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EVALUATION OF SERUM ALPHA-1-ANTITRYPSIN IN COMPARISON WITH CD4+ T CELLS AS MARKER FOR HIV INFECTION AMONG HIV PATIENTS IN IYI-ENU MISSION HOSPITAL, OGIDI, ANAMBRA STATE

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ABSTRACT

This study aims to assess the levels of serum Alpha-1 antitrypsin (A1AT) and $CD4^+$ T cells in HIV infected patients in Iyi-Enu Mission Hospital Ogidi, Anambra State of Nigeria. Sixty (60) diagnosed HIV patients (28 males and 32 females) and forty (40) healthy subjects (20 males and 20 females) were used. Serum A1AT was assayed by Turbidimetric method while that of CD4 count was performed by the method of BD FAC Scan flow cytometer. The results showed that the observed low CD4 and serum A1AT in patients with HIV when compared with controls showed that CD4 count of patients with HIV was significantly low ($p<0.05$) while the serum A1AT level of patients with HIV shows no statistical significant difference ($p>0.05$) when compared with the control subjects. There is a positive correlation that is associated with serum A1AT concentration and HIV infection which shows that the reduction in serum A1AT concentration leads to an increase in HIV infection. The CD4 count in male patients with HIV was significantly low ($p<0.05$) while the serum A1AT levelof male patients with HIV infected showed no statistical significant difference ($p>0.05$) when compared with the female patients with HIV. Female have higher CD4 cell counts than male, whether HIV-infected or not. The results showed no significance statistically ($p>0.05$) in the sex related distributions and stratified age groups. It was concluded from this study that decreased serum A1AT and low CD4 count, may be a valuable index in the diagnosis and monitoring of patients with HIV.

Keywords: Alpha-1-Antitrypsin, $CD4^+$ T-Cells, HIV, Patients, Infection

INTRODUCTION

Alpha-1 Antitrypsin (A1AT) deficiency is a hereditary condition, first described in 1963 (Laurell and Eriksson, 2012). Intense research over the past 40 years has led to a detailed understanding of the structural genetic abnormalities, pathophysiology of associated pulmonary emphysema, and liver disease and therapeutic approaches for treating the deficiency and managing the associated diseases (Larsson, 2008). The most recent data indicate that cirrhosis and carcinoma of the liver affect about 30–40% of patients with A1AT deficiency over the age of 50 years and are a significant cause of death in nonsmoking individuals with the PI*ZZ phenotype (Elzouki and Eriksson, 1996). A1AT is the protease inhibitor most prevalent in serum. It normally circulates in serum in concentrations of 120–200 mg/dl and was named for its ability to inhibit trypsin. (Brantly*et al*., 2013).

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However, its major biological role is to inhibit neutrophil elastase (NE), an enzyme that degrades elastin, basement membrane and other matrix components (Sandhaus *et al.*, 1997). A1AT is synthesized by hepatocytes and belongs to the serpin family. Of the deficiency alleles, PI*Z (protease inhibitor Z), is most common and in the homozygous form (PI*ZZ) results in low serum concentrations of A1AT protein, usually below 50 mg/dl (Tobin *et al.*, 2003). The Z variant of the molecule, which is the phenotype most frequently associated with lung disease, results in normal mRNA and rate of synthesis of antitrypsin, but only 15% is released into the circulation (Brantly *et al*., 2013).

Moreover, the deficiency occurs because about 85% of abnormal A1AT are synthesized and also blocked in the terminal secretory pathway of the hepatocytes. In the null variants of A1AT deficiency, where no A1AT protein is produced, there are no inclusions and liver disease is not reported. Through the technique of isoelectric focusing, about 100 genetic variants of A1AT have been identified to date. The alphabetical designation to these variants is based on their mobility in an electrophoretic field at alkaline P^H. The rapidly migrating variants are designated by the early letters of the alphabet and those migrating more slowly by the later letters, with the Z variant being slowest. The predominant normal phenotype is PI*MM (medium mobility), present in 94–96% of Caucasians (Cox, 2012). Approximately 2–3% of the Caucasian population are heterozygous (PI*MZ). A1AT deficiency has been reported in the Far East and Africa, but is relatively rare. On the basis of a large survey of studies regarding the occurrence of A1AT deficiency worldwide, de Serres estimates that worldwide, 117 million individuals have the PI*MS and PI*MZ phenotypes and that 3.4 million individuals have the PI*ZZ, PI*SZ, or PI*SS phenotype (de Serres, 2002).

