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APPLICATION OF FACTORIAL DESIGN FOR THE OPTIMIZED PRODUCTION OF ANTISTAPHYLOCOCCAL METABOLITE BY *AUREOBASIDIUM PULLULANS*

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Abstract

Background: Antimicrobial substances are mainly produced by bacteria and lower fungi, and have great roles in the treatment of most infectious diseases.
Purpose: Production of antistaphylococcal metabolite from A. pullulans by development of a cultural medium using response surface methodology.
Methods: Production of antistaphylococcal metabolite from Aureobasidium pullulans was optimized in shake flasks using a statistical experimental design approach. Effect of various components in the basal medium, glucose, peptone, KH_2PO_4 as well as initial ph and temperature were statistically combined using an experimental 2^4 fractional factorial two-level design and tested for their influence on maximal antistaphylococcal metabolite production. results were analyzed using response surface methodology (RSM) software.
Results: Optimum production of antistaphylococcal metabolite occurred at glucose 2.0 %, peptone 2.5%, KH_2PO_4 0.15%, pH 4.0 and temperature 30^0C. The maximal amount of antistaphylococcal metabolite 900 U/flask from about 0.85 g of dry weight biomass was extracted.
Conclusion: The antistaphylococcal activity of A. pullulans seemed to be associated with primary metabolite rather than secondary metabolite. However, this conclusion should be taken with caution because both secondary metabolites as well as antibiotics are heterogeneous group and our knowledge regarding the exact definitions and of secondary metabolite / antibiotics are far from the perfection.
Key words: Aureobasidium, antistaphylococcal activity, production, factorial design

INTRODUCTION

Aureobasidium pullulans (de Bary) is cosmopolitan yeast like fungus that occurs in diverse habitats including the phyllosphere of many crop plants and due to production of melanin, it is popularly known as black yeast (1-3). Literature survey shows few reports on production of antimicrobial compounds from Aureobasidium pullulans (3-4).

Despite extensive use of antibiotics and vaccination programs, infectious diseases continue to be leading cause of morbidity and mortality worldwide. Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problem (5). The need for safe and effective antimicrobial compounds increases in parallel with the expanding number of immuno-compromised patients at risk for invasive fungal / bacterial infections.

One of the commonest operations in the study of production of antimicrobial agents by microorganisms is the development of a medium to obtain maximum cell and metabolic product yield (6-7). The selection of media for microorganism's growth and metabolic products is usually based on a combination of experimentation and logic (8). Often such medium screening strategies involve the "one factor at a time" technique. This approach is tedious and time consuming, especially for a large number of variables.

Moreover, it does not guarantee the determination of optimal conditions (9). The experimental design constitutes an efficient tool and is well adapted for treating problems with a large number of variables. In particular response surface methodology can be used when presence of complex interaction is suspected (9).

In our preliminary studies in the development of the production medium, various parameters were found to be important factors in enhancing the antistaphylococcal metabolite formation. However, no systematic study to achieve optimum medium composition and process conditions has been reported for the production of antistaphylococcal metabolite. This work reports production of antistaphylococcal metabolite from *A. pullulans* by development of a cultural medium using response surface methodology.

Materials And Methods

Antistaphylococcal metabolite from *A. pullulans* was carried out as described in our previous article (3).

Optimization of media for production of antistaphylococcal metabolite from *A. pullulans* using factorial design

A two level factorial design-experiment was carried out for five variables viz. glucose (0.4, 2.0 and 4.0 g%), peptone (0.5, 2.5 and 5.0 g%), KH_2PO_4 (0.015, 0.15 and 0.3 g%), pH (3.0, 4.0 and 5.0) and temperature (25, 30 and 35^{0}C) affecting the production of antistaphylococcal metabolite by *A. pullulans*.

RESULTS

Optimization of media for production of antistaphylococcal metabolite by *A. pullulans*

To observe the effect of five variables namely glucose, peptone, KH_2PO_4, pH and temperature on production of antistaphylococcal metabolite, statistically designed experiments were performed. The variables having significant effect on production were evaluated by conducting 40 experiments which included two replicates of a 2^4 factorial experiments with all the four factors and eight center points. Results were analyzed using response surface methodology (RSM) software. Effect of temperature could not be established because at 35^{0}C there was no response in terms of production of antistaphylococcal metabolite. Therefore, we dropped it from the analysis. Table 1 gives the responses obtained in the form of production of antistaphylococcal metabolite (U/flask). The experimental results obtained showed that all the variables had significant effect on production of antistaphylococcal metabolite from *A. pullulans* strain. Based on the identification of variables by the 2-level factorial design, a central composite design was developed for variables significantly affecting production of antistaphylococcal metabolite.

These studies revealed that the optimum production of antistaphylococcal metabolite occurred at 2.0 % glucose, 2.5% peptone, and 0.15% KH_2PO_4, pH 4.0 and temperature 30^{0}C.

The responses obtained were statistically evaluated and the model was built based on the variables with confidence levels more than 95% (Table 2). The model generated was of the quadratic type, the selected P-values of linear and interactive variables have been mentioned in Table 3. The standard error in production of antistaphylococcal metabolite was estimated to be 35.75.

Figs. 1 a, b, c indicate that glucose-peptone, KH_2PO_4- peptone, pH-peptone, KH_2PO_4- glucose, pH-glucose, pH- KH_2PO_4 have a quadratic relationship. With increase in any of them the antistaphylococcal metabolite production increased.

The model has a high correlation coefficient (R^2=0.8719), a significant F-value (17.3212), an insignificant lack of fit F-value (1.8966) and standard

error less than 10 in all the factors. Based on the model equation, three-dimensional surface plots were constructed, which gave the optimal level of the variables and their linear, interactive or quadratic responses. The plots represent interaction of two variables while keeping others constant.

TABLE 1: 2- LEVEL FACTORIAL DESIGN FOR PRODUCTION OF ANTISTAPHYLOCOCCAL METABOLITES BY *A. PULLULANS*

RUN	A	B	C	D	RESPONSE* (UNITS / FLASK)
1	-1	-1	-1	-1	200
2	1	-1	-1	-1	170
3	-1	1	-1	-1	240
4	1	1	-1	-1	210
5	-1	-1	1	-1	190
6	1	-1	1	-1	170
7	-1	1	1	-1	230
8	1	1	1	-1	200
9	-1	-1	-1	1	190
10	1	-1	-1	1	150
11	-1	1	-1	1	190
12	1	1	-1	1	220
13	-1	-1	1	1	180
14	1	-1	1	1	0
15	-1	1	1	1	180
16	1	1	1	1	200
17	-1	-1	-1	-1	210
18	1	-1	-1	-1	180
19	-1	1	-1	-1	220
20	1	1	-1	-1	190
21	-1	-1	1	-1	200
22	-1	-1	1	-1	230
23	-1	1	1	-1	190
24	1	1	1	-1	240
25	-1	-1	-1	1	180
26	1	-1	-1	1	260
27	-1	1	-1	1	160
28	1	1	-1	-1	260
29	-1	-1	1	1	240
30	1	-1	1	1	0
31	-1	1	1	1	220
32	1	1	1	1	220
33	0	0	0	0	380
34	0	0	0	0	420
35	0	0	0	0	460
36	0	0	0	0	480
37	0	0	0	0	430
38	0	0	0	0	380
39	0	0	0	0	450
40	0	0	0	0	420

*RESPONSE IS IN TERMS OF PRODUCTION OF ANTISTAPHYLOCOCCAL METABOLITE PER FLASK. THE DATA OF RUNS IS THE MEAN OF THREE INDEPENDENT EXPERIMENTS.

A= GLUCOSE C= KH_2PO_4

B= PEPTONE D= pH

TABLE 2: ESTIMATES OF THE MODEL COEFFICIENTS WITH THEIR *P*- VALUES:

VARIABLES		ESTIMATE OF COEFFICIENT	*P*– VALUES*
A	GLUCOSE	-10	0.4363
B	PEPTONE	19.375	0.2542
C	KH_2PO_4	-10.625	0.4175
D	pH	-13.125	0.3546
AB	GLUCOSE – PEPTONE	16.875	0.2872
AC	GLUCOSE - KH_2PO_4	-13.125	0.3546
AD	GLUCOSE - pH	-4.375	0.6872
BC	PEPTONE - KH_2PO_4	10	0.4363
BD	PEPTONE - pH	8.75	0.4784
CD	KH_2PO_4 - pH	-12.5	0.3687
AA	GLUCOSE - GLUCOSE	-59.0625	0.0492
BB	PEPTONE - PEPTONE	-59.0625	0.0492
CC	KH_2PO_4 - KH_2PO_4	-59.0625	0.0492
DD	pH- pH	-59.0625	0.0492

A = GLUCOSE, B = PEPTONE, C = KH_2PO_4, D = pH

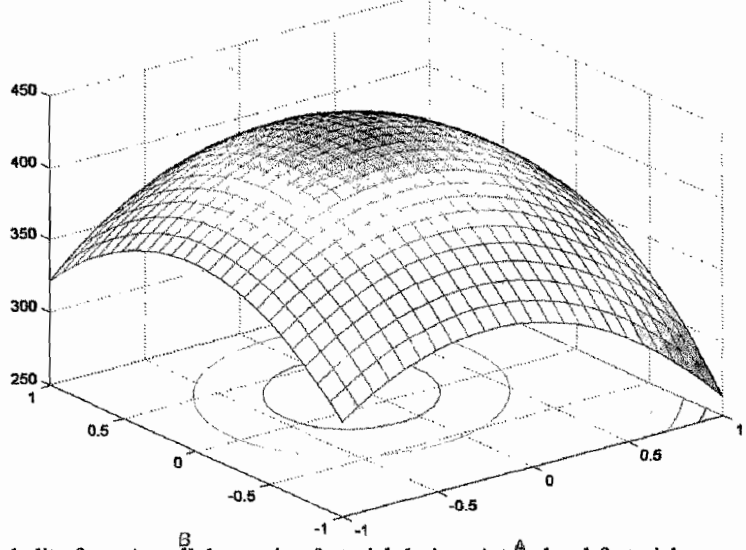
* *P*- VALUES LESS THAN 0.05 INDICATE SIGNIFICANT VARIABLES.

