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SEROEPIDEMIOLOGICAL STUDY OF PREVALENCE OF MALARIA IN VILLAGE SOLANA, UTTAR PRADESH, INDIA

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The roles of causative factors responsible for prevalence of malaria in the village of Solana, India, were studied. Mosquitoes and larvae density in and around the area were measured by process of random sampling and counting their numbers under microscopy. Malaria in population of the village was diagnosed by standard ELISA method and malaria antibody capturing level were measured against three *Plasmodium falciparum* antigens. The effect of insecticides for the control of malaria was also evaluated. Results of study showed that more than two third of village human populations (75%) were suffering from malaria, with 67.14% being children below 14 years of age. Similarly vectors identification study showed *Anopheles culicifacies and Anopheles stephensi* as the main source for infection transmission. Sporozoite positive rate estimated in *Anopheles culicifacies* was found to be 1.26%. Both vectors were resistant to DDT and Malathion insecticides. Antibody capturing by three different *Plasmodium falciparum* antigens study showed that glycophospholipid antigen (GPL) was able to capture and detect highest amount of malarial antibody followed by sonicated *Plasmodium falciparum (Pf)* antigen and ring infected erythrocyte surface antigen (RESA) i.e. 0.69±0.25, 0.60±0.22 and 0.59±0.23 respectively. Age specific antibody levels was found to gradually increase from lowest to highest age groups i.e. 0.29-1.18 for GPL, 0.26-0.94 for RESA and 0.25-0.97 for Pf. The study showed that infants and children are highly prone to malaria attacks than the adult population, which may be as a result of low level of Plasmodium antibody in their circulation.

Key words: Solana village, endemicity, malaria incidence, antibodies, ELISA, insecticides.

INTRODUCTION

Malaria endemicity is one of the major public health problems of India. Recent World Health Organization (WHO) report suggests that about 7 million human populations in India suffer from malaria annually (1). Incidence of *Plasmodium falciparum* is gradually rising according to National Malaria Eradication Program (NMEP) (2), and human death rate are increasing i.e. from 500-600 to more than 1000 in recent years. Epidemiological study of malaria also showed that *P. falciparum* infection is more

common in Uttar Pradesh, Bihar, Orissa and Northeastern states than other regions of India (3). In addition to this, malaria is a major cause of mortality and morbidity in developing countries with immense economic consequences on the afflicted population. These include considerable direct (medical consultations, hospitalizations, laboratory tests and medications) and indirect (work day lost) costs for households that are already socio economically disadvantaged (4,5,6,7).

Several studies have shown malaria infection to be dependent upon life style and literacy in these areas. It has also been observed that human malaria parasites are becoming resistant to common anti-malaria drugs (8,9). For the first time in 1973, resistance to chloroquine in *P. falciparum* was detected in Assam with several hundred deaths occurring due to resistant malaria. Similarly *P. vivax* resistant cases were found in Bombay and South Bihar India (10). Recently, Chloroquine resistant *P. vivax* has been observed in different parts of the tropical region of the world (11,12,13). In spite of the increasing numbers of resistant strains, chloroquine is still recommended by the National Malaria Eradication Program of the Government of India as standard therapy for all types of malaria. Chemoprophylaxis with chloroquine, mosquito coils, insecticide sprays and bed nets are generally used for prevention. It has also been observed that mosquitoes are developing resistance to various insecticides, which is causing difficulties in the eradication of malaria (14). Poor socio economic conditions in certain areas are also causing immense set back in malaria eradication programs.

Transmission of malaria is predominantly common in rural India but also there is a growing concern about the urbanization of malaria (3). Unplanned growth of the cities without public services and hygiene has been found to be favourable conditions for anopheles breeding and appropriate environment for disease transmission (15). Risk factors associated with the transmission of urban malaria may be different from those identified in rural areas. However, most studies that have been carried out in rural areas throw very little light on the

failure of malaria eradication program and provide little information of the causative factors and no reasons for the continued spread of infection. It is important to understand the risk factors and reasons responsible for uncontrolled transmission of malaria infection in the rural areas so that remedial measures for prevention of disease can be suggested. Therefore, this epidemiological and experimental study was done in Solana village of Uttar Pradesh (UP), a village of India where eradication program for malaria started in 1941, in order to understand the causative factors responsible for the continued malaria transmission and to suggest methods for prevention of malaria in these areas.

MATERIALS AND METHODS

Study area: The study was conducted in Solana village of District Meerut, Uttar Pradesh situated near the river Ganga (Fig. 1). A canal has been built from river Ganga for irrigation of fields and the village is situated 500 meter from the west side of the riverbank. This canal irrigates the village fields and supplies water for drinking. Also, four water pond surround the village area and the village surroundings are fully covered with trees, water plants, grasses and household garbage. One pond has been cleaned and used for fish culturing. The village is not connected to the city road system and mode of transportation is bullock cart, horse driven cart, bicycles and tractors. There is no hospital or public health center in the village. There are about 500 families living in the village with a population of 2000 to 3000. Ninety percent of the villagers are farmers and about 10% are petty shopkeepers, labourers and civil servants. Several jaggery-manufacturing units are located in the west side of the village. The main cultivable crops of the area are, sugar cane, paddy, mustard, animal feeding plants and beans. Three temples and one mosque are located in the village. Two primary schools and one middle school are present in the village and one high school is located 5 kilometer from the village. Most of the village children attend primary and middle education in the village only. Almost all families maintain one or more domestic animals with most families keeping their animals inside the house

95% of the population did not use mosquito net. Previous general clinical survey had shown most villagers to be suffering from fever, headache, cough, eye infection and polio.

Mosquito collection: Mosquitoes in and around cow sheds and houses were collected with the help of cattle bate CB and human bate HB for 20 minutes by WHO sucking tube between 4 and 7am. Identification of the mosquito types (genera and species) was done according to WHO guidelines (16, 17, 18, 19, 20, 21).

Mosquito larva collection: Mosquito larvae were collected from 10 locations in each pond and creek.

Blood fed Mosquitoes collection: Blood fed mosquitoes resting on the wall under the bed and corner of the house and also outdoors were collected. All mosquitoes' blood were collected on a 3 mm Whatman filter paper and dried in room temperature and stored at -20^0C till use.

Human blood collection: Finger prick, thick and thin blood smear on glass slides, and blood soaked filter paper samples were collected from subjects with symptoms of malaria. Thick smear was used for the detection of malaria parasites, thin smear for identification of malaria parasite species, and filter paper sample for ELISA immunoassay. Thick and thin blood films were stained with 10% Giemsa stain for 10 minutes and slides were washed with buffer solution and dried in room temperature. Blood films were examined under high power oil immersion lens of Olympus microscope. Parasites were counted against 300 white blood cells. ELISA immunoassay was performed on the filter paper blood using GPL (glycolipid) antigen, Pf sonicated antigen and RESA synthetic peptide antigen. A 1:100 dilution of serum was made with phosphate buffered saline (PBS) and standard ELISA procedure performed as described by Roy *et al* (23). Final agglutination results were read using LP 300 Pasteur ELISA reader.

Spleen examination: All febrile subjects were clinically examined by qualified physicians for splenic enlargement according to Hackett's Index (22).

Mosquito blood meal examination: Blood meals were collected from recovered mosquitoes for de-

tection of animal and human blood. ELISA dots method was used to determine human and animal inoculation rate according to Roy and Sharma (24).

Mosquito sporozoite detection rate: Mosquitoes were dissected in 5% normal saline for sporozoite detection in salivary gland under dissecting microscope after species identification.

Insecticide susceptibility test: The WHO method (25) was used. Test kits provided by WHO/TDR were used and batches of anopheline mosquitoes were exposed to standard insecticide impregnated papers with 4% DDT, 0.1% Icon, 0.025% Deltamethrin and 5% Malathion. The standard exposure time of one hour was used.

Statistical analysis of data: Data obtained were statistically analyzed using student 't' test and p values were calculated for level of significance with cut off value as control mean + 2 SD.

RESULTS

The results of epidemiological study of malaria in Solana village are presented in Table 1. It was found that 44% of the village population were suffering from different types of malaria infection and 79% of those infected were found to be positive for *P. falciparum* malaria. 18.7% for *P. vivax* and 2.3% were from mixed infections. Children of age group below 14 years showed high prevalence of malaria i.e. infant parasite rate was found to be more than 65%. Gamatocyte prevalence rate in the blood smear of the subjects were found to be 2.06%. Total parasite density index (PDI) was also high i.e. 5.3%. Enlargement of spleen in children below age 2-9 years was 51.4%.

Vector/larvae identification survey: Four Anopheles species and one Culex species were found i.e. *An. culicifacies, An. stephensi, An. annularis, An. sapitus* and *Culex quenquefasiastus. An. culicifacies* and *An. sapitus* were found in maximum numbers during 4 to 7 am indoor resting collections (Table 2). Three *An. culicifacies* mosquitoes were found positive to sporozoite detection study. All the four types of Anopheles and the Culex mosquito larvae were found in three of the four village ponds, pools and creeks (Table 3). The fourth pond, which has been

cleansed and used for culturing fishes, did not contain mosquito larvae.

Insecticide susceptibility test: *An. annularis* and *An. sapitus* were found to be 100% sensitive to 4% DDT, 0.1% Icon, 0.025% Deltamethrin and 5% Malathion but *An. culicifacies* and *An. stephensi* showed 30-40% resistance to 4% DDT and 40-50% resistance to 5% Malathion.

Mosquitoes inoculation rate (Man biting rate): 86 blood fed mosquitoes were collected indoors and outdoors. Animal/human blood identification test found 24 mosquitoes containing human blood meal in their body. Human inoculation rate was 27.91%.

Immunological sensitivity study: A total of 389 blood samples were collected out of which 205 were from fever cases and the remaining from patients having no history of malaria/fever. ELISA results of the three antigens showed very high OD value in subjects above 15 years, moderate among subjects 10-14 years age group and a very low value in 0-11 months age group. The ELISA values of antigens titer are presented in Table 4.

Table 1: Microscopic examination of age specific parasite, gametocyte and spleen positive

Clinical findings of subjects					Microscopic examination for malaria				
Age group	No	Spleen +ve	Parasite +ve	Age specific +ve	Parasite Species			% +ve	Pf G +ve
					Pf	Pv	Mixed (Pf+Pv)		
0-11 mon	6	-	4	66.67	3	1		2.33	
12-23 mon	4	1	2	50.00	1	1		1.16	
2-4 yrs	26	19	10	38.46	8	2		5.81	1
5-9yrs	114	60	59	51.75	42	15	2	34.30	3
10-14yrs	84	42	40	47.62	34	5	1	23.25	1
15 and above	155	38	57	36.77	48	8	1	33.14	3
	389	160 41.13%	172 44.21%	44.21	136 79.06%	32 18.60%	4 2.33%	100%	8 2.06
2-9 yrs	140	79 56.43%	69 49.29%	49.29%	50 72.46%	17 24.64%	2 2.9%	100%	4 2.86%

Table 2: Details of mosquitoes/larvae and sporozite positive rate.

Details of mosquito/larvae			
Mosquito/Larves	No. of larvae	No. of mosquitoes	Sorozoite +ve rete (%)
An culicifacies	184	238	3(1.26)
An stephensi	97	169	0
An sapitus	283	305	0
An annularis	166	147	-
Culex quenquefasciatus	209	156	
	939	1015	3(0.3492)

Table 3: Mosquitoes larval collection and identification of breeding

S. No	Name of areas	Types of mosquitoes species emerge from Larvae
1	Pond (1)	*An annularis, An sapitus and Culex quenquefasciatus*
2	Pond (2)	*An stephensi, An annularis, An sapitus and Culex quenquefasciatus*
3	Pond (3)	*An annularis, An sapitus and Culex quenquefasciatus*
4	Pond (4) (Fish culture pond)	Larvae absent
5	Creek	*An culicifacies, An stephensi, An annularis.*

Table 4: Age specific ELISA OD value of fever cases vs *P. falciparum* antigens.

Particular of subjects (Age Group)	Fever cases	Positive cases	ELISA OD value of antigen		
			GPL	RESA	Pf
0-11 months	4	3	0.29	0.26	0.25
12-23 months	2	2	0.37	0.38	0.35
2-4 yrs	15	10	0.59	0.50	0.48
5- 9yrs	71	58	0.78	0.65	0.62
10-14 yrs	50	40	0.92	0.87	0.86
15 and above	63	59	1.18	0.94	0.97
Total	205	172	0.69±0.25	0.6±0.22	0.5[illegible]±023

DISCUSSION

Application of DDT was started in Solana area of District Meerut by malaria control team in 1941. The establishment of NMCP in 1953 and NMEP in 1958 showed drastic decline in malaria and density of mosquitoes in these areas. Also, *P. vivax* and *P. falciparum* endemicity in these regions were also reduced considerably due to extensive DDT spray. In 1960s, NMEP epidemiological survey reported for the first time that *Anopheles culicifacies* was resistant to DDT. Analysis of malaria situation revealed that malaria was never eradicated but had only declined to low levels and returned with increased vigor (2). Furthermore, reversions from consolidation and maintenance phases coincided with the DDT shortages. In the next decade (1970s), these problems further multiplied and as a result malaria resurgence was widespread. But in the 1980s and 90s, malaria problem became serious and several large scale out breaks of malaria were reported. Exophilic vector behaviour became more pronounced and indoor residual spraying (IRS) had poor impact on vectors. NMEP also identified hard-core areas of about 51 million populations where indoor residual spraying (IRS) had failed (3).

In our study, only two malarial parasite species i.e. *P. falciparum* and *P. vivax* were present in village Solana and *P. falciparum* was the predominant parasite specie in the area. Several epidemiological studies also showed the presence of *P. falciparum*, *P. vivax* and *P. malariae* in Terai region of UP (26,27). Mosquito larvae of *An. culicifacies*, *An. stephensi*, *An. annularis*, *An. sapitus* and *Culex quenquefastiatus* larvae were found in three main ponds and in all creeks but no larvae of mosquitoes were seen in cleaned fishpond.

Infant parasite rate was found to be very high i.e. 66.67% in the area and common in both male and female population of village. Dev and Sharma (28) found in three years study, an infant parasite rate between 25.81 – 41.59% and the highest in 1991 at Sonapur, Assam. A similar result was shown by different authors in endemic area of Myanmar with 75% in Oktwin village of Myanmar (29). The gametocyte positive rate

in this study was found to be 2.06%, which is much higher than 0.85% in the Myanmar study (30).

Four types of mosquitoes were collected from cowsheds and houses of village Solana. In the mosquito collection study maximum number of *An. culicifacies* and *An. stephensi* were recovered. *An. culicifacies* is main vector for transmission of malaria in rural and semi rural areas of India and accounts for about 60% malaria cases annually (31). The sporozoite positive rate was found to be 1.26% in *An. culicifacies* by salivary gland dissection study. This is lower than studies of other workers, which showed salivary gland sporozoite positive rate of 4.04% (4/99) in *An. dirus* in Oktwin village, Myanmar (30).

Serological study of village population for presence of malaria and antibody titer showed a very high ELISA OD values with GPL and Pf antigens but RESA-R1 antigen showed low OD titer. RESA-R1 antigen has been used for the measurement of endemicity level of malaria and anti-RESA-R1 antibody levels correlate well with level of endemicity (32, 33). This study showed village Solana as a mesoendemic area according to RESA-R1 ELISA OD results, but spleen diagnostic test showed more than 50% enlargement of spleen in children between 2-9 years of age pointing toward high endemicity (22). There is indication from the study that children above the age of 4 do not have repeated infection due to high level of antibody present in their body contributing to protection against *P. falciparum* malaria. Also, recent GPL antigen studies of Remasamy and Reece (34) and Schofield *et al* (35) showed that GPL and GPI antibodies help in protection against *P. falciparum* malaria.

CONCLUSIONS

This study has identified several factors responsible for the high endemicity of malaria in the village Solana UP, India. The village and surrounding area is near a major riverine swampy area with large amount of shrubs and bushes, which form a favourable breeding site for mosquitoes. Also, there is no proper sanitation system and no proper disposal of household garbage, animal and human excreta. The primary health care (PHC) cen-

ter of the village is ill equipped with no qualified doctor or trained medical technician. Chloroquine is given as a treatment for most of the fever cases at PHC without any laboratory investigation, so that drug resistant strain of parasite might have developed in the village population. In addition vector mosquitoes have been found to be resistant to various insecticides further complicating the situation. It is suggested that proper cleaning of surrounding area, good sewage disposal and hygiene, which are main principles for healthy and good living should be strictly followed. Cattle shed should be located outside human residence. Regular use of Deltamethrin impregnated bed nets and /wall residual spay (with Icon) may reduce the incidence of parasite transmission. Lastly, malaria biomedical engineering procedures of NMEP and WHO should be implemented for better management and prevention of malaria.

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ORAL PROTOZOA IN A NIGERIA POPULATION

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A study aimed at establishing the occurrence of oral protozoa in a Nigeria population was carried out over a 6-month period, January 1998 to June 1998. A total of 203 dental patients attending the dental clinics of the University of Nigeria Teaching Hospital (UNTH), Enugu were involved. Scraping of plaque were taken from the buccal surface of T16 and T36 (near the gingival margins) and placed on individual glass microscope slides. To each was added a drop of saline, which was mixed with the plaque and covered with a coverslip and then examined immediately. Thirty-three (16.30%) of the patients harboured protozoa in their mouths. Of these, 10 (4.9%) had *Trichomonas tenax*, while the majority 23 (11.3%) had *Entamoeba gingivalis*. No patient had both species of protozoa in their mouths. The associations of age, sex, teeth cleaning and other dental parameters with prevalence of protozoa were recorded.Our findings suggest that poor oral hygiene, calculus, old age and loss of attachment of periodontal fibers are factors which favour the proliferation of *Entamoeba gingivalis*.

INTRODUCTION

Entamoeba gingivalis and *Trichomonas tenax* were first described from the human mouth in 1849 and 1850 respectively (1). Studies from different parts of the world have confirmed that both organisms parasitize in an oral cavity changed by inflammation, yet also in a healthy mouth (2,3,4). Their highest occurrence rate has been recorded in adults with periodentesis and atrophy of the periodentium, somewhat lower in adults with gingivitis (2). No study has been conducted on the topic in Eastern Nigeria. The present study was carried out in the dental clinics of the University of Nigeria Teaching Hospital, Enugu.

MATERIALS AND METHODS

A temporary laboratory was set up in the Dental Clinic of the University of Nigeria Teaching Hospital, Enugu, Nigeria and all patients attending the clinics were included in the survey. A prepared chart was used to record information on age, sex, dental parameters, teeth cleaning and protozoa isolated. The dental parameters were recorded by the dentists and were taken from 6 index teeth " T16, TU, T24, T31 and T44 or where absent, from the neighboring tooth" as follows: presence or absence of bacterial dental plaque, subgingival calculus, gingival bleeding. Patients were interviewed to obtain information about the instrument used to clean their teeth (toothbrush, chewing stick (atu), or both) and the frequency of cleaning teeth (once a day, more than once, sometimes).

Scrapings of plaque were taken from the buccal surface of T16 and T36 (near the gingival margins) and placed on individual glass microscope slides. To each was added a drop of saline, which was mixed with the plaque and covered with a coverslip. Similar preparations were made of scrapings from any posterior carious lesion. The wet smears were examined immediately under a 40X objective for the presence of motile amoebae or flagellates and their presence or absence recorded.

RESULTS

Out of a total of 203 patients examined (123 females and 80 males), 33 (16.3%) harboured oral protozoa. Of these 14 (42.4%) were males, while 19 (57.6%) were females. Thus 17.5% of males were infected while 15.5% of females were infected. Ten (4.9%) harboured *Trichomonas tenax*, while 23 (11.4%) harboured *Entamoeba gingivalis*. Individuals aged 20 years or less were one third as infected (33.3%) as those above age 20 years (66. 7%) Table 1.

The highest overall prevalence was in the age group > 50 years (33.3%) followed by the age group 11-20 years (19.6%). The age group < 10 years had the lowest prevalence (10.5%) Table 1. Also with respect to sex, the highest prevalence was in the > 50 years age group. There was no increase in prevalence with respect to age. Seventy eight percent had plaques present on the index teeth while

58.3% had calculus. Individuals with calculus on 5 index teeth were more infected (23.2%) than those without calculus (P<0.25).

Although oral hygiene was generally poor, 51.5% claimed that they clean twice a day. There was no association between the occurrence of oral protozoa and the type of cleaning instrument used or the frequency of cleaning (Table II.)

Table 1: Age in relation to Prevalence of Protozoa

	<10	11-20	21-30	31-40	41-50	>50
Positive for protozoa	2	9	7	5	3	7
Negative for protozoa	17	37	53	40	19	14
Percentage Positive	10.5	19.6	11.7	14.3	13.7	33.3

Table 2: Teeth cleaning habits in relation to prevalence of protozoa

Frequency	Toothbrush	Chewing stick	Both
2 x day	9(47.4%)	4(50%)	3(50%)
1 x day	10(52.69)	3(37.5%)	3(50%)
Sometimes		1(12.5%)	

DISCUSSION.

The prevalence of *Entamoeba gingivalis* (11.4%) amongst the patients in the study was much lower than that reported in Kenya (1) and in American and European studies (16). As suggested by others (1), the proportion would have been higher if a culture technique (7) had been used for diagnosis. Although *Trichominas tenax* was encountered less commonly than *Entamoeba gingivalis*, the prevalence of 4.9% though higher than the 2.8% recorded in Kenya, is much lower than reported in other places (3,6,8,). This study thus suggests that *E. gingivalis* is more common than *T. tenax* in Nigerians

Although the highest prevalence in this study was found in the age group above 50 yrs, there was no overall increase in preva-

lence of protozoa with age as has been reported in other studies (5, 6). A possible explanation for this, may be due to the fact that there was not much difference in oral hygiene between the different age groups except in the > 50 yrs group, where oral hygiene was particularly poor coupled with greater prevalence of calculus in the age group. Chinge *et al* (1) and Wantland and colleagues (3) found a positive association between age and prevalence of protozoa in males. Such an association was not observed in the present study. The explanation of the previous authors was that older men tend to be less careful about oral hygiene than women and suggested that this may account for the higher prevalence of protozoa in men.

Several authors (1,4,9) have implicated the state of oral hygiene as being an important factor. In this study both the amount of calculus and the loss of attachment of periodontal fibres were associated with the presence of protozoa. Such an association had earlier been reported by others (1, 9) who found that infection with protozoa was directly proportional to the amount of calculus and to the progression of periodontal disease. No association was found between gingival bleeding and the presence of protozoa, similar to the study by Chinge *et al* (1) but in contrast to the report by others (10). Leo *et al* (11) explained that since gingival bleeding may occur as a result of bacterial dental plaque remaining in contact with the gingival tissues for a relatively short period, it's presence may not necessarily reflect a state of prolonged poor oral hygiene.

There was no association found between frequencies of teeth cleaning or the instrument used for cleaning and the occurrence of oral protozoa. The finding of dental plaque in 78.8% of the patients suggests that oral hygiene was generally poor.

These findings suggest that poor oral hygiene, calculus, old age and loss of attachment of periodontal fibres are factors, which favour the proliferation of *E. gingivals* similar to the report by others (1).

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EPIDEMIOLOGICAL STUDY OF URINARY SCHISTOSOMIASIS AMONG PRIMARY SCHOOL PUPILS IN EKITI STATE, NIGERIA

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The prevalence of *Schistosoma haematobium* infection was investigated among primary school pupils in Ekiti State by questionnaire survey in 601 schools between 1997 and 1998. A total of 9,551 (24.4%) were positive by the survey. 3483 (22.4%) of the girls and 6,069 (25.7%) of the boys were infected. The prevalence of this infection between girls and boys shows a significant difference ($x^2_{15} = 59.5$; $p<0.05$). Ekiti South West local government had the highest prevalence of S. *haematobium* infection of 69.0% while Ikole local government had the lowest prevalence of infection of 2.0%. Out of 1,049 pupils with clinical and laboratory examination, 280 (50.9%) of the 550 boys and 184 (36.9%) of the 499 girls were infected. Chi-square analysis shows a significant difference of S. *haematobium* infection between the girls and boys ($X^2_{11} = 86.2$; $p<0.05$). Chi square analysis showed that questionnaire survey could be used to predict the laboratory epidemiological data ($X^2_1 = 3.84$; $p<0.05$).

Key words: Schistosomiasis, Epidemiology, Infection, Bulinus globosus

INTRODUCTION

Schistosomiasis is a pathological condition caused by infection with Schistosoma parasites (1). The infection is widespread with a relatively low mortality rate but very high morbidity rate from severe debilitating illness in people all over the world (2,3). It is second to malaria in its socio-economic and public health implication while it remains about the most important of all water impounding diseases in tropical countries such as Nigeria (4).