Meanwhile, it has recently been shown that alpha-1 antitrypsin and a specific fragment there of inhibit HIV-1 infection *in vitro*, suggesting that this abundant serine protease inhibitor may play a protective role in HIV-1-infected individuals. In the present study we report the first case of an HIV-1-infected patient with alpha-1 antitrypsin deficiency (Brantly *et al*., 2013). The medical history of this individual suggests a very rapid loss of CD4 T cells and thus the need for antiretroviral therapy shortly after infection. To assess the role of this serine protease inhibitor in the course of HIV-1 infection further the authorstosearch for more HIV-1-infected individuals with alpha-1 antitrypsin deficiency. Genetic host factors are known to influence the clinical course of HIV infection. Alpha-1 antitrypsin deficiency is an inherited disorder on chromosome 14 associated with emphysema and liver cirrhosis. It effects approximately one in 3000 inhabitants in Scandinavia and one in 10 000 inhabitants in other countries (Shapiro *et al.*, 2011). Alpha-1 antitrypsin is the most abundant circulating serine protease inhibitor and its main function is to protect the lung against proteolytic damage from neutrophil elastase (Shapiro *et al.*, 2011).

A few number of scientific papers have been published on Alpha-1 Antitrypsin (A1AT) deficiency in human immunodeficiency virus (HIV) and their relationship with Cluster of differentiation(CD)4^+ T cells, but none of these publications is related to Anambra State of Nigeria. Hence, this study aims to assess the levels of serum Alpha-1 antitrypsin (A1AT) and cluster of differentiation $CD4^+$ T cells in HIV infected patients in Iyi-Enu Mission Hospital Ogidi, Anambra State of Nigeria.

MATERIALS AND METHODS

Geographical descriptions of the study area and population: The area of study was Ogidi is an Igbo town, the headquarters of Idemili-North Local Government Area, Located at latitude $6^0$10N and longitude $6^0$47^{1}E. It is a moderate population with most of the inhabitants' traders, banker, civil servants and students.

Iyi-Enu Mission Hospital, Ogidi, Anambra State is permanent site and fully functional. It is located 10

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kilometres from Onitsha City along Old Enugu road. The Hospital site covers an area of about 7acres.

Definition of subjects: The group of patients was recruited from Iyi-Enu Mission Hospital, Ogidi, Anambra State. Includes: Sixty (60) HIV patients, twenty eight (28) male and thirty two (32) female aged 15 to 60 years. Forty (40) apparently healthy volunteer control subjects (twenty (20) males and females) aged 15 to 60 years, that served as the group of which was recruited from anywhere in Ogidi, Anambra state. Clinical diagnosis of patients (HIV infected patients) was confirmed by enzyme-linked immunosorbent assay (ELISA).

Research design: Ethical approval was obtained from the management of Iyi-Enu Mission Hospital, Ogidi, Anambra State, for the collection of samples for the purpose of this project work and one on one discussion was carried out with HIV infected patients as test and non- HIV infected patients control subjects.

Inclusion criteria: Already diagnosed HIV infected patients and control subjects inside the above age bracket (15-60) and those who gave their consent were included in this study.

Exclusion criteria: HIV infected patients and control subjects outside the above age bracket were excluded from this study and those who did not give their consent were excluded.

Ethical considerations: The profile was submitted to the Health Research Ethical committee, Iyi-Enu Mission Hospital, Ogidi who gave their approval in writing to conduct the study. During the study, instrument that proved the anonymity of the respondents was distributed. The respondent's sincere and honest cooperation was solicited while their freedom to discontinue the study if they wished was emphasized. The study was carefully explained to the subjects and their informed consent was obtained before they were recruited into the study.

Sample collection: Venous blood samples (4.0ml) were collected from each subject using a 5.0mls sterile disposable syringe. 1.5mls of the venous blood was dispensed into a 5.0mls plain sample container while 2.5mls of the whole blood were transferred to EDTA (Ethylenediamine Tetracetic acid) sample container, labeled with the subject's Laboratory number, age and sex. The blood in the plain sample container was spun for 5 minutes at 3000 rpm. The serum was separated from the red cells using a dry clean pasteur pipette into a sterile plain specimen container. Serum Alpha-1 Antitrypsin assay was carried out while level of whole blood $CD4^+$ T cell was estimated immediately after sample collection.

Analytical methods: The tests were performed in batches daily alongside with standards.

Alpha-1-antitrypsin: The serum A1AT was estimated by the method of Turbidimetric, with kit assay system (Spinreact, Spain).

$CD4^+$ Assay: The whole blood CD4 was estimated by the method of BD FAC Scan flow cytometer (2005), with kit assay system (BD Biosciences, United State).

Statistical analysis: The results obtained in this study were analyzed statistically. The mean and Standard deviation values were calculated in each case. Student's t-test statistical method was employed for comparisons using a computer programme (SPSS) for "Windows Release 18.0". The comparison was done at 95% confidence level, a p-value equal to or less than 0.05 ($p \leq 0.05$) were considered statistically significant.