TABLE 3: ANALYSIS OF VARIANCES OF MODEL:

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARE	F	*P* VALUE
MODEL	11	407435	37039.5455	17.3212	0
LINEAR	4	24337.5	6084.375	2.8453	0.0426
A	1	3200	3200	1.4965	0.2314
B	1	12012.5	12012.5	5.6175	0.2049
C	1	3612.5	3612.5	1.6894	0.2043
D	1	5512.5	5512.5	2.5779	0.1196
INTERACTIONS	6	25887.5	4314.5833	2.0177	0.0967
AB	1	5512.5	9112.5	4.2614	0.0484
AC	1	5512.5	5512.5	2.5779	0.1196
AD	1	612.5	612.5	0.2846	0.5967
BC	1	3200	3200	1.4965	0.2314
BD	1	2450	2450	1.1457	0.2936
CD	1	5000	5000	2.3382	0.1375
QUADRATIC	2	357210	178605	83.523	0.0003
AA	1	357210	357210	167.046	0
BB	1	0	0	0	1
CC	1	0	0	0	1
DD	1	0	0	0	1
PURE ERROR	7	8950	1278.5714	0	0
LACK OF FIT	21	50925	2425	1.8966	0.196
ERROR	28	59875	2138.3929	0	0
TOTAL	39	467310	0	0	0

* *P* – VALUES LESS THAN 0.05 INDICATE SIGNIFICANT VARIABLES.

A =
GLUCOSE
B = PEPTONE

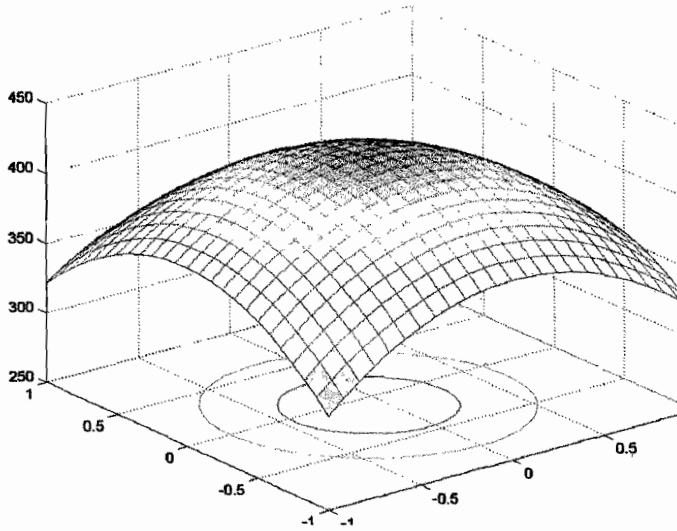


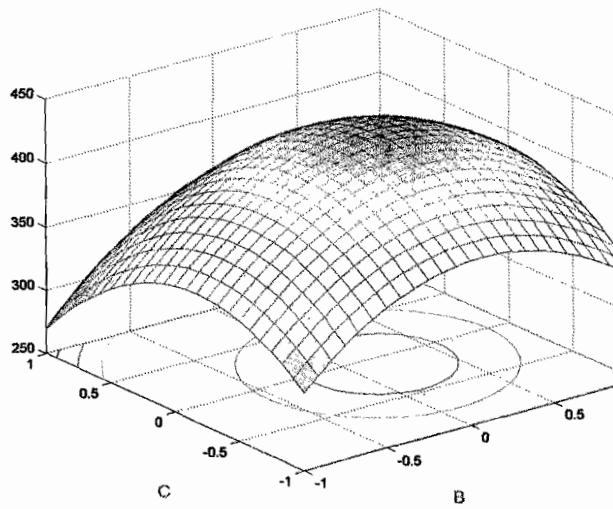
ACTIVITY (UNIT / ML)

ACTIVITY (UNIT / ML)

Fig. 1.a. Optimization of antistaphylococcal metabolite from A. pullulans using factorial design. A two level factorial design experiments was carried out for optimization of production of antistaphylococcal metabolite for four variables viz glucose (0.4, 2 and 4 %), peptone (0.5, 2.5 and 5%), KH2PO4 (0.015, 0.15 and 0.3%) and pH (3, 4 and 5).

A = GLUCOSE
B = PEPTONE
C = KH_2PO_4
D = pH





ACTIVITY (UNIT / ML)

ACTIVITY (UNIT / ML)

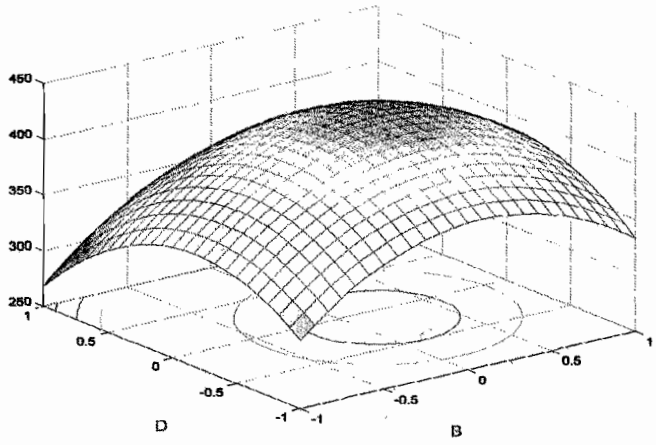
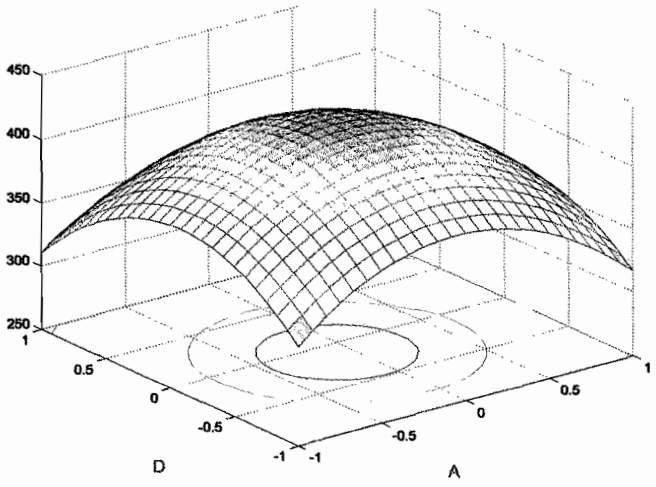


FIG. 1.B. OPTIMIZATION OF ANTISTAPHYLOCOCCAL MET

UNDER FIG. 1.A.

B = PEPTONE
C = KH_2PO_4
D = PH

ACTIVITY (UNIT / ML)



ACTIVITY (UNIT / ML)

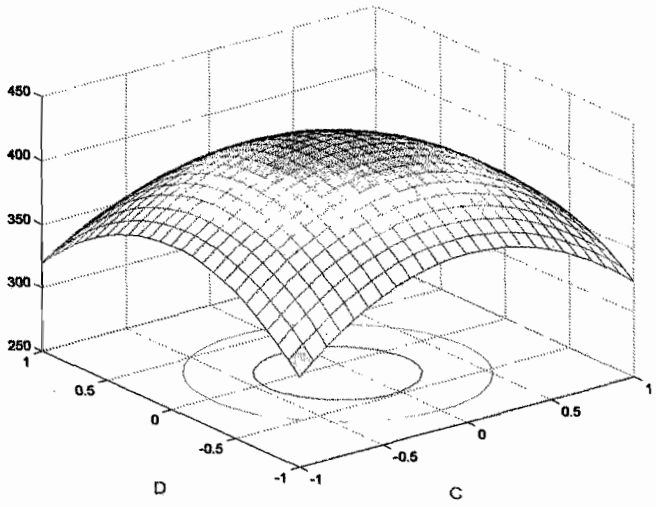


FIG. 1.C. OPTIMIZATION OF ANTISTAPHYLOCOCCAL METABOLITE FROM A. PULLULANS USING FACTORIAL DESIGN. AS

UNDER FIG. 1.A.

DISCUSSION

In order to optimize the parameters of production of antistaphylococcal metabolite by *A. pullulans* factorial design was used. In the traditional methods of optimization, since each parameter is independently investigated, the interaction effect is missed. Moreover, it is tedious and time consuming , especially for a large number of variables (10). According to **Adinarayana and Ellaiah** factorial experiments are a good way of judging the relative significance of the influencing factors and give a quantitative measure of the contribution of each factor to the overall response (11).

In the present investigation, a 2^4 factorial design was chosen to investigate the effect of parameters namely; glucose, peptone, KH_2PO_4, temperature and pH. Under optimized conditions *A. pullulans* produced 900 units of antistaphylococcal metabolite from the biomass grown in 100 ml medium under the optimum conditions.

The production of antistaphylococcal metabolite was parallel to log phase of growth, though, the initial lag of one day was observed before the beginning of accumulation of intracellular antistaphylococcal metabolite. The antistaphylococcal activity of *A. pullulans* seemed to be associated with primary metabolite rather than secondary metabolite. However, this conclusion should be taken with caution because both secondary metabolites as well as antibiotics are heterogeneous group and our knowledge regarding the exact definitions and of secondary metabolite / antibiotics are far from the perfection.

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THE EPIDEMIOLOGY OF MALARIA IN UNIVERSITY OF AGRICULTURE MAKURDI HEALTH CENTRE, MAKURDI, NIGERIA.