Infection of man occurs mainly during washing of clothes or house utensils, swimming, bathing, fetching of water or other recreational activities in a body of infested water (5). The distribution and epidemiology of schistosomia-

sis has been documented in some parts of the world. Schistosoma infection is the second most prevalent tropical disease and leading cause of severe morbidity in several foci in Africa (2). Cases of *Schistosoma haematobium* infections in the dominant form are frequently encountered in schools (6,7,8), hospital and clinics in various parts of Nigeria (5,9) due to different forms of water supply, water contact and human behaviour (5,10,11).

Schwartz (12) associated the high incidence of squamous cell carcinoma of the bladder and cervix with urinary schistosomiasis. This is of great health concern. Pugh and Gilles (13) reported 13.5% prevalence of *Schistosoma haematobium* infection in the Ruwon Sanyi village area and 18.5% in Kuwungate, Kaduna, Nigeria. Ezejie and Adeserrano (11) observed over all prevalence of between 24.0% and 65% in school children in Ajara community of Badagary, Lagos. In Lagos State of Nigeria, 13.4% was reported for *Schistosoma haematobium* infection (14). Dennis *et al* (15) also reported an overall prevalence of 4.8% in school children in Liberia.

Since surveillance of diseases is an indispensable part of every successful disease control program, this study therefore aimed to investigate the epidemiology of urinary schistosomiasis among primary school pupils in Ekiti State, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in Ekiti state of Nigeria, which was created out of the old Ondo state in October 1996. The state lies between latitude 7°N and 8°N of the equator and longitude 4°E and 5°E (Fig.1). The study area is characterized by the presence of hills and rocks in addition to numerous rivers, springs and streams and the vegetation is mainly rainforest. The main stay of economy is farming. The state is divided into 16 local government areas with 601 primary schools and 286,501 pupils (Table 1).

Visitation and Health Talk

Prior to the collection of urine samples from primary school pupils, permission was obtained from Local Government Area Primary Education Board (SPEB) chairmen, to have health talks with the teach-

ers during their weekly regular meetings holding at the secretariat on the aetiology, mode of transmission, manifestation and health implication, pathogenesis and pathology of urinary schistosomiasis. Thereafter, statistics of the number of primary schools in each local government were collected from the head-teachers.

Administration of Questionnaire

After the health talks, all the headmasters agreed to educate the pupils in preparation for questionnaire. Subsequently, questionnaires were administered with the help of the headmaster and class teachers in each of the 601 primary schools with the aim of determining the prevalence rate of schistosomiasis.Thus, the individuals studied were mainly primary school pupils.

Laboratory Examination

One school was selected from endemic areas in each local government area. Urine samples were collected from school pupils between 11.00 and 14.00 hours, a period when the eggs of *Schistosoma haematobium* are concentrated in the urine. Pupils involved in this study were randomly selected using the class register in each endemic school to avoid bias.

Examination of the urine specimens was done qualitatively by using the haematrix strips. 10 ml of the urine was collected in a specimen bottle and the strip was dipped into the urine specimen for 20-30 seconds. The tip of the strip was then observed for blue colouration and this was compared with the chart to ascertain positivity or negativity of the test (16).

Detection of intermediate host

Freshwater habitats in the different communities in all local government areas were surveyed for the presence of potential intermediate snail host especially where water contact activities were pronounced.

Data Analysis

The data generated in the study were analyzed using Chi-square analysis.

RESULTS

In this study, 601 schools were surveyed to determine the occurrence and prevalence of urinary schistosomiasis in sixteen local government areas of Ekiti State, Nigeria. Two approaches were used; a survey by questionnaire and the laboratory examination of urine

specimens collected from pupils in selected schools. A total of 39,191 pupils surveyed by questionnaire included 15,552 females and 23,639 males. Of these, 9,551 (24.4%) were positive for *S. haematobium*. 3,483 (22.4%) of girls and 6,068 (25.7%) of the boys were infected (Table 1). Chi-square analysis showed that there is a significant difference in the prevalence of *S. haematobium* infection with sex by questionnaire survey. (X^2_{15} = 59.5; $p < 0.05$).

Table 1 also shows the prevalence of *S. haematobium* by sex and local government. Pupils in Ekiti Southwest had the highest prevalence (69.9%), Ekiti East (43.3%), Ilejemeje (32.0%) and Moba (26.0%) while Ikole had the lowest prevalence of 2.0%.

For the laboratory analysis, a school each was selected from the local government areas found to be endemic based on the result of the questionnaire survey. Samples were collected from pupils in each selected school in the twelve local government areas. Of 1,049 pupils samples examined, 464 (42.2%) had eggs of *S. haematobium* in their urine. 280 (50.9%) of the 550 boys and 184 (36.9%) of the 499 girls studied had schistosoma infection as depicted in Table 2. Statistical analysis using chi-square shows that there is a significant difference in the prevalence of *S. haematobium* infection between the male and female pupils (X^2_{15}= 86.2; $p< 0.05$) by laboratory test. A test of independence using a comparative chi- square analysis between positive result of laboratory and questionnaire survey of schistosomiasis shows that there is no difference in the outcome; hence survey can be used to predict laboratory epidemiological data ($x^2_1 = 3.84$; $p<0.05$).

Table 1: Schistomiasis survey by questionnaire in Ekiti State.

S/N	LGA	Total No of School	Total No of Pupils	Total No of pupils tested	No of Male	No of Female	Total No of pupils positive (%)	No of Male positive (%)	No of female positive (%)
1.	Ise Orun	27	34.595	1640	920	720	115(7.0)	58(6.3)	57(7.9)
2.	Gboyin	28	16.781	2087	1087	1000	164(7.9)	90(8.2)	74(7.4)
3.	Ekiti S/W	48	24.155	3863	2363	1500	2674(69.2)	1374(58.1)	1300(86.7)
4.	Ado-Ekiti	58	34.595	5129	2730	2399	845(16.5)	652(23.9)	193(8.0)
5.	Ikere-Ekiti	82	20.398	2978	1588	1390	666(22.4)	316(19.9)	350(25.2)
6.	Ekiti East	22	13.112	1820	1220	600	779(42.8)	480(39.3)	299(49.8)
7.	Ekiti West	45	19.947	3053	2066	987	369(12.1)	249(12.1)	120(12.2)
8.	Emure	25	8.448	1593	1071	522	57(3.6)	30(2.8)	27(5.2)
9.	Ikole	41	24.057	3238	1950	1288	62(1.9)	31(1.6)	31(2.4)
10.	Oye	40	19.944	3085	2085	1000	91(2.9)	60(2.9)	31(3.1)
11.	Irepodun/Ifedore	31	20.360	2507	1453	1054	104(4.1)	70(4.8)	34(3.2)
12.	Efon	19	10.253	1023	623	400	123(12.0)	70(11.2)	53(13.3)
13.	Ido-Osi	41	17.167	2167	1183	984	87(4.0)	57(4.8)	30(3.0)
14.	Ilejemeje	9	4,037	607	420	187	194(32.0)	52(12.4)	142(75.9)
15.	Ijero	53	22,359	2559	1559	1000	461(18.0)	250(16.0)	211(21.1)
16.	Moba	32	17,422	1842	1321	521	479(26.0)	259(19.6)	220(42.2)
	TOTAL	601	286,501	39,191	23,639	15,52	9551(24.4)	6068(25.7)	3483(22.4)

Table 2: Prevalence of *Schistosoma haemotobiun* among primary school children determined by laboratory test in Ekiti State

S/N	LGA	No of male examined	No of male infected (%)	No of female examined	No of female infected (%)	Total no Examined	Total no infected (%)
1.	Ado-Ekiti	49	38(77.6)	41	24(58.5)	90	62(68.9)
2.	Gboyin	41	18(43.9)	44	16(36.4)	85	34(40.0)
3.	Ekiti S/W	54	51(94.4)	39	27(69.2)	93	78(83.0)
4.	Ikere-Ekiti	50	40(80.0)	40	21(52.5)	90	61(67.8)
5.	Ekiti East	56	37(66.7)	41	34(82.9)	90	71(78.9)
6.	Ekiti West	48	27(56.30)	42	34(81.1)	90	61(67.8)
7.	Emure	44	9(20.5)	39	1(2.6)	83	10(12.1)
8.	Ikole	50	4(8.0)	40	0(0.0)	90	4(4.4)
9.	Oye	39	4(35.9)	44	6(13.6)	90	10(11.1)
10.	Irepodun/Ifedore	42	14(33.3)	38	10(26.3)	80	24(30.0)
11.	Efon	39	18(46.2)	51	2(3.9)	90	20(22.2)
12.	Ise/Orun	38	20(52.6)	40	9(22.5)	78	29(37.2)
	TOTAL	550	280(50.9)	449	184(36.9)	1049	464(42.2)

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Table 3: Survey of freshwater habitats for *Bulinus globosus* in the study areas

S/N	LGA	Total No of stream in the LGA	Total No of stream positive for *Bulinus globosus (%)*
1.	Ise-Orun	304	10(3.3)
2.	Gboyin	448	81(18.1)
3.	Ekiti S/W	503	72(14.3)
4.	Ado-Ekiti	745	163(21.9)
5.	Ikere-Ekiti	402	62(15.4)
6.	Ekiti East	271	10(3.7)
7.	Ekiti West	534	82(15.4)
8.	Emure	203	40(19.7)
9.	Ikole	557	71(12.7)
10.	Oye	502	80(15.9)
11.	Irepodun/Ifedore	441	70(15.9)
12.	Efon	197	52(26.4)
13.	Ido-Osi	495	72(14.5)
14.	Ilejemeje	86	19(22.1)
15.	Ijero	600	81(13.5)
16.	Moba	349	60(17.2)
	TOTAL	6,637	1025(15.4)

DISCUSSION

This study has shown that schistosomiasis is prevalent in Ekiti State, Nigeria. This is in consonance with earlier reports (17,18) that *S. haematobium* is known to be endemic throughout Nigeria. The prevalence of infection varied from one locality to the other as observed elsewhere; 24.0% (11); 26.6% and 36.8% (19), although these prevalent rates reported were however lower than the peak values of 83.0% of infection recorded in this study. The variation in the rate of infection in the different local government areas and between the sexes may suggest that there are differences in their socio-cultural background in terms of provision of potable water in individual community (20). The preva-

lence of *S. haematobium* infection is higher in males than their female counterpart in the population sampled. This confirms the activities of the boys at the active transmission sites observed in most of the local government areas especially in Ekiti Southwest local government areas where males were frequently seen participating in swimming, washing, fishing, bathing and recreating in the streams (21,22). In contrary, Edungbola *et al* (5, 23) recorded a greater participation of females in Babaena district of Kwara State, Nigeria.

It was noted that the high level of illiteracy, ignorance and conservative beliefs paved way for continuity in the transmission of the infection in the study areas. It is an old belief that when black dog urinates in the streams, which is later crossed by an individual, such individual would pass blood in his urine. In view of this, basic health education on the transmission must be the focus of prevention before any measure can be implemented. This may be followed by the provision of pipe-borne water, which actually has been the demand of the populace, even though according to their belief, water is linked with the transmission of schistosomiasis. Mass treatment of all infected individuals with the symptoms through free medi-care by Government in the three tiers would save lives and help to eradicate the disease in the area.

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STUDIES ON THE BEHAVIOUR OF SOME IONS IN THE HEART OF RATS INFECTED WITH TRYPANOSOMA BRUCEI BRUCEI

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Behaviour of Sodium [Na^+], Potassium [K^+] and Phosphorous [$PO4^{3-}$] ions was studied in the heart of albino rats infected with *Trypanosoma brucei brucei* and the parasitaemia level monitored. Post infection shows a significant rise [$p < 0.05$] in Na^+, K^+, and Ca^{2+} ions concentration with a significant decrease ($p < 0.05$) in the phosphorous ions concentration as the disease progresses. At high parasitaemia level, there is a slight increase in Na^+ and $PO4^{3-}$ ions while K^+ concentration remains constant and Ca^{2+} ions concentration was significantly reduced ($p < 0.05$). The reasons and mechanism responsible for these are unknown although their increased concentrations in tissues generally have been shown to be as a result of damage to cells and tissues during infection.

Key words: Trypanosoma brucei brucei, Parasitaemia level, Concentration of ions.

INTRODUCTION

Some ions such as sodium, potassium and calcium have been discovered to be the most important components of extra-cellular and intracellular fluids of most tissues in animal. They are involved in various physiological roles and their concentrations in pathological states of animals have served as a good parameter in clinical diagnosis especially in *Trypanosoma b. brucei* infection where for example the parasite infectivity potential and toxicological effects have been based on maintenance of calcium

homeostasis (1, 2, 3).

With a unique ability to maintain the cellular contents and ions stable concentrations, *Trypanosoma b. brucei,* to which humans have become refractory on the basis of a high density lipoprotein that is toxic to the parasites, ravages cattle and sheep over millions of square miles in Africa (1). Although, these ions have been established as necessary requirement by the infecting parasite, their concentrations in the host are equally affected during infection.

MATERIALS AND METHODS

Seventeen Albino rats (*Rattus norvegicus*) of mixed sexes weighing between 200-300g obtained from animal house, Biochemistry Department, University of Ilorin were used for this work. These were randomly selected into three groups: uninfected (control), infected (test) and infected but used to monitor the parasitaemia level.

The parasite strain (ILRAD 1807 maintained in monkey host) were obtained from the Veterinary Pathology Department, University of Ibadan and used for inoculation of rats with phosphate saline glucose (PSG). This serves as a buffer for keeping the trypanosome alive and to dilute the parasitaemia level obtained from the rat blood samples to 2 – 5 trypanosome per view, which was used to inoculate the animal intra peritoneally with syringe containing 0.5 ml of the inoculum. Feeding and other sanitary conditions were maintained as it was before inoculation. Parasitaemia level was observed on daily basis through blood smears.

Animals were anaesthesized with chloroform and sequentially sacrificed every other day. Organs were then collected after sacrificing the animal and placed in 0.25M sucrose solutions for homogenization using pestle and mortar for subsequent analysis for sodium, potassium, calcium and phosphorous ions in the heart.

Cations determination in heart homogenate was carried out by flame photometry for Na^+ and K^+ based on the principle that these ions emit light when aspirated into a burner. The light emitted passes through a filter into a photosensitive element to produce current. The amount of current produced is proportional to the concentration of the ions in the sample. The calcium

ions were determined by complexometric method (4). Calcium reacts with cresophthalein comphexone in alkaline medium to give a purple colour, which is estimated colorimetrically at 580nm. Phosphorus ions in heart homogenates were determined by the method of Fisher and Subbarows (5). Phosphate reacts with acid molybdate to form phosphomolybdic acid. The hexavalent molybdenum of the phosphomolybdic acid then react with malachite green (reducing agent) to give a green colour read at 620nm, the intensity of which is proportional to the concentration of phosphate ions present in the sample.

RESULT

The parasitaemia level as infection progresses is shown in Fig 1. Day 0 represents the day inoculation was done. The pre-patent period was approximately 48 hours, an objective measurement susceptible to experimental analysis (6). The graph is an indication of alternating rise and fall in parasitaemia level until eventual death of the animal. Fig 2, 3, and 4 shows the level of sodium, potassium and calcium ions concentration in the heart homogenate upon infection. The results shows significant rise ($p < 0.05$) in the ion concentration while this is followed by fluctuations in the levels until the eventual death of the animal. Fig. 5 shows phosphorus ions concentration in the heart homogenate with a significant decrease ($p < 0.05$) in its concentration followed by a period of increased level and eventual fluctuation in concentration.

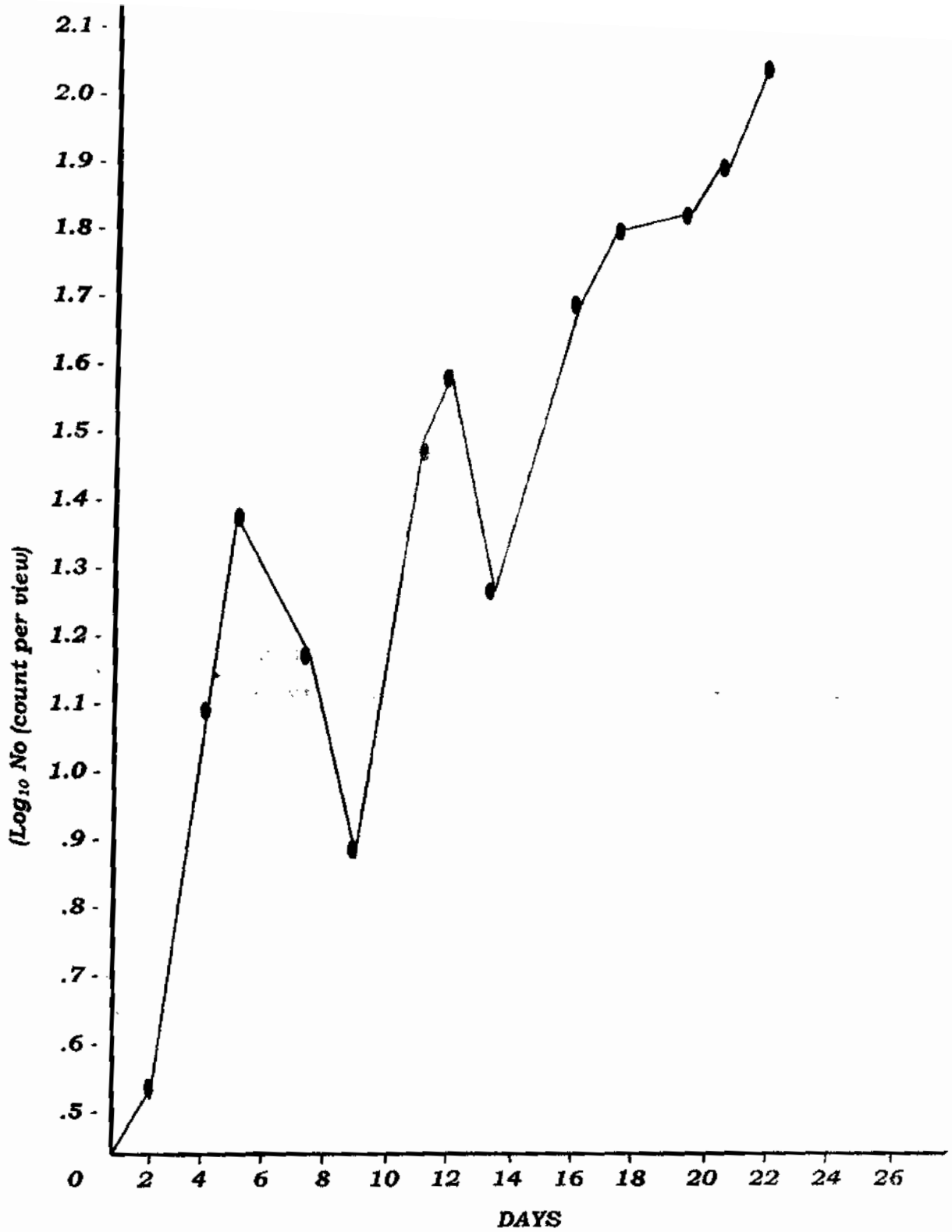

Fig. 1: A Plot of parasitaemia level as disease progresses

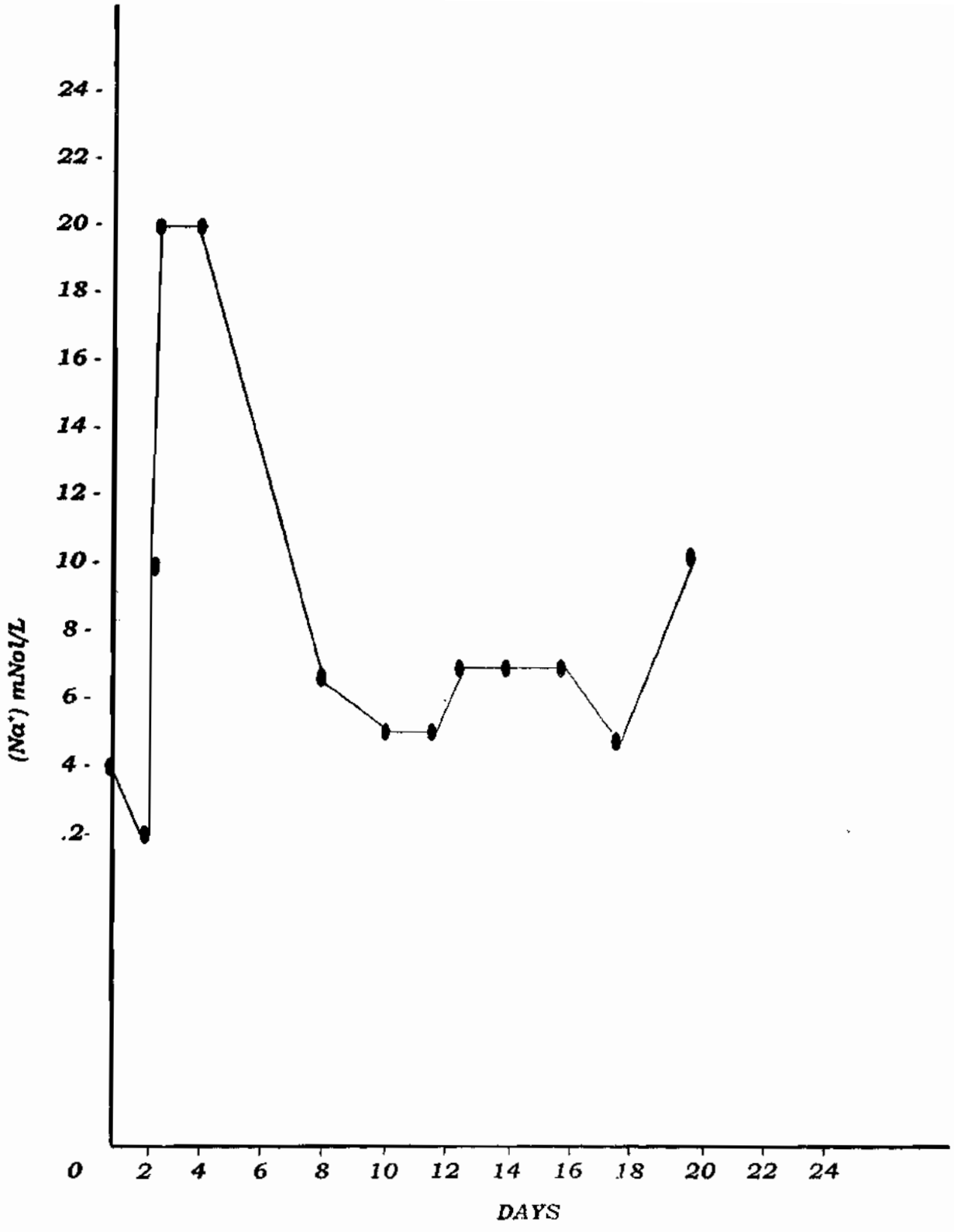

Fig. 2: Sodium ion concentration in heart during Trypanosome infection in rats

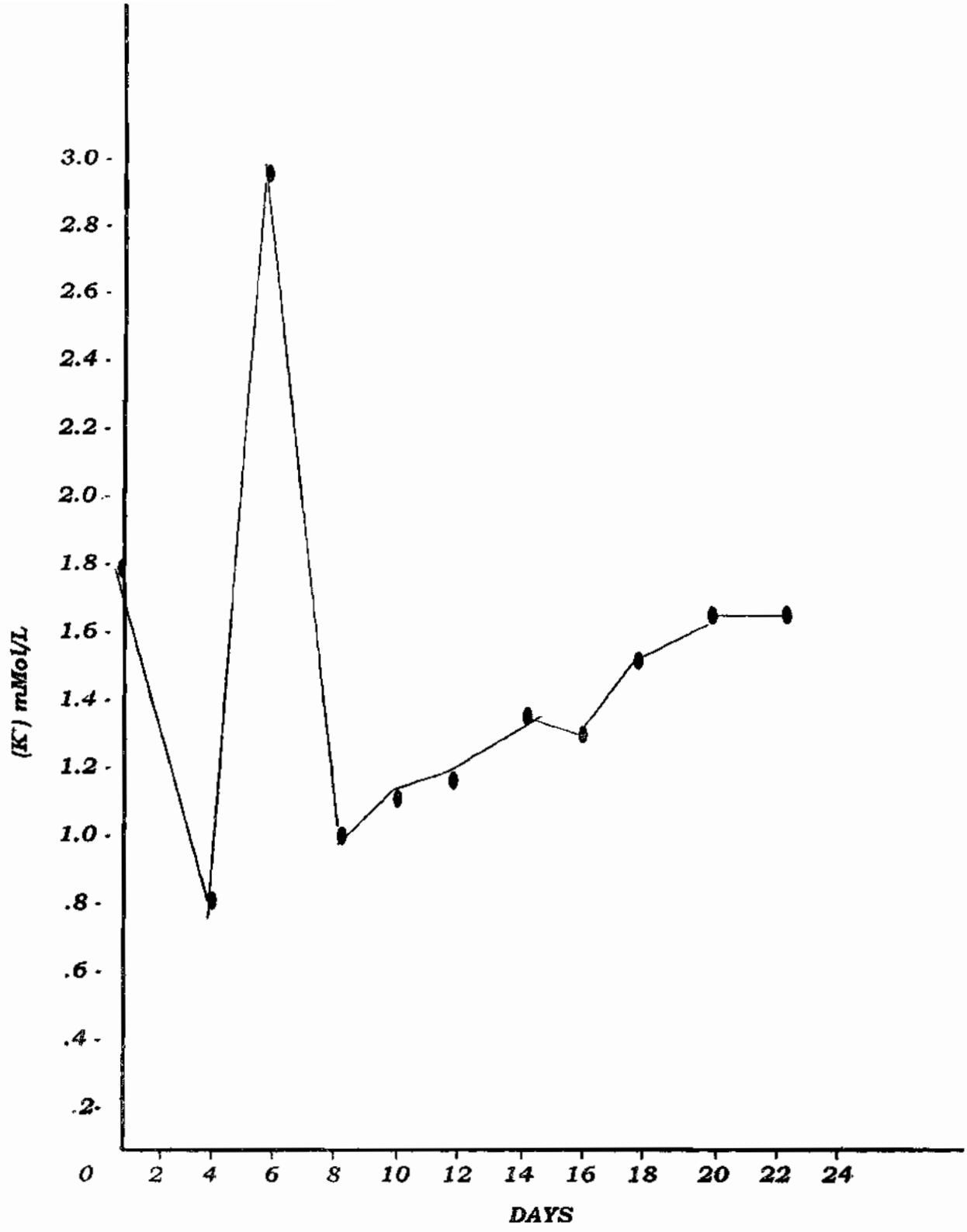

Fig. 3: Potassium ion concentration in heart During Trypanosome infection in rats.

