IMMUNOLOGICAL/HAEMATOGICAL AND OPPORTUNISTIC INFECTIONS MONITORING OF RESPONSE OF SOME HIV POSITIVE INDIVIDUALS TO HAART ADMINISTRATION IN IMMANUEL HOSPITAL, EKET, NIGERIA.

[1]ETOK, N. A., [2]MBOTO, C. I. AND [3]EPOKE, J.
[1]*State Public Health Laboratories*
Idongesit Nkanga Secretariat, Uyo,Nigeria.
[2]*Microbiology Dept. University Calabar, Nigeria*
[3]*Faculty of Allied Medical Sciences*
University Of Calabar, Calabar, Nigeria.

ABSTRACT

This paper monitored the Immunological/haematological and opportunistic infections response of some HIV positive individuals to HAART administration in Immanuel Hospital Eket, Akwa Ibom State. A total of 188 stool and blood samples were respectively collected from HIV infected individuals within a period of 6 months. Seventy control subjects (individuals non-reactive to HIV antibodies test) were equally recruited. Macroscopic and microscopic analysis were deployed to identify intestinal parasites. The serial algorithm was employed for HIV screening while the cyflow counter and CYTECH CD4 Easy Count Kits were employed in the estimation of CD4. In all age groups, greater proportion of the study subjects were in the moderate immunity (CD4200-499). A higher incidence of both HIV and intestinal parasites (60% for HIV and 65% for intestinal parasite) was recorded by female participants. CD4 counts varied widely with the duration of HAART administration - the highest response of 62% being recorded by subjects who had been on HAART for over 1 year. Moderate to severe anaemia was a common feature among the HIV seropositive individuals with 89.9% having Hb blow 12.5gld. This observation was however not statistically significant (at $p \leq 0.05$, with P value 0.1939), but the observations of opportunistic infections namely *Trichomonas hominis* (p = 0.99998) and *Stongyloides stercoralis* (p = 0.9998) were significant. The research findings have shown that monitoring of HIV positive individuals on chemotherapy is essential in order to fully exploit its benefits and also identify development of resistance and adverse reactions among the patients.

INTRODUCTION

UNAIDS and WHO estimate that AIDS has killed more than 25million people since it was first recognized in 1981, making it one of the most destructive epidemics in recorded history. Despite recent, improved access to antiretroviral treatment and care in many regions of the world, the AIDS epidemic claimed an estimated 2.8million lives in 2005 of which more than half a million (570,000) were children (FMOH, 2006). Globally between 33.5 and 46 million people currently live with HIV. In 2005, between 3.4 and 6.2 million people were newly infected and between 2.4 and 3.3 million people living with AIDS died. Sub Sahara Africa remains by far the worst-affected region with an estimated 21.6 to 27.4 million people leaving with HIV (FMOH, 2006)

The presence of HIV infection in individuals can be ascertained only through the use of laboratory tests on body fluids such as blood, plasma/serum etc. The understanding of the transmission of HIV has given rise to various interventions which can prevent the occurrence of new cases. Reduction of viral load by efficient retroviral therapy is also a powerful tool in the overall interventions against HIV.

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HIV 1 viral load measurement has been found to be useful in monitoring treatment. It requires the establishment of a baseline plasma viral load before starting ART. The viral load in the case of successful ART becomes undetectable in 4 to 6 months after therapy. The assessment of viral load though possible is a very expensive, complex and sophisticated procedure more so in a developing economy. This work therefore seeks to use the prevalence of intestinal parasites in combination with haematological/immunological parameters to monitor the response of some HIV positive individuals to HAART Administration in Immanuel Hospital Eket, Nigeria.

MATERIALS AND METHOD

The study centre was the Immanuel Hospital Eket; Eket is a major city in the Niger Delta region of Nigeria. It is the second largest city in Akwa Ibom State after Uyo, the State Capital. Akwa Ibom State is located on the South-southern part of Nigeria, lying between latitude $4^0 32^I$ and $5^0 33^I$ North and longitude $7^0 25^1$ ad $8^0 25^1$ East and has a land mass of approximately 8, 412 sqkm. The study subjects were patients who were consecutively referred to the HIV/AIDS screening centre of the hospital by the consultants. A total of 188 patients who made an informed decision to participate were enlisted for the study between January and June, 2008.