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Abstract

The epidemiological study of Malaria in University of Agriculture Makurdi health center was carried out between January 1998 and December 2005. The Malarial cases were sorted out from other medical cases. Data of males were separated from those of females. Data for each year under age groups 0 - 9, 10 - 19, 20 - 29, 30 - 39, 40 - 49, 50 - 59, 60 - 69, were differentiated. The results revealed the highestincidence of malaria in 2005, but males were more affected during the period under review ($p<0.05$). Age wise, those in age group (20 - 29) had the highest incidence (149.80^0) followed by those in age group (0 - 9), which had (78.20^0), then age group (30 - 39), which had (70.80^0) and lastly the age group (10 - 19) had (60.10^0). But those in the age groups (40 - 49), (50 - 59) and (60 - 69) had very low malarial infection rates (0.8^0), (0.3^0) and (0.0^0) respectively. The phenomenon is referred to as beneficial and risky reverse opportunities in favour of the old ones as against the young ones. This however depends on environment..

Keywords: **Epidemiology, Malaria, Nigeria.**

INTRODUCTION

Human malaria has been recognized since the earliest period, and the occurrence of mosquitoes trapped in amber suggests its prevalence in pre-historic times (1). The first evidence that plasmodium was the etiologic agent of malaria was recognized by Charles laveran in 1880 as he scanned a live mount of a febrile soldier's blood at Constatine Hospital in Algeria (2). Malaria in man is caused by four species of plasmodium, *P. vivax, P. malaria, P. ovale and P. falciparum* (3). The last named is not only the most common in Africa but is the most virulent and enjoys the reputation as the greatest killer of mankind, being particularly dangerous to children (4) and responsible for all serious complications and deaths (5).

According to World Health Organization (WHO), each year 300 to 500 million people living in the tropics and sub-tropics become infected with malaria. Nearly 3 million, mostly children die. About 1.5 billion people live in the regions where malaria is endemic. Those regions are Africa, India, South East Asia, and South America (6,7,8,9). About 93% of the 550 million people living in Africa are at risk of malaria and over 90% of the 300-500 million clinical cases are reported from Africa (10). Ukpai and Ajoku 2001 reported high prevalence rates of malaria in

Owerri (75%) and Okigwe (85.5%) (11). Malaria is directly responsible for one in five childhood deaths in Africa and its resurgence in Africa contrasts dramatically with the global decline in mortality since 1900 (12).

Fifty percent (50%) of Nigerian population experience at least one episode of malaria each year, the financial implication could amount to ₦400 million every year (13).

In Nigeria today, malaria affects more people than it did in the 60s. The Federal Ministry of Health reports that one in four people suffer from malaria fever at one time or the other, while up to 1.2 million children (under the age of five) still die of malaria annually (3,4) and the numbers affected are growing "remorselessly" (14). Saganuwan and Abdul (15) reported 48.7% prevalence of malaria among Katcha people in Niger State which they attributed to incessant use of chloroquine (15). It was also reported that in Owerri, the age group (0-10) years had the highest rate of infection (79.31%) while the age group (41-50) years had the lowest rate of infection (68.96%). But in Okigwe the age group (41-50 years) had the highest rate of infection (90.32%) while age group (0-10 years) had the lowest rate of infection (80-76%) (11).

Malaria causes wide spread premature death and suffering imposing financial hardship on poor household and holds economic growth and development in living standards (12). The rapid spread of resistance to antimalarial drugs present a potentially devastating threat. For decades, chloroquine was the main drug used, but increasing resistance forced its replacement in parts of Asia and South America during the 1980s, and in the 1990 African countries are starting to follow suit (12). In Eastern Nigeria, 40-60% of malaria cases have been reported not to respond to treatment with the drug (3). The persistence of malaria as a public health problem is partly as a result of resistance of malaria parasites to antimalarial drugs and to insecticides by anopheles mosquitoes (16). Although, Saganuwan and Yatswako (17) reported that the keys to its eradication remains improvement in the standard of living, reduction in poverty, enlightenment campaign by Non-governmental Organization (NGO) and the use of polypharmacy in malarial chemotherapy (17).

However in view of the prevalence, holoendemicity, epidemicity of malaria and its devastating consequences on economy of highly endemic countries like Nigeria, there is need to find out the distribution of malaria in each geopolitical zones of Nigeria. Bearing in mind that Makurdi in which University of Agriculture Health Centre is located is among malarial zones. Hence, the present study was aimed at determining the epidemicity of malaria in University of Agriculture Health Centre, Makurdi, Benue State, Nigeria using age and sex as factors under consideration.

MATERIALS AND METHODS

The sampling station was University of agriculture Makurdi Health Centre located at North-core of the

University. The University is located about 10km away North of Makurdi town and about 1km from upper part East of River Benue. The vegetation of the University is typical that of savannah region in tropic located in the middle belt of Nigeria. The University has network of streams apart from being closed to river Benue. All the data of malarial cases registered, identified and diagnosed using clinical signs like paroxysm of fever, loss of appetite, headache, lassitude, muscle pains, chills, thirst, nausea, vomiting, delirium and convulsion in children as well as microscopy were collected. The period under review is from January 1998 to December 2005. Malarial cases for each year were sorted out from other cases as the data of males for each year were sorted out from those of females. Data were sorted according to age groups 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, their data for each year under review. The actual numbers of the affected males and females in their respective age groups for each particular year as well as their grand total for each year starting from January 1998 to December 2005 were determined.

Statistical Analysis – Table, graph and pie chart were used to analyze the results (18). Significant levels of differences between the values of males and females as well as among all the age groups were determined at 5% (19).

RESULTS

Out of 8,992 malaria cases registered between January 1998 to December 2005, nine hundred and twenty eight (928), 886, 1096, 1076, 913, 1158, 1360, 1575 suffered from malaria in the years 1998, 1999, 2000, 2001, 2002, 2003, 2004 and 2005 respectively. The highest malaria incidence was in 2005, and the lowest in 1999. When considering the epidemicity of malaria within the total period under review, a total of 4503 (50.5%) of malaria cases were males as differentiated from those of females 4414 (49.5%), signifying higher distribution in males as compared to females. But in 2005 alone a total of 813 malaria cases were against males signifying the year more males were affected that year. In all other years under review males had higher distribution of malaria as compared to females see table 2 below and fig. 1.

Sex wise, the females had low distribution throughout the period under review as compared to males who had higher distribution, although in 2001, the gap of distribution between males and females was wider fig. 1.

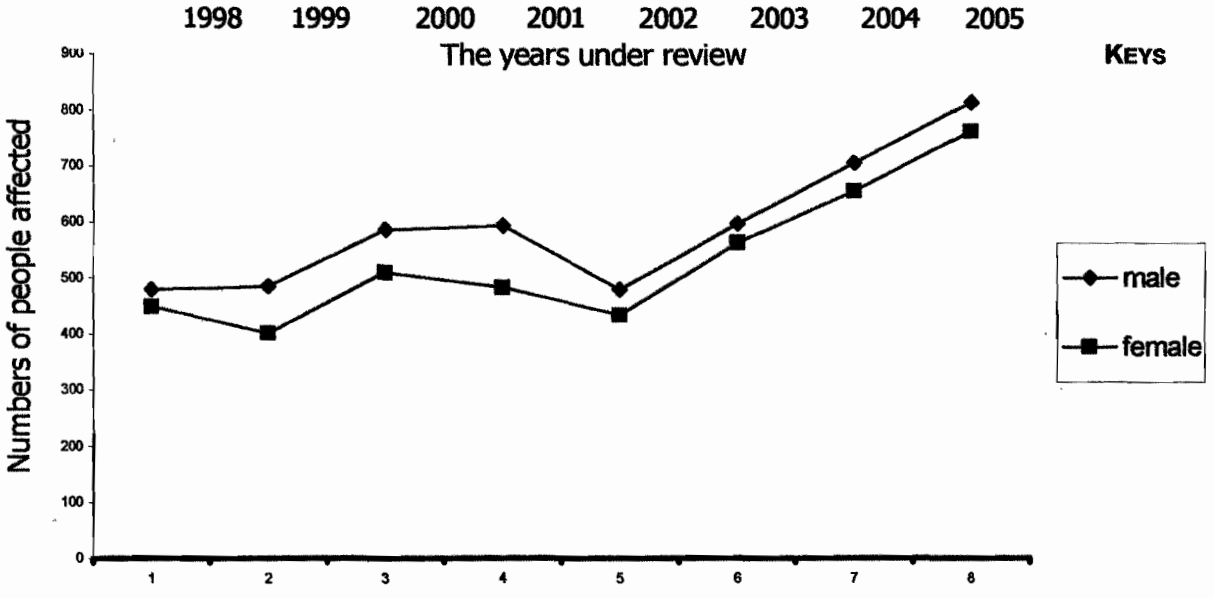
Table 1. Yearly Incidence of Malaria by sex

Year	1998		1999		2000		2001		2002		2003		2004		2005	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Age																
0-9	110	101	103	65	120	122	152	130	81	74	124	120	151	149	177	175
10-19	102	95	74	70	100	110	90	63	68	56	90	86	113	103	142	139
20-29	150	135	235	150	263	175	241	191	262	246	262	248	297	271	325	290
30-39	113	116	72	114	100	102	110	96	66	57	116	107	141	132	167	158
40-49	4	2	1	2	1	-	-	-	2	1	2	1	3	-	-	-
50-59	-	-	-	-	2	-	-	3	-	-	2	-	-	-	2	-
60-69	-	-	-	-	-	1	-	-	-	-	-	-	-	-		
Total	479	449	485	401	586	510	593	483	479	434	596	562	705	655	813	762
Grand total	928		886		1096		1076		913		1158		1360		1575	

