

## BLOOD CHEMISTRY AND PLATELET SEROTONIN UPTAKE AS ALTERNATIVE METHOD OF TRACKING HIV/AIDS

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A cross sectional study was conducted to investigate the blood chemistry and platelet serotonin uptake as alternative method of determining HIV disease stage in HIV/AIDS patients. Whole blood was taken from subjects at the Human Virology of the Nigerian Institute of Medical Research. Subjects were judged suitable for the various investigations by means of a questionnaire. The Genie II HIV diagnostic kit was used to confirm HIV positive status. HIV positive subjects were grouped in to two: those receiving antiretroviral therapy were referred to as the ARV group and those not on antiretroviral therapy were designated as non-ARV group. Each group was further subdivided according to the Centers for Disease Control 1993 classification of HIV disease. HIV negative subjects must have been tested no later than two months to the sample collection date and must not lead a high-risk lifestyle. Serum was used to assay for blood chemistry activities with Randox analytical reagents. Blood platelets were prepared from one milliliter of whole blood and platelet serotonin uptake rates were determined. The serum glutamic-oxaloacetic transaminase (SGOT) of non-ARV subjects was the only blood chemistry parameter that showed any significant variation from normal ( $p < 0.05$ ). The mean activity of this enzyme was  $28.4 \pm 5.29$  U/L compared to a normal value of 12 U/L. A disease stage-related variation was observed. Platelet serotonin uptake rates of the two HIV positive groups showed no significant difference with the HIV negative control. The data obtained showed that serum glutamic-oxaloacetic transaminase activity is significantly increased in HIV/AIDS patients in a manner that is disease stage related. However, serum glutamic-pyruvic transaminase, bilirubin, triglycerides, amylase, serum creatinine, and alkaline phosphatase showed no significant variation from normal values. Platelet serotonin uptake of HIV subjects was not significantly different from the control.

**Key words:** Blood chemistry, platelet serotonin uptake, HIV/AIDS

### INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a progressive disease that gradually, if untreated, overwhelms the immune systems of infected patients (1). Many rapid and easy-to-use diagnostic kits are available for easy detection. Antiretroviral treatment in most cases is dependent on the disease stage of the patient. This is determined primarily by the CD4+ T-lymphocyte counts (2). The testing kits for this count is relatively expensive, costing a minimum of five dollars per test. The equipment and reagent storage facilities required can also be quite expensive. Those factors make the procedure inaccessible to most Third World inhabitants who are

bearing the brunt of the current global epidemic. We studied eight of the commonest and most routinely performed blood chemistry parameters and platelet serotonin uptake rates with a view of finding alternative methods of determining HIV disease stage. Blood chemistry analyses are regularly carried out to monitor patients' tolerance for drugs and as pathological markers. Serotonin uptake by platelets has been used as an indirect measure of the rate of serotonin intrasynaptic inactivation in depressed patients (3,4,5).

## **SUBJECTS AND METHODS**

### **Study design**

This was a cross sectional study involving 67 HIV seropositive subjects recruited and confirmed positive at the Nigerian Institute of Medical Research (NIMR) Human Virology Laboratory in Lagos, Nigeria. Ten HIV seronegative persons served as the control group. The subjects were representative of the geo-political, ethnic, economic, religious and educational diversity of Nigeria. The purpose of the study was clearly explained to them. Consent was obtained and counseling given before blood samples were taken. Participants who were on multivitamins or prolonged non-AIDS related treatment were excluded from the study. The age range was between 20 and 60 years. The HIV positive group was sub-divided into two: those who were on antiretroviral therapy (ARV) at the Institute and those who had not commenced any form of antiretroviral therapy (non-ARV). The HIV disease-stage classification was according to the Centers for Disease Control revised 1993 classification for HIV infection among adolescents and adults (2).

### **Blood collection**

Blood samples were collected between 08:30h and 09:30h. Six ml of blood were collected by venous puncture. Five ml were put into plain bottles for blood chemistry analyses while the remaining 1 ml was put into potassium EDTA bottles for platelet preparation. Another 4 ml were collected for CD4+ T-lymphocyte count which was done within 6 h of sample collection. Sera and platelets were prepared within 3h of sample collection and the assays done within 24h.

