Susceptibility of Human Blood Groups to Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) Infections.

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Abstract

Susceptibility of blood groups to HIV/AIDS and Hepatitis B virus infections in human subjects was studied. One hundred and fifty adult male and female patients who came for routine check up at the Heart to Heart Centre, General Hospital Calabar and the General Hospital Ogoja in Cross River State were used for this investigation.The patients were classified into three groups with group I consisting of sixty (60) normal subjects (i.e. non HIV/AIDS and non HBV patients) serving as control. Group II was made up of sixty (60) HIV/AIDS infected patients only and group III had thirty (30) patients co-infected with HIV/AIDS and HBV. Blood and serum samples were collected and screened for both HIV/AIDS and HBV. The blood and serum samples were assayed for totalprotein and bilirubin levels, aspartate aminotransferase(AST), alanine aminotransferase(ALT) activities, antioxidants vitamins levels and determination of blood groups. The results showed that group O+ had increased levels relative to control and others,suggesting that patients with blood group O+ are more susceptible to HIV/AIDS and HBV infections. The results showed that blood group B+ had decreased level relative to the control and other blood groups, suggesting that patients with blood group B+ may resist HIV/AIDS and HBV infections. The results also revealed that AST and ALT activities were significantly increased, suggesting hepatic peroxidation and liver damage. The results also showed that the antioxidant vitamins(vitamin A and vitamin C) were significantly decreased, suggesting high level of free radicals leading to hepatic peroxidation and liver damage.

Key Words: HIV/AIDS infection, HBV infection, Aspartate aminotransferase, Alanine aminotransferse

Introduction

The Acquired Immunodeficiency Syndrome (AIDS) is global problem caused by Human Immunodeficiency virus(HIV) which can be contracted through sexual contact ,exposure to blood including sharing contaminated needles and syringes and by blood products or other body fluids. Human immunodeficiency virus infection/Acquired immunodeficiency syndrome has been the leading cause of death among young adults in the United States and has a devastating impact on people in developing countries (Horowitz *et al.,* 1998).

AIDS was first reported by Gottlieb *et al.,* (1981) in the USA, with daily new

infections of about 800,000 and high mortality rate (Goulder and Watkins, 2004). Human immunodeficiency belongs to a retrovirus of the lentivirus subgroup. There two major types. HIV- 1 and HIV- 2. HIV-2 is found more prominently in West Africa (Hoffbrand, 2002). Diagnosis is confirmed by the presence of antibodies to HIV or detection of HIV RNA in plasma. The blood count reveals a progressive lymphopenia and fall of CD4:CD8 ratio from the normal value of 1.5-2.5: 1 to less than 1:1. There is also a rise in serum immunoglobulins (Hoffbrand, 2002). Other available methods for screening HIV include ELISA kits with a confirmatory test using Western blot technique.

Hepatitis B virus is one of the five groups of hepatitis virus so discovered. The term hepatitis refers to inflammatory processes of the liver. The most common causes are hepatitis A, B, C, D and E viruses. Other causes of hepatitis include alcohol and drugs. (Attah, 2002). Hepatitis B virus (HBV) is a double stranded DNA virus of the Hepadnaviridae family. It was identified in the 80's and found to be responsible for 70-90% of post transfusion hepatitis in many countries (Lok *et al.*, 2007). The virus is transmitted by blood and blood products. The low rate of HBV infection in high risk groups such as homosexuals and prostitutes suggest a limited role in sexual transmission. An estimated 2000 million people are infected with this virus worldwide (Has Lett, 2002).

Co-infection with human immunodeficiency virus type 1(HIV-1) and hepatitis B virus (HBV) is common and of increasing clinical relevance. Relatively rapid progression of HBV- mediated liver disease has been described in individuals with HIV-1 related immunodeficiency (George *et al.*, 2001). In co-infected individuals HBV viral loads tend to be higher than those infected with HBV alone. This may accelerate progression from HIV-1 infection to AIDS (George *et al.,* 2001).