Table 2:
The Distribution of malaria between males and females for the period under review

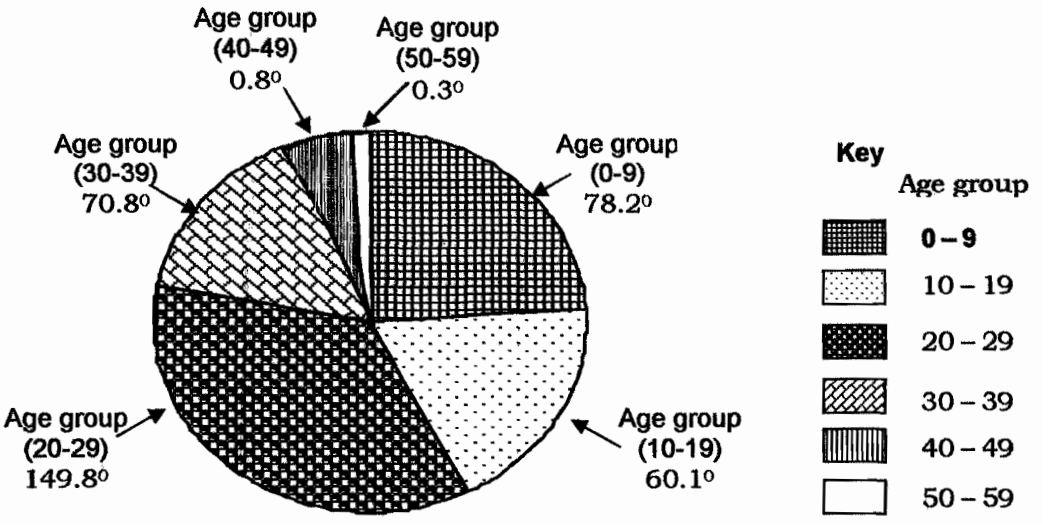
Year	Males	Female
1998	479	449
1999	485	401
2000	586	510
2001	593	483
2002	479	434
2003	596	562
2004	705	655
2005	813	762

Fig. 1 Graphical distribution of malaria using sex for the period under review



Age wise, malaria patients in age group (20 – 29) had the highest incidence 3,741 (41.6%) as those of age group (60 –69) had the lowest 1 (0.00%). But those in age groups (0–9), (10–19), (30–39), (40–49), (50–59), (60–69), had malaria distribution of 1954 (21.7%), 1501 (16.7%), 1769 (19.9%), 19 (0.00%) and 7 (0.00%) respectively. Although for a particular year the males in age group of (20–29) had the highest distribution 325 as against the females in the age group (60–69) who had the lowest (table 1 above)

Fig. 2: Pie chart distribution of malaria using age for the period under review



Age wise, the malarial patients within the age group (20-29) had the highest distribution (149.80^0) followed by those in age group (0-9), which had (78.20^0) then age group (30-39), which had (70.80^0). But those in age group (10-19) had (60.10^0) as those in age groups (40-49), (50-59), and (60-69) had (0.80^0), (0.30^0) and (0.0^0) respectively. There was association between males and females distribution as 5.4% more of males were affected when compared.

DISCUSSION

The highest epidemicity of malaria, was experienced in 2005 (table 1). Males were more affected than females (table 1, Fig. 1). Those in age group (20-29) had the highest incidence. This is a (Fig. 2) clear confirmation of the report of other workers (6,7,8,9) that each year 300-500 million people living in the tropics and subtropics became infected with malaria and that about 1.5 billion live in the regions where malaria is endemic of which Africa is one of them. Our finding is also supported by another report of (12) that the resurgence of malaria in Africa contrasts dramatically with the global decline in mortality since 1900 (6,7,8,9). Our report is further supported by (4) that in Nigeria malaria affects more people than it did in 6Os and that Federal Ministry of Health reported that one in four people suffered from malaria at one time or the other. However, our finding was confirmed by the report of (14) that the number of people affected in Nigeria is growing remorselessly as Saganuwan and Abdul (15) reported 48.7% prevalence in the Middle Belt (15). Our finding of highest epidemicity of malaria in University of Agriculture Makurdi health centre in 2005 is further supported by the report of (10) that about 93% of the 550 million people living in Africa are at risk of malaria and over 90% of the 300-500 million clinical cases are reported from Africa (10).

Nevertheless, the higher epidemicity amongst males (table 2, fig. 1) as well as the highest distribution among those of age groups (20-29) followed by (0-9), then (30-39) and (10-19) may suggest the devastating economic consequence of malaria. Because those in the age groups (20-29) and (30-39) are adults who are suppose to be energetic and responsible for one family responsibility or the other as many Africans marry early. This is supported by the report of (12) that malaria causes wide spread premature death and suffering imposing financial hardship on poor household and holds economic growth and development in living standards (10). Our finding is further supported by the report of (4) that 50% of Nigerian population experience at least one episode of malaria each year, the financial implication could amount to ₦400 million every year (13).

Nonetheless, the higher epidemicity amongst males (table 1, fig. 1) and the highest distribution amongst those of age group (20-29) may be suggestive of more exposure of those people to mosquito as males always go out to earn their living so also those in age group (20-29) as some of them may be students and junior staff. But lowest distribution (fig.2) in those of age group (60-69) and very low epidemicity in those of age groups (40-49) and (50-59) (fig.2) may be suggestive of decrease exposure of those people to mosquito which consequently leads to low epidemicity. Connotatively the higher the age the lower the chance of malarial infection and the lower the age, the higher the chance of malarial infection which i may refer to as "beneficial and risky reverse opportunities in favour of the old ones as against the young ones" although pending on the environment. There was association between males and females' distributions as 5.4% more of males were affected when compared. Furthermore, there was no association among malarial patients of all the age groups.

If nothing is done to fight against high malaria endemicity in university of Agriculture Makurdi health centre, the higher malaria epidemicity between men and females as well as higher distribution among those of the age group (20-29) will continue to be on increase.

CONCLUSION

Our findings revealed highest malaria distribution in 2005; males experienced higher epidemicity through out the period under review. But generally, the young ones experienced higher malaria infection rate than the old ones because of possible more exposure of young ones to mosquito as compared to the old ones. The phenomenon is refer to as "beneficial and risky reverse opportunities in favour of the old ones on one side as against the young ones on the other side. This may however depend on the environment.

RECOMMENDATION

It is recommended that mosquito nets be used regularly. NGOs should intensify efforts at the control programme to face the global challenges imposed by high malaria prevalence. There should be good water drainages in the environment where there are water bodies, bush clearing and health education should be

embarked upon by Community Extension Worker (CHEW) on the importance of environmental cleanliness as being embarked upon by Benue State Government at the end of every month.

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A STUDY OF BACTERIAL ISOLATES IN CASES OF OTITIS MEDIA IN PATIENTS ATTENDING OAUTHC, ILE-IFE

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ABSTRACT

Bacteriology examinations were carried out on one hundred and seven (107) ear swabs of patients attending Ear, Nose and Throat (ENT) clinic as well as those sent to Medical Microbiology and Parasitology department of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife between February 2004 and January, 2005. Of the one hundred and seven ear swabs from patients in all age groups and had been provisionally diagnosed of Otiti Media (OM), ninety three ((93) specimens yielded growth out of which eleven (11) showed mixed bacterial growth. A total number of one hundred and four (104) isolates were recorded with the following prevalence. Pseudomonas aeruginosa accounting for the highest 40 (38.5%), Staphylococcus aureus 32 (30.8%), Proteus mirabilis 16 (15.4%) Klebsiella species 12 (9.6%) and E.coli 4 (3.8%). Eighty (76.9%) cases occurred in children of 0-14 years of age while twenty four (23.1%) occurred in older age. This difference is statistically significant (P<0.05) using the T-test. Only the common forms of Otitis Media cases were seen in this study which included Acute Otitis Media (AOM), Acute suppurative Otitis Media (ASOM) and Otitis Media with Effusion (OME). The in-vitro antibiotic susceptibility tests showed that the isolates were more sensitive to Gentamicin (33.3% - 100%) and Ofloxacin (25% - 100%) than to other drugs tested. This work has further confirmed the diverse nature of bacterial aetiology of otitis media and revealed their high resistance to the commonly used antibiotics. This consequently underscores the need for culture and antibiotic susceptibility in the management of OM.

Keywords: Otitis media, bacteriology, anitibiotic susceptibility

INTRODUCTION

Otitis media as described by Sehnert (1) and Michael et al (2) is the inflammation of the middle ear. It often results from dysfunction of the Eustachian tube (ET) while other sources of ear contamination are from infected water during bath or swimming, vomiting or aspiration of food or drink due to palatal paralysis and milk feed of infants held in horizontal position among others.

OM is seen in all age groups but has been reported to be more prevalent in infants and Children (2-4). Symptoms associated with OM include pain, fullness of the ear, fever, headache, anorexia, irritability, vomiting and diarrhea. There may be a discharge from the middle ear. Infants with OM intermittently touch their ears while most of them have nasal congestion. Meningitis can complicate OM on rare occasions and Elaine et al (5) have shown that deficiency in phonological skill often follows in children with recurred or persistent OM. Furthermore, infections may result in loss of hearing in a high percentage of children below 3 years of age (1).

A Racial prevalence had earlier been reported to exist while a recent study that control socio-economic and other factors showed equal incidence in black and white races(6)

The pathogens most frequently encountered in cultures of ear infections are Pseudomonas spp., Staphylococcus anreus, Proteus spp., Streptococcus spp. Haemophilus spp., and coliforms (7 – 11) with varying prevalence.

