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A SURVEY OF BACTERIAL AND FUNGAL OPPURTUNISTIC INFECTIONS AMONG HIV CLIENTS IN KANO METROPOLIS

***Usman, A.D.[1] and Uba, A.[2]**
[1]Department of Biological Sciences, Bayero University Kano, Nigeria.
[2]Biological Sciences Programme, Abubakar Tafawa Balewa University Bauchi, Nigeria
*Correspondence author

ABSTRACT
Ethical clearances from Aminu Kano Teaching Hospital Kano (AKTH) and Hospitals Management Board, Kano State were obtained and 600 patients were randomly selected, their informed consents obtained and tested for HIV by rapid tests using serial algorithm as recommended by World Health Organization and adopted by the Federal Ministry of Health. The biodata of the patients were collected confidentially and anonymously. All those confirmed to be HIV positive were further tested for CD4 cell count by flowcytometry technique (Pertec®) and were selected and screened for opportunistic bacterial and fungal pathogens using sputum samples. The bacterial pathogens were isolated using Blood and Chocolate agar plates and identified biochemically except the Acid Fast Bacilli (AFB) which was tested in all the HIV positive samples by Ziehl Neelson staining technique. The fungal pathogens were isolated using Sabouraud Dextrose Agar (SDA) with antibiotics and Brain Heart Infusion (BHI) Blood agar also with antibiotics and identified morphologically by wet microscopic mount. Results of this study showed that out of the 600 blood samples tested, 72 (12 %) were HIV positive, 46 (7.7%) were male while 26 (4.3%) were female. On the basis of age groups, 30 – 40 years were found to have the highest number of HIV positive (40%, n = 72), then followed by 15-20 years (40%, n = 72), while the lowest number was recorded among 5 – 14 years. CD4 cells count categorization by WHO showed highest number of HIV positive among those with CD4 cells of ≥ 500 cells/ml with 37 cases (51% n = 72), then followed by those in the third category with CD4 count of ≤ 200 cell/ml (28%, n = 72) while in the category of 200 – 499 cell/ml, 15 cases (21% n= 72) were recorded. The overall organisms isolated among the 72 HIV positive patients were 139 as follows: Streptococcus pneumoniae (4%), Klebsiella pneumoniae (11%), Pseudomonas aerugenosa (19%), Haemophilus influenzae (4%), Acid Fast Bacilli (23%), Candida albicans (26%), Aspergillus species (11%), Cryptococcus neoformans (1.4%), and Histoplasma capsulatum (0.6%). Highest number of the opportunistic pathogens was recorded in the ≤200 cell/ml CD4 category with 61 organisms while the other two categories both recorded 39 organisms each. In this study therefore, the number of the opportunistic pathogens isolated among HIV positives indicates significant co-existence of polymicrobial infection due to immune suppression ($p < 0.05$). Also significant association was found between low CD4 cells count of ≤200 cells/ml and the occurrence of major opportunistic bacterial and fungal pathogens such as Mycobacterium tuberculosis and Candida albicans ($p < 0.05$) respectively.

Key words: HIV, AIDS, Bacteria, Fungi, Opportunistic pathogens, CD4 cells.

INTRODUCTION

Human Immunodeficiency virus (HIV) is a lentivirus, one of the sub-families of *retrovirus,* that can lead to acquired Immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life – threatening opportunistic infections (Coffin *et al.,* 1986).

The CD4 T lymphocytes also known as T helper cells; a subpopulation of the lymphocytes which are co-coordinators of the body's immune response, for example, providing help to the B cells in the production of antibody, as well as in augmenting cellular immune response to antigens. CD4 is a cell surface molecule found mostly on lymphocytes that is important for the activation of the lymphocytes when they are stimulated by antigens. Since CD4 is a major receptor for viral surface glycoprotein, (gp120), T cells expressing this surface marker are primary targets for HIV infection (Audu *et al.,* 2007). Cells expressing these markers are important in regulating the immune response necessary for controlling pathogens and neoplasm. Therefore marked reduction in the number of this cells leads to an immune-compromised state that eventually leads to disease. In Nigeria CD4 counts in healthy individuals, have been found to range from 323/mm^3 to 1160/mm^3 of blood (Audu *et al.,* 2007).
Most patients die from opportunistic infections or malignancies associated with the progressive failure of the immune system (Lawn, 2004). Rate of clinical disease progression varies widely between individuals and has been shown to be affected by many factors such as host susceptibility and immune function (Tang and Kaslow, 2003), health care and coinfections (Lawn, 2004) as well as factors relating to the viral strain (Campbell *et al.,* 2005).