Sample Collection

Stool samples were collected in clean wide mouthed, leak-proof containers earlier provided for the patients. While blood samples were collected as 8ml venous blood into two K_5EDTA containers for haemotological parameters, HIV screening and CD4 count evaluation.

Stool Analysis

Each stool sample was macroscopically examined for the presence of worms or worm parts. Precisely 1gm of sample (if sample was formed) was emulsified in 10ml of normal saline or 1ml (if watery) and mixed with 9ml of normal saline. Microscopic examination was done using both the direct smear and concentration techniques. The concentration method of choice was the formal ether technique. For the direct smear, a drop each of the earlier emulsified sample was placed on either ends of a microscope slide respectively. To one drop, a drop of Lugols iodine was added mixed and covered with a cover slip, to the other drop, nomal saline was added and equally mixed and covered with covership. Both smeers were then examined first with 10x objective and thereafter 40 x objective for the presence of parasites.

HIV Screening

The serial testing procedure was employed (FMOH, 2006). Three rapid test kits; determine HIV 1/2 (ABOTT, Japer Co. Ltd), Chembio HIV 1/2 stat pak (Chembio Diagnositic Inc., USA), and Bundi HIV 1/2 (Bundi International Diagnostic Ltd, Nigeria) were selected. A double ELISA result was considered confirmatory.

Haematological Analysis

Three parameters; Haemoglobin estimation (Hb) / packed cell volume (PCV); total and differential white blood cell count were assessed. The methods of Sood (2003) were employed.

CD4 Counts Estimation

The CD4 estimation was performed by the automated flow cytometry analyzer FACS (Beckton Dickson) CDC, 1997 CDC, 2003)

Statistical Analysis

The incidence of intestinal parasite according to age and gender, response of study subjects to HAART administration using immunological/haematological and parasitological monitoring were analysed, statistically using the Odds Ratio (OR) and probability at 95% confidence interval.

RESULTS

The distribution of HIV among the subject according to gender showed a higher incidence of HIV among females 61% than males 39%. This pattern was observed also in the incidence of

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intestinal parasites with 65% among females and 35% among males (Fig 1). Incidence of HIV according to age showed that age group 0-10 had 0% while 11-21 years had 4%. The highest with 63(30.9%) was recorded by age group 21 – 30 years. This was followed by 31-40 years and greater than 50 years with 19% and 22% respectively; and 41-50 years with 24%.

Incidence of intestinal parasites also varied, widely with the highest (35%) among age group 41-50 yrs and 0% among 20 years and below. Age groups 21 – 30 years, 31 – 40 years and greater than 50 years had 25%, 30% and 10% incidence respectively (Fig. 2). The most frequent intestinal parasite was *Entamoeba histolytica* (35%) followed by *Ancylostoma duodenale* and *Ascaris lumbricoides* (25.0%) while *Trichuris trichiura* was 5.0%. The opportunistic infections were *Trichomonas hominis* 5.0% and *Strogyhoidies stercoralis* 5.0%. (Table 1)
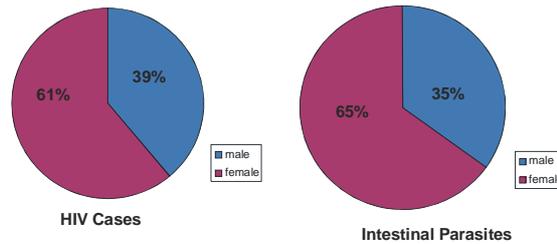

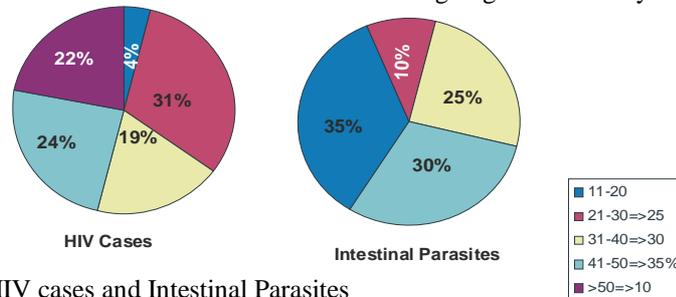Figure 1: Incidence of HIV cases and Intestinal Parasites according to gender of study subjects

