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MENINGOCOCCAL CARRIAGE AND CEREBROSPINAL MENINGITIS AFTER *MENAFRIVAC* MASS IMMUNIZATION IN BURKINA FASO

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ABSTRACT

The aims of this study were to evaluate the impact of conjugate vaccine A, MenAfriVac, on *Neisseria meningitidis* (Nm) asymptomatic carriage and cerebrospinal meningitis in three health districts (Bogodogo, Kaya, and Dandé) of Burkina Faso. Asymptomatic carriage of Nm was assessed by performing cross-sectional studyrepeated (rounds 1 to 10) before and after introduction of the conjugate vaccine against serogroup A of *N. meningitidis* (NmA), MenAfriVac. In each round at least 1,500 people were enrolled in each district for a month. Data oncases of meningococcal meningitis in the three studied health districts were collected through meningitides epidemiological surveillance of Burkina Faso.Nm was identified in680 of 23,885 throat swabs before vaccination (2. 84%)withNmYasthe dominant serogroup(1.87%). During the same period (2009 and 2010), 891 cases of suspected meningitis were reported in the three health districts among whom 42 were due toNm (4.71%) withNmX (3.70%) asthe most frequently identified serogroup. After vaccination, Nm was identified in 1117 of 27,245 pharyngeal samples (6.42%); NmX (4.42%) wasthe dominantserogroup. From 2011 to 2013, 965 cases of suspected meningitis were reported in all health facilities in the three studied health districts located in the geographical study area; 91 was due toNm (9.43%) andNmWasthe most commonserogroup(52 cases= 5.38%).After introduction of conjugate vaccine A (MenAfriVac), the NmAserogroup almost disappeared both in asymptomatic carriers and in patients with cerebrospinal meningitis. However the presence of the NmW and NmXserogroups, which appear to have replaced serogroup A, is very worrying with regard to meningitis prevention and control in Burkina Faso. It appears necessary to strengthen surveillance and laboratory diagnosis of the different meningococcal serogroups circulating in Africa.

Keywords: meningococcal meningitis, serogroups W and X, meningococcal carriage, MenAfriVac.

PORTAGE DU MENINGOCOQUE ET MENINGITES CEREBROSPINALES APRES IMMUNISATION DE MASSE PAR LE *MENAFRIVAC* AU BURKINA FASO

Ky-Ba[1,*], A., Tranchot[2], J., Sanou[3], I., Christiansen[4], P., Ouedraogo[2], A. S., Ouattara[5], K., Kienou[5], M., Tamboura[6], M., Kambiré[6], D.,Ouédraogo-Traoré[6], R., Sangaré[5], L.

RÉSUMÉ

Objectifs: Evaluer l'impact du vaccin conjugué A, MenAfriVac, sur le portage asymptomatique du *Neisseriameningitidis* (Nm) et sur la méningite cérébrospinale dans trois districts sanitaires (Bogodogo, Kaya, et Dandé) du Burkina Faso.

Matériel et Méthodes: Le portage asymptomatique de Nm a été évalué à travers une étude transversale avec plusieurs passages (round 1 à 10) avant et après l'introduction du vaccin conjugué contre le *N. meningitidis*sérogroupe A (NmA), MenAfriVac. À chaque round, au moins 1.500 personnes ont été enrôlées dans chaque district pendant un mois. Les données sur les cas de méningites à méningocoque dans les trois districts sanitaires ont été recueillies à travers la surveillance épidémiologique des méningites du Burkina Faso.

Résultats: Le Nm a été identifié dans 680 des 23 885 prélèvements de gorge avant la vaccination (2. 84%) et le NmY était le sérogroupe dominant (1,87%). Au cours de la même période (2009 et 2010), 891 cas suspects de méningites ont été notifiés dans les trois districts sanitaires, 42 cas étaient dus au Nm (4,71%) et NmX (3,70%) était le sérogroupe le plus fréquemment identifié. Après la vaccination, 1117 Nm (6,42%) ont été identifiés des 27 245 prélèvements pharyngés, le NmX (4,42%) était le sérogroupe dominant. De 2011 à 2013, 965 cas suspects de méningites ont été enregistrés dans toutes les formations sanitaires des trois districts sanitaires étudiés; 91 étaient liés au Nm (9,43%), le sérogroupeNmW était le plus fréquent (52 cas, 5,38%).

Conclusion: Après l'introduction du vaccin conjugué A (MenAfriVac), le sérogroupe A (NmA) a presque disparu à la fois chez les porteurs asymptomatiques et chez les malades atteints de méningites cérébro-spinale. Toutefois, la présence des sérogroupesNmW et NmX, qui semblent avoir remplacé le sérogroupe A, est un très inquiétante en ce qui concerne la

prévention et le contrôle de la méningite au Burkina Faso. Il apparaît nécessaire de renforcer la surveillance et le diagnostic en laboratoire des différents sérogroupes de méningocoques qui circulent
Mots clé: méningite cérébrospinale; serogroupes W et X, portage du méningocoque, MenAfriVac.

INTRODUCTION

Meningococcal meningitis is a serious public health problem, especially in the part of sub-Saharan Africa called the meningitis belt of Lapeyssonnie. This area stretches from Senegal to Ethiopia and has an estimated population about 500 million people. This belt has become a ramp that includes other African countries (1). Every year, an upsurge in cases of meningitis occurs during the dry season in countries located in this area, which thus have a high endemic background (1, 2, 3).

According to the World Health Organization (WHO), there have been 700,000 cases of meningococcal meningitis globally over the last 15 years,with an estimated lethality rate of more than 10% and a considerable proportion of sequelae, sometimes reaching 20%(4).

During epidemics, about 90% of cases are attributable to *Neisseria meningitides* serogroup A, W, X (3). Since records began, meningococcal serogroup A has been the dominant cause of epidemics of meningococcal meningitis in this region; however, NmW and NmX have also been responsible for epidemics (5, 6, 7, 8).

Burkina Faso, a landlocked West African country with a population of roughly 18 million, is one of the few countries entirely located within the meningitis belt and has hyperendemic rates of meningitis (3, 9, 10). From 2003 to 2009, there were 78,518 reported cases of meningococcal meningitis with 8,568 deaths (11%) in Burkina Faso. Among the fatal cases, 5.569 (65%) were caused by group A *N. meningitides* (11).

From 2010 to 2012, NmX has been responsible for meningitis epidemics in Burkina Faso, causing 59% of the confirmed cases of meningococcal meningitis in this country in 2011(12).The lethality rates of meningitis caused by this serogroup were as high as those reported for NmA(12); children aged 1–9 years being the most frequently affected age group (12).

The mechanisms leading to the spread of infections linked to meningococcal infections and to epidemics of meningococcal meningitis remain unknown. The rate of asymptomatic carriage may reach 15% in Africa during epidemics (13, 14, 15). The environmental conditions present in the meningitis belt of sub-Saharan Africa during the dry season, particularly the high temperatures, very low humidity, and the Harmattan (dusty wind that blows from the Sahara), and respiratory co-infections related to degradation of mucosal barriers defenses are considered to contribute to the increase in sensitivity to meningococcal disease (16,17,18, 19). Several studies have shown the importance of the dynamics of asymptomatic carriage in individuals and communities and the effect of the season on colonization by meningococci (20, 21, 22, 23).

Over the past three decades, control of meningitis epidemics in the African meningitis belt has been confined to reactive vaccination with polysaccharide vaccine when the incidence in a given administrative area has reached a critical incidence (4). Implementing reactive vaccination with polysaccharide vaccine at the beginning of an epidemic has saved many lives; however, it does not decrease the frequency of outbreaks because polysaccharide vaccines confer protection for a limited time, especially in children, and have little or no impact on asymptomatic carriage (20). Conjugate vaccines, in which polysaccharidesare linked to a supportprotein, are likely to be more effective in preventing epidemics because they induce immunological memory and decrease pharyngeal carriage (21).

As a prelude to the introduction of the conjugate vaccine A, Burkina Faso initiated a study of nasopharyngeal carriage of meningococcus in three health districts (Bogodogo, Dandé, and Kaya). After the introduction of immunization, other carriage studies have been conducted, mainly to evaluatethe impact of the vaccine on serogroup A and on the carriage of the other two main *N. meningitides* serogroups: X and W.

In December 2010, the meningococcal A vaccine, MenAfriVac, was introduced in Burkina Faso through a mass vaccination program aimed at reducing the frequency of occurrence of cases and outbreaks related to serogroup A, which is the usual causative agent in the African cerebrospinal meningitis belt. Considering the results obtained elsewhere byadministering anti-meningococcal conjugate vaccines (24,25, 26), MenAfriVacwas expected to impact the rate of both carriage and the occurrence of meningitis due to NmA in Burkina.

The aim of this study was to evaluate the impact of conjugate vaccine A, MenAfriVac, on the frequency of occurrence of clinical cases of cerebrospinal meningitides and NmA carriage, and possibly cases associated with other common serogroups in Burkina Faso.

MATERIALS AND METHODS

Ethical Considerations

This study was approved by the Ethics Committees of Health Research in Burkina Faso, the Regional Committee for Research insouthern Norway, and the Commission of Internal Revision of the Centers for Disease Control in Atlanta, GA, USA.Written informed consent was obtained from all study participants or their parents or guardians.

Study Sites

The study was conducted in three health districts of Burkina Faso (Bogodogo, Kaya, and Dandé). Villages or sectors in each district were chosen randomly. In BogodogoDistrict, an urban district located in the capital of Burkina Faso, oropharyngeal samples and cerebrospinal fluid wereprocessed by the laboratory of Charles De Gaulle pediatric hospital. In Kaya District, which is a rural district located in north-eastern Burkina Faso, samples were sent to and processed by the laboratory of the Regional Hospital of Kaya (CHR) and the bacteriology laboratory of the university teaching hospital of Yalgado Ouedraogo. Finally, in the rural district of Dandé, which is located in the western part of the country, the Bacteriology Laboratory of the university teaching Hospital of Bobo Dioulasso, Souro Sanou Hospital, was in charge of bacteriological analysis of samples.

Type and period of the study

This descriptive cross-sectionalstudy was performed foranalytical purposes and comprisedfive separate rounds 3 monthsbefore and after vaccination. The study was conducted from January 2009 to November 2011.During this period, specimens were collected to obtain data on asymptomatic carriage of *N. meningitidis*. However, data related to cerebrospinal meningitis cases was collected continuously between 2009 and2013 in the three health districts studied.

Sampling for meningococcal carriage

A sample representing persons aged 1 to 29 years was obtained by cluster sampling at several levels as follows:

Eight villages were selected randomly in each of the two rural districts studied (Dandé and Kaya). For each campaign, 42 concessions per village were selected by simple random sampling from maps showing the Global Positioning System (GPS) coordinates of all concessions in the selected villages, these maps having been prepared before the start of the study. All persons in the target group who lived in one of the randomly selected concessionswere invited to participate in the study.

In the urban district studied (Bogodogo), all residential blocks were identified on a geographical map of the district. Sixteen blocks per campaign were selected by simple random sampling and mapped with GPS coordinates. During each campaign, all households in the selected blocks were visited and eligible subjects invited to participate in the study.

Sampling for cases of cerebrospinal meningitis

The sample of cases of cerebrospinal meningitis comprised all cases registered in the Ministry of Health database (National Epidemiological Surveillance System) during the period of the study.The cases of cerebrospinal meningitisin the three health districts studied (Bogodogo, Dandé, and Kaya) were selected according to the WHO definition of acute bacterial meningitis, which classifies cases as 'suspected'or'laboratoryconfirmed'.

Data collection for meningococcal carriage

The residents of the study sites were first informed of the project by local health workers and community leaders. Each randomly selected household was visited by the study staff and questionnaires administered to all target family members after they had signed individual writteninformed consent forms. The consent of a parent or guardian was obtained for children aged less than 18 years. Oropharyngeal specimens were obtained by swabbing the posterior wall of the pharynx with a sterile cotton swab, these samples being collected by technicians who had previously been trained to take such swabs.Each participant received a paper bracelet with a bar code corresponding to a unique identification number linked to the questionnaire.

Data collectionfor cases of meningococcal meningitis

Data was collected on an especially designed form for each case. The primary tools for collecting these data were clinical records, recordsofnotification of cases, the documents that accompanied the samples, and results of laboratory analysis of cerebrospinal fluid (CSF).

RESULTS

Rate of carriage of meningococcus in the selected three health districts of Burkina Faso before the introduction of meningococcal A conjugate vaccine MenAfriVac

The overall carrier rate of serogroup Nm before vaccination was 2.84% (680/23885) (Table I). In the Bogodogo health district, the overall carriage rate was 1.08% (93/8596) and the carriage rates of NmA0.11%, of NmX0.32%, of NmY0.51%, and of NmW0.12%. In the Dandé health district, the overall carriage rate was 3.14% (270/8582) and the carriage rates of NmA0.18%, ofNmX0.27%, of NmY2.49%, and of NmW 0.20%. Finally, in the Kaya health

district, the overall carriage rate was 4.72% (317/6707) and the carriage rates of NmA 0.83%, of NmX 0.79%, of NmY 2.81%, and of NmW 0.28%.

Notably, the prevalence of NmY (65.7%) prior to vaccination was 4-fold that ofNmX (15.3%), 5-fold that ofNmA (12.1%) and more than 9-fold greater than that ofNmW (6.9%)(Table II).

Types of meningococcus responsible for cerebrospinal meningitis in the three studied health districts before introduction of the anti-meningococcal A conjugate vaccine

From 2009 to 2010 (before the vaccination campaign), 891 cases of suspected bacterial meningitis were reported in the three studied health districts; 97 of these cases (10.88%) having been confirmed by laboratory analysis. Among the 92 confirmed cases, 42 (43.2%) were caused by*N. meningitidis*, 51 (52.57%) by *Streptococcus pneumonia* and four (4.12%) by *Haemophilus influenzae*. The distribution of serogroups of *N. meningitidis* was as follows: five cases (5.15%) of NmA, 33 (34.02%) of NmX, and four (4.12%) of NmW. All the NmA strains isolated were from theDandé district, whereas NmX was isolated in 16 cases (16.49%) in Kaya, nine (9.27%) in Bogodogo, and eight (8.24%) in Dandé health districts. Finally, NmW was isolated in two cases in Kaya (2.06%) and two in Dandé (2.06%).

TABLE I: CARRIAGE RATE OF MENINGOCOCCAL IN THE THREE HEALTH DISTRICTS BEFORE VACCINATION

Sites	Rounds	Number of participants	Sérogroup A	Sérogroup X	Sérogroup Y	Sérogroup W
Bogodogo district	R1	1710	2	0	4	0
	R2	1716	4	1	15	1
	R3	1717	3	0	6	3
	R4	1720	1	4	12	4
	R5	1733	0	23	7	3
	Total 1	8596	10	28	44	11
Dandé district	R1	1663	4	0	47	6
	R2	1709	5	2	55	3
	R3	1763	2	0	37	1
	R4	1742	2	0	42	2
	R5	1705	3	21	33	5
	Total 2	8582	16	23	214	17
Kaya district	R1	1663	19	1	40	8
	R2	1714	18	29	70	7
	R3	1643	7	18	38	1
	R4	1687	12	5	41	3
	Total 3	6707	56	53	189	19

TABLE II: MENINGOCOCCAL CARRIAGE RATE IN THE THREE HEALTH DISTRICTS AFTER VACCINATION

Sites	Rounds	Number of participants	Sérogroup A	Sérogroup X	Sérogroup Y	Sérogroup W
Bogodogo District	R6	1750	0	11	1	1
	R7	1713	0	9	0	14
	R8	1736	0	9	5	7
	R19	1724	0	4	2	3
	R10	1645	1	3	0	25
	Total1	8568	1	36	8	50
Dandé District	R6	1748	0	20	50	6
	R7	1728	0	12	30	11
	R8	1725	0	10	31	31
	R19	1704	0	4	24	14
	R10	1656	0	6	2	282
	Total2	8561	0	52	137	344
Kaya District	R5	1706	0	365	12	2
	R6	1697	0	244	17	0
	R7	1678	0	214	8	0
	R8	1668	0	106	5	7
	R9	1683	0	86	6	2
	R10	1684	0	14	5	28
	Total3	10116	0	1029	53	39

Meningococcal carriage rate in the three studied health districts of Burkina Faso after vaccination with MenAfriVac

The overall carriage rate Nm after vaccination was 6.42% (1749/27245)(Table II). In the health district of Bogodogo, the overall carriage rate was 1.10% (95/8568), comprising one case (0.01%) of NmA, 36 (0.42%) of NmX, eight (0.09%) of NmY, and 50 (0.58%) of NmW.In the health district of Dandé, the global carriage rate was 533/8561 (6.2%), comprising52 (0.60%) of NmX, 137 (1.60%) of NmY, and 344 (4.01%) of NmW. No NmA serogroup was isolated in this district. In the health district of Kaya, the carriage rate was 11.08% (1121/10 116), comprising1029 (10.17%) of NmX, 53 (0.52%) of NmY, and 39 (0.38%) NmW. No NmAserogroup was isolated in this district.

After immunization, the prevalence of NmX was almost three-fold (63.9%) that of NmW (24.8%) and five-fold that ofNmY (11.3%). However, NmA was isolated in only 0.1% of participants.

Meningococcus responsible for cerebrospinal meningitidis in the three studied health districts after vaccination with MenAfriVac

The MenAfriVac vaccine was introduced in Burkina Faso December 2010; thus, the period from 2011 to 2013 was considered the post-vaccination period. During this period, 965 suspected cases of meningitis were reported in the three studied health districts, 179 (18.54%) of these cases being laboratory-confirmed. Among the confirmed cases, 91 (50.83%) were caused by*N. meningitidis*, 83 (46.36%) by *S. pneumoniae*, and five (2.79%) by *H. influenzae*. The distribution of *N. meningitides* serogroups was as follows: 58 cases (32.40%) of NmX, 52 (29.05%) of NmW, one (0.55%) of NmY, and none of NmA (Table III).

TABLE III: SUMMARY TABLE OF THE MENINGITIS DATA OF 2009, 2010, 2011, 2012 AND 2013 IN THE HEALTH DISTRICTS OF BOGODOGO, DANDÉ AND KAYA

Districts		Year				
		2009	2010	2011	2012	2013
Bogodogo	Hib	2	1	1	0	0
	Negative	99	120	105	55	39
	NmA	0	0	0	0	0
	NmW	0	0	4	7	5
	NmX	0	9	1	2	0
	NmY	0	0	1	0	0
	Sp	10	17	16	1	4
	Total	111	147	128	65	39
Dandé	Hib	0	0	1	0	0
	Negative	15	140	121	163	29
	NmA	0	5	0	0	0
	NmW	0	2	2	15	10
	NmX	0	8	0	3	0
	NmY	0	0	0	0	0
	Sp	3	5	9	1	12
	Total	18	160	133	182	41
Kaya	Hib	1	0	1	1	1
	Negative	137	289	44	102	165
	NmA	0	0	0	0	0
	NmW	0	2	3	5	1
	NmX	6	10	15	12	5
	NmY	0	0	0	0	0
	Sp	7	9	16	11	13
	Total	145	310	61	131	185

As to sites, the single NmY strain was from Bogodogo health district. NmX and NmY strains were identified in all three study sites.In Kaya, 32 cases (17.87%) of NmX and nine (5.02%) of NmW were identified; in the Dandé district, 27 cases (29.67%) of NmWandthree (3.29%) of NmX, and in the Bogodogo district, 16 cases (17.58%) of NmW and three (3.29%) of NmX.

DISCUSSION

Cerebrospinal meningitis is a major public health problem in countries of the meningitidis belt such as Burkina Faso. With the aim of reducing the negative impact of this disease, there havebeen vaccination campaigns in Burkina Faso and many other African countries that are subject to outbreaks of this disease.

TheMenAfriVac vaccine wasspecifically developed to targettheNmAserogroup. Prior to the vaccination campaign, the prevalence of asymptomatic carriage of this serogroup was 0.11% in the Bogodogo district, 0.18% in the Dandé district, and 0.83% in the Kaya district. Post-vaccination, a single NmA carrier was identified intheBogodogo district; this carrier had not received the MenAfriVac vaccine. Thus, this study showed that NmA carriage had almost total disappeared from the three study sites, confirming that the conjugate vaccine MenAfriVac greatly reduces the prevalence of NmA carriage (23, 27, 28, 29, 30). A study conducted in Brazil in 2010 also showed that the conjugate vaccine reduces the prevalence of rhinopharyngeal colonization by Nm (31). Our findings are similar to those of a study conducted in Chad from 2009 to 2012, which also showed that the MenAfriVac vaccine led to a

reduction in the prevalence of rhinopharyngeal colonization byNmA (30).

Before the 2009–2010 MenAfriVacvaccination campaign, the NmAserogroup was only isolated from CSF of patients with cerebrospinal meningitis in the Dandé district (5.15% of laboratory-confirmed cases). Despite the presence of asymptomatic carriage of NmA in the Bogodogo (0.11%) and Kaya districts (0.83%), NmAwas not isolated from the CSF of any patients from these two health districts before the vaccination campaign. This may be attributable to the effect of the reactive mass vaccination (polysaccharide vaccine A/C and A/W/Y/C) performed in Kaya in 2007 and 2008 and in Bogodogo in 2008 (9). Of note, people in the Dandédistrict had not received vaccination in 2009 with the polysaccharide vaccine, which provides short-lived immunity (2–3 years) against the serogroups that it contains and is less active in children aged less than 2 years (32).

However, post-vaccination, NmA was not isolated from the CSF of any patients with cerebrospinal meningitis in any if the studydistricts. This disappearance of NmA may be associated with the effect of the MenAfriVac conjugate vaccine on this serogroup. This finding is similar to those of several other studies (33, 34).

Of note, we found an increase in prevalence of NmX carriage post-vaccination in the Bogodogo and Kaya districts, from 0.32% to 0.42% and from 0.79% to 10.17%, respectively. In contrast with patients with meningitis in Bogodogo, the prevalence of NmXwas higher in the CSF of patients with meningitis in the Kaya district post-vaccination, doubling from 16 to 32 cases compared with the pre-vaccination era. The particularly high prevalence of asymptomatic carriers of NmX in theBogodogo and Kaya districts and of laboratory-proven NmX cerebrospinal meningitis in theKaya health district could be related to the close proximity of these districts to Niger, where outbreaks caused by this serogrouphaveoccurred since 2006 (7, 35, 36).

Also of note, the post-vaccination NmW carriage rate in the Dandé health district was 20-fold that before introduction of the vaccine (0.20% vs. 4.01%). Similarly, the post-vaccination prevalence of laboratory-confirmed NmW meningitis in this district was 9-foldthat found in the pre-vaccination era.This very significant presence of the NmW serogroup in the Dandé district post-vaccination is likely related to the proximity of this city to Mali,in which cases of laboratory-confirmed NmW meningitis were reported in 2007 and 2009 (34, 37).
A post-vaccination increase in the prevalence of asymptomatic carriers of NmWwas also seen in the health district of Bogodogo, the rate there increased from 0.12% to 0.58%. Also, whereas this serogroup had not been isolated from patients with meningitis in this district pre-vaccination, post-vaccination there were 16 such cases (17.58%). Of note, the capital of Burkina Faso (Ouagadougou), in which theBogodogo district is located, is a crossroads through which many people continuously move into the country for diverse reasons. The significant presence of the NmW serogroup in this town could be attributable to thisinflux of people, including some from areas to the west thathave borders withMali.

Studies by Paul and colleagues have shown that the NmW observed in Burkina Faso post-vaccination belong to the NmW clone ST-11, which hadlast been seen in the country in 2006 but reappeared after the MenAfriVac nationwide mass vaccination campaign (38). This clone has been identified both in carriers of NmW non-invasive strains and in patients with meningitis (23, 34,39). Thus far, the ability of NmW to cause large epidemics has been associated with the hyper-virulent ST-11 clone (34, 40,41, 42). In our study, we identified persistence of the NmW serogroup in 2013 in patients in the Bogodogo and Dandé districts. This may imply that a new strain of hyper-virulent NmW emerged after the mass vaccination with MenAfriVac, which resulted in the disappearance of NmA carriers and patients with laboratory-confirmed NmA meningitis in the three studied health districts.

