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Immunoglobulin Levels in HIV Patients And Abo Blood Group: Is There A Relationship?

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ABSTRCT

This study aims at determining relationship between the ABO blood group and the level of immunoglobulin classes in patients infected with Human immunodeficiency virus type 1 (HIV-1). One hundred and fifty three (153) confirmed HIV-1 positive subjects were enrolled in the study. These comprise of 62 (40.5%) males and 91 (59.5%) females. Sex and age-matched HIV-negative control were also recruited. The subjects comprise of adults aged 18-55 years with 36 years as the mean age. The study was conducted at the CDC-UNTH ART clinic Enugu after obtaining ethical approval from the relevant authority.The total protein, globulin, albumin IgG, IgM and IgA were assayed for both the HIV-positive patients and control subjects. The standard tube technique for both cell and serum grouping was used in determining the blood groups of the patients and control group under the study. The total protein, albumin and immunoglobulins levels were all determined using the Roche/Hitachi 902 automated analyser .The assay principle of the total protein and albumin was colorimetric assay while that of immunoglobulins was turbidimetric. Globulin assay was determined by finding the difference between total protein and albumin values. Descriptive statistics which include means, standard deviation (SD), frequency and percentage were used to analyze categorical and continuous variables. Differential statistics which include chi-square was used to test association between categorical variable while two-way analysis of variance (ANOVA) was used to compare means of continuous variables. All analyses were done using the Statistical Package for Social Sciences (SPSS) software version 18.0.The mean levels of the total protein, globulin, albumin, IgG ,IgM and IgA for the HIV-positive subjects were 84.92g/l, 40.13g/l, 44.96g/l, 19.31g/l, 1.56g/l and 2.41g/l respectively. The mean levels of the total protein, globulin, albumin, IgG, IgM and IgA for the control group were 75.94g/l, 30.19g/l,45.80g/l, 18.78g/l, 0.88g/l, 2.12g/l respectively. Our work did not establish any statistically significant relationship ($p<0.05$) between ABO blood group and HIV infection. There is no significant difference in the mean levels of total protein, globulin, IgG, IgM and IgA across the blood groups (A,B,AB and O). We recommend the inclusion of IgM, IgG, IgA, globulin and total protein assays as baseline study in addition to the well established $CD4^+$ cell count and viral load assay in assessing the Immune status of people infected with HIV at baseline. More study is recommended.

KEY WORDS: HIV, infection, Immunoglobulin, ABO Blood group.

Introduction

Immunoglobulins or antibodies circulate in the blood and serves the immune functions of searching out and neutralizing or eliminating antigens. Isotypes of immunoglobulins are of five classes namely, immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin D (IgD) and immunoglobulin E (IgE) and they fulfill different roles in immune defense. Increased polyclonal immunoglobulin may be seen with liver cirrhosis, infections, severe malnutrition, inflammatory disorders and autoimmune disorders while monoclonal increase are seen in tumors such as multiple myeloma, lymphoma and chronic lymphocytic leukemia. Decrease in immunoglobulin is seen in conditions such as agammaglobulinemia and chronic lymphoblastic leukemia. Out of the five existing isotypes of antibodies, those of HIV-1 specific antibodies are mostly IgG and to a lesser extent IgM and IgA (Constantine et al, 2005). The antibody response to HIV-I does not appear in the plasma until approximately 2 – 5 weeks after transmission. Generally neutralizing antibodies to HIV-I becomes detectable 12 weeks or more after transmission. HIV belongs to the retrovirus family and sub-family lentiviridae. Two major types of HIV have been reported- HIV-1 and HIV-2. The third type, HIV-3 is not popular. HIV-1 is more prevalent, potent and worldwide than the others and has been used as the prototype in the