Ikeh et. Al (12) incriminated Corynebacterium diphtheriae, Actinomyces isrealii,

Mycobacterium tuberculosis in their findings while Bailey and Scoth (7) had earlierreported other Mycobacteria and ycoplasma pneumoniae. A recent report by Hiroshi et al (13) implicated Chlamydial pneumonia.

It is assumed that knowledge of the occurrence rate, the nature and type of organisms incriminated in the various forms of Otitis Media will go a long way in helping to choose the type and duration of therapy so that relapse rate will be reduced and cure will be automatically effective.

Though the treatment of OM is controversial and subject to change particularly in the developing countries, the antibiogram of these organisms has been reported to vary with time and geographical area as well as continent to continent, probably due to the use and abuse of antibiotics among other factors. Hence the need for periodic update of antibiogram for effective chemotherapy and management of OM cannot be overemphasized. Therefore this study was undertaken to know the new trend of prevalence and antibiogram profiles of bacteria agents of OM in our community.

MATERIALS AND METHODS

Study Design:

All cases of provisionally diagnosed otitis media (OM) at the ENT clinic of OAUTHC as well as those sent to the Medical Microbiology and Parasitology department of OAUTHC between February 2004 and January 2005 were studied and swabs were obtained. There was no age or sex barrier as all individuals of any age group presented with cases of OM were included in this study. The study included the documentation of age and sex of the patients.

Sample Collection:

A total number of one hundred and seven (62 males and 45 females) ear swab samples were collected from neonates, children, adults of all age groups presenting with various forms of OM as earlier explained. None of these patients had been on any antibiotics therapy prior to the collection of specimens. Before sample collection, the external ears were cleansed with sterile cotton swabs moistened with sterile normal saline Processing of Samples:

All samples were inoculated on blood agar (BA) chocolatre agar (CA) and MacConkey agar (MCA) plates before smears for Gram staining were made on clean microscope slides. The BA and MCA plates were incubated aerobically at 37^0C for 24 hrs while the CA plates were incubated under 5% CO_2 at 37^0C for 24hrs. the growths were examined macroscopically and biochemically to identify the isolates as recommended by Cowan and Steel (14). Antibiotic sensitivity was performed on the isolates and identified organisms by the disc diffusion method using Diagnostic Sensitivity Test (DST) agar as described by Stokes and Ridway (15). Control organisms used were Stephylococcus auteus (NCTC 6571), E. coli (NCTC 10418) and Pseudomonas (NCTC 1066)

RESULTS

From the 107 ear swabs processed, 93 representing 86.9% yielded bacterial growth while 14 (13.1%) showed no growth. Of the 93 growth, 11 (11.8%) showed mixed bacterial growth. One hundred and four (104) isolates were recorded with Pseudomonas aeruginosa accounting for the highest percentage occurrence 40 (38.5%) and the lowest with E.coli 4 (3.8%) as seen in table 1. Other predominant isolates include S.aurens 32 (30.8%), Klebsiella spp 12 (9.6%) and Proteus mirabilis 16 (15.4%).

The distribution of isolates in relation to Gram reaction as presented in table 1 shows that Gram

negative organisms were more prevalent than Gram positive organisms 72 and 32 respectively. Prevalence of pathogens in relation to sex as seen in table 2 shows a ratio of male to female to be 44:49 (1:1.11) that is not significantly different.

The prevalence of OM among different age groups is shown in table 3 with 80 (76.9%) occurring in children (0-14yrs). This indicates a statistically significant difference ($P<0.05$) using T-test between prevalence of OM in children (0-14yrs) in comparison with older ages.

Pie chart showing the frequency of isolates is shown in table 4

The antibiotic susceptibility pattern of the various isolates is presented in table 5.

The most prevalent organism, P.aeruginosa shows a high susceptibility to Gentamycin (80%) and ofloxacin (70%) and the only Gram positive organism S.aureus showed moderate sensitivity to erythromycin (75%) Gentamycin (62.5%) and Streptomycin (65.6%). It should be noted that S.aureus showed resistance to penicillin.

Table 1: Prevalence of pathogens in relation to sex and Gram reaction

ISOLATES	FREQUENCY (%)[A]	MALE N(%)[B]	FEMALE N(%)[B]
GRAM NEGATIVE BACTERIA			
PSEUDOMONAS AERUGINOSA	40 (38.5)	28 (70.0)	12 (30.0)
PROTEUS MIRABILIS	16 (15.4)	8 (50.0)	8 (50.0)
KLEBSIELLA SPECIES	12 (11.5)	0 (0)	12 (100)
ESCHERICHIA COLI	4 (3.8)	4 (100)	0 (0)
TOTAL	72 (68.2)		
GRAM POSITIVE BACTERIA			
STAPHYLOCOCCUS AUREUS (87.5)	32 (30.8)	4 (12.5)	28
GRAND TOTAL	104 (100)	44 (42.3)	60 (57.7)

[A]PERCENTAGE BASED ON TOTAL NUMBER OF ISOLATES

[B]PERCENTAGE BASED ON DISTRIBUTION OF STRAINS OF EACH ISOLATES BY SEX OF PATIENTS

Table 2: Sex pattern of positive culture

SEX	NO	%
MALE	44	47.3
FEMALE	49	52.7
TOTAL	93	100.0

Table 3a: Age distribution of otitis media cases and bacterial isolates

AGE RANGE		CHILDREN 0-14YRS	ADULT >14YRS
ISOLATES	FREQUENCY	N (%)	N(%)
PSEUDOMONAS AERUGINOSA	40	28 (70)	12 (30)
STAPHYLOCOCCUS AUREUS	32	24 (75)	8 (25)
PROTEUS MIRABILIS	16	16 (100)	0 (0)
KLEBSIELLA SPECIES	12	8 (67)	4 (33.3)
ESCHERICHIA COLI	4	4 (100)	0.(0)
TOTAL	104	80 (76.9)	24 (23.1)

Table 3b: Bacterial isolates from Children and Adults.

ISOLATE	**FREQUENCY**	**ANGLE SUBS TENDED**
PSEUDOMONAS AERUGINOSA	40	40/104 x 360/1 = 138.5°
STAPHYLOCOCCUS AUREUS	32	32/104 x 360/1 = 110.8°
PROTEUS MIRABILIS	16	16/104 x 360/1 = 55.4°
KLEBSIELLA SPECIES	12	12/104 x 360/1 = 41.5°
ESCHERICHIA COLI	4	4/104 x 360/1 = 13.8°
TOTAL	104	360°

P.AERUGINOSA 138°

KLEBSIELLA SP. 41.5°

P.MIRABILIS 55°

S.AUREUS 110.8°

E.COLI 13.8°

Table 4: Pie chart showing the frequency of isolates

	PSEUDO SPP	*S.AUREUS*	*PROTEUS SPP*	*KLEB SPP*	*E.COLI*
TOTAL NO. OF ISOLATES	40	32	16	12	4
ANTIBIOTICS	N(%)	N(%)	N(%)	N(%)	N(%)
OFLOXACIN (OFL)	28(70)	8(25)	16(100)	0(0)	4(100)
COTRIMOZAXOLE (COT)	16 (40)	8 (12.5)	0(0)	0(0)	0(0)
GENTAMICIN (GEN)	32(80)	20(62.5)	0(0)	4(33.3)	0(0)
CEFUROXINE (CXM)	4(10)	0(0)	0(0)	0(0)	0(0)
STREPTOMYCIN (STR)	0(0)	21(65.5)	0(0)	0(0)	0(0)
CHLORAMPHENICOL (CHL)	0(0)	16(50)	0(0)	0(0)	0(0)
PENICILLIN (PEN)	0(0)	0(0)	0(0)	0(0)	0(0)
ERYTHROMYCIN (ERY)	0(0)	24 (75)	0(0)	0(0)	0(0)

DISCUSSION

Most of the patients seen in this study had the various common forms of OM ranging from AOM, ASOM, CSOM to OME which usually follow poorly managed or untreated OM.

The observed prevalence of 38.5% 30.8% and 15.4% for P.aeruginosa, S.aureus and P.mirabilis respectively correlates with those of Devan et al (16) who reported 48% and 22% for P.aeruginosa and Proteus spp respectively while Ogisi and Osamor (10) recorded prevalence of 31% and 24% for Pseudomonas spp and Proteus spp respectively. This is however in contrast to the findings of Watson (17) and Michael et al (2) who recorded Haemophilus influenza, Streptococcus pneumonia and Moraxella catarrhalis as predominant organisms for OM cases. However, results from this work agrees with that of Azeez (18) who reported P. aeruginosa, S. aureus and Proteus spp in his work at Oyo (Nigeria) while Brobby and Zachik (19) had earlier concluded that H.influenza and S. pneimoniae do not play important role in the pathogenicity of OM in the topics.

Brobby (4) and other authos (1, 7, 10, 19) have reported that the aetiologic organisms of OM vary from continent to continent i.e. locality to locality. This variations can be attributed to the emergence of increasing antimicrobial resistance, difference in social cultural practices, nutrition and socio-economic factors among others. The diverse nature of bacterial aetiology of OM reported in this study therefore confirms previous studies.

In line with this study, Hashisaki (20) reported Paeruginosa as the most commonly recovered organism from the chronically draining ear while other researchers have also recorded high prevalence of P.aeruginosa 48%, 38% and 31% by Devan et.al.. (16), Coker et al. (11) and Ogisi and Osamor (10) respectively. Since Pseudomonas does not normally inhabit the upper respiratpry tract, its

presence in the middle ear cannot be ascribed to an invasion through ET, it must be considered as secondary invader gaining access to the middle ear via defect in tympanic membrane.