Opportunistic infections are late complications of HIV infection, for the most part occurring in patients with less than 200 CD4+ T cells per microliter. (Sudha, 2003).

Infections with opportunistic pathogens have been one of the hallmarks of the acquired immunodeficiency syndrome since the beginning of the epidemic. An abundance of research and literature has been dedicated to these opportunistic fungi, viruses, and parasites. Less attention has been given to the bacterial infections complicating the course of persons infected with HIV.

Fungal infections caused by ; Aspergillus species, Candida species, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Penicillium marneffei* and *Pneumocystis jirovei* are among the most common opportunistic infections caused by microbes in HIV patients.

MATERIALS AND METHODS

Sample sites

Blood specimens were collected from some of the HIV care and support centres in Aminu Kano Teaching Hospital, Kano (AKTH) , Infectious Disease Hospital (IDH) Kano and Murtala Mohammed Specialist Hospital (MMSH) kano . Based on the 2001 population census, and considering the acceptable error margin of 2.5%, and the level of confidence (95%) a minimum sample size of 384 was calculated using a statistical software Open-epi, 2.2.1 version (2008).

Blood and data collection

An unlinked anonymous method of testing was used, as provided by the National policy on HIV/AIDS and Sexually transmitted infections, Federal government of Nigeria, April, 2002. Blood samples were collected and labeled without identifying the names of the patients, cutting across all age groups. Each sample was transported to the laboratory while maintaining cold chain.

The complete biodata for each patient were collected using a questionnaire while the anonymity of the patients was maintained.

Statistical Analysis

Open-epi 2.2.1 version statistical software was used to analyse the data collected.

HIV tests

Three rapid test kits were used while adopting serial algorithm for testing HIV as recommended by Federal Ministry of Health, (2007). It involves first step testing using Determine®. If positive the second test kit (Unigold®) was used. If the two gave discordant results, the third test kit (Stat-pak®) was used as a tie breaker. All were tested according to the manufacturers instructions.

CD_4 T – cells count

Pertec ® Cyflow (Flow Cytometry Method)

Whole blood lysis method for the analysis of lymphocyte populations was used. The blood was collected in EDTA and processed on the same day. About 50µl of phosphate Buffer solution was dispensed into tubes. This was followed by the addition of 10µl of each fluorescent antibody and 50µl of whole fresh blood. The preparation was mixed by vortexing the tube and incubating for 10 – minutes. This was followed by lysis of red blood cells and fixation of cells. Analysis was done immediately using the CD_4 cells counting system; a product of **Pertec** ® Made in Germany.

Laboratory Diagnosis of AIDS Related Opportunistic Infections

Inclusion Criteria

Only patients that were HIV positive and with or without clinical symptoms of AIDS were tested for fungal and bacterial pathogens associated with pulmonary infections using sputum sample for each.

Laboratory Diagnosis of Bacterial Opportunistic Infections

Tuberculosis (Cheesbrough, 2004)

Ziehl Neelson staining Technique for sputum specimen was used.

Method

A thick smear of purulent sputum was made on a clean grease – free microscope slide. The smear was allowed to Air dry. This was followed by heat – fixing of the smear. The smear was then flooded with carbol fuchsin stain, and passed over a gentle flame until steaming for 5 minutes. This was washed and decolorized using 1% acid alcohol for 1 minute. It was then washed and counter – stained with methylene blue for 3 minutes. It was then washed, air dried and viewed under the microscope using oil immersion objective.

Streptococcus Pneumoniae (Cheesbrough, 2004)

This was done by culturing sputum specimen on Blood and chocolate agar plates. The isolates were identified both morphologically by Gram's staining and Biochemically by the use of Optochin disc (Oxoid)

Haemophilus influenza (Cheesbrough, 2004)

This was done by culturing sputum specimen on Blood and chocolate agar plates. The isolates were identified both morphologically by Gram's staining and Biochemically.