majority of the studies on HIV pathogenesis and treatment. HIV-1 is phylogenetically divided into three groups — 'M', 'N' and 'O', with the M group further split into 9 subtypes (A, C, D, B, H, E, F, G) and 15 circulating recombinant forms. Today, group M has a near global distribution, whereas groups N and O are restricted to individuals of West African origin. HIV-2 is also most common in individuals from West Africa and is composed of eight subtypes (Clades) - A, B, C, D, E, F, G,H (Sharp et al.,2011).It is widely believed that HIV-1 and HIV 2 originated in African primates. One of the earliest observations made about AIDS is its extensive variation both within an individual and among host making it one of the fastest evolving of all organisms. HIV-I uses various mechanisms to evade the immune system including the infection and killing of $CD4^+$ T lymphocytes. Immunoglobulin activation and impaired responses are co-existent features of AIDS and the interactions between HIV and the host responses are complex (Nagase et al., 2001). Seven distinct phases of the life cycle are commonly recognized; binding, fusion, reverse transcription, integration, replication, assembly and budding (aidsinfo, 2015). Entry of HIV into target cells ($CD4^+$ cells) involves the: binding of virions (gp120 env protein) to receptors on target cells followed by fusion of the viral envelope (gp41) with the plasma membrane of the target cells. The binding of $CD4^+$ cells with gp120 initiates a change in the $CD4^+$ molecule to align gp120 with co-receptors (these are chemokine molecules; CXCR4 and CCR5) that facilitate HIV attachment and fusion. However, the virus can also infect by a different mechanism, many other types of human cells that lack the $CD4^+$ molecule. This mechanism occurs via a glycolipid-type, galactosyl ceramide receptor (Constantine *et al.*, 2005). A number of factors affect the risk of disease progression and they include the following - viral factors, host genetic factors and interactions between these two. The relationship between HIV replication and disease progression however is complex. For instance, disease progression is seen at significantly lower HIV RNA levels in women than in men (Sterling *et al.,* 2001). In a limited number of cases, the presence of different HIV strains and quasispecie may explain differences in rates of disease progression. Host genetic factors further determine the magnitude of HIV replication. For example, individuals with mutation in the allele coding HIV immune co- receptors (CCR5 or CXCR4) have demonstrated delay progression/resistance to HIV/AIDS (Morgan et al., 2002).

Immunoglobulin M (IgM) is pentameric and the first immunoglobulin class produced in response to an antigen and its high valency makes it more efficient than other isotypes in binding antigens and triggering complement. It is usually shortlived. IgM to HIV can be detected as early as 1-2 weeks after infection, but usually between 25 and 33 days on the average.

Immunoglobulin G (IgG) is monomeric and the major antibody in serum and non-mucosal tissues. The HIV-specific antibodies are largely IgG.

Immunoglobulin A (IgA) is the predominant immunoglobulin class in external secretions. Maternal IgA antibodies enter cord blood via placenta microtransfusion and are of good specificity in assessing the HIV status of infants, 3 months of age and above (Constantine,2005).

The results of the tests for IgG, IgA, and IgM levels are usually evaluated together. Abnormal test results usually indicate that there is something affecting the immune system and suggest the need for further testing. This test is not specifically diagnostic but can be a strong indicator of a disease or condition. It is pertinent to note that intravenous immunoglobulin (IVIg) is an important component in treating some immunodeficiency as well as autoimmune diseases. Documented studies showed that prophylaxis or treatment with IgG might be beneficial if sufficient levels of agent-specific antibodies were present (Kumar et al., 2006). Neutralizing serum antibody has the potential to prevent HIV-1 infection, as demonstrated by passive transfer of protective antibody to rhesus macaques challenged with simian human immunodeficiency virus (Baba *et al*., 2000) and had being investigated as a means of passive immunization for prevention and therapy.

Approximately three hundred (300) blood group systems have been described but the ABO blood group system is the most important blood group system in human blood transfusion. The discovery of ABO blood group system is credited to the Australian scientist Karl Landsteiner who found three (3) distinct blood types in 1900. Landsteiner described A, B and O while Alfred von Decastello and Adriano Sturli discovered the fourth type, AB, in 1902. The molecular basis of the ABO blood group system was highlighted in 1990 (Yamamoto et al, 1990).The gene encodes a glycosyltransferase, which transfers *N*-acetyl D-galactosamine (group A) or D-galactose (group B) to the nonreducing ends of glycans on glycoproteins and glycolipids. The group O phenotype results from inactivation of the A1 glycosyltransferase gene. Blood groups have been reported by many researchers in different parts of the world as factors predisposing some disease conditions.The ABO blood group have shown some association with various non infectious and infectious diseases. There is evidence supporting the view that blood group O provides a selective advantage against severe malaria (Rowe et al.,2009). There is paucity of information on the immunoglobulin levels in HIV patients and its relationship with ABO blood group.

This study was embarked upon with the following aims and objectives:

AIMS AND OBJECTIVES

1. To find out if there is association between HIV-1 infection and ABO blood group.
2. To compare the mean levels of total protein, globulin, albumin, immunoglobulins (IgG, IgM and IgA) in people who are seronegative and seropositive for HIV-1.
3. To compare the mean levels of immunoglobulin classes (IgG, IgM and IgA), total protein, globulin and albumin in HIV-1 infected subjects of different ABO blood group.

SAMPLE COLLECTION

One hundred and fifty three confirmed HIV-1 positive patients aged 20-50 years were enrolled for the study. These comprise 62 (40.5%) males and 91 (59.5%) females. The same number of control (non-HIV-infected) were also recruited. The following were excluded; the HIV-1 positive patients with co-infection of Tuberculosis, HCV and HBV infection, pregnant HIV positive females.