In general, we believe it is important to note that the unusual emergence of NmX and NmW serogroups post-vaccination was associated with the virtual absence of NmA. This finding is particularly relevant in light ofthe observed increase in overall prevalence of meningococcus after vaccination with MenAfriVac conjugate vaccine in the sites of our study among asymptomatic carriers (from 2.84% to 6.42%) and patients with meningitis (from 43.2% to 50.83%). Of note, there was also an increase in the global meningococcal carriage rate in the threestudied districts. Indeed, in the Bogodogo district it increased from 1.08% to 1.10%, in Dandéfrom 3.14% to 6.2%, and in Kaya from 4.72% to 11.08%. This increase in prevalence of asymptomatic carriers of meningococcuspost-vaccination was linked with a very low rate of carriage of NmA. Paul et al. reported similar findings (43).

In contrast with these two serogroups, we identified a significant decrease in the rate of carriage of NmY within the three studied districts post-vaccination. The prevalence of this serogroup dropped from 0.51 % to 0.09% in Bogodogo, from 2.49% to 1.60% in Dandé, and from 2.81% to 0.52% in Kaya. This reduction may reflect the effects of group immunity, including immunity against NmA. Despite the very high prevalence of carriage of this serogroup in all the health districts, analysis of CSF from patients

with meningitis in all three studied districts post-vaccination revealed a single patient from whom NmYwas isolated, this patient being in the Bogodogo health district. Serogroup Y meningococcal meningitis does not appear to be associated with outbreaks, but rather occurs sporadically (44).

CONCLUSION

In this study, we identified the disappearance of both NmA carriage andserogroup A meningococcal meningitis since the introduction in Burkina Faso of the MenAfriVac anti meningococcal A conjugate vaccine in December 2010. Despite the introduction of this vaccine, Burkina Faso remains vulnerable to outbreaks related to serogroup X, for which no vaccine is currently available, and to re-emergence of serogroup W, because the prevalence of these two serogroups both in asymptomatic carriers and in patients with meningitis has increased post-vaccination in the three studied health districts. It is therefore essential to review strategies against acute bacterial meningitis. Strengthening surveillance is essential to monitoring these changes, detectingepidemics in a timely manner and remaining reactive to those caused by any Nm serogroup.

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DYNAMICS OF GERMS RESPONSIBLE FOR ACUTE BACTERIAL MENINGITIS IN BURKINA FASO IN THE LAST TEN YEARS (2005-2014)

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ABSTRACT

The aim of this study was to analyze ten (10) years of epidemiological surveillance data of meningitis in Burkina Faso for high risk germs patterns identification in order to contribute to the strengthening of prevention strategies.

A retrospective study of the past decade (2005- 2014) of cases of acute bacterial meningitis occurred in the thirteen health regions, collected through epidemiological surveillance data meningitis in Burkina Faso. From a total of 88 057 suspected cases of acute bacterial meningitis, we recorded 9134 deaths. From the laboratory confirmed cases, the identified germs were as follows: 56.79% of *Neisseria meningitidis*, 41.09% of *Streptococcus pneumoniae* and 2.13% of *Haemophilus influenzae*. Among the meningococcus isolated, we observed the following distribution: 23.11% of NmA, 58.84% of NmW and 18% of NmX.

Mortality associated with acute bacterial meningitis remains still high in Burkina Faso despite the complete disappearance of NmA since 2012, after the conjugate vaccine A (MenAfriVac) has been introduced in this country. However the emergence of NmX, the reemergence of NmW and the persistence of high prevalence of *Streptococcus pneumoniae* are a major concern in the fight against meningitis in Burkina Faso. So, it is necessary, in addition to the strengthening of surveillance, diagnosis and case management to develop and make available and accessible a conjugate trivalent vaccine against NmA the NmX and NmW serogroups.

Keywords: meningococcal meningitis, W and X serogroups, *Streptococcus pneumoniae*, MenAfriVac.

DYNAMIQUE DES GERMES RESPONSABLES DES MENINGITES BACTERIENNES AIGÜES AU BURKINA FASO DANS LES DIX DERNIERES ANNEES (2005-2014)

RÉSUMÉ

Contexte : l'objectif de cette étude était d'analyser les données de la surveillance épidémiologique des méningites des dix (10) dernières années afin de dégager les profils de germes à risque en vue de contribuer au renforcement des stratégies de prévention

Méthodes: Une étude rétrospective des dix dernières années (2005- 2014) sur les cas de méningites bactériennes aigues des treize régions sanitaires ; recueillies à travers les données de surveillance épidémiologique des méningites du Burkina Faso.

Résultats: Sur un total de 88 057 cas suspects de méningites bactériennes aigues, nous avons enregistré 9134 décès. Parmi les cas confirmés au laboratoire, les germes identifiés se répartissent comme suit : 56.79% de *Neisseria meningitidis*, 41.09% de *Streptococcus pneumoniae* et 2.13% d'*Haemophilus influenzae*. Parmi les méningocoques, nous avons observé 23.11% de NmA, 58.84% de NmW et 18% de NmX.

Conclusion: La mortalité associée aux méningites bactériennes aigues demeure toujours élevée au Burkina Faso malgré la disparition totale du NmA depuis 2012 suite à l'introduction du vaccin conjugué A (MenAfriVac). Cependant l'émergence de NmX, la réémergence de NmW, et la persistance de la forte prévalence du *Streptococcus pneumoniae* constituent une

préoccupation majeure dans la lutte contre la méningite au Burkina Faso. Il s'avère donc nécessaire, en plus du renforcement de la surveillance, du diagnostic et de la prise en charge des cas de mettre au point et de rendre disponible et accessible un vaccin trivalent conjugué couvrant le NmA, le NmX et le NmW.

Mots-clés: méningite à méningocoques, sérogroupes W et X, Streptococcus pneumoniae, MenAfriVac.

INTRODUCTION

Acute bacterial meningitis is a major public health problem in the south-Sahara African area and particularly in Burkina Faso (1). This disease is a serious disease that can cause death within hours or may leave significant neurological sequelae (1, 2). Meningococcus is responsible for major epidemics, usually every 5-10 years, causing many cases of deaths in the population (3). More than half of the cases of meningococcal *Neisseria meningitidis* in the world occur in the African south-Sahara countries (4); they represent the 4th cause of mortality in under 15 years children, after malaria, diarrheal and respiratory diseases (3); according to the reports of the Directorate for the Fight against Disease (DLM), Burkina Faso recorded from 2006 to 2007 a lethality rate of 8% for meningitis (5).

Prevention and response strategies are usually based on epidemiological surveillance, communication, proper case management and mass vaccination. In addition to serogroup A that was most responsible for epidemics before the MenAfriVac vaccine introduction, other serogroups have led to serious epidemics in Burkina Faso. This study aimed to analyze the ten (10) past years ie 2005-2014 data of the epidemiological surveillance of meningitis in order to study trends and identify germs profiles at high risk for the upcoming years. This study could enable the Ministry of Health of Burkina Faso, especially the DLM in strengthening prevention of epidemic outbreaks.

MATERIALS AND METHODS

Study sites

The study was conducted in thirteen (13) health regions of Burkina Faso: the Boucle of Mouhoun, the Cascades, the Centre, the Centre-East, the Centre-North, the Centre-West, the Centre-South, the East, the Hauts-Bassins, the North, the Plateau Central, the Sahel and the South-West regions. The country has a National Reference Laboratory for meningitis (NRL) that is the Charles De Gaulle University teaching Hospital laboratory of Bacteriology-Virology and four (4) national laboratories located in the University teaching Hospital of Yalgado Ouedraogo, the University teaching of Hospital Souro Sanou, the Centre Muraz and the National Laboratory of Public Health. Each region, through its health districts is affiliated to a laboratory for CSF analysis and isolation of germs.

Type and period of the study

The study was descriptive retrospective and was performed for analytical purposes. It covered ten years period, from January 2005 to December 2014.

Sampling and sample

The sampling method was exhaustive: the sample comprised of all cerebrospinal meningitis cases registered in the Ministry of Health database (national epidemiological surveillance system) during the study period.

Cases of cerebrospinal meningitis of all health regions were selected and classified as suspected and confirmed cases, in accordance with the WHO definition of acute bacterial meningitis.

Data collection

Data was collected for analysis purpose on an especially designed form for each case. Clinical records, case filings forms, sampling bulletins and CSF analysis results were the primary tools for data collection.

Results

From 2005-2014, Burkina Faso recorded a lethality rate of 10.37 %. 5775 (6.55 %) of these suspected cases have been laboratory confirmed.

TABLE I- DISTRIBUTION OF MENINGITIS CASES AND DEATHS (LETHALITY) ACCORDING THE YEAR (N = 88,057)

Year	Suspected cases (N)	Deaths (N)	Lethality (%)
2005	3623	751	20.72
2006	19162	1677	8.75
2007	25695	1865	7.25
2008	10345	1068	10.32
2009	4878	693	14.20
2010	6837	989	14.46
2011	3878	588	15.16
2012	7022	739	10.52
2013	2984	367	12.29
2014	3633	397	10.92
Total	88 057	9 134	10.37

The 2010 lethality rate was almost the double of that observed in 2007 or 2006

Prevalence of identified germs from 2005 to 2014

Among the confirmed cases, 3280 (3280/5775, that is to say 56.79%) were *Neisseria meningitidis*. The serogroups distribution was as follows: 23.11% (758/3280) of NmA, 58.84% (1930/3280) of NmW and 18% (591/3280) of NmX. *Streptococcus pneumoniae* and *Haemophilus influenzae* represented respectively 41.09% (2373/5775) and 2.13% (123/5775) of laboratory-confirmed cases.

TABLE II: GERMS DISTRIBUTION OF ACCORDING TO THE YEAR (N =5775)

	NmA (%)	NmW (%)	NmX (%)	Spn (%)	Hib (%)	Total
2005	41 (68.33)	0 (0)	0 (0)	17 (28.33)	2 (3.33)	60
2006	244 (89.37)	3 (1.09)	0 (0)	20 (7.32)	6 (2.19)	273
2007	253 (89.71)	4 (1.58)	0 (0)	23 (8.15)	2 (0.70)	282
2008	156 (89.14)	0 (0)	0 (0)	19 (10.85)	0 (0)	175
2009	40 (27.21)	4 (2.72)	0 (0)	100 (68.02)	3 (2.04)	147
2010	20 (7.46)	2 (0.74)	207 (77.23)	36 (13.43)	3 (1.11)	268
2011	4 (0.35)	111 (9.96)	158 (14.18)	798 (71.63)	43 (3.86)	1114
2012	0 (0)	1357 (64.95)	201 (9.62)	502 (24.03)	29 (1.38)	2089
2013	0 (0)	236 (37.76)	23 (3.68)	351 (56.16)	15 (2.40)	625
2014	0 (0)	213 (28.70)	2 (0.27)	507 (68.32)	20 (2.69)	742
Total	758 (13.12)	1930 (33.42)	591 (10.23)	2373 (41.09)	123 (2.13)	5 775

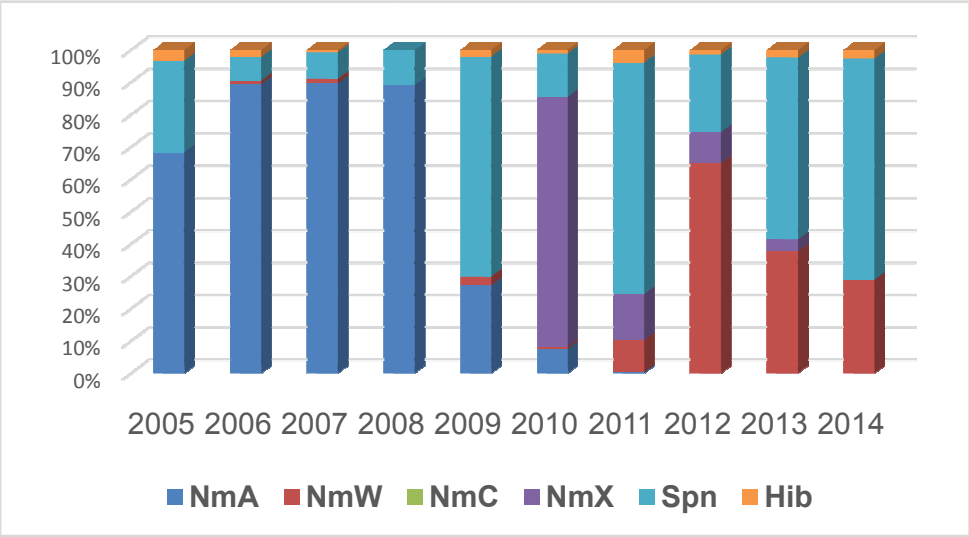

FIGURE 1: GERMS DISTRIBUTION ACCORDING TO THE YEAR, FROM 2005 TO 2014

TABLE III: DISTRIBUTION OF GERMS ACCORDING TO HEALTH REGIONS FROM 2005 TO 2014 (N = 5775)

Health region	NmA	NmW	NmX	Spn	Hib	Total
Boucle of Mouhoun	57	254	55	271	8	**645**
Cascades	9	140	20	98	3	**270**
Centre	149	24	16	93	8	**290**
Centre-East	10	262	70	272	18	**632**
Centre-North	75	77	29	168	11	**360**
Centre-West	80	148	25	236	13	**502**
Centre-South	47	140	63	124	5	**379**
East	16	154	24	214	17	**425**
Hauts-Bassins	61	353	81	311	3	**809**
North	88	204	149	231	13	**685**
Plateau Central	94	75	43	220	11	**443**
Sahel	9	60	4	70	12	**155**
South-West	63	39	12	65	1	**180**
TOTAL	**758**	**1930**	**591**	**2373**	**123**	**5 775**

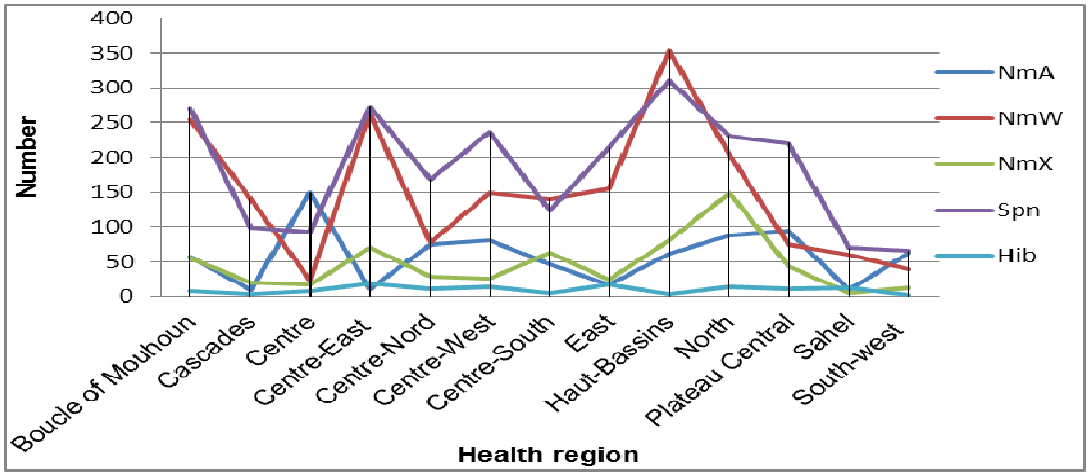

FIGURE 2: DYNAMICS OF GERMS DISTRIBUTION ACCORDING TO THE HEALTH REGION

DISCUSSION

Among the suspected cases, the bacterial meningitis were laboratory-confirmed for 5775 patients, equivalent to about one patient in 15. One limitation of our study is related to the low rate of laboratory confirmed cases: less than 7% of cases have been laboratory confirmed. Several studies reveal the insufficient use of the laboratory in meningitis surveillance in the meningitidis belt countries (6, 7). This situation could be explained by the integrated surveillance system set up in Burkina Faso before the introduction of the serogroup A anti meningococcal conjugate vaccine, the MenAfriVac in 2010. Since mass vaccination with MenAfriVac, the country adopted a surveillance system in which the CSF analysis of each suspected case is mandatory. In addition, since 2010 the use of real-time PCR increased the laboratories capacities for *N. meningitidis* detection.

During the 2005 to 2014 period, 88 057 meningitis suspected cases have been recorded in the 13 health regions of Burkina Faso. The years 2013 and 2014 were relatively calm with fewer cases (6617) in contrast to years 2006 and 2007 when the number of reported cases was 6 times higher (44,857).

Analysis of epidemiological surveillance data from 2005 to 2014 showed that *Neisseria meningitidis* was the most responsible of meningitis in Burkina Faso, about 57% of laboratory-confirmed cases. So this finding confirms what Leon Lapeyssonnie described since 1963 on germs that cause acute bacterial meningitis in the countries of the meningitidis belt in which Burkina Faso is entirely included (4, 8).

The NmA has been the dominant serogroup till 2008 with its peak in 2007: about 90% (253/282) of NmA was recorded among confirmed cases of the year. In addition to Burkina Faso, other countries located in the meningitidis belt were hit hard in 2007 by the NmA including Sudan and Uganda that notified from January 1st to March 16th, 2007, respectively 7149 and 3297 cases, mainly due to NmA (7).

Of all NmA isolated over the past 10 years, the Centre health region which houses the capital of Burkina Faso is one that recorded the highest prevalence of serogroup with about 20% (149/758) of NmA. This observation could be explained by the fact that this region is the main convergence area for both local and foreign populations in order to engage in economic activities.

Of note, the country experienced from 2010 to 2012 an abrupt emergence of a new serogroup, the NmX that was, until that period absent from the country where 566 cases of NmX with 207 cases in 2010 and 201 serogroup 2012 have been recorded. This serogroup had been reported in other countries of the belt meningitidis since 2006 including Niger, Togo, Ghana and Kenya (9, 10, 11, 12, 13). A part from Kenya, Niger, Togo and Ghana are Burkina border countries at the east and the south and thus, population movement between Burkina Faso and its border countries could explain the spread of this serogroup in almost all the 13 health regions that have been affected. Contrarily to the other epidemic serogroups, no vaccine is yet available against the NmX; in effect since December 2010, Burkina Faso, like other countries of the meningitidis belt introduced nationwide a conjugate vaccine against serogroup A, the MenAfriVac. Since 2011 we have seen the almost total disappearance of NmA: only 0.35% of NmA (4/1114) and no case of this serogroup have been reported since this year. Several studies have shown the impact of this

vaccine on cerebrospinal meningitis as well as on the NmA asymptomatic carriage.(14, 15, 16, 17).

Further, the study revealed the re-emergence of the NmW serogroup after vaccination. From 2011, a progressive increase in this serogroup was observed with a peak in 2012: approximately 65% (1357/2089) of germs confirmed during this year (2012). The number of cases declined in 2013 and 2014 but remained still high: respectively 236 and 213 cases. Out of all the affected regions, the regions of Hauts Bassins, Center-East and boucle du Mouhoun were the most affected with respectively 18.29% (353/1930), 13.57% (262/1930) and 13.16% (254/1930) of meningitidis cases caused by NmW from 2005 to 2014. Of note, in addition to Burkina Faso, countries such as, Benin, Mali, Nigeria, Gambia, Guinea and Sudan have experienced epidemics due to the NmW after MenAfriVac vaccination (18, 19, 20, 21). The proximity of some health regions with neighboring countries such as Hauts Bassins and Boucle du Mouhoun regions that are adjacent to Mali as well as the center-East region, that is near to Benin; could explain their high exposure to the NmW. Of note, this serogroup was isolated for the first time in Burkina Faso in 2002 - 2003 causing high morbidity and mortality (22, 23). Our study showed a very high lethality rate in 2012 where about 65% of the isolates have been identified as NmW. Indeed in 2012, we recorded a lethality rate 10.52% with 739 deaths out of 7022 cases as well as in 2010 when the lethality rate was 14.46% with 989 deaths out of 6837 cases of meningitis. This year, 77.23% (207/268) of the isolates were related to NmX. In general, we found that the lethality due to NmX and NmW is equivalent or even higher than that caused by NmA; this finding has been revealed by other authors (21, 24, 25).

Besides the *Neisseria meningitidis*, the study has shown the significant proportion of *Streptococcus pneumoniae* (41.09%) in the occurrence of cerebrospinal meningitis during the past decade in Burkina Faso. This germ, well-known for its high lethality (26, 27) has been observed since 2009; however it had a net increase after vaccination with MenAfriVac. In 2011, it represented almost 72% (798/1114) of the isolates; during the same year we have recorded 3878 cases of meningitis with 588 deaths, that is to say a lethality rate of 15.16%. Since 2011, there has been noticed a decrease of cases but the number of cases remained high from 2012 to 2014: it was respectively 502 cases in 2012, 351 in 2013 and 507 cases in 2014.

In addition to the three (03) most affected health regions by the NmW, other regions such as the Centre-West, North and Plateau Central regions had shown a high number of cases Spn.

CONCLUSION

Cerebrospinal meningitis continues to cause high mortality in Burkina Faso population although no NmA case has been isolated since 2011, reflecting effective action of MenAfriVac vaccination against this serogroup. However the emergence of NmX serogroup in 2010 and re-emergence of NmW serogroup since 2012 remain a major concern in the fight against meningococcal meningitis. In addition, the study has identified *Streptococcus pneumoniae* as a significant cause of high mortality of cerebrospinal meningitis in Burkina Faso. If a vaccine against NmX has not yet been developed , a conjugate vaccine against pneumococcus, which takes into account most of Spn serotypes encountered in Burkina Faso has been introduced in the childhood immunization program since 2013,. Similarly, polysaccharide vaccine against the NmW serogroup exists but has a limited access for developing countries populations such as Burkina Faso. Given this change in the epidemiological situation that is heterogeneous and dynamic geographically and through years, highlighting the emergence of NmX, the reemergence of NmW, and the persistence of high prevalence of *Streptococcus pneumoniae*, it appears imperative to reinforce preventive and case management strategies.

Thus, in addition to the strengthening of the surveillance which is essential to monitor these changes and remain able to detect epidemics caused by every serogroup of Nm; it is imperative to develop a trivalent conjugated vaccine covering NmA, NmX, and NmW serogroups and to make it available to the population. Such vaccine would be particularly useful for the prevention of meningococcal disease in the African meningitis belt and could protect against more than 90% of invasive cases of meningococcal meningitis. The major challenge after the vaccine development would be for the country to integrate it systematically into the national immunization program.

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PREVALENCE AND DISTRIBUTION OF INTESTINAL PARASITE INFECTIONS IN HIV SEROPOSITIVE INDIVIDUALS ON ANTIRETROVIRAL THERAPY IN VOM, PLATEAU STATE NIGERIA

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ABSTRACT

Background:The immunologic status of an individual can determine outcomes of treatment and their capacity to combat opportunistic infections. Co-infection with other parasites will confound the situation; however there is inadequate information on the interaction of HIV and helminth infections. We wanted to establish the relationship of the immunologic status and the prevalence of intestinal parasites in HIV/AIDS patients enrolled for antiretroviral therapy at the Vom Christian health centre. Materials & Methods: With their consent, stool samples of 205 subjects were collected and examined parasitologically by direct microscopy and concentration techniques. Their most resent $CD4^+$ cell counts were obtained at the centre. The demographic characteristics of the subjects were determined from their response to a questionaire. Results: Out of the 205 subjects examined 61.9% of them had various parasites with helminthes occurring in 51.9% of the cases. The age group of 31-40 years was the most significantly infected (P< 0.05). Hookworms and *Schistosoma mansonii* were most frequent with prevalence rates of 18.1% and 16.5% respectively. The highest prevalence of parasites occurred in HIV/AIDS people with CD4+ cells between 101-200 cells/µl and those with counts below 100 cells/µl.Occupation was highly associated with parasitic infections (p< 0.05). Conclusion: Parasitic infection remained highly prevalent among the subjects examined in spite of ART treatment and in the case of intense infection in the immunocompromised, treatment outcome may be compromised.

Key Words: HIV/AIDS, Parasites, ART, Coinfection

LA PREVALENCE ET LA DISTRIBUTION DES INFECTIONS INTETINALES PARASITES CHEZ LES INDIVIDUS SEROPOSITTIFS AU VIH SUR LA THERAPIE ANTIRETROVIRALE A VOM, ETAT DE PLATEAU AU NIGERIA

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RÉSUMÉ

Contexte : L'état immunologique d'un individu peut déterminer les résultats du traitement et leur capacité a lutter contre les infections opportunistes. Co – infection par d'autres parasites va confondre la situation ; cependant il n'y aucune information adéquate sur l'interaction entre VIH et les infections helminthiques. Nous voulions établir la relation de l'état immunologique et la prévalence des parasites intestinaux chez les patients au VIH/SIDA qui se sont inscrits pour la thérapieantirétrovirale au Centre de Sante de Chrétien de VOM.

