysis was undertaken by the use of computer software.

RESULTS

Demographic characteristics

Among the hospitalized patients during 3 years, the elderly patients constituted approximately 18% of all hospital admissions. A high percentage (51.5%) was found in the age group of 55-64 years. The percentage of hospitalized elderly of all admissions for both sexes of all age groups in each year were 16.74%, 18.24% and 18.09% respectively. Male hospitalized elderly were found to be approximately 1.4 times more than that of female patients in each year (Table 1).

Table 1. Percent distribution of yearly hospitalized elderly by age and sex

Age groups		1985		1986		1987		Total %
		Male	Female	Male	Female	Male	Female	
55-64		52.2	51.7	50.4	54.3	49.7	52.1	51.5
65-74		33.9	33.8	34.0	30.8	33.9	33.0	33.4
75+		13.9	14.5	15.6	14.9	16.4	15.0	15.1
Total	%	16.74		18.24		18.09		
	n	6492		7191		7337		

% of hospitalized elderly = $\frac{21020}{118746}$ x 100 = 17.7%

Male : Female = 12355 : 8665 = 1.43 : 1

Table 2. Distribution of hospitalized elderly patients by ward and sex (1985-1987)

Ward	Male		Female		Total	
	No.	%	No.	%	No.	%
Medical	4051	55.2	3292	44.8	7343	36.6
Surgical	2735	60.6	1776	39.4	4511	22.1
Trauma	1597	53.4	1393	46.6	2990	14.7
Uro	1727	82.2	375	17.8	2102	10.3
Cancer	648	45.6	772	54.4	1420	6.9
Cardiac	714	59.2	492	40.8	1206	5.9
Neuro	467	56.1	366	43.9	833	4.1
Sub-total	11939	58.5	8466	41.5	20405	100.0
Unknown and not recorded					615	
Total					21020	

Regarding admission to different wards in all 3 years, most of the diseased elderly (36.6%) were admitted to medical ward, followed by surgical, trauma, urology, cancer, cardiac and finally neuro ward. Female elderly admitted to urology ward was found one-fifth of that of male hospitalized elderly. On the other hand, female elderly hospitalization to cancer ward was 1.2 times more than that of male elders (Table 2).

Morbidity pattern

ICD code was used to reveal the prevalent diseases of elderly patients in hospital admission. Grouping of ICD was made so as to identify most leading occurrent diseases in hospital admission. With regard to the ICD group, the highest percentages of hospitalized elderly for neoplasms, circulatory system diseases, digestive system diseases and injury and poisoning were found at the age group of 55-64 years and for genitourinary system diseases, it was found at the age group of 65-74 years. The lowest percentages for all these ICD groups were found at 75 years and above (Table 3). It was noted that, on the whole, the mean duration for hospital admission was 21 days.

Among the various diseases of admit-

Table 3. Percent distribution of hospitalized elderly by ICD group and age group (1985-1987)

ICD group & diseases		Age groups 55-64	65-74	75 +	Total
2 - Neoplasms		56.3	33.5	10.2	100
7 - Circulatory system diseases		48.6	35.4	16.0	100
9 - Digestive system diseases		56.01	32.3	11.7	100
10 - Genitourinary system diseases		6.8	40.1	23.1	100
17 - Injury and poisoning		52.3	28.9	18.8	100
Others		54.0	32.3	13.7	100
Total	%	51.5	33.4	15.1	17.7
	n	10832	7014	3174	21020

ted elderly patients of both sexes, neoplasms, circulatory system diseases, injury and poisoning, digestive system diseases and genitourinary system diseases were found to be the leading causes of morbidity. Seventy eight percent of all hospitalized elderly in 3 years were found in these causes (Table 4).

Table 4. Leading causes of morbidity among hospitalized elderly for both sexes (1985-1987)

ICD group & diseases	Total hospitalized elderly	%
2 - Neoplasms	3968	18.9
7 - Circulatory system diseases	3925	18.7
17 - Injury and poisoning	3544	16.9
9 - Digestive system diseases	2687	12.8
10 - Genitourinary system diseases	2285	10.9
Others	4611	21.8
Total	21020	100.0

Mortality pattern

The case fatality rate for hospitalized elderly was 12.8% for the 3 year period. Out of the total hospitalized elderly deaths, deaths due to infectious and parasitic diseases, neoplasms, circulatory system diseases, digestive system diseases and injury and poisoning were found highest in 55-64 year age group and the lowest in 75 years and above (Table 5).

The most prevalent diseases contributing to deaths in YGH for the 3 year period were circulatory system diseases, neoplasms, digestive system diseases, infectious and parasitic diseases, and injury and poisoning (ie., ICD groups 7, 2, 9, 1 and 17). These leading causes accounted for 80% of all total deaths during the 3 years in YGH (Table 6).

DISCUSSION

Compared with the hospitalized elderly aged 65 and over from acute hospitals in the United States of America, diseases of the circulatory system (29.3%), diseases of the digestive system (12.7%), neoplasms (11%) and diseases of the respiratory system (9.9%) were the leading causes of morbidity [1].

Compared with other countries, morbidity pattern in UK, Netherlands, Switzerland and France were similar to ours [1]. Compared with community study conducted in Myanmar, arthritis was the most frequent reported disease (35.9%), followed by lung diseases (24.7%), high blood

Table 5. Percent distribution of hospitalized elderly deaths by ICD group and age group (1985-1987)

ICD group & diseases		Age groups 55-64	65-74	75 +	Total
1 - Infectious and parasitic diseases		54.3	32.8	12.9	100
2 - Neoplasms		58.8	31.2	10.0	100
7 - Circulatory system diseases		40.9	38.5	20.5	100
9 - Digestive system diseases		50.0	33.9	16.1	100
17 - Injury and poisoning		40.5	33.5	26.0	100
Others		39.6	38.7	21.6	100
Total	%	46.7	35.6	17.7	12.8
	n	1254	957	476	2687

Table 6. Leading causes of death among hospitalized elderly for both sexes (1985-1987)

ICD group & diseases	Total deaths	%
7 - Circulatory system diseases	833	31.0
2 - Neoplasms	532	19.8
9 - Digestive system diseases	310	11.5
1 - Infectious and parasitic diseases	287	10.7
17 - Injury and poisoning	85	6.9
Others	540	20.1
Total	2687	100.0

pressure (19.2%) and heart conditions (8.9%) [2]. It is evident that, there are different morbid patterns in hospital and community. This difference may be evident as most of the hospitalized elderly patients were chronic and severe cases, while in community survey, the pattern was mainly obtained from self reporting.

Since this study was conducted at YGH only, it may explain the low percentage of female hospatilized elderly to uro ward. According to estimated number of deaths by WHO for South East Asia region in 1980, infectious and parasitic diseases including certain respiratory diseases was the first leading cause of deaths, followed by circulatory system diseases and neoplasms [3]. It is quite different from our hospital data. In our study, the cases of infectious and parasitic diseases seemed to be very few, as most of these patients were usually admitted to the Infectious Diseases Hospital (IDH).

On the whole, in Myanmar, the leading causes of morbidity and mortality were similar for the study period, except in rank position.