Pseudomonas aerugenosa, Klebsiella pneumonia (Cheesbrough, 2004)

These were isolated by culturing sputum specimen on Blood and chocolate agar plates. The isolates were identified both morphologically by Gram's staining and Biochemically.

Laboratory Diagnosis of Fungal Opportunistic Infections

Cryptococcosis (WHO/SEARO, 2007)

Sputum sample of the suspected patients was cultured on Sabouraud's Dextrose Agar with antibiotics, observed for colonial appearance and microscopic identification of *Cryptococcus neoformans* using India ink.

Candidiasis (Oropharyngeal and pulmonary) (Rapid Identification of germ tube test) (WHO/SEARO, 2007).

This is a rapid screening test where the production of germ tubes within two hours in contact with the serum was considered as indicative of *Candida albicans* after culturing sputum on Sabouraud's Dextrose Agar with antibiotics. One colony of suspected *Candida albicans* was picked and inoculated into a sterile serum allowed to stand for two hours, observed under the microscope for the production of germ tubes.

Germ tubes appear as filaments that are not constricted at their point of origin or the parent cell.

Histoplasma capsulatum (Cheesbrough, 2004)
Sputum sample of the suspected patients was cultured on Brain Heart Infusion Blood agar with antibiotics, observed for colonial appearance and microscopic identification of *Histoplasma capsulatum.*

Aspergillus species (Cheesbrough, 2004)
Sputum sample of the suspected patients was cultured on Sabouraud's Dextrose Agar with antibiotics, observed for colonial appearance and microscopic identification of *Aspergillus species.*

RESULTS AND DISCUSSION

In this study, blood samples of 600 patients were collected and screened for HIV by rapid tests using serial algorithm. Out of the patients screened, 72 (12%) were positive for HIV. Out of the 72 HIV positives, 46 (64%) were male while 26 (36%) were female as shown in Table 2. Although the males have highest overall prevalence of HIV (7.7%) against the female with 4.3% as shown in Table 1, the number of male screened 383 (63.7%) were not equal to that of female which was 217 (36.3%). Presently, female are more predisposed to HIV/AIDS accounting for 50% of all the adults living with HIV/AIDS in the world and 58% in sub-Saharan Africa (Nancy *et al*., 2005).

On the basis of age groups, highest number of HIV positive cases were recorded in 30 – 40 years and 15 – 29 years with 29 (40%) and 26 (36%) cases respectively due to the fact that these groups were at their sexually active age. Table 3 shows AIDS case definition as categorized by WHO, on the basis of CD4 cell count. Out of the 72 HIV positive cases, 37(51%) were the highest category and considered to be asymptomatic with CD4 cell count of ≥500 cell/ml, while 20 (28%) were categorized AIDS patients and the second most highest with CD4 cells count of ≤200 cells/ml. The second category with CD4 count of 200 – 499 cells/ml were found to be 15 (21%), and considered as AIDS indicator group. Although this category is considered AIDS indicator, recent studies showed that counts of 350 cell/ml should be considered as the baseline for treatment with antiretroviral drugs as implemented in India and other South east Asian countries. This is not yet implemented in the HIV/AIDS policy of Nigeria. Also based on the case definition of AIDS by CDC (1992), some conditions may be considered as AIDS defining even if the CD4 count is higher.

Table 4 shows the occurrence of opportunistic bacterial and fungal pathogens among the HIV positive patients while Table 5 shows the distribution of bacterial and fungal pathogens based on the WHO CD4 categorization. Bacterial opportunistic pathogens constituted 61.2% (85 cases) of the total pathogens detected (*n = 139*) while the fungal pathogens were 38.8% (54 cases), all responsible for pulmonary infections among HIV patients. Testing CD4 count, Viral load, opportunistic infections as well as clinical symptoms are very important parameters used in establishing AIDS.

