



Original Research Article

Analysis of blood parameters in HIV positive patients

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ARTICLE INFO

Article history:

Received 25-07-2019

Accepted 16-07-2020

Available online 28-09-2020

Keywords:

Hematological parameters

Anemia

HIV positive patients

ABSTRACT

Background: Human immunodeficiency virus (HIV), the causative agent in AIDS has been a challenge to medical fraternity from the time since it was first discovered in 1983. About 40 million people are living with HIV infection globally and 99% of the infected people are in South East Asia (ESA). Close monitoring of HIV infected patients is essential in reducing the morbidity and mortality of HIV patients. The monitoring HIV disease progression & deciding the time to initiate anti retro viral therapy requires evaluation of T CD4 cell counts and HIV/RNA viral load at regular intervals along with monitoring of biochemical parameters and hematological parameters in HIV Seropositive patients.

Objective: The objective of the study was to evaluate some biochemical parameters and hematological parameters in newly diagnosed HIV seropositive patients (primary stage).

Materials and Methods: A total of 300 newly diagnosed HIV patients and 200 age and sex matched healthy individuals as control were tested for CBC, lipid profile, renal function tests, liver function tests, electrolytes and some minerals (copper, zinc, iron). Our observations were subjected to statistical analysis Mean \pm standard deviation calculated. Comparison was done by student t-test.

Results: HIV positive patients had significantly ($p < 0.001$) decreased mean Hb (10.22 ± 1.51) and RBC count (3.99 ± 0.75) compared with HIV negative subjects with Hb (12.52 ± 1.21), RBC count (5.08 ± 0.42). We observed a decrease in total cholesterol, HDL cholesterol and LDL cholesterol, and an increase in triglyceride and VLDL levels. HIV patients increasingly require careful monitoring and evaluation for altered renal function, to prevent co morbidities of treatment and non-treatment related nephrotoxicity. In this study patients with HIV showed significant increase in the mean serum copper level when compared with the control subjects respectively, zinc and iron levels significantly decreased compared with normal subjects.

Conclusion: It was concluded in the present study that, hematological manifestations of the HIV infection are complication associated with the disease, anaemia being a very common finding. This complications are not related to age or the sex of the patients. The lipid profile of the patients is also seen to be altered by the disease process. Decreased Lipid levels are more pronounced in anemic groups than non-anemic group.

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1. Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a medical diagnosis for a combination of symptoms which results from a breakdown of the immune system. Immune system poses the first defense against the invading organisms. The immune deficiency in AIDS is caused by a virus. “Acquired” means something which is not naturally

present in the body and developed due to some infection or condition. “Immune Deficiency” means the deficiency in the immune system resulting in its weakening and “Syndrome” a condition which has a variety of symptoms leading to various disorders and a set of diseases. It is not a curse or a punishment.¹ AIDS is caused by a virus named HIV (Human Immunodeficiency Virus). The disease process is slow and usually takes years after the infection for a person to notice that he/she has been infected, when the effects of the weakened immune system manifest

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themselves. The presence of the HIV in the blood of the person indicates that the person is HIV positive.² So far, two viruses have been identified to cause AIDS: HIV 1 and HIV2. It is a member of the retroviruses family. It is a very small virus which resembles a porcupine which is wrapped up. HIV presents a couple of strands of RNA as well as an enzyme, reverse transcriptase. They are present in the form of a cone. This conical structure is covered by an envelope which is studded with a knob like protein stuck from the surface, responsible for the typical appearance of the virus. In healthy individuals, infections are deferred by virtue of multiple cells in the body which essential component of the immunity. The most important components are the White Blood Cells present in the blood and lymphatic system including the lymph glands. These cells fight foreign invaders in the body and produce antibodies which neutralize/kill the invaders. How exactly HIV weakens the immune system is still being researched. As per the widely adopted view, HIV enters the WBCs in the blood. This virus attacks CD4 variety of WBCs which is an essential component of immune system. In this case, HIV enters the white blood cell. Once inside the nucleus, it gets incorporated in to the host DNA and starts multiplication. These cells now attack other white blood cells. This leads to the reduction in the count of the WBCs leading to compromised immunity. HIV remains practically immune to counter attacks, since it hides inside the attacked cells which are also the cells that are supposed to attack HIV. Eventually, AIDS develops when killed CD4 cells can no longer be replaced. It was observed that per day production of new HIV in an AIDS patient is around 10 billion.³