Matériaux et Méthodes : Avec leur consentement, échantillons de selles de 205 sujets ont été recueillis et examines parasitologiquement par des techniques de microscopie et de concentration directes. Leurs plus récent taux de CD4+ont été obtenus au Centre. Les cultures caractéristiquesdémographiques des sujets étaientdéterminésà partir de leur réponses a un questionnaire.

Résultats : Sur les 205 sujets examines, 61,9% d'entre eux avaient divers parasites avec les helminthes qui se produisent dans 51,9% des cas. Le groupes des 31 – 40 ans d'âge a été la plus infectée de manière significative (P<0,05). Ankylostomes et *Schistosoma masonii*étaient les plus fréquents avec des taux de prévalence de 18,1% et 16,5% respectivement. La plus forte prévalence de parasites survenus chez des personnes au VIH/SIDA les cellules CD4+ entre 101 – 200 cellules /µ/ et ceux avec les chiffres ci – dessous de 100 cellules. L'occupation a été fortement associée a des infections parasites (P<0,5).

Conclusion : L'infection parasitaires est restéetrèsrépandue parmi les sujets examines en dépit du traitement ART et dans le cas de l'infection intense chez les personnes immunodéprimées, le résultat du traitement peut être compromise.

Mots clés : VIH/SIDA, Parasites, ART, Co – infection.

BACKGROUND

Human immunodeficiency virus (HIV) causes progressive impairment of the body's cellular immune system leading to increased susceptibility to infections, tumors and fatal conditions of AIDS. HIV is an enveloped RNA virus, on infection the DNA the viral genome becomes integrated in the DNA of the infected cell ensuring permanent infection and replication of the virus. The virus becomes established with the cells bearing CD4 glycoprotein in their plasma membrane. The whole T cell population in the body begins to decline, leaving the patient open to opportunistic infection. A decrease in CD4+ T-lymphocyte counts is responsible for the profound immunodeficiencies that lead to various opportunistic infections in HIV infected patients. Since the beginning of the AIDS pandemic, opportunistic infections have been recognized as common complications of HIV infection. The spectrum of opportunistic infections in the HIV infected subjects varies from one region to another (1).

The aetiologic spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi and viruses[2]. Gastrointestinal problems resulting from opportunistic parasitic infections in HIV and AIDS infected subjects often present as diarrhea and significant disease has been recorded in 50-96% of cases worldwide with 90% prevalence rate reported in Africa[3]. Either backed by HIV or independently, intestinal parasitic infections have continued to be major cause of morbidity and mortality in humans (4). Diarrhoea accounts for 50 million deaths worldwide and it ranks third among diseases responsible for human mortality globally (5). Intestinal parasitic infections are among the most common infections world-wide. It is estimated that some 3.5 billion people are affected, and 450 million are ill as a result of these infections (5). The rate of infection is remarkably high in Sub-Saharan Africa, where the majority of HIV and AIDS cases are concentrated (6). In developed countries diarrhea occurs in 30-60% of AIDS patients and 90% in the developing countries (7).

Diarrhea and weight loss are independent predictors of mortality (8). Acute and chronic diarrhea has been associated with different species of gastrointestinal parasites, which are responsible for considerable morbidity and mortality in HIV/AIDS patients (9). Many of the opportunistic infections that ultimately plague such individuals involve infectious agents that are normally checked by the mucosal barriers which include *Cryptosporidium spp, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides,* hookworm infection, *Schistosoma spp* and *Strongyloides stercoralis* are important cosmopolitant intestinal parasites that are common among children and immunocompromised individual (10).

Parasitic infections in HIV-infected patients are common in many regions and populations across Nigeria and represent a lasting public health challenge. There are a number of studies on parasites among HIV/AIDS patients in Nigeria with different prevalence rates. Due to the importance of intestinal parasites in HIV+/AIDS patients and because there are only few studies regarding the prevalence of intestinal parasites and their association with CD4+ cell counts in this population are available in Jos. This study was carried out to determine the prevalence and parasitic profile of intestinal infections in HIV/AIDS patients in relation to their immunologic status.

MATERIALS AND METHODS

Study design

This cross sectional survey involved interviewing of the HIV/AIDS patients using structured a questionnaire and also laboratory analysis of stool specimen for protozoa and helminth from the respondents. The study was carried out in Vom Christian Hospital, located in Jos- south of Plateau State Nigeria. The APIN laboratory was established in 2007 and it renders services to people from surrounding towns and villages like Turu, Vwang, Kuru, Farin lamba, Bukuru and other environs. Patients were enrolled from the months of March - April 2010.

Study Population

Stool samples were obtained from a total of 205 people who were confirmed to be HIV positive and were enrolled for antiretroviral therapy. Questionnaires were administered to them to obtain their demographic characteristics and gender status.

Ethical consideration

Ethical approval was obtained from the research ethics committee for the Vom Christian hospital. Written informed consent was obtained from all the study participants, and human experimentation guidelines of the hospital were followed.

Sample collection

A total of 205 stool specimens were collected in labeled, clean, leak-proof and wide mouth plastic containers from the patients enrolled for the study. The most recent CD4 T-cells counts of the participants were obtained from their ART fellow-up record in the hospital.

Sample processing

Direct microscopy of smears was performed for the detection of ova, larvae, trophozoites as described by Cheesebrough (1999) and cysts of intestinal parasites and formol-ether concentration technique for helminthic ova and larva (12). A drop of stool concentrate was also stained by modified Ziehl-Neelsen staining technique for oocysts of *Isospora belli*, *Cryptosporidium* and *Cyclospora species* (13).

RESULT

The prevalence of intestinal parasites among HIV/AIDS infected people in Jos was 61.9% with 3.1% having multiple parasites infection. More of the people who enrolled for the survey were women and they also had the highest prevalence of 40% intestinal parasites while men recorded a prevalence rate of 22% infection. Both male and female HIV/AIDS patients within the age range 30-39 had the highest prevalence rates of intestinal infection.

During this survey eight different species of intestinal parasites were identified from the population studied. Majority of the intestinal parasites were geohelminths which recorded a prevalence of 59.1%. Hookworm recorded the highest prevalence rate of 18.1% followed by *Schistosoma mansoni* with a prevalence of 16.5% while the lowest parasite reported was *Trichuris trichiura* with a prevalence rate of 6.3%. HIV/AIDS patients who who said their occupation was farming and house wives had the highest rates of intestinal parasites.

The minimum CD4+ cell count was 13cells/µl and the maximum count was 711cells/µl. the highest prevalence of parasites occurred in HIV/AIDS people with CD4+ cells between 101-200 cells/µl and those with counts below 100cells/µl.

TABLE 1: AGE AND SEX DISTRIBUTION OF HIV/AIDS SUBJECTS WITH INTESTINAL PARASITES IN VOM

Age Range	Males		Females		Total	
	Examined	Positive (%)	Examined	Positive (%)	Examined	Positive (%)
≤ 19	6	4 (1.95)	17	11 (5.37)	23	15 (7.32)
20-29	23	13 (6.34)	39	24 (11.7)	62	37 (18.1)
30-39	25	17 (8.29)	32	21 (10.2)	57	38 (18.5)
40-49	18	8 (3.90)	27	17 (8.29)	45	25 (12.2)
≥ 50	5	3 (1.46)	13	9 (4.39)	18	12 (5.85)
Total	77	45 (21.9)	128	82 (40.0)	205	127 (61.9)

p-value = 0.696 (sex); p-value = 0.066 (age group)

TABLE 2: SINGLE AND MULTIPLE HELMITH/PROTOZOA INFECTIONS IN HIV/AIDS INFECTED SUBJECTS IN VOM

TYPE OF PARASITE INFECTION	NUMBER POSITIVE (%)
Protozoa	48(37.8%)
Helminth	75(59.1%)
Multiple infections	4(3.1%)
TOTAL	127(61.9%)

TABLE 3: PREVALENCE AND DISTRIBUTION OF HELMINTH/PROTOZOA OVA IN HIV/AIDS INFECTED PATIENTS ON ART IN VOM

Parasites (Ova/ Cyst)	Prevalence Rate (%)
Entamoeba histolytica	18 (8.78)
Cryptosporidium parvum	17 (5.37)
Entamoeba coli	15 (7.32)
Trichuris trichiura	8 (3.90)
Hookworm	23 (11.2)
Ascaris lumbricoides	16 (7.80)
Schistosoma mansoni	21 (10.2)
Hymenolepsis nana	9 (4.39)

TABLE 4: DISTRIBUTION OF INTESTINAL PARASITES AND $CD4^+$ CELL COUNTS IN HIV/AIDS INFECTED PATIENTS IN VOM

CD_4^+ cell counts (cells/µl)	E	A	C	S	En	T	H	Hy	Total (%)
≤ 100	5	3	3	6	4	0	3	0	24(11.7)
101-200	4	5	4	3	5	3	6	3	33(16.1)
201-300	0	0	0	0	0	0	0	0	0 (0.00)
301-400	3	3	1	5	1	2	2	3	20(9.75)
401-500	2	2	5	3	2	1	5	2	22(10.7)
501-600	3	1	2	3	2	2	4	0	17(8.29)
≥ 601	1	2	2	1	1	0	3	1	11(5.37)
Total	18	16	17	21	15	8	23	9	127(61.9)

KEY : E- *Entamoeba histolytica;* **A-** *Ascaris lumbricoides;* **C-** *Cryptosporidium parvum;* **S-** *Schistosoma mansoni;* **En-** *Entamoeba coli* **T-** *Trichuris trichiura;* **H-** *Hookworm;* **Hy-** *Hymenolepsis nana*

TABLE 5: PREVALENCE OF INTESTINAL PARASITE INFECTION IN HIV/AIDS PATIENTS IN VOM WITH RESPECT TO OCCUPATION

Occupation	Number of Respondents	Number Positive (%)
Civil servants	32	23 (11.2)
Farmers	47	28 (13.7)
House wives	72	33 (16.1)
Artisan and Students	17	16 (7.80)
Unemployed	30	24 (11.7)
Medical workers	04	02 (0.98)
Long distance driver	03	01 (0.49)
Total	205	127 (61.9)

P value=0.001

DISCUSSION

HIV infection is believed to be a significant risk factor for acquiring intestinal parasitic infection (14). Our study was carried out in Vom where most of the participants are rural dwellers. The prevalence of intestinal parasitic infection among HIV/AIDS infected persons was 61.9% in this study which confirms several similar reports in Nigeria. Inabo *et al.,* (15) had reported prevalence rates between 60-70% in HIV/AIDS infected persons in

Zaria Nigeria, 69.2% in Ethiopia[16], 63.9% in Rio de Janeiro (17). Lower rates were however reported in Benin (14), Nassarawa Toto (18), Apulia, Italy (19), in East Delhi (20), in Abuja and Abeokuta, respectively (261, 22, 23). However higher prevalence rates of 89.5% has been reported in Lagos [24] among HIV infected subjects. The varying prevalence rates may be due to geographical variation of the study locations and is likely to depend upon the endemicity of that particular parasite in the community (25).

In this study 3.1% of the HIV infected people had multiple intestinal parasitic infections lower than it was reported by Inabo *et al* (15) in Zaria. Female HIV patients had a higher prevalence rate of intestinal parasitic infection (40%) than the HIV infected men (21.9%). Gender was not significantly associated with the rate of parasitic infection in this study population (p=0.696). Akinbo *et a* (14) had reported that gender was significantly associated with the prevalence of intestinal parasitic infections among HIV-infected patients although that study found more HIV intestinal parasitic infection in men while Kipyegen *et al* (26) reported no significant association between intestinal parasitic infection and gender. Age was not a significant factor associated with parasitic infection in this study (p=0.066) even though in both male and female HIV infected patients, those within the age range 30-39 had the highest rate of intestinal parasites. Kipyegen *et al* (26) reported no significant association between age and parasitic infection.

The HIV/AIDS patients in this population had a higher prevalence of helminthes infections (59.1%) than protozoan infections (37.8%). This is not in consistent with most findings; Kipyegen *et al* (26) reported a higher prevalence of protozoan infections among HIV patients in Baringo, Kenya. Inabo *et al* (1) also reported higher rate of coccidian parasites than helminthes. Among the helminthes, hookworms were more prevalent (18.1%) followed by *Schistosoma mansonii* (16.5%). Kipyegen *et al* (26) reported hookworms as the least prevalent parasite in HIV infected subjects in in Kenya ((1.3%), Abaver *et al* (18) also reported Hookworms (8.5%) and *S. mansonii* (5.7%) as the most prevalent intestinal helminth in HIV infected patients in Toto, Nigeria. Akinbo *et al* (14) reported *Ascaris lumbricoides* (33.1%) to be the most prevalent intestinal parasite in HIV infected subjects in Benin followed by hookworm (20.6%). The prevalence of hookworm in this study population is lower than that reported in Benin. In Ilorin Nigeria *S. stercolaris* was the most prevalent helminth in HIV infected persons (27).

Entamoeba histolytica/dispar was the only pathogenic parasite found in this study (8.78%). Studies indicate increased risk for invasive amoebiasis among HIV infected persons (28).

In this study with HIV subjects in Vom, *Cryptosporidium parvum* was the only opportunistic emerging parasite observed. Amatya *et al*[29] also found a higher prevalence of coccidian parasite amongst HIV infected persons in Nepal with Cryptosporidia as the most frequent coccidian parasite. Even though studies have highlighted *Cryptosporidium species* as the predominant pathogen with significant association to diarrheal cases (30) and occurrence of Cryptosporidium in both symptomatic and asymptomatic cases indicates high risk of infection in this parasite. The parasitic infections detected in this study have been reported in Nigeria and other African countries among HIV/AIDS infected subjects. The low prevalence of pathogenic protozoa and opportunistic emerging parasites (coccidian) may be due to the time this study was carried out as the organisms have been reported to be prevalent in humid temperatures.

On the socioeconomic factors, occupation of the subjects was found to be a significant factor associated with the prevalence of HIV/AIDS co-infection among the respondents in Vom (p=0.001).House wives, farmers and Civil servants had 16.1%, 13.7% and 11.2% prevalence respectively. Akinbo *et al (14)* had also reported a significant association between parasitic infection and occupation among HIV patients in Benin Nigeria with artisans, farmers and security guards having high prevalent rates.

Out of the 127 subjects with HIV/AIDS and intestinal parasite co-infections, 22.1% had CD_4^+ T cell count ≥ 501 cells/µl, 33.1% had CD_4^+ T cell count between 200-500 cells/µl and 44.9% had CD_4^+ T cells ≤ 200 cells/µl. studies have reported that most infections with opportunistic parasites and pathogenic parasites were associated with CD_4^+ cells below 200 cells/µl. Even though most of the parasites isolated were in the group of patients who had CD_4^+ T cell counts below 200 cells/µl. This study shows that, parasite infection was not directly associated with CD4 T-cell counts, as parasite infection was observed in patients across all the ranges of CD4 cell counts however, the intensity of the infection may influence treatment outcome in patients who are immunocompromised.

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HEMATOLOGICAL DERANGEMENT PATTERNS IN NIGERIAN DOGS INFECTED WITH *TRYPANOSOMA BRUCEI*: A SIMPLE PROTOTYPE FOR ASSESSING TOLERANCE TO TRYPANOSOME INFECTIONS IN ANIMALS

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RUNNING TITLE: HEMATOLOGY OF *T.BRUCEI* INFECTED DOGS

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ABSTRACT

The haematology of Nigerian local puppies experimentally infected with the Federe strain of *Trypanosoma brucei* was studied in a total of six 9-weeks old puppies born to two local bitches. Four were randomly selected and inoculated with about 0.8 x 10^6 of *T .brucei* subcutaneously and the remaining two served as the uninfected control. The parasitaemia was monitored daily using wet mount microscopy. The packed cell volume (PCV), red blood cell (RBC) counts, total and differential white blood cell (WBC) counts and rates of both red blood cell and white blood cell loss per day and per parasitaemia log equivalent value(LEV) were monitored twice in a week . Parasitaemia was detected in the infected group four days after infection which was followed by an acute disease course, though with low fatality rate in the dogs. The anemia was characterized by a fluctuating PCV decrease from the pre-infection value of 29.5±4.5% and 15.3±3.3% at two weeks after infection when one of the dogs died. There was a mild decrease in the overall erythrocyte values which was attributable to trypanotolerance in the local breed of dogs. The post infection hematological derangement pattern was characterized by an overall post-infection RBC count drop of 1.92±0.23(x 10^{12}/µl) (39.0%), mean daily drop of 0.07±0.05 (x 10^{12}/µl) and an overall drop per LEV of 0.69(x 10^{12}/µl). The overall mean post-infection total WBC count drop was 0.61±0.15(x10^9/µl)(43.6%) with a mean daily drop of 0.02±0.14(x10^9/µl), and an overall drop per LEV of 0.22±0.44(x10^9/µl). There was an overall higher post infection leukocyte drop compared to erythrocyte. The result poses fundamental research questions on the likelihood of differential surface sialic acid contents of erythrocytes and leukocytes and the possible roles of trypanosome sialidase in creating this difference as well as enhancing pathogenesis of leucopenia in the dogs . It was concluded that the patterns of hematological derangements demonstrated as erythrocyte and leukocyte drop (loss) rates and drop per parasitaemia Log Equivalent Values could serve as a prototype for comparing susceptibility to animal and human *T. brucei* infections and, other trypanosome species.

Key words: Federe, *Trypanosoma brucei*, haematology, derangement, patterns.

MODELES DE DERANGEMENT HAEMATOLOGIQUES CHEZ LES CHIENS NIGERIANS INFECTES PAR TRYPANOSOMA BRUCEI : UN PROTOTYPE SIMPLE POUR L'EVALUATION DE LA TOLERANCE AUX INFECTIONS TRYPANOSOME CHEZ LES ANIMAUX.

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RÉSUMÉ
L'hématologie de chiots locales nigérianesinfectéesexpérimentalement avec la souche de FEDERE de *Trypanosomabrucei* a été étudiée dans un total de 6 – 9 semaines chiots néesà deux chiennes locales. Quatre ont été choisis au hasard et inoculés avec

environ 0.8 x 10^6 de *T. brucei* sous – cutanée et les deux autres ont servi de témoin non infecté. La parasitemie àétécontrôlée quotidiennement en utilisant la microscopie humide montage. L'hématocrite(PCV), numération globulaires rouges(RBC), numération et différentiel des globules blancs(WBC) et les taux de la perte des globules rouges et globules blancs par jour et par la parasitemie connecter valeurs équivalentes ont été contrôlées deux fois par semaine. La parasitemie àété détecter dans le groupe infecté quatre jours après infection à été suivie par une évolution de la maladie aiguë, mais avec faible taux de mortalité chez les chiens. L'anémie à été caractérisée par une diminution variante de PCV de la valeur pré – infection de 29,5 ± 4,5% et 15,3 ± 3,3% à deux semaines après l'infection quand l'un des chiens est mort. Il y avait une légère diminution de l'ensemble des valeurs érythrocytes ce qui est attribuable à la trypanotolerance dans la race locale des chiens. La modèle de dérangementhématologiqueaprès l'infection a été caractérisée un ensemble de la baisse des numérations globules rouges de 1, 92±0,23 (x10^{12} /µl) (39,0%), la baisse moyenne quotidienne de 0,07±0,05(x10^{12}/µl) et une baisse globale par LEV de 0,69 (x10^{12}/µl). La baisse moyenne globale totale après infection du WBC a été 0,61±0,15 (x10^9/µl) (43,6%) avec une baisse moyenne quotidienne de 0,02±0,14 (x10^9/µl), et une baisse globalepar LEV de 0,22±0,44 (x10^9/µl). Il avait une plus baisse leucocytes globalesaprès infection par rapport aux érythrocytes. Le résultatpose des questions de recherche fondamentale sur la probabilité de surface différentielle de rôles possibles de sialidase trypanosomes dans la création de cette différence ainsi que l'améliorationde la pathogenèse de la leucopénie chez les chiens. Il a été conclu que les tendances des dérangementshématologiquesdémontrées comme le taux de la baisse (perte) d'érythrocyte et la baisse par la parasitemie connecter valeur équivalente pourrait servir de prototype pour comparer la sensibilité aux infections *T. brucei* chez les animaux et les humaines et d'autres espèces de trypanosomes.

MotsClés : FEDERE, *Trypanosomabrucei*, hématologie, dérangement, tendances

INTRODUCTION

Pancytopenia is one of the consistent pathological features of trypanosomiasis in man and animals whose severity is often dependent on parasite and host factors (1). Host's ability to control parasitaemia and development of anemia had been identified as hallmarks of tolerance to trypanosomiasis (2, 3). The African trypanosomiasis arising from animal infective *Trypanosoma brucei* and the human infective sub species, *T. brucei gambiense and T.b.rhodesiense* are recognized as stage dependent pathologies, characterized by the early and late stage syndromes in infected hosts (4, 5) and are characterized by anemia. Its socio-economic impact arises principally from the characteristic wasting nature of the disease as it causes severe losses in production due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion (6). The disease had been recognized as a major barrier to the development of African continent (6).

The disease in dogs, arising from *T. brucei* is generally acute and fatal (7, 8) and resulting to fever, anemia, myocarditis, corneal opacity, marked body edema, and central nervous system disturbances. The *T. brucei* group of trypanosomes generally localize in solid tissues of various organs causing extensive degenerative disease and anemia (4). The major processes associated with pathogenesis of *T. brucei* infection were described by (7). These include; extravasation of parasites into body tissues leading to severe lesions, vasculitis, increased vascular permeability and thrombosis, direct toxic damage caused by biological active substance produced by dead or living trypanosomes and increased erythrophagocytosis, resulting to excess destruction of erythrocytes .

In the last decade, canine trypanosomosis arising from tse-tse transmitted *T. brucei* and *T. congolense* had been identified as an important threat to hunting dogs(9) as well as exotic and local city dogs(10, 11), in Nigeria. Due to the absence of sustainable surveillance, the exact prevalence situation and socio-economic impacts of the disease in Nigeria are not well known. Furthermore, the trypanosusceptibility status of most African dog breeds, against the backdrop of emerging new trypanosome strains, is not well known. We reported mild anemia in *Trypanosoma congolense* infected Nigeria puppies characterized by a slight drop in packed cell volume (PCV), hemoglobin and red blood cell counts which did not occur until the last half of 8 weeks post infection period which was attributable to trypanotolerance in the local breed of dogs (12). The Federe strain of *T. brucei* is a newly emerging and highly pathogenic trypanosome, often producing acute disease. The drop in number of new cases of *T. b. gambiense* sleeping sickness in Nigeria which share etiological, structural and host properties with animal infective *T. brucei* coupled with emergence of this strain has generated research interest in the Federe strain of *T. brucei* (13 – 15).

The aim of this study was to investigate the hematological derangement patterns as a basis for assessing the susceptibility of young Nigerian young dogs to the Federe and other strains of *Trypanosoma brucei*. This is with the view to providing additional hematological data needed for the characterization of this strain and assessing of tolerance to *Trypanosoma brucei* infections in animals and man.

MATERIALS AND METHODS

Experimental Animals

A total of six-9 weeks old local puppies of mixed sexes weighing 3.0 to 4.0 kilograms body mass were used. The six puppies were whelp by four different mothers in the same village near the Federal University of Agriculture, Makurdi, Nigeria. The bitches were local dogs and mounted by other local male dogs from within the area. The puppies were acclimatized for one week at the Veterinary Teaching Hospital, Federal University of Agriculture, Makurdi before use. During this period they were dewormed with Albendazole suspension, 25mg/kg against round worms, tapeworms and hook worms. The puppies were fed with pap made from millet (3/4) and fish (1/4), rice, yams, beans, milk, fish, and occasionally biscuit bones, while water was provided *ad libitum.*

Trypanosome specie

The trypanosome specie used was *T. brucei* Federe strain, obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The parasite was isolated from cattle, cryopreserved in liquid Nitrogen and sub-passaged into the donor albino rats prior to use.

