Detection of Hepatitis C Virus Antibodies among HIV patients on HAART attending a General Hospital Northern- Nigeria.

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Abstract: Hepatitis C virus is a life threatening viral infection of the liver, while co-infection with human immunodeficiency virus (HIV) is a growing public health concern. The study was conducted among confirmed HIV infected patients attending the Talata - Mafara General Hospital. One hundred and Ninety two (192) serum samples were assayed for the presence of antibodies to HCV, using a third generation enzyme linked immunsorbent assay. A well-structured questionnaire was administered to volunteer subjects after consent approval to obtain demographic and other relevant Data. Overall result showed that 37(19.3%) of the subjects screened had antibodies to HCV, the prevalence of HCV was higher among males subjects with 25(13%) positivity compared to the females subjects with 12 (6.3%). Considering prevalence based on age, individuals of the age group 21-30 and 31-40 recorded a higher prevalence of 7(2.6%) and 10(5.2%) respectively. Based on marital status a high prevalence of 14(7.2%) was recorded among widows and widowers. Educational status showed that subjects with secondary education recorded a prevalence of 12(6.2%) positivity. From this study it is paramount that adequate diagnosis of the Hepatitis C Virus be carried out in people with HIV infection so as to reduce further complications among co-infectious subjects in the population.

1. **Introduction**:

Human Immunodeficiency Virus (HIV) is a major public health concern and cause of death in Africa. Although Africa is inhabited by over 14.7% of the world's population, it is estimated to have more than 88% of people living with HIV and 92% of all AIDS death in 2007 (UNAIDS, 2008). Life expectancy of patients with HIV has increased and focus has now shifted to the management of concurrent illness such as chronic HCV infection and other co-infections which have the potential to increase long term morbidity and mortality (Munshi *et al.,* 2008). Hepatitis C virus (HCV) is an RNA virus with envelope, a single stranded, of positive sense that belongs to the family flaviviridae (Lauer and Walker, 2001). It is RNA dependent, RNA polymerase, small measuring between 30-60nm in diameter. 170 million people (about 3%) of global population are chronically infected with HCV, and most of these cases occur in Africa with the highest prevalence (WHO, 2004). HCV strains exhibit considerable variation in genomic structure. Hepatitis C virus (HCV) is mounting a global health challenge, causing a significant proportion of chronic liver disease around the world. In understanding the long term outcomes of HCV infection among HIV patients, clinicians may need to identify the patients at risk for HCV-related complication and offer treatment to prevent further morbidity and mortality, (Choo *et al.,* 1989). Chronic hepatitis C is the most common cause of chronic liver disease and cirrhosis, and the most common indication for liver transplantation in the United State, Australia, and most of Europe (Wasley and Alter, 2000). About 60-70% of patients with acute HCV infection may progress to chronic infection. Similarly, Infection with HIV leads to a progressive distribution in the number of T-cells possessing CD4+ receptors (Ryu *et al.,* 1994). Progressive depletion of CD4+ T-cell lymphocytes is the cardinal events in the pathogenesis of infection by the HIV-1. Enumeration of CD4+ lymphocytes may aid in the diagnosis or prognosis of immunodeficiency as AIDS. Not only is the CD4+ T-cells enumeration important in classification of disease severity, it is equally important is assessment of response to HAART (Akanmu *et al*., 2003, Awalu and Chukwedo, 2008). The lower the CD4+ count (normal counts are usually between 500cell/µl – 1600 cells/µl), the greater the likely damage of HIV to such individuals (CDC, 2008). It has also been discovered than Hepatitis C Virus, which shares a common mode of transmission is prevalent in HIV positive individuals,(Santiago, 2005).

2. Materials And Methods

Study area

The project was carried out amongst HIV – infected individuals whose CD4+ count were determined when they are confirmed HIV-positive at General Hospital Talata-Mafara in Zamfara State. The work was carried out between July – September, 2010.

Subjects

Confirmed HIV patients attending (GHAIN) laboratory in General Hospital Talata Mafara within the locality.

Sample Size

A total of 192 seropositive HIV patient attending General Hospital Talata Mafara, Zamfara state within the age bracket of 11-60years.

Questionnaire

Structural questionnaire comprising of subjects information on their biodata e.g. sex, age, marital status, family history on HCV were administered simultaneously to the subjects so as to know their demographic data in the analysis of the results obtained.