HIV positive person does not show any external signs of infection until the progression to AIDS, which may take up to 10 years or even more. Such person is well capable of transmitting the infection to the others. A person with HIV is called "having AIDS" when his immune system is totally broken down and killed CD4 cells no longer be replaced in the desired numbers AIDS has to be acquired. HIV can transmit from one person to the other by physical sexual contact, transfusion of blood or blood products, infected needles or syringes or via vertical transmission from mother to fetus during pregnancy, delivery or breast feeding. Hence one has to take the necessary precaution. AIDS does not discriminate a person by sex, religion or caste.⁴

2. Materials and Methods

The present cross-sectional, case control study was carried out in the Clinical Biochemistry department, Saraswati medical college, Unnao, U.P India, during 2017 to 2019. The patients attending OPD/IPD of Saraswati Hospital were selected for the study. 300 newly diagnosed HIV patients and 200 age and sex matched healthy individuals as control were included in the study. Ethical clearance was obtained from Institutional Ethics committee at the

Saraswati medical college (No. SMC/IEC/12-2017, dated 19/01/2017). A written informed consent was obtained from all the subjects participating in the present study after explaining the details of the study in their local language. The participants of the study were 300 patients including men and women between 18-65 yrs. Quality control samples were run along with patient sample to assure the quality of instrument function.

Participants able to understand and provide informed consent, with documented HIV infection (By any licensed ELISA and confirmation by Western Blot), >18 years, absence of AIDS related comorbidities, with the CD4 cell count >500 cells/ul or the percent of CD4 cells >29% of all lymphocytes were included in the study. Whereas the patients with the history of chronic or clinically significant hematologic abnormalities, pregnancy or venereal diseases were excluded.

Blood was collected in a sterile EDTA containing tube & plain bulb and processed following the established laboratory protocol and by universal precaution as per the guideline of National AIDS control organization (NACO, India).

The blood samples of the subjects enrolled in the study were analyzed for hemoglobin, total RBC count and related indices, reticulocyte count, differential WBC count, platelet count, PCV and ESR. CBC was estimated BY SYSMEX KX-21 cell counter. Samples for ESR were analyzed using Westergren method. Commercially available kits were used to assay ALT and AST (NADH, Kinetic UV, IFCC recommended kits). Urea estimation was done by Urease-GLDH kinetic method and Creatinine by jaffe's kinetic method. Bilirubin was measured directly by use of Quantitative determination of bilirubin. Total cholesterol was determined using Cholesterol oxidase – Peroxidase method while HDL was determined after precipitation of LDL with phosphotungstate methods. Serum sodium, potassium, chloride levels was determined by Easylyte analyser works on ISE. Copper, Zinc and Serum Iron estimation by colorimetric methods.

Manufacturer's procedure for each test was followed accordingly. The findings obtained by the usage of the test kits were taken as the normal ranges.

SPSS, version 20.0 was uses for the statistical analysis of the results of the present study. Categorical variables were reported as numbers and percentages and continuous variables as means with standard deviations (SD). Differences between two groups were analysed with independent t-tests for normally distributed continuous variables. Pearson's correlation analysis was performed to assess the association between Variables.