### **HIV confirmation**

Subjects were screened for HIV status at various centers outside the Institute but confirmation was done at the Human Virology laboratory with the Genie II HIV confirmation kit. CD4+ counts were performed with the Dynabeads method.

### **Determination of Blood Chemistry/Enzyme Activities**

The chemical analysis of total bilirubin, direct bilirubin, triglyceride, amylase and creatinine levels in the sera of the HIV/AIDS patients were determined using the Synchron CX5 automated spectrophotometer (Beckman, Switzerland). The activities of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase and alkaline phosphatase in HIV/AIDS patients were also carried out using the Synchron CX5 automated spectrophotometer.

### **Determination of Platelet Serotonin Uptake**

Platelets were prepared from the collected blood according to the method of Oxenkrug (4) as modified by Ebuehi and Akinwande (5). Platelet viability was confirmed using the method of Oxenkrug (4). Briefly, 8 tubes containing 1 ml of platelet-rich plasma each were placed in a shaking water bath at 37°C for 5 min. 0.4ml of  $4 \times 10^{-6}$  M serotonin creatinine sulphate was added to each of the first five tubes and 0.4 ml of 0.9% NaCl to each of the other three tubes. Incubation was stopped 5 min after addition of serotonin and the tubes were transferred into an ice-bath. Platelets were then prepared using the method of Oxenkrug (4). The isolated platelets were suspended in 2.5 ml of 0.4M HClO<sub>4</sub> and then centrifuged at 1500g for 15 min. To 2 ml of the resulting supernatant (containing the discharge serotonin in 0.4M HClO<sub>4</sub>) was added

0.5 ml concentrated HCl. The concentration of platelet serotonin was determined spectrophotometrically. Protein concentration in platelet-rich plasma and in the discharged serotonin, were determined by the method of Lowry *et al* (6). The difference between serotonin concentration of platelets in the five experimental tubes and in the three control tubes was taken as serotonin uptake by platelets within 5 min of incubation. The rate of serotonin uptake was expressed per mg protein.

### Statistical Analysis

Data obtained were analyzed by two-tailed student's t-test (7). A p-value of <0.05 was considered statistically significant. Calculations were done using Microsoft Excel 2000 statistical tools.

### RESULTS

Of the eight blood chemistry parameters investigated, only the serum glutamic-oxaloacetic transaminase (SGOT) activity of the non-ARV group gave a significantly ( $p < 0.05$ ) higher value than the normal. SGOT activity for these patients had a mean of 28.4 U/L as compared to the normal value of less than or equal to 12 U/L (Table 1).

The two CD4<sup>+</sup> count classification for which subjects were available in the non-ARV group also showed significantly higher SGOT activity values than the normal. Those with a CD4<sup>+</sup> count of less than 200 cells/ $\mu$ L had SGOT activity of 34.76 U/L while those with a CD4<sup>+</sup> counts of between 200-499 cells/ $\mu$ L had activity value of 21.06 U/L. These values showed significant difference at  $p < 0.05$  (Table 2). The platelet serotonin uptake rate of HIV positive subjects showed no significant difference from the HIV negative subjects (Table 3).

**TABLE 1**

Blood Chemistry Parameters of Non-ARV and ARV HIV Positive subjects<sup>1</sup>

Blood Chemistry Parameter	Normal Serum Value	Non-ARV Subject	ARV Subject
Serum glutamic-oxaloacetic transaminase (U/L)	$\leq 12$	28.4 $\pm$ 5.29	13.65 $\pm$ 1.95
Serum glutamic-pyruvic transaminase (U/L)	$\leq 12$	15.25 $\pm$ 4.68	9.25 $\pm$ 1.74
Total bilirubin (mg/dl)	$\leq 1$	0.58 $\pm$ 0.05	0.52 $\pm$ 0.05
Direct bilirubin (mg/dl)	$\leq 0.25$	0.24 $\pm$ 0.04	0.26 $\pm$ 0.04
Triglyceride (mg/dl)	60-150	81.86 $\pm$ 9.39	81.09 $\pm$ 8.81
Amylase (U/l)	$\leq 52$	27.39 $\pm$ 4.23	27.35 $\pm$ 3.15
Creatinine (mg/dl)	$\leq 1.3$	1.01 $\pm$ 0.06	1.02 $\pm$ 0.04
Alkaline phosphatase (U/l)	9-35	31.18 $\pm$ 5.06	40.04 $\pm$ 7.01

<sup>1</sup>Values represent mean  $\pm$  standard error of mean. Source of normal serum levels of parameters: Randox Laboratory Manuals.