Experimental Design

The six dogs were randomly selected and tagged numbers 01, 02, 03, 04, 05, and 06. The numbers 01, 03, 04 and 06 constituted the infected group and were inoculated with 0.8 x 10^6 of the parasites via the subcutaneous route. The remaining two dogs served as the uninfected control group. Parasites for inoculation were estimated as described by Lumsden *et al;* (16). Daily parasitaemia was estimated from wet mount preparation made through ear puncture. However, the packed cell volume (PCV) was determined twice in a week.

Blood Collection

Blood for hematology was obtained through venipuncture of the cephalic vein using 23 guage hypodermic needles and 5ml syringes. The blood was collected into ethylene diamine tetraacetate (EDTA) bottles prior to use. A total of 2ml of blood was collected into each EDTA bottles.

Hematological Techniques

Commercially heparinized capillary tubes were ¾ filled with blood, sealed on one end with plasticine, and centrifuged for 5 minutes in a microhaematocrit centrifuge at 12,000 rpm to deterime the PCV. The packed cell volume was read off the microhaematocrit reader (17, 18). The erythrocytes and leukocytes were enumerated using the Neubaur haemocytometer (18). Thin blood smears were made and stained with Giemsa stain (18). 100 cells were counted and differentiated per slide. The patterns of hematological derangements were determined arithmetically using the Post Infection Drop (PostID), Daily Drop (DD), Overall Percentage Drop(OPD) and Drop Per Parasitaemia LEV(DPPL) in PCV , RBC and the total WBC count values of the infected group as follows:

$$DD = \frac{\text{PostID}}{\text{Number of Post-infection Days}}$$

Post ID = Mean Pre-infection Value(MPreIV) – Mean Post-infection Value(MPostIV).

$$OPD = \frac{\text{PostID}}{\text{MPreIV}} \times 100$$

$$DPPL = \frac{\text{MPostID}}{\text{Mean Parasitaemia LEV}}$$

In the course of the experiment, terminally sick animals were given painless chemical euthanasia as described by Severidt *et al*., (19) for necropsy and the remaining infected dogs given curative treatment with diminazene aceturate at the end of the study.

The data collected in this study was statistically analyzed to test significant difference using the student t-test (20) and the P value of less than 0.05 was regarded as significant.

RESULTS

The Nigerian local dogs infected with *T. brucei* became parasitaemic 4 days post-infection (PI). The parasitaemic pattern of the infection is shown on Fig. 1. Between the pre-infection (Day 0) and 29 P.I., the parasitaemia attained peak values four times. These were on days 6, 11, 18 and 25 P.I. with mean log equivalent values of 4.30±0.00, 4.10±0.40, 3.50±0.70, and 4.30±0.00 respectively. Following parasitaemia, the dogs exhibited pyrexia, slight weight loss and anemia. The pre-infection mean PCV of the infected

group was 29.5±4.5 (%) while that of the control group was 26±8.5 (%) (Fig.2). After infection, the PCV did not differ significantly until day 8 PI when it dropped to 18.5±5.8(%) and then to 15.3±3.3(%) on day 11 ($P<0.05$), followed later by apparent increase to 17.7±5.5(%) when the experiment was terminated.

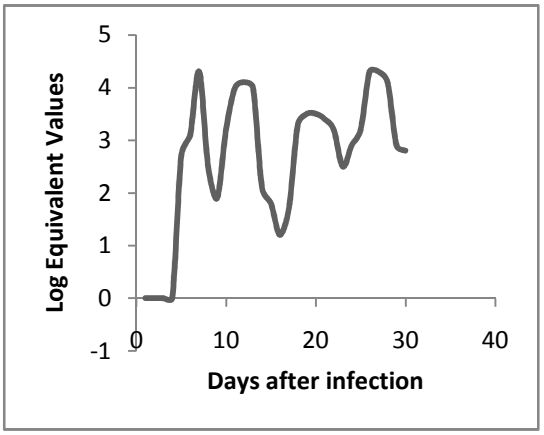


Fig 1. Daily parasitaemia of the *T. brucei*-infected dogs

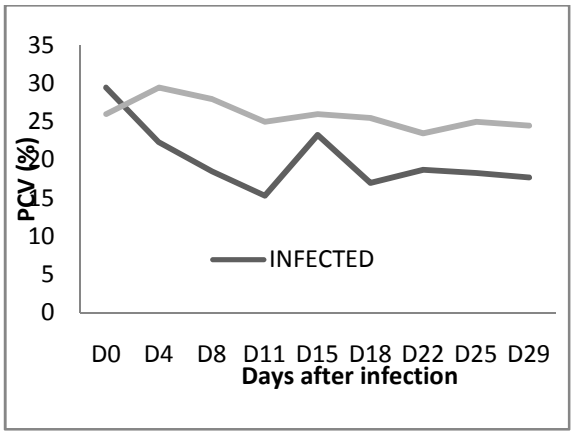
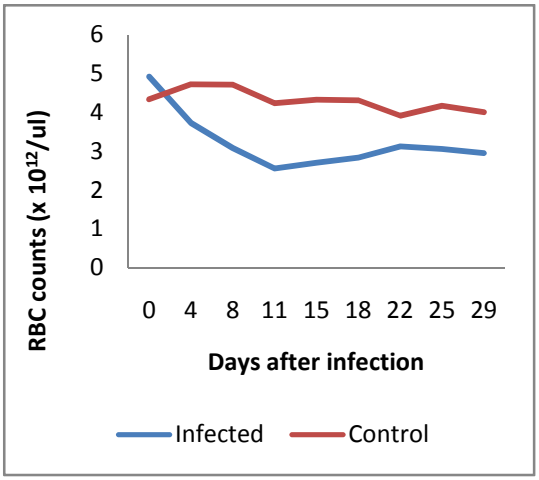


Fig 2. Mean variation in daily PCV of *T. brucei*-infected dogs

This translated to an overall mean post infection drop of 10.6(%), daily drop of 0.37(%) and an overall drop of 3.82% per LEV (Table 1). The mean PCV of control animals fluctuated within normal range. The pre-infection mean RBC count of infected group was 4.92±1.0 ($x10^{12}/\mu l$) while that of the control group was 4.33±0.21($x10^{12}/\mu l$) (Fig.3). After infection, the RBC count of the infected group dropped progressively to 2.55±0.3 ($x\ 10^{12}/\mu l$) on day 11PI and thereafter fluctuated to 2.95±0.47($x10^{12}/\mu l$) ($P<0.05$) on the 29th day before termination of observations. This translated to post-infection value of 3.00 ± 0.13($x\ 10^{12}/\mu l$), post-infection drop of 1.92 ± 0.23($x\ 10^{12}/\mu l$) (39.0%), daily drop of 0.07 ± 0.05 ($x\ 10^{12}/\mu l$) and drop per LEV of 0.69 ($x\ 10^{12}/\mu l$) (Table 1). The RBC values of the infected group only fluctuated within normal range.

TABLE 1: BLOOD CELL LOSS RATES IN *T.BRUCEI*-INFECTED YOUNG DOGS.

Blood cell parameters	Packed Cell Volume(%)	RBC ($x10^{12}/L$)	Total WBC ($x10^{9}/L$)
Mean Pre infection Value	29.5±4.5	4.92±0.44	1.40±0.44
Mean Post Infection Value	18.9±4.5	3.00±0.25	0.79±0.15
Post Infection Drop(%)	10.6(36.0)	1.92(39.0)	0.61(43.6)
Daily Drop	0.37	0.07	0.02
Drop per Parasitaemic LEV	3.82	0.69	0.22



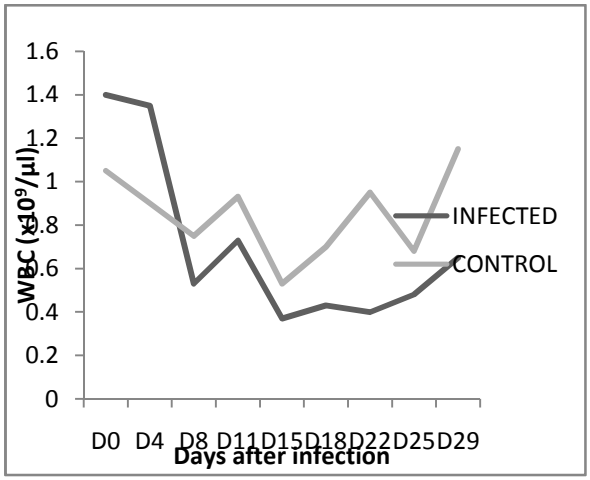


Fig 3: Variation in mean RBC counts in *T.brucei*-infected dogs

Fig4: Variation in mean daily total WBC of T. *brucei*-infected dogs

The mean total WBC count of the dogs before infection was 1.4±0.44(x10⁹/µl), and 1.05±0.77(x10⁹/µl) for the infected and control groups respectively. After infection the total WBC counts dropped to 1.35±0.44(x10⁹/µl) on day 4 (P<0.05) and plunged further to 0.53 ±0.17(x10⁹/µl) on day 8 (P<0.05) with subsequent fluctuating values slightly lower than those of the control group (P>0.05) until day 29(Fig.4). This translated to an overall mean post-infection value of 0.79±0.25(x10⁹/µl), post-infection drop of 0.61±0.15(x10⁹/µl) (43.6%), daily drop of 0.02±0.14(x10⁹/µl), and drop per LEV of 0.22±0.44 (x10⁹/µl),. The Control values fluctuated between 1.15±0.28 (x10⁹/µl), and 0.53±0.04 (x10⁹/µl), within this period.

The absolute differential count values of *T. brucei* infected and control dogs is shown on Table 2. The lymphocyte values of infected and control dogs on Day 0 was 0.57±0.15(x10⁹/µl) and 0.56±0.09(x10⁹/µl) respectively. After infection, there was an apparent increase in the infected group to 0.72±0.10(x10⁹/µl) (P>0.05) on Day 4 but thereafter plumped to fluctuating values below those of Control group until Day 29(P>0.05). The neutrophil values of infected and control groups were 0.44±0.07(x10⁹/µl) and 0.31±0.09(x10⁹/µl) respectively. After infection, the neutrophil values of infected group dropped progressively to 0.21±0.06(x10⁹/µl), 0.18±0.07(x10⁹/µl), and 0.10±0.06(x10⁹/µl) on Days 8, 11 and 15 respectively (P<0.05), but thereafter showed apparent fluctuating improvement to 0.38±0.09(x10⁹/µl) on Day 29. After infection, the eosinophil counts in the infected group increased from the pre-infection value of 0.02±0.00(x10⁹/µl) to 0.05±0.01(x10⁹/µl), on Day 4 and to 0.03±0.00 (x10⁹/µl) on Days 18 and 25 respectively (P<0.05). The Eosinophil values of Control group ranged from 0.00 ±0.00 to 0.02±0.00(x10⁹/µl) within the period. The post-infection monocyte counts increased from the pre-infection value of 0.02±0.00(x10⁹/µl to 0.04 ±0.01(x10⁹/µl) and 0.05 ±0.01(x10⁹/µl) on Days 11 and 15 respectively which was statistically significant (P<0.05). Values of the Control group ranged from 0.03±0.00 to 0.00±0.00(x10⁹/µl) within the period.

TABLE 2: MEAN DIFFERENTIAL ABSOLUTE LEUKOCYTE VALUES OF YOUNG DOGS INFECTED WITH *T.BRUCEI*.

Leukocyte Parameter	0	4	8	11	15	18	25	27
	Days of Parasitaemia							
Lymphocytes (x10⁹/L)	0.57±0.15 (0.56±0.09)x	0.72±0.10 (0.69±0.15)	0.24±0.07 (0.25±0.15)	0.48±0.11 (0.30±0.09)	0.20±0.05 (0.25±0.06)	0.19±0.07 (0.28±0.09)	0.24±0.07 (0.39±0.10)	0.22±0.09 (0.29±0.07)
Neutrophils (x10⁹/L)	0.44±0.07 (0.31±0,09)	0.27±0.10 (0.34±0.08)	0.21±0.06 (0.49±0.10)	0.18±0.07 (0.59±0.09)	0.10±0.06 (0.39±0.09)	0.20±0.04 (0.39±0.08)	0.19±0.06 (0.34±0.10)	0.38±0.08 (0.36±0.09)
Eosinophils(x10⁹/L).	0.02±0.00 (0.02±0.00)	0.05±0.01 (0.02±0.00)	0.02±0.00 (0.00±0,00)	0.02±0.00 (0.00±0.00)	0.02±0.00 (0.00±0.00)	0.03±0.00 (0.01±0.00)	0.03±0.00 (0.01±0.00)	0.02±0.00 (0.02±0.00)
Monocyte (x10⁹/L)	0.02±0.00 (0.01±0.00)	0.11±0.09 (0.01±0.00)	0.02±0.00 (0.00±0.00)	0.04±0.01 (0.03±0.00)	0.05±0.01 (0.01±0.00)	0.03±0.00 (0.03±0.01)	0.01±0.00 (0.02±0.00)	0.03±0.00 (0.02±0.00)
Besophils (x10⁹/L)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00 ±0.00)	0.00±0.00 (0.00±0.00)	0.00±0.00 (0.00±0.00)

Note : x- control values in brackets.

DISCUSSION

The Federe strain of *T. brucei* used in this study was pathogenic to the Nigerian local puppies even though they were expected to exhibit tolerance to the infection as a consequence of genetically determined phenomenon(3) and passive maternal antibodies(21,22). This is at variance with our observations in an earlier study on Nigerian local puppies infected with *Trypanosoma congolense* (23) in which the young dogs did not develop anaemia until 4 weeks later. After infection, parasitaemia developed 4days later. Although this is consistent with most experimental infections due to *T. brucei* sub species (4, 24), the parasitaemic peaks attained in *T. brucei*-infected puppies occurred much earlier and with shorter intervals compared to the observations in *T. brucei* infected ewes (25). This points the higher pathogenicity antigenic properties of this strain of trypanosome resulting to severer disease course in the dogs.

The erythrocyte values in *T. brucei* infected dogs varied with the level of parasitaemia as they returned to or near pre-infection values when parasitaemia waves were low and then plumped when parasitaemia was high. The pre-infection PCV was 29.5±4.5(%) but by the 4th day P.I. which coincided with parasitaemia onset , dropped to 22.3±4.5(%) and then further to 15.3±3.3(%) by the 11th day , two days after which puppy No.4 died. The PCV fluctuated with parasitaemia until the 29th day when the study was terminated. In the previous work of Abenga *et al;* (12), *T. congolense* infected Nigerian puppies died at 15% PCV while others were unable to walk. The low PCV values occurring concomitantly with the peak parasitaemia in this study may be attributed to the hemolysis arising from the activities of the parasite (1). Although several factors such as, immunological mechanisms, hemolytic factors, adherence of trypanosomes to red blood cells, RBC fragmentation, high body temperature and hyperactivity of the mononuclear phagocyte system (1, 26) had been associated with events leading to anemia in

trypanosomosis. Advances in research on sialic acids had given credence to the fact that sialidases produced by trypanosomes play leading roles in hemolysis of erythrocytes and pathogenesis of trypanosome induced anemia through cleavage of erythrocyte surface sialic acid thereby leading to RBC senescence and destruction through erythrophagocytosis and development of anemia (27, 28). This was demonstrated by increase in serum sialic acid concentration following cleavage of erythrocyte surface sialic acid and subsequent development of anemia in *T. vivax* infected cattle (27). The slight recovery in erythrocyte values observed between times of high parasitaemia was probably due to compensatory erythropoietic hyperplasia which is often evidenced by reticulocytosis, normoblastaemia, macrocytosis and erythroid hyperplasia of the bone marrow (1).

The hematological derangement patterns are seldom reported in African trypanosomiasis even though they have relevance in assessing sialidase activities of infecting trypanosomes and trypanotolerance. Earlier works (29, 30) had demonstrated that erythrocyte surface sialic acid concentration of trypanotolerant N'dama breed of cattle were five times higher than that of trypanosusceptible Zebu breed. In this study, the overall post infection drop in the PCV of the infected puppies was 36.0% with average daily drop of 0.37% and an overall drop of 3.82% per parasitaemia LEV. The overall post infection percentage drop in RBC counts was 19.8% with an average daily drop of 0.02 ($x10^{12}/\mu l$) and an overall drop of 0.20 ($x10^{12}/\mu l$) per parasitaemia LEV. These drop rates logically represent a measure of sialidase activities of the infecting trypanosome which is specie dependent. This is consistent with responsive anemia in African trypanosomiasis (1, 31).

Although decreases in the total WBC counts of infected dogs did not differ significantly from those of the control group ($P>0.05$), they were observable. This differs widely from our observations on the same parasite strain in pigs leading to overwhelming lymphocytic leucocytosis throughout the observation period (13). The percentage overall mean post infection total WBC drop in the dogs was 43.6% with the average mean daily drop of 0.02 ($x10^{9}/\mu l$) and overall drop per parasitaemic LEV of 0.20 ($x10^{9}/\mu l$) (Table 1). Although leucopenia occurs in experimental and naturally occurring *T. brucei* infections (Anosa 1988), leucocytosis freguently occurs(13, 32) depending on parasite antigenic properties. Leucopenia observed in the infected puppies may have arisen from massive peripheral utilization, phagocytosis in the bone marrow and other organs such as liver and spleen as well as general depression of granulopoiesis which had been identified as some of the common causes of leucopenia in African trypanosomiasis (8). Although literature on the role trypanosome sialidase activities in the pathophysiology of leucopenia in trypanosomiasis is lacking, it suffices to assume, their roles here may be similar to those earlier described(27, 28), thereby leading to the cleavage of leukocyte surface sialic acids, senescence and phagocytosis by macrophages in tissues. This being the case, rates of leukocyte loss observed in the *T. brucei* infected dogs may be a reflection of sialidase antigenic property of the parasite strain. This was characterized by, a sporadic lymphocytosis on Day 4 and then lymphopenia, neutropenia, eosinophilia and monocytosis. Whereas

Neutropenia is believed to arise from bone marrow granulocyte hypoplasia as a result of significant depression of precursor cells in early part of trypanosome infection (1), as well as phagocytosis of these cells and their precursors in the bone marrow and elsewhere (33), initial lymphocytosis followed later by lymphocytopenia, is believed to result from trypanosome antigenic challenge leading to an increased proliferation of immunocompetent cells into antibody and or lymphokine producing cells(34) but followed later by the depletion of the earlier hyperplastic lymphoid follicles and germinal centers, resulting to ultimate lymphopenia (35). This phenomenon may have played out in the *T. brucei* infected dogs. Eosinophilia and monocytosis are common features of African trypanosomiasis. Monocytosis had been reported to be matched by proliferation of macrophages in several tissues in trypanosome infected animals whose proliferation and activation are believed to be stimulated by increased demands to remove particulate matter, including trypanosomes, red blood cells, leukocytes and dead tissue cells (1). *T. brucei* being a tissue invasive parasite is therefore likely to generate monocyte in circulation as demonstrated in this study. We (36, 37) had earlier demonstrated monocyosis in *T. brucei gambiense* infection of rabbits and vervet monkeys respectively. Eosinophilia on the other hand is a response to massive inflammation in tissues (38) in the *T. brucei* infected puppies.

On the whole, the overall post infection percentage drop in leukocyte values was slightly higher than those of erythrocytes. This suggests that leukocyte loss outweighed erythrocyte loss in *T. brucei* infected young dogs, and that, immunosupression may be one of the vital antigenic properties that has placed the Federe strain above other strains of *T. brucei* in Nigeria in terms of pathogenicity. Although the trypanosome sialidase and sialyltransferase activities

were not investigated here, since it has been speculated that resialylation (putting back of sialic acids) of erythrocyte surface by the enzyme Sialytransferase occurs in *T. brucei* infected hosts, thereby resulting to self- cure from anemia (27), it suffices to assume this phenomenon was responsible for the relatively lower post infection percentage drop in the erythrocyte values of the puppies. This being the case, this study poses four important research questions; firstly, what is the leukocyte surface sialic acid concentration in comparison to that of erythrocytes?, secondly, do sialidases play any roles in the pathogenesis of leucopenia in African trypanosomiasis?, thirdly is there any sialyl transferase preferential activities that cause self-cure in *T. brucei* induced anaemia but not leucopenia?, and fourthly, are there leukocyte surface molecules that antagonize resialylation in trypanosome infections?

It is concluded that *Trypanosoma brucei* caused anaemia, which began in the first week post infection but fluctuated throughout the observation period, signifying the susceptibility of the young local dogs to the infection. The overall higher post infection leukocyte drop or loss compared to erythrocyte which raises fundamental questions on the differential surface sialic acid content of erythrocytes and leukocytes and roles played by trypanosome sialidase in creating the difference. The patterns of haematological derangement demonstrated as erythrocyte and leukocyte drop (loss) rates and drop per parasitaemia Log Equivalent Values could serve as simple means of comparing susceptibility to *T. brucei* and other trypanosome species. Trypanotorelance would in this case be expressed as lesser numbers of erythrocyte or leucocyte loss per parasitaemia log Equivalent Value.

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REVIEW ARTICLE

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SPECTRUM OF ASPERGILLOSIS: PATHOGENESIS, RISK FACTORS AND MANAGEMENT

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ABSTRACT

This article reviews comprehensively the spectrum of diseases (aspergillosis) caused by *Aspergillus spp*, the commonest pathogenic form being the *A.fumigatus*. *Aspergilus spp* are ubiguitous in the environment and the respiratory tract is the portal of entry in most cases. Aspergillosis is associated with significant mortality and morbidity, the prevalence appears to be on the increase. About 10million people are at risk of aspergillosis, and 50% would die even with treatment. Immunodeficiency, especially neutropenia is central to the pathogenesis of aspergillosis. Diseases caused by *A. fumigatus* include;1) Invasive aspergillosis seen mostly in stem cell and organ transplant recipients, patients with haematological malignancies, cancer patients on chemotherapy and patients with AIDS. Invasive aspergillosis is life threatening, it affects the lungs and sinuses but could disseminate to affect the CNS, eye, skin and kidney. 2) Chronic pulmonary Aspergillosis occurs in the setting of previous cavitatry lung disease, most commonly tuberculous infections. 3) Allergic bronchopulmonary aspergillosis (ABPA) affects people with asthma and cystic fibrosis. *A. fumigatus* is also implicated in the exacerbation of asthma. The clinical symptoms of aspergillosis depend on the type and the systems affected; respiratory symptoms are more common as the respiratory tract is disproportionately affected in aspergillosis. Diagnostic features and treatment also depends on the type of aspergillosis. Diagnostic testing for aspergillosis includes radiologic tests, culture tests, galactomannan testing in body fluids, immunologic tests to detect*Aspergilus* -specific immunoglobulins. Treatment modalities include surgery, use of antifungals and immunomodulatory therapy with cytokines.

SPECTRE DE L'ASPERGILLOSE: PATHOGENESE, LES FACTEURS DE RISQUES ET LA GESTION.

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RÉSUMÉ

Ce document, comprehensivement, fait un compte rendu du spectre des maladies (aspergillose) causées par *Aspergillus spp.*,la plus courante de la forme pathogénique était le *A. fumigatus* . *L'Aspergillus spp*estomniprésent dans l'environnement et dans la plupart des cas, les voies respiratoires sont les portails d'entrée. L'aspergillose est associe à un significatif de mortalités et morbidité, la prévalenceparait d'être en augmentation. A peu près 10 million des personnes sont en danger d'aspergillose et 50% mourrait même avec le traitement, déficience immunologique en particulierneutropénie est centrale àla pathogenèse de l'aspergillose. Les maladies causées par *A. fumigatus*comprennent :

1. **L'aspergillose invasive qui est trouvé principalement dans les cellules souches et des receveurs de greffes d'organes, les maladies avec les magnites hématologiques, les patients cancéreux sous chimiothérapie et les patients atteints du SIDA. L'aspergillose invasive est dangereuse pour la vie. Il affecte les poumons et les sinus, mais pourrait diffuser à affecter le système nerveux central, l'œil, la peau et le rein.**
2. **L'aspergillose pulmonaire chronique se produit dans le cadre de la malade de poumon précédente, fréquemment les infections tuberculeuses.**
3. **L'aspergillose broncho – pulmonaire allergique (ABPA) affecte les gens atteints d'asthme et mucoviscidose. *L'A.fumigatus* est aussi compris dans l'exacerbation d'asthme.**

Le symptôme clinique d'aspergillose dépend du type et les systèmesaffectés ; les symptômes respiratoires sont plus courants la voie respiratoire est affectée de manière disproportionnée dans l'aspergillose. Les caractéristiques diagnostiques et traitement dépendent aussi du type d'aspergillose. L'analyse diagnostique pour l'aspergillose comprend les tests radiologiques, testes culture, le test galactomannan pour fluides organiques, testes immunologique pour détecter

***l'Aspergillus* – immunoglobuline spécifique. Les modalités de traitement comprennent la chirurgie, l'usage des antifongiques et la thérapie avec des cytokines immuno modulatrices.**

1. INTRODUCTION

Fungal infections have become very prevalent with associated increase in mortality and morbidity. This is especially so for life-threatening invasive fungal infections as a result of increase in immunodeficiency disorders such acquired immune deficiency syndrome (AIDS), cancer and cancer treatment, and immunosuppressive therapy following transplantation. The result of these is an increased risk of invasive aspergillosis which has a mortality of 30% even with treatment [1,2]. *Aspergillus* also complicates other chronic medical conditions; allergic bronchopulmonary aspergillosis affects people with asthma and cystic fibrosis while chronic pulmonary aspergillosis occurs in the setting of previous tuberculosis infection.