The range of S.aureus prevalence in OM can be said to be wide since 30.8% was recorded in this work while Ikeh et. al (12) reported 44% and Azeez (18) 25%

Anaerobic investigation was excluded in this study since anaerobic cultivations are not routinely done for OM plus the fact that very few reports suggest that anaerobic bacteria may cause OM and studies of gas tension in middle ear show that the middle ear cleft poorly support anaerobic growth (12). Giebin(3) had earlier reported that middle ear effusion culture are sterile for anaerobic bacteria.

It is estimated that 70% of children would have had one or more episodes of OM by their third birthday(12) and in agreement with this, children accounted for 76.9% as against 23.1% for adult. This high prevalence in children is statistically significant ($P<0.05$) using T-test and correlates with several other reports (2,3,18). That OM is predominantly an early childhood disease can be explained by several factors including the immature and short ET, malnutrition, immature immune response, frequency of upper respiratory tract infection in children, poverty, poor hygiene, overcrowding, group daycare attendance, bottle feeding and postural (horizontal) feeding practices: such as night breast feeding and that whereby the baby is forced to swallow watery pap or liquid drugs by intermittent closure of the baby's nose.

The ratio of male to female was insignificant (1:1.11) with female slightly higher. This is in contrast to Pukander et. al (22) and Azeez(18) who recorded more males than females. The incidence, of more females in this work can be explained by their frequency of using cotton buds to clean ears possibly resulting in the introduction of organisms into the middle ear.

The overall percentage sensitivity of the organisms to Gentamycin and Ofloxacin are comparable to the findings of Ikeh et. at (12) and Azeez (18). Ofloxacin may however not serve a useful purpose in children among whom the disease is most common since it belongs to quinolone group of antibiotics which are usually contraindicated as paediatric regimens(18) However, S.aureus, the only Gram positive isolate and second in prevalence rate showed good sensitivity to some of the commonly used drugs like Erythromycin, Streptomycin and Chloramphenicol. And this agrees with the work of Ako-Nai et. al. (23).

This work has recorded a high resistance of bacterial isolates to the commonly used and cheaper antibiotics. This underscores the need for more effort to be geared towards new drug formulations that will cater for children of all ages and the need to intensify the campaign against drug abuse.

RECOMMENDATIONS

It has been reported that children who were breast-fed for 12 months or more had significantly less frequent ear disease related to OM than did infants who were bottle fed at birth or within the first months of life(2), therefore, the campaign on baby friendly programme of breast feeding should be intensified while postural (horizontal position) feeding of children be discouraged and emphasis should be on upright position of feeding. Mothers attending both antenatal and postnatal clinics can be enlightened on the dangers of the wrong position of feeding.

Infants should be cared for at home instead of daycare centres since it has been reported that infants cared for in group daycare centres have higher episodes of AOM(2).

The government is therefore implored to extend maternity leave period from the present 3 months to 6 months in Nigeria. Other recommendations include: General good hygiene practices, avoiding overcrowding, good balance diet, appropriate use of antibiotics, bringing cases of OM early enough to the hospitals. The

bacteriology of OM underscore the need of monitoring the changing trends in etiology and multi-resistant strains of causative agent of OM. Bacteriology of OM has helped to discover cases of OM that only antibiotic treatment can take care of instead of painful and resource wasting surgery that has been mistakenly done in the past for common OM.

Lastly if the ear fails to dry up upon application of antibiotics or is unresponsive to antibiotic treatment, it must not be forgotten that such cases could be due to etiologic agents like fungi such as Aspergillus niger as well as Candida albicans and even viral agents.

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ORIGINAL ARTICLE

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TREND IN TUBERCULOSIS MORTALITY IN NIGERIA

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E-mail: sojidaniel@yahoo.com. +2348033774418, Footline: TB, DOTS, Mortality, HIV.

ABSTRACT

Background Tuberculosis continues to cause considerable morbidity and mortality especially in sub Saharan Africa in spite of the availability of effective antimicrobial agents. This study aimed to assess the trend and factors associated with deaths in Sagamu Nigeria.

Design: A retrospective case control study of TB patients dying before completing the directly observed treatment short course (DOTS) at the Ogun State University Teaching Hospital; Sagamu.

Result: Of the 946 patients registered at the outpatient DOTS programme between January 1997 and December 2003, 53(5.6%) died before completing treatment. The death rate from tuberculosis rose from 5.4% in 1997 to 8.6% in 2003, an increase of 59.3%. HIV prevalence rate among TB patient increased from 7% to 25.2% during the same period. The highest age specific case fatality rate of 12% was in the age group 50-59 years. HIV positive status and history of previous TB treatment were significantly associated with dying during treatment. Neither sputum smear negativity nor pre-admission weight was associated with the risk of death. The median survival time from the commencement of treatment was 8 weeks. There was no significant difference in the survival time in relation to the HIV status.

Conclusion: This study has demonstrated an upward trend in mortality from TB infection which is associated with HIV co-infection and previous TB treatment failure. This calls for the strengthening of HIV and TB control measures, combined with strategies aimed at making antiretroviral drugs cheaper and more affordable for people living with HIV/AIDS in resource poor countries.

INTRODUCTION

Tuberculosis continues to cause considerable morbidity and mortality especially in sub Saharan Africa despite availability of effective antimicrobial agents. About 2 million people die from this disease annually with severe social and economic consequences. It cannot be ignored that TB is a disease of poverty with 95% of cases and 98% of deaths due to it occurring in the developing nations[1].Factors such as multi-drug resistance human immunodeficiency virus infection and delayed therapy have been implicated[2]. In several African countries, TB is still a major cause of morbidity and mortality[3,4]. HIV has been observed to be an important predictor of death in patients with tuberculosis[5,6]. Among medical in patients at the Olabisi Onabanjo university teaching hospital, Sagamu, TB accounted for 10% of medical admissions. TB was the commonest infectious disease

causing death and the third most common cause of death after cerebro-vascular accident and renal failure[7]. This study aimed to assess the trend and factors associated with deaths among patients attending tuberculosis and leprosy control centre in Sagamu, Nigeria.

MATERIALS AND METHODS

SETTINGS

The study was carried out in Sagamu Local Government area in Ogun State of Nigeria. The town is a semi-urban area with an estimated population of 200,000 people[8]. It is located about 50km from Lagos and Ibadan. The Tuberculosis and Leprosy control centre attached to the department of community medicine and primary care, Olabisi Onabanjo University Teaching Hospital is the major referral centre for the treatment of TB. The German Leprosy Relief Association (GLRA) was responsible for the provisions of free drugs for the programme. The centre provides DOTS for the treatment of tuberculosis on out- patient basis

TB CONTROL PROGRAMME

Patients with suspected or confirmed diagnosis of TB are referred to the TBL unit. Diagnosis of TB was based on finding at least 2 out of the three samples positive for acid fast bacilli by Ziehl Neelson stain. Patients who are sputum smear negative but suspected to have TB are further evaluated with a chest radiograph, mantoux skin reaction, erythrocyte sedimentation rate and clinical assessment. Patients are then categorised as new patients (smear positive or smear negative), treatment after default. relapse and treatment failure case according to the national tuberculosis and leprosy control treatment guidelines. All smear positive and very sick smear negative TB patients are commenced on short course chemotherapy with daily rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months of intensive treatment followed by ethambutol/thiacetazone and isoniazid on outpatient basis for 6 months. Patients on re-treatment regime receive a 3 months intensive phase with the addition of streptomycin to the four drugs in the intensive phase mentioned above. The continuation phase is last 5 months with rifampicin, isoniazid and ethambutol three times a week. All patients routinely had pre and post test counselling for HIV screening using two methods namely the immunocombs II HIV 1&2 Bispot test kit (Orgenics, France) and the Capillus HIV-1/HIV-2 kit(Cambridge diagnostics, Ireland). A positive test is considered only when the blood sample is positive for the two test kits.

STUDY DESIGN

The study is a retrospective study of patients attending the tuberculosis and leprosy control unit of the Olabisi Onabanjo university teaching hospital Sagamu, between January 1997 to December 2003.All the patients admitted into the DOTS programme who were subsequently traced home and confirmed dead by relatives, family members and co-patients or patients under going treatment at the TBL unit who were admitted and died at the medical ward of the Olabisi Onabanjo university teaching hospital were regarded as 'dead'. Patients who died during treatment were matched for age and sex with those who completed their T.B treatment during the same period ('Alive') on the ratio 1:2. Patients on antiretroviral drugs during anti-tuberculosis treatment were excluded from the study.

DATA ANALYSIS

Data entry was done with Epi-info 6.04 statistical software. The dependent variable in the analysis was whether patient died or was alive at the end of eight months of anti-tuberculous treatment. A variety of independent variables such as HIV status of patient, sex, age, body weight at presentation, previous treatment of tuberculosis, sputum smear result were selected for potential inclusion in the model to discriminate between the outcome of dead or alive after treatment. Descriptive statistics were generated

for each variable; bivariate analysis were then conducted between each independent variable and outcome of treatment (i.e. whether Dead or Alive), using 2 independent sample t test andX2 analysis for continuous and discrete variables respectively. Variables that were statistically significant (P< 0.05) in the bivariate analyses were entered into a multiple logistic regression. Meaningful 2 way interaction effects between significant independent variables were investigated using contingency table analyses. The level of statistical significant in bivariate and multivariate analyses was taken at P≤0.05 Data was analysed using EPI 2002. The level of significance was set at p≤0.05 at 95% confidence limit.

RESULTS

A total of 946 patients were admitted into the TBL programme between January 1 1997 and December 31 2003 with a yearly average of 135 patients. A male: female ratio of 1:1 was observed in the study. A total of 53 deaths occurred during the study period giving a case fatality rate of 5.6%. Thirty one males and 22 females died with a ratio of 1.4:1 deaths. The death rates were lowest in 2002 and highest in 2003. The increase in the rate of death with tuberculosis rose from 5.4% in 1997 to 8.6% in 2003,an increase of 59.3%(Table 1).