There have been reports of people with certain blood groups being more susceptible to certain diseases such as group O (Ganong, 1997). The membrane of human red cells contains a variety of blood group antigens which are also called agglutinogens. The most important and best known of these are the A and B antigens. The A and B antigens are inherited as Mendelian dominants and individuals are divided into four major blood types on this basis. Type A individuals have the A antigen, type B have the B antigen, type AB have both and type O have neither A or B antigen. (Ganong, 1997).

It has been reported that certain conditions have higher incidence in certain ABO blood groups. For instance, group O subjects have high risk of gastrointestinal tract ulcers (Ganong, 1997). Thus certain blood group may be more susceptible to HIV/HBV infections. The levels of mineral elements and vitamins have been shown to decrease in chronic hepatitis B infection. This could be due to poor metabolism of these mineral elements by the damaged liver (Javier et al, 1990). Liver enzymes, aspartate aminotrasferase and alanine aminotrasferase and bilirubin levels have been shown to increase in patients infected with hepatitis B virus and human immunodeficiency virus in cases where there is co- infection (Attah, 2002).

The present research is aimed at finding a link between blood groups and HIV/HBV infections as well as the role of antioxidants and their status in HIV/HBV patients.

Materials and Method

Sixty HIV positive patients attending weekly routine clinical checkup at the Heart to Heart centers in General Hospitals Calabar and Ogoja in Cross River State of Nigeria were recruited for this study. The blood samples of sixty HIV free blood donors were also used as the control. Thirty patients with both HIV and HBV infections were also used in this study. The blood serum samples were collected at intervals of two days and refrigerated at – 7ºC before analysis.

Experimental Design

The design consisted of sixty (60) HIV positive patients (comprising of 30 males and 30 females) assigned group I. The control was

made of sixty (60) HIV negative subjects (30 male subjects and 30 female subjects) assigned group II and thirty (30) HIV and HBV patients (co- infected patients of 15 male and 15 female patients) assigned group III. The determination of HIV status was done using ELISA kits method described by Weniger *et al*. (1994).

Methodology

Determination of Total Serum Protein Level.

Total serum protein level was determined by the Biuret method described by Gornall *et al.* (1949). To 0.5ml of the sample solution was added 1.0ml distilled water to bring the volume to 1.5 ml in each tube. Tube 1, the blank received 1.5ml distilled water. The suspension was mixed and 0. 2ml of 5 % sodium deoxycholate (DOC) in 0.01N KOH was added and mixed to make the suspension more soluble. Then 1.5ml of Biuret reagent (1.5g $CUSO_4$. $5H_2O$, 6.0g sodium potassium tartrate and 300ml of 10% NaOH per litre) was added (including the blank). The contents in each tube were mixed in a vortex mixer and the absorbance read at 50nm in a 6400/6405 spectrophotometer (Jenway Essex, England) against the reaction blank. The concentration of the standard bovine serum albumin (BSA) was 2mg/ml (1.0g BSA was dissolved in 500ml H_2O). 10% NaOH was prepared by dissolving 100g NaOH in 1 litre of distilled water.

Determination of Alanine Aminotrasferase and Aspartate Aminotransferase

The measurements of the concentrations of these enzymes were done by spectrophotometric determination of their absorbances, using analytical grade laboratory reagents. The laboratory reagent kits from Biosystems laboratories (S.A Costa Brava, Barcelonia, Spain) were used to assess the concentration of alanine aminotransferase and aspartate aminotrasferase in the serum.

Determination of Serum Bilirubin concentration.

Reagent kits from Randox Laboratories (United Kingdom) were used to assess the concentration of bilirubin in the serum. All absorbances readings were taken with DREL 300 HACH model spectrophotometer.

Determination of vitamin A

The spectrophotometric method adopted by Toro and Akerman (1975) was used. Vitamin A in the sample was extracted by treating a 3ml portion of the sample with 3ml of absolute ethanol followed by 6ml of pure hexane. After centrifugation the upper hexane layer was used for analysis as described below.