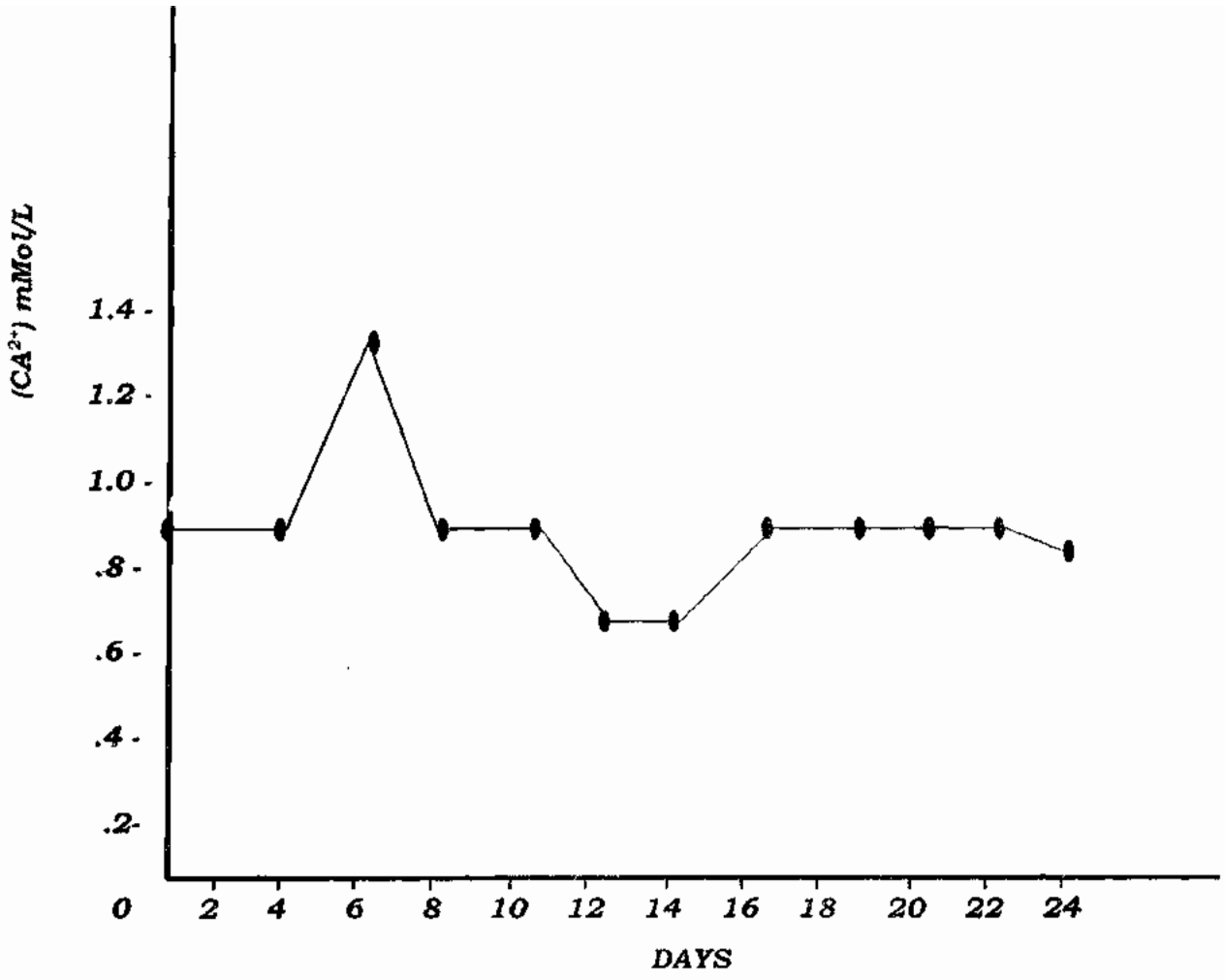

Fig. 4: Calcium ion concentration in heart During Trypanosome infection in rats.

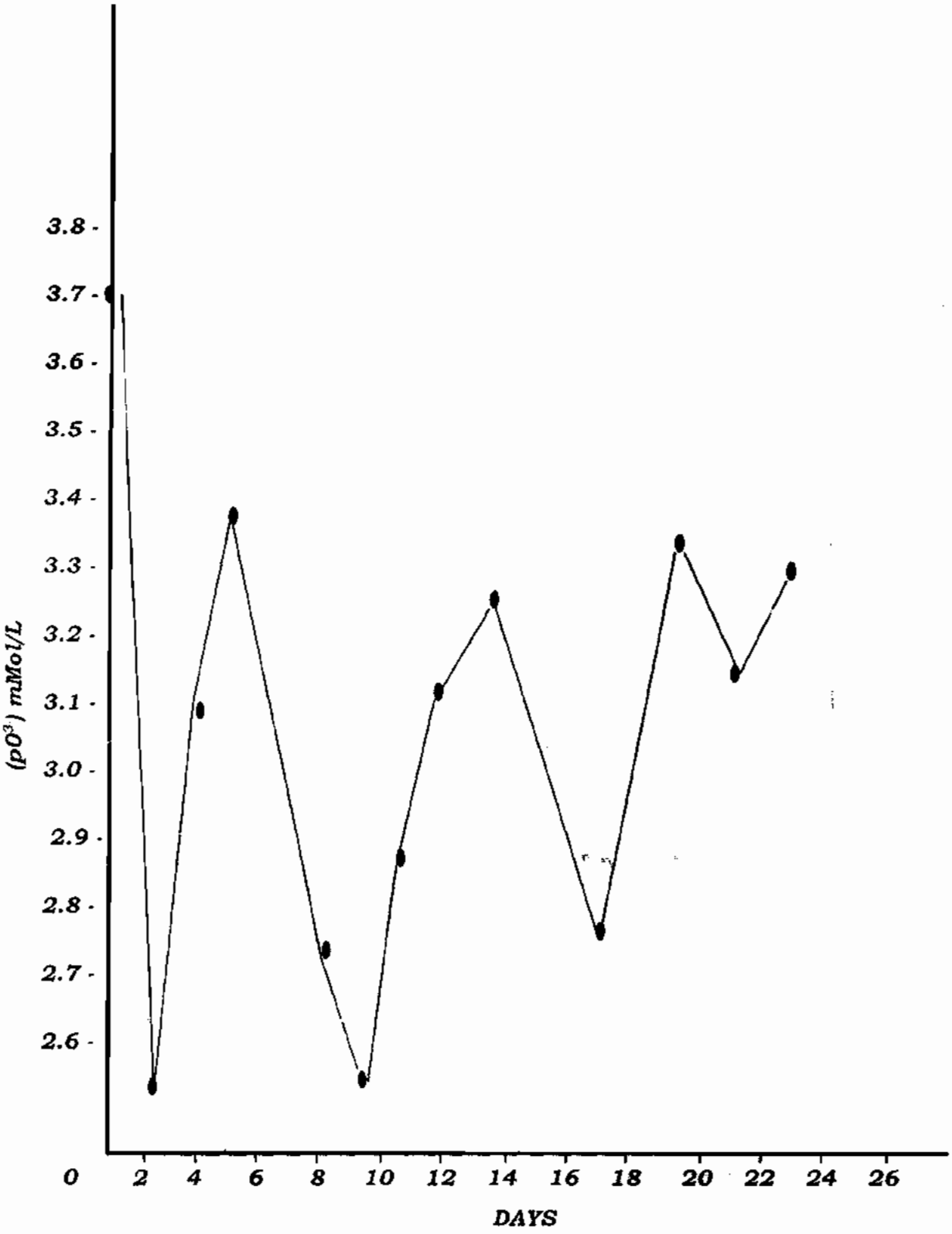

Fig. 5: Phosphorus ion concentration in heart During Trypanosome infection in rats.

DISCUSSION

The parasitaemia level as monitored on daily basis in Fig 1 after the pre patent period shows a significant increase in the first few days of infection and this is followed by periods of fluctuations and remissions in which the parasite population were decreased. This decrease might have been due to the production of antibodies, which suppresses further production of the trypanosomes. However after this initial stage, a significant rise ($p < 0.05$) in parasitaemia level was noted leading to the death of the animals. This is probably due to the immune evasion mechanism of the parasites, which renders the immune response to earlier generation of trypanosomes ineffective (1,10)

The significant rise ($p < 0.05$) of Na^+, K^+ and Ca^{2+} ions concentration shown graphically post infection is an indication of damages done to the cells and tissues of the host being infected by trypanosomes (7, 8). The decrease and fluctuations in concentration of these ions as the disease progresses may be explained by the antigenic variation property of the parasite in which as variants of the parasite are being destroyed by the host, others undergo a genetic rearrangements to escape the host immune system for a while usually through changes in membrane components in successive generations. The consequent response of the host immune system on recognizing these variants leads to killing of these parasites due to its earlier stimulation by the parasite (1).

At high parasitaemia level with consequent death of the animal, Na^+ and $PO4^{3-}$ ions were not significantly increased while K^+ ions remain constant as Ca^{2+} ions concentration also decrease. The reasons responsible for these were unknown, although calcium ions have been shown to have influence on the cytoskeleton and morphology of the nucleolus in *T. b. brucei* (9). This study has shown the need to understand the basis and mechanisms responsible for behaviour of these ions during infection by *T. b. brucei*, which can be used as clinical parameters for diagnosis in higher animals.

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KALA-AZAR IN A NIGERIAN: REPORT OF A CASE WITH A FATAL OUTCOME

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A case of visceral leishmaniasis (Kala-azar) in a 60-year-old Nigerian female is presented. The clinical findings were fever, weight loss, lympadenopathy, hepatomegaly, and self-healing cutaneous ulcers. Laboratory findings included severe anaemia, lymphocytosis and amastigotes in the blood smear. The patient died before she could be commenced on the pentavalent antimonial specific for the disease.

INTRODUCTION

Leishmaniasis is caused by protozoa of the leishmania species. It is usually zoonotic and involves rodents, canines and various forest mammals. It is transmitted by the phlebotomine sand flies with incubation period of several months. The disease in man is usually cutaneous, mucocutaneous or visceral (Kala-azar). In Africa there are only few accurate statistics on visceral leishmaniasis (1). It is endemic in rural Sudan, and Kenya. The disease is rare in sub-Saharan West Africa. We therefore report here the first documented case of Kala-azar in Nigeria.

CASE REPORT.

A.D (Hospital No. 185654) was a 60-year old housewife and a farmer from one of the Southwestern states. She was admitted with a 9-month history of high-grade intermittent fever, excessive sweating, and difficulty in swallowing. Other symptoms included recurrent self-healing crusting lesions on the face and upper trunk. She also had progressive weight loss. She had not noticed similar skin lesion in her local community. Examination revealed a wasted elderly woman. She was pale and febrile (T-38.8°c). There were multiple ulcers on the face and upper trunk. Some were fresh, some were covered with

scabs and some already healed leaving hypopigmented patches on the skin. There were both axillary and cervical adenopathy. The respiratory rate was 20 breaths/min and the lung fields were initially clear. The pulse rate was 88 beats/min and the blood pressure was 110/70mmHg with normal first and second heart sounds. The abdomen was soft and non-tender and there was hepatomegaly of 5cm below the right coastal margin. The spleen and kidneys were not palpably enlarged. A tentative diagnosis of disseminated tuberculosis was made with differential diagnoses of leishmaniasis (visceral and cutaneous) and deep mycotic infection. Her laboratory results were as follows: PCV-17%, WBC-10.3 x 10^9/L (neutrophils-30%, lymphocytes-70%) and ESR-27mm/hr. Her electrolytes, urea and creatinine estimation were within the normal ranges. The chest X-ray was normal; barium swallow without fluoroscopy monitor was normal. Antibody against human immune deficiency virus infection (HIV) using Enzyme Linked Immunosorbent Assay (ELISA) technique was negative. Buffy coat blood smear was teaming with amastigotes on two occasions.

Fig 1 is a Giemsa stained buffy coat blood smear from our patient as seen under oil immersion of a microscope (X1000). The macrophages contained numerous amastigotes.

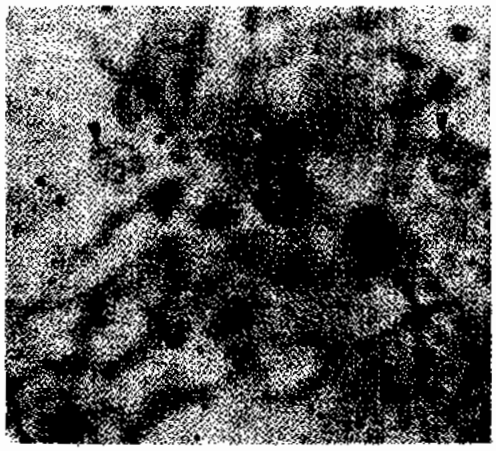

Fig 1

Cutaneous ulcer scraping and swabs were negative for amastigotes as well as for acid and alcohol fast bacilli.

Treatment outcome

The treatments given were both supportive and specific. She was transfused with 3 units of packed red cells with 80 mg of intravenous Frusemide (Lasix) preceding each unit over 4-6 hours. She also had high protein diet with multivitamins supplementation. She had oral Levamisole 120 mg stat, oral

Mebendazole 100 mg twice daily for 2 weeks and oral Biltricide 2.4 gm stat. These were given pending the availability of Antimony Sodium Stilboglucuonate (10mg/kg daily for 3 weeks), which she never had due to non-availability coupled with financial constraints. Pentostam was very expensive and not readily available locally. The patient succumbed to superimposed chest infection before the arrival of the Pentostam ordered from abroad. This was 2 months after the diagnosis was established. A request for postmortem examination was denied by the relatives.

DISCUSSION.

Visceral leishmaniasis (Kala-azar) is caused by *Leishmania donovani.* It is endemic in Asia, the Mediterranean, South America, East and North Africa. It is relatively rare in sub-Saharan West Africa with occasional cases from Sene-Gambia (2), Togo (3) and Chad Republic (4). There are about 500,000 new cases of Kala-azar reported annually (5). It is endemic in about 62 countries around the world. It is spreading in several new areas owing to epidemiological changes, such as urbanization and mass migration of people. There are various clinical forms of Kala-azar in different localities. Two forms of transmission have been observed; the urban form where transmission is primarily human-to-human, and the rural form where transmission is primarily zoonotic. In Africa, a rural form of transmission is generally seen. The patient in this report is presumed to have acquired the infection by zoonotic means from rodents or ground squirrel, which are the usual reservoir of the parasites in this part of the world. The infection in her is also a sporadic human infection that is characteristics of zoonotic infection. The Africa Kala-azar differs in several ways from those seen elsewhere. Skin lesions are characteristic. These may represent healed ulcerations at the sites of the initial infection, or in some cases represent parasites reinvasion of the skin producing macular and nodular skin lesions. This is called dermal leishmanoid. Because of the dermatotrophic nature of *L. donovani* strain in this area, leishman skin test is often positive and parasites may be found in these dermal lesions. There were multiple superficial ulcers on the face and upper trunk of

this patient, but amastigotes could not be recovered from them. This may be as a result of paucity of parasites in the superficial lesions. However, in Sudan where a number of oral lesions were associated with visceral leishmaniasis, parasites were found in abundance in the oral lesions, but not in the enlarged spleen or liver. Such cases may represent intermediate condition of parasites virulence and host resistance between typical Kala-azar and cutaneous leishmaniasis (6). This case report presented with the symptoms and signs of African form of Kala-azar i.e. fever, weight loss, anaemia, visceromegaly, polyadenopathy and multiple skin lesions. Some infected patients may remain asymptomatic for a long time until they have their immunity lowered by malnutrition or some other tropical conditions and more recently HIV infection. Indeed overlapping of visceral leishmaniasis and AIDS had been documented (7). The risk of co-infection in Africa is less. Only one case each has been reported in Cameroon and Guinea-Bissau (8). However, this risk may soon be heightened in the sub region because of mass migration of people due to civil wars, famine and high rate of prostitution among the populace. By extension we may start to see more cases in Nigeria. Laboratory diagnosis of leishmaniasis is generally based on smears or histopathology and additional clinical information to characterize the species. Molecular studies with restriction endonucleases and isoenzymes pattern should ultimately provide a sound biochemical basis for identification and differentiation (9,10). Morphologic diagnosis is the most accepted for the identification of leishmania, which are intracellular parasites. They are typically found in the vacuoles of mononuclear cells or macrophages. In tissue section or smear stained with Giemsa, the parasites are identified by the presence of dark staining kinetoplast and a lighter staining nucleus. *L. donovani* is usually diagnosed in specimens from liver, spleen, bone marrow or lymph node. Scrapings from cutaneous or mucocutaneous ulcers must be taken from active margin of the lesions (11). Culture of the blood in Kala-azar aspirates or scraping in cutaneous lesions is definitive (12). Serologic tests may be of value in visceral leishmaniasis, but is of limited value in cuta-

neous disease. The most frequently used serologic test is indirect immunofloresence (IIF). The older complement fixation (CFT) test using mycobacterial antigen is also useful (13). The diagnosis was established in our patient by Giemsa staining of the buffy coat smear, in keeping with the fact that parasites were frequently found in the peripheral blood film of Africans with Kala-azar. For example, parasitaemia was detected in 15 (75%) of the 20 patients with Kala-azar in Kenya (14). The rarity of this condition in our environment is underlined by the non-availability of appropriate medication in most cities in our areas of practice. With increased awareness and diagnosis of Kala-azar in Nigerian patients, it is expected that the relevant drugs will be more readily available. In conclusion, practicing physicians in Africa are advised to consider Kala-azar in cases of pyrexia of unknown origin (PUO) especially now that it has been established to be an opportunistic infection in patients with AIDS.

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MANIFESTATIONS OF AGGRESSIVE ATYPICAL KAPOSI'S SARCOMA [AAKS] IN HIV DISEASE PATIENTS SEEN IN MAIDUGURI, NORTH-EASTERN NIGERIA.

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Infection by the human immunodeficiency virus (HIV) has since the mid-1980's been known to distinguish atypical, aggressive Kaposi's sarcoma (AAKS) from the endemic type in Africa. In our series at the University of Maiduguri Teaching Hospital, we recorded 44 patients with AAKS, 35 of them male and 9 female, giving an M: F ratio approximately 4:1. The peak age groups for the males were 21-30 years, and 31-40 years, while for the females it was 21-30 years. The site distribution of AAKS lesions was predominantly the lower limb (70.5%) followed by the upper limb (9.1%); those with multiple site lesions (nose, face, oral cavity, penis and trunk) also accounted for 9.1% of the cases. The commonest clinical features manifested by the patients were fever (100%), weight loss (86.8%) skin nodules (86.4%), and diarrhoea (55.3%). Virtually, all occupational groups were affected, with students, civil servants and businessmen topping the list.

Key words: Atypical Aggressive Kaposi's sarcoma, HIV infection

INTRODUCTION

From the mid-1980's aggressive, atypical Kaposi's sarcoma (AAKS) has been known to be associated with HIV infection in some African countries (1-3). In a comprehensive review on the AIDS virus, Gallo in 1987 (4) traced the history of evolution of observations that led to the conclusion that KS is associated with HIV infection. According to Gallo's review, KS was the first sign that a new disease was afoot in the USA. This was because KS, in an aggressive form, was appearing in young, middle-class homosexual men in the USA. Hitherto endemic KS, a slowly developing cancer, was only known to occur among the elderly in Europe, the Mediterranean, and parts of Africa. We report here on a series of patients with HIV-associated KS seen

in Maiduguri, North-eastern Nigeria from September 1994 to March 2003, studied prospectively.

SUBJECTS AND METHODS

Study area

Clinical and laboratory investigations were carried out at the University of Maiduguri Teaching Hospital (UMTH) located in Maiduguri. Maiduguri is the capital of Borno State, one of the 36 states, which together with the Federal Capital Territory, make up the Federal Republic of Nigeria. The UMTH is the major referral centre for the six states that constitute the North-eastern geopolitical zone of Nigeria. In addition, patients come to UMTH from Chad and Cameroon Republics, two countries that share borders with Borno State.

HIV serology

Two tests for the presence of antibodies against HIV were carried out on the serum of each study patient. A patient was regarded as HIV-seropositive if he or she tested positive by both tests. About half of the study subjects were tested by a combination of ELISA and Western blot (WB), while the rest were tested by a combination of a latex agglutination test and an immune chromatographic test. Kits for all four types of tests had been evaluated in Nigeria and approved for use by the AIDS Control Programme of tne Federal Ministry of Health. About 5ml of venous blood was drawn from each patient. The blood was allowed to clot at room temperature, and then centrifuged at 1,000xg for 10 minutes. Serum was then aspirated into a cryovial and stored at -20^{0}C until tested.

a. ELISA plus WB

The first 23 patients were tested by this combination. ELISA test was carried out using Wellcozyme HIV 1+2 kits manufactured by Murex Diagnostics Ltd, Dartford, England. Sera found positive by ELISA were subsequently subjected to the Western blot (WB) test. Kits for WB test were Bio-Rad Novapath HIV immunoblot manufactured by Bio-Rad Clinical Division, Hercules, California, U.S.A. for HIV-1, and New Lavblot II from Diagnostics Pasteur, Marnes-la Coquette, France for HIV-2.

b. Agglutination plus immune chromatography

Each serum was initially tested by Capillus HIV-1/HIV-2, which is a latex agglutination test. The kit is a product of Trinity Biotech PLC. Brag Co Wicklow, Ireland. Sera

positive by Capillus were then tested by the Abbott Determine HIV-1/2 test system, a product of ABBOTT laboratories, Diagnostics Division, Abbott Park, Illinois, USA.

Histological examination

The tissue specimens of all patients were fixed in 10% formal saline; several blocks of tissues were embedded in paraffin wax and 5-micron sections were cut. The sections were stained with haematoxylin and eosin (H&E) stains. The diagnosis of Kaposi's sarcoma is based on the 1985 histologic classification system (5), which states the variants as: (a) Granulomatous KS, (b) Angiomatous KS, (c) Sarcomatous KS and (d) Mixed KS.