The elderly are an integral part of society and are entitled to their fair share of health and social services that are provided but there is still lack of special health care services to their needs in many countries. In addition to that, with the higher burdens of morbidity and disability in older aged group, there will be increased demands on the health care system. So it is the right time to tackle the health problems of elderly to promote their health status.

Conclusion

1. The elderly hospitalized patients contribute some proportion of hospital admission.
2. There should be Primary Health care approach for early diagnosis and prevention of commonly prevalent diseases in elderly

population.

3. Special care unit for elderly patients should be considered in specialist hospitals.

ACKNOWLEDGEMENT

We thank the Medical Superintendent and the Medical Record Officer, YGH for their kind co-operation in data collection. The staff from Epidemiology Research Division, DMR are much appreciated for their help in data analysis.

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Accepted for publication 12 Dec 1995

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

The value of different special stains in the identification and semiquantitation of Helicobacter pylori: a histopathological study

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The recent identification of Helicobacter pylori overlying the gastric mucosa is strongly associated with active chronic gastritis which is not responsive to treatment with antacid alone. The routine Haematoxylin and Eosin stain together with Modified Giemsa stain, Carbol fuchsin stain and Gram stain were used for identification of these organisms. Each of them has their different value in supporting the diagnosis. In this study, a total of fifty cases were collected during May to September 1993. Endoscopic biopsy specimens were taken from these patients from the gastric antrum, fundal and duodenal regions. They were patients attending the outdoor clinic of New Yangon General Hospital, presenting with dyspepsia. Modified Giemsa stain and Carbol fuchsin stain are superior to Gram stain in the sense that a clear and distinctive spiral morphology is identified. Moreover, they are less expensive, easily available and the techniques are uncomplicated and rapid to perform compared to that of the Warthin - Starry silver stain mentioned by others. However, Gram stain is useful for exclusion of other Gram positive and negative organisms and H & E stain for correlation of the severity of gastritis with bacterial colonization. A semiquantitation of bacterial colonization can also be done at the same time with identification. All of these staining methods can be applied in routine hospital laboratories without difficulty.

INTRODUCTION

Recently, spiral or curved bacilli are commonly found colonizing the surface epithelium and superficial glands of the gastric mucosa. They are found mostly in patients presenting with gastritis or dyspepsia. They are thought to have important association with gastrititis and peptic ulcer. There are different diagnostic methods that are widely used for the detection of these bacilli. In this study, we tried to establish the best method for bacterial identification which will be applicable in our routine hospital laboratories and to find out the simplest method for semiquantitation.

PATIENTS AND METHODS

A total of fifty cases were collected during May to September 1993. They were patients attending the outdoor clinic of New Yangon General Hospital. They were cases presenting with gastritis and dyspepsia. Endoscopic biopsy specimens were taken from gastric antrum, fundal and duodenum regions. Specimens were fixed in 10% buffered formalin in separate bottles, transported to the Pathology Research Division, Department of Medical Research, sectioned at 3-4 um thickness and stained with routine Haematoxylin and Eosin as well as Carbol fuchsin, Modified

Giemsa and Gram Stain.

H & E stain - A conventional method of H & E staining was done.

Carbol fuchsin stain

This was done according to the method described by Rocha et al [1]. Dewaxed tissue were taken to water and stained for five minutes in carbol fuchsin solution. After rinsing in tap water the sections were briefly decolorised with acetone. Although the author has mentioned that it need not be mounted, we prefer mounting for long term preservation. The slides were examined under oil immersion lens.

Modified Giemsa stain

This was done according to Gray et al. [2]. Dewaxed sections were taken to water and then incubated in 2% Giemsa solution in distilled water for 30 minutes at room temperature. After rinsing in tap water the sections were quickly dehydrated through ethanol solutions before being cleared with xylene and mounted in DPX.

Gram stain

This was done according to MacCallum [3]. Dewaxed sections were taken to water and then treated with Good Pasture's solution for 10 minutes. It was then rinsed in distilled water, fixed in formalin for a few minutes, washed again, treated with picric acid for 5 minutes, rinsed again in distilled water, differentiated in 95% alcohol for 30 sec, rinsed in distilled water and treated with Stirling's gentian violet solution for 3 min. It was again rinsed in distilled water, treated with Gram's iodine solution for 1 min, rinsed in distilled water, differentiated in a solution of equal parts of aniline and xylene (several changes), until sections appeared light purplish red. It was then mounted with DPX.

Semiquantitative estimation of H. pylori

This was done under the light microscope as described by Karttunen et al [4]. Bacterial colonization was counted and scoring was done according to Appendix (1) [5].

Grading of gastritis

This was done according to Appendix (2) [5].

RESULTS

The results are as shown in the table.

Table. Distribution of H. pylori and severity of inflammatory reaction

	No. of cases detected		
H. pylori +ive bacterial count grades	Antrum	Fundal	Duodenum
0	16	24	45
1	19	16	0
2	8	4	0
3	5	0	1

Total number of cases = 50

	No. of cases detected		
Severity of duodenitis/ gastritis grades	Antrum	Fundal	Duodenum
0	2	1	0
0.5	12	14	9
1.0	16	22	25
1.5	16	5	12
2.0	2	0	0
3.0	0	0	0
4.0	0	0	0

H & E staining

With H & E staining, different degrees of gastritis can be graded. Majority of the cases were within chronic superficial gastritis of different degrees.

Carbol fuchsin staining

With carbol fuchsin, positive sections showed spiral bacteria as dark red bodies on gastric mucosa anainst a reddish background.

Modified Giemsa staining

With this technique, purplish spiral bacilli are clearly identified on the surface epithelium as well as in the gastric pit and lumen (Figure 1 to 4).

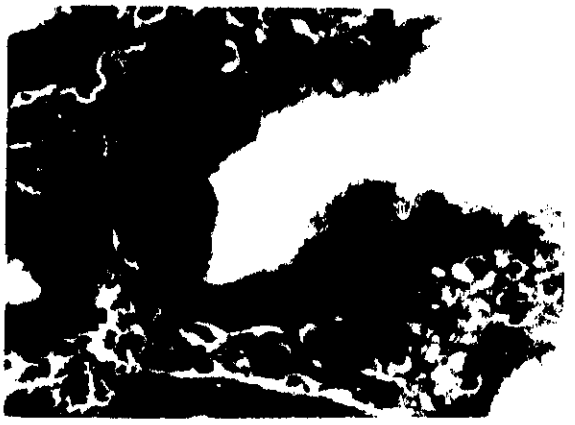

Fig. 1. Photomicrograph of intense mononuclear cells infiltration in the lamina propria of gastric mucosa with H. pylori infection (H & E stain 100X)

Gram staining

Gram stain is not superior to the above two but is useful for exclusion of other Gram positive and negative organisms.