Ethical Clearance

Ethical clearance was sought for and obtained from the Hospital Talata Mafara management through the chairman, ethical clearance committee.

Specimen Collection and Storage

4mls use whole blood specimen collected by venipuncture in evacuated blood collection K2EDTA or K3EDTA containers. Samples that are not immediately used should be stored at 18-220C but the test should be performed within 48hours but no later than 72 hours after the blood specimen (kept at room temperature) is drawn. The CD4+ count of 190 blood specimen of HIV – infected persons were estimated and monitored after period of 3 months using the facscount machine.

Scope of Study

They CD4+ count of 192 blood specimen of confirmed HIV-infected person undergoing HAART; highly active antiretroviral therapy and suspected hepatitis C case of those infected patients were estimated and monitored after a period of 3months using the facscount and 3rd generation enzymes – linked immune sorbent assay for testing of hepatitis C virus.

Principle of enzyme immunoassay

Multiple epitopes of HCV proteins are bound to the microtitre wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and attach to the solid phase. No reactive antibodies are removed with the wash buffer. Human lgGs bound to the antigen are reacted with goat – anti – human lgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue colour. No colour or very pale blue colour indicates a negative reaction. The intensity of the reaction is photometrically quantities.

Specimen Collection and Preparation for sreening:

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid haemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at 20^{0}C or colder may be used. Avoid repeated freeze thaw.

3. Result

192 samples were screened and confirmed seropositive and their CD4 lymphocyte count were estimated. From the result 115 female patients tested positive, while about 75male patient were reactive to the test (HIV seropositive).

Among the 192 HIV positive subjects 37(67.5%) tested positive for HCV antibodies with 29 males and 12 (32.4%) females, as seen in Table1.

Table 2 showed distribution based on sex ,a higher rate of infection were recorded among male subject aged 31-40, with 5.6% prevalence while females aged 21-30 recorded 2.6 positivity.

Table 3 showed distribution of HCV based on marital status with the high prevalence rate of 7.2% in males, while the females recoded (0%) rate.

Table 1: Overall Sample Distribution Of Patients Based On Sex

Sex	No of subject screened (HIV)	HCV screened	% positive
Male	77(40.1%)	25	13.0
Female	115(59.8%)	12	6.3
Total	**192(99.9%)**	**37**	**19.3**

Table 2: Age Distribution Of Hcv Positive Subjects

Age (Years)	Male	Female	Total
11-20	(0%)	1(0.5%(	1(0.5%)
21-30	5(2.6%)	7(3.6%)	12(6.2%)
31-40	10(5.2%)	2(1.0%)	12(6.2%)
41-50	8(4.1%)	2(1.0%)	10(5.1%)
51-60	2(1.0%)	–(0%)	2(1%)
Total	**25(12.9%)**	**12(6.1%)**	**37(19%)**

Table 3: Distribution Of Hcv Positivity Based On Marital Status

Marital status	Male	Female	total
Monogamy	6(3.1%)	8(4.1%)	14(7.2%)
Polygamy	3(1.5%)	3(1.5%)	6(3.1%)
Widow/widower	14(7.2%)	0(0%)	14(7.2%)
Divorce	1(0.5%)	1(0.5%)	2(1%)
Single	1(0.5%)	(0%)	1(0.5%)
Total	**25(12.8%)**	**12(6.1%)**	**37(19%)**

Table 4:Distribution Of Hcv Based On Educational Status Of Positive Patients

Educational status	Male	Female	Total
Non-Formal Education	(0%)	2(1.0%)	4(1.0%)
Primary Education	10(5.2%)	2(1.0%)	12(6.2%)
Secondary Education	12(6.25%)	8(4.1%)	20(10.3%)
Tertiary Education	3(1.5%)	(0%)	3(1.5%)
Total	**25(12.9%)**	**12(6.1%)**	**37(19%)**

Table 4 which was based on Educational status shows its highest rate of 6.25% with those having secondary Education, while it is 0% in females.