3. Results

This present study was conducted at Saraswati general Hospital, Unnao, U.P. As defined in materials and methods,

the study group consisted of 300 newly diagnosed HIV patients and 200 age and sex matched healthy individuals as control. Table 1 shows that there is no significant difference between males and females of controls & HIV positive patients ($P=0.49$ & $P=0.16$). Proportion of male patients in this study is significantly high than females in HIV positive patients. ($p < 0.01$). Table 2 shows that a large number of HIV+ patients 132 (44.0%) were aged between 31-40 years, 99 (33.0%) aged 18-30 years and the least 2(0.6%) aged 61-70. Majority of the HIV- patients 86 (43.0%) were aged 31-40 years followed by 18-30 years 63(31.5%). Table 3 shows that neutrophils, PCV & ESR were significantly increased in HIV positive subjects compared to normal subjects and hemoglobin, total RBC count, total WBC count, lymphocytes, platelets, MCV, MCH, MCHC were significantly decreased compared with normal subjects. Table 4 shows that mean blood urea, serum creatinine, AST, ALT, triglyceride levels were significantly increased in HIV subjects compared with normal subjects, cholesterol was significantly decreased in HIV subjects compared to normal subjects, and no significant difference in total bilirubin, HDL and serum sodium, potassium and chloride. Table 5 shows that serum copper was significantly increased in HIV positive subjects compared to normal subjects and serum zinc & serum iron was significantly decreased in HIV positive subjects compared to normal subjects. Table 6 shows that neutrophil count, PCV and ESR were significantly increased in HIV positive males than normal male subjects whereas Hb, total WBC, lymphocytes, total platelets, MCV, MCH, MCHC and RBC count were significantly decreased in HIV positive males than normal male subjects and there was significant difference in hematological parameters between HIV positive females and normal females subjects. The neutrophil count, PCV and ESR was also significantly increased in HIV positive females than normal female subjects. Hb, total WBC, lymphocytes, platelets MCV, MCH, MCHC, totals RBC count is decreased significantly in HIV positive females than normal female subjects. Table 7 shows that serum urea, serum creatinine, AST, ALT and triglyceride levels were significantly increased in HIV positive males compared to normal male subjects. Total cholesterol and HDL levels were decreased significantly in HIV positive males compared to normal male subjects. No significant difference was found in total & direct bilirubin levels between HIV positive males compared to normal male subjects. Serum urea, serum creatinine, AST, ALT, and triglyceride levels were significantly increased in HIV positive females compared to normal female subjects. Serum cholesterol and HDL levels were significantly decreased in HIV positive females compared to normal female subjects. There was no significant difference in serum electrolyte levels between HIV positive females and normal female subjects. Table 8 shows that the serum copper was significantly increased

in HIV positive males compared to normal male subjects. Serum zinc & serum iron were significantly decreased in HIV positive males compared to normal male subjects. Copper was significantly increased in HIV positive females compared with controls. Zinc & iron was also significantly decreased in HIV positive females compared with normal female subjects. Table 9 shows that prevalence of anemia (Hb < 10.0 gm %) was higher in HIV positive patients (60%) ($P=0.0002$). It was also found that hemoglobin, total WBC, MCV, MCH, MCHC and RBC count was significantly decreased in anaemia patients with Hb < 10.0 gm% compared to patients with Hb > 10.0 gm%. There was no significant difference in neutrophils, lymphocytes, platelet count, PCV and ESR in anemic patients with Hb < 10.0 gm% compared to patients with Hb > 10.0 gm%.

4. Discussion & Conclusion

AIDS, the acquired immunodeficiency syndrome is a fatal illness caused by retrovirus known as HIV which breaks down the body's immune system, leaving the patients vulnerable to a host of life threatening opportunistic infection, neurological disorders, or unusual malignancies. AIDS patients are often associated with aberration of biochemical parameters like renal profile, liver profile, thrombocytopenia and severe anemia with high ESR. Hematological manifestations of the HIV infection are well recognized complication of the disease and may be clinically important. Anemia is very common finding in patients with HIV infection, particularly in individuals with more advanced disease. HIV infection alone, without other complicating illness, may produce anemia in some patients. A study of serum immunoreactive erythropoietin in HIV infected patients in various stage of illness showed that level of the hormone failed to rise commensurately with increasing anemia. Suggesting that insufficient amounts of erythropoietin may be one cause of anemia in this setting.⁵ Other studies have suggested that soluble factors in the serum of HIV infected patients may inhibit hematopoietic or that direct HIV infection of marrow progenitor cells may play a role in producing anemia and other hematological abnormalities associated with HIV infection.^{6,7}