DISCUSSION

With routine use of H & E stain, H. pylori can be visualized in histological

Fig. 2. Photomicrograph of dense H. pylori colonization on the surface epithelium and superficial gastric mucosal glands (Carbol fuchsin stain 100X)

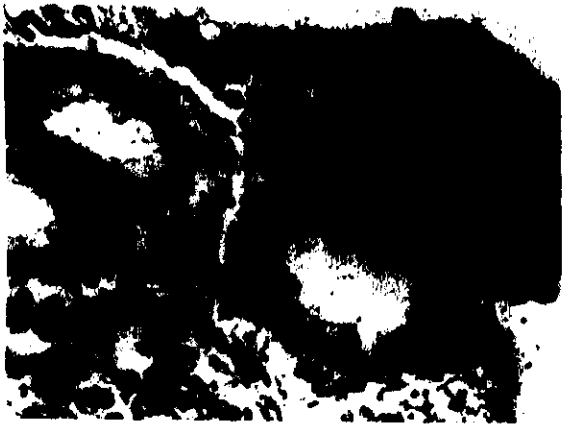

Fig. 3. Photomicrograph showing dense H. pylori colonization on the surface epithelium and superficial gastric mucosal glands (Modified Giemsa stain 100X)

sections by an experienced microscopist and the type and severity of gastritis can be determined at the same time [6]. However, it is necessary to confirm the spiral bacilli, and so far, special staining is the only way to diagnose these bacteria in tissue sections. In the very first report of H. pylori, Warthin-Starry silver stain was used [7]. This staining is difficult and time consuming to perform. Among different variety of staining procedures, like acridine

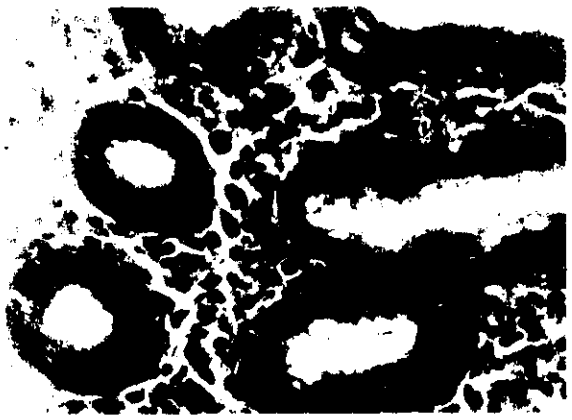

Fig. 4. Photomicrograph of moderate H. pylori colonization in the superficial gastric glands (Gram Stain 100X)

orange [8], Modified Giemsa [2], Gimenez [9], cresyl fast violet [10], and half-Gram [11], we prefer the more rapid and simple staining methods as they are easy to perform, uncomplicated and the reagents are readily available and inexpensive. We have found out a good result with all of the methods applied. We prefer carbol fuchsin and Modified Giemsa stain because of its superiority in that a clear and distinct bacterial morphology is obtained and the semi-quantitation of bacterial colonization can be done without difficulty. However, Gram stain is still a useful method for exclusion of other bacterial colonization.

Occasionally, Gram negative tightly spiral organisms larger than helicobacters can be seen in stained samples [12]. For these bacteria the name Gastrospirillum hominis has been proposed. They are much rarer than H. pylori, occurring about once for every 1000 H. pylori [12-15].

Although a strong correlation between H. pylori and active chronic gastritis is clear and accepted, the exact mechanism or pathogenesis is far from known. Cell damage can be caused by cytotoxin leading to vacuolization [16] and depletion of microvilli [17]. It will be of great benefit if further techniques like immunostaining with monoclonal [8,9] or polyclonal antibodies [20], electron microscopy [21] and DNA hybridization (DISH) [22] can be applied. Although these are more laborious than routine staining techniques, they may be more sensitive as they detect bacteria more easily even when sections are not counterstained or containing bacteria in low numbers and when bacteria which do not hybridize are equivocally identified as H. pylori.

In conclusion, based on this small research work we can roughly estimate the incidence and prevalence of H. pylori in our country and establish the applicable staining procedures for identification of H. pylori in tissue sections.

ACKNOWLEDGEMENT

We express our sincere thanks to Director-General Dr. Myint Lwin, Deputy Director-General Dr. Than Swe, Director Dr. Myint Lwin, all the staff of Pathology, Bacteriology and Clinical Research Division, DMR and the staff of NYGH who participated in this research work.

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Accepted for publication 8 Jan 1996

Appendix 1. Semiquantitative estimation of *H. pylori* by light microscopy

Grade 0	Absent	
Grade 1	Focal bacterial colonization in small amount	< 20 microbes/HPF
Grade 2	Moderate amount either focally or diffusely	20-60 microbes/HPF
Grade 3	Large amount/marked colonization diffusely at the surface of gastric epithelium	> 60 microbes/HPF

4-6 fields were examined
If HP was patchy, the grade of HP colonization was classified according to the most pronounce grade of colonization.

Appendix 2. Grading of gastritis

Grade 0	Normal	No inflammation, no loss of glands
Grade 0.5 Grade 1.0 Grade 1.5	Mild Moderate Severe	Superficially gastritis, chronic inflammation in lamina propria without loss of normal glands without atrophy.
Grade 2.0 Grade 2.5 Grade 3.0	Mild	Atrophic gastritis, chronic gastritis in lamina propria with coexisting loss of normal glands

Antagonism of enterotoxigenic Escherichia coli heat-labile toxin by quinacrine in rats

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Escherichia coli strain W 165-1/82 which produce heat-labile toxin (LT) was used in preparation of enterotoxin. Ligated intestinal rat model using Wistar rats were used to obtain the maximum secretory response after challenging with the toxin. It was found that quinacrine inhibited the secretory response and also reduced the cyclic adenosine monophosphate (cAMP) level in the intestinal fluids. The maximum secretory response was obtained with 125 ug of crude LT toxin in 61-80 grams of rats. It was also noted that 500 ug of quinacrine produced a maximum inhibition in rats.

INTRODUCTION

The mechanism of watery diarrhoea has been extensively studied and it has been made clear that LT activates adenylate cyclase of the enterocyte plasma membrane and this results in fluid secretion into the intestinal lumen which causes diarrhoea. The treatment of diarrhoea has been greatly improved by the oral administration of the mixture of fluid electrolyte However this is only a supplement of fluid and salt and the patients are still debilitated and unable to carry on normal activities during the episode. Inhibitors for adenylate cyclase and for prostaglandins have also been examined for use in treatment, but these drugs are not in practical use as yet.

Jacoby and Marshall [1] reported that some anti-inflammatory compounds inhibited cholera toxin-induced accumulation of fluid in the rat intestine. Dreyfus [2] also reported that quinacrine significantly reduced intracellular levels of cyclic AMP in isolated cells treated with STa and suggested that activation of archidonic acid is involved in the mechanism of action of STa. Thus, it would be interesting to know the effect of quinacrine and to determine whether quinacrine is effective on the LT-induced fluid accumulation in rat intestine.

MATERIALS AND METHODS

Experimental animal

Three-month-old Wistar rats (Wistar Institute, England) bred at the Department of Medical Research were used throughout the study. Three groups of rats were catagorised according to their weight which were 41-60 g, 61-80 g and 81-100 g respectively. For experimental purposes each group of rats comprised 5 to 20 in number for control and for drug testing.