Aspergilli are saprophytic fungi found worldwide in soil and decomposing vegetable materials. There are over 350 accepted species of Aspergillus, and the commonest disease-causing species is *A.fumigatus,* followed by the *A. flavus.* Other disease-causing species include *A. amstelodani,A. terreus,A. niger,A. avenaceus*, and *A.nidulans* [3]. *A. fumigatus* is fast growing and sporulates abundantly, and the spores or conidia are released into the environment. The conidia is small(about 2-3um in diameter) , and can withstand extreme atmospheric condition because of their outer protein coat being hydrophobic. The fungus is thermophilic; it can grow at temperatures of up to 77^0C, but grows best at ~ 37^0C.

The respiratory system is disproportionately affected by deep-seated fungal infections. *A. fumigatus* is the most prevalent airborne fungal pathogen[4]. *Aspergillus* spp. cause a wide spectrum of pulmonary infections including acute invasive, chronic and allergic, as well as implantation disease such as fungal keratitis. This review highlightsthe pathogenesis, risk factors, clinical features, diagnosis and treatment of diseases caused by *A. fumigatus* as reported in literature.

2. VIRULENCE FACTORS OF *A.FUMIGATUS*

The ability of fungi to cause disease and their virulence factors are borne out of strategies to overcome and survive in the harsh environment of the host. Primary pathogens cause disease in immunocompetent hosts; A. *fumigatus* is an opportunistic pathogenand cause disease in immunocompromised persons. This distinction however, is not clear cut, as primary pathogens such as *C. immitis* may cause virulent disease in immunocompromised persons and opportunistic fungi such as*C. neoformans* may occasionally cause disease in immunocompetent persons.

*A .fumigatus*is able to cause disease by a number of virulence factors. These factors include structures (adhesins) that enable them to adhere to tissues so as to avoid being cleared or swept away by ciliary movement or mucous. Conidia of *A.fumigatus* are covered with hydrophobic proteins known as rodlets. These rodlet proteins are encoded for by RODA and RODB genes and, mediate adhesion of the conidia to albumin and collagen. Receptors on the surface of hyphae include galactomannan and chitin of *A. fumigatus* which mediate adhesion to complement, fibrinogen, immunoglobulin, and surfactant A and D[4]. Ability to grow at elevated temperature is another virulence factor. Fungi that cause systemic infections are able to grow at body temperature and even at febrile temperatures of 38-42^0C. *A. fumigatus* is particularly thermophilic, and can grow at temperatures of up to 55-77^0C[5]. HSP 70 is thought to be required by fungi to adapt to high temperatures [6].

A.fumigatus secretes proteases (serine and aspartic protease, metalloprotease) and phospholipases which degrade elastin present in lung tissue. The serine proteases degrade collagen, fibrin and fibrinogen [7]. Production and release of degradative enzymes enable them to establish disease and disseminate, and also protects from the effects of oxidation. *A. fumigatus* produces three catalases; Cat- A associated with conidia, and Cat 1p and Cat 2p associated with hyphae [4] as well as superoxide dismutases (containing Mn, Cu and Zn) that protect it from oxidative damage [8].Melanin is also synthesised by *A. fumigatus* from acetate using a 6 genes pathway [4].Melanin protects against harsh conditions and ROS [8].The ability to obtain Fe from the storage or transport forms in the host is another virulence factor.*A. fumigatus* uses three mechanisms of Fe uptake; reductase Fe uptake, siderophore-mediated Fe uptake and ferrous Fe uptake mechanisms [9]. *A. fumigatus* secretes a number of toxins such as aflatoxin and gliotoxin. Aflatoxin does not have any bearing on virulence of *A. fumigatus*, it is hepatotoxic and carcinogenic. Gliotoxin is immunosuppressive and inhibits phagocytosis by macrophages and T-cell activation [7]. It also slows ciliary movement thus making it difficult for the fungal cells to be swept away, and causes damage to the epithelia [10]. Calcineurin acts as a sensor for*A. fumigatus*, it is said to influence the expression of several virulence factors [11].

TABLE I: VIRULENCE FACTORS OF PATHOGENIC ASPERGILUS SPP.

VIRULENCE FACTOR	ROLE IN PATHOGENICITY	REFERENCE
Galactomannan, Chitin , Rodlet	Adhesion	4
Proteases and Phospholipases	Degradation of elastin in lung tissue and tissue damage	7
Catalases and SOD	Protection from oxidative damage	4,8
Melanin	Protection from oxidative damage and harsh conditions	4,8
Fe-siderophores (Sid-A genes)	Fe uptake and growth.	9
Gliotoxin	Immunosuppression	7,10
Calcineurin CNAA-gene	Growth of fungi and tissue invasion	11
Ability to grow at 37-42^{0}C	Survival in host tissue, ability to cause systemic infection	5,6

3. DISEASES CAUSED BY *ASPERGILLUSSPECIES* (ASPERGILLOSIS)

The portal of entry in most cases is the respiratory tract. Humans inhale conidia in the environment which can get to the lung alveoli because of their small size and hydrophobic coat (aerodynamics) [12]. However, most immunocompetent persons are able to eliminate the spores by innate immune mechanisms. Those who are at great risk of developing disease are people with immune defects such as patients with neutropenia, and people with neutrophil and macrophage dysfunction. Other susceptible hosts are patients on chemotherapy and long term corticosteroid therapy, patients with haematological malignancies such as leukaemia, bone marrow transplant recipients, solid organ transplant recipients and AIDS patients. Apart from immunosuppression, steroids have been shown to accelerate the growth rate and doubling time of *A. fumigatus* [3]. The diseases caused by *A. fumigatus* are invasive aspergillosis, chronic pulmonary Aspergillosis (including aspergilloma), and allergic bronchopulmonary aspergillosis (ABPA). *A. fumigatus* is also implicated in the exacerbation of asthma [13].

3.1CHRONIC PULMONARY ASPERGILLOSIS (CPA)

CPAis also known as pulmonary aspergillosis with cavities. CPA occurs in immunocompetent people who have had previous cavitatry lung diseases, with tuberculosis being the underlying disease in most cases[14, 15]. About 30-44% of patients with CPA had underlying TB [15, 16]. Other predisposing diseases are sarcoidosis [17], bronchiectasis, pulmonary infarcts, lung abscesses, bronchial cysts [18], ABPA, emphysema, prior treated lung CA [16] and cavities formed by other fungal infections [19, 20]. CPA occurs in different forms; *chronic cavitatry pulmonary aspergillosis* (CCPA), *chronic fibrotic pulmonary aspergillosis* (CFPA), and *pulmonary aspergilloma*. *Chronic necrotising pulmonary aspergillosis* (CNPA) involves hyphal invasion of tissues and is thus considered as a sub-acute form of IPA, it occurs in people with mild impairment of immunity such as defects in mannose binding lectin (MBL), diabetes mellitus (DM) and corticosteroid use[21, 22]. Patients with CCPA have multiple cavities which increase over time by expansion or formation of new cavities. Pulmonary aspergilloma occurs as fungal balls in CCPA. CFPA is the end result of CNPA or more commonly untreated CCPA, there is marked fibrotic reaction within the cavities [21].

In pulmonary aspergilloma, *A. fumigatus* over grows on the surface of these cavities forming a spheroidal mass of hyphae with inflammatory cells, fibrin, mucous and tissue debris. These appear as intracavitatry spherical structures with a surrounding area of translucency on radiographs [18]. Complex pulmonary aspergilloma may be synonymous with CCPA, though not all CCPA contain fungal balls [21]. Simple pulmonary aspergilloma occur as isolated thin-walled cysts in persons who do not have any underlying lung disease, this makes up about 18% of pulmonary aspergilloma cases [14]. *A. fumigatus* colonising the bronchial tree is said to secrete digestive enzymes which creates space for the fungal ball to grow [23].

The commonly affected site is the upper lobe, and some patients may be asymptomatic with the disease picked up during routine chest radiograph. It frequently affects middle-aged people with a predominance of males [14, 21]. However, symptomatic persons present frequently with haemoptysis, with bleeding from the bronchial arteries that is usually self-limiting [24]. Local invasion of these blood vessels or the release of endotoxin or trypsin-like proteolytic enzymes is said to cause haemoptysis [18]. Recurrent large volume haemoptysis is associated with poor outcome. Other symptoms are cough, dyspnoea, chest pain, malaise, shortness of breath, and weight loss [21]. Diagnostic features include radiologic demonstration of 1 or more cavities and/or fungal balls, precipitating antibodies to *A. fumigatus*, and positive culture tests.

The outcome of patients with CPA depends on the presence and severity of underlying lung condition, the fungal balls do not respond to antifungals. Amphotericin B and itraconazole show some benefits and IFN-γ is used as an adjunct [21]. Increasing *Aspergillus*-specific IgG titre, size and number of lesions are associated with poor outcome [18]. Treatment is thus surgical involving lobectomy or resection [15]; however, there could be recurrence [14] and associated post-operative complications such as pleural aspergillosis, fistula, respiratory failure or disseminated disease [15, 25-27]. The annual mortality rate of CPA is 15% (range 5-25%) and patients usually die from respiratory failure or pneumonia [18]. Factors which have been associated with poor prognosis include severe underlying lung disease and recurrent haemoptysis [14].

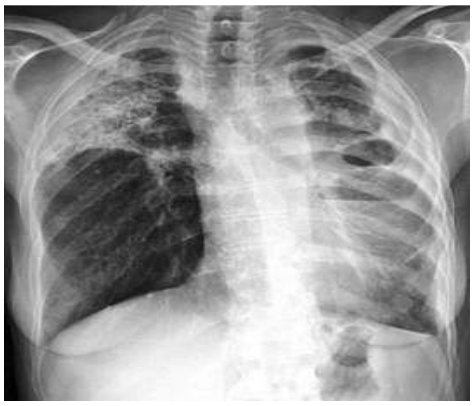

FIGURE I CHRONIC PULMONARY ASPERGILLOSIS Severe bilateral chronic pulmonary aspergillosis with the left upper lobe replaced by one large and several smaller cavities and a fluid level (which on aspiration grew a pure growth of Aspergillusfumigatus). There is also extensive disease of the right upper lobe with a more consolidation-type appearance, but containing multiple small cavities.© LIFE @http://life-worldwide.org

3.2ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

ABPA is a severe allergic pulmonary disease seen commonly in patients with cystic fibrosis and asthma, with an incidence estimated to be about 2.5% in adults with asthma from five referral cohorts[28, 29]. It is a hypersensitivity reaction to *Aspergillus* antigens, about 28% of asthmatic are shown to be sensitised to the antigens from studies done[30,31]. Sensitisation to *Aspergillus* antigen apart from increasing the risk for ABPA increases the severity of asthma in this group of people. Among patients with cystic fibrosis the presence of atopy predisposes to the development of ABPA [32]. ABPA has also been described in people with allergic fungal sinusitis [28], chronic granulomatous disease and hyper IgE syndrome [34].

Central to the pathogenesis of ABPA is an IgE-mediated hypersensitivity reaction and specific IgG-mediated type III hypersensitivity reaction[35]. Impaired mucociliary action in CF, and inflammation in the airways in asthma makes the inhaled *Aspergillus* allergens to persist. *Aspergillus* attaches and grows on the bronchial epithelial cells, producing allergens including several proteases and these proteases detach the epithelial cells and elicit release of pro-inflammatory mediators [36]. These mediators cause damage to the airways over years resulting in bronchiectasis, and recruitment of inflammatory cells [37]. There is also a specific Th-2 CD4+ response seen in people with ABPA [38]. There seem to be some genetic predisposition to ABPA as familial clustering has been shown amongst asthmatics and cystic fibrosis patient, about 5% of ABPA patients showed familial clustering in one study [39,40]. The cystic fibrosis Trans membrane regulator (CFTR) gene is also thought to have an etiologic role in ABPA in cystic fibrosis patients[41,42].

ABPA presents clinically as worse asthma with wheezing, pleuritic chest pain, fever, and expectoration of brown mucus plugs [43]. Diagnostic criteria of ABPA in asthmatics are increased levels of allergen specific IgE and total serum IgE (>417kIU/L), pulmonary infiltrates on chest radiograph, and proximal bronchiectasis. This group of ABPA patients are known as ABPA – central bronchiectasis. Most ABPA patients also have a positive skin reactivity test to *Aspergillus* antigens, increased serum specific IgG antibodies and eosinophilia [43, 44]. A second diagnostic group of ABPA patients has all features except central bronchiectasis; this group is known as ABPA-seropositive. The diagnostic criteria for ABPA in cystic fibrosis is slightly different, a worsening of clinical condition is one of them, central bronchiectasis is not a criterion and the total serum IgE concentration should be > 1000kIU/L [44].

There are five stages described for ABPA based on radiographic infiltrates and total serum IgE; stage I is the acute stage with infiltrates in the upper or lower lobe involvement and a markedly elevated IgE. Stage II is a remission stage without infiltrates and a normal or elevated IgE, while stage III is an exacerbation stage with features same as the acute phase. Patients with infiltrates could also be in stage IV when they have corticosteroid –dependent asthma, and stage V is the end stage with fibrotic or cavitatry lesions [45]. Both oral and inhaled steroids are used to reduce the inflammation in ABPA[44]. Antifungals such as itraconazole are given concomitantly to eradicate fungal growth in the

lung and minimise the production of allergens, reducing inflammation, and risk of invasive aspergillosis [46,47].

3.3SEVERE ASTHMA AND *A. FUMIGATUS*

Some patients with asthma tend to have more severe symptoms than others, this is characterised by prolonged hospital stay and increased use of bronchodilators. Fungal sensitisation has been linked to severe asthma, although many fungal allergens may cause this, *Aspergillus* allergens are important in the exacerbation of asthma [13,48]. *A. fumigatus* is a major indoor aeroallergen [49], with proteases which are implicated in the hypersensitivity reactions in the lung [50]. This *Aspergillus* protease has also been shown to cause damage to the airway epithelium, and release of inflammatory cytokines-IL6 and IL8 [51]. *Aspergillus* specific Serum IgG antibody level which is a diagnostic feature for aspergillosis was found to be associated with severe asthma [52].

3.4 INVASIVE ASPERGILLOSIS

Invasive aspergillosis (IA) commonly affects people with neutropenia with the risk for IA more after the third week of the neutropenia. It is seen in immunosuppressed people such as those on chemotherapy and/or long-term steroids, recipients of bone marrow transplant or solid organ transplant, with those who receive heart and lung transplant at an increased risk, and patients with hematologic malignancies such as leukaemia. IA is also common in persons with congenital immunodeficiency disorders such as CGD and acquired immunodeficiency disorders [53-55]. It is rare in immunocompetent hosts. Invasive aspergillosis is responsible for 30% of fungal infections seen in cancer patients [56]. Despite treatment, the mortality rate of IA was 75-90% in leukaemia patients in the 1980s and 1990's but has now fallen to ~30% [1,2] and up to 25% of leukaemia patients develop IA [57-59]. This makes it a major cause of death in leukaemia patients, and also in bone marrow or organ transplant recipients.

There are four groups of IA; acute or sub-acute invasive pulmonary aspergillosis, tracheobronchitis and obstructive bronchial disease, invasive Aspergillus sinusitis, and disseminated disease. These occur almost exclusively in immunocompromised patients, with the portal of entry being the respiratory tract. Invasive aspergillosis can also occur by direct invasion of wounds and burns on skin, the cornea (keratitis) or by association with in-dwelling catheters [3, 54]

Invasive pulmonary aspergillosis (IPA) is the most common type of IA (80-90%) [3], the acute type being more common than the sub-acute type. There are two pathologic entities of IPA; angio-invasive or non-angio-invasive. The angio-invasive is seen in neutropenic patients and manifests as vascular invasion by hyphal elements with coagulative necrosis and haemorrhage. While in non-angio-invasive IPA seen in non-neutropenic patients, there is a pyogranulomatous inflammation and necrosis without evidence of vascular invasion [60]. IPA has been increasing in incidence, with a prevalence of about 56% of all invasive mycoses found on autopsy[57, 61]. Neutropenia is a strong risk factor, the degree and duration contributing greatly to the risk of developing IPA [53]. Patients with leukaemia have neutropenia, while those with CGD have dysfunctional neutrophils. Neutropenia, immunosuppression and prolonged hospital stay make bone marrow transplant recipients at great risk; about 5% of bone marrow recipients develop IPA and this risk is higher with allogeneic stem cell transplantation[59, 62, 63]. Solid organ transplantation especially, that of heart and lung carries a high risk for IPA, with an incidence of 19-26% [59, 63].

Sub-acute IPA occurs in people with AIDS, CGD [64], and in apparently immunocompetent persons especially those with chronic obstructive pulmonary disease (COPD) [65]. Low CD4 count (<50 cells/mm3), co-existing neutropenia and steroid therapy are associated risk factors for AIDS patients [66, 67]. COPD patients are susceptible because of long term therapy with steroids and other factors resulting from their treatment and hospital stay [68]. Steroids impair the phagocytic functions of neutrophils and macrophages [3]. Co-morbidities such as DM, alcoholism, malnutrition and asthma also predispose COPD patients to sub-acute IPA [69].

The symptoms of IPA depend on the immune status of the host; immunocompetent individuals have more prominent symptoms which may last over weeks or months, while immunocompromised persons tend to have less symptoms but rapid progression of disease. Symptoms are non-specific; cough dyspnoea, pleuritic chest pain, haemoptysis and fever that do not subside with antibiotics use. Fever is however absent in patients on corticosteroid therapy, and patients with chronic IPA also have malaise and weight loss [51]. AIDS patients with IPA have an increased incidence of tracheobronchial involvement in addition to the symptoms described [67,70].

Early diagnosis and prompt use of antifungal agents such as amphotericin B or voriconazole could reduce mortality rate. However, rapid diagnosis is difficult and there are treatment limitations with amphotericin B and voriconazole due to toxicities [71, 72]. Chest radiograph in the early stages of the disease is usually non-specific;

the hallmark of diagnosis is histological examination of lung tissue [73]. Detection of *Aspergillus* antigens such as galactomannan in body fluids can diagnose the disease even before the appearance of clinical signs and symptoms [74]. Bronchoscopy and bronchoaveolar lavage (BAL) is done to obtain fluids for culture and detection of Aspergillus antigens and PCR[60,75], especially in patients with diffuse lung involvement [69]. Prognosis of IPA also depends on removal of the underlying defects, for example, restoration of neutrophil counts and function. Immunomodulatory therapy such as colony-stimulating factors and interferon-γ could be used as adjuvant therapy; IFN-γ was shown to accelerate cure without clinical toxicity in renal transplant recipients with IPA [76, 77].

Aspergillus tracheobronchitis is isolated to the tracheobronchial tree. The risk factors are same as IPA, but tracheobronchitis is more common in AIDS patients [67, 70] and lung transplant recipients [78]. In about a quarter of patients, no apparent immunosuppression is observed [70].There are three forms of the disease; an obstructive type with limited inflammation but with production of thick mucus plugs full of *Aspergillus*, the ulcerative type which affects a limited area of the tracheobronchial tree and is common in recipients of lung transplant, and the pseudomembranous type with extensive inflammation of the membranes [79] .Most patients present with symptoms of cough, fever, chest pain, dyspnoea and haemoptysis[3, 69].

Mortality is high (78%) especially in the pseudomembranous and obstructive types, patients die from respiratory failure[3, 80]. Dissemination or tracheal perforation may complicate the disease [3][6]. Diagnosis is based on characteristic findings on bronchoscopy and microscopic demonstration of the fungus from respiratory specimens. Outcome is good with antifungal therapy, especially with the ulcerative type [69].

Invasive Aspergillus sinusitis may manifest as acute rhinosinusitis, chronic sinusitis or as a paranasal Aspergillus granuloma[3]. It is very uncommon in patients who receive solid organ transplants, but the *acute rhinosinusitis* is common in bone marrow transplant recipients, and patients with neutropenia [81,82]. *A. flavus* is implicated more often in invasive Aspergillus sinusitis [83]. Common sites affected are the maxillary, ethmoid and mastoid sinuses. Patients present with fever, local pain, nasal discharge, epistaxis and headaches. It can occur alone or with pulmonary aspergillosis, but CNS involvement is quite common [3,84] .The disease can spread to surrounding structures such as the palate, orbit or brain, and this is usually fatal[85, 86]. Diagnosis is by demonstration of fluid opacities in the sinuses on CT-scan and a positive culture. Patients are usually managed with amphotericin B and voriconazole [3], surgery is also important [87].

Chronic Aspergillus sinusitis occurs in immunocompetent persons or those with mild suppression in immunity such as diabetics, alcoholics and people living with HIV [62, 87-89]. The mucosa and other tissues are invaded by the *Aspergillus* hyphae, and the bone may be destroyed. Symptoms include diplopia and visual impairment, headaches, and nasal stuffiness; fever is absent[3, 69]. *Aspergillus* sinusitis may be complicated by osteomyelitis [90], and brain abscess or stroke if the sphenoid sinus is involved [91]. Radiological features (similar to that seen with acute form) and positive culture are diagnostic. Treatment involves antifungal therapy and surgical debridement, the disease usually runs a chronic course and may relapse [3].

Disseminated aspergillosis involves the central nervous system in most cases but also affects organs such as the eye, kidney, skin and heart[92, 93]. About 10-40% of bone marrow transplant recipients are affected, and at autopsy 6-15% of patients who died from haematological malignancies had CNS aspergillosis. CNS aspergillosis is also common in HIV patients ; and neutropenia and steroid use are additional risk factors [93].The disease progresses rapidly with a fatal outcome, and early diagnosis is difficult.

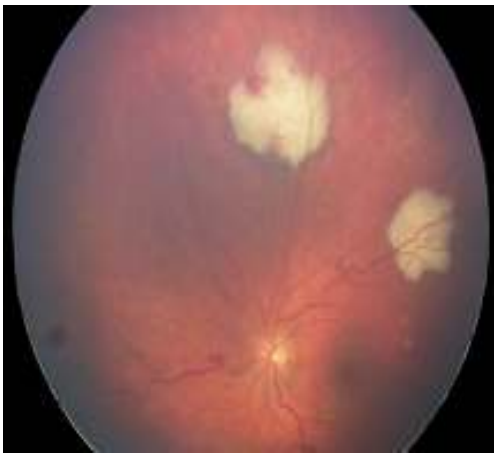

FIGURE II: ASPERGILLUSENDOPHTHALMITISAspergillusinfection of the retina of the eye following dissemination from the lung.© LIFE @http://life-worldwide.org

Cutaneous aspergillosis may occur with disseminated invasive aspergillosis, but it can also occur directly at intravenous catheter sites in neutropenic patients [3]. Cutaneous aspergillosis can also arise from an adjacent affected tissue such as the sinus. A. flavus is most often associated with

primary cutaneous aspergillosis [94]. It has been reported in diabetic patients and apparently immunocompetent persons [95].The lesions are usually distributed over areas of terminal circulation such as the limbs [96].Aspergillus can also cause dermatitis in premature new-borns [97]; it also invades burns and surgical wounds [3].