Hematological abnormalities are among the most common complications of HIV which involves all lineages of blood cells.⁸ Results from a thesis showed that HIV infection affects hematological indices of patients regardless of age, sex. Majority of the HIV positive patients were males 234 (78.0%) whilst majority of the randomly selected HIV negative patients were males 142 (71.0%). HIV positive patients had significantly ($p < 0.001$) decreased mean Hb (10.22 ± 1.51) and RBC count (3.99 ± 0.75) compared to HIV negative patients with Hb (12.52 ± 1.21), RBC count (5.08 ± 0.42). This confirms research by Dangana et al.⁹ who also observed a significant drop in Hb levels ($p < 0.05$). Friel & Scadden et al¹⁰ observed

Table 1: Frequency of Male and female subjects in Normal subjects and HIV Positive subjects

			Group		Total
			Normal subjects	HIV positive subjects	
Gender	F	Count	58	66	124
		% within Group	29%	22.0%	24.8%
	M	Count	142	234	376
		% within Group	71.0%	78.0%	75.2%
Total	Count	200	300	500	
	% within Group	100.0%	100.0%	100.0%	

Table 2: Distribution according to age groups in HIV positive and HIV negative subjects

Age (years)	HIV positive subjects (300) (N%)	HIV negative subjects (200) (N%)	P-value
18-30	99 (33%)	63 (31.5%)	0.799
31-40	132(44%)	86 (43%)	0.897
41-50	54 (18%)	37 (18.5%)	0.981
51-60	13 (4.3%)	13 (6.5%)	0.378
61-70	2 (0.6%)	1 (0.5%)	0.642

Table 3: Comparison of haematologic parameters between HIV positive and normal subjects

Variable	Units	Normal subjects (n):200	HIV positive subjects (n):300	P value
HB	g\ dL	12.52 ± 1.21	10.22 ± 1.51	<.0001
Total WBC	Thousands	8089.00 ± 1274.59	7151.33±2700.48	<0.0001
Neutrophils	%	61.85 ± 5.29	64.08±8.95	= 0.001
Lymphocytes	%	31.47 ± 4.39	28.48±6.25	<0.0001
Platelets	Lakhs	3.05 ± 0.72	1.81 ± 0.94	<0.0001
PCV	%	43.91 ± 3.01	48.60 ± 7.43	<0.001
MCV	%	87.20 ± 2.99	81.21 ± 9.30	<0.0001
MCH	Picogram/cell	29.77 ± 1.73	27.16 ± 4.67	<0.0001
MCHC	%	33.47 ± 3.63	31.34 ± 4.06	<.0001
RBC COUNT	Mil/μL	5.08 ± 0.42	3.99 ± 0.75	<.0001
ESR	mm/hg	10.58±3.10	45.79±14.32	<.0001

Table 4: Comparison of biochemical parameters among HIV Positive subjects and normal subjects

Variable	Units	Normal subjects (N:200)	HIV positive subjects (300)	P value
Serum Urea	mg/dL	33.06 ± 7.0	39.30 ± 13.98	<0.0001
S. Creatinine	mg/dL	1.01 ± 0.20	1.19 ± 0.57	<.0001
AST	U/L	29.36 ± 6.22	35.30 ± 16.09	<.0001
ALT	U/L	29.85 ± 7.09	40.57 ± 17.92	<.0001
S.TB(total bilirubin)	mg/dL	0.72 ± 0.17	0.78 ± 0.40	0.06
S. Direct Bilirubin	mg/dl	0.44±0.15	0.48 ± 0.33	0.11
S. Cholesterol	mg/dL	162.83±15.25	148.32 ± 25.78	<0.001
S. Triglyceride	mg/dL	109.41±19.80	172.71 ± 52.69	< 0.0001
S.HDL	mg/dL	45.06 ± 5.78	45.89 ± 7.02	0.166
S.Sodium	meq/dL	139.18 ± 3.30	139.81 ± 8.01	0.289
S.Potassium	meq/dL	4.39 ± 0.61	4.26 ± 1.06	0.100
S.Chloride	meq/dL	103.82 ± 4.02	103.47± 9.18	0.61