Drug

Quinacrine hydrochloride (mepacrine), N^4-(6-chloro-2-methoxy-9-acridinyl), N^1, N^1-diethyl-1, 4-pentane diamine dihydrochloride with molecular weight of 472.88 was used. It is composed of bitter bright yellow crystals and one gram can be dissolved in about

35 ml of distilled water. It is a product of Sigma Company Ltd. of Australia.

Bacterial strain

Enterotoxigenic E. coli W165-1/82 which produces only heat-labile toxin, isolated from acute diarrhoeal case from Yangon Children's Hospital was used.

Preparation of crude heat-labile toxin

E. coli was cultured in nine litres of CAYE medium, which constitutes 2 per cent casamino acid (Difco), 0.6 per cent yeast extract (Difco), 0.25 per cent sodium chloride, 0.871 per cent dipotassium hydrogen phosphate, 0.25 per cent glucose and 0.1 per cent trace salt solution ($MgSO_4$ 5 per cent, $MnCl_2$ 0.5 per cent, $FeCl_3$ 0.5 per cent and H_2SO_4 0.001 per cent). The medium was supplemented with lincomycin hydrochloride to enhance the synthesis of LT and shaken in Takasaki shaker (280 rpm/min) at 37°C overnight. The bacterial culture was then centrifuged at 10,000 rpm for 20 minutes at 4°C and pelleted cells were suspended in approximately 300 ml of 0.01 M Tris (hydroxymethyl) aminomethane (Tris-hydrochloride buffer) pH 8.6, containing 0.9 per cent sodium chloride. The suspension was sonicated in an ultrasonic disruptor sonicator (Tomy Seiko Co. Ltd.) for five minutes. The sonicated suspension was spinned at 15,000 rpm for 30 minutes and again the supernate was spinned for 3 hours at 20,000 rpm. The supernate was collected and solid ammonium sulphate was added to give 65 per cent saturation. The precipitate was collected by spinning at 10,000 rpm for 30 minutes and this was suspended in TEAN buffer pH 7.4 which constitutes 1 mM ethylenediamine tetraacetic acid (EDTA), 3 mM sodium azide NaN_2 and 0.2 M sodium chloride. It was then dialysed twice against 2 litres of TEAN buffer and used as crude LT [3].

Detection of toxin by Ouchterlony double gel diffusion method

The crude LT toxin was assayed by Chinese Hamster Ovary cell (CHO) assay for morphological change. Sensitivity and specificity of LT was tested by Ouchterlony double gel diffusion method [4] using LT antisera.

Determination of protein

The protein concentration of crude LT toxin was measured by the method of Lowry, 1951 [5].

The ligated loop model

This was done according to the method of Klipstein 1979 [6]. Three groups of rats weighing 41-60 g, 61-80 g, and 81-100 g respectively were used. Prior to injection of LT toxin, rats were fasted for one day. Intramuscular Nembutal 0.05 ml per rat was given as an anaesthetic agent. The abdomen was shaved and opened. A small intestinal loop, about ten centimeters in length was tied with double knots. Different concentrations of toxins contained in 0.5 ml ie. 370 ug, 185 ug, 120 ug, 90 ug, 75 ug and 60 ug per rat were administered by injecting directly into each ligated loop with a 27 G needle. If the anaesthesia induced by Nembutal was not significant, diethyl ether was also given. The rats were then kept at RT (20°C) without food and water. After 16 hours, rats were sacrificed, the ligated loop was taken out and secretory response was measured and weighed. The secretory response in terms of gram per 10 centimeters length was weighed with a balance (Chyo, Japan) which has a sensitivity of ± one milligram. The weight and length of loops were recorded and its ratio

(gut weight per length) was determined. The volume of fluid was also measured.

Administration of quinacrine

Quinacrine tablet (25 mg) was dissolved in five ml of saline and diluted to obtain concentrations of 500 ug/ml, 250 ug/ml and 125 ug/ml. Soon after the injection of LT toxin 0.1 ml of quinacrine was given directly into the ligated loops.

Measurement of cyclic AMP

As soon as the rats were sacrificed the ligated loops were taken out and placed on ice. The fluid was measured for maximum secretory response and the loops were cut open and the mucosa was scraped and placed in Tris buffer. Cyclic AMP was measured by using the assay kit supplied by Amersham United Kingdom, according to the method of Lin et al., 1974 [7].

RESULTS

Purity and specificity of heat-labile (LT) was done by Ouchterlony double gel diffusion and by Chinese Hamster Ovary (CHO) cell assay [8]. The crude LT possesses a protein concentration of 736 ug/ml.

Secretory response to different doses of toxin is shown in Fig. 1 in three different weight-groups of rats. It was observed that the maximum secretory response occurred with 125 ug of LT in all the three tested groups. It was also noted that rats weighing 61 to 80 grams showed a good response when compared with the other 2 groups. Antagonism of enterotoxigenic E. coli LT toxin by quinacrine in rats is shown in Fig. 2. It was shown that 125 ug of LT, when antagonised with 500 ug of quinacrine resulted in a decrease in secretory response as well as in

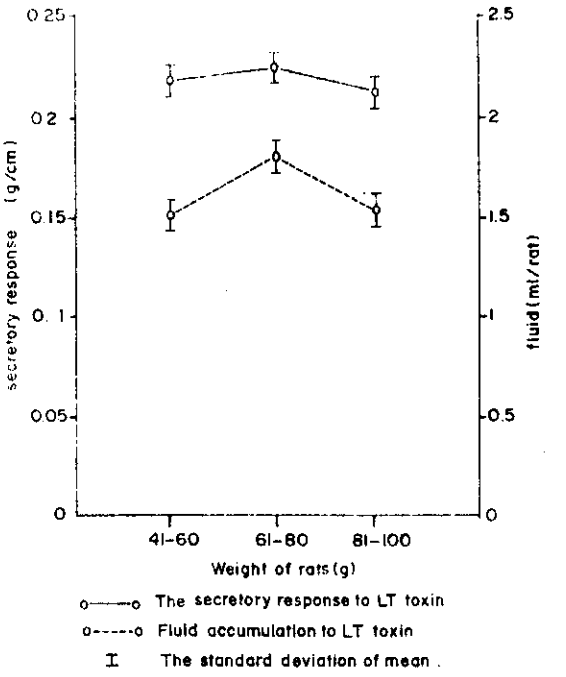

Fig. 1. The secretory response and fluid accumulation in different weight groups after 125 ug/ml LT injection

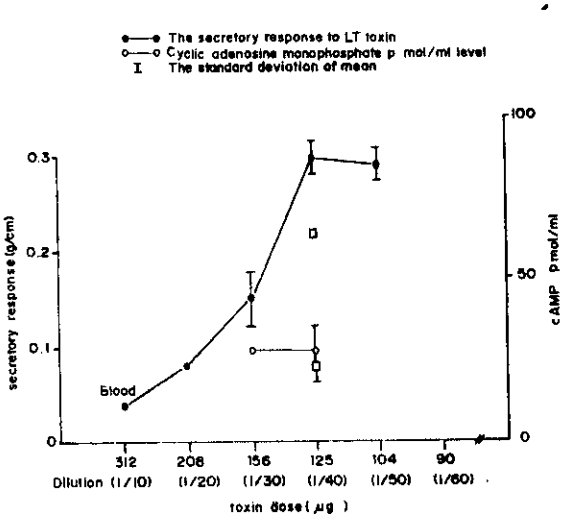

Fig. 2. Antagonism of enterotoxigenic E. coli LT toxin by quinacrine in rats

cyclic AMP levels. The experiment was repeated in rabbits and the results confirmed that of the previous

rat experiments (Fig. 3). The secretory response fell from 0.224 g/cm to 0.187 g/cm in rats. In both cases it was found that although there was no secretion to high doses of toxin it could damage the intestinal wall and intestinal bleeding occurred.