Figure 2: Prevalence of HIV cases and Intestinal Parasites

Table 1: Intestinal parasites found in stool of HIV infected patients

Organism	Status	Number	Percentage	C.I	P
Ancylostoma duodenale	Non oi	5	25	95	0.99864
Ascaris lumbicoides	Non oi	5	25	95	0.99864
Trichuris trichura	Non oi	1	5	95	0.99998
Entamoeba histolylica	Non oi	7	35	95	0.99380
Trichomonas hominis	oi	1	5	95	0.99998
Stongyloides stercorhalis	oi	1	5	95	0.99998

The full blood counts (FBC) reports are shown in Table 2. The haemoglobin (Hb) of the study subjects ranged between 3 and 13g/dl; 89.9% of which fell within the classification for anaemia while 10.1% were normal. The total white blood cells counts (WBC) ranged between 3.3 and 8,200/L. The differential white blood cell count showed that the WBC population comprised neutrophils eosinophils, lymphocytes and monocytes basically.

Besides the 100% recorded by few study subjects in age group 0 -10 years at the low immunity category, all age groups recorded their highest percentage participants in the moderate immunity category (Fig. 3). Female subjects with 21% recorded the highest number of low immunity subjects but virtually tallied at high immunity level with 23.4% and 23.7% respectively for male and female (Fig. 4).
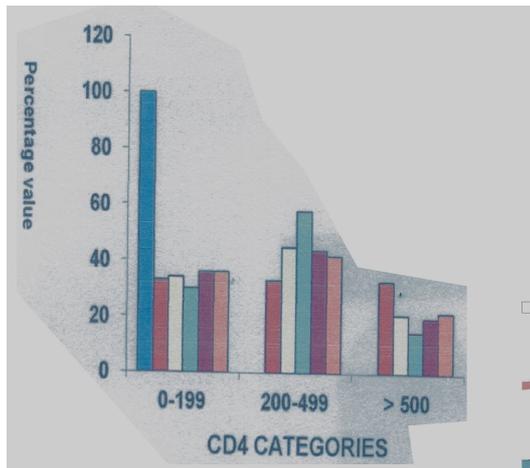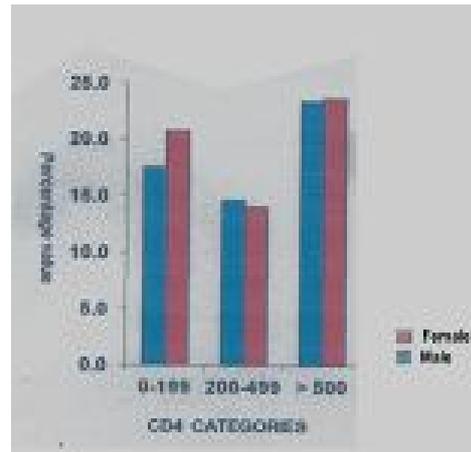
Figure 3: Influence of age on CD4 Counts

Figure. 4: Influence of gender on CD4 Counts

Table 2: Full blood count parameters

Parameters	No. Low	No. Normal	No. High	OR 8.89474	P
Hb	169(89.9%)	19(10.1%)	0	0.11243	0.1939
PCV	169(89.9%)	19(10.%)	0	0.11243	0.1939
WBC	15(8.0%)	173(92.0%)	0	0.08671	0.1542
Newtropuals	22(11.7%)	166(88.3%)	0	0.13253	0.2234
Eosinopuals	0	88(46.8)	100(53.2)	0.88	0.9183
Lymphocytes	0	0	0	NA	NA
Monocytes	188(100%)	188(100%)	0	NA	NA
Basophal	0	188(100%)	0	NA	NA

KEY

Parameters	Low	Normal	High
Hb	<12.5	12.5-14	>14
PCV	<38	38-42	>42
WBC	<4000/L	4000-10,000/L	>10,000/L
Neulrophile	<55%	55-76%	>70%
Lymphocytes	<25%	25-40%	>40%
Monocytes	<2%	2-8%	>8
Basophile		0-1%	