RESULTS

The result assesses the levels of serum A1AT and $CD4^+$ cells in HIV infected patients, comparing the results among the subject, control, age groups and gender in the study area.

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Table 1: CD4 count and serum A1AT level in HIV infected patients and controls in the study.

	Patients (n = 60)	Controls (n = 40)		
Parameters	**Mean ± SD**	**Mean ± SD**	**t-value**	**p-value**
CD4 Count	425.67±249.08cell/µL	1119.13±339.20cell/µL	11.78	< 0.05 (S)
Serum A1AT level	189.97±43.07mg/dL	199.18±23.33 mg/dL	1.2	> 0.05 (NS)

Table 1 above reveals the mean±SD of CD4 count and serum A1AT level of patients with HIV infected patients and the control subjects. The mean CD4 count and serum A1AT level of patients with HIV was found to be 425.67±249.08cell/uL and 189.97±43.07mg/dL respectively. The control subjects were found to be 119.13±339.20cell/uL and 199.18± 23.33mg/dL respectively. This table also showed the CD4 count of patients with HIV was significantly low ($p<0.05$) while the serum A1AT level of patients with HIV were low ($p<0.05$) but showed no statistically significant difference ($p>0.05$) when compared with the control subjects
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Table 2: CD4 Count and Serum A1AT Level in Male and Female Patients With HIV In The Study

	Male (n = 28)	Female (N = 32)		
Parameters	**Mean ± SD**	**Mean ± SD**	**t-value**	**p-value**
CD4 Count	307.36 ± 208.89cell/µL	529.19 ± 237.43 cell/µL	3.82	<0.05 (S)
Serum A1AT level	189.44 ± 60.75 mg/dL	190.43 ± 17.80 mg/dL	0.09	>0.05 (NS)

The table above denotes the sex distribution of the mean±SD CD4 count and serum A1AT level in male and female patients with HIV. From this table, the mean CD4 count and serum A1AT level in male patients with HIV was found to be 307.36±208.89cell/uL and 189.44±60.75mg/dL respectively. That of the female patients with HIV were found to be 529.19±237.43cell/uL and 190.43 ± 17.80 mg/dL respectively. This table also showed the mean CD4 count in male patients with HIV was significantly low ($p<0.05$) while the serum A1AT level of patients with HIV were low but showed no statistically significant difference ($p>0.05$) when compared with the female patients with HIV.

Table 3: CD4 count and serum A1AT level in male and female control subjects

	Male(n = 20)	Female (n = 20)		
Parameters	**Mean ± SD**	**Mean ± SD**	**t-value**	**p-value**
CD4 Count	937.70 ± 229.21 cell/µL	1300.55 ± 338.15 cell/µL	3.90	<0.05 (S)
Serum A1AT level	187.96 ± 26.38 mg/dL	210.39 ± 12.50mg/dL	3.40	<0.05 (S)

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Table 3 reveals the sex distribution of the mean±SD CD4 count and serum A1AT level in male and female control subjects. From this table, the mean CD4 count and serum A1AT level in male control subjects were found to be 937.70±229.21cell/uL and 187.96±26.38 mg/dL respectively while female control subjects were found to be 1300.55±338.15 cell/uL and 210.39±12.50mg/dL respectively. This table also showed that the mean CD4 count and serum A1AT level in male control subjects was significantly low ($p<0.05$) when compared with female control subjects.

Table 4: Age related distribution of cd4 count and serum A1AT level in HIV patients in study

	18 – 30 (Years) (n = 14)	31 – 60(years) (n = 46)		
Parameters	**Mean ± SD**	**Mean ± SD**	**t-value**	**p-value**
CD4 count	412.71 ± 240.43cell/µL	429.61 ± 254.12cell/µL	1.40	>0.05 (NS)
Serum A1AT level	176.04 ± 66.28 mg/Dl	194.20 ± 32.93 mg/dL	0.22	>0.05 (NS)

From the above table, the stratified age groups of the mean±SD CD4 count and serum A1AT level of the age groups 15-30 years and 31-60 years in patients with HIV. The mean CD4 count and serum A1AT of the age groups 15-30 years was found to be 412.71±240.43cell/uL and 176.04±66.28mg/dL respectively while the age groups 31-60 years in patients with HIV were found to be 429.61±254.12cell/uL and 194.20±32.93mg/dL respectively. This table also discloses the mean CD4 count and serum A1AT level of the age groups 15-30 years and 31-60 years in patients with HIV were low but showed no statistical significant difference ($p>0.05$) in the values obtained of age group 15-30 years when compared with age group 31-60 years.