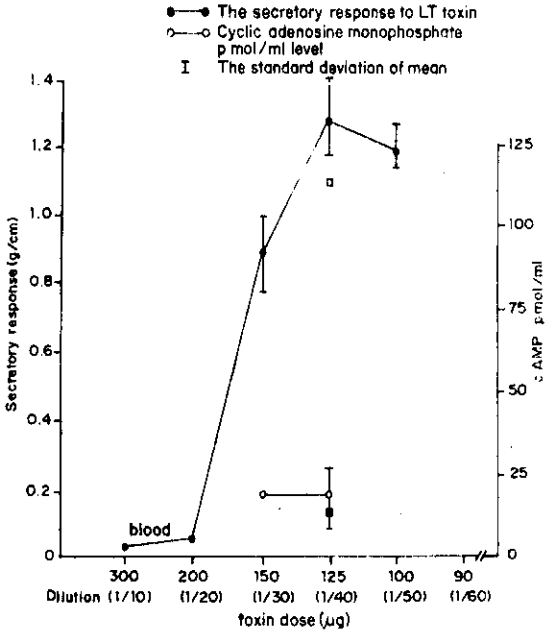

Fig. 3. Antagonism of enterotoxigenic E. coli LT toxin by quinacrine in rabbits

DISCUSSION

The treatment of cholera by intravenous fluid and electrolytes replacement is well established and, if instituted before dehydration and shock are irreversible, can completely protect the patient. The outpouring of fluid and electrolytes into the small intestine in cholera seems to be due to stimulation of secretion and not to interference with sodium or glucose absorptive capacity. It was stated by Carpenter [9] that the mechanism for this activity was not clear. Klipstein et al. [6, 10-15] studied the protective effects of primary and booster immunization with E. coli heat-labile enterotoxins in rats and reported that the degree of protection against the toxin correlated with that against viable bacteria and with elevated serum antitoxin titres.

Chlorpromazine inhibits adenylate cyclase enzyme in several tissues [16-18]. It has been shown that chlorpromazine reduces adenyl cyclase activity and fluid production in the intestine of mice which had been exposed to cholera toxin (CT), Escherichia coli heat-labile enterotoxin (LT) and prostaglandin E_1. In piglets, diarrhoea due to heat-labile E. coli (LT) was reduced by chlorpromazine and was used successfully in Asiatic cholera [19]. Holmgren et al. [20] has shown that chlorpromazine is a potent inhibitor of cAMP-mediated intestinal secretion. Anti-inflammatory agents such as aspirin, indomethacin, dexamethasone, prednisolone, phenylbutazone and sodium salicylate may inhibit secretory product or stimulate absorptive mechanism. They may block receptor sites for the toxin, or they may alter its detoxification. Recent studies have shown that aspirin and indomethacin inhibit the synthesis of PGF_{2a} from archiodonic acid and reduce prostaglandin released from the spleen [21, 22]. Many investigators have conducted studies on secretory response induced by ST and by inhibitors [23-26]. Quinacrine, indomethacin and chlorpromazine are inhibitors of phospholipase A2, cyclooxygenase and calmodulin mediated reaction respectively, and reduce secretory response of suckling mice to submaximal doses of STa, but had no effect when animals were challenged with maximal doses of STa [27-32].

Regarding the molecular mechanism of LT and CT induced diarrhoea, and mode of action, the reported knowledge is based on studies using cell-free systems being adenyl cyclase

activation by LT and CT, independent of archidonic acid and prostaglandins. However, studies using enzyme (phospholipase and cycloxygenase) inhibitors, would suggest that archidonate pathway is somehow involved (Fig. 4). In this study the decreased cyclic AMP levels in intestinal fluids in rats and rabbit after treatment with quinacrine suggest the inhibition of phospholipase A_2 to release archidonic acid. It may be involved in the mechanism of action of inhibitors of prostaglandin E_1 synthesis and/or due to direct inhibition of enterocyte adenylate cyclase.

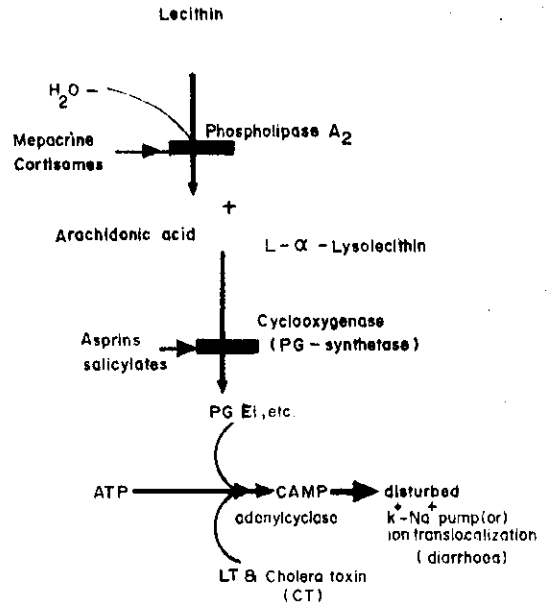

Fig. 4. The reaction catalyzed by phospholipase A_2

ACKNOWLEDGEMENTS

We are indebted to Professor U Hla Myint for providing us with quinacrine hydrochloride. We would like to express our gratitude to Dr.Aye Kyaw, Deputy Director of Biochemistry for analysing the cAMP levels in rats and to the staff of animal house for providing the required animals. We would also like to thank our Director Dr. Myint Lwin for his expert suggestions and last but not least Dr. Myint Lwin, Director-General of Department of Medical Research for his encouragement in research activities.

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Accepted for publication 8 Jan 1996

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

Study of venom neutralising efficacy of monospecific cloudy liquid antivenom

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Venom neutralizing efficacy of a batch of monospecific cloudy liquid antivenom H 93723 expiry 8-9-97 was assessed retrospectively on seven systemic Russell's viper bite cases. Each received 40 mls (4 ampoules) of antivenom which includes one to four ampoules of cloudy antivenom. Venom antigen and antivenom levels before and after the antivenom were followed up to 72 h by enzyme immunoassay technique. Results indicated that in severe envenomed cases (venom level >80 ng/ml) (n=4), venom antigen remained detectable up to 8 to 12 h and antivenom was not detected until 4 to 10 h (12-20 h in 2 cases) after the antivenom. Dose related neutralising efficacy of cloudy antivenom was observed. Five out of 7 patients were fatal. Use of cloudy or precipitated antivenom should be discontinued.