The case fatality rates increased progressively from 3.9% in the 20-29year age group to 12.2% in the 50-59year age group. Higher mortality was seen in older patients from 50 years and aboce (Table II)

Table III. summarises the factors associated with dying during tuberculosis treatment in the study population. Those patients who died were significantly more likely to be HIV sero positive (p≤0.05) and to have had a previous TB treatment (p≤0.05). Other factors such as sputum smear acid fast bacilli negative result and preadmission weight were not significantly associated with mortality during TB treatment. About 60% of deaths during TB treatment was in the first two months. The interval between commencing treatment and dying was not significantly associated with HIV status (Table I II).In the multivariate analysis HIV remained the only predictor of death in the study population (OR 6.53 95% CI 2.80-15.3' P=0.00001) as shown in Table V.

Figure I shows the trend of both the annual HIV prevalence and mortality rates among TB patients during the study period.

TABLE I: YEARLY ADMISSION AND DEATHS AND ANNUAL DEATH RATES

YEAR	NO OF PATIENTS REGISTERED			NO OF DEATHS			ANNUAL DEATH RATE		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL
1997	63	66	129	5	2	7	7.9	3.0	5.4
1998	64	65	129	4	3	7	6.3	4.6	5.4
1999	53	44	97	5	1	6	9.4	2.3	6.2
2000	65	81	146	3	6	9	4.6	7.4	6.2
2001	75	79	154	5	2	7	6.7	2.6	4.6
2002	82	58	140	3	1	5	3.7	1.7	3.6
2003	72	79	151	6	7	13	8.3	8.7	8.6
TOTAL	474	472	946	31	22	53	6.5	4.7	5.6
M:F RATIO	1:1			1.41:1			1.41:1		

TABLE II: ADMISSION, DEATHS AND FATALITY RATES BY AGE AND SEX

AGE (YEARS)	NO OF REGISTERED PATIENTS			NO OF DEATHS			FATALITY RATES		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL
≤20	92	93	185	5	3	8	5.4	3.2	4.3
20-29	163	192	355	8	6	14	4.9	3.1	3.9
30-39	102	100	202	5	7	12	4.9	7.0	5.9
40-49	52	39	91	5	1	6	9.6	2.6	6.6
50-59	25	16	41	3	2	5	12	12.5	12.2
≥60	42	30	72	5	3	8	11.9	10.0	11.1
TOTAL	476	470	946	31	22	53	6.5	4.7	5.6

TABLE III: INTERVAL BETWEEN ADMISSION INTO THE TBL DOTS PROGRAM AND DEATH

TOTAL INTERVAL	HIV POSITIVE N=21	HIV NEGATIVE N=32	TOTAL (%)	CUMULATIVE FREQUENCY (%)
0-4 WEEKS	4	13	17(32.1)	17(32.1)
5-8 WEEKS	7	8	15(28.3)	32(60.4)
9-12 WEEKS	1	1	2(3.8)	34(64.2)
≥12 WEEKS	9	10	19(35.8)	53(100)
TOTAL	21	32	53(100)	

$X^2 = 2.72$

P =0.44

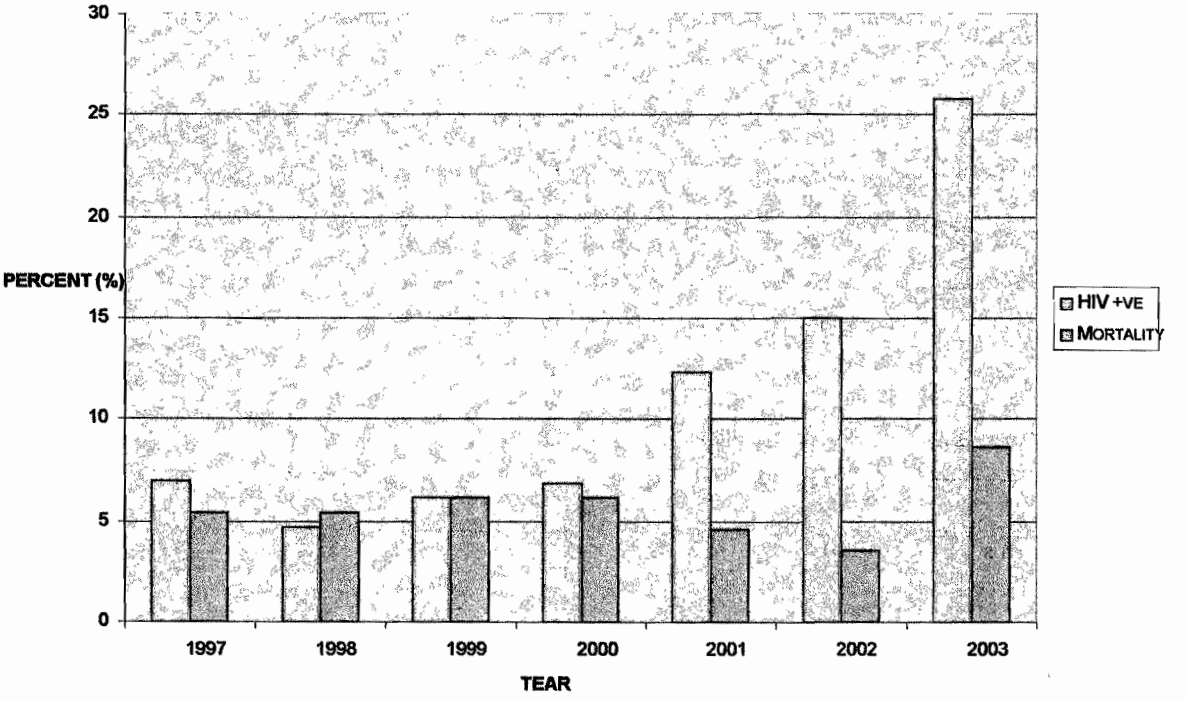
ABLE IV: FACTORS ASSOCIATED WITH DEATH DURING TB TREATMENT

FACTORS	DEAD N=53	ALIVE N=106	ODDS RATIO (C.I)	P VALUE
HIV POSITIVE STATUS	22(41.5)	10(9.4)	2.71 (1.84-3.79)	0.0001
SPUTUM SMEAR NEGATIVE	17 (32.1)	23(21.6)	1.70 (0.76-3.80)	0.12
PREVIOUS TB TREATMENT	10(18.9)	6(5.9)	3.88 (1.20-12.94)	0.009
PRE-ADMISSION WEIGHT	46.4±13.1	48.7±14.8	-	0.34

TABLE V: FACTORS ASSOCIATED WITH DEATH DURING TB TREATMENT

FACTORS	ODDS RATIO (C.I)	P VALUE
HIV POSITIVE STATUS	6.46 (2.7-15.3)	0.0001
SPUTUM SMEAR NEGATIVE	2.08 (0.89-4.87)	0.09
PREVIOUS TB TREATMENT	1.40 (0.44-4.5)	0.5
SEX	0.98 (0.47-2.1)	0.9

FIGURE I: HIV PREVALENCE AND MORTALITY RATES AMONG TB PATIENTS IN SAGAMU 1997-2003



DISCUSSION

The case fatality rate in this study was 5.6%. This is similar to an earlier study in the eastern part of Nigeria, which recorded an overall case fatality rate of 5.8%[9], but in contrast to 33% and 40% respectively in studies in Malawi[10,11] .The high death rate among tuberculosis patients in the later studies may be related to the high HIV prevalence rate of 84-89% among TB patients in the above mentioned countries.

Case fatality rate in this study was observed to increase with age. Although death rate was highest in the 50-59 year age group, 75% of the total deaths still occurred in individuals less than 50 years. This is in contrast to earlier studies in the developed countries where more deaths occurred in individuals who are 65 years and above[12] . This underscores the difference in the epidemiology of TB in developed countries compared to developing countries. People in developing counties are exposed to TB earlier in life and so die younger due to several factors including inadequate and inaccessible diagnostic and treatment facilities, overcrowding, and poverty[13]. However, recent studies in many developed countries show a new peak spanning the ages 20-49 years accompanying the pre-existing peak in the elderly[14]. The AIDS epidemic has been observed to be responsible for the significant increase in the number and rate of tuberculosis death in younger adults[15].

HIV co-infection was associated with increasing mortality in this study. The annual case fatality rate rose from 5.4% in 1997 to 8.6% in 2003, an increase of 59.3%.These observation have also been reported in similar studies[4].

Another factor associated with mortality was history of previous treatment of TB. Patients previously treated who did not comply strictly with their medications are at risk of developing multi-drug resistant strains that are difficult to cure with the current anti-TB regimen in the DOTS programme and this may contribute to mortality in these patients.. Other factors such as sputum smear negativity for acid

fast bacilli and pre-admission weight were not significantly associated with mortality in our study. Other studies however observed that smear negative AFB was significantly associated with mortality[10,11]. HIV infection has been associated with atypical presentation such as sputum smear negative result or extra pulmonary TB which may lead to diagnostic delay thereby contributing to mortality[17]. The mortality in smear negative patients may however be due to diseases other than TB. The time interval between commencement of TB treatment and death was not related to HIV status. Other studies however reported that non HIV infected patients died early in treatment compared with TB/HIV positive patients[10,11] .The interval between diagnosis and death in the present study was much longer compared with other studies. This may be as a result of late presentation in studies done among medical in patients[9,18]

The factors contributing to death could not be ascertained in this study. as most of the patients died at home being unable to afford hospital admission. This is in keeping with earlier observation that majority TB deaths takes place outside the hospital settings[19].

In conclusion the present study which analysed deaths from tuberculosis in patients attending an out patient TBL control centre *in* Sagamu observed an upward trend in mortality and also found HIV infection to be the only predictor of death in multivariate analysis.