- An aliquot of hexane extract (2ml) was evaporated to dryness over a water bath at 600ºC and the residue was dissolved in 0.5ml chloroform. The solution was then treated with 3 ml of trifloroacetic acid – chloroform reagent, after mixing well the absorbance was read in a spectrometer at a wave length of 620nm against a reagent blank at zero. Similarly, a standard vitamin A solution was prepared and diluted to a chosen concentration (0.4µg/dl). 1 ml of the standard vitamin A solution was treated as discussed for the sample and its absorbance was also read at the same wave length (620nm). The vitamin A concentration was calculated from the readings using the relationship below.

Vitamin A= 100/V x au/as x C
Where
V = volume of sample used
au= absorbance of sample
as= absorbance of standard vitamin A solution
C= concentration (µg/dl) of the standard vitamin A solution
Normal value for vitamin A ranges between 25 – 70 µg/dl (Toro et al, 1975)

Determination of vitamin C

In spectrometric method described by Toro and Ackerman (1975).2ml of the sample was used to assay for vitamin C and 3ml of methaphosphoric acid solutions are mixed well then centrifuged. 2ml of the supernatant was then treated with 0.5ml of 0.15M Nacitrate solution. Similarly, 2ml of vitamin

C standard solution was treated with Nacitrate solution in a separate tube to serve as standard while reagent blank was set up 0.3ml of distilled water was added to each tube mixed well and followed by 1ml of working dye solution (indophenols) and mixed well. The absorbance was read in a spectrometer at 520nm with the reagent blank at zero.

A few crystals of ascorbic acid were added to each of the tubes and the colour decolorized. Their respective absorbance was read at the same wave length. The obtained readings were subtracted from the initial reading, to obtain the current readings using the relationship below to calculate the concentration of vitamin C.

Vitamin C (mg/dl) = au/as x C
au= absorbance of sample
as= absorbance of standard vitamin C solution
C= concentration of standard vitamin C
Normal values for vitamin C range between 0.5- 2.0mg/dl (Toro et al).

Statistical Analysis

Data collected were expressed as mean ± SEM. Statistical significant between the groups was determined by analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

Results

Table 1 presents the percentage distribution of blood genotype groups among non-HIV/HBV subjects (control group), HIV patients and, HIV and HBV co-infected patients. The percentage distribution of group A in the control versus the HIV infection group was not statistically significant. The value did not change even in HIV and HBV co-infection group. Blood group B showed significant decrease ($p< 0.05$) in HIV infection. The control group versus HIV and HBV co- infected group showed significant decrease. 25.00% versus 1.30% ($p< 0.05$). The results showed that patients with blood group AB were more infected when compared with control and other study groups. The AB blood group population in the HIV infected group increased significantly.

($p< 0.05$) when compared with the control. The values obtained were (control group versus HIV infected group) 3.40% versus 25.00%. Also the HIV and HBV co-infected group showed significant ($p<0.05$) increase in AB blood group population when compared to the control group. The values obtained (control group versus HIV and HBV co-infected group) were 3.40% versus 16.60%.

Table 2 shows the effect of HIV and HBV infection on enzyme activities, bilirubin concentration and total serum protein. The aspartate aminotransferase (AST) levels in the HIV group and the HIV/HBV co-infected group showed an increased ($p<0.05$) when compared with the AST levels in the normal non HIV/ non HBV group. The alanine aminotransferase (ALT) levels in the HIV and the HIV/HBV co-infected group showed a remarkable increase ($p<0.05$) when compared to the control group. The HIV group showed a significant increase ($p<0.05$) in ALT levels when compared to the control group (control group versus HIV group) 21.90± 1.7(u/l) versus 43.70±2.6(u/l). The HIV and HBV co-infected group showed a significant increase ($p<0.05$) in ALT levels when compared with the control group. The values obtained were (control group versus HIV and HBV co-infected group) 21.90±1.7(u/l) versus 43.80±1.5 (u/l).

The results showed that there was significant increase ($p<0.05$) in the serum total protein levels of the HIV infected and HIV/HBV co-infected when compared with the control. Table 2 also showed that the bilirubin levels in the HIV group and HIV/HBV co-infected group increased ($p<0.05$) when compared to the control. However, the increases in bilirubin levels in both cases were not statistically significant.