4. CONCLUSION

The global burden of aspergillosis

Aspergillosis as described are important causes of morbidity and mortality worldwide, affecting mostly the immunosuppressed. The incidences and associated mortalities of invasive aspergillosis have increased as a result of advancement in treatments, and cancers. Prolong hospital stay, frequent hospital visits, prolong duration of treatment and high costs of antifungals are all factors that make aspergillosis to exert a huge financial burden on the economy. Aspergillosis complicating other chronic medical conditions increases the morbidity and mortality from these conditions. Coupled to these is the difficulty with diagnosis especially in developing nations.

About 10million people are at risk of aspergillosis, and 50% would die even with treatment. Most invasive diseases are in stem cell and organ transplant recipients, greater than 75,000 people receive these transplants annually with about 10% at risk of developing invasive disease [98] As discussed, ABPA affects asthmatics and people with cystic fibrosis. About 4 million out of the 193 million with asthma are affected, while 15% of people with cystic fibrosis develop ABPA. Chronic pulmonary aspergillosis has a worldwide prevalence of about 3million, about a third of these cases occur in the setting of previous tuberculosis infection [16]. This is of importance as the rate of tuberculosis seems to be on the increase with AIDS especially in sub-Saharan Africa. Allergic fungal sinusitis and rhinitis may not be associated with mortality, but they affect the quality of life with significant loss of work or school days and reduced performance. They affect 12 million people at any time [99]. Fungal eye infections affect 1million people worldwide, causing about 10% of avoidable blindness [100].

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ORIGINAL ARTICLE

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PULMONARY CANDIDIASIS AND CD_4 COUNT IN HIV POSITIVE PATIENTS SEEN IN JOS, NORTH CENTRAL NIGERIA

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ABSTRACT

Background: Accurate and reliable diagnosis of HIV opportunistic infections plays a central role in effective HIV intervention programmes. Pulmonary infections are the leading cause of morbidity and mortality in HIV infected individuals.
Objectives: We set out to determine the prevalence of Pulmonary candidiasis by isolating *Candida* species from the sputum of HIV sero-positive patient's presenting to hospital with complaint of cough for more than two weeks and related the level of CD_4 count to Pulmonary candidiasis.
Methods: Using sterile wire loop, each sputum sample was inoculated into duplicate SDA (Thermo Scientific, UK); one tube without antibiotics, another tube supplemented with Chloramphenicol (0.05%) and Cycloheximide (0.5%) antibiotics. The patient's CD_4 count was determined using a Cyflow machine (PARTEC^R, Germany).
Result: Fifty two (54.2%) female and 44(45.8%) male HIV positive subjects were compared with a control group made up of, 52(54.2%) female and 44(45.8%) male HIV negative subjects. Twenty one (21.9%) HIV positive subjects had *Candida* species in their sputum samples compared to 12(12.5%) in the HIV negative subjects. Among HIV positive subjects, 17(17.7%) had *Candida albicans* isolated from their sputum, 11(11.5%) of whom had a CD_4 count of <200 cells/ul.
Conclusion: We concluded that, there is a risk of pulmonary candidiasis occurring in HIV infected patients with CD_4 count <200cells/ul and that, *Candida species* contributes to chronic cough experienced by HIV infected patients. We recommend that HIV positive patients who have chronic cough and whose CD_4 count is <200cells/ul be placed on systemic antifungal medication.

Keyword words: Candida, Cough, Nigeria, Sputum

CANDIDOSE PULMONAIRE ET NUMERATION LYMPHOCYTES CD_4 CHEZ LES PATIENTS VIH OBSERVES AU NORD – CENTRALE DE JOS, NIGERIA.

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RÉSUMÉ

CONTEXTE : Diagnosticprécis et fiable des infections opportunistes joue un rôle central dans l'efficace du programme d'intervention de VIH. Les infections pulmonaires sont les causes principales de morbidité et mortalité chez les individus infectés par le VIH.

OBJECTIFS : Nous avons cherché à déterminer la prévalence de Candidose Pulmonaire en isolant les espèces de *Candida* des expectorations des patients VIH séropositifsprésentant a l'hôpital les plaintes de toux pendant plus de deux semaines et lie le niveau de numération lymphocytes CD_4 a la Candidose Pulmonaire.

METHODES : Utilisant boucle de fil stérile, chaque échantillon d'expectoration a été inoculé en double exemplaire SDA (Thermo Scientific, UK) ; un tube sans antibiotiques, un autre tube complété d'antibiotiques Chloramphénicol (0,05%) et Cycloheximide (0,5%). La numération lymphocytes CD_4 du patient a été déterminé en utilisant un Machine CyFlow (PARTEC^R, Germany).

RESULTAT : Cinquante – deux (54,2%) sujets féminins et 44(45,8%) sujets masculins VIH positifs ont été comparés à un groupe contrôlé composé de 52(54,2%) sujets féminins et 44(45,8%) sujets masculins VIH

négatifs. Vingt et – un(21,9%) sujets VIH positifs ont eu des espèces*Candida* dans leurs échantillons d'expectoration comparativement à 12(2,5%) dans les sujets séronégatifs. Parmi les sujets séropositifs, 17 (17,7%) ont eu *Candida albicans*isolé de leur expectoration, 11(11,5%) d'entre eux ont eu la numération lymphocytes CD_4 de <200 cellules/ul.

CONCLUSION : Nous avons conclu qu'il ya une risque de Candidose Pulmonaire se produisant chez les patients infectés par le VIH de numération lymphocytes CD_4 de <200 cellules/ul et que, *Candida species* contribue à une toux chronique vécué par les patients infectés par le VIH. Nous recommandons que les patients séropositifs qui ont une toux chronique et dont la numération lymphocytes est <200 cellules/ulêtre place sur l'antifongique systématique médicament.

Mots – Clés : Candida, La toux, Nigeria, Expectoration.

INTRODUCTION

Candida species have been increasingly recognized as a source of fungal pneumonia in patients with acquired immune deficiency syndrome (1,2). Candidiasis is a common endogenous opportunistic yeast infection (3). Candidiasis may present as an acute, chronic, superficial or disseminated infection (2). It is a worldwide infection affecting all age, sex and occupational groups. Pulmonary infections are the leading causes of morbidity and mortality infections in HIV infected individuals (4,5). Pulmonary candidiasis is characterized by low grade fever, cough with mucous and sometimes bloodstained sputum as well as pleural effusion. Of the causative agents, the most common is *Candida albicans* (3). While other *Candida* yeast may occasionally cause clinical disease, *Candida albicans* is the organism isolated from most patients (6). *Candida* species are true opportunistic pathogens that exploit recent technological advances to gain access to blood circulation and deep tissues (7,8).

Soon after the Acquired Immunodeficiency Syndrome (AIDS) was first described in 1981, it became clear that opportunistic infections (OIs) occurred with remarkable frequency and caused substantial morbidity and mortality among patients with AIDS (9,10). Many of the OIs in adults are usually secondary to activation of "innocent" pathogens, which were commensals existing passively at a time when host immunity was intact, before the acquisition of HIV infection (11). Clinical AIDS is a common finding in many HIV health care facilities in Nigeria (12,13).

Among the various OIs, respiratory infections account for up to 70% of AIDS defining illnesses and their relative importance differs in different parts of the world (14,15). Respiratory OIs are a common manifestation of HIV/AIDS in Nigeria (16). Sixty percent of patients seen in the Jos University Teaching Hospital (JUTH) with AIDS presented with respiratory tract infections, ranging from sinusitis, to upper and lower respiratory tract infections (17).

A diagnosis of *Candida* pneumonia is difficult to establish in immune compromised patients (1). Making a diagnosis of candidiasis may often be difficult, but the risk factors associated with the condition are well known and are commonly found in the intensive care units (18). The presence of one or more risk factors should therefore heighten clinical suspicion (14). Clearly, a thorough understanding of the natural history of AIDS related OIs and a comprehensive analysis of the pace and quality of immune recovery in each patient is required for optimal management of this condition (15).

In tropical medical practice, chronic cough, fever and weight loss presenting in a clinic, raises a suspicion of pulmonary tuberculosis (PTB) until ruled out, given the prevalence of PTB in the environment. Therefore sputum acid fast bacilli (AFB) examinations as well as chest x-ray (CXR) are ordered for; the patient is often started on anti-tuberculosis therapy immediately. In the absence of determination of other cause, even when sputum returns negative for AFB, treatment is continued empirically anyway (17). When the response to the anti-tuberculosis treatment is poor or absent, the tendency is more likely to be to doubt the patients drug compliance, genuineness of the drugs used or suspect drug resistant PTB. Cases like this are common in tropical medical practice and pulmonary mycosis is rarely thought of as a likely cause of these symptoms (18,19).

The HIV/AIDS patients presenting with respiratory tract symptoms like cough, breathlessness and chest pain will need detailed investigations including sputum microscopy (Gram and AFB staining), culture and chest X-ray (CXR).

To date, few, if any, rigorous studies on the causes of chronic cough in African, treatment-naive, HIV infected patients have been performed (20,21). As regards the diagnosis of pneumonia, there is little information about the role of Candida species isolated from respiratory samples, and criteria for the diagnosis of Candida species pneumonia are still to be defined (22,23). Such analyses are helpful for determining the preventive medications and treatment needed for HIV- infected persons (24).

The aim of this study was to determine the prevalence of Candida species in the sputum of HIV sero-positive patients and relate their Cluster

of Differentiation (CD_4) cell count to presence or otherwise of Candida species in the subject.

MATERIALS

Patients from 18 years old who presented to JUTH ARV treatment clinic and who have been coughing for at least two weeks were referred to a TB Reference Laboratory for sputum AFB microscopy, culture and sensitivity. These were the candidates that were approached to be recruited and asked for consent to be included in this study.

At the TB reference laboratory, patients included were selected statistically, using simple random sampling method (25,26). Patients on anti-tuberculosis or anti mycoses treatment, were excluded as well as any patient who had ever smoked or is currently smoking cigarette, any who presents with pedal edema or history of heart failure. Written informed consent was obtained and it was made clear to each patient that he/she was free to opt out from the study at any time.

Sputum samples were obtained from 96 HIV sero positive patients (case) who have been coughing for at least two weeks. A marching patient based on sex and age group presenting on the same day, who is HIV negative from other clinics presenting to the TB reference laboratory, at the same period are selected as a control. Patients who later failed to produce up to three fresh sputum samples as instructed were excluded. One hundred and five patients who fulfilled the inclusion criteria were recruited for the study; the extra 9 patients were added to make room for attrition during the study. Baseline blood samples were taken for CD_4 lymphocyte count. Ethical clearance for the study was obtained from the Jos University Teaching Hospital's Ethics Committee.

Two hundred and eighty eight (96x3) sputum samples and 96 baseline blood samples for CD_4 count were obtained from these patients. Similarly 288 sputum samples were collected from HIV negative control patients who had chronic cough, fever and weight loss.

LABORATORY PROCEDURE

The patients' confidence was boasted through explanation of the reasons for the collection of the sputum. Three separate sputum specimens were collected from each patient (spot, morning, spot) (27,28). A gram stained smear was immediately prepared from each freshly produced sputum sample submitted to assess the quality of the sputum before it was accepted for the study. Then using a sterile wire loop, the sputum was inoculated into duplicate Sabouraud Dextrose agar tubes (SDA, Thermo scientific, UK); a tube without antibiotics, a tube supplemented with Chloramphenicol (0.05%) and Cycloheximide (0.5%) antibiotics. Yeast growth to be analysed is collected and emulsified in sterile water to McFarland 0.5 equivalent. The analytic profile index (API) 20C Aux (Biomerieux, France) was then used to identify the yeast encountered (29). This was guided by the accompanying manufacturers database (V4.0) – with the API looked up and the numerical profile determined from the list of provided profiles.

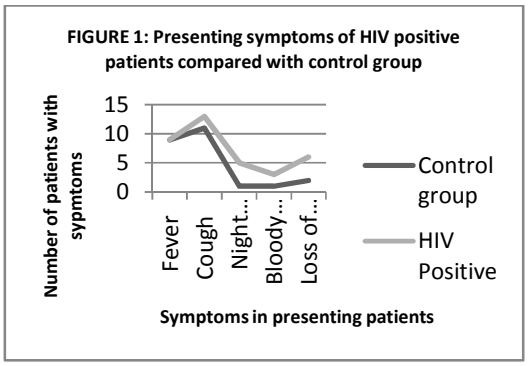
Using lavender coloured EDTA vacutainer, about 5mls of blood was withdrawn using the cubital fossa vein of each of the selected patient. The patient's CD_4 count was determined using a Cyflow machine (PARTEC^R, Germany), observing peaks generated and the count read after the run from the Cyflow monitor screen. Data was analysed using SPSS statistical package. Proportions were compared using X^2 test, differences at the 5% level being regarded as significant (25,26).

RESULTS

Fifty two (54.2%) females and 44 (45.8%) males HIV positive patients were compared with a control group made up of, 52(54.2%) females and 44(45.8%) males HIV negative patients. Among the cases 49 (50.7%) females and 34 (35.6%) males were below the age of 45 years. Above the age of 45 years, 3 (3.1%) females and 10 (10.4%) males presented. Cases between the ages of 30-39 years formed the largest age group presenting with 25 (26.0%) females and 20 (20.5%) males.

Of the cases studied, 95 (99.0%) presented with cough compared to 93 (96.9%) in the control group, 75 (78.1%) presented with fever compared to 73 (75.0%) in the control group, 6 (6.3%) with Loss of weight compared to 46 (47.9%) in the control group, 4 (4.1%) with Bloody sputum compared to 20 (20.8%) in the control group and 3 (3.1%) with Night sweats compared to 41 (42.7%) in the control group.

Twenty one (21.9%) HIV positive patient's sputum samples had *Candida* species compared to 12 (12.5%) samples in the control group. Seventeen (17.8%) patient's sputum samples out of the 96 HIV positive patient's samples had *Candida albicans* compared to 10 (10.4%) in the control group. All the 17 HIV positive patients who had *Candida albicans* isolated, from their sputum presented with cough, 69.2% presented with fever and only one patient presented with Bloody sputum (Figure 1).



Nine (9.4%) of the HIV positive patients who had Candida *albicans* isolated from their sputum were between the ages of 30-39 years, all were females. At P<0.05 these results are statistically significant. The age group of 30-39 contributed 9 (9.4%) out of 45 (46.9%) of patients from whom *Candida albicans* was isolated. This was followed by 4 (4.1%) out of 18 (18.8%) patients within the age group of 30-34 (Table 1).

TABLE 1: Distribution of *Candida albicans* isolates within the age grouping of HIV positive patients.

Age group	With Candida Total (%)	Without Candida Total (%)
15-19	0 (0.0)	0 (0.0)
20-24	3 (3.1)	10 (10.4)
25-29	3 (3.1)	12 (12.5)
30-34	4 (4.2)	13 (13.5)
35-39	5 (5.2)	21 (21.8)
40-44	0 (0.0)	11 (11.4)
45-49	2 (2.1)	7 (7.3)
50+	0 (0.0)	5 (5.2)
Total	17 (17.7)	79 (82.3)

Among the 17(17.7%) HIV positive patients who had *Candida albicans* isolated from them, 11 (11.5%) had a CD_4 count < 200 cells/ul (8 females, 3 males). At P<0.05 these results are statistically significant (Table 2).

TABLE 2: Distribution of CD_4 count by *Candida albicans* isolated among HIV positive patients

CD_4 count(Cell/ul)	*Candida albicans* isolates from Males	Females	Total (%)
<200	3	8	11 (64.7)
200-500	4	2	6 (35.3)
Total	7	10	17 (100)

$X^2 = 6.9$ d.f. = 1 P< 0.05

DISCUSSION

The study set out to determine the prevalence of Pulmonary candidiasis by isolating *Candida* species from the sputum of HIV sero-positive patient's presenting to hospital with complaints of cough for more than two weeks and relate the level of CD_4 count to Pulmonary candidiasis. There were more female HIV positive patients reporting to clinic than were male patients. The most common presenting symptom among Pulmonary Candidiasis patients was cough, closely followed by fever and loss of weight.

The main finding in this study was that HIV positive patients in Jos had *Candida* species in their sputum, compared to the HIV negative control group. This figure varies from findings of other parts of the world, where similar studies were carried out. A recognized difficulty in studies of this type is the method of specimen collection and the sterility of the sputum container used (4,7). In our study the patients were provided with sterile bottles and only the patients whose three sputum sample pairs yielded *Candida* species were accepted as positive for *Candida species*.

Our finding is much lower than the 35.5% *Candida* species isolated by Nwabuisi and Ologe in Ilorin, Nigeria (30). Castro and Martinez in 2008 detected 47.5% candidiasis in Brazil, and 60% was reported in the USA (2,14). The finding is however higher than that found in Taiwan, and India (31,32).

Fungi isolated from sputum may represent either pathogens or saprophytes (32). The prevalence and prognosis of pulmonary fungal infection has been difficult to evaluate since diagnoses was seldom confirmed (31).

This study agrees with established finding of other studies that *Candida albicans* is the most commonly isolated strain of Candida, both as a colonizer in the general population and as a pathogen in patients infected with HIV (3,4,32).

In this study the median CD_4 count among HIV positive patients was lower than found in Zaire (15). Many (64.7%) of our patients who had CD_4 count of <200cell/ul, had chronic cough and their sputum yielded *Candida albicans* (12,15). This finding in our study suggests that the severity of HIV infection is directly proportional to the degree of immune deficiency as indicated by the CD_4 count of the patient (8,11,14).

CONCLUSION

We concluded that a high number of HIV infected patients had *Candida species* isolated from their sputum and had CD_4 count <200cells/ul. This suggests that, *Candida species* contributes to chronic cough experienced by HIV infected patients and that, the most predominant Candida species isolated was *Candida albicans*.

LIMITATIONS

The limitation of the study was that only HIV positive patients who presented to the clinic were captured in the study, other HIV positive patients who were too sick were admitted directly for in-patient care from the emergency care unit.

RECOMMENDATIONS

We recommend that HIV positive patients who have chronic cough and whose CD_4 count is <200cells/ul be commenced on prophylactic systemic antifungal medications until their CD_4 count reaches 500cells/ul.

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HISTOLOGICAL AND BIOCHEMICAL MARKERS OF THE LIVER OF MALE WISTAR RATS ON ORAL ADMINISTRATION OF NEVIRAPINE SUSPENSION

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ABSTRACT

Background: Mechanism of action of nevirapine in the prophylaxis treatment and treatment of HIV-1 may involve elevations in levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and other biomarkers of liver function. This study presents the hepatotoxic effect of nevirapine suspension using animal model.

Methods: A total number of 15 male Wister rats were fed normal chow and antiretroviral drug (Nevirapine) for a period of six weeks. The liver organ of the rats were obtained and subjected to histological procedures and biochemical analysis using enzyme assay obtained from Randox Laboratories Limited, Antrim United Kingdom (BT294QY).

Results: The wistar rats showed no significant mean body weight difference when compared with the control group. However there was significant difference in the mean values of AST (77.77±3.03) and ALT (89.37±3.19) of the treated rats. Nevirapine treated rats showed significant difference in AST, ALT, and ALP in the single (77.77± 3.03, 31.80±1.73, 43.81 ±1.54) and double (89.37±3.19, 33.38±2.01, 34.64 ±1.02) doses when compared with the controls (75.14 ±2.00, 29.16±0.17, 45.44 ±1.85) respectively. Mild vascular congestion, infiltration of sinusoids by inflammatory cells, and haemorrhage were induced by nevirapine as compared with the control group showing normal vessels without congestion, normal sinusoids appearing normal without infiltration.

Conclusion: The liver histology of the rats fed with Nevirapine suspension showed diffused hepatocellular necrosis. Routine check of the drug effect is important as it provides effective life management of HIV infected individuals.

Keywords: Nevirapine, Wister rat, Hepatotoxicity, Liver, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP).

HISTOLOGIQUES ET MARQUEURS BIOCHIMIQUES DU FOIE DE RATS MALES WISTAR PAR ADMINISTRATION ORALE DE LA NEVIRAPINE SUSPENSION

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RÉSUMÉ

Contexte: Mécanisme d'action de la névirapine dans le traitement de prophylaxie et le traitement du VIH-1 peut impliquer élévations des taux d'alanine aminotransférase, aspartate aminotransférase, la phosphatase alcaline et d'autres biomarqueurs de la fonction hépatique. Cette étude présente l'effet hépatotoxiques de la suspension de la névirapine en utilisant un modèle animal.

Méthodes: Un nombre total de 15 rats mâles Wistar ont été nourris chow normal et médicament antirétroviral (névirapine) pour une période de six semaines. L'organe du foie des rats ont été obtenus et soumis à des procédures histologiques et analyse biochimique utilisant un dosage de l'enzyme obtenue à partir de Randox Laboratories Limited, Antrim Royaume-Uni (BT294QY).

Résultats: Les rats Wistar ont montré aucune différence significative de poids corporel moyen en comparaison avec le groupe témoin. Cependant il y avait de différence significative dans les valeurs moyennes d'AST (77,77 ± 3,03) et ALT (89,37 ± 3,19) des rats traités. Névirapine chez les rats traités ont montré de différence significative dans AST, ALT et ALP dans la seule (77.77 ± 3.03, 31.80 ± 1.73, 43.81 ± 1.54) et double (89.37 ± 3.19, 33.38 ± 2.01, 34.64 ± 1.02), des doses en comparaison avec les contrôles (75.14 ± 2.00, 29.16 ± 0.17, 45.44 ± 1.85), respectivement. Légère congestion vasculaire, infiltration des sinusoïdes

par des cellules inflammatoires, et des hémorragies ont été induites par la névirapine par rapport au groupe de contrôle montrant les vaisseaux normaux sans congestion, sinusoïdes normales apparaissant normale sans infiltration.
Conclusion: L'histologie hépatique des rats nourris avec névirapine suspension a montré une nécrose hépatocellulaire diffuse. Contrôle de routine de l'effet du médicament est importante car elle permet une gestion efficace de la vie des personnes infectées par le VIH.

Mots-clés: Névirapine, Wister rat, Hépatotoxicité, Foie, de l'alanine aminotransférase (ALT), d'aspartate aminotransférase (AST), la phosphatase alcaline (ALP).

INTRODUCTION

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used for prophylaxis and treatment of Human Immunodeficiency Virus (HIV) infections [1,2]. It acts by reversibly inhibiting the activity of HIV-1 reverse transcriptase, an enzyme which directs the polymerization of DNA from viral RNA, a necessary component for HIV-1 replication [3]. The inhibition of reverse transcriptase-directed polymerization of DNA from viral RNA has been an important therapeutic target for the treatment of HIV-1 infection, which was initially reported with the nucleoside analogue AZT [4]. Unlike the nucleoside analogues, NVP binds directly to the reverse transcriptase at amino acid residues 181 and 188. This site is close to but not directly at the polymerase catalytic site on the large subunit of the heterodimeric reverse transcriptase. The binding of NVP to reverse transcriptase occurs primarily through hydrophobic interactions to a pocket formed by seven strands, as a result the rate of the chemical reaction catalysed by the reverse transcriptase is significantly slowed [5]. NVP does not exhibit activity against other viral polymerase, including HIV-2 and simian immunodeficiency virus reverse transcriptase. NVP has been studied in several combination regimens for the treatment of HIV [6].

As nevirapine dose administered to mother and infant has been widely used to prevent mother-to-child transmission of HIV-1 in resource-limited settings [7], it has also been associated with severe skin and hepatic hypersensitivity reactions that have hampered its use particularly for HIV prophylaxis [8]. Hepatotoxic effect of NVP is common in patients with higher CD4 counts and also in the first three weeks of NVP treatments in HIV infected subjects [9,10]. The immune pathways that consequently cause the liver damage have been likened to the pathogenesis of liver injury in diseases such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infections where activated cell-mediated immunity is incriminated for the liver damaged [11,12]. The fact that NVP-induced hepatotoxicity is common in patients with higher CD4 counts imply that increased stimulation of the cell-mediated immune system response in some HIV-positive patients may predispose them to NVP-induced hepatotoxicity. According to Stern *et al*., [13] and Dieterich *et al*., [14], the mechanism of NVP-induced hepatotoxicity remains unknown, however, it was postulated to be immune mediated. Such immune mediation has already been proven in animal models for NVP-induced skin reactions [15,16]. Hepatotoxicity, a case of liver dysfunction or liver damage, is sometimes associated with an overload of drugs or xenobiotics, producing a wide variety of clinical and histopathological indicators of hepatic injury, however, the measurement of some level of substances that may be present in the blood helps in initial detection [17,18,19].