A summary of the influence of HAART on CD4 counts showed that 48% of the participants on HAART were still on low immunity (0-199/ul), 33% progressed to moderate immunity (200 – 499) while only 20% could graduate to high immunity level (≥500/ul) (Fig 5)

CD4 counts were found to vary widely with duration of HAART administration (Fig. 6). The results of 42% subjects who had been on drug below one month still had low immunity. High and moderate immunity respectively were 27% and 30% respectively. Similar observations were made on low, moderate and high immunities respectively at other time intervals 52%, 35% and 6% for those who had been on HAART upto 6 months but less than one year; 30%, 24% and 34% respectively for those who had lasted up to one year, 23%, 8% and 58% for those who had been on HAART for over one year.

Control subjects were individuals with confirmed HIV seronegative status having normal full blood counts compliments at an Haemoglobin (Hb) level of 13.0g/dl and above. Results are shown in Table 3. A total of seventy (70) volunteers were used as control with 39% intestinal parasites incidence.

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Table 3: Control

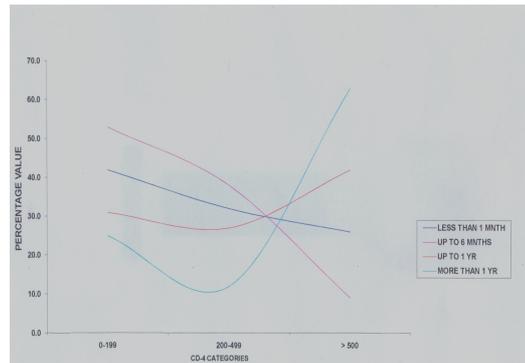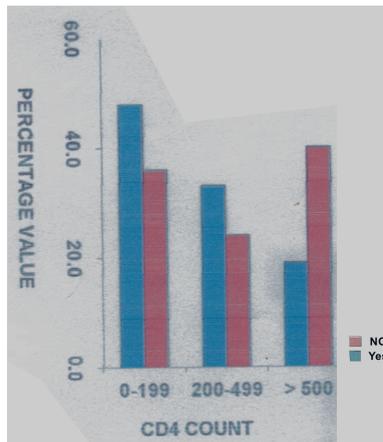| Organism	Status	Number	Percentage	C.I(%)	P
Ancylostoma duodenale	Non 01	7	26	95	0.15209
Ascaris lumbicoides	Non 01	9	33	95	0.16709
Trichuris trichura	Non 01	5	19	95	0.1351
Entamoeba histolylica	Non 01	6	22	95	0.14391
Trichomonas hominis	01	0	0	NA	NA
Stongyloides stercorhalis	01	0	0	NA	NA
Total		27	100		

Figure 5: Influence of HAART on CD4 Counts Fig. 6: Summary of Influence of Duration of HAART

DISCUSSION

Monitoring of patients on chemotherapy is essential in all infectious diseases. It is of greater importance in HIV because of the severity of illness, the potential of the virus to mutate and become resistant to drugs and the cost of treatment. Hence Laboratories are expected to play a critical role in the successful implementation of Anti Retroviral theraphy. Various areas are recommended for monitoring. Our findings underscore the significance of parasitological, haemotological and immunological monitoring of response of HIV positive individuals to HAART administration.

The patients were found to habour various species of helminthes, protozoans and flagellates. The overall incidence of intestinal parasites was found to be significantly higher in HIV patients than HIV non-infected controls. This is probably due to easy detection of parasite in patients infected with HIV. Since parasite establishment is facilitated, it will increase the load of parasites, hence they can be easily detected and identified in stool examinations. Opportunitic parasites were found exclusively in HIV/AIDS patients and not in HIV seronegative controls.

There is much circumstantial and some direct evidence to suggest that parasite are an important or most obligatory factor in AIDS. First, AIDs has not emerged as an over whelming epidemic among heterosexual, in the developed (and largely parasite free) countries of North America, Europe and Australia. This strongly suggests that there is an environmental components to AIDS in the tropical regions of the world (Redfield 1980 and Peace 1989). Second Ettiopians with HIV died two to three time more quickly than those infected in the West who were without parasites. (Kalinkovich, 1996). Third, AIDS was induced in cats raised and housed under pathogen free conditions by infusing plasma pooled from cats with fenal immunodeficiency virus (FIV) (Diehl *et al*, 1995).