INTRODUCTION

Antivenom therapy is the mainstay of treatment in the management of snakebite. Myanmar Pharmaceutical Factory manufactures liquid and lyophilised antivenoms. The Central Medical Depot distributes antivenoms to hospitals throughout Myanmar. Because of lack of cold storage facility and failure of guaranteed constant power supply, liquid antivenoms are often kept at room temperature resulting in cloudiness and precipitation denoting denaturation of the globulin [1]. However, shortage of antivenom leads one to use cloudy or precipitated liquid antivenom in various township hospitals. The effects of storage temperature on the efficacy of liquid antivenoms had been studied [2]. The aim of the present study was to monitor the venom neutralising efficacy of cloudy liquid antivenom used in treating Russell's viper bite cases.

MATERIALS AND METHODS

While studying the antivenom (ASV) kinetics of the Russell's viper bite cases of Taungdwingyi hospital, we came across 7 systemic cases treated with cloudy ASV. It is a retrospective study of cloudy ASV treated Russell's viper bite cases, who received 1 to 4 ampoules (amps) of the cloudy ASV out of a total of 4 infused intravenously. Routinely, 40 mls (4 amps) of ASV is administered to Russell's viper bite cases. The batch number of ASV used was H93723 expiry 8-9-97 which had no obvious precipitates but turned cloudy on mixing. ASV used were transported to the hospital without cold storage facility and were kept at room temperature in the main store of the hospital until these were needed. Because of misuses, only some were transferred to +4°C refrigerator for emergency use. Venous samples were collected at 0, ½ hrly for 4h, 2 hrly up to

18h, then at 24, 36, 48 and 72h after the ASV. Venom antigen and antivenom levels were measured by enzyme immunoassay technique (EIA) [3]. Cases were selected at random by the medical officer. Clinical features and the outcomes were also recorded in a standard proforma.

RESULTS

A total of 7 systemic envenomed cases, 4 had a venom level of >80 ng/ml (severe envenoming) and 3 cases with a venom level of <65 ng/ml (mild envenomed) were studied. Antigen clearance of severe envenomed cases (infused with 4 amps of ASV containing 2 to 4 amps of cloudy ASV) was delayed for 8 to 12 h and ASV could not be detected 4 to 20h (mean 11.5h, n=4) after the ASV (data not shown). In mild envenomed cases, the dose of coludy ASV did not appear to influence the venom antigen clearance and ASV kinetics (data not shown).

Venom antigen and antivenom clearances of 4 systemic envenomed cases treated with a total 4 amps of ASV containing 1 to 4 amps of cloudy ASV are shown in figure 1.

Clinical features and the outcome of 7 cases are presented in table 1. Five out of 7 cases died, secondary to shock (2 cases) and renal failure (3 cases). Only two patients presented with haemorrhagic manifestations.

DISCUSSION

A retrospective analysis of data on performance of ASV in snakebite cases of Taungdwingyi gave us an opportunity to study venom neutralising efficacy of a batch of cloudy ASV which happened to be given to 7 systemic envenomed cases. Precipitated ASV were not used for treating patients in this hospital.

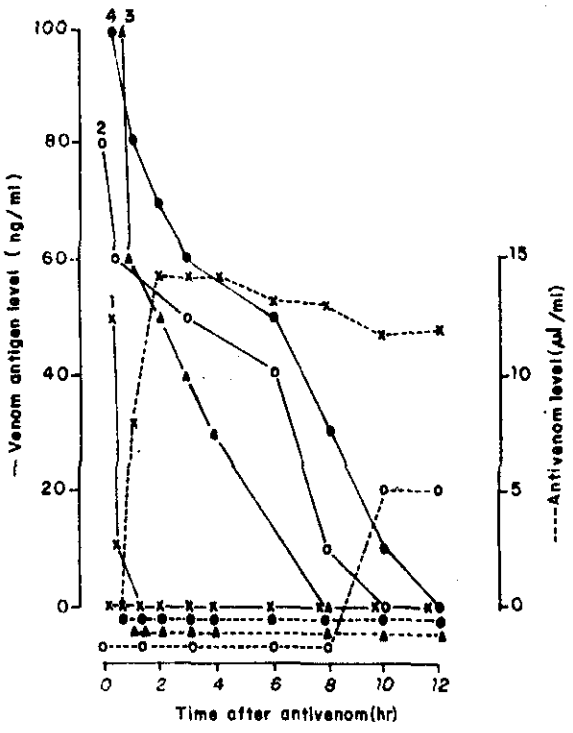

Fig. 1. Venom antigen and antivenom clearances of 4 systemic envenomed cases treated with a total 4 amps of antivenom containing 1 to 4 amps of cloudy ASV indicated by number 1 to 4 in the figure

The expected inverse relationship between venom neutralisation and the dose of the cloudy ASV was confirmed in this study. Failure to eliminate venom antigen and delay appearance of antivenom after ASV indicated that either the ASV used failed to completely neutralise the incoming venom antigen or denatured venom specific IgG failed to recognise the whole venom used in EIA. However, 4 amps of the cloudy ASV administered to a mild envenomed case (DH) showed presence of some venom neutralising efficacy in it (data not shown). Failure of neutralisation of venom antigen by precipitated liquid ASV had been reported [2,4].

Table 1. Clinical features and outcomes of 7 cloudy antivenom treated patients

Code	TK	KHS	UMM	TZ	NL	PT	DH
Age/Sex	57M	13F	55M	25M	11M	25M	68F
Bite hour (h)	6.45	3.20	24.00	16.10	3.45	8.40	11.00
Adm. venom level (ng/ml)	100*	100*	100	80	30*	50*	60
No. cloudy ASV	4	3	2	2	2	1	4
Local swelling	+	+	+	-	+	+	-
Epigast. pain			+D2	+	+		+
Renal angl. pain				+	+		+
Shock	+	+D2		+		+	
Renal failure			+	+		+	+
Conj. oedema						+	
Malena						+	
Haematemesis			+D5			+	
Haematuria						+	
Fatality	+	+	+	+		+	

* after ASV
Adm. = admission
D = day

An earlier study on ASV showed that in severe envenomed cases (venom level >60 ng/ml), 40 mls of ASV may not be sufficient to completely neutralise venom antigen in a proportion of cases [5] and therefore precipitated or cloudy ASV will have no place for treating these cases. However, in mild envenomed cases, inclusion of 1 to 2 amps of cloudy ASV in a total of 4 had little effects on venom neutralising efficacy suggesting that 2 amps of potent ASV could be used for treating these cases.

Application of local pad and immobilisation was found to be useful in inactivating venom locally in mild envenomed cases (10-20 ng/ml) [6]. It was observed that 20 mls of antivenom could neutralise a venom level of 10-30 ng/ml in 1h (n=7) (unpublished observation). It is suggested that application of local pad and immobilisation and or administration of 20 mls rather than 40 mls ASV used routinely for treating mild envenomed cases, should be evaluated and considered in future which will result in substantial saving of ASV.