Several enzymes that trigger important chemical reactions in the body are produced and found within the cells of the liver, however, damage or injury to the liver cause elevations to the liver enzyme levels. These enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function [20]. Other measurable liver function is reflected in albumin and total protein concentration and the prothrombin time, which are the markers of liver biosynthetic capacity [3,5].

As HAART can substantially extend an HIV patient's life, one of the major problems are its adverse systemic and oral effects. To examine the mechanism of effect of nevirapine, an examination of the histological and biochemical markers of liver using wistar rats administered nevirapine were compared to those who were not given for a period of six weeks.

MATERIALS AND METHODS

Animals

Fifteen male adult wistar rats were obtained from a breeding stock maintained in the animal house of the Agricultural Science Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, and housed in well ventilated plastic cages in animal house in Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The rats were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e. L:D;12h:12h photoperiod) at room temperature, allowed unrestricted access to water and rat chow and acclimatized for 7 days before the

commencement of the experiment. The body weights of the rats ranged between 180 and 300g.

Drugs and Source

The antiretroviral drug (Nevirapine) used was produced for Evans Medical Plc Nigeria by CIPLA Limited, Verna Indl. Estate Goa 403 722 India with Batch No.G10930 and National Agency for Food Drug Administration and Control (NAFDAC) reg. No. 04-9498.

Experimental procedure

Fifteen male wistar rats were randomly distributed into three groups with five rats per group. Corresponding therapeutic doses for rat models were calculated and aqueous solutions formed were administered daily as follows. Rats in groups 1 (Control) received 0.9% food and normal saline. Group 2 (single dose) received 0.01% of Nevirapine, food and normal saline. Group 3 (double dose) received double dose of Nevirapine, food and normal saline.

Animal sacrifice and sample extraction

Animals were weighed and sacrificed after six weeks treatment. Blood samples were obtained by cervical dislocation and collected into EDTA bottles. Serum was used for the hepatic enzymes activities (ALT, AST and ALP).

Histological Procedures and Analysis of the liver

The liver organs were cut on slabs (0.5cm thick) and fixed in 10% formol saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before transferred into two changes of molten paraffin wax for 20min each in an oven at 57^{o}C. Serial sections of 5µm thick were obtained from a solid block of tissue and stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven- dried. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK) to demonstrate the cytoarchitecture of the liver.

Biochemical Analysis

The analysis of the result for ALT, AST and ALP were done using SPSS program for windows (17.0 version). The reagents used for the enzyme assay were obtained from the Randox Laboratories Limited, Antrim United Kingdom (BT294QY).

RESULT

The mean body weight gain of the rats treated with antiretroviral drug as shown in Table 1 has no significant differences when compared with the control group that received distilled water and food for 6 weeks**.**

Table 2 shows significant differences in ALP, AST and ALT. AST and ALT mean values increased as compared to the control, however, ALP mean value decreased when compared with the control.

TABLE 1: MEAN BODY WEIGHT OF RAT

Group/Day	1-7	8-14	15-21	22-28	29-35	36-42
1	158.09 ± 0.39	166.07 ± 0.43	154.04 ± 0.34	124 ± 0.17	144.04 ± 0.25	157.06 ± 0.34
2	159.06 ± 0.41	142 ± 0.21	156 ± 0.38	164.05 ± 0.42	138 ± 0.28	140.02 ± 0.20
3	160 ± 0.38	145 ± 0.30	155.04 ± 0.35	200.6 ±0.46	240.03 ± 0.51	234 ± 0.48

Key: Group 1- Control Group 2 - Single dose Group 3-Double dose

TABLE 2: EFFECTS OF NEVIRAPINE ON LIVER FUNCTIONS

Biochemical Markers	Group1	Group2	Group3
AST (U/L)	75.14 ±2.00	77.77± 3.03	89.37±3.19
ALT (U/L)	29.16±0.17	31.80±1.73	33.38±2.01
ALP (U/L)	45.44 ±1.85	43.81 ±1.54	34.64 ±1.02

Key: Group 1-control Group 2-single dose Group 3-Double dose.

Plates 1, 2 and 3 show the gross morphology of liver for rats fed with NVP as compared with the control. Those fed with NVP suspension showed diffused hepatocellular necrosis in about 90% of the rats. No such changes were observed in the control.

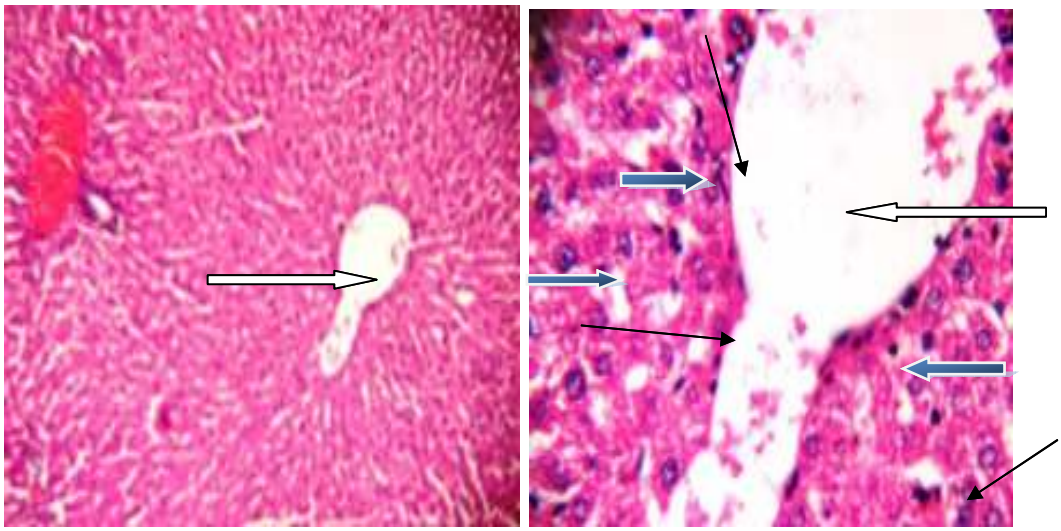

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PLATE 1: MICROGRAPH OF LIVER SECTION OF RATS IN THE CONTROL GROUP

Normal vessles without congestion (white arrow), the sinusoids (slender arrow) appear normal without infiltration, the hepatocytes show normal morphology (blue arrow). No pathological lession seen.

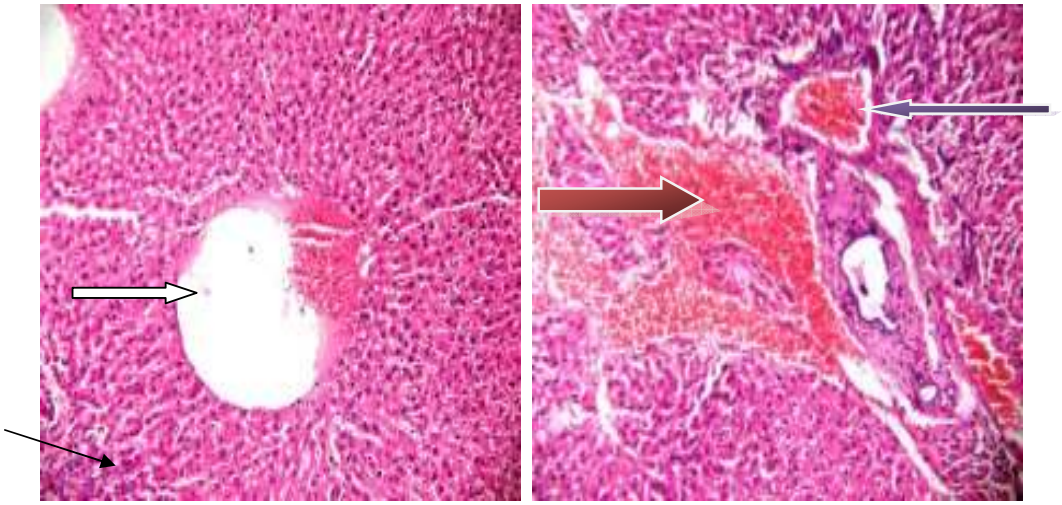

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PLATE 2: MICROGRAPH OF LIVER SECTION OF RATS FED WITH SINGLE DOSE OF NEVIRAPINE SUSPENSION

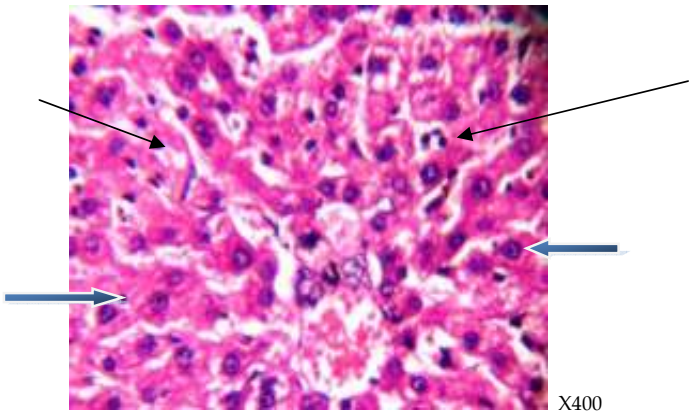

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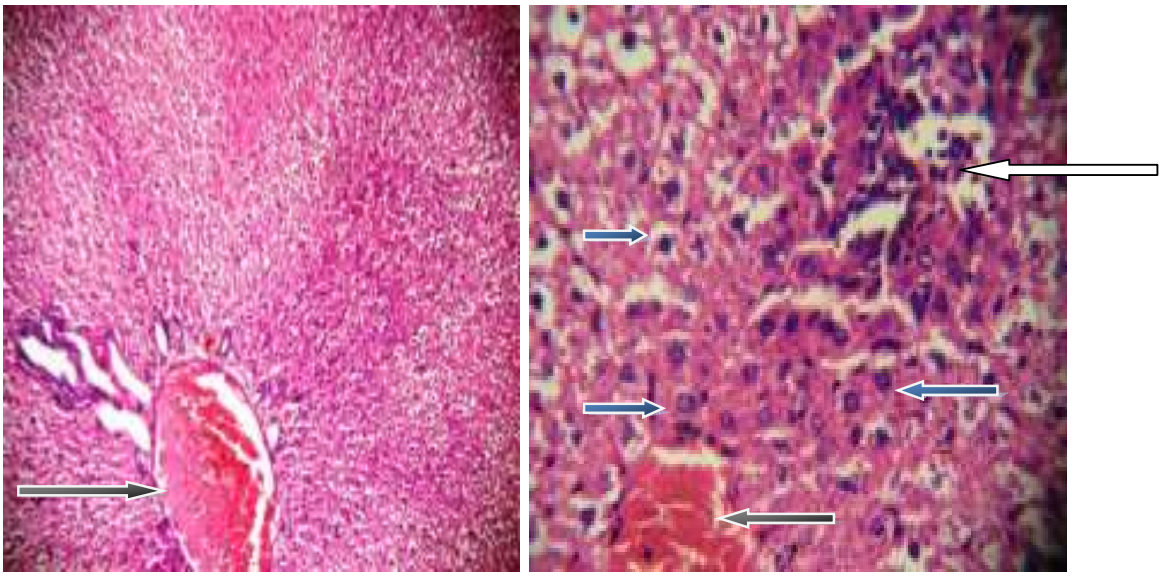
PLATE 2(CTD.): MICROGRAPH OF LIVER SECTION OF RATS FED WITH SINGLE DOSE OF NEVIRAPINE SUSPENSION

The central vessel appear normal (white arrow), there is mild congestion of the portal vein (black arrow) and focal area of mild haemorrhage (red arrow), the sinusoids shows mild infiltration of inflammatory cells (slender arrow) the hepatocytes show normal morphology (blue arrow).



X100 X400

PLATE 3: MICROGRAPH OF LIVER SECTION OF RATS FED WITH DOUBLE DOSE OF NEVIRAPINE SUSPENSION

There is mild vascular congestion (black arrow), the sinusoids show mild infiltration by inflammatory cells, there is focal granuloma within the liver parenchyma (white arrow), the hepatocytes show normal morphology (blue arrow).

DISCUSSION

Effects of administration of NVP given orally on morphology of the liver of albino wistar rats were studied. Nevirapine is one of the most widely used antiretroviral drugs in the treatment of human immunodeficiency virus infections. Its mechanism of action is not limited to treatment of HIV-1 but also in the reduction of mother-to-child transmission of HIV-1 which may involve reduction of maternal viral load as well as prophylaxis of infants [2]. The drug can be used as a single-dose or as a combination therapy with other antiretroviral drugs including lamivudine, stavudine and Zidovudine [21,22,23,24]. Nevirapine administration has been associated with severe skin and hepatic hypersensitivity reactions that have

hampered its use particularly for HIV prophylaxis [25,26,8] as well as oral adverse effects, including whitish plaque in the lips and bilateral buccal mucosa, burning, taste disturbance, and xerostomia [1].

From this study, no significant difference was recorded in the mean body weight of the rats fed with NVP as compared with those in the control group. This supports the findings from a similar study by Ayeni *et al*., [24].

As revealed from the result in this study, Nevirapine is associated with significant activities of ALT and AST. Elevated activities of these enzymes indicate hepatic damage which results from several mechanisms including generation of toxic species and peroxidation of membranes [21]. The results showed that AST mean values were significantly increased in rats treated when compared with the control values. This elevation in Nevirapine treated rats agrees with earlier reports of Sule *et al*., [23], Johnson and Baraboutis [27] and Martinez *et al*., [9]. However, Nevirapine treated rats showed significant reduction in the values of ALP when compared with the control. According to Dufour *et al*., [28,29], ALT activity is the most frequently relied biomarker of hepatotoxicity in that it plays a vital role in amino acid metabolism and gluconeogenesis. The estimation of this enzyme is more specific for liver abnormalities since it is primarily located in the liver [30]. Aspartate aminotransferases (AST) is another liver enzyme found in the liver and other organs including heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level [30]. It helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury [31]. It can also signify abnormalities in heart, muscle, brain or kidney [28,29]. According to Nathwani *et al*., [30], the ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage. Alkaline phosphatase (ALP) may be elevated if bile excretion is inhibited by liver damage. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepatobiliary effects and cholestasis [32]. In humans, increased ALP levels have been associated with drug-induced cholestasis [33]. As revealed from the result in this study, nevirapine is associated with significant elevated activities of ALT and AST. The result shows that AST mean values were significantly increased in rats treated with the antiretroviral drug when compared with the control values, which agrees with earlier reports of Umar *et al*. [21], Sule *et al*., [23], Johnson and Baraboutis [27] and Martinez *et al*. [9]. They concluded that nevirapine could be associated with hepatotoxicity. Nevirapine hepatotoxicity could be associated with some risk factors including gender, CD4 cell count, co-infection with hepatitis B or C and pregnancy [34,35,36,37,2,38]. Several drugs known to induce hepatotoxicity, like nevirapine, in association with an activated immune system include diclofenac [39], paracetamol [40], bacterial lipopolysaccharide (LPS) plus ranitidine [41] and trovafloxacin [42]. The elevation of enzymes activity, especially in ALT in Nevirapine-treated rats is indicative of liver injury. This agrees with reports that severe hepatic reactions of HAART was attributed to Nevirapine component of HAART [27,9]. Sulkwosk *et al*., [43] also reported hepatotoxicity as a major side effect of all antiretroviral classes with Nevirapine having the highest risk. Liver converts drugs into reactive forms and hence results in toxicity [21].

Histological examination of NVP administration on the morphology of some organs of the body have been reported and series of reports as regards the toxicity of NVP a non-nucleoside reverse transcriptase inhibitor on the small intestine, kidney, spleen, mitochondria, bile, muscle and bone had been recorded [44,45,46,47,24]. The gross morphology of the liver from rats fed NVP observed in this study agrees with that reported by Umoren and Osim [3]. From result of this study as shown in Plate 1 revealed a normal healthy state of the liver with the portal tract intact. There are normal vessels without congestion, periportal hepatocytes arranged in plates and sinusoids appeared normal without infiltration, within the portal tract were the portal vein, hepatic artery and bile duct, no pathological lesion is seen. From plate 2, the liver of the rats showed disorganized cyto-architecture with sinusoidal and central vein endothelial desquamation. It revealed mild congestion of the portal vein and focal area of mild haemorrhage, the sinusoids showed mild infiltration of inflammatory cells, mild perivascular infiltration, the sinusoids are mildly infiltrated by inflammatory cells even though the hepatocytes showed normal morphology. Plate 3 revealed poor architecture of the liver. There was mild vascular congestion with the sinusoids showing mild infiltration by inflammatory cells. A focal area of granuloma within the liver parenchyma was seen. There was mild congestion of the portal vein which shows that much damages was done in the liver of the rat administered with double dose of Nevirapine. The present result agrees with studies of Degott [48] who reported that liver damage is associated with alteration in bile secretion and Akerlund *et al*., [49] who showed that there is always an increase in cholesterol synthesis when there is a disturbance in bile release and utilization due to liver damage.

Umoren and Osim [3] earlier reported the effects of NVP administration given through oral gavage on biliary secretion and its biochemical composition in

albino wistar rats. The result obtained showed significantly decreased biliary secretions resulting in insignificant increase in conjugated bilirubin but significant elevations in total cholesterol, total bilirubin, and unconjugated bilirubin. Also, significant decreases in biliary electrolytes concentrations were observed.

Furthermore, Poirier *et al*., [50] and Ayeni *et al*., [24] reported that NVP can cause acute kidney injury as a result of severe mitochondrial dysfunction and lactic acidosis induced as well as acute renal failure after the initiation of NVP during the study of the effects of NVP on foetal parameters, kidney and spleen of dams.

Conclusion

The above results suggest that NVP administration may cause liver hepatotoxicity in albino wistar rats.

The effect of antiretroviral drugs on biochemical indices of liver function is of paramount importance and should not be overlooked. This is to ensure that the liver function is not impaired in the process of managing a particular health problem in case of HIV patient, hoping to minimize the duplication of this virus in the human system, while a lot of harm is being done to the liver. The observation that it takes some weeks to develop liver injury means that NVP itself plays a role in the initiation of the lesion. Therefore, it can be concluded that NVP activates the cell-mediated immune response leading to liver injury that is propagated by the drug itself or the immune system.

Following the results in this study and various reports on related studies, it could be concluded that the lives of HIV patients on regular use of HAART containing Nevirapine are prone to risk.

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CASE REPORT

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HEPATITIS C VIRUS-ASSOCIATED PORPHYRIA CUTANEA TARDA: A CASE REPORT

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ABSTRACT

Porhyria cutanea tarda (PCT) is a rare, inherited or acquired disorder due to decreased activity or deficiency of uroporphyrinogen decarboxylase (UROD), one of the enzymes in the haem synthetic pathway. It is characterized by cutaneous manifestations such as erosions, blisters and bulae in the dorsum of the hand, forearm, elbows and knees; and painful indolent sores that heal with dyspigmented and scarring lesions. A 25 year old sales man presented with a 7 month history of recurrent blistering of the skin of the extremities- hands, elbows, knees and feet which occurred spontaneously or following trivial trauma. There was no family history of similar skin symptoms. Examination showed broken and fresh blisters of varying sizes with some healed lesions on the dorsum of the hands, over the elbow and knee joints, and toes. Serum ferritin was 360µg/L (40-340 µg/L), urine uroporphyrinogen was positive (+++) and Hepatitis C antibodies screening was positive. Some improvement of the cutaneous lesion was noticed following commencement of therapeutic phlebotomy.

Key words: Porhyria, blisters, Hepatitis C virus, uroporphyrinogen.

LE VIRUS HEPATITE C – PORPHYRIE CUTANEE TARDIVE ASSOCIE : LE RAPPORT D'UN CAS.

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RÉSUMÉ

Porphyrie cutanée tardive (PCT) est rares troubles héritée ou acquis en raison d'activitéréduite ou d'insuffisance d'Uroporphyrinogene décarboxylase (UROD), l'une des enzymes dans la voie de synthèse de l'hème .C'est caractérisé par les manifestations cutanées telles que :l'érosion, les ampoules et *bulae* dans le dos de la main, l'avant – bras, les coudes et les genoux ; les plaies douloureuses indolentes qui guérissent avec les lésionsdyspigmentees et cicatrisées. Un vendeur de 25ans présenté d'une histoire de 7 mois de formations d'ampoules de la peau récurrentes des extrémités – les mains, les coudes, les genoux et les pieds qui a eu lieu spontanément ou suite àun traumatisme trivial. Il n'y avait pas d'histoire familiale de symptômes de la peau similaires. L'examen a montréles ampoules diverses et fraîches de taille différentes avec des lésionscicatrisées sur le dos des mains, au-dessus des coudes et les articulations du genou et les doigts de pieds. Ferritinesérique était 360µg/L (40 – 340 µL), Uroporphyrinogene urine était positif (+ + +), et le dépistage de l'hépatite C était positif. Une certaine amélioration de la lésioncutanée a été remarquée au commencement des saignées thérapeutiques.

Mots clés : Porphyrie, les ampoules, virus Hépatite C, Uroporphyrinogene.

INTRODUCTION

Porphyrias are a group of metabolic disorders resulting from inherited or acquired defects in any of the enzymes involved in the synthesis of haem (1). They are generally rare conditions.[2,3] However porphyria cutanea tarda (PCT) is the most common type occurring in both sex and all ethnic groups. It is due to decreased activity or deficiency of uroporphyrinogen decarboxylase (UROD) in the

liver.(1,4) Other factors such as hereditary haemochromatosis, alcohol and hepatitis C infections have been associated with PCT(2,5,6). It is characterized by cutaneous manifestations such as erosions, blisters and bulae in the dorsum of the hand, forearm, and other pressure points such as elbows and knuckles of the toes; and painful indolent sores, that heal with dyspigmented and scarring lesions (1,7).

Failure of progression of haem synthesis caused by decreased in UROD activity results in accumulation of porphyrin by-products first in the liver and subsequently disseminate in the plasma into other organs. They are excreted primarily in the kidney but some are excreted in feces. Porphyrins are photoactive molecules and when exposed to light in the skin they mediate oxidative damage to biomolecular targets and increase mechanical fragility causing cutaneous lesions (1,8).

This case is reported in view of the rarity of this disorder and the challenges in it diagnosis and management in our environment.

CASE REPORT

E.B is a 25 year old trader who was referred to the Dermatology clinic at Bingham University Teaching Hospital (BHUTH) with a 7 month history of recurrent easy blistering of the skin of the extremities-hands, elbows, knees and feet. The blisters occurred spontaneously or following trivial trauma such as washing of clothes and wearing tightly fitting shoes. There was no associated bleeding, itching, or fever. Patient was on vitamins C, A, E and B complex prescribed at the general outpatient department (GOPD) where he was first seen. He is allergic to chloroquine. There was no family history of similar skin symptoms. Patient does not drink alcohol or smoke cigarette.

Examination showed a young man with broken and fresh blisters of varying sizes with some healed lesions on the dorsum of the hands, over the elbow and knee joints, and toes. (see figures 1 and 2) The cardiopulmonary system was stable. The liver and spleen were not palpable.

His haematocrit was 40%. The WBC and platelets counts were 8.4 $x10^9$/L and 220 x 10^9/L respectively. Total serum protein, albumin, bilirubin were normal. Serum alanine and aspartate transaminases, as well as serum urea and creatinine were all within the normal limits. Serum ferritin was 360µg/L (40-340 µg/L) Urine uroporphyrinogen was positive (+++). Histology of the skin lesion showed bullous dermatitis as characterized by a keratinizing stratified squamous epithelium with intraepidermal bullae giving a diagnosis of bullous dermatitis. No notable inflammatory reaction. Hepatitis C antibodies screening was positive while HBsAg and HIV screening were negative. Other relevant investigations such as liver iron stores, UROD enzyme assay and genetic studies were not done due to lack of facilities.

Patient was advised to avoid exposing his body to sunlight and activities that will traumatize his skin. He was also placed on vitamins C and E. There was no improvement. Chloroquine was contraindicated as patient was allergic to it. Weekly therapeutic phlebotomy was commenced on the patient. He had only 3 sessions and absconded despite some improvement.