RESULTS

A total of 44 cases of AAKS were diagnosed within the period of study. There were 35 males and 9 females, giving a male: female ratio 3.9:1. The peak age group is the 3rd and 4th decades of life, which accounted for 40.9% and 36.4% respectively. The lesion is rare before the 1st and after the 5th decades of life. The youngest patient was a 3-year-old boy who presented with inguinal lymph node enlargement (Table-1 and Figure 5). The site distribution of AAKS is predominantly the foot and leg (lower limb), which accounted for 31 cases (70.5% of all cases); 59% (26 cases) were males while 11.4% (5 cases) were females. The upper limb accounted for 4 cases (9.1% of all sites) with no sex bias. Four cases of multiple sites were seen in only males. Three cases of lymph nodes (2-axillary and one cervical lymph nodes) were seen (Table 2).

On the whole, 44 patients were studied, but information on fever, weight loss and diarrhoea is available in respect of only 38 patients. Fever was the most frequent clinical feature in the patients. Weight loss was also a frequent occurrence seen in 86.8% of the patients, and 86.4% of them had skin nodules at various sites. Diarrhoea and oral lesions occurred in 55.3% and 45.5% respectively. Lymphadenopathy was the least common presentation, seen in only 6 patients (13.6%). Table 3 summarizes these observations. All occupational groups were virtually affected, with students, civil servants, and businessmen topping the list as shown in Table 4.

Table 5 shows the various histological patterns of Kaposi's sar-

coma. Mixed patterns accounted for 63.6% of the cases. Spindle cell predominance accounted for 27.3%, while angiomatous and granulomatous variants accounted for 4.5% each.

Figures 1 to 5 show representative sites of manifestations of AAKS in our series. Figures 1 and 2 are the same patient, a 38 yr old man, presenting with multiple-site nodules. Figure 3 is predominantly on the buttocks and trunk. Figure 4 shows a fungating (ulcerated) nodule on the leg of a 30 year old man. Figure 5 is a 3-year-old boy presenting with inguinal lymphadenopathy, biopsy of which revealed KS

Table 1: Age and sex distribution of AAKS cases

Age Group	Male	Female	Total	%
0-10	1	1	2	4.5
11-20	3	1	4	9.1
21-30	12	6	18	40.9
31-40	15	1	16	36.4
41-50	3	0	3	6.8
51>	1	0	1	2.3
Total	35	9	44	100

Table 2: Sex and site distribution of AAKS cases

Site	Male	Female	Total	%
Upper Limb	2	2	4	9.1
Lower limb	26	5	31	70.5
Trunk	2	0	2	4.5
Lymph node	1	2	3	6.8
Multiple	4	0	4	9.1
Total	35	9	44	100

Table 3: Clinical features of the patients

Features	Patients	No manifesting	% manifesting
Fever	38	38	100
Weight Loss	38	33	86.8
Skin nodules	44	38	86.4
Diarrhoea	38	21	55.3
Oral lesions	44	20	45.5
Lymphadenopathy	44	6	13.6

Table 4: Occupational groups of the patients

Occupation	n
Student	8
Civil servant	7
Business	6
Driver	3
Farmer	2
Lawyer	2
Teacher	2
Banker	2
Pupil	2
Military	1
Mechanic	1
Unemployed	2
NA	6
Total	**44**

NA = Not available

Table 5: Histological patterns of 44 biopsies of AAKS

Histologic patterns	Cases	
	n	%
Spindle cell predominant	12	27.3
Angiomatous	2	4.5
Mixed	28	63.6
Granulomatous	2	4.5

Figure 1. A 38-year-old man presenting with nodules on the face (plus oral cavity, chest and legs)

Figure 2. The same patient as in Fig. 1, here showing nodules with severe oedema on the left leg.

Figure 3. A 36-year- old man presenting with generalized, multiple-site nodules, predominantly on the buttocks and trunk.

Figure 4. A fungating, ulcerated nodule on the leg (popliteal fossa) of a 30-year-old man.

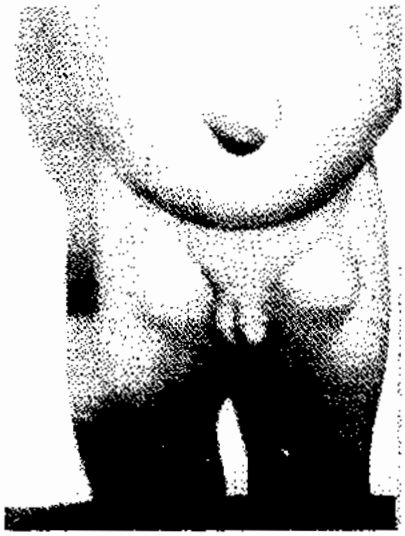

Figure 5. A 3-year-old boy presenting with inguinal lymphadenopathy. Biopsy revealed KS. The boy and both of his parents

DISCUSSION

AIDS-associated KS and other forms of KS have similar histologic features. The clinical history, physical features, and result are the basic parameters possibly suggesting the type of KS. However, KS associated with AIDS commonly presents with variable histologic features based on the stage of the disease and this may have other differential diagnoses. Whether early KS lesions represent a transitional stage of preneoplastic and hyperplastic nodules that will evolve into a true neoplasm is still unresolved and raises interesting issues about the biogenesis of KS. Much evidence has accumulated in favour of the concept that KS begins as a hyperplastic lesion of activated cells that may regress or progress, depending on the host's immune status and the availability of continuous proliferate stimuli. HIV could provide both the mitogenic stimulation through chronic antigenic stimulation and a dysfunction in the host's immune defense mechanisms such as cytotoxic T–Lymphocytes (6). Epidemic KS has been recognized since 1981 in homosexual men and has been associated with HIV infection and was first described in young male homosexuals (7). It affects both sexes with predominance of males over females in Africa, probably due to socioeconomic behaviour in the African society. The pattern of AIDS-associated KS in our study favours the lower extremities that are similar to the classical type. Four of our patients had multiple site involvement, which include the tip of the nose, face, oral cavity, glans penis and trunk. The frequency of large fungating, florid tumour mass with extensive ulceration, haemorrhage and gross oedema of the foot should probably be taken as evidence of aggressive lesion with silent involvement of the deeper tissues. This aggressive lesion is rarely seen in the endemic

KS. When the epidemic KS exclusively involves the lymph nodes, it is more likely that internal organs are also involved and this is probably considered as evidence of aggressive disseminated lesion. In this study we also report three cases with two axillary and one inguinal lymph node KS in adults and a child respectively. However, lymph node involvement has been reported in endemic KS without skin lesion, mainly in children usually with a rapidly fatal course (8,9). It has been reported in association with lymphoreticular tumours and immunosuppressive therapy (10). The behaviour of AAKS ranges from slowly indolent, relatively benign skin nodules, simulating the classic endemic KS, to more commonly aggressive disseminated malignancy, which has been seen in 50% of patients, the average survival of approximately 18 months. KS progresses in an orderly fashion from few nodules in the early stages to innumerable, rapidly progressing nodules, and finally to systemic lesions. HIV infections is associated with generalized aggressive or atypical KS, but not with endemic KS (1-4).

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A REVIEW OF PSOAS ABSCESS

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Psoas abscess is an uncommon clinical entity that can be primary, following haematogenous dissemination of an aetiologic agent, the source of which is usually occult, or secondary, as a result of local extension of an infectious process near the psoas muscle. The triad of presentation; fever, loin pain and limitation of hip movement may not be found in all patients. The correct diagnosis can be made with a vigilant clinical examination, epidemiological, microbiological and radiological investigations. The main stay of treatment is medical and or surgical drainage of abscess and treatment of the underlying illness. With the resurgence of tuberculosis, consequent upon the HIV/AIDS pandemic, there has been an increase in the number of psoas abscess due to mycobacterial organisms. This is a review of the literature on psoas abscess with highlights on the mode of presentation, diagnosis and treatment modalities.

Key words: psoas, abscess, review

INTRODUCTION

Psoas abscess arises mostly from spread of infections from surrounding anatomical structures. Diseases of the gastro-intestinal system are the most common causative factors, although haematogenous spread in the immunocompromised patients also account for some cases (1-3). Psoas abscess is relatively uncommon with a reported incidence of 0.4/100,000 per year in the United Kingdom (2), but with a mortality rate of 18-25% (1-3). Any age group may be affected, as some cases have been reported in the neonates (4), but general review showed the preponderance in the older age group in whom predisposing factors and the source of infection are more prevalent (1-3, 5).

Psoas abscess was virtually synonymous with tuberculosis of the spine or sacroiliac joint before

the introduction of streptomycin for the treatment of tuberculosis. In India and Africa where TB is endemic (6), 5% of patients with spinal tuberculosis (Potts disease) develop a psoas abscess (7). In the period between 1950 and 1985 when tuberculosis was largely mastered in the Western World (8), a tuberculosis abscess tracking down the psoas sheath was a rarity. However with the resurgence of TB, consequent upon the HIV/AIDS pandemic, tuberculous psoas abscess has once again become a focus of attention (9,10,11).

This article reviewed the literature on psoas abscess and discussed the technological milestones in the clinical and radiological diagnosis, and treatment of this condition.

SURGICAL ANATOMY OF PSOAS MUSCLE

The psoas muscle consists of the psoas major and minor. The psoas major is a long muscle on either side of the lumbar vertebra column and the pelvic brim (12). It arises from the anterior surfaces and lower borders of the transverse process of all five lumbar vertebrae, five interdigitation each from bodies of two adjoining vertebrae and intervertebra disc and bodies of five lumbar vertebrae between the digitations. It descends along the pelvic brim posterior to the inguinal ligament but anterior to the capsule of the hip joint. It converges into a tendon and receives the fibres of the iliacus muscle and attached to the lesser trochanter of femur. A large subtendinous bursa, which occasionally communicates with the cavity of the hip joint, separates it from the capsule of the hip joint in 15% of the population. In the abdomen, the muscle is related to the peritoneum, kidneys and ureters. On the right, it is related to the inferior venacava and the ileocaecal junction. On the left, it is related to the colon. In the groin, it is related behind to the hip joint capsule and laterally to the femoral nerve. The psoas major acts together with the iliacus to flex the thigh upon the pelvis. When there is fracture of the neck of femur, it acts as a lateral rotator of the femur. The psoas minor lies anterior to the psoas major, entirely within the abdomen. It originates from the intervertebra disc between T12 and L1 and is attached to the pecten pubis and iliopectineal eminence. It is absent in 40% of the population

and acts as a weak flexor of the trunk (12).

An abscess may destroy part of the psoas muscle but is likely to be confined within the psoas fascia. The fascia is attached medially to the spine above and to the brim of the pelvis below. Laterally it blends with the fascia covering quadratum lamborum and the iliacus; a psoas abscess can extend further laterally than the outer border of the muscle. In the groin, the iliopsoas fascia is attached to the inguinal ligament and the iliopectineal eminence; the femoral vessels are medial to this deep attachment.

AETIOPATHOGENESIS OF PSOAS ABSCESS

Psoas abscess is of two varieties. The primary type is caused by haematogenous dissemination of bacteria or spread through lymphatics, the source of which is usually occult. This type is seen in about 30% of cases and especially in immunocompromised patients such as diabetics and alcoholics (13, 14). The commonest organism implicated in this type of psoas abscess is *Staphylococcus aureus* (15,16,17), though other types of organism have been isolated (14,18).

Secondary type occurs as a result of local extension of infective process and is responsible for about 70% of psoas abscess. Intraperitoneal inflammatory processes and spinal pathology are the two most important conditions leading to psoas abscess formation. The intraperitoneal lesions include diverticulitis of the colon, appendicitis, pancreatitis and Crohn's disease (19,20,21). Several reports have shown Crohn's disease to be the single most common bowel lesion associated with psoas abscess (21-25). Other bowel lesions include carcinoma and actinomycosis of the colon. Microorganisms recovered from the inflammatory processes causing secondary psoas abscess are varied and include aerobic and anaerobic enteric gram-negative bacilli such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae* and other organisms such as *Clostridium difficile*, Diphtheroid bacilli and *Bacillus spp* (21,26). Tuberculosis of the spine and sacroiliac joint is another major cause of secondary psoas abscess usually following rupture of thoracolumbar abscess (11). Pyogenic osteomyelitis, sacroilitis and

spondylitis are other spinal inflammatory lesions leading to secondary psoas abscess. Other rarer lesions associated with secondary psoas abscess include renal calculus (27), carcinoma of the bladder and cervix, and anastomotic dehiscence (28).

Bacteria invasion of the muscle initiates an inflammatory or a granulomatous response, which results in the formation of purulent exudates, made up of neutrophil and macrophages. Hydrolytic enzymes released by neutrophil and macrophages into the surrounding tissues leads to tissue digestion, liquefaction and suppuration, with pus tracking down the muscle sheath.

CLINICAL MANIFESTATIONS

The triad of presentation comprises flank pain in 80% of patients, limitation of hip movement from painful psoas spasm in 45% of patients and fever in 40% of cases. These three symptoms when seen together are termed the triad of psoas abscess, and are seen in less than 50% of cases (2,3). Other features include back pain and limitation of back movement, abdominal pain extending to the loin, painful hip stress test, tender, soft and dull compressible mass in the iliac fossa, lumbar fullness with swelling extending below the groin, which can be emptied. Features of the predisposing illness may be apparent.

Clinically, psoas abscess must be differentiated from renal abscess, ruptured epigastric artery, femoral hernia, saphenous varix, lipoma, rupture of adductor longus with haematoma, femoral aneurysm, iliac artery aneurysm, iliac lymphadenopathy, chondrosarcoma of ilium and osteoarthritis of the hip.

DIAGNOSIS OF PSOAS ABSCESS

Diagnosis of psoas abscess is premised on a high index of suspicion, meticulous clinical examination, and radiological, microbiological and other ancillary investigations. A complete blood count may show evidence of leucocytosis and neutrophilia with raised C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), suggesting an inflammatory process. Plain abdominal radiograph of the lumbosacral region may show obliteration of the psoas shadow on the affected side. Plain X-ray is of low diagnos-

tic accuracy (29). Ultrasonography is diagnostic in 70% of cases and shows enlarged psoas or hypoechoic mass in the psoas region although small sized abscesses may be missed (29). Computerized Tomographic (CT) scan is diagnostic in 91% of cases and lesion shows as hypodense mass in the psoas region (29). It also has the advantage of imaging surrounding structures like vertebra, kidney, pancreas and the bowel. Although gallium scan is diagnostic in only 80% of cases, it is superior to CT-scan in demonstrating concomitant bony infective focus (30). CT-guided aspiration of abscess provides specimens for microbiological evaluation and may be used as a form of therapy. Magnetic Resonance Imaging (MRI) better delineates the extent of inflammatory changes and demonstrates abscess distinct from surrounding soft tissue without the need for contrasts (29,31). Bone marrow infiltrate in osteomyelitis of the vertebrae is also better demonstrated.

TREATMENT OPTIONS

The management of psoas abscess involves the treatment of the abscess and the predisposing or underlying illness. Treatment options include medical (15) or surgical approach but in most cases a combination of the two is required (15,16,17,18). If detected early, antibiotics may be used as a sole treatment option (15,18) but this is usually for long and therefore rarely used alone. Antibiotic selection should be based on result of microbiological cultures and sensitivity (15-18). Since *Staphylococcus aureus* appears to be the commonest cause of primary abscess, third generation cephalosporin or vancomycin may be used pending the result of culture and sensitivity. Percutaneous ultrasound or CT-guided needle aspiration followed by culture/sensitivity and the use of appropriate antimicrobial agents is a very popular therapeutic approach (10,14,32,33). Percutaneous drainage with catheter left in situ is advisable particularly when bowel pathology is suspected (32,33). Open drainage is the preferred option when gastrointestinal diseases are the cause of psoas abscess. This option allows simultaneous treatment of the underlying illness and allows for adequate debridement of necrotic psoas muscle and drainage of the abscess cavity. Drainage can be performed through several

approaches such as via the petit triangle. This triangle is by the side of the abdomen and is bounded by the iliac crest, lattismus dorsi and the external oblique muscle (34). The lateral approach is through mid one-third of the iliac crest. Anterior approach is underneath the inguinal ligament while the Ludluff incision is medial approach to the hip. Treatment of the underlying cause is important, for example, transabdominal resection may be performed in Crohn's disease or carcinoma of the colon. Failure to completely drain abscess may occur when the abscess is multiloculated or when there is underlying bowel lesions or when there is muscle involvement without liquefaction. Open drainage is preferred in this situation where bony or cartilaginous sequestra in the tract or the diseased vertebrae can be removed (34). Supportive treatments that may be necessary include anticoagulation, because of the increased risk of pulmonary embolism in these patients (13). Arthrotomy of the hip may be necessary when iliopsoas abscess lies adjacent to the hip capsule (20).

COMPLICATIONS FROM DELAY DIAGNOSIS

Complications that may arise from delay in the diagnosis of psoas abscess include iliac vein thrombosis due to compression by the abscess (13). This may lead to pulmonary embolism. Hydronephrosis due to compression of the ureters and renal failure may occur. Atrophy of the psoas muscle may occur in protracted case (5). There may be flexion contracture of the hip. Recurrence of psoas abscess may occur if underlying predisposing cause is not removed. Further dissemination of organisms especially in immunocompromised patients may also occur.

CONCLUSION

Although psoas abscess is a rare entity, occasional cases are seen now and then. It requires that clinicians must have high index of suspicion and be conversant with the diagnostic imaging techniques, common aetiologic agents and surgical techniques employed in the management of this condition.

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ASYMPTOMATIC SIGNIFICANT BACTERIURIA AMONG PREGNANT WOMEN IN ADO-EKITI, EKITI STATE, NIGERIA

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Microbiological culture examination of urine samples from 502 pregnant women attending antenatal clinic resulted in the isolation of seven genera of bacterial species. They were *Staphylococcus aureus* 16 (21.3%), *Escherichia coli* 12 (16.0%), *Staphylococcus spp* 11(14.7%), *Klebsiella spp* 8 (10.7%), *Pseudomonas aeruginosa* 7 (9.8%), *Streptococcus faecalis* 6 (8.0%), and *Citrobacter spp* 1 (1.3%). Asymptomatic significant bacteriuria occurred in 16 (12.22%), pyuria in 10 (2.0%) while significant bacteriuria with pyuria occurred in 1(0.2%). The antibiogram indicated that ofloxacin, ciprofloxacin and nalidixic acid were in that order the most effective of the antibiotics tested. All isolates showed multiple resistance to most of the antibiotics tested. Plasmid DNA was detected in *Ps. aeruginosa* and *E. coli* with an estimated molecular weight of between 4.5 and 6.5 kb. The result indicated a significant rise in the frequency of S. *aureus* in asymptomatic bacteriuria.

Keywords: Asymptomatic bacteriuria, urinary tract infections, pregnant women

INTRODUCTION

Asymptomatic bacteriuria in women is a frequent occurrence that results when urinary tract pathogens such as *Escherichia coli*, amongst others enter the bladder without causing symptoms (1). The pathogens, which are usually eliminated by host defense factor, may persist for a short, but rarely a long time resulting in symptomatic urinary tract infection (UTI). Studies on asymptomatic bacteriuria in healthy women and school girls showed no adverse outcomes, whereas adverse outcomes have been reported in well defined groups such as pregnant women, catheterized or elderly patients (2.)

Asymptomatic bacteriuria during pregnancy has been associ-

ated with an increased risk of developing pyelonephritis (3,4,5), maternal and infant morbidity, preterm labour and low birth weight (6,7,8). Adverse outcomes have been prevented with antimicrobial drug therapy. However, the efficacy of antimicrobial drug treatment is limited by increased antimicrobial resistance in community-acquired strains of *E. coli* particularly to ampicillin, sulphonamides, and trimethoprim-sulfamethoxazole (9).

The present study examined the incidence of asymptomatic bacteriuria in pregnant women in Ado-Ekiti, Ekiti State, Nigeria. The antibiotic resistance pattern of the isolates and the presence of plasmid DNA were also investigated.

MATERIALS AND METHODS

Collection of samples

Sample of clean-catch early morning voided mid-stream urine were collected from 502 pregnant women attending antenatal clinic at the state specialist hospital, and Okesa maternity centre, Ado-Ekiti, Ekiti state, Nigeria. The patients were apparently healthy and symptom-free at the time of sample collection. All samples were collected aseptically, stored at 4°C and examined within two hours of collection. Information about patients such as age, gestation age, occupation, blood group and parity were collected from the hospital case note. Informed consent of individual involved in this study was obtained.

Microscopic examination of urine

Three loopful of well-mixed uncentrifuged urine were placed on a clean grease-free slide and covered with a coverslip. The preparation was examined under the microscope for bacteria and pus cells.

Isolation of Bacteria

Samples were examined using standard methods (10). Briefly a calibrated wire loop capable of delivering 0.001 ml of urine was used for culturing on Cysteine-Lactose Electrolyte Deficient (CLED) and MacConkey agar. The culture plates were incubated aerobically at 37°C for 24 hours Culture plates without visible growth were further incubated for additional 24 hours before being discarded. The number and types of colonies growing on the medium (CLED) were recorded as being insignificant, doubtful (contaminated) or significant when 1 or less colony, < 10 colonies or 10 or more colonies

were counted respectively.

Identification of isolates

Identification of bacterial isolates was based on the combination of cultural, morphological and biochemical characteristics (11).

Antimicrobial Assay

Antimicrobial susceptibility testing of the isolates was done by the Kirby-Bauer disc diffusion technique (12), with the following antibiotics; Ampicillin, Cotrimoxazole, Gentamycin, Chloramphenicol, Nalidixic acid, Vibramycin, ofloxacin and ciprofloxacin.

Plasmid Analysis

Isolation of plasmid from the isolates that showed multiple resistance to antibiotics was done as described by Birnboim and Doly (13). This plasmid DNA was resolved on agarose gel electrophoresis using lambda DNA cleaved with *Hind* III restriction enzyme as the molecular weight marker.

RESULTS

Of the 502 urine samples examined, 16 (12.2%) yielded significant growth, 228 (45.4%) had insignificant growth, 15 (2.9%) had mixed growth while 193 (38.45) had no growth (Table 1). There was pyuria in 10 (2.0%) urine samples while significant bacteriuria with pyuria occurred in 1 (0.2%) (Table 2). Table 3 shows the various bacterial isolates with their percentage distribution. *S. aureus* was the most common organism with an isolation rate of 21.3% followed by *E. coli* with 16.0% isolation rate while *Salmonella spp* had the least prevalence rate. Table 4 shows the susceptibility patterns of the bacteria pathogens to the eight antimicrobial agents employed. Ofloxacin was the most effective drug on all the tested organisms followed by ciprofloxacin and vibramycin. Most of the isolates exhibited multiple antibiotic resistance (MAR) patterns. *Providencia* and *Enterobacter spp* showed the single R-type with 100% resistance to both cotrimoxazole and ampicillin. All the other organisms showed multiple resistant pattern ranging from 2 to 8 antibiotics. *Ps. aeruginosa, Citrobacter* and *Salmonella spp.* were 100% multiple R-type while *E. coli* and *Proteus spp.* demonstrated 91.7% and 83.3% resistance respectively and *S. aureus* showed 81.3% resistance (Table 5).

Ps. aeruginosa, which was resistant to four antibiotics, harboured three plasmid bands. *E. coli* resistant to two antibiotics had one plasmid DNA band. All other isolates had no detectable plasmid (Table 6).

Table 1: Occurrence of significant bacteriuria in pregnant women in Ado-

CASES	TOTAL NO OF PATIENTS	%
Significant growth	61	12.2
Insignificant growth	228	45.4
Mixed growth	15	2.9
No growth	193	38.4
Growth of *Candida albicans*	5	0.9
TOTAL	502	100

Table 2: Relations between pyuria and significant bacteriuria

CASES	TOTAL NO OF PATIENTS	%
Significant bacteriuria	61	12.2
Pyuria	10	2.0
Significant bacteriuria with pyuria	1	0.2
Significant bacteriuria without pyuria	60	11.9
Pyuria without significant bacteriuria	9	1.8
TOTAL	502	100

Table 3: Profile of bacteria isolated from cases of significant bacteriuria in pregnant women.