A.Estimation of Test Parameters

1.Immunoglobulin Estimation: This was done using automated immunoturbidimetric principle assay of Roche/Hitachi 902 analyzer.

2. Total Protein Estimation: This was done using the automated biuret colorimetric assay method of Roche/Hitachi 902 analyzer.

3.Albumin Estimation: This was done using automated albumin bromcresol green photometric method of Roche/Hitachi 902 analyzer.

4.Globulin Estimation: This was done using the difference between total protein and albumin values.

5.Blood Grouping: Tube technique: The standard tube technique for both cell and serum grouping was used in determining the blood groups of the patients under the study.

6.HIV Infection: The diagnosis was determined by Western Blot test (Immunetics).

B. Statistical Analysis: All analyses were done using the Statistical Package for Social Sciences (SPSS) software version 18.0.Chi-square was used to test association between categorical variable while two-way analysis of variance (ANOVA) was used to compare means of continuous variables.

P value of 0.05 or less (P=<0.05) was assumed to be significant in this study.

RESULT

Table 1: Prevalence of HIV-1 infection and association across ABO blood group

Blood group	HIV + (%)	HIV - (%)	Total (%)
A	31 (20.3)	32 (20.3)	63 (20.3)
B	31 (20.3)	20 (15.2)	51 (18.5)
AB	5 (3.3)	0 (0)	5 (2.2)
O	86 (56.2)	101 (64.6)	187 (59.1)
Total	153 (100)	153 (100)	306 (100)

*X^2 = 3.920, P = 0.270

The above table indicates:

- The prevalence rate.
  There is no significant statistical relationship or association between ABO blood group and HIV infection (p>0.05).

Table 2: Comparison of mean levels of total protein, globulin, albumin, lgG, lgM and lgA in HIV positive patients across the ABO blood group

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	lgG (g/l)	18.351[a]	6	3.058	1.454	.195
	lgM (g/l)	29.541[b]	6	4.923	3.144	.006
	lgA (g/l)	14.148[c]	6	2.358	2.340	.033
	TP (g/l)	4497.090[d]	6	749.515	12.245	.000
	ALB	196.662[e]	6	32.777	1.617	.143
	GLOB	5416.067[f]	6	902.678	10.342	.000
Intercept	lgG (g/l)	28981.438	1	28981.438	13782.226	.000
	lgM (g/l)	131.343	1	131.343	83.872	.000
	lgA (g/l)	418.752	1	418.752	415.584	.000
	TP (g/l)	508862.023	1	508862.023	8313.502	.000
	ALB	160514.685	1	160514.685	7919.791	.000
	GLOB	97625.133	1	97625.133	1118.459	.000
Bgrp	lgG (g/l)	2.461	3	.820	.390	.760
	lgM (g/l)	.935	3	.312	.199	.897
	lgA (g/l)	1.543	3	.514	.511	.675
	TP (g/l)	237.885	3	79.295	1.295	.277
	ALB	109.677	3	36.559	1.804	.147
	GLOB	50.234	3	16.745	.192	.902
Group	lgG (g/l)	10.108	1	10.108	4.807	.029
	lgM (g/l)	21.415	1	21.415	13.675	.000
	lgA (g/l)	5.179	1	5.179	5.140	.024
	TP (g/l)	3622.520	1	3622.520	59.183	.000
	ALB	42.199	1	42.199	2.082	.150
	GLOB	4678.128	1	4678.128	53.596	.000
Bgrp * Group	lgG (g/l)	.338	2	.169	.080	.923
	lgM (g/l)	3.470	2	1.735	1.108	.332
	lgA (g/l)	5.276	2	2.638	2.618	.075
	TP (g/l)	98.511	2	49.256	.805	.449
	ALB	26.849	2	13.424	.662	.517
	GLOB	228.433	2	114.216	1.309	.272
Error	lgG (g/l)	473.133	225	2.103		
	lgM (g/l)	352.352	225	1.566		
	lgA (g/l)	226.715	225	1.008		
	TP (g/l)	13772.049	225	61.209		
	ALB	4560.197	225	20.268		
	GLOB	19639.215	225	87.285		
Total	lgG (g/l)	85414.529	232			
	lgM (g/l)	791.924	232			
	lgA (g/l)	1482.389	232			
	TP (g/l)	1572965.720	232			
	ALB	479737.060	232			
	GLOB	338293.180	232			
Corrected Total	lgG (g/l)	491.484	231			
	lgM (g/l)	381.892	231			
	lgA (g/l)	240.863	231			
	TP (g/l)	18269.139	231			
	ALB	4756.858	231			
	GLOB	25055.283	231			

a. R Squared = .037 (Adjusted R Squared = .012)
b. R Squared = .077 (Adjusted R Squared = .053)
c. R Squared = .059 (Adjusted R Squared = .034)
d. R Squared = .246 (Adjusted R Squared = .226)
e. R Squared = .041 (Adjusted R Squared = .016)
f. R Squared = .216 (Adjusted R Squared = .195)