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CASE REPORT

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DISSEMINATED ENDEMIC KAPOSI'S SARCOMA IN A YOUNG MAN WITHOUT EVIDENCE OF HIV INFECTION.

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ABSTRACT

We report a case of disseminated endemic Kaposi's sarcoma in an HIV- negative young man, involving the head, face, eye, tongue, upper and lower limbs, trunk, glans penis and peripheral lymphadenopathy. Tests for human immunodeficiency virus were negative. The clinical and histologic features with absence of immunosuppressive drugs in patient history confirmed the African endemic Kaposi's sarcoma. To the best of our knowledge this might be the first case reported in this part of the world.

KEYWORDS: Disseminated, Endemic, Kaposi's sarcoma, HIV-negative.

INTRODUCTION

In 1872, Moritz Kaposi, a Hungarian dermatologist, described odd skin tumors in five men in their sixth and seventh decades of life as "idiopathic multiple pigmented sarcoma of the skin **(1)**." There are five subtypes of Kaposi's sarcoma which include: 1) classic; 2) endemic African cutaneous; 3) endemic African lymphadenopathic; 4) acquired immunodeficiency virus-associated; and 5) those associated with immunosuppressive therapy. Although it is not understood, epidemiologic and biologic evidence has suggested that the pathogenesis of classic Kaposi's sarcoma involves the transmission of an infectious viral agent such as human herpes virus 8 or some other unique agent that is transmitted independently **(2,3)**.

The most common form that is associated with Acquired Immunodeficiency Syndrome has been well documented since first described in the early 1980's **(4)**. This form appears in up to 40% of AIDS patients and may account for up to 90% of all cancers found in the AIDS population **(5)**. The African type, primarily found in regions of equatorial Africa, is a much more common entity within its geographic setting, with findings of up to 9% of all malignancies in eastern Zaire **(6)**. While usually less severe than the AIDS-associated condition, the African-type is the only form known to regularly occur in children **(6)**. African-Endemic Kaposi sarcoma is the second most prevalent malignancy in Africa representing about 10% of all malignancies **(7)**.

We report a young man with aggressive Kaposi sarcoma with no evidence of HIV infection.

CASE REPORT

A 30-year-old male farmer presented with a history of progressively asymptomatic multiple skin nodules on the left lower limb and face of 10 months duration. This is followed shortly by similar lesions on the ipsilateral leg and foot, forehead, face, right eye, right hand, glans penis and tongue. No associated itching or preceding history of trauma. No past history of immunosuppressive therapy. Significant peripheral

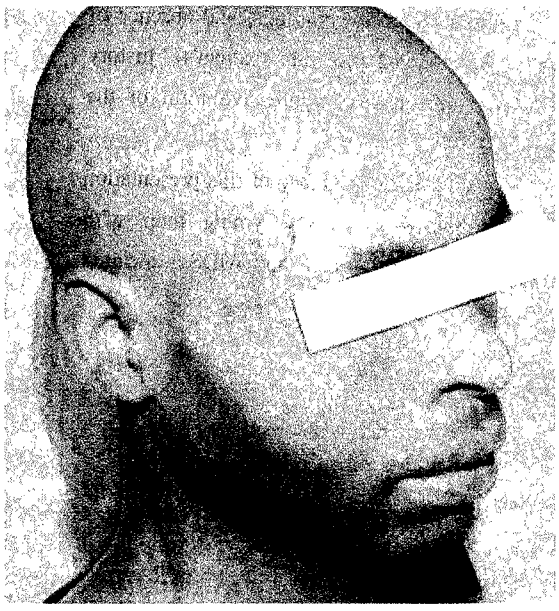
lymphdenopathy (cervical and inguinal), oedema of the left lower limb (fig.1and 2) were observed. Chest, cardiovascular, abdominal and central nervous system examinations were essentially normal. A complete blood count and metabolic profile were within normal with the exception of mild normochromic anaemia (Packed Cell Volume=30%), Scanning of abdomen and pelvis did not show any abnormality. Biopsy of one of the skin nodules (fig. 2) showed Kaposi's sarcoma (fig. 3). The patient was screened for HIV by the following tests. Table I.

Table I. Tests for HIV infection

KIT NAME	TYPES OF TEST	DESIGNED TO DETECT	RESULT
DETERMINE	RAPID IMMUNO-CHROMATOGRAPHY	ANTIBODIES TO HIV-1 AND HIV-2	NEGATIVE
CAPILLUS	RAPID AGGLUTINATION	ANTIBODIES TO HIV-1 AND HIV-2	NEGATIVE
GENIE II	RAPID IMMUNO-CHROMATOGRAPHY	ANTIBODIES TO HIV-1 AND HIV-2	NEGATIVE
ORAQUICK	RAPID IMMUNO-CHROMATOGRAPHY	ANTIBODIES TO HIV-1	NEGATIVE
GENSCREEN	ENZYME – LINKED IMMUNOSORBENT ASSAY	ANTIGEN P24 OF HIV-1, ANTIBODIES TO HIV-1 GROUPS M AND O, AND ANTIBODIES TO HIV-2	NEGATIVE
NEW LOVBLOT 1	IMMUNOBLOT (WESTERN BLOT)	ANTIBODIES TO SPECIFIC HIV-1 ANTIGENS	NEGATIVE(NO VISIBLE BAND)
AMPLICOR HIV-1 MONITOR	NUCLEIC ACID AMPLIFICATION	HIV-1 RNA	NEGATIVE (VIRAL RNA NOT DETECTED)

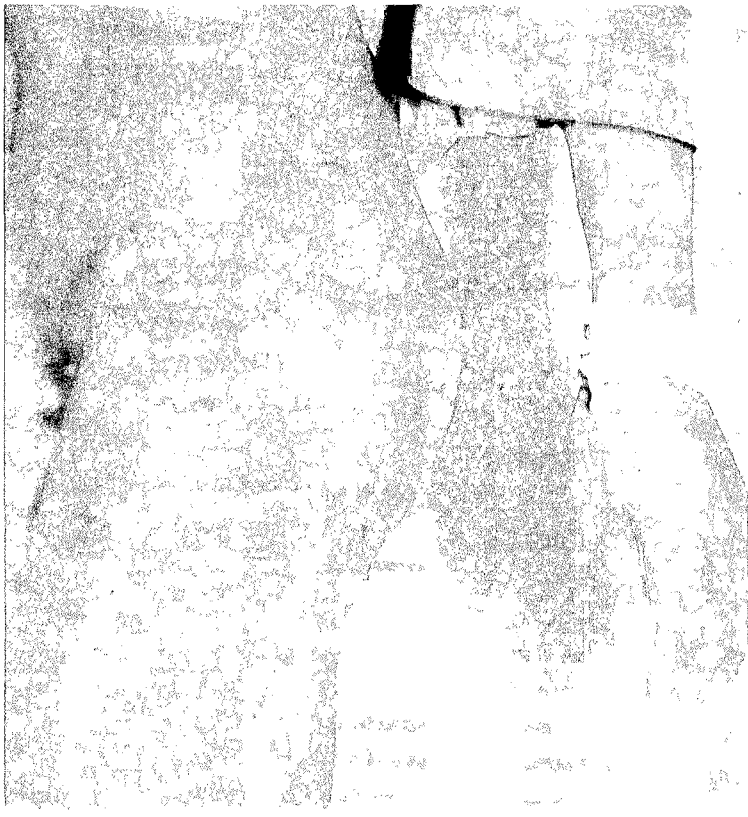
LEGEND 1

Photograph showing multiple nodules of Kaposi's sarcoma on the head and face.



LEGEND 2

Photograph showing multiple nodules of Kaposi's sarcoma on the left lower limb and severe oedema.



LEGEND 3

PHOTOMICROGRAPH OF KAPOSI'S SARCOMA. (H&E X40).

DISCUSSION

The pattern of AIDS-associated Kaposi sarcoma favours the lower extremities that are similar to the classical type. However, in our previous study, we reported aggressive KS in AIDS patients with multiple lesions on the body (8). This patient also presented with features of aggressive KS but was HIV-negative with the various tests of HIV at our center, table I Esu-Williams et al (9) carried out a study on the distribution of 330 seropositives patients and found 314 (95.2%) for HIV-1 group M, 1 (0.3%) for HIV-1 group O, 3 (0.9%) for HIV-2 and 12 (3.6%) for dual HIV-1 and HIV-2. This patient had similar tests to rule out HIV-1 group O that may be HIV negative by other routine tests.

The presentation of this patient is widespread Kaposi sarcoma involving the head and face, right eye (conjunctiva), tongue, right hand, left leg, glans penis and cervical and inguinal lymph nodes. The short course of this disease and wide spread of the lesions made it aggressive. In contrast to classic Kaposi sarcoma, which evolves very slowly and runs a benign course, even in the face of extracutaneous disease (10), the African endemic lymphadenopathic Kaposi sarcoma affects mainly children (6). This patient is a young man (30 years), which is also in contrast to the classic KS that was seen in elderly (older than 60years).

Kendrich et al (11) reported a similar case of widespread Kaposi's sarcoma in an elderly HIV-negative African-American. In the same center, Kagu et al (12) also reported a case of African Endemic Kaposi Sarcoma involving only the lower extremity in a 62-year-old farmer. Both types of African KS typically affect the lower extremities with the cutaneous type comprising 90% of cases (7).

The histological features of classic KS are not different from other forms of the disease (2). The short course of disease, and clinical features in our patient led to diagnosis of aggressive KS, with a negative HIV serology, normal laboratory findings, and absence of immunosuppressive drugs in patient's history confirmed the diagnosis of aggressive form of the disease.

In conclusion, the importance of this presentation is that of a negative-HIV young man with aggressive KS as seen in AIDS-associated Kaposi's sarcoma patients.

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