BACTERIAL ISOLATES	TOTAL NO OF ORGANISM	%
Staphylococcus aureus	16	21.3
Escherichia coli	12	16.0
Staphylococcus spp.	11	14.7
Pseudomonas aeruginosa	7	9.3
Streptococcus faecalis	6	8.0
Klebsiella spp.	8	10.7
Proteus spp.	6	8.0
Citrobacter spp.	3	4.0
Enterobacter spp.	2	2.7
Providencia spp.	3	4.0
Salmonella spp.	1	1.3
TOTAL	75	100

Table 4: Pattern of resistance of bacterial isolates to antimicrobial agents

Bacterial isolate	No of isolate	AMP	CHL	COT	GEN	NAL	CIP	OFX	VIB
S. aureus	16	10(62.5)	7(43.7)	7(43.7)	5(31.3)	3(18.8)	1(6.3)	0	1(6.3)
E.coli	12	6(50.0)	2(16.7)	3(25.0)	4(33.3)	1(8.3)	1(8.3)	0	7(58.3)
Staphylococcus spp.	11	6(54.5)	1(9.1)	9(81.8)	2(18.2)	0	1(9.1)	1(9.1)	3(27.3)
Klebsiella spp.	8	5(62.5)	1(12.5)	7(100.0)	2(25.0)	2(25.0)	1(12.5)	1(12.5)	0
P. aeruginosa	7	7(100.0)	7(100.0)	7(87.5)	4(57.1)	7(100.0)	0	0	4(57.1)
S. faecalis	6	5(83.3)	4(66.7)	2(33.3)	2(33.3)	2(33.3)	0	0	1(16.7)
Proteus spp.	6	3(50.0)	2(33.3)	6(100.0)	2(33.3)	1(16.7)	0	0	1(16.7)
Citrobacter spp.	3	1(33.3)	2(66.7)	2(66.7)	2(66.7)	0	0	0	2(66.7)
Providencia spp.	3	0	0	2(66.7)	0	0	0	0	0
Enterobacter spp.	2	1(50.0)	1(50.0)	0	1(50.0)	0	0	0	0
Salmonella spp.	1	1(100)	1(100)	1(100)	1(100)	0	0	0	1(100)
TOTAL	75	45	28	46	25	16	4	2	20

AMP = Ampicillin COT = Cotrimoxazole GEN = Gentamycin CHL = Chloramphenicol
NAL = Nalidixic acid OFX = Ofloxacin CIP = Ciprofloxacin VIB = Vibramycin
( ) = % resistance

Table 5: Prevalence of multiple antibiotic resistance (MAR) strains among bacterial isolates from pregnant women

Bacterial isolate	Total No isolate	Single R-type (%)	Antibiotics	Multiple R-type (%)	Antibiotic range	Antibiotics to which multiple resistance was demonstrated
S. aureus	16	1(18.8)	Chl, Amp	13(81.3)	2-5	Chl, Gen, Nal, Cip, Cot
E.coli	12	1(8.3)	Amp	11(91.7)	2-5	Amp, Chl, Gen, Nal, Cot, Cip
Klebsiella spp.	8	2(33.3)	Amp, Cot	6(66.6)	2-4	Amp, Nal, Gen, Cot, Cip, Ofx
P. aeruginosa	7	0(0)		7(100)	4-6	Amp, Nal, Vib, Chl, Cot, Gen, Ofx
S. faecalis	6	1(16.7)	Cot	5(83.3)	2-4	Amp, Cot, Nal, Gen, Chl
Proteus spp.	6	1(16.7)	Cot	5(83.3)	2-4	Gen, Cot, Vib, Amp, Nal, Chl
Staphylococcus spp.	11	3(27.3)	Cot	8(72.7)	2-4	Gen, Cot, Amp, Vib, Chl, Ofx, Cip
Citrobacter spp.	2	-	-	2(100)	4-5	Vib, Gen, Chl, Cot, Amp, Vib
Providencia spp.	2	2(100)	Cot	0	-	-
Enterobacter spp.	2	1(50)	Amp	0	-	Gen, Chl.
Salmonella spp.	1	0(0)	-	1(100)	5	Vib, Gen, Chl, Cot, Amp.

AMP = Ampicillin COT = Cotrimoxazole GEN = Gentamycin CHL = Chloramphenicol
NAL = Nalidixic acid OFX = Ofloxacin CIP = Ciprofloxacin VIB = Vibramycin
( ) = % resistance

Pg. 70 — 85

Table 6: **Plasmid analysis of some of the bacterial species from asymptomatic significant bacteriuria of pregnant women**

Bacterial isolate	Antibiotic resisted	No of bands/plasmid	Estimated molecular weight of plasmid kilobase pair less than 6.5
E.coli	Vib, Cot	1	Less than 6.5
E.coli	Amp	-	
Proteus spp.	Vib, Gen; Cot, Amp	-	
Proteus spp.	Cot	-	
Klebsiella spp.	Cot, Amp	-	
Klebsiella spp.	Cot, Chl	-	
Citrobacter spp.	Vib, Gen, Chl, Cot, Amp	-	
P. aeruginosa	Chl, Amp, Cot, Vib	3	Between 4.5 - 6.5

DISCUSSION

Urinary tract infection (UTI) is one of the most common bacterial infections in both pregnant and non-pregnant women. It is estimated that 10-20% of all women suffers from UTI at some point in life (14). The prevalence of bacteriuria in female varies from less than 1% in infants to 10% and even more in older women. The prevalence of asymptomatic bacteriuria in pregnancy ranges from 4% to 7% depending on the population studied (15). The overall isolation rate of bacteria in the population studied was12.2%. Famurewa (16) earlier reported significant bacteriuria in 12 (2.5%) non-pregnant women, 108 (22.2%) pregnant women and 120 (24.7%) asymptomatic women. Olusanya *et al* (17) also reported significant bacteriuria in 122 (23.9%) pregnant women and 37 (12.2%) non-pregnant women in Sagamu, Ogun State, Nigeria. The result of this study further affirms that variation in frequency may be ascribed to population characteristics such as age, parity, socio-economic status, sexual activity (multiple sexual partners) and health care during pregnancy (17, 18, 19,20).

S. aureus was the most prevalent bacterium (21.3%), which agrees with the report of earlier workers (16, 17, 21,22). It is important to note that *E. coli* which is implicated in 75% of out-patients with urinary tract infections is the second most prevalent in this study. Anderson *et al* (23) observed that although *E. coli* was not among the organisms found in the periurethral area, yet it was one of the common organisms causing significant bacteriuria because of its ability to outgrow other organisms that could cause similar problems. Akinyemi *et al* (24) reported the occurrence of coagulase negative staphylococci (CNS) and their importance as pathogens rather than contaminants in the urinary tract. This has been confirmed in this study and in reports from USA (25), Sweden (26), UK (27) and Nigeria (16). *S. saprophyticus* has been considered the most frequent agent of urinary tract infection in young sexually active women after *E. coli* (28).

The *in vitro* susceptibility pattern of the isolates revealed that the quinolone class of antimicrobials is highly effective against all isolates demonstrating less than

10% resistance. The newer quinolones (ciprofloxacin, ofloxacin) are excellent agents for treatment of UTI (20) although the quinolones are not generally safe in pregnancy and should probably be avoided (29). The high prevalence of resistance to β-lactam (ampicillin) has long been appreciated. This has been confirmed by this study and as such the continued use of β-lactams should be discouraged as much as possible in the empirical treatment of uropathogens. Of considerably greater concern is the increasing prevalence of resistance to trimethoprim-sulfamethoxazole (co-trimoxazole) as observed in this study. This data suggests that this drug also may not be aeceptable for empirical therapy. The resistance pattern seen in this study further affirms the need to discourage empirical treatment of UTI. The prevalence of resistance among uropathogens has been shown to be on the increase. The prevalence of resistance to trimethoprim-sulfamethoxazole rose from more than 9% in 1992 to more than 18% in 1996 for *E. coli*. It rose from 8% to 16% among all isolates combined while for ciprofloxacin, it did not change significantly during the 5 years period (9). The high resistance to these commonly used and relatively cheap antibiotics (ampicillin, co-trimoxazole etc) in Nigeria could be a reflection of widespread and indiscriminate usage of these drugs. Of obvious concern is the likelihood that increased use of fluoroquinolones for the treatment of UTI in women may likely promote the emergence of resistance to these drugs. A rapid development of resistance to nalidixic acid in Ontario, Canada after widespread use has been reported (30), but resistance to this drug is relatively low from this study. Inappropriate use of antimicrobial agent has been identified as a major risk factors for the development of resistance (31), hence the correlation between antimicrobial resistance and antimicrobial use.

An important finding in the current study is the lack of plasmid-mediated resistance to quinolones. Despite an increased widespread usage, resistance has not emerged (32). Although other studies have reported increasing fluoroquinolone resistance amongst *E. coli* (33, 34), low resistance rate was observed amongst the *E. coli* in this study. The presence of plasmid

DNA in some of the isolates shows that this antimicrobial resistance could be plasmid mediated. Co-transfer of plasmid borne resistance amongst *E. coli* from UTI has been reported (30). Plasmid bands were detected in both *Ps. aeruginosa* and *E. coli*. The plasmid DNA in both organisms had identical size and both isolates were resistant to vibramycin, suggesting that this is likely to be plasmid-mediated resistance. The increase in antibiotic resistance among community-acquired isolates is worrisome and new approaches to possibly prevent or control the emergence of resistance should be developed. Hence, the World Health Organization (35) recognized the importance of studying the emergence and the need for strategies for its control.

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PENICILLIN-RESISTANT STREPTOCOCCUS PNEUMONIAE – A REVIEW

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Since the first report in 1967, the incidence of Penicillin Resistant *Streptococcus pneumoniae* (Pneumococcus) has risen steadily worldwide, and now complicates diagnostic and treatment strategies for infections due to this organism. More worrisome is the fact that in areas where Penicillin Resistant *Streptococcus pneumoniae* (PRSP) has become established, resistance to other antimicrobial agents such as cephalosporins, sulphonamides and macrolides is also common. This development has a grave implication for therapy of life threatening pneumococcal infections like meningitis and septicaemia, with the extended spectrum cephalosporins, such as ceftriaxone and cefotaxime, and the newer macrolides, azithromycin and clarithromycin. The mechanism of resistance to β-lactam antibiotics is decreased binding of drug to the bacteria cell wall brought about by genetic transformation in bacterial chromosome. Recently, using molecular techniques that can index overall relatedness of the drug resistant pneumococcal isolates, it has been possible to establish clonal dissemination of drug resistant pneumococci across continents, with acquisition of additional drug resistance determinants as a result of "local" antibiotic selective pressures. This is a review of literature on the epidemiology, mechanism of resistance, laboratory identification, treatment, prevention and control of Penicillin Resistant Pneumococci (PRP), with emphasis on the problems of identification and reporting in developing countries.

Key words: penicillin, *Streptococcus pneumoniae*, resistant, extended spectrum cephalosporins.

INTRODUCTION

Streptococcus pneumoniae infections are among the leading causes worldwide of illness and death in young children, persons with underlying illness, and the elderly. In the United States of America alone, *Streptococcus pneumoniae* is a leading cause of morbidity and mortality, resulting each year in an estimated 3,000 cases of meningitis, 50,000 cases of bacteraemia, 500,000 cases of pneumonia and 7,000,000 cases of otitis media (1, 2). In developing countries, 50% of the estimated 4 million child deaths annually from

pneumonia are caused by *Streptococcus pneumoniae* (3).

In the past, *Streptococcus pneumoniae* was almost uniformly susceptible to penicillin, allowing most physicians to treat persons who had severe infections with penicillin alone and without testing for resistance. Since the 1960s however, resistance to penicillin and most other antimicrobial agents, has spread rapidly. Penicillin Resistant *Streptococcus pneumoniae* (PRSP) was first reported in 1967 in Australia (4), in New Guinea in 1969 (5) and in South Africa in 1977 (6), and since then in many countries throughout Africa, America, Asia and Europe (7-26).

In Africa, with the exception of South Africa, literature on the occurrence of PRSP appears sparse. The few reports have documented low prevalence rate, which is probably due to gross underreporting. Poor or absent antibiotic resistance surveillance and infection control progammes, and poor laboratory backup in many health institutions are some of the factors responsible for underreporting of the occurrence of PRSP and other resistant organisms in these countries. This article reviewed the epidemiology, diagnostic and therapeutic difficulties, and preventive measures for PRSP and emphasizes the need to increase surveillance for these organisms in developing countries.

HISTORICAL/EPIDEMIOLOGICAL PERSPECTIVES

The German pathologist, Klebs in 1875 described *Streptococcus pneumoniae* in the fluid from the lungs of a man dying with pneumonia (27). In 1881, the organism was concurrently identified in the old and new worlds, by Pasteur in France, who named it *Microbe Septicemique du salive*, and Sternberg in the United States, who called it *Micrococcus pasteuri* (27,28). By late 1880s, the term pneumococcus was generally used because this bacterium has come to be recognized as the most common cause of lobar pneumonia (28). It was also recovered from several body sites such as cerebrospinal fluids, synovial fluids, kidney, middle ear, blood and the pericardium (27,28).

In 1890s, Felix and klumperer showed that immunization

with killed pneumococci protected animals against subsequent pneumococcal challenge, and further, that protection could be transferred by infusing serum from immunized mice into naive recipients (29). Felton prepared the first purified pneumococcal capsular polysaccharides for immunization of human subjects (30), and a preparation of type 1 polysaccharide was used to abort an epidemic of pneumonia at a State Hospital in Worcester, Massachusetts in 1938 (31). MacLeod and coworkers further confirmed this during the World War II when they found that vaccinating military recruits with capsular material from several serotypes of *Streptococcus pneumoniae* greatly reduced the incidence of pneumonia due to serotypes present in the vaccine, but not other pneumococcal serotypes (32). Serotypes of pneumococci were earlier recognized, based on the observation that injection of killed organism into a rabbit stimulated the production of antibody that agglutinated and caused capsular swelling of the immunizing strains, as well as of some, but not all, other pneumococcal isolates (29). Capsule swelling (Quellung) reaction became the basis of the American and Danish serotyping schemes of *Streptococcus pneumoniae*.

The name Diplococcus was adopted in 1926 based on the presence of paired cocci on Gram stained sputum. In 1974 it was renamed *Streptococcus pneumoniae* based on the presence of chains when grown in liquid medium (27). *Streptococcus pneumoniae* was the subject of pioneering genetic research work by Griffith (33), when in 1928, he demonstrated genetic transformation in *Streptococcus pneumoniae*. In the 1930s, it was recognized that a large proportion of healthy population carried pneumococci in the nasopharynx and that this was often a source of disease in contacts of these asymptomatic carriers (28,34).

In the pre antibiotic era, mortality from untreated pneumococcal disease was 77% (35). It was 50% in patients treated with specific antipneumococcal serum (35). Following the introduction of sulphonamides in 1930 and penicillin in 1945, mortality decreased to about 25% (36) but remained unchanged at this rate for about three decades of antibiotic therapy (37,38,39).

During this period (1940s to 1970s) however, occasional strains of pneumococcus exhibiting increased resistance to penicillin (4,40), tetracycline (41,42), erythromycin and lincomycin (43,44) had emerged.

The first documented evidence of resistance to pneumococcus was in 1912 by Morgenroth and Kaufman (45) when optochin (ethylhydrocuprein hydrochloride) resistant pneumococci were obtained from experimentally infected mice treated with optochin. Optochin resistant pneumococci were then reported among clinical isolates obtained from patients treated with optochin in 1915 (46). Sulphonamide resistant pneumococci were reported in patients with meningitis and lobar pneumonia between 1939 and 1943 (47). Penicillin resistance was first described in 1945 among mutant strains of pneumococci *in vitro* (40) shortly after the introduction of penicillin into the market. Clinical isolates of *Streptococcus pneumoniae* with reduced susceptibility to penicillin were first reported in Boston in 1965 (48). Penicillin minimum inhibitory concentration (MIC) of 0.1 and 0.2 µg/mL were reported in two of the two hundred isolates tested. Despite these findings, the investigators failed to recognize the clinical significance of their discovery. The first pneumococcal strain with reduced susceptibility to penicillin (MIC 0.6 µg/mL), for which clinical relevance was recognized, was reported in 1967 (4) after being isolated from a 25-year old Australian woman. During the next decade, several alarming reports were published documenting worldwide spread of pneumococci with reduced susceptibility to penicillin (MIC 0.1 – 1 µg/mL).(5-9). In 1977 (10), pneumococci exhibiting high level penicillin resistance (MIC ≥ 2.0 µg/mL) were isolated from young African children admitted to King Edward VIII Hospital, Durban, South Africa, with meningitis, septicaemia, otitis media, pneumonia and empyema. By the early 1980s, worldwide distribution of multidrug resistant pneumococci have been described with reports from New Guinea, Israel, Poland, South Africa and the United States of America (11-15).

The incidence and pattern of penicillin resistance among pneumococci remained fairly stable in the early 1980s (14), but due to the various degree of resistance en-

countered and the various nomenclatures used, the Centre for Disease Control and Prevention (CDC), in 1995 suggested a standardized classification of resistance level (49). CDC defines susceptibility of *Streptococcus pneumoniae* to Penicillin as MIC ≤ 0.06 µg/mL. All isolates for which the MIC is ≥ 0.1 µg/mL are regarded as non-susceptible. Isolates that are non-susceptible are characterized further as Penicillin intermediate (*Peni*; MIC 0.1-1 µg/mL) or Penicillin resistant (*Penr*; MIC ≥ 2.0 µg/mL). Isolates for which MIC is ≥ 2.0 µg/mL were previously referred to as displaying high-level penicillin resistance (50). This terminology is no longer advocated by CDC (49).

Between 1979 and 1987, non-susceptible pneumococci accounted for approximately 5% of the strains recovered in the United States. During the same period, Penicillin resistant strains (*Penr*) were rare, approximately 0.02% (1 of 4585) of pneumococci sterile-site isolates submitted to the CDC Sentinel Hospital Surveillance system (51). By the early 1990s, however, a dramatic increase in the frequency of isolation of non-susceptible pneumococci was reported (52-57), with a corresponding increase in Penicillin resistant (*Penr*) strains. For example in 1991 - 1992, 2.6% of all isolates were Penicillin resistant as against 7.3% in 1992-93 (52, 53) and 9.5% in 1994-95 (57). Similarly, several other countries reported increasing incidence of Penicillin non-susceptible strains with corresponding increase in Penicillin resistant (*Penr*) strains during this period (16-26). The common serotypes of pneumococci resistant to penicillin (MIC ≥ 2.0 µg/mL) and other β-lactam agents encountered include serotypes 6A, 6B, 19A, 19F, 14 and 23 (13). Others include serotypes 1, 3, 5, 15, 31 and 35 (15). In the United States, outbreaks in daycare centres were caused mainly by serotypes 6B, 14, 19F and 23 (58, 59).

In Africa, only few surveys have been reported except in South Africa, where resistant rates are close to 20% (60). Surveys carried out in Nairobi, Kenya in 1981 (61) and during 1991 to 1992 (62) gave 26% prevalence rates. In Tunisia, a rate of 10% was reported (63). Reports from Zambia (64), Senegal and Ivory Coast (63) have posted

rates below 5% and less than 2% in Morocco and Egypt (63). A survey in Nigeria in 1978 reported a 20% prevalence rate for Penicillin Resistant Pneumococci (65). The relatively few surveys carried out by African countries do not give a true picture of the occurrence of PRSP in this continent. More surveys will be necessary to know the true prevalence in these countries.

Penicillin Resistant Pneumococci have been recovered more frequently from children five years or younger than from other age group (1, 2, 9-13,64,67,68,73,74). Although young children are still at risk for resistant infection, an increased frequency of drug resistant pneumococci has been encountered in adults (60). Risk factors for an infection secondary to a resistant pneumococcal strain include hospitalization, prior exposure to antimicrobial agents, underlying illness and tobacco use (6-13,15,19,23,39,67,68,69,71).

In areas where PRSP has been established, resistance to other antimicrobials, such as cephalosporins (67-71), sulphonamides (15, 60, 73) and macrolides (66, 74) is also common. The identity of drug-resistant isolates within a country or in different countries has been investigated using techniques such as polymerase chain reaction (BOX PCR), pulse field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MLEE), penicillin binding protein (PBP) profiling and multilocus sequence typing (MLST) to index their overall relatedness (75, 76). This has illustrated the extreme diversity of drug resistant pneumococci particularly in countries such as Spain and Africa, where resistant isolates have emerged rather than being imported. However, superimposed on this diversity is the emergence and clonal spread of resistant isolates that are presumably fitter than other isolates. Thus in Spain although there is a great divergence in the relatedness of resistant isolates, more than 60% of all Penicillin resistant isolates belong to four major clones; Spain^{23F}-1, Spain^{6B}-2, Spain^{9V}-3 and Spain^{14}-5 (77). Two of these clones have been extensively studied. The first major clone, Spain^{23F}-1 (resistant to penicillin, tetracycline, chloramphenicol and sometimes erythromycin) probably arose from Spain in the early 1980s and since then has spread across more than six other

countries in three continents (78). The spread of this clone has been accompanied by the emergence of variants that have acquired additional drug resistance determinants as a result of "local" antibiotic selective pressures. The second major Spanish clone, Spain^{6B}-2 is also resistant to penicillin, tetracycline and chloramphenicol and has become prevalent in Iceland and the United Kingdom (79), other European countries (80,81) and in Asia (82). Extensive human population mobility is the major factor in the global dissemination of these resistant clones.

MECHANISM OF RESISTANCE IN PRSP

Pneumococcal resistance to β-lactam agents, like Penicillins and Cephalosporins, is due to changes in the target sites of the enzymes called Penicillin Binding Proteins (PBP). These high molecular weight proteins are believed to catalyze the terminal stages in peptidoglycan (murein) synthesis (83). There are six PBPs found in susceptible strains of *Streptococcus pneumoniae;* PBP 1a, 1b, 2a, 2x, 2b and 3 (84). All are high molecular weight proteins except PBP3, which is probably not too involved in β-lactam mediated cell lysis (84, 85). β-lactam compounds inhibit these enzymes by covalently binding to their active sites (84).

The altered PBPs in pneumococcus have low affinity for penicillin and related β-lactam compounds (85), a mechanism which only occurs in organisms that are naturally transformable. Altered PBPs also play a role is resistance to Penicillin in other naturally non-transformable strains like *Staphylococcus aureus* and enterococci (86), but in these cases, it is due to acquisition of new abnormal PBPs, rather than decrease in the affinity of the normal PBPs. Pneumococcal isolates with high Penicillin MIC seems to be entirely due to the expression of low affinity forms of PBP 1a, 2a, 2b, 2x and perhaps 1b (87). There is a reduction in the affinity of at least three of these five PBPs. For example, resistance to at least 8 μg/mL can be achieved by alterations in only PBP 1a, PBP 2x and PBP 2b. Since PBPs in these strains also have decreased affinities for other β-lactam antibiotics, most PRSP have increased resistance to third generation cephalosporins including ceftriaxone and cefotaxime. High- level resistance

to cephalosporins requires reduction in the affinities of only PBP 2x and PBP 1.

The reduction in the affinity of PBPs for β-lactam compounds results from the appearance of the so-called "mosaic" amino acid sequences of the proteins (85). Over the last few decades, the high selective pressures provided by antibiotics in the environment of the bacteria have selected for strains that have these new or changed PBPs, less able to bind β-lactam antibiotics. What we see today are pneumococci that have PBP encoding chromosomal genes that are hybrid molecules made with DNA from *Streptococcus mitis* and other yet to be identified streptococcal species (88). Many different "mosaic" genes have been sequenced to date. It is difficult to calculate the exact events that gave rise to these variants PBPs. To complicate matter is the fact that these "mosaic" genes have been transferred to other viridians streptococci such as *Streptococcus sangius* and *Streptococcus oralis* (87). As a result of the gene flow between these naturally transformable streptococci, it is difficult to determine the events that occurred to produce these mosaic PBPs now found in resistant pneumococci.

PRSP with MIC ≥ 2 μg/mL are also more likely to be resistant to non β-lactam agents such as chloramphenicol, trimethoprim-sulphamethoxazole, erythromycin, tetracycline and aminoglycosides (15,60,66,74,75). Resistance to chloramphenicol, tetracycline and erythromycin appears to be chromosomally mediated (89). A 7.244 kb defective transposon Tn *1207.1* containing *mef* (A) gene has been found inserted in the competence *cel* B chromosomal region of *Streptococcus pneumoniae* conferring resistance to macrolide (M phenotype) via an active efflux mechanism (90). Another 25.3 kb conjugative transposon, Tn *1545* has also been found inserted into the chromosome of resistant pneumococcus (91). This transposon carries the *erm* (B) gene that confers resistance to erythromycin and the structurally unrelated macrolide, lincosamide and streptogramin B (MLS_B phenotype), through methylation of 23S rRNA, the common target of these agents. This transposon also carries the *tet* (M) gene

that codes for tetracycline resistance via the production of a protein that binds to the ribosome and blocks protein synthesis (92). Resistance to trimethoprim-sulphamethoxazole is attributed to trimethoprim and occurs by a decrease in the affinity of trimethoprim for its target enzyme, dihydrofolate reductase (93). Chloramphenicol resistance is due to the production of an inducible chloramphenicol acetyltransferase (94).