Table 5: Age related distribution of CD4 count and serum A1AT level in control subjects in study

	18 – 30 (Years) (n = 23)	31 – 60(years) (n = 17)		
Parameters	**Mean ± SD**	**Mean ± SD**	**t-value**	**p-value**
CD4 count	1202.61 ± 307.322cell/µL	1006.61 ± 356.40cell/µL	0.60	>0.05 (NS)
Serum A1AT level	201.08 ± 19.27 mg/Dl	196.60 ± 28.35 mg/dL	1.90	>0.05 (NS)

Table 5 unveils the stratified age groups of the mean±SD CD4 count and serum A1AT level of age groups 15-30 years and 31-61 years in control subject. From this table, the mean CD4 count and serum A1AT level of the age groups 15-30 years was found to be 1202.61±307.322cell/uLand 201.08±19.27mg/dL respectively while the age groups 31-60 years in control subjects were found to be 1006.61±356.40cell/uL and 196.60±28.35mg/dL respectively. This table also indicates that the mean CD4 count and serum A1AT level of age group 15-30 years and 31-60 years in control subjects were high but showed no statistical significant difference ($p>0.05$) in the values obtained from the age group 15-30 years when compared with age groups 31-60 years.

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DISCUSSION

Alpha-1-antitrypsin (A1AT) deficiency results from mutations on the Protease Inhibitor (PI) locus located in chromosome 14 and has been associated with pulmonary early-onset emphysema and chronic obstructive pulmonary disease (COPD). African populations show a lower prevalence of A1AT deficiency compared to Europeans.

The observed low CD4 and serum A1AT in patients with HIV when compared with controls showed that CD4 count of patients with HIV was significantly low ($p<0.05$) while the serum A1AT level of patients with HIV were low ($p<0.05$) but shows no statistical significant difference ($p>0.05$) when compared with the control subjects. These results obtained in this study are in agreement with those reported by Zamani *et al.,* (2010); Sabina *et al.,* (2011).

The low level of CD4 in HIV infected patients observed in this study can be attributed to very rapid loss of CD4 T cells caused by hypoalbuminemia, hypogammalobulinemia and lymphopenia most severely affecting $CD4^+$ T cells (Zamani *et al.,* 2010 and Sabina *et al.,* 2011). The observed low level of serum A1AT were in agreement with those reported by Palcia *et al,* (2012) which may be due to reduced serum levels of A1AT contribute to the development of chronic obstructive pulmonary disease (COPD) and the accumulation of abnormally folded A1AT protein that can increase the risk for live diseases usually associated HIV infected patients. The association between reduced serum A1AT concentration and HIV infection is consistent with a role for A1AT as an endogenous HIV suppressor.
The CD4 count in male patients with HIV was significantly low ($p<0.05$) while the serum A1AT level of male patients with HIV infected showed no statistical significant difference ($p>0.05$) when compared with the female patients with HIV. These findings agree with the reports of Prins *et al.,*(1999); Maini, *et al.,* (2007), Bennett *et al.,* (2012), Jensen-Fangel *et al.,* (2012);

The observed low CD4 count in male subjects with HIV infected and non – HIV infected when compared with that of female subjects with HIV infected and without HIV infected were in agreement with those reported by Prins *et al.,*(1999); Maini, *et al.,* (2007), Bennett *et al.,* (2012), Jensen-Fangel *et al.,* (2012) who explained in general, that female have higher CD4 cell counts than male, whether HIV-infected or not. The differences in CD4 cell response observed in the present study suggest that female repopulate their peripheral CD4 cells in response to virus suppression more quickly than male. Female may have increased peripheral redistribution of memory CD4 cells from lymphoid tissue in response to decreases in virus load induced by highly active antiretroviral therapy (HAART). And also that thymic output of naive CD4 cells in response to HAART, an important contributor to later CD4 count increases, may be greater for female than for male. The results of sex related distributions of serum A1AT in both male subjects with HIV infected and non - HIV infected when compared with that of female subjects with HIV infected and without HIV infected showed no significance statistically ($p>0.05$). This agrees with the reports of David, *(*2014).

The results of stratified age groups (15–30 and 31–60) showed no significance statistically ($p>0.05$). This agrees with the reports of David, (2014) and Vila *et al., (*2014).

CONCLUSION

It can be concluded from this study that: decreased serum A1AT and low CD4 count were observed when compared with the control subjects. This low in CD4 count, which is the same in both male and female patients infected with HIV which may be dependent on and reflect rapid loss of CD4 T cells caused by low level of albumin (hypoalbuminemia) and lymphocyte (lymphopenia) most severely affecting $CD4^+$ T cells and decrease in serum A1AT concentration, which are not gender or age related

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reflect accumulation of abnormally folded A1AT protein.

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AUTHORS CONTRIBUTION:

All authors contributed in one way or the other to make this research a success.

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