Five out of seven deaths were probably due to severe degree of envenoming, use of denatured ASV and to the late arrival for treatment. The sequelae of snake bite observed were attributed to use of the cloudy denatured ASV which failed to neutralise the circulating venom antigen for 8 to 12 h.

Enzyme refined liquid ASV on storage at 37°C became cloudy after 6 months of storage and precipitated out by the end of a year. Cloudiness or opacity signifies loss of potency and is not recommended for injecting into human [1]. Lyophilised preparation is recommended to be used in tropical countries [7]. It has been suggested that rapid turnover of fresh stock of liquid antivenom should be practised if reliable cold storage cacility is not available and precipitated or cloudy ASVs should no longer be used to treat snakebite cases [2].

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Accepted for publication 17 Jan 1996

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

A comparative study of YGH index and Wayne's index in the diagnosis of thyrotoxicosis

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This study was done during 1992 to 1994. The patients were in-patients of Wards 1 & 2 and those referred from the Nuclear Medicine Department of Yangon General Hospital. They all were clinically thyrotoxic patients confirmed by either high uptake of radioactive iodine or high serum T_3 and T_4 levels. We took proper history taking and did thorough clinical examination for all patients. Then we recorded the findings and counted the scores for each patient according to YGH index and WAYNE index for diagnosis of thyrotoxicosis. The total number of patients studied was 138. Mean age was 36.5 years. The sensitivity of YGH index was 93.47%. The sensitivity of WAYNE index was 89.85%. The difference between the sensitivities of two scoring systems was not significant statistically. Therefore, both scoring systems can be applied with the nearly same sensitivity in the clinical diagnosis of thyrotoxicosis.

INTRODUCTION

The clinical syndrome of hyperthyroidism is one of the most dramatic in clinical medicine. Almost all systems of the body may be affected in hyperthyroidism but the emphasis often falls more strikingly on one eg. cardiovascular or neuropsychiatric and clinicians may easily miss the correct diagnosis if they are unaware of the signs and symptoms of thyrotoxicosis. A high index of suspicion is required to diagnose atypical cases with masked thyrotoxicosis. Therefore, many medical centres have proposed several diagnostic indices for clinical diagnosis of thyrotoxicosis. The aim of the study is to compare the usefulness of YGH index with Wayne's index in the clinical diagnosis of thyrotoxicosis.

MATERIALS AND METHODS

The study period was a two year study, starting from January 1992. The patients were in-patients of Wards 1 & 2 and those referred from Nuclear Medicine Department of YGH. They all had clinical features suggestive of thyrotoxicosis and were confirmed by high serum T_3 and T_4 levels and high radioactive iodine uptake. A proper history taking and thorough clinical examination were performed.

We recorded the findings and counted the scores for each patient according to YGH index and Wayne's index for diagnosis of thyrotoxicosis. Statistical analysis was performed by Chi-square test. YGH index scoring system is shown in table 1. Wayne's index scoring system is shown in table 2.

RESULTS

The total number of patients studied was 138, 120 females and 18 males. The female to male ratio was 6.67:1.

Table 1. The YGH index

Score	+3	+2	+1	0	-1	-2	-3
K = Skin	Hot and wet	Warm and moist	Warm and moist	Normal	Cool or dry	Cool and dry	Thick, coarse and dry
L = Lethargy or hyperkinesis	Severe hyperkinesis	Moderate hyperkinesis	Mild hyperkinesis	Normal	Mild lethargy	Moderate lethargy	Severe lethargy
M = Metabolism	Weight loss 30%	Weight loss 20-30%	Weight loss 10-20%	Normal	Weight gain 10-20%	Weight gain 20-39%	Weight gain 30%
N = Nervous symptoms	Excitability and strong finger tremor	Coarse finger tremor	Find finger tremor	Normal	Lassitude or slowness	Lassitude and slowness	Lassitude, slowness, poor memory
O = Circulation and pulse rate	130/min A.F. or heart failure	111-130/min	91-110/min	70-90/min	60-69/min	60/min	Heart failure
P = Exophthalmos	Severe exophthalmos with lid lag	Moderate exopthalmos with lid retraction	Mild exophthalmos or stare	Normal	Mild anophthalmos	Moderate anophthalmos	Severe anophthalmos
Q = Thyroid gland	Enlarged with bruit and thrill	Enlarged with bruit	-	Normal	-	-	-

(+6) and above = Toxicity

(+4) to (+5) = Equivocal range

(+3) and less = Euthyroid

Table 2. Weighting factors allocated to the symptoms and signs of thyrotoxicosis (Wayne's index)

Symptoms of recent onset or increased severity	Present score	Absent score	Signs	Present score	Absent score
Dyspnoea on effort	+1		Palpable thyroid	+3	-3
Palpitation	+2		Bruit over thyroid	+2	-2
Tiredness	+2		Exophthalmos	+2	
Preference for heat		-5	Lid retraction	+2	
Preference for cold	+5		Hyperkinetic movements	+4	-2
Indifference to temperature	0		Lid lag	+1	
Excessive sweating	+3		Hands: Hot	+2	-2
Nervousness	+2		Moist	+1	-1
Appetite increased	+3		Casual pulse rate:		
Appetite decreased		-3	Atrial fibrillation	+4	
Weight increased		-3	Regular rate: 80/min		-3
Weight reduced	+3		80-90/min	0	
			Over 90/min	+3	

(+10) = Euthyroid
(+11) to (+19) = Equivocal range
(+20) = Thyrotoxic

The youngest patient was 11 years old and the oldest patient was 70 years old. Mean age of the patients was 36.5 years. Majority of patients (45%) were in the 30-39 year age group (Fig. 1).

Fig. 1. Age and sex distribution of thyrotoxic patients

According to YGH scoring system 129 out of 138 patients scored more than 6 (thyrotoxic level), 2 patients scored 4-5 (equivocal) and 7 patients scored less than 3 (euthyroid). The sensitivity of YGH index was 93.47 (table 3).

When Wayne's index scoring system was used, 124 patients got more than 19 (thyrotoxic level), 8 patients scored 11-19 (equivocal) and 6 patients scored less than 11 (non-toxic). The sensitivity of Wayne's index was 89.85 (table 4).

Comparison of results of two indices is shown in table 5. The P value for difference between two indices was (>0.05). Therefore the difference between the sensitivities of two scoring systems was statistically not significant.

DISCUSSION

Thyrotoxicosis is a common endocrine disorder in Myanmar. There are several indices for clinical diagnosis of the thyrotoxicosis (eg. Wayne's index, Silinks T pathotype, Newcastle

Table 3. Result of YGH index

YGH Scores	Number of Patients
6	129
4-5	2
3	7
Total	138
Sensitivity	93.47%

Table 4. Result of Wayne's index

Wayne's scores	Number of Patients
19	124
11-19	8
11	6
Total	138
Sensitivity	89.85%

Table 5. Comparison of the result of two indices

Diagnostic indices	Toxic	Equivocal	Euthyroid
Wayne's	124	8	6
YGH	129	2	7

index and YGH index) [1-4]. Among them Wayne's index is the most popular one. It requires both symptoms and signs to complete the scoring system. However, symptoms of thyrotoxicosis may not help much in diagnosis as most of them could be attributed to other diseases. Therefore in 1974 Daw Kyin Aye proposed a YGH index for clinical diagnosis of the thyrotoxicosis. It was based on the Silinks T pathotype index (table 6). Both YGH and Silinks indices depend only on the signs of thyrotoxicosis. Thus they are less time consuming and have fewer errors.