Table 3: Table of the mean levels of total protein (TP), globulin, albumin, lgG, lgM and lgA in HIV positive patients across the ABO blood group

Parameters	Groups	Blood group				Total#
		A	B	AB	O	
lgG (g/l)	HIV +	19.2703	19.1819	18.7080	19.4136	19.3146
	Control	18.8306	18.6875	-	18.7851	18.7795
	Total*	19.1206	19.0440	18.7080	19.1796	19.1324
lgM (g/l)	HIV +	1.8755	1.6094	1.6300	1.4251	1.5604
	Control	.7007	1.0308	-	.9040	.8821
	Total*	1.4756	1.4479	1.6300	1.2311	1.3294
lgA (g/l)	HIV +	2.8741	2.1539	2.4460	2.3360	2.4117
	Control	1.9544	2.1133	-	2.1777	2.1227
	Total*	2.5610	2.1426	2.4460	2.2771	2.3133
TP (g/l)	HIV +	85.8242	85.1135	78.6800	84.8809	84.9165
	Control	74.0750	75.5333	-	76.6275	75.9443
	Total*	81.8245	82.4400	78.6800	81.8085	81.8613
ALB	HIV +	44.2484	44.4194	40.9400	45.6523	44.9641
	Control	45.1375	46.5500	-	45.8255	45.7962
	Total*	44.5511	45.0140	40.9400	45.7168	45.2474
GLOB	HIV +	41.6968	40.6903	37.7400	39.5028	40.1303
	Control	28.3312	28.8583	-	31.0824	30.1873
	Total*	37.1468	37.3884	37.7400	36.3682	36.7446

**The row total means were compared across the blood groups*
 # The column total means were compared between groups (HIV + and control (HIV -))
***The means in the cells are the interaction between the two factors.*

The above table shows that there is no significant difference in the mean levels of protein, Globulin, Albumin, lgG, lgM and lgA across the blood groups (A, B, AB and O). However, the mean levels of protein, globulin, lgG, lgM and lgA, except albumin were higher with significant statistical difference in HIV-1 positve patients when compared with control group.
The interaction between the two factors - blood group and HIV status, does not have a significant effect on the levels of protein, Globulin, Albumin, lgG, lgM and lgA. This implies that whatever differences that exist in the levels of the parameters between the HIV positives and negatives were independent of the blood groups.

DISCUSSION:
Several workers have reported the existence of an association between blood group and diseases as shown in our introduction. However, our work did not establish any significant statistical relationship between ABO blood groups and HIV infection and this agreed with work done by Ukaejiofo and Nubila, (2006). Our work also failed to establish any statistical difference in the mean levels of total protein, globulin, albumin, IgG, IgM and IgA across the different ABO blood groups. Higher mean levels of total protein, globulin, IgG, IgM and IgA with significant statistical difference ($p < 0.05$) were observed in the HIV-1 positive subjects when compared with the control group. Lower mean levels of albumin without statistical difference were recorded for the test group when compared with the control group This agrees with work done by Akinpelu *et al*, (2012) who documented polyclonal hyperglobulinemia in HIV-positive subjects. Our work also partially agreed with that of Ifeanyichukwu et al., (2009) who submitted that IgG and IgA were significantly increased in symptomatic HIV infection compared with asymptomatic HIV infection while the level of IgM were similar in both groups.
Total protein is used in the diagnosis and treatment of diseases involving the liver, kidney or bone marrow and other diseases, thereby assessing the risk of disease progression. No significant statistical difference in mean levels was observed for total protein, globulin, albumin, IgG, IgM and IgA across the different ABO blood group of the HIV-1 positive subjects enrolled in the study.

CONCLUSION

We found in this study that there is no statistical relationship between ABO blood group and HIV-1 infection There was also no significant difference in the mean levels of protein, Globulin, Albumin, lgG, lgM and lgA across the blood groups (A, B, AB and O). Higher mean levels of total protein, globulin, IgG, IgM and IgA with significant statistical difference ($p < 0.05$) were observed in the HIV-1 positive subjects when compared with the control group. There was no significant statistical difference in the levels of albumin between test and the control group

We recommend the inclusion of IgM, IgG, IgA, globulin and total protein assays as baseline study in addition to the well established $CD4^+$ cell count and viral load assay in assessing the Immune status of people infected with HIV at baseline. More study is recommended.

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