Table 3 presents the results of the antioxidant vitamins (vitamin A and vitamin C) levels. The results showed that there was significant decrease ($p<0.05$) in their values when compared to those of the control. The vitamin C values obtained (non-HIV/non-HBV group versus HIV infected group) were 17.8±0.1 mg/dl versus 10.4±0.4mg/dl. Also the vitamin C levels in the HIV/HBV co-

infected group showed a significant decrease ($p<0.05$) when compared to the control. The values obtained (non-HIV/non-HBV versus HIV and HBV co-infected group) were 17.8±0.1 mg/dl versus 10.2±0.8mg/dl.

The vitamin A level in the HIV infected group showed a significant decrease ($p<0.05$) when compared to the control group. The vitamin A levels in HIV and HBV co-infected group also showed a significant decrease ($p<0.05$) when compared to the control.

Table1: Percentage distribution of blood genotype groups

Type of sample	Blood Group A+	Blood Group B+	Blood Group AB+	Blood Group O+
Control	16.60%	25.00%	3.40%	55.00%
HIV infected group	16.00%	3.00%*	25.00%*	56.00%
HIV and HBV co -infected group	16.60%	1.30%*	16.60%*	65.30%

% n=60, HIV and HBV n=30; *significantly different from control at $p<0.05$

Table 2: Effect of HIV/HBV infections on enzyme activities, bilirubin concentration and total serum protein

Type of sample	Serum AST	Serum ALT activities(μ/l)	Bilirubin(μmol/l)	Serum protein(g/dl)
Control	29.5±0.5	21.9±1.7	12.6±0.8	66.6±1.2
HIV infected group	31.7±3.0	43.7±2.6*	16.5±3.3	76.7±1.4
HIV and HBV co - infected group	34.5±0.6	43.8±1.5*	18.4±0.5	74.8±3.0

Mean ± SEM n=60,HIV/HBV n=30; * significantly different from control at $p<0.05$ using ANOVA and t test.

Table 3: Effect of HIV and HBV infections on antioxidant vitamins

Type of sample	Vitamin C(mg/dl)	Vitamin A(μg/dl)
Control group	17.8±0.1	56.2±0.4
HIV infected group	10.4±0.4*	31.2±1.5*
HIV and HBV co-infected group	10.2±0.8*	30.7±0.4*

Mean± SEM n=60, HIV/HBV n=30 * significantly different from control at $p<0.05$ using ANOVA and t test.

Discussion

The results showed that serum bilirubin and protein levels as well as aspartate aminotransferase and alanine aminotransferase activities showed an increase in patients infected with either HIV or HBV or both infections. Aminotransferases are released from hepatic cells when damage occurs. The serum levels of these enzymes are particularly elevated in hepatocellular disease resulting in cytoplasmic leakage of these enzymes into circulation (Tredger *et al.,* 1997).

The study showed that blood group O has the highest prevalence of infections, suggesting that this blood group could be more susceptible to HIV/HBV infections when compared to other groups. This result is in agreement with the findings of Clark et al (1959). On the other hand, subjects with blood group B show decreased susceptibility to HIV infection. It may be assumed that B genotype persons have highest degree of natural resistance against HIV infections (Clark *et al.,* 1959).

The result from this study shows a significant reduction in the values of vitamins A and C in HIV and HBV patients compared to the control group. (Allard *et al.* 1998) showed that naturally occurring antioxidants, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, vitamin C,

E and A normally protect against damage to cells caused by free radicals. During HIV infection, it has been suggested that an abundance of free radicals are produced in an environment in which the antioxidant compounds are progressively depleted (Allard *et al*. 1998). Supplementation with vitamins C, E and A increased the antioxidant vitamins (Allard *et al.* 1998).

Conclusion

This study shows that certain blood groups are more susceptible to HIV/HBV infections than others, and that HIV/HBV infections may increase hepatic aminotransferase activities which may result from liver damage. The study also shows that HIV/HBV infections may deplete antioxidant vitamins resulting in hepatic per oxidation.

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