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There is good evidence that if the level of HIV remains suppressed and CD4 count remains greater than 200, then the quality and length of life can be significantly improved and prolonged. However, a relatively high CD4 count (greater than 200/µl) is sometimes but not inevitably associated with superior viral responses and a CD4 cell count less than 50/µl has a relatively poor probability of a good virologic response (Mastroianni *et al* 2000). Our findings showed that at the time of study 33% of the subject had graduated from low to moderate (200 – 499) immunity while 20% recorded high immunity (greater than 500/µl). The probability is that these patients at these levels had started experiencing marked reductions in their viral load counts. Barllett, (2000) who reported an inverse correlation between baseline CD4 cell count and probability of achieving a viral load less than 500c/ul with HAART. Although 33% and 20% appear low, it could be considered reasonable since Stanley and Hams 2003 in a Swiss cohort study reported that there is a reverse correlation between response and the extent of prior antiretroviral therapy with regard to number of agents, number of classes and duration of treatment. For example, the probability of achieving a viral load less than 500c/ml with HAART therapy was 91% in treatment naive patients compared with 75% in treatment experienced patients. Similar arguments possibly hold for changes in CD4 counts with changes in the duration of HAART administration. Since our study population was already exposed to HAART, a low rate of progression from low CD4 counts to high as time progressed was apparent. Stanley and Hams (2003) said that among patients who achieve undetectable virus, the probably of maintaining a viral load less than. 500c/ml at 2 years is 80% for treatment naïve patients compared with 62% for treatment experienced patients. The conclusion of many authorities is that the initial regime is the most important because it is associated with the greatest probably of achieving prolonged viral suppression (Amuron *et al* 2007).

Variations of results according to sex and age tended to follow a similar pattern for all of HIV, intestinal parasites and CD4 counts. The high prevalence of HIV/intestinal parasites among females than males, though in contrast with the results of Savioloh *et al* (1992) was in consonance with the work of Mbuagbaw *et al* (2006), and Vant Wout, *et al* (1996). All age groups had their best performance in the moderate CD4 category (200-499/µl). This indicates the efficacy of the Anti Retromoral Therapy. However age group 30-39 years demonstrated the highest value of 58% which suggest that given the availability of HAART, the middle age responds faster to treatments.

Anemia is defined as a reduction below normal levels of calculated red blood cells or the quantity of hemoglobin. In this study patients with Hb $\leq$ 12.5 were considered anaemic (Blockman, 1990; Amegor *et al*, 2009). For years Anemia is an important clinical profile in HIV/AIDS and frequently seen in the advanced stages of the disease. In the late 1980s to early 1990's blood transfusion used to treat anemia in setting HIV disease were found to be associated with a significant increase in mortality in patients with AIDS, and recombinant human erythropoietin (epoetin alfa, procrit) was approved as an alternative treatment option for anemia in HIV/AIDS (Amergor *et al*, 2009).

CONCLUSION AND RECOMMENDATION

HIV related anaemia can involve multiple casual mechanisms, and often several of these are operative in the same patient at the same time. These casual mechanism include HIV itself, which can induce chronic inflamation and slow red blood cell production. In addition drug toxicities, opportunistic infections, malabsorbtion syndromes leading to folate or Vit B12 deficiency, blood loss, iron deficiency, lymphoma and other AIDS related malignancy can all cause or contribute to anaemia.

With the increase of HAART, there is a decline in malignancies, and inflamation related malabsorbtion syndromes. Also, HAART, may reduce HIV chronic inflamation. All of these factors can improve anaemia, and are most likely responsible for decrease in case of severe anaemia noted during the HAART era. This study indicates that inspite of these advantage mild

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to moderate anaemia remained common among the study subject and so underscores the importance of monitoring anaemia and maintaining normal hemoglobin levels as a treatment goal even as anti retroviral therapies continue.

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