It appears that pneumococci, in addition to having incorporated DNA from non-pneumococcal streptococci, may have also shared DNA with Gram-negative microbes in other to acquire additional drug resistance. The genes that encode resistance to erythromycin, tetracycline and aminoglycosides, *erm* (B), *tet* (M) and *aph* (A3) respectively, identified in PRSP have also been found in *Escherichia coli* and *Klebsiella spp*, *tet* (M) in resistant *Haemophilus* and *Neisseria spp*, and *aph* (A3) in *Staphylococcus aureus*, *Enterococcus faecalis* and *Helicobacter spp* (89).

Although the quinolone antimicrobials have improved spectrum of activity against Gram-positive organisms, they do not possess sufficient activity to be clinically useful against *Streptococcus pneumoniae*. One of the genes responsible for resistance to quinolones is *gyr* (A), which encodes the A-subunit of DNA gyrase, the site of action of quinolones (95). Newer quinolones may be potent against *Streptococcus pneumoniae* despite mutations in the *gyr* (A) and *par* (C) genes and may be useful in treating infections by this organism (96).

To date, β-lactamase producing pneumococci have not been reported. The mechanism of resistance is entirely chromosomal with horizontal transfer of resistant genes via transformation and conjugation.

LABORATORY IDENTIFICATION OF PRSP

Streptococcus pneumoniae is a fastidious organism requiring particular attention to proper laboratory procedures for identification and *in vitro* susceptibility testings (97). Routinely in the laboratory, pneumococci are identified by three reactions; the so-called alpha-haemolysis on blood agar with flat or concentrically ringed colonies, catalase negativity and solubility in bile salt or susceptibility to ethyl-

hydrocupreine (Optochin). Occasional strains may form rounded, rather than flat or concentrically ringed, colonies or may lack capsules and hence mis-identified as viridian streptococci. These atypical strains are likely to be encountered from sites with normal flora or among Penicillin resistant strains (98). Strains with zone diameter of inhibition ≥ 10 mm to optochin disk can be presumptively identified as pneumococci. Incubation in air with added CO_2 caused decreased zone size, which is reversed when pneumococci, but not viridian streptococci, are incubated in air (101). In recent years, a number of isolates have been found to be optochin resistant (102,103), which has led cautious microbiologists to rely more on the use of bile solubility for definitive identification.

IN-VITRO SUSCEPTIBILITY TESTING

Recent reports have emphasized the importance of accurate susceptibility testing of all clinically significant isolates of *Streptococcus pneumoniae*, the need for new agents, and periodic revaluation of existing drugs (1,97,104). Testing is complicated by the fact that there are currently no automated microbroth dilution MIC systems available for susceptibility testing of *Streptococcus pneumoniae*.

Laboratory may choose to use agar disk diffusion on Mueller-Hinton agar supplemented with 5% sheep blood, incubated in 5% CO_2 to screen for Penicillin resistance using the 1 µg oxacillin disk (49,97,98) or 5 µg methicillin disk (99). The cut off zone diameter of inhibition for oxacillin is 20 mm (49) and for methicillin, 25 mm (99). Oxacillin is preferred to methicillin, because of the enhanced resistance of oxacillin to degradation during storage (100). This method is also acceptable for testing other oral agents including trimethoprim/sulphamethoxazole, erythromycin, clindamycin and tetracycline as well as vancomycin for parenteral use (49,105). Penicillin disk is not used because it gives inaccurate results (98).

Susceptibility to Penicillin can be used to predict susceptibility to all other β-lactams. However, to distinguish between Penicillin intermediate and Penicillin resistant isolates, and to obtain susceptibility information for cephalosporins, a quantitative MIC test must be

done (1,49,97). The agar dilution method is regarded as the reference method for determining the MIC for pneumococcus (49,98). This is carried out in cation-adjusted Mueller Hinton agar supplemented with 5% whole defibrinated horse or sheep blood or 5% lysed and centrifuged horse blood for sulphonamides (98,106). The inoculum size is 10^4 CFU per spot and plates are incubated in air or added 5-10% CO_2 overnight (49).

Recently, the E-test (AB Biodisk, Solna, Sweden) has become popular. This is a method of determining MIC based on diffusion of an antimicrobial gradient from a calibrated antibiotic impregnated plastic strip applied onto the surface of an inoculum coated agar plate. The antibiotic gradient produced results in an ellipse of inhibition. The point at which the ellipse meets the strip is the MIC. This technique has become widely used in clinical laboratories for quantifying MICs for penicillin and third generation cephalosporins (1,49,107). Evaluation of the E-test has shown excellent correlation with agar dilution and broth microdilution method for Penicillin G, Cefotaxime, Ceftriaxone, Amoxicilin, Chloramphenicol, Erythromycin and Tetracycline, though, the MIC for Penicillin G tends to be slightly lower resulting in some resistant strains being categorized as intermediate (107,108).

TREATMENT OF PRSP INFECTIONS

Opinion differs on how to treat infections caused by Penicillin Resistant Pneumococcus. There are very few randomized controlled clinical trials of antimicrobial agents for the treatment of these infections. Schreiber and Jacobs (1), in a recent review, stressed the need for more controlled trials to determine optimal antimicrobials or other intervention necessary to treat infections due to PRSP. To optimize initial or empiric therapy for pneumococcal infections, clinical health-care providers must be informed of the prevalence and patterns of resistance among isolates in their community. The degree of resistance, variability in drug levels at different sites, particularly in the CSF and middle ear, natural history of the disease at different sites and in different age groups, stage of infection at which initial or appropriate therapy was instituted and presence of underlying ill-

nesses such as malnutrition, immunodeficiency, or malignancy, are some of the factors affecting treatment outcome (15,98).

The consensus among recent reviews is that Penicillin should no longer be used in the initial treatment of pneumococcal meningitis (109-110). Several authors advocate monotherapy with third generation cephalosporin, either ceftriaxone or cefotaxime (110-112), while others suggests initial therapy should include the combination of ceftriaxone or cefotaxime with vancomycin (111,112). A clinical study by Viladrich *et al* in Barcelona, Spain (114) showed cefotaxime and ceftriaxone to be reasonable first agent for meningitis. Although, Penicillin was potentially effective against sensitive strains or even intermediate resistant strains in high doses, they recommended that it should not be used as first line agent in view of the poor clinical outcome in their patients. Vancomycin has also been evaluated for the treatment of PRSP associated meningitis (115), but concern about penetration into the cerebrospinal fluids in adults prompted studies of combination regimens. Vancomycin and ceftriaxone combination was found to be synergistic even against strains with high penicillin and cephalosporin MIC (116). Ceftriaxone and rifampin was also found to be effective in adults given dexamethasone as adjunctive therapy (117). In adults treated with adjunctive dexamethasone, ceftriaxone plus rifampin is the preferred empiric combination regimen because dexamethasone reduces the penetration of vancomycin into the CSF in adults but not in children (118).

In areas with low prevalence of Penicillin Resistant Pneumococci therefore, empiric initial therapy with a third generation cephalosporin is advocated. In areas where pneumococci resistant to extended spectrum cephalosporins are prevalent, empiric therapies with vancomycin and an extended spectrum cephalosporin should be considered, until culture and susceptibility results are known. If the Penicillin MIC for the agent is < 0.1 µg/mL, then therapy can be changed to Penicillin 500,000 units/kg/day alone, which will most often be less expensive and carry less risk of promoting resistance to third generation cephalosporin and vancomycin. Alterna-

tively, the cephalosporin may be continued alone. For intermediate resistant isolate (MIC 0.1-1.0 µg/mL), third generation cephalosporins should be considered alone with vancomycin discontinued. When the MIC equals or exceeds 2.0 µg/mL or when there is little or no clinical improvement, the combination of cephalosporin and vancomycin should be continued. Vancomycin should not be used alone in the treatment of *Streptococcus pneumoniae* associated meningitis (115). Also, chloramphenicol is no longer recommended for use in the treatment of pneumococcal meningitis. Friedland and Klugman (119) demonstrated unfavourable outcome, defined as death, severe neurologic deficit or poor clinical response in 80% (20 of 25) of patients with PRSP meningitis treated with Chloramphenicol, 75-100 mg/kg/day, as initial therapy. Similarly in Dallas, 12 of 16 penicillin resistant isolates of *Streptococcus pneumoniae* from blood or CSF were associated with chloramphenicol minimum bactericidal concentration (MBC) of 8 µg/ml or more, resulting in poor clinical response (120).

In the treatment of otitis media due to PRSP, the elevated MIC for oral β-lactam agents including the new cephalosporins, the relatively low serum concentrations and poor penetration of antimicrobials into the middle ear combined to complicate therapy of otitis media due to these organisms (1, 119, 121). Amoxicillin has been advocated as the drug of choice for the initial treatment of acute otitis media, even in regions with high prevalence of PRSP (109,110,125). Studies have demonstrated relatively high clinical success rate in patients with PRSP associated otitis media ranging from 63% with Amoxicillin in a rural Kentucky study (122) to 82% with Amoxicillin Clavulanate potassium in another large multicentre open labeled trial in the United States of America (123). The clinical efficacy of second-generation cephalosporins, cefuroxime axetil and cefprozil, against pneumococci, have also been demonstrated in some studies (124-126). Barry *et al* (125) recorded 81% (43 of 53) clinical success rate in children with PRSP associated acute otitis media and 92% (152 of 166) in PSSP group.

Gehanno *et al* (126) also reported success rate of 75% for Penicillin resistant strains, 90% for Penicillin intermediate strain and 93% in Penicillin susceptible strain of pneumococcal acute otitis media in children under five years of age. Based on these studies, it is advocated that Amoxicillin 40 mg/kg/day should be the first line agent in the empiric treatment of acute otitis media in children and adults. In children with recurrent otitis media, who have not responded to Amoxicilin, Amoxicillin-Clavulanate (40 mg/kg/day Amoxicillin and 10 mg/kg/day Clavulanate) or second-generation cephalosporin, such as cefuroxime axetil (30 mg/kg/day) should be considered. For strain refractory to oral agent, injectable second or third generation cephalosporin or vancomycin may be indicated.

In treating Pneumococcal pneumonia due to resistant organism, opinion also differs over the best initial agent. This is as a result of few controlled trials designed to document the outcome in these patients. In a study by Pallares *et al* (127) and the report of the American Thoracic Society (128), underlying disease appeared to be a more significant risk factor for mortality than the susceptibility to the infecting organism. Hence some authors continue to emphasize the use of injectable Penicillin as a first line agent, claiming that treatment failure is much less likely than in meningitis caused by a strain with the same level of drug resistance (111, 129, 130). Others recommend initial use of cefuroxime, cefotaxime or ceftriaxone (109,111). Based on the available literature (109,111,113,128), it is currently advocated that initial treatment of pneumococcal pneumonia in patients requiring hospitalization should consist of cefuroxime, ceftriaxone or cefotaxime. Therapy can be altered on the basis of the clinical response and not solely on the MIC. If a patient is infected by a non-susceptible strain but is responding to treatment, no change in antimicrobial therapy is necessary. In patients with underlying disease or in community with high prevalence of PRSP, initial therapy should consist of cefotaxime or ceftriaxone and vancomycin. Therapy may be changed depending on the susceptibility of the organism and patient's clinical response.

CLINICAL SIGNIFICANCE OF PRSP INFECTION

The clinical importance of Penicillin resistance among pneumococci appears largely uncertain. Some reports (127,130) seem to suggest that patient outcomes are similar in individuals with PRSP and PSSP infection, even when the initial therapy consists of a β-lactam antibiotic. Older age and underlying disease appears to be more important factors influencing death from invasive pneumococcal disease than β-lactam susceptibility (127,128,131).

However, increase dosages of β-lactam agents is required to produce adequate bactericidal concentration (in view of the elevated MIC) particularly in the CSF and middle ears. Though patient outcome may not differ significantly from sensitive cases, financial costs may be influenced by the large doses required. Patients with hospital acquired non-susceptible pneumococcal infection were shown by Weis *et al* (132) to cost an institution approximately $16,000 more to treat than patients with Penicillin susceptible bacteria ($P < 0.05$). The difference in treatment costs was attributed to increased patient care requirement such as intensive or critical care beds and nursing services. In a nutshell, infection with resistant organism tend to increase the overall cost of therapy at both individual and institutional level and also increase the risk of toxicity from the increase use of potentially toxic drugs like vancomycin.

PREVENTION OF PNEUMOCOCCAL INFECTION

Patients who are at high risk of acquiring infections by pneumococci such as splenectomized patients, sickle cell anaemia patients, patients with immunoglobulin deficiencies or haematological malignancies should benefit from prophylactic Penicillin V or Erythromycin (98). Bacteraemia with PRSP may however occur in these groups of patient (133).

The use of multivalent polysaccharide vaccines in selected groups such as the elderly has been recommended. The 14-valent pneumococcal vaccines is no longer in use because of its lack of efficacy in children under 2 years of age and only 64% efficacy in children greater than 2 years (134). The currently available 23-valent pneumococcal vaccines contain purified

capsular polysaccharide antigens from 23 serotypes of *Streptococcus pneumoniae,* representing 85-90% of the serotypes responsible for invasive disease in children and adults in the United States (135,136). Of the seven serotypes most commonly associated with drug resistance, six are represented in the vaccine. Some degree of protection is provided against serotype 6A that is absent in the vaccine because of serologic cross-reactivity with serotype 6B that is present in the vaccine (137). Because of the emergence of drug resistant pneumococcal infection, there is need for adherence to the recommendation of the Advisory Committee on Immunization Practices (ACIP) that persons 2 years and above, with medical conditions placing then at increased risk for pneumococcal infection and all persons 65 years and above, should receive the 23-valent pneumococcal vaccines (138,139).

Children under the age of 2 years are especially susceptible to invasive pneumococcal infections and are at an increase risk for drug resistant infection. Commercially available polysaccharide vaccines are not able to elicit adequate immune response in young children under 2 years of age. This has led to the development of pneumococcal capsular polysaccharide-protein conjugate vaccine that employ the same principle used in *Haemophilus influenzae* type b vaccine; coupling the polysaccharide to a carrier protein, which increases immunogenicity (140,141).

Preliminary antibody titre result shows these vaccines, containing many serotypes, to be safe, and consistently elicit an immunologic response in infants as young as two months (140,141). In February 2000 (142), a conjugate vaccine for seven pneumococcal serotypes was licensed for use in infants and children, and is now recommended in the United States for all children less than 2 years of age, with catch-up vaccination schedules suggested for children 2 to 4 years of age (143).

OTHER CONTROL MEASURES

Surveillance for drug resistant *Streptococcus pneumoniae* should be initiated in all institutions and communities. In some states in the United States of America (56), state-wide surveillance for drug resistant *Streptococ-*

cus pneumoniae, as a notifiable condition, has been initiated. The Centre for Disease Control and Prevention (CDC), in collaboration with the Council of State Territorial Epidemiologists and Public Health Laboratory Directors, is helping to develop strategies for collecting information on PRSP in other states and for preventing morbidity and death associated with infection with these strains. Eradication of carrier states may also be an option to reduce level of resistance in community (60). Attempt at eradication with rifampicin and erythromycin carried out mainly in South Africa was successful in 96% of carriers while only 74% success rate was recorded with vancomyin (144). In areas with high prevalence of PRSP, there is at present, no rationale for treatment of carriers, as its value in outbreak situations remains largely unproven (15).

PROBLEMS OF PRSP IN AFRICA

Little is known about the prevalence of PRSP in Nigeria and many other African countries apart from South Africa. Most health institutions in Africa lack active antimicrobial resistance surveillance, drug monitoring and infection control programmes. There is therefore apparent lack of awareness by health care providers, of the occurrence of PRSP and other resistant organisms, and their clinical significance. Added to this is the poor laboratory service in many centres, to identify and perform susceptibility testing (145).

With poor socioeconomic situations in many African countries, occasioned by bad governance, there is gross under funding of the health sector. Little attention is paid to infectious disease surveillance and control programmes, an aspect of medicine that has not been well appreciated by many authorities. Superimposed on this, is the lack of regulation on antibiotic prescription and usage. Self-medication and over-the-counter prescription of antibiotics is widespread in Nigeria and many African countries (145,146). The problem of drug resistance is therefore expected to be enormous in these countries. A limited survey in 1978 gave a PRSP prevalence rate of 20% in Nigeria (65). This has a grave implication for therapy of serious pneumococcal infection in this country. Based on this and the

available literatures from other Africa countries (10,13,61,71,98) and elsewhere (109-114), penicillin will no longer be recommended in the initial (empiric) therapy of serious pneumococcal infections in Nigeria. Ceftriaxone and cefotaxime are the preferred agents in the initial empiric therapy of pneumococcal infections of the lungs, blood stream and the central nervous system.

There is the need to increase surveillance for PRSP in health institutions and communities to determine the true prevalence and evaluate their susceptibility to newer agents. Antibiotic prescription practice should be regulated by law, with outright ban on over-the-counter sale of antibiotics. Laboratories should make available, reports of susceptibility pattern of common pathogens in the environment to the physicians and other health care providers on a regular basis. There should be a coordinated action between the various health institutions and the National Infection Control Centre, which should be responsible for storing data on susceptibility and occurrence of PRSP and other resistant organisms.

CONCLUSION

Since there is no doubt that imprudent use of antimicrobials promotes the spread of drug resistance in both the hospital and the community, the emergence of drug resistant *Streptococcus pneumoniae* is hardly surprising. Although appropriate antimicrobial use has unquestionable value, providing antimicrobials for viral infections of the upper respiratory tract does not benefit patients, it rather increases the likelihood that resistant organisms will be selected. Many instances of "presumed bacterial infection" are likely to be of viral aetiology but are misdiagnosed because of inadequate diagnostic criteria used by the physician. Physician concern over inadequate treatment for presumed bacterial infection, combined with patient pressure for prescribing antimicrobials, further complicates the problem as does the use of more expensive, broad spectrum agents, which may not be necessary unless indicated by organisms' identification and susceptibility.

The primary responsibility for identification, management and control of the spread of drug resistance pneumococcal infection lie

with the primary care physician and diagnostic laboratory. Laboratories must be equipped to isolate and perform susceptibility tests and must participate in external quality control programmes. The presence of Penicillin intermediate and resistant *Streptococcus pneumoniae,* as well as the status of other drug classes must be known in each community and updated frequently to help guide empiric therapy of infections potentially caused by these organisms since organism detection and *in vitro* testing may not be available most of the time.

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A SURVEY OF HOSPITAL ACQUIRED INFECTIONS IN OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITAL, ILE-IFE

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A well-structured infection control programme plays a vital role in reducing mortality, morbidity and cost resulting from nosocomial infections in hospitalized patients. However infection-control activities in developing countries is severely constrained by lack of infection control infrastructure and lack of strong commitment by hospital clinicians and administrators as well as the level of socio-political and economic development prevalent in the developing world. The Infection Control Program (ICP) of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) was established in 1995. An analysis of data of a hospital wide surveillance obtained between January 1995 and December 1999 is hereby presented, highlighting our experience with the pattern of nosocomial infection seen in this hospital. From January 1995 to December 1999, a total of 19,471 patients were discharged during this period. Of this, 515 cases of infection were recorded while on admission, giving an annual prevalence rate of 2.7% for nosocomial infection per year. (X^2 = 47.34, df = 4, p = 0.000). The highest infection rate was recorded from the orthopaedic ward (12.8%), followed by the intensive care unit (ICU)(8.4%) while the neonatal ward (NNW) and the paediatric ward (PW) recorded relatively low figures (0.5%) and (0.4%) respectively. Gram-negative rods (GNR) 491(78.8%) were commonly encountered as pathogens implicated in hospital-acquired infection (HAI) followed by *Staphylococcus aureus* and "other" organisms 17(2.7%). Current methods are inadequate because phenotypic typing alone has limited discriminatory power. DNA typing method is now the 'gold' standard for epidemiological and routine investigation of HAI.

INTRODUCTION

A safe and appropriate health care delivery should be an important pre-occupation in hospitals. One way of achieving this is the establishment of Infection Control Committees (ICC), which has the primary task to investigate and design measures to control all forms

of Hospital Acquired Infection (HAI), thereby ensuring that nosocomial infections are within reasonable control. However, it is also true that the effectiveness of infection prevention and control depends largely on the level of sophistication of health care services and the prevalence of diseases (1).

Well-structured infection control has a vital role in reducing mortality, morbidity and cost resulting from nosocomial infections in hospitalized patients (2, 3). The United States Study on the Efficacy of Nosocomial Infection Control (SENIC) showed that a properly conducted program of surveillance and control could prevent 32% or nearly one third of cases (1,4). Hence the incidence of HAI and the problem of resistant organisms are low in developed countries with well established infection control programs (1, 3). Whereas report from the developing countries south of the Sahara have put the nosocomial infection rate to vary from 3-15% (2,5), in Latin America, infection rate range from 10-26% with increased mortality and morbidity and a consequent economic burden (2,6,7).

Moreover, infection control activities in developing countries are severely constrained by lack of infection control infrastructures and a strong commitment by hospital clinicians and administrators (7,8), as well as the level of socio-political and economic development prevalent in the developing world (9). The Infection Control Program (ICP) of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) was established in 1995 with an Infection Control Team (ICT) reporting to an Infection Control Committee (ICC). An analysis of data of a hospital wide surveillance obtained between January 1995 and December 1999 is hereby presented, highlighting our experience with the pattern of nosocomial infection seen in this hospital and the challenges ahead.

MATERIALS AND METHODS

A systematic program developed by the hospital but based on the Centres for Disease Control protocol and WHO (2,5,9,10) for active surveillance for the collection routine input data was adopted by the hospital at the onset of infection control activities in 1995 to date. The hospital has 2 Infection Control Nurses (ICN) based primar-

ily in the Medical Microbiology and Parasitology department. The ICN work closely with the Consultant Medical Microbiologist, who in turn reports the findings of the ICT to the ICC. The ICC is responsible for policy formulation and recommendation to the hospital. The hospital in Ile-Ife has a bed space of approximately 400.

The microscopy, culture and sensitivity result of all specimens received in the laboratory are scrutinized by the ICN before the commencement of routine visit to the wards. The CDC guidelines for determining the presence and classification of infection, determine which event is HAI (4,5). All specimens in the laboratory and bacterial agents associated with HAI were investigated by standard microbiological methods (12).

Primary data were collected with the aid of forms, designed for active surveillance. This contained essential identifying data like patient's name, age, sex, hospital identification number, ward or location within the hospital, service and date of admission. Others are date set of infection, the site of infection, and the organism(s) isolated from specimens and antimicrobial susceptibility pattern of isolates. Monthly summary constitutes secondary data and the following are considered essential: service unit, site of infection and causative agent(s). These are further analyzed at the end of every year to generate a third level of data for the overall frequency and distribution pattern of HAI in our hospital, which is the basis of the present report.

RESULTS

From January 1995 to December 1999, a total of 19,471 patients who were previously on admission for various ailment or disease conditions were discharged during this period. Of this, 515 cases of infection were recorded while on admission, giving a prevalence rate of 2.6% of infection per patient discharged per year (x^2 = 47.34 df=4, p=0.000). Table I shows a break down and the prevalence of HAI by year. The highest infection rate was recorded in 1996 (3.6%) and the lowest was 1991 (1.2%), while Table II shows the summary and trend of infection according to service unit. The highest infection rate was recorded from the orthopaedic ward (12.8%), fol-

lowed by the intensive care unit (8.4%) while the neonatal ward (NNW) and the paediatric ward (PW) recorded relatively lower figures (0.5% and 0.4%) respectively. The pattern of HAI by service area is summarized in Table III. When the frequency and type of infection were compared, wound infection from the surgical service units was highest with 260 (50.5%), next was urinary tract infection (UTI) 204 (39.6%) followed by bacteraemia 34 (6.6%). Respiratory infections and miscellaneous infections were 5 (1.0%) and 12 (2.3%) respectively. However, when the patterns of HAI between surgical and medical services were compared, Chi square showed a significant difference (X^2 = 46.4, df 4, $p<0.05$).

Table IV shows the frequency of pathogens and hospital associated infections. Gram-negative rods (GNR) 491(78%) were commonly encountered as pathogens implicated in HAI, followed by *S. aureus* 115(18.5%) and 'other' organisms 17(2.7%). The individual Gram negative rods implicated in HAI is shown in Table V. The highest recoverable pathogen was from the genus *Pseudomonas* 1693(4.4%), followed by *Klebsiella* 151(30.8%), *Proteus* 84(17.1%) and *Escherichia* 58(11.8%) successively while the least was *Citrobacter* 3(0.6%). Gram-negative pathogens that presented difficulty with identification were reported as 'coliform 26 (5.3%). Figure A is a histogram illustrating Gram- negative rods and HAI.