Table 5: Comparison of trace elements among HIV positive subjects and normal subjects

Variable	Units	Normal subjects (200)	HIV positive subjects (300)	P value
S.Copper	μg/dL	114.99 ± 17.77	137.05 ± 45.90	<.0001
S.Zinc	μg/dL	99.24 ± 15.75	64.89 ± 23.48	<.0001
S.Iron	μg/dL	88.20 ± 13.39	66.68 ± 25.77	<.0001

Table 6: Comparison of hematological parameters between HIV positive male subjects and normal male

Variable	Normal Male (N:142)	HIV positive male subjects (234)	P value
Hemoglobin	12.69 ± 1.21	10.45 ± 1.50	<.0001
Total_WBC	8166.19± 1282.55	7288.88± 2606.96	0.0001
Neutrophils	61.80 ± 5.29	64.42 ± 8.44	0.001
Lymphocytes	31.26 ± 4.43	28.55 ± 6.25	<.0001
Platelets	3.11 ± 0.70	1.76 ± 0.88	<0.0001
PCV	43.73± 2.96	48.78 ± 7.51	<0.0001
MCV	87.25 ± 2.77	82.12 ± 8.28	<.0001
MCH	29.80 ± 1.62	27.39 ± 4.62	<.0001
MCHC	33.66 ± 3.64	31.39 ± 3.81	<.0001
RBC_Count	5.08±0.43	3.96 ± 0.73	<.0001
ESR	9.12±2.16	33.7±10.4	<0.001

Table 7: Comparison of biochemical parameters between HIV positive males and normal male subjects

Variable	Normal male subjects (N:142)	HIV positive males (234)	P value
S.Urea	32.98 ± 6.80	39.61 ± 14.00	< 0.0001
S.Creatinine	1.01 ± 0.21	1.20 ± 0.58	0.0002
AST	28.79 ± 6.19	34.92 ± 15.85	<0.0001
ALT	29.92 ± 7.17	40.14± 17.87	< 0.0001
S.TB(Total bilirubin)	0.7275±0.1718	0.77 ± 0.39	0.167
S. DB (Direct Bilirubin)	0.4401±0.15	0.4752±0.3331	0.237
S.Cholesterol	162.29± 15.33	148.20 ± 24.25	<0.0001
S.Triglyceride	109.02 ± 19.66	171.79 ± 52.89	<0.0001
S.HDL	45.35 ± 5.65	38.07±7.07	<.0001
S.Sodium	139.02± 3.42	139.81 ± 8.17	0.273
S.Potassium	4.42 ± 0.61	4.31 ± 1.10	0.310
S.Chloride	103.95 ± 4.07	103.60± 9.23	0.671

Table 8: Comparison of trace elements between HIV positive male and normal male subjects

Variables	Units	Normal male subjects (N:142)	HIV positive male (234)	P value
S.Copper	µg/dL	114.97±17.74	137.44±45.195	<.0001
S.Zinc	µg/dL	98.45±16.23	65.45±24.05	<.0001
S.Iron	µg/dL	88.04±13.07	65.98 ± 25.61	<.0001

Table 9: Prevalence of Anemia (Hb <10 gm%) in HIV positive subjects (300 cases)

Total No. of HIV positive subjects	HIV + patients with Hb <10.0 gm%	HIV + patients with Hb > 10.0 gm%
300	180 (60.0%)	120 (40%)

that the yearly incidence of developing anemia increases with disease progression affecting 5% of all patients with asymptomatic HIV infection. The low RBC parameters could be as a results of decreased red blood cells production or ineffective erythropoiesis. Anemia has been shown with statistically significant predictor of progression to AIDS and is independently associated with increased risk of death in patients with HIV.¹¹ The significant decrease in MCV, MCH and MCHC (P<0.001) compared to HIV negative patients indicates that HIV positive experiences microcytic hypochromic anaemic conditions.

Total leucocyte (WBC) counts of HIV positive patients were found to be significantly (p<0.005) lower ($3.99 \pm$

0.75) as compared with HIV controls (5.08 ± 0.42). This observation is in agreement with the findings of the other researchers. Leucocytopenia was observed in 68 (22.66%) cases in our study.