In this study, out of the 72 HIV positive patients, 139 pathogens were isolated which indicates co-existence of polymicrobial infection due to immune suppression. *Candida albicans* was the highest organism detected with 36 cases (26%). It can be isolated in HIV patients even if the CD4 cell count was above 500cells/ml (Jha, *et al*., 2006). Acid fast bacillus (AFB) was the second highest organism detected with 32 cases (23%) which is less than the previous studies of 56% in Malaysia (Cheong, *et al*., 1997), 32% in Brazil and 76% in India (Hira *et al*., 1998). This difference in HIV/TB co-infection may be due to regional variation. Still the 23% recorded cases of AFB positive in this study is significant because of the complications of HIV/TB co – infection, resulting in wasting syndrome and subsequently death. Tuberculosis ranks as the most common infection seen in the developing countries (Shailaja *et al*., 2004).

Pseudomonas aerugenosa is known to be a stubborn bacterium due to its resistance to wide range of broad spectrum antibiotics. It was significantly isolated in 27 cases (19%) as the third most common opportunistic pathogen in this study. *Pseudomonas aerugenosa* infection in patients with HIV is often community acquired and is associated with mortality (Shailaja *et al*., 2004). It was the most common organism isolated under the third WHO category (≤ 200 cells/ml). This indicates very serious threat in the management of AIDS clients.

Table 1: Incidence of HIV among sexes (n = 600)

SEX	Total no. examined	HIV positive cases
Males	383 (63.7%)	46 (7.7%)
Females	217 (36.3%)	26 (4.3%)
Total	**600 (100%)**	**72 (12%)**

Table 2: Distribution of HIV positives patients by age group and sex (*n = 72)*

Age group	SEX		
(Years)	Males	Females	**Total**
5 - 14	3 (4%)	2 (3%)	**5** (7%)
15 – 29	14 (19%)	12 (17%)	**26** (36%)
30 – 44	20 (28%)	9 (13%)	**29** (40%)
> 45	9 (13%)	3 (4%)	**12** (17%)
Total	**46** (64%)	**26** (36%)	**72** (100%)

Table 3: Distribution of HIV positive cases based on CD4 + cells count (n = 72)

CD4 + cell count (Cell/ ml)	No. of HIV Positive cases	% of HIV Positive Cases	Clinical Status
≥ 500	37	51	Asymptomatic
200 - 499	15	21	AIDS Indicator
≤ 200	20	28	AIDS
Total	**72**	**100**	

Table 4: Occurrence of opportunistic pathogens among HIV positive Patients (n = 139)

Opportunistic Pathogen	No. of Cases	(%)
(Bacteria)		
Streptococcus pneumoniae	6	4
Pseudomonas aerugenosa	27	19
Klebsiella pneumoniae	15	11
Haemophilus influenzae	5	4
Acid Fast Bacillus (AFB)	32	23
(Fungi)		
Candida albicans	36	26
Aspergillus species	15	11
Cryptococcus neoformans	2	1.4
Histoplasma capsulatum	1	0.6

Table 5: Distribution of Oppurtunistic pathogens based on CD4+ cells categorisation (n = 139)

	CD4+ cells Categorisation (cells/ml)			
Opportunistic pathogen	**≥ 500**	**200 – 499**	**≤ 200**	**Total**
(Bacteria)				
Streptococcus pneumoniae	0	0	6	**6**
Pseudomonas aerugenosa	2	9	16	**27**
Klebsiella pneumoniae	8	7	0	**15**
Haemophilus influenzae	2	1	2	**5**
Acid Fast Bacillus (AFB)	7	10	15	**32**
(Fungi)				
Candida albicans	16	7	13	**36**
Aspergillus species	1	5	9	**15**
Cryptococcus neoformans	2	0	0	**2**
Histoplasma capsulatum	1	0	0	**1**
Total	**39**	**39**	**61**	**139**

Conclusion and recommendations

Significant association was found between low CD4 cells count of ≤200 cells/ ml and the occurrence of major opportunistic bacterial and fungal pathogens such as *Mycobacterium tuberculosis* and *Candida albicans* respectively.

Recommendations

- Routine screening of oppurtunistic pathogens along with AFB In the management of HIV positive clients.
- Strategy should be developed by the HIV care and support centre to ensure compliance to Antiretroviral (ARV) treatment otherwise a lot of fluctuations in the CD4 cells count as well as Viral load may be misleading in the management of HIV positive clients.

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