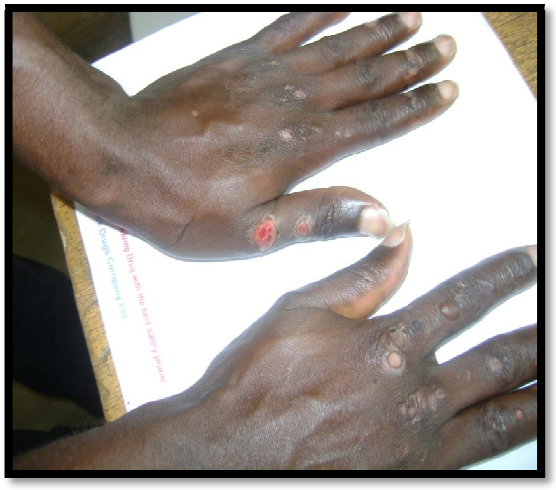

Fig 1: Porphyria cutanea skin lesion on the dorsum of the hands of the patien

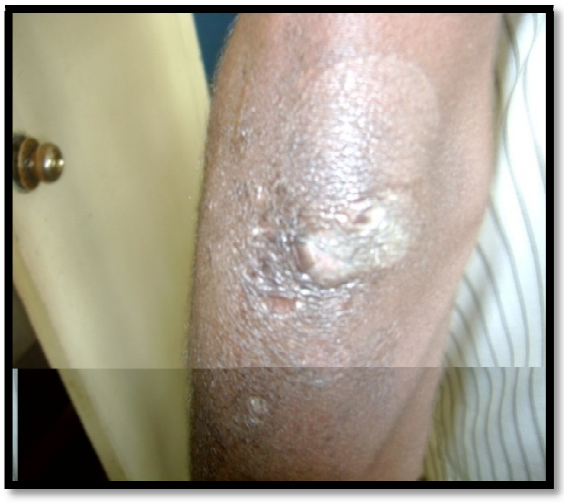

Fig 2: Porphyria cutanea skin lesion on the elbow of the patient

DISCUSSION

The diagnosis of PCT in this patient we described was based on the characteristic cutaneous lesions he presented with. This was substantiated by the positive urine uroporphyrinogen (+++), the high serum ferritin levels and most importantly the positive HCV antibodies which has a strong association with PCT (5,9). Diagnosis of PCT is confirmed by assaying the UROD enzyme activity in red blood cells and carrying out mutation analysis of genes encoding UROD especially in the familial forms.[4] Patients with PCT have abnormally high levels of porphyrins in their urine, serum or plasma specimens and the faecal coproporphyrin fraction is often abnormally high too (7). Other laboratory work up include screening for those factors associated with the condition such as HFE gene mutations and iron profile.[5] About 80% of all cases of PCT are acquired while the remaining 20% are inherited (8). Although assay of UROD enzyme or studies of UROD and HFE gene abnormalities were not done, our patient is likely to be suffering from acquired or sporadic PCT in view of the age of onset and a negative family history.

The care of patients with PCT is multidisciplinary, involving consultations with hepatologist, haematologist, dermatologist, gynaecologist, oncologist etc. (8). Our patient could have benefited from further evaluation by a hepatologist but we had none in our centre, couple with the patient's financial constraints. Educating the patient about the disease, its precipitating factors and the treatment modalities available is very important. Patient must avoid exposure to sun light, trauma, alcohol or tobacco use and may have to discontinue oestrogen therapy if on any. The use of topical sun screen creams, consumption of vitamin C rich diets and avoidance of iron-rich dietary supplements may be of help.[8] Therapeutic phlebotomy is one of the treatment modalities especially in patients with increased iron stores. It involves the removal of one unit of whole blood from twice a week to once in 2-3 weeks with the aim of reducing the iron stores (1,10). Oral Chloroquine phosphate, 125-250mg or hydroxychloroquine sulfate, 100-200mg 2-3 times per week, is given to patients who cannot tolerate therapeutic phlebotomy or whose iron overload is relatively mild (11). Our patient was adequately educated and counseled. However intervention with chloroquine was not an option for him because he is allergic to it and could cause itching which is a form of mild trauma that may exacerbate his condition. Therapeutic phlebotomy was the major viable treatment option left which he reluctantly accepted because he was scared of being bled despite much assurance of the minimal risk and likely improvement he stands to benefit from the option.

In conclusion PCT is a rare condition. We hope that this report will bring increase awareness on the existence of PCT in our environment and hence an increased index of suspicion and prompt referral to avoid misdiagnosis and mismanagement.

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SERO-EPIDEMIOLOGICAL SURVEY AND RISK FACTORS FOR HEPATITIS B VIRUS (HBV) INFECTION AMONG PREGNANT WOMEN IN LOGO LGA, BENUE STATE, NIGERIA

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ABSTRACT

Hepatitis B virus (HBV) infection is associated with chronic liver diseases, cirrhosis and hepatocellular carcinoma, liver failure and death. The prevalence of Hepatitis B virus carrier and infectivity status and social characteristics among three hundred and ten pregnant women in Logo LGA, Benue State, Nigeria, was determined through random anonymous testing of volunteers attending antenatal clinics of different hospitals within the community. Thirty of three hundred and ten blood samples tested positive for HBV infection. 11.9% were in the 3rd trimester of their pregnancy and 58.1% were within the age bracket of 21-30 years. Illiterates women constituted 14.4% of those sampled while civil servants were 6.7%. HBV carrier status was determined by the presence of Hepatitis B surface antigen (HBsAg). Repeated reactive samples were confirmed by Enzyme linked immounosorbent assay (ELISA) Kit (Diagnostic Automation, Inc., USA). Maternal HBV infectivity status was determined by testing all HBsAg positive samples for the presence of hepatitis B e antigen (HBeAg). A total of thirty (9.7%) pregnant women identified as carriers of HBV and eleven of the thirty tested positive for HBeAg. Hence, 3.6% (11/310) of the entire study population was found to have high viral replication as well as high risk of transmitting HBV to their neonates. The frequency of HBV carrier did not vary with age, however, it varies significantly with the previous use of contraceptives and the anaemic status of the subjects ($P< 0.05$) .This study demonstrates the endemicity of HBV infection in Logo and high infectivity rate, suggest that HBV is likely to be acquired by both vertical and horizontal means of transmission. Testing for HBsAg is recommended for all pregnant women at first prenatal visit so that positive mothers receive prompt intervention.

Key words: Hepatitis B e antigen, Hepatitis B surface antigen, seropositivity, pregnant women, neonates.

ENQUETE SERO – EPIDEMIOLOGIQUE ET LES FACTEURS DE RISQUE POUR L'INFECTION DU VIRUS DE L'HEPATITE B (VHB) CHEZ LES FEMMES ENCEINTES A LA ZONE DE GOUVERNEMENT LOCAL DE LOGO DANS L'ETAT DE BENUE, NIGERIA.

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RÉSUMÉ

L'infection de virus de l'hépatite B (VHB) est associée aune malade chronique du foie, la cirrhose et carcinome hépatocellulaire, insuffisance hépatique et la mort. La prévalence du porteur de virus de l'hépatite B et le statut de l'infectiosité et de caractéristiques sociales chez trois cent dix femmes enceintes a la zone de gouvernement local de Logo dans l'état de Benue, Nigeria, a été déterminé par des tests anonymes aléatoire des volontaires fréquentant les consultations prénatales des divers hôpitaux à l'intérieur de la communauté. Trente de trois cent dix échantillons de sang ont été positifs de l'infection de VHB. 11,9% étaient au 3ème trimestre de leur grossesse et 58,1% étaient dans la tranche d'âge de 21 – 30 ans. Les femmes analphabètes constituaient 14,4% de celles échantillons alors que 6,7% étaient fonctionnaires. Le statut de porteur de VHB a été déterminé par la présence de l'antigène de surface de l'hépatite B (HBsAg). Echantillons réactifsrépétés ont été confirmés par enzymes liée Kit de dosageimmun/kit de dosage enzymatique d'immunosorbent lie (ELISA) (Diagnostic Automation, Inc., USA). La maternelle VHB statut d'infectiosé a été déterminée en analysant tous les échantillons HBsAg positifs pour présence de l'antigène de l'hépatite B (HBsAg). Une totales de trente (9,7%) femmes enceintes ont été identifiées comme porteuses de VHB et onze de ces trente ont été testées positives pour HBsAg. Donc, 3,6%(11/310) de l'ensemble de la population d'étude est trouvé d'avoir une réplication virale élevée ainsi qu'une risque élevée de transmettre à leurs nouveau – nés. La fréquence du porteur du VHB ne varie pas avec l'âge, néanmoins, elle varie

de façon significative avec l'utilisation antérieure de contraceptifs et l'étatanémique des sujets (P<0,05). Cette étudedémontre l'endémicité de l'infection par le VHB à Logo et le taux d'infectioséélevée, suggèrent que le VHB est probablement d'êtrecontracte par les deux moyens verticaux et horizon taux de transmission. Test pour HBsAg est recommandé pour toutes femmes enceintes à la première visite prénatale pour que les mères séropositives reçoivent une intervention rapide.

Mots – clés : L'antigène de l'hépatite B, l'antigène de surface de l'hépatite B, séropositivité, femmes enceintes, nouveau – nés.

INTRODUCTION

Hepatitis B virus is a blood borne and sexually transmitted pathogen that is spread through percutaneous and mucosal exposure to infected blood and body fluids. The virus was first discovered as "Australia antigen" later named Hepatitis B surface antigen (HBsAg), in patient blood. Hepatitis B e antigen (HBeAg) was identified several years later as a marker for patients at high risk for transmission of the disease (1). The virus has caused several epidemics in parts of Africa and Asia (2) and approximately 350 million persons worldwide are infected with the virus (3, 4, 5, 6), resulting in 2 million deaths annually.

When a pregnant woman is infected with HBV there is a chance she may infect her foetus. It has been reported that 10-20% of women seropositive for HBsAg transmit the virus to their neonates, but in women who are seropositive for both HBsAg and HBeAg; vertical transmission is approximately 90% (7, 8).

Nigeria is classified among the countries highly endemic for viral hepatitis. Currently about 18 million Nigerians are infected (9, 10). Many of these people may not be aware of the infection and hence fail to seek appropriate medical attention therefore progressing to chronic liver disease, cirrhosis and hepatocellular carcinoma, liver failure and death. Similarly when pregnant women are involved they constitute a serious health risk not only to their unborn child but the society at large.

Although, studies have been carried out on HBV in other parts of the country, information is scarce on the prevalence of HBV among pregnant women in the rural parts of Benue state, in particular Logo LGA. The aim of this study therefore is to determine the prevalence of hepatitis B virus carrier and infectivity status of pregnant women attending ante natal clinics in Logo LGA of Benue state, Nigeria.

MATREIALS AND METHODS

AREA OF STUDY

This study/research was conducted in the various health centres within the locality of Logo Local Government of Benue State which is located in an area covering about 1,408 Km ^{2} and a population of 169,063 in the 2006 census, in North-Central Nigeria. Its average annual Rainfall is 1200mm and the average annual maximum temperature is 33.3^{O}C. Logo is located on latitude 9^{o}37'N and longitude 6^{o}33'E and it is bordered in the East by Katsina Ala Local Government, in the North by Ukum Local Government, in the West by Taraba State and in the South by Buruku Local Government. Agriculture is the mainstay of its economy with the production of varieties of cash crops throughout the year.

STUDY DESIGN

The study was a hospital based descriptive cross sectional survey conducted between 1st July, 2012 and 30th April, 2013 at the antenatal clinics within Logo LGA, Benue State Nigeria. On every antenatal day, the pregnant women were given health talk on HIV/AIDS and hepatitis infections and were advised on the need to know their status. Only consenting attendees were recruited and included in the study.

SUBJECTS

Three hundred and ten pregnant women attending antenatal clinics in different hospitals in Logo Local Government were randomly selected from the clinic centres. Their specimens were retrieved and reviewed for analysis after informed consent. Information obtained from the case note included demographic characteristics, risk factors, blood group, haematocrit, and results of serological markers for hepatitis B virus.

Pre-structured questionnaires were administered to three hundred and ten consenting pregnant women. Each questionnaire was designed to obtain demographic data such as age, occupation and educational status. Risk factors information were also obtained which include the stages of pregnancy, history of blood transfusion, and history of STIs, whether or not respondents share sharp objects like razors and toothbrushes.

ETHICAL CONSIDERATIONS

Ethical approval was gotten at the Benue state Ministry of Health and Human Services, Makurdi through the Medical Director of the General Hospital Ugba and the Logo Local Government Health Department.Informed consent was obtained from the from the antenatal clinic attendees with assurance that all information obtained would be treated as confidential and would be used for the purpose of this study only.

SAMPLE SIZE AND ITS DETERMINATION

Using the Kish (11) formula: $n= (z^2pq/d^2)$ for determining adequate sample size and further correcting for population less than 10,000 using nf = n/1+(n/N) (12), 310 respondents were enrolled for this survey.

COLLECTION OF BLOOD SAMPLES / SERUM PREPARATION

Hepatitis B surface antigen (HBsAg) detection was done using the in vitro diagnostic kit manufactured by Cal – Tech Diagnostic, Inc. USA. The test kit (dipsticks) is a rapid immunochromatographic assay designed for qualitative determination of HBsAg in human serum or plasma. Assays were carried out at room temperature. The sera samples were removed from the freezer and left at room temperature to thaw. The test strips were removed from their foil pouches and immersed into serum samples with arrows pointing towards the samples. The strips were taken out after about 10secs and placed on a clean, dry, non-absorbent surface. This is to allow time for the reaction to take place. It was observed that the specimen was absorbed into the test strips and moved by capillary action upward towards the control line. Results were read after 10mins post immersion. Positive samples generated a colour band in the test region of the strips and another in the control region while negative samples had a colour band in the control regions only.It utilizes a combination of monoclonal and polyclonal antigen body to selectively detect elevated level of HBsAg in serum/plasma. The test was carried out and interpreted according to the manufacturer's instructions and in the laboratories of the hospitals were the samples were collected.

Reactive samples were stored in a freezer and further confirmed for HBsAg in the Innovative Biotechnology Laboratory Keffi where HBsAg positive samples were tested for HBeAg, associated with infectivity and active virus replication; using commercially available enzyme linked immounosorbent assay kit ELISA (Diagnostic Automation Inc. USA)

DATA ANALYSIS

Data from the questionnaires were analyzed using SPSS version 15.0. Chi-square was used to compare significant differences between HBV prevalence and risk factors. Significance was determined at $P < 0.05$ at 95% Confidence interval.

RESULTS

The results of the Seroprevalence study are presented in table 1, showed that of the three hundred and ten (310) pregnant women tested, 30 (9.7%) pregnant women were seropositive for Hepatitis B surface antigen (HBsAg). Eleven (11) out of the thirty (30) pregnant women were identified as HBeAg positive. Hence, 3.6 % (11/310) of the entire study population was positive for HBeAg.

With respect to age, the results showed that there is an increase in HBsAg titres with increase in age up to 30 years followed by a decline. Statistically, however, there was no significant association (x^2 =1.960; p = 0.5808) between age and seroprevalence of HBV infection.

The level of educational attainment and occupation and seroprevalence of the pregnant women are presented in table 2.It showed that there is an inverse relationship between educational attainment of the women and seroprevalence of HBV infection. Details show that women with high prevalence of the infection are illiterates (14.4%) while those with some levels of education had lower prevalence even though there was no significant association (x^2 = 4.213 p = 0.239). Similarly, the results revealed that house wives had higher prevalence (13%) than the other women considered in this study. Despite this observation, no significant association (x^2 =5.86 p = 0.119) between HBV infection and the occupation of the women. There was no significant association between infection and pregnancy stages of the women(x^2 = 2.239 p = 0.326), (Table 3).

TABLE 1: AGE SEROPREVALENCE OF HEPATITIS B SURFACE ANTIGEN AND HEPATITIS B E ANTIGEN AMONG THE PREGNANT WOMEN.

Age group	No. examined	HBsAg seropositivity (%)	HBeAg seropositivity (%)
11-15	10	1 (10)	1 (20)
16-20	90	7 (7.8)	4 (4.4)
21-25	100	11 (11)	4 (4)
26-30	80	9 (11.3)	1 (1.3)
31-35	25	2 (8)	1 (4)
36-40	3	0 (0)	0 (0)
41-45	2	0 (0)	0 (0)
Total	310	30 (9.7%)	11(3.5%)

X^2=1.960, p = 0.5808 X^2 = 2.222, p = 0.5276.

TABLE 2: SOCIAL CHARACTERISTICS AND HEPATITIS B VIRUS SEROPOSITIVITY AMONG THE PREGNANT WOMEN.

Social characteristics	No. examined (n = 310)	No. positive (n=30) (%)
Education		
Illiterate	104	15 (14.4)
Primary	99	5 (5.1)
Secondary	65	6 (9.2)
Tertiary	42	4 (9.5)
Occupation		
Civil servant	15	1 (6.7)
House wife	200	26 (13.0)
Students	17	1 (5.9)
Business	78	2 (2.6)

Education; x^2 = 4.213 p= 0.239; Occupation; x^2 = 5.862 p = 0.119

TABLE 3: HEPATITIS B VIRUS SEROPOSITIVITY AND STAGE OF PREGNANCY AMONG THE PREGNANT WOMEN.

Trimester	No examined	No positive (%)
First trimester (1-3 months)	39	3 (7.7)
Second trimester (4-6 months)	86	5 (5.8)
Third trimester (7-9 months)	185	22 (11.9)
Total	310	30 (9.7)

X^2 = 2.239 p = 0.326

The frequency of HBV carriers did not vary significantly with blood transfusion, sharing needles, Alcoholic consumption, Herbal medicine use and history of STI(s). However, there was a significant frequency variation in the distribution of HBsAg and contraceptive use subjects (x^2 = 7.212 p = 0.007) and anaemic and non- anaemic subjects (x^2 = 7.143 df = 1 p 0.008) (Table 4). HBV carriers also decrease as anaemia decreases significantly (x^2 = 15.048 df = 6 p = 0.0199), (Table 5). Among all the contraceptive devices used previously by the subjects, only users of oral pills were positive for HBeAg. (Table 6).

DISCUSSION

This study showed that the prevalence of hepatitis B virus (HBV) infection in apparently healthy pregnant women attending antenatal clinics in the major hospitals within Logo LGA, Benue State was 9.7%, falls within figures reported for other African countries. This corroborates the World Health Organization (13) report for Nigeria as highly

endemic area with prevalence greater than 8%. The HBsAg seropositivity of 9.7% among pregnant women in Logo shows that Logo like other areas in Nigeria is endemic for HBV infection.

In related studies in different parts of Nigeria, higher prevalence rates of 13.8% were reported among pregnant women in Lagos (14) 11.6% in Maiduguri (15), 12.6% in North central Nigeria (10) 11% in (16) and 12.3% in Minna (17).

Other findings were, 8.3% in Zaria (18), 2.19% in Benin City (19), 4.3% in Port Harcourt in 2005 (20) and 2.89% in 2006 (21) and 5.7% in Ilorin (22). The result of this study is higher than the 6.3% reported in pregnant women in Tanzania (23) and 3.7% in Ethiopia (24), showing that variations exist within the same continent of Africa.

Similar studies in other parts of the world were 10% in India (25), 12% in Taiwan (26) and 17.3% in Burkina Faso (27) agreeing with Juszozyk (28) that the global prevalence of chronic HBV infection varies, highest in Africa, Asia and the western Pacific (>8%) to lowest in western Europe, North America and Australia.

TABLE 4: DISTRIBUTION OF HBSAG POSITIVE CASES ACCORDING TO RISK FACTORS AMONG PREGNANT WOMEN.

Risk Factors		No. Examined	Seropositivity (%)	X^2, df=1 p value
Blood transfusion	Yes	15	3(20)	
	No	295	27 (9.2)	1.453 0.228
Sharing needles	Yes	270	21(7.8)	0.015 0.0903
	No	40	9 (22.5)	
Contraceptive use	Yes	109	18(16.5)	7.212 0.007
	No	201	12 (6)	
Alcoholic consumption				
	Yes	105	11(10.5)	0.095 0.758
	No	205	19 (9.3)	
Herbal medicine use	Yes	200	22(11)	0.938 0.333
	No	110	8(7.3)	
Anaemic status				
	Anaemic	45	10(22.2)	7.143 0.008
	Not Anaemic	265	20(7.5)	
History of STI(s)	Yes	50	5 (10)	0.006 0.939
	No	260	25 (9.6)	

TABLE 5: AGE DISTRIBUTION OF HBSAG AND HBEAG AMONG ANAEMIC PREGNANT WOMEN

Age group	No. examined	Anaemic (%)	HBsAg positivity (%)	HBeAg positivity (%)
11-15	10	6 (60)	1 (10)	0 (0)
16-20	90	14 (15.6)	4 (4.4)	0 (0)
21-25	100	12 (12)	3 (3)	3 (3)
26-30	80	10 (12.5)	2 (2.5)	2 (2.0)
31-35	25	2 (8)	0 (0)	0 (0)
36-40	3	1 (33.3)	0 (0)	0 (0)
41-45	2	0 (0)	0 (0)	0 (0)
Total	310	45(5%)	10(3.2%)	5(1.6%)

$x^2 = 15.048$ p =0.0199 $X^2 = 2.801$ p =0.8334 $x^2 = 15.048$ p =0.0199 $X^2 = 2.801$ p =0.833

TABLE 6: DISTRIBUTION OF HBSAG AND HBEAG AMONG PREGNANT WOMEN THAT USED CONTRACEPTIVES PREVIOUSLY.

Contraceptives	No examined	HBsAg positivity	HBeAg positivity
Oral pills	75	14 (18.7)	5(6.7)
Inject able	26	2 (7.7)	0 (0)
Loop	1	0 (0)	0 (0)
Pills/inject able	7	2 (28.6)	0 (0)
Total	109	18 (16.5)	5 (4.6)

$X^2 = 4.128$ p = 0.6594

HBeAg seroprevalence of 3.6% in the entire study population implies that one out of every three HBV carriers (11/310) is at high risk of transmitting HBV to her neonate as well as higher chances of chronicity. This is alarming but tallies with the result of Olubuyide *et al* (29), who found 3.4% among HBsAg positive doctors and dentist at university teaching hospital Ibadan. However, it is very high when compared with 0.8% found by Madzine *et al*, (30), in Zimbabwe as well as 6.64% and 1.39% obtained by Harry *et al* (15) among blood donors and pregnant women in Maiduguri respectively. Hence the issue of vertical transmission in sub-Saharan Africa cannot be ignored.

High prevalence of HBV carriers among teenagers (11-25) and 26-30 age groups and the corresponding high HBeAg prevalence among the same age group further show the severity of the infection in the community.

Analyses showed that out of the 310 respondents (33.5%) were illiterates out of which 50% tested positive for HBsAg. There is an inverse association between educational status and HBsAg positivity with less educated women showing the highest positivity, indicating the positive influence of education and public enlightenment/ awareness on the carrier rate of HBV infection. Although HBV infection is considered one of the most important occupational infectious hazards in developed countries (31), result from this study did not reveal statistical significance between HBV seropositivity and the different occupations of the pregnant women studied.

Most of the studied women (185, 59.7%) were in the 3rd trimester of gestation. This group also had the highest HBsAg seropositivity 11.9% (22/185),

followed by those in the 2nd trimester of gestation, results revealed no significant association between infection and pregnancy stages of the women.

The distribution of HBsAg and HBeAg among teenagers and 26-30 years age groups as shown in table 4 and 5 could be due to anaemia which significantly decreases with increasing age. It could also be attributed to the effect of oral pills table (6) towards higher expression of HBeAg (Table 1) among the teenagers and 26-30 age groups. Oral pills are steroid hormone prepared in tablet form and have slower but longer action in the body stimulating the immune system for a longer period of time. They have been reported to immunosuppress (blind) lymphocytes by reducing the reactivity of T-lymphocytes and reduce immunoglobulin secretion (32; 33). Hence anaemia, effect of oral pills and other unanalyzed factors such as HIV and malaria may have contributed immensely to the endemicity of HBV infection among pregnant women in the study community, Logo LGA.

CONCLUSION

Since the virus can be transmitted from infected mother to the offspring especially at birth, the presence of HBV infection in women in general and pregnant women in particular calls for concern. Free screening of all pregnant women should be incorporated in the antenatal and post-natal infection programmes in hospitals to prevent potential infection of the infants by their infected mothers. The use of other HBV infection serological markers such as anti HBs, anti HBc, and HBe as well as HIV co-infection and cases of HBsAg positive women and the effects of the positivity on their babies is advocated for further studies.

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