4. Discussion

Anti-retroviral drugs has transformed HIV/AIDS from a uniformly fatal illness into a manageable chronic infection and has been shown to be able to restore CD4 + cells in HIV infected patients (Rathbun *et al.,* 2006). The gains of HAART could be compromised by co-infection with hepatitis viruses as they are known to have adverse effects on the prognosis of HIV and hepatitis infections (Feld *et al.,* 2005) consequently increased attention has to be paid on co-infection of hepatitis viruses and HIV especially in the developing countries like Nigeria where these groups of viruses are endemic. In this study out of the number of patient screened (39.0%) were male with HIV positive status while (59.8%) were positive for female. It shows that there is a higher prevalence of infection in the female folk compared to the male in Zamfara State which could be as a result of mass illiteracy among the females , early marriage, and polygamous lifestyle , which encourage the spread of the virus. The study also reveals HCV seroprevalence rates of (19.3%) in group of HIV – 1 infected individuals samples, with a (13.0%) HCV positivity for the male and (6.3%) for female, which is similar to the study comes carried out in Jos by Alabi *et al.,*2007),who recorded 11.1% seropositivity among the subjects.

Previous studies in Nigeria has reported an overall HCV prevalence of (2.9%) among blood donors in Rivers State of Nigeria (Kaote *et al.,* 2005), (Agwale *et al.,* 2004) also recorded an HCV seroprevalence of 8.2% among HIV infected Nigerians. There is a clear indication of increased HCV infection in HIV infected individuals in Nigeria. It is known that HIV/HCV co-infected individuals accelerate rapidly to end – stage liver disease, AIDS

defining clinical event and death (Greub, 2000, Manga *et al.,* 2001).

In Table 2 Age distribution of HCV positive subjects shows a marked prevalence among subject aged 31-40 in males (5.2%) compared to the female (3.6%) of age group 21-30. In our location of study it was observed that the male subject in this study inclined to drug abuse such as IVDU from the use of syringes, sharing of infected instrument like razor, clipping of nails with unsterilized manicure instruments, while is a propable avenue for the spread of the virus as transmission involves blood contact. Table 3 showed distribution of HCV positive individuals based on marital status with a high prevalence rate of HCV among the widow/widower, having (7.2%) prevalence, there is no significant rate with the divorce and single group with a (0.5%) and (0%) respectively, however the monogamous and polygamous group both have (3.1%) for the males and (4.1%) for the females.

Table 4, shows prevalence rate distribution based on educational status with a high rate of infectivity recorded among those with Secondary Education for the male subjects with (6.25.%)positivity compared to the females with (4.1%). The result obtained in this study based on educational distribution; is similar to the work of (Kuo *et al.,* 2006) where it was observed that despite the high HCV prevalence less than 50% had any formal education, out of these less than 20% was aware of HCV, which underscore the need for expanded education and public enlightenment on the hepatitis C virus. While HIV and HCV educational prevention programmes be focused to reduce the stigma HIV and HCV co-infection of those having secondary education. The average CD4 count for the female shows (279 cell/μ) compared to the male of (1509 cell/μ). Infection with HIV/HCV has been reported to have therapy (Ranieri *et al.,* 2002) as a case seen a 35years old female client co-infected with HIV and HCV who virologically failed therapy (CD+ decline from 199 to 66) after four months of HAART, there is also records that, the rate of increase in CD4+ cells post – HAART does not change in HIV and hepatitis co-infection. Although there have been a case reports of clearance of HCV viraemia after initiation of HAART (Ranieri *et al.,* 2002), majority of available data indicates that HAART results net increase in HCV viraemia (Change *et al.,* 2002). Triple coinfected individuals i.e. HIV/HRV/HCV are more likely to present with lower CD4+ counts and therefore reduced host immunity. Triple co-infection is therefore a growing problem in Nigeria and needs careful attention owing to its adverse effects on HIV treatment response, unfortunately however, no effective vaccines has been developed against HCV infection. The study therefore confirms the endemicity

of hepatitis C (HCV) anti body detection among HIV seropositive individuals.

Conclusion

Based on the analysis and result obtained it is recommended that, upon confirmation of HIV infection, all patients should also be tested for HCV infection while CD4+ FACS counting facility be employed to enable easy determination of patient CD4+ count. Combined therapeutic regimen should be administered to individuals co-infected with HIV and HCV immediately to avert further complications. Importantly free voluntary testing at no cost should be made available and accessible to patient who wishes to know their HCV status.

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