Table 7. Comparison of the result of two indices (Dr. Daw Kyin Aye)

Diagnostic indices	Toxic	Equivocal	Euthyroid
Wayne's	13	15	6
YGH	23	11	0

She also compared the Wayne's index and YGH index in 1974, studying 34 proven thyrotoxic patients confirmed by high uptake of I^{131} (table 7). In her study 67% of patients fell in thyrotoxic range using YGH index and with Wayne's index, only 38% fell into the toxic category. Therefore YGH index was more sensitive than Wayne's index in her study.

However in our study the difference between the sensitivies of both indices was not statistically significant. This may be due to (1) our study included larger sample size (2) we used both I^{131} uptake test and serum T_3, T_4 levels for confirmation of thyrotoxicosis.

CONCLUSION AND RECOMMENDATIONS

YGH index for diagnosis of thyrotoxicosis is simple, easy to apply, rapid to score and practical. Since it is as sensitive as other existing indices eg. (Wayne's index), we suggest that it could serve as a useful scoring system in areas where there are no facilities for thyroid function tests.

Moreover it can be used as a screening test before patients are submitted to expensive investigations even in centres where there are endocrine laboratories and nuclear medicine facilities.

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Table 6. Silink's T Pathotype (1964)

Score	6	4	2	0	-1	-3	-5
K = Skin		Warm and moist	Warm or moist	Normal	Cool or dry	Cool and dry	Myxaedema
L = Adynamia	Severe adynamia	Moderate adynamia	Mild adynamia	Normal	Mild adynamia	Moderate adynamia	Severe adynamia
M = Metabolism	60%	40 to 60%	20 to 40%	0 to 20%	-15 to 0%	-30 to 15%	-
N = Nervous symptoms	Excitability and irritability, strong tremor	Coarse tremor, nervousness	Mild tremor	Normal	Lassitude or slowness	Lassitude & slowness	Lassitude, slowness, poor memory
O = Circulation and pulse rate	120 or A.F. or decompensation	101-120	81-100	62-80	60	-	-
P = Exophthalmos	Marked exophthalmos	Moderate exophthalmos and lid retraction	Mild exophthalmos with stare or stare only	Normal	Mild anophthalmos	Moderate anophthalmos	Severe anophthalmos

Toxicity = Even scores

Normal = 0

Hypothyroid = Odd scores

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Accepted for publication 24 Jan 1996

The Myanmar Health Sciences Research Journal, Vol. 7, No. 3, 1995

SHORT REPORT

Perception towards preventive practices of HIV infection/AIDS among mother attendants at Yangon Children Hospital

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AIDS is a major cause of infant and childhood mortality in certain regions of the world. It is documented that the predominant mode of HIV transmission worldwide at present, is heterosexual transmission. The Global AIDS Policy Coalition (GAPC) estimated that by the year 1995, 6.9 million people (5.7 million adults and 1.2 million children) will become newly infected and of all new HIV infections the largest proportion is in sub-Saharan Africa (64%), followed by South East Asia (10%) [1]. With the AIDS pandemic, women often have been viewed as reservoirs of infections, posing a threat to men, and also to their babies, since vertical transmission was recognized. In Myanmar, the magnitude and spread of HIV infection has been increasing since 1988 [2]. This situation reflexes the importance of HIV/AIDS prevention and control programmes which will need to develop appropriate education and other public measures to respond specifically to the growing problem of this threatening disease in the community. Although a series of studies on HIV infection/AIDS has been documented in Myanmar, there is still a gap of information regarding perception towards HIV infection/AIDS among attendants or caretakers who are mostly mothers in the family.

Study area and population

Yangon Children's Hospital was the area of study where a total of 596 mothers of sick or operated children admitted to medical, surgical, nutrition and neonate wards were selected. Face-to-face interview was undertaken after pretesting the questionnaire by trained research assistants from May to November, 1992.

Demographic variables

Study groups were mainly at the age group of 25-29 years (30.5%) followed by 30-34 year age group (22.5%) and 35-39 year age group (19.6%). Only 2.3% was found under 19 years and 6.5% were at the age of 40 years and above. By educational status, 43.3% were able to read or write i.e. at the level of 3Rs, followed by 30% who passed middle grade and 15.4% passed high school level. Some 8.6% were university and college level and only one mother of 596 (0.2%) has professional degree. The majority (70.5%) were dependents and unskilled workers whereas 20.6% were working mothers e.g. clerks and shop keepers. A few mothers were skilled and professional ones e.g. manager, teachers and doctors at 7.7% and 1.2% respectively.

Perception towards AIDS

Regarding perception towards AIDS, 76.2%revelaed that it can be prevented and 75% expressed that it is an important problem in our country. Almost all of them (96%) were afraid of getting this disease themselves or in their family, and 94.5% were willing to take care of HIV infected children.

Only 41.3% of the respondents answered that handling infected materials like blood and body fluid with gloves is a preventive practice (Table). Over 70% said that AIDS can be prevented by avoiding unnecessary injections, by screening blood before donation, by using sterile equipment for injections or by avoiding intravenous drugs. One striking feature was found that 22.3% accepted condom as a preventive measure for disease spread whereas 62.8% did not give the answer and 14.8% did not think that condom utilization is effective in AIDS prevention.

Table. Preception towards prevention of AIDS through practice

Statements	Percentage		
	Yes	No	Don't No
Handling infected materials with gloves	41.4	3.8	54.8
Avoiding unnecessary injection	77.3	1.2	21.5
Screening blood before transfusion	75.0	0.7	24.3
Using sterile needle/ syringe	72.3	2.2	25.5
Avoiding intravenous drugs	72.3	1.9	25.8
Using condoms during sexual intercourse	22.4	14.8	62.8

Most of the mothers perceived that *HIV infection/AIDS is an important* urgent health problem in our country. They are in favour of taking care of infected children. Thus, mothers seem to have a good outlook towards this disease.

Change in the behavioural attitudes toward the use of condom is still essential. This study shows that counselling is a need to be promoted to change the unwanted behaviour and it will provide more effective ways of prevention of AIDS transmission.

It is highly recommended to promote safe motherhood for all women by an appropriate and effective health education through health services and non-governmental associations like Maternal and Child Welfare Association, Myanmar Medical Association etc.

ACKNOWLEDGEMENT

We like to thank the Director-General of the Department of Medical Research, for allowing us to undertake this study by DMR grant. Jimmy Mang, Technician Grade 3 from Epidemiology Research Division is much appreciated for his effort in data analysis.

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Accepted for publication 24 Oct 1995

MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

Department of Medical Research

Ministry of Health, Yangon, Myanmar

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