Table 1: INCIDENCE AND PATTERN OF NOSOCOMIAL INFECTION IN OAUTHC ILE IFE

YEAR	NUMBER OF DISCHARGE	NUMBER OF INFECTIONS N(%)
1995	4805	107(2.2%)
1996	3411	122(3.6%)
1997	4185	127(3.0%)
1998	3703	119(3.2%)
1999	3367	40(1.2%)
TOTAL	19471	515(2.6%)

X^2 = 47.34, df = 4, P = 0.000

Table 2: SUMMARY AND TREND OF INFECTION BY SERVICE

SERVICE	DISCHARGES	INFECTION
SURGICAL WARD	3670	150(4.1%)
ORTHOPAEDIC WARD	876	112(12.8%)
OBST & GYNAE	5531	138(2.5%)
MED. WARD	3961	68(1.7%)
PAED	3638	15(0.4%)
NNW	1498	7(0.5%)
ICU	297	25(8.4%)
TOTAL	19471	515(2.6%)

Table 3: PATTERN OF HAI SERVICE AREA 1995-1999

SERVICE	BACTEREMIA	WOUND INFECTION	UTI	RESPIRA-TORY INFECTION	OTHERS
SURGICAL WARD	7	77	54	3	9
ORTHO WARD	-	85	25	2	-
OBST WARD	1	72	65	-	-
MED. WARD	12	10	46	-	-
PAED WARD	9	2	4	-	-
NEONATAL	4	-	3	-	-
ICU	1	14	7	-	3
TOTAL	34	260	204	5	12
515(100%)	(6.6%)	(50.5%)	(39.6%)	(1.0%)	(2.3%)

$X^2 = 46.4$, df = 4, P = 0.000 (p<0.005)

* For the purpose of analysis "surgical ward", "orthopaedic ward" and "Gynaecology" were combined as surgical, Medical ward and Paediatric ward were combined as Medical and Neonatal and ICU were also combined.

** The columns "Respiratory infections" and "others" were not included in the analysis due to the small number of samples in these groups.

Table 4: HOSPITAL ASSOCIATED INFECTIONS BY SITE AND PATHOGEN 1995-1999

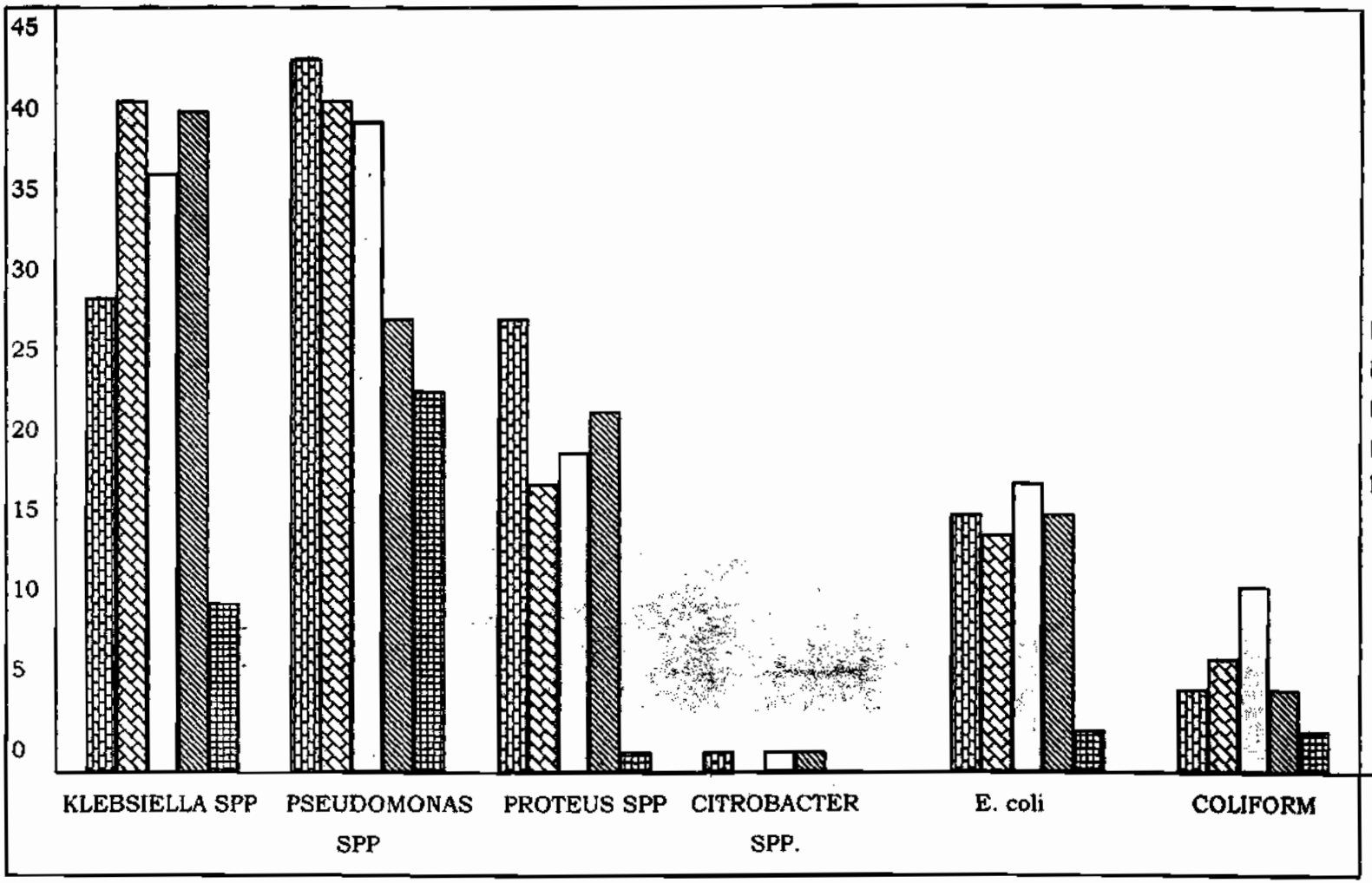
	GRAM NEGATIVE	S. aureus	*OTHERS
BACTEREMIA (n = 34)	17	16	2
WOUND INFECTION (n = 260)	286	83	14
UTI (n = 204)	177	16	-
RESPIRATORY (n = 5)	6	-	-
OTHERS (n = 5)	5	-	1
TOTAL 515	491	115	17

*Others
Coagulase negative *Staphylococcus* = 9
Streptcoccis faecalis = 8

Table 5: GRAM NEGATIVE RODS IMPLICATED IN HAI 1995-1999

ORGANISM	YEAR					
	1995	1996	1997	1998	1999	TOTAL
PSEUDOMONAS SPP.	42	40	39	27	21	169 (34.4%)
KLEBSIELLA SPP.	28	40	35	39	9	151 (30.8%)
PROTEUS SPP.	27	16	18	22	1	84 (17.1%)
CITROBACTER	1	-	1	1	-	3 (0.6%)
E. COLI	14	13	15	14	2	58(11.8%)
COLIFORM	4	6	10	4	2	26(5.3%)
	*	*	*	*	*	491(100%)

GRAM NEGATIVE RODS IMPLICATED IN HAI (1995-1999)



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DISCUSSION

The ultimate objective of establishing an ICP in any hospital is to translate surveillance efforts into infection prevention (1,5). In our experience, the overall average prevalence rate of HAI in this hospital is 2.6%. This figure is low when compared with 3.78% reported by Ogunsola *et al* (13) for Lagos University Teaching Hospital, and lower still for annual prevalence rate of 3-15% reported for hospitals in developing countries (5,6). Differences in matters of protocols and standard definition of cases of nosocomial infection may be responsible for differences in prevalence rate from one hospital to another which make a direct comparison difficult (4). This is why the CDC definition of nosocomial infection includes clinical and laboratory information and requires updating (2). However the investigation of HAI has been greatly aided by the development of DNA typing methods in the developed countries, tools that are currently not available to us at the moment.

Urinary tract infection (UTI) is the most common form of HAI accounting for between 30-45% (10,15), however, in our survey, UTI accounted for 39.6% of HAI after wound infection, which was 50.5%. Nearly all nosocomial UTI occurred in patients with recognized risk factors, such as urethral catheterization and other forms of instrumentation (13,15,16), this was also the case in our experience. The surgical service department recorded a high frequency of wound infection compared to the medical departments. This observation may reflect the extreme factors before, during and after operation of patients seen during this period. A prospective study done in this hospital on postoperative wound infection, reported an infection rate of 15.1% (17). However, this was restricted to general survey without orthopaedic and obstetrics and gynaecological cases taken into consideration, which are included in this survey. Our observation on the pattern of wound infections is consistent with report from other developing countries (16).

Bacteraemia as a form of HAI is most often secondary to urinary, surgical wound or lower respiratory tract infection, or to extra luminal cannular related sepsis (5). We observed that bacteraemia accounted

for 6.6% of total HAI seen in our hospital while a study from Trinidad and Tobago reported 8% (11). The underlying disease condition in the patients may have contributed to the observed level of bacteraemia in this survey.

Gram-negative rods were more implicated in HAI in this survey, more commonly, *Pseudomonas spp*, which is a recognized opportunistic pathogen in the hospital setting. The pattern of hospital associated infections by site and pathogen is similar to what has been reported by other studies (5,6,7,16). Fig. A shows modest achievement recorded on individual Gram-negative rods from 1995-1999 due to intervention method instituted like hand washing, increased disinfectant and soap rations to the wards and personal contacts to the clinical service departments.

Our survey shows that we need to improve on our infection control infrastructure and to encourage strict adherence to infection control practices such as hand washing techniques and improvement of facilities in the hospital environment (18). Finally, the effective prevention and control of HAI in the developing countries lies in the introduction and application of molecular epidemiology into surveillance activities. Current methods are inadequate because phenotypic typing alone has limited discriminatory power. DNA typing method is now the 'gold' standard for epidemiological and routine investigation of HAI (16,19).

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CARE SEEKING PRACTICES ON DIARRHOEA IN A RURAL COMMUNITY IN NIGERIA

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Although diarrhoea is a preventable disease, it still remains a major cause of morbidity and mortality among Nigeria children. A Nigerian child under age of five has an average of 4.3 diarrhoea episodes each year. This translates to 70 million episodes of diarrhoea in children under five, based on the 1991 census. With case fatality rate of 0.4% (1), Nigeria records 300,000 diarrhoea related deaths each year in children under five years of age. This community survey was conducted in the south west of Irepodun Local Government Area of Kwara state, Nigeria, to enable us determine care-seeking and diarrhoea management practices in a typical rural setting. Four thousand and sixty one (4,061) children under five year of age from nine villages were studied using the standard WHO questionnaire on diarrhoea case management and morbidity. The survey focused mainly on children who had diarrhoea in the 24-hour period prior to the study. Of the 4061 children who were 5 years or below, 876(21.6%) had diarrhoea two weeks prior to the study. There were 207 children (5.1%) who had diarrhoea within 24 hours prior to the study. The rate of use of salt sugar solution (SSS) was 16%, while that of oral rehydration salt (ORS) was 6%. Seventy three percent of mothers interviewed did nothing for the treatment of diarrhoea, nor understood what to do. 16% used various drugs. 69% of the health facilities in these rural districts used antibiotics as their first line anti-diarrhoea treatment. Health education on Oral rehydration therapy (ORT) needs to be intensified at the grassroot level.

Key words: Diarrhoea, Children, Care, Rural

INTRODUCTION

The word "diarrhoea" originates from the Greek terms *dia* (through) and *rhein* (to flow) (2). Diarrhoea is one of the leading causes of death among children in

the resource-poor countries including Nigeria. Nigeria records about 300,000 diarrhoea related deaths annually among children aged five years and below. Its effect on the social and economic life of the family is immense. Most of the diarrhoea deaths result from dehydration, from excessive loss of water, salt and electrolytes from the body. It has been observed that, malnourished and low birth weight babies (LBW) are more vulnerable to diarrhoea diseases than infants weighing 2500 grams or more at birth. Although malnourished and well-nourished children have equal numbers of diarrhoeal episodes, these episodes are more severe or prolonged in the malnourished children (3). Similarly, children with LBW or malnutrition are also at higher risk of becoming dehydrated during a diarrhoea episode than children with normal birth weight. One possible explanation for this increase severity is that infections in the proportionally greater intestinal surface area of infants and children result in larger stool losses per kilogram of body weight (4).

Diarrhoea is a clinical syndrome of diverse etiology associated with frequent passage of loose watery stools, vomiting and fever (5). It could be a symptom of infection by many different bacterial, viral and parasitic agents. Essentially, diarrhoea occurs when one or more of the following are present: abnormal increase in daily stool weight or increase in stool fluidity or increase in stool frequency. Diarrhoea is often accompanied by urgency, perianal discomfort, incontinence or a combination of all three (1). Diarrhoea can also occur in association with other infectious diseases such as malaria and measles. Approximately 70-80% of the vast number of sporadic diarrhoea episodes in people reporting at health centre in resource-poor countries can be diagnosed by aetiology. However, very few laboratories can identify all the newly described pathogens.

From a practical stand point, diarrhoeal diseases can be divided into six clinical presentations: simple diarrhoea, managed by oral dehydration with solution containing water, glucose and electrolytes, with specific aetiologic agent unimportant in management; bloody diarrhoea or dysentery caused by organisms such as *Shigella, Escher-*

ichia coli 0157:H7 and certain other organisms; persistent diarrhoea that last at least 14 days; severe acute diarrhoea as seen in cholera; minimal diarrhoea associated with vomiting, typical of some viral gastroenteritis and illness from the toxin such as *Staphylococcus aureus, Bacillus cereus* or *Clostridium perfringens* and hemorrhagic colitis with watery diarrhoea containing gross blood but with no fever or faecal leukocytes. Multiple episodes of diarrhoea can weaken children's resistance and increase susceptibility to other illnesses leading to death. One reason for continued mortality due to diarrhoea is lack of proper information and knowledge regarding the disease aetiology. Lack of proper information leads to false beliefs and myths and by extension faulty care-seeking practices. This study focused on simple diarrhoea managed by ORT and the care-seeking practices of mothers of children with diarrhoea in these communities.

METHODOLOGY

The survey was done using the WHO standard questionnaire on diarrhoea case management and morbidity (WHO/CDD: SER 86.2 Rev.1). The study involved 9 rural communities in the South-West of Irepodun Local Government Area of Kwara State of Nigeria. Four thousand and sixty-one (4,061) children under five years of age were surveyed in nine villages in a rural community in Nigeria. Information was collected by interviewing mothers of these children who had diarrhoea 2 weeks and 24 hours prior to the survey. Children under five years were identified using a cluster sampling method. Households were chosen randomly using a chance selection. The interviewers went from house to house and identified the children who had diarrhoea, two weeks and 24 hours before the date of interview. Standard questions asked include; the amount of food given during episodes of diarrhoea, the signs and symptoms that would prompt them to seek help from a health worker, and the use of drugs in treatment before seeking help. The questions were asked in the local languages. The interviewers also watched mothers prepare ORS and home fluids.

RESULTS

Demographic figures showed 4061 children under age of five years in the 9 villages surveyed. Of this, 876 (21.6%) reportedly had diarrhoea two weeks before the study. There were 207 children who had diarrhoea in the 24 hours prior to the study, giving a point prevalence of 5.1%. The rate of use of SSS was 16% while ORS was 6.7%. The incidence rate of diarrhoea per child was 4.3%. A large proportion (73%) of mothers interviewed did nothing for the treatment of diarrhoea disease because they conceived that it was a normal growth process that must take place, and needed no type of treatment. 16% sought non-SSS treatment by using drugs such as Kaolin, Morphine and Nalidixic acid purchased from local pharmacy shops. Eight hundred and fifty-three (21%) of the mothers, in addition, gave rice-based drinks and or corn-based drinks to their children. Only 1,746 (43%) of the mothers continued to feed sick children normally while 36% increased the amount of fluid during the episode.

Among the 1,879 mothers who were still breast-feeding before the diarrhoeal episodes, 1,538 (82%) continued to breast feed their babies while 341 (18%) stopped breast-feeding. Further findings showed 69% of the health facilities in the surveyed communities offered antibiotics as first line anti diarrhoeal treatment. 18 (2%) of the 876 children who had diarrhoea two weeks before the study died.

DISCUSSIONS

As a matter of fact, the use of ORT should be started early at the onset of the disease. In the acute stage, the patient is encouraged to drink freely and to take bland semi-solid and oral rehydration therapy (ORT) should be started early (6).

Mortality from diarrhoea in infants and children could be greatly reduced if ORT is correctly used and often for enough period. The incidence rate of 4.3% reported in this study compares favourably with findings elsewhere in the country (1), but the 2% fatality within two weeks in the nine villages studied is five times higher than the national average of 0.4%. That 73% of mothers did nothing to control or prevent diarrhoea in their children indicates a high level

of unawareness and call for intensive diarrhoea education and ORT use within the communities.

Most cases of acute diarrhoea, if handled properly and early enough at home will not even require treatment with ORS (7). Home management should be based on giving extra amount of safe fluids together with continued feeding with the child's normal diet. That mother stopped feeding their children with diarrhoea on the premise that "if they eat nothing they stool nothing" shows another mythical misconception that needs to be urgently corrected. However, chronic diarrhoea in young children poses special management problems because large fluid and electrolytes loses are tolerated poorly (1).

The choice of home fluid depends on local circumstances. In some of the communities studied, SSS has been used successfully, but in other, there have been problems with dangerous and ineffective solution caused by incorrect mixing. In some of the communities, the use of cereal-salt solution has been advantageous. Those made from rice and or corns have been comparatively cheap, handy, and culturally acceptable. They were also more likely to be given in the illness. However, the finding that some mothers merely diluted the child's usual foods (pap) to make fluids is wrong. Homemade cereal based ORT solution should not be confused with food. Furthermore, home-made food-salt solution are likely to promote sodium absorption more effectively than sugar-salt solution because they contain more of the carrier molecules (glucose and amino acids derived from starch and protein) necessary for water and salt absorption in the gut. This means that food-salt solution may decrease stool out-put and reduce loss of salt and water.

About 60% of the mothers who reportedly gave SSS were able to prepare it correctly. The commonest errors in preparation were those of over concentration. A lot of drugs have been inappropriately used in treating diarrhoea diseases. Thus, a thorough inventory of medication is essential to the accurate diagnosis of induced diarrhoea. Cardiotonic agents, such as digitalis, and cardioregulatory drugs, such as quinidine, are particular offenders (8). The use of in-

jections and antibiotics in the treatment of diarrhoea was common in these communities. Sometimes, as much as four different drugs had been used, the commonest being kaolin, ampicillin, nalidixic acid and chloramphenicol. World Health Organization has continued to emphasize the dangers of the unnecessary or careless use of drugs in the treatment of acute diarrhoea in children. Compared to the ORS, these drugs are expensive, dangerous and useless (9). Poor families spend more money they cannot afford to do harm rather than good to their children. Considering the frequency of presentation, it has been noted that acute transient diarrhoea disease, apparently of infectious origin, is an extremely commonly encountered entity in both the infants and the adults (10).

RECOMMENDATION

Health education regarding diarrhoea diseases and the use of ORT need to be intensified to create lasting awareness among mothers. Emphasis should be on the importance of cheap simple ORT as the first remedy (and often the only remedy required) for acute diarrhoea. The crucial information must become common thinking at all levels of all societies and be backed up by all health providers, including local drug peddlers and herbalists.

Above all, further research is necessary in the areas of the significance thinking and thus about diarrhoea. The hygiene component of diarrhoea disease, control and prevention should also be stressed among the communities and society at large. This is important because people are likely to have fewer diarrhoea episodes if they have access to clean drinking water and good sanitation facilities. If however, hygiene behaviour including hand washing is poor, the health benefits resulting from provision of improved sanitation and water supplies will be limited. Clean drinking water and better sanitation typically reduce diarrhoea incidence by only 25%(11).

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ANTI BACTERIAL ACTIVITY OF SOME SELECTED DISINFECTANTS REGULARLY USED IN HOSPITALS.

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The antibacterial activities of three commercial disinfectants: Dettol, Robert and Savlon against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella spp.* and *Bacillus spp.* were investigated. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the well technique of media diffusion method. The highest MIC of 1:10 against *Pseudomonas aeruginosa* by Roberts and the lowest MIC of 1:60 by Savlon against *Staphylococcus aureus* were observed. The highest minimum bactericidal concentration (MBC) of 1:10 against *Escherichia coli* and *Pseudomonas aeruginosa* was by Robert. All test organisms were susceptible to various dilution of Savlon used.

INTRODUCTION

Since microorganisms were identified as agents of infection, various methods have been described to either eliminate them totally or just restrict the number of viable cells. Some workers (1) observed that environmental surfaces are epidemiological important reservoir of nosocomial bacterial species. Disinfection is defined as the selective elimination of certain undesirable organisms in order to prevent their transmission (2). This is achieved by the use of chemical substance called disinfectants. Disinfectants are used in hospitals as pre-operative and surgical scrubs, general disinfection of surfaces and for disinfecting cleaning equipment.

It is well established that concentration have a great influence on the effectiveness of disinfectant i.e. a bactericidal disinfectant may become bacteriostatic at a lower concentration. The in-use topping of old dilutions and use of disinfectant concentration lower than recommended concentration

have been identified as dangerous practices (3). Usually disinfectants are referred to as bactericidal or bacteriostatic without defining the concentration, the identity state of the organism and condition under which the two come in contact (4). The mode of actions of disinfectants is thought to be linked to destruction of proteins, lipids or nucleic acids in the cells or its cytoplasmic membrane, although microorganisms differ in their sensitivity to chemical germicides (5). Some researchers (6) have reported the survival in and contamination of working dilutions of some disinfectants in hospital by some microorganisms. This study therefore investigates the antibacterial activity of some commonly used disinfectant against some pathogenic bacteria commonly encountered in the environment. The nature of antibacterial activity, minimum inhibitory concentration and minimum bactericidal concentration were examined.

MATERIALS AND METHOD

Six bacterial species: *Staphylococcus aureus, Bacillus spp, Klebsiella spp, Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli* obtained from the medical microbiology and parasitology laboratory of the University of Ilorin Teaching Hospital were used as test organisms. Varying concentrations of the disinfectant were prepared by dilution (7). The media diffusion method was employed in the determination of the minimum inhibitory concentration (MIC) of the disinfectants while the media dilution method was employed in the determination of the minimum bactericidal concentration (MBC) (8).

RESULTS

The minimum inhibitory concentrations (MIC) of the test disinfectants are shown in Table 1. The organisms showed varying sensitivity to the disinfectants. The lowest bacteriostatic concentration was 1:20 dilution of Robert against *Klebsiella spp.* while the highest was 1:60 dilution of Savlon against *Staphylococcus aureus.* Table 2 shows the minimum bactericidal concentration (MBC) of disinfectants. The highest bactericidal concentration was 1:20 dilution of Dettol against *Bacillus spp.* while the

lowest was 1:10 dilution of Robert against *Pseudomonas spp.* and *E. coli.* Savlon appeared to be the most potent against the organisms.

Pseudomonas spp. and *Klebsiella spp.* appeared to be the most resistant of the organisms while *Staphylococcus aureus* appeared to be most sensitive.

Table 1: The minimum Inhibitory Concentration of Disinfectants

Test Organism	Dettol	Robert	Savlon
Klebsiella spp.	1:40	1:20	1:80
Pseudomonas spp	1:40	1:40	1:80
Escherichia coli	1:60	1:40	1:80
Bacillus spp	1:60	1:60	1:100
Salmonella typhi	1:60	1:60	1:100
Staphylococcus aureus	1:60	1:60	1:160

Table 2: The minimum Bactericidal Concentration of the Disinfectants

Test Organism	Dettol	Robert	Savlon
Klebsiella spp.	1:20	1:20	1:180
Pseudomonas spp	1:20	1:10	1:180
Escherichia coli	1:40	1:10	1:180
Bacillus spp	1:120	1:180	1:180
Salmonella typhi	1:180	1:180	1:180
*Staphylococcus aureus**	1:60	1:100	1:180

Key. * = Susceptible to all Concentration used .

DISCUSSION

Result obtained confirmed the submission that concentration affects the activity of disinfectants. The different active component could have contributed to the difference in activity of the disinfectants. The disinfectants appear to have broad spectrum of activity, showing activity against Gram-positive and Gram-negative bacteria. It has been recommended that disinfectants for general use should be able to kill a wide range of common or potential pathogens (9). The media component could also have affected the outcome of the activity testing; the presence of organic matter has long been identified as a factor that affects the action of disinfectants.

Pseudomonas spp. has been consistently reported as a problematic organism, in showing resistance not only to antibiotic but also to disinfectants (6, 9, 10) although the resistance factor has not been elucidated in the case of resistance to disinfectants. On the other hand, the emergence of *Klebsiella spp.* showing resistance to the disinfectant is of interest as it has not been previously shown to survive in and contaminate disinfectants (6).