**TABLE 2**

Serum Glutamic - Oxaloacetic Activity of Non-ARV Subjects Classified according to CD4<sup>+</sup> Counts<sup>1</sup>.

CD4 <sup>+</sup> Count (cells/ $\mu$ l)	SGOT (U/l)
<200	34.76 $\pm$ 8.95
200-499	21.06 $\pm$ 3.83
$\geq$ 500	-*

<sup>1</sup>Values represent mean  $\pm$  standard error of mean

\*There was only one patient in this category

## DISCUSSION

Serum glutamic -oxaloacetic transaminase (SGOT) activity of non-ARV group was significantly raised above the normal value. Laboratory data have shown elevated levels of muscle enzymes, including creatine kinase, aldolase and SGOT in HIV disease (8). These findings are typical of polymyositis. Inflammatory muscle disease has been reported to occur often in persons with HIV disease than in the general population (9). In some patients polymyositis is the initial manifestation of HIV infection (10).

Polymyositis may be diagnosed months before the onsets of AIDS. Patients usually complain of progressive proximal muscle weakness involving upper and lower extremities, with increasing difficulty rising from a chair, walking upstairs and using arms for any length of time.

Detailed analysis of the SGOT values for non-ARV subjects showed a relationship with the HIV disease stage as determined by the CD4<sup>+</sup> count. SGOT is a serum enzyme that is used in the clinical assay of the liver damage.

**TABLE 3**

Platelet Serotonin Uptake of HIV Positive and HIV Negative Subjects<sup>1</sup>.

Subject	Uptake Rate (nMol serotonin/mg proten/5min)
HIV positive	2.00 $\pm$ 0.76
HIV negative	2.21 $\pm$ 0.52

<sup>1</sup>Values represent mean  $\pm$  standard error of mean

In HIV/AIDS patients, SGOT, along with other blood chemistry parameters studied in this work, is normally used to monitor the reaction of patients to antiretroviral drugs. From the present findings, SGOT may be used, along with other tests, as an early detection method for AIDS. As the enzyme activity was found to also be disease-stage related, it could also be used to follow the progress or stage of the disease in patients who have been diagnosed as AIDS patients.

The other blood chemistry parameters, studied showed no significant difference with the normal values. Sinicco *et al* (11) report that hepatic enzymes might be elevated to as much as ten times normal in acute hepatitis in HIV disease, but return to normal within six weeks. Lebovics *et al* (12) and Schneiderman *et al* (13) also reported that neither the pattern nor the extent of elevation of these serum enzymes correlates with specific findings in the liver. SGOT levels seem to correlate with the severity of HIV disease. This suggests that SGOT could be used as a rapid indicator of HIV disease stage, which could aid patient monitoring in rural and field settings.

The platelet serotonin uptake observed from HIV positive subjects showed no significant difference with the serotonin uptake of HIV negative subjects. These findings suggest that HIV/AIDS does not affect platelet serotonin uptake and rate of intrasynaptic inactivation. Serotonin uptake by blood platelets has been used as an indirect measure of the rate of serotonin intrasynaptic inactivation in depressed patients (3,5) and in the rats (14). A possible explanation for the lack of significance in the results obtained in this study for platelet serotonin uptake may be that most of the HIV positive subjects were getting confirmation of the HIV status for the first time. Follow-up study of the post-confirmation depression might reveal more interesting facts.

In conclusion, serum glutamic-oxaloacetic transaminase (SGOT) activity holds promise as a tracking method for HIV disease progression in patients with HIV/AIDS. However, larger study will be required to confirm this.

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