Klebsiella spp., Pseudomonas aeruginosa and *Escherichia coli* were pointed out as organisms that have been well documented as agents of nosocomial infection and suggests interruption of transmission or cross contamination, and appropriate disinfection as part of measures of controlling nosocomial infections. That the tested disinfectant showed activities against these organisms is thus cheering information. In view of the importance of disinfection in clinical practice and domestic hygiene, and the danger of development of resistance by the organisms exposed to the disinfectant, it will be in the overall interest of all to ensure that only fresh preparations of disinfectants are used routinely and dilution should be restricted to the concentration ranges that have been found to have definite activity against the organisms.

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HELICOBACTER PYLORI SEROLOGY AND EVALUATION OF GASTRODUODENAL DISEASE IN NIGERIANS WITH DYSPEPSIA

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***Helicobacter pylori* (*H. pylori*) has been strongly associated with various gastroduodenal diseases worldwide with only a few studies emanating from developing countries. The objectives of this study were to determine the prevalence of serum Immunoglobulin G (IgG) and underlying gastroduodenal pathology in Nigerian patients with dyspepsia and ascertain the usefulness of *H. pylori* IgG screening in decreasing endoscopic workload in dyspeptics in Nigeria. Fifty-five patients with dyspepsia and 55 age and sex-matched apparently normal control were screened for *H. pylori* IgG using Immunocomb^{R}II kits. Each of the 55 patients was also examined endoscopically with biopsies taken appropriately. Serology was positive in 94.5% and 92.7% of dyspeptic patients and controls respectively. Gastroduodenal inflammation was the commonest endoscopic finding, 43 (78.18%). Other findings were malignant gastric tumour 6(10.9%), reflux oesophagitis 3 (5.45%), gastric ulcer 2 (3.64%), and duodenal ulcer in 1 (1.82%). Chronic gastritis was the main histopathologic finding in the dyspeptic patients. It is concluded that serum *H. pylori* IgG cannot be used as a screening procedure to reduce endoscopic workload in Nigerian patients with dyspepsia.**

Keywords: dyspepsia; *Helicobacter pylori* serology; gastroduodenal disease

INTRODUCTION

Dyspepsia, which has been defined as pain or discomfort centered in the upper abdomen (1,2), is a common gastrointestinal complaint in Nigeria (3). In Ibadan, Nigeria, over 50% of dyspeptic patients have been shown to have non-ulcer dyspepsia (4). Since the landmark discovery by Barry and Marshal of the association between *Helicobacter pylori* and gastritis in 1982 (5), several studies have confirmed (6,7) or doubted its association with gastroduodenal disease (8) and yet some suggested its association with non-gastrointestinal disease (9). Serology for *H. pylori* infection has been found to be accurate, rapid, cost-effective (10,11) and capable of significantly decreasing endoscopic workload in

patients with dyspepsia (12). We investigated a total of 110 subjects at the University College Hospital Ibadan, Nigeria to determine the prevalence of *H. pylori* IgG serology and underlying gastroduodenal disease in patients with dyspepsia and normal controls.

MATERIAL AND METHODS

One hundred and ten adult subjects of both sexes, aged between 18 to 74 years consisting of 55 patients with dyspepsia (34 males; 21 females) with no previous treatment for *H. pylori* and 55 apparently normal control with no previous or present history of dyspeptic symptoms (33 males; 21 females) gave informed consent to participate in the study. Dyspeptic patients were consecutively selected from the pool of patients attending our Gastroenterology clinic, based on consent and fitness to undergo eosophagogastroduodenoscopy (OGD). The control groups were apparently normal individuals selected from office workers and individuals attending the hospital for routine check ups and hypertension. The control group did not undergo OGD screening. Patients and control were aged between 18 and 74 years and the study period lasted for eighteen months. Excluded from the study were patients who have been on non-steroidal anti-inflammatory drugs (NSAIDs) in the last 3 months, chronic liver disease (all the dyspeptic patients were negative for hepatitis B and human immunodeficiency viruses 1 and 11) as well as pregnant women.

Five milliliters of venous blood was collected from each subject, into unheparinised bottles. The serum was separated after centrifugation and frozen immediately at -20°C till time of analyses after the study period. Sera were analyzed for *H. pylori* IgG using Immunocomb^{R}II (Manufacturer- ORGENICS, Yvane, Israel. Website: http://www.orgenics.com) with sensitivity and specificity of 95.8% and 76% respectively. Upper gastrointestinal endoscopy was carried out in all the 55 patients with multiple biopsies taken from the gastric antrum, duodenum, upper and lower margins of cancerous lesions and other suspicious sites in the upper gastrointestinal tract. The control subjects did not undergo eosophagogastroduodenoscopy. Tissue specimens were promptly

fixed in 10% formalin and subsequently processed in the histopathology laboratory where paraffin sections were stained with routine haematoxylin and eosin stain for histological examination.

RESULT

Serology

Fifty-two (94.5%) and 51 (92.7%) of the dyspeptic patients and normal controls were seropositive for *H. pylori* IgG receptively. Seropositivity was similar in both males and females. All the patients with gastric carcinoma were positive for *H. pylori* IgG antibody.

Endoscopy

Endoscopic findings were mainly gastroduodenitis 32(58.18%), chronic gastritis 9 (16.36), and chronic duodenitis 2 (3.6%) Table 1. Atrophic gastritis was observed in 11 patients with gastroduodenal inflammation. Six patients aged 35 to 67 years had gastric tumours (5 antral; 1 cardial) and consisted of 4 males and 1 female. The only female patient among them was 35 years old while the men were between 46 and 67 years with a mean age of 58 years. Reflux oesophagitis was found in 2 of the patients with gastric cancer. Submucosal hemorrhage was found in 5 of the patients with gastritis. Three patients had oesophagitis while 12 (28%) of the 43 patients with gastroduodenal inflammation also had bile reflux.

Histopathology

Only 45 (18.8%) of the dyspeptic patients eventually had histopathologic assessment performed on the biopsy specimens (other specimens were lost in transit). Of these, 36 patients had gastric tissue present while 27 patients had duodenal tissue included in the biopsy specimen for assessment. Following histological assessment, there were 5 cases of gastric adenocarcinoma. Thirty-four of 36 (94.4%) gastric biopsies assessed had histological evidence of varying grades of chronic gastritis and one case of normal gastric mucosa was recorded. All duodenal biopsies examined show histological evidence of chronic duodenitis, but none had *H. pylori* on histology. *H. pylori* colonization was observed at histology in 14 (41.2%) of the 34 cases of chronic gastritis, while one of the five cases of gastric adenocarcinoma showed *H. pylori* colonization in the background gastric tissue.

Table 1: Endoscopic features in patients with dyspepsia

Endoscopic features	Number	Percentage
Gastroduodenitis	32	58.18
Chronic gastritis	9	16.36
Gastric tumour	6	10.9
Chronic oesophagitis	3	5.45
Chronic duodenitis	2	3.64
Gastric ulcer	2	3.64
Duodenal ulcer	1	1.82

DISCUSSION

Helicobacter pylori has been shown by various studies to be causally linked with various gastroduodenal disease (5,13,14,15). Most of these studies were done in developed countries of the world, with only a few studies emanating from developing countries. This cross-sectional study has shown a high seroprevalence of *H. pylori* both in dyspeptic patients and apparently normal control with no current or previous symptoms of dyspepsia. Previous studies in Nigeria and other parts of Africa have shown a similar high prevalence of *H. pylori* infection in normal and dyspeptic population (16,17,18). These findings further strengthen the multifactorial concept of aetiopathogene sis of dyspepsia as well as the fact that in a developing country like Nigeria where there is no significant difference in the infection in dyspeptic and normal populations, *H. pylori* may well be an innocent bystander or an opportunist that cashes in when other factors have rendered the mucosa susceptible to damage. Also, the well known fact that many people infected with *H. pylori* never show symptoms of disease (19) and finding of a similar prevalence of *H. pylori* infection in geographical area where incidence of dyspepsia is high in one and low in the other (20) tend to whittle down the significance of *H. pylori* infection in the pathogenesis of

acid peptic disease in these regions. Recently, it was shown that there has been an increase in peptic ulcer disease unrelated to *H. pylori* infection in developed countries (21). This finding also attests to polycausality of peptic ulcer disease. From the foregoing the magnitude of contribution of *H. pylori* to dyspepsia in the studied group is difficult to determine, as prevalence of infection in asymptomatic controls was not significantly different from those who had dyspepsia. The variation in host and microbial factors, which are known to determine the development of disease as well as the multifactorial nature of dyspepsia, may account for this. The extent of contribution of *H. pylori* in the population needs to be determined by further studies.

Dyspeptic patients in Nigeria could be safely presumed to be *H. pylori* positive until otherwise proven, as 94.5% of the dyspeptic patients were sero-positive. This prevalence in however slightly higher than 85% documented by Malu *et al* in blood donors in Jos, Northern Nigeria using a similar method. Endoscopic finding of mainly gastroduodenal inflammation in our study has been a typical finding among Nigerian patients with dyspepsia with incidence of frank ulceration much lower than findings in developed countries of the world (4, 22,23,24). This is most likely due to rampant abuse of over-the-counter drugs which in Nigeria include antibiotics, antacids and antisecretory drugs, which patients take without prescription and only presenting for hospital care if there is no improvement or with incomplete resolution of their symptoms. This abuse of therapy may cause some degree of ulcer healing before endoscopy is carried out and may account for the low incidence of ulcer recorded in these studies. Some of the patients with dyspepsia and gastroduodenal inflammation were found in addition to have doudenogastric reflux (bile reflux) but the degree of contribution of this to the dyspeptic pain is difficult to quantify. Endoscopic oesophagitis seen in 3 (5.5%) patients, 2 of whom had reflux in addition, suggest that gastric acid reflux may be contributory to dyspeptic pain in our patients. None of them, however, presented with classical symptoms of gastro-oesophageal reflux disease (GORD)

before endoscopy. Six (10.9%) of the patients were found to have gastric carcinoma (5 antral; 1 cardial) with age range 35 to 67 years. All the patients with gastric adenocarcinoma were positive for *H. pylori* serology but only one was positive on histology. The histological finding of *H. pylori* in 41.2% of the gastric tissue of patients with chronic gastritis is lower than IgG in the serum. This difference may be attributable to the patchy pattern of *H. pylori* colonization in gastric mucosa. Also previous studies have show that identification of *H. pylori* is less sensitive when routine elementary and eosin preparation alone is applied [as was the case in this study] than when it is combined with special techniques such as Giemsa or Steiner staining (25). It is instructive that one of the patients who had a normal gastric histology was also negative for *H. pylori* IgG. This we consider as a case of non-ulcer dyspepsia type II.

CONCLUSION

It is concluded that *H. pylori* seropositivity is similarly high among both normal people and dyspeptic patients in the study group and therefore the test is not discriminatory between the two groups and as such cannot be used to confirm dyspepsia. Also, gastroduodenal inflammation remain the commonest cause of dyspepsia in Nigeria and *Helicobacter pylori* is commonly associated with chronic gastritis in Nigeria as is the case in the rest of the world.

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PITYRIASIS VERSICOLOR – POSSIBLE GENETIC BASIS, PROBABLE TRANSITION FROM COMMENSALISM TO PARASITISM AND THE IMPLICATION ON TREATMENT APPROACH

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Non-occurrence of Pityriasis versicolor (PV) in spouses of individuals with this superficial fungal infection despite several years of cohabitation suggests that heredity might play an important role in those affected. Forty subjects who were married were studied in two phases. The first phase involved using a detailed structured format to obtain information on the presence of PV in subjects, their spouses and siblings. In the second phase the family of an index case was studied over a 2-year period from a clinical and mycological aspect. Seventy percent of subjects interviewed in the first phase had at least one family member with PV. In the second phase, 4 other siblings of the index case studied all had PV. Of the 3 that were married (index case inclusive), 2 had PV but none of their spouses had. This study supports the role of heredity in the individuals with PV.

Keywords: Pityriasis versicolor, Hereditary, Treatment

INTRODUCTION

Pityriasis versicolor (*various colours)* (PV) is a superficial fungal skin disease presenting with finely scaly patches in 'geographical' patterns often found in areas of maximum sebum production such as the trunk, the neck, face and thighs after puberty. The observation that married adult patients who recurrently suffers from Pityriasis versicolor but whose spouses never had the entity despite many years of living together prompted a study to consider the role of hereditary and the interplay of other factors in the appearance of this clinical entity.

Malassezia furfur (MF) or *Pity-*

rosporum orbiculair is a commensal on the skin. The skin in up to 96% of post-pubertal adults harbours the yeast form (1). Studies of normal and affected skin of patients with PV support a dimorphic concept: that MF becomes pathologic when it changes from the yeast to a filamentous form (2). PV is rare before puberty (3). An apparent clustering of PV in families has been noted for decades and the probability of genetic basis has been postulated (4). PV is commoner in the tropics and subtropics than in the temperate countries with prevalence as high as 40% in adults in some small communities (5). There is thus a need to look more at this entity in the West African sub region.

SUBJECTS AND METHOD

A predominantly epidemiological study was conducted using questionnaires. We decided to study family units. The study focused on adults and on marriages of not less than two years duration. Cohabitation of couples at least twice a week or any other prolonged skin to skin contact was the most important inclusion criterion. Information on parents, brothers, sisters and children (where indicated) of the index PV case seen in the clinic was documented. The study excluded the extended family to ensure more reliable information. The study was carried out in two phases.

Phase 1

A detailed structured questionnaire was utilized to obtain demographic data on the index cases as well as information on the presence of PV in spouses and siblings. This had the advantage regarding cost of the study and the relative ease of obtaining information. Information could be obtained on family members not physically around – while in the boarding schools, or on a job out of town. As PV fluctuates from one season to another and flares up in response to various aggravating factors, active cases may thus not always be available for physical assessment at the time of visitations to the families as in one of the cases of Robert's (4). We were encouraged in the use of questionnaires for phase one due to the ability of many people to correctly diagnose the condition in our environment. From unpublished data, in the skin

clinic at University College Hospital, Ibadan, eight out of every ten cases of PV patients were right about the diagnosis they made. PV is a well-known entity attested to by the existence of distinct names for it in many Nigerian languages (6). A few tribal names for PV in Nigeria are: 'Aikhumosele' – affliction of the beautiful in Bini (Edo), 'Ifo' – spots on light complexioned people in Yoruba, 'Ndiong' – rash of the beautiful in Efik, 'Ngwo ndi nma' – spots of the beautiful in Igbo and 'Kyasfi' – spots on young people in Hausa.

Phase 2

This involved closely studying, over a 2-year period, family members of an index case thoroughly from the clinical and mycological aspects. This was considered to be essential since other cutaneous conditions like erythrasma, pityriasis rosea, atypical seborrhoeic dermatitis or dermatophytosis could be confused with PV, even by the health workers. It also allowed us to observe fluctuations and the possibility of contagion over the period. It was more demanding in term of cost and time hence the smaller number families for this phase.

RESULTS

Phase 1

Of the 40 index cases (diagnosed from clinical features and mycology), 19 were males and 21 were females. They were aged 18 – 70 years. The responders have been married for 2 – 50 years. One of the sets of couples had a spouse who had PV. PV in him had been present for years, long before marriage or bodily contact with the spouse. In 70% of the index PV cases interviewed there was one or more family members (parents, children or siblings) with PV.

Phase 2.

Figure 2 shows the family tree of an index case and the presence of PV over a two-year period in family members. The pattern of involvement is suggestive of some hereditary involvement.

FIGURE 1

PREDISPOSING FACTORS FOR PITYRIASIS VERSICOLOR.

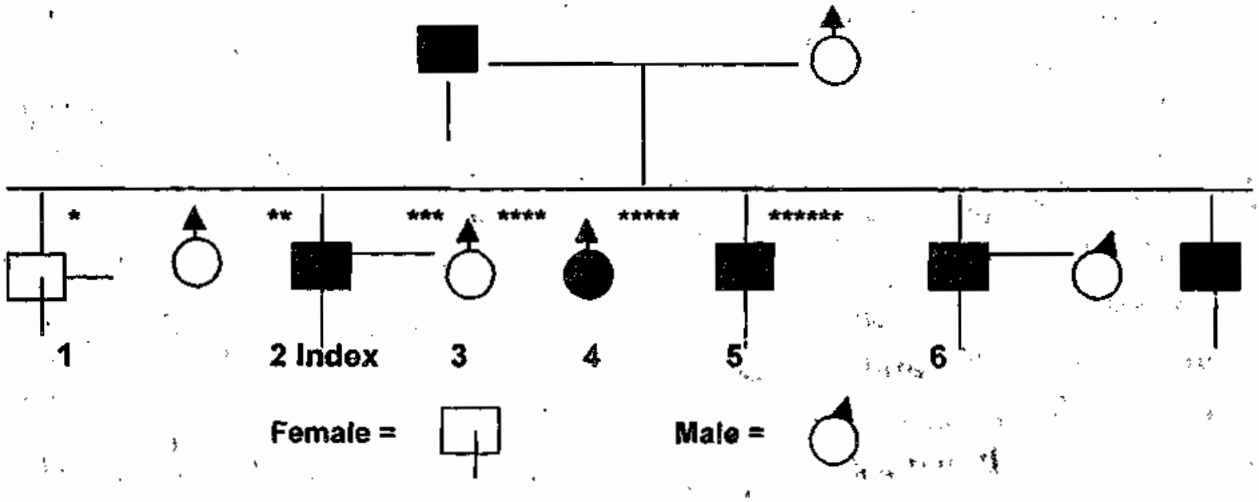
iAge 0-5 yrs (Yeast form (Colonization 0-10%) Increase in SEBUM **ii) Age 15 yrs-Adult** (Yeast form (colonization-96%) **(iii)** Genetic Predisposition (Aggravating factors) (A) (B) (C) (D)

(v) Clinical state (Pityriasis versicolor) **(iv)** (Pathogenic filamentous form.

(A) High humidity, heat, CO2, Sweat, occlusion (14)
(B) Steroid (iatrogenic or Cushing's disease) (10)
(C) Reduced body immunity-pregnancy, malnutrition, **malignancy**, intensive care setting (8)
(D) More amino acids on the skin, shorter alkali neutralization time, Reduced degree of water spreading (15).

FIGURE 2

FAMILY OF A CASE OF *PITYRIASIS VERSICOLOR* STUDIED CLOSELY FOR TWO YEARS HISTORICALLY FOR PV AND WITH CLINICAL AND MYCOLOGICAL CONFIRMATION FOR PITYROSPORUM ORBICULARE



Black fill effect = Pityriasis versicolor

* Siblings of the family being studied – 5 females and one male youngest = 35 years (******)

1* = First born 2 Second born etc. of family being studied**
2 index case: age 46 years, female already married for 23 years**
Husband still has not had pityrisis versicolor.

DISCUSSION

The present study is a preliminary one. The advantage of the present study using questionnaire mainly, apart from financial, is the avoidance of the problem of examining women in purdah (where the husband and patient are strict moslems). Secondly a visit may not yield result since PV might have cleared in a spouse at the time of visitation, as in one of the 3 cases of Roberts (4). Clinical diagnosis of PV from our experience agrees with the patients' impression in about 8 out of every 10 cases in this environment and from a questionnaire tested before the commencement of the present study. It is thus considered justifiable to use the questionnaire in gathering of data in the study of this chronic skin disease exhibiting periods of exacerbation and remission.

In the present study, one of the patients had a spouse who had PV. It was found that he had PV long before marriage or bodily contact with the index case, the wife. It cannot be proven conclusively from this study that the current infection is not from the wife. It will require complex mycological studies to show that the strains from the couple are the same.

The present study has limitations. There could be missed diagnosis especially where the spouses are not very observant and where the extent of the lesions is small. More positive responses i.e. the presence of PV in 70% of blood relations of the patients suggests some genetic or familial predisposition. Genetic factors have been adduced in a study (7). The continued reappearance of PV in such blood relations (who do not live with the index case after the latter has moved away following marriage for example and where spouses remain unaffected after long periods of staying together and having bodily contact) suggests more of a genetic rather than familial predisposition.

Faegerman and Fredrikson scraped apparently normal skin from 85 infants younger that 5 years of age and observed no *Pityrosporum orbiculaire* (PO) colonization (1). They recovered the yeast however from 10%, 23% and 93% of children aged 5, 10 and 15 years respectively. It was postulated that the development of sebaceous gland, reaching a maximum at about puberty and increased skin

fatty acid content must account for the increased yield with age. The common site on the trunk, the onset of PV at puberty and the lipophylic nature of the organism in culture support the need for lipids for organism sustenance. Lipid content does not explain it all since infants are also affected in some special situations. Although Maples (5) did not indicate the medical status of 2 infants with PV seen in his survey of a village, the high prevalence of infants' colonization with PO appears to be unique to the intensive care setting. It was not possible to recover PO from 50 normal newborn infants within the first 3 days of life – demonstrating that PO is not perinatally transmitted (8). In contrast, skin colonization of low birth weight infants with *Candida spp.* appear to occur frequently during labour and delivery (9). The flare up of PV in pregnancy, malignancy, steroid (iatrogenic or Cushing's disease) (10) and the colonization in infants hospitalized in intensive care units suggest some relationship to the level of body immunity. Sweating and occlusion in a hot tropical setting are some of the aggravating factors and possibly contribute to the high prevalence in some tropical areas with up to 40% in some series compared to less than 1% in temperate countries (11).

Studies of normal and affected skin of patients with PV support the dimorphic concept that *Pityrosporum orbiculaire* becomes pathogenic when it changes from yeast to a filamentous form (3). Since about 95% of the skin in adults harbours the yeast form, the absence of clinical PV in the spouses of the patients screened by questionnaire in this study, as well as the presence of PV in 70% of members of the families of patients point to hereditary factors in the predisposition to the filamentous form, while the factors mentioned earlier on act as adjuncts (Fig. 1).

Pityriasis versicolor responds to most of the antifungal agents available in most countries; Whitfield ointment (Benzoic acid/ salicylic acid mixture), polyenes, imidazoles, selenium sulphide, sulphur ointment, sodium thiosulphate and recently triazoles like itraconazole (sporanox). There is often recurrence however after cessation of the agents some time later either weeks or months. Even the use of ketoconazole which when

taken orally gets to all skin surfaces, removing the possibility of missed or skipped lesions does not prevent recurrence of PV. In a trial of ketoconazole by Borelli (12), 78 of 82 patients were considered cured. He concluded that this result indicated that ketoconazole is effective in the treatment of pityriasis versicolor, but problem of protecting susceptible persons from infection and re-infection remains unsolved. PV thus appears not to be readily contagious like other fungal entities e.g. dermatophytosis since the causative organism is a normal commensal in about 96% of pubertal and post pubertal individuals. It will appear that the clinical condition known as PV results from the interplay of features as shown in figure 1.

The principles/approach to treatment will thus involve: maintenance therapy (continuous or intermittent) to reduce the population of the fungi by the cheapest (i.e. effective treatment at an affordable price) and safest drugs. Griseofulvin should never be used as it has no effect whatsoever on the organism. Apart from the wasting of money, it may also result in headache (a common side effect) to the unfortunate patient. Oral medication can be tried in extensive lesions. There will thus be no skipped areas (common with the use of topical agents) to areas beyond the reach of the patients' hands. Itraconazole and ketoconazole have been tried this wise. Ketoconazole 400 mg as a single dose for 1-2 days can be used to treat widespread infections. Fluconazole is also effective. Topical imidazole (creams) applied twice daily may be helpful but are much expensive for the developing countries. A shampoo with fungicidal activity such as ketoconazole (Nizoral shampoo by Janssen/Cilag) is good especially as it can be applied to a large area of the body including the hair. The latter harbours the causative organism (PO), as well as *Pityrosporum ovale*. Ketoconazole (Nizoral) shampoo application should be twice weekly in the first phase for a period of 2-4 weeks leaving the shampoo in contact with the skin for 5-10 minutes before rinsing. In the prophylactic phase once week application as above will be adequate. Selenium sulphide and Zinc pyrithione have also been in use. Avoidance of heat and very moist/humid areas will be

beneficial. The use of corticosteriod creams as cosmetics, a common practice in the West Africa (13) and as skin bleaching agent should be discouraged since steroids encourage proliferation of PO. Also greasy/oily cosmetic should also be avoided. Since the organism is lipophylic, the use of coconut oil on the skin has been found to increase the prevalence of PV in some villages. Good skin hygiene should be encouraged. The patient's mind should prepared to expect the hypo or hyper pigmentation to lag behind therapy by weeks or months depending on sun tanning exposure, skin colour and other factors.

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