Mean platelet count of HIV positive patients (1.81 ± 0.94) was significantly lower than HIV negative patients (3.05 ± 0.72) in the present study. 39% of the HIV positive cases showed thrombocytopenia which was in agreement with previous works.¹² Thrombocytopenia is known to complicate HIV infection according to Sullivan et al.¹³ Thrombocytopenia may be as a results of increase platelets destruction or decreased platelet production in subjects not on antiretroviral treatment, which may affect the normal

haemostasis.

The mean ESR of HIV positive patients was significantly higher (45.79 ± 14.32) compared with HIV control patients (10.58 ± 3.10) due to decreased erythrocyte count (anemia) and haematocrit which is consistent with other studies, Dangana et al., has reported utility of ESR.^{10,14} In predictor of the development of AIDS, ESR is important when coupled its value with a Count of <500 cells/ μL and an elevated $\beta 2$ -Microglobulin in predicting the progressing to AIDS.

We report a high proportion of male HIV-infected individuals. Urea and creatinine levels were significantly higher in males than females. This is in line with standards and reference ranges established for both sexes for these analytes. The findings in the present study are notable with high prevalence of mild renal impairment in the study population.

Kamga HLF et al. showed that the mean serum urea & creatinine were significantly higher in HIV negative participants than in HIV positive ($p < 0.0001$). Many studies have suggested increased susceptibility to the development of renal disease in HIV patients.

The present study showed that lipid profile was altered in HIV infected patients. Alteration in the lipid profile occurred even during the early stages of HIV infection and, more so, as the disease progressed. Previous studies have demonstrated that patients with AIDS exhibit highly abnormal total lipid concentrations in serum.¹⁵ A few authors^{16,17} who determined the levels of serum triglycerides, total cholesterol and HDL cholesterol in HIV infected individuals by the level of immunological deficiency, also came to the same conclusion that, with an increase of immunological deficiency and clinical development of HIV infection, lipid profile disorders, indicated by an increase in triglyceride level and decreased Concentrations of HDL cholesterol intensified as well. Consistent with earlier reports, our study also showed similar findings in which the decrease in total cholesterol, HDL cholesterol and LDL cholesterol, and an increase in triglyceride and VLDL levels. Lipid levels are slightly higher in females than in males. Decreased Lipid levels were more pronounced in anemic groups than non-anemic group hence the finding in the present study are comparable with previous above studies.

Kereveur A et al stated that hypercholesterolemia observed in early and hypertriglyceridaemia in later stage of the infection are due to cytokine effects on different enzymes of lipid metabolism.¹⁸ Fernandez et al. reported that IFN α , 2 micro globulin and TNF α were related to the level of total cholesterol, VLDL and HDL cholesterol.¹⁹

HIV infection is a condition caused by the human immunodeficiency virus (HIV). The condition gradually destroys the immune system, which makes it harder for the body to fight infections. Considerable evidence indicates

that copper, zinc and iron are necessary component of many metabolic pathways.^{20,21} In these studies patients with HIV revealed significant increase in the mean serum copper level when compared with the control subjects respectively and zinc and iron levels were significantly decreased compared with normal subjects, and males and females HIV positive cases and respective controls. Serum copper was significantly increased and serum zinc and serum iron were significantly decreased. These findings are consistent with previous study done by Neil et al, who reported that there was a significant increase in the mean of serum copper concentration when compared with control group. Also, the results of the present study showed that, there is significant decrease in the mean serum zinc levels in patients when compared with mean serum zinc level of control group with ($p < 0.05$).

5. Limitation of the Present Study

The association of health seeking behavior of HIV positive subjects and the state of hematological & biochemical manifestations need to be studied in detail for further understanding initial, intermediary & final stage of HIV in order to increase the life expectancy of HIV patients. Our Study has Limitation as patients with mild renal and hepatic impairment should have been subjected to biopsy (FNAC).

6. Conclusion

The association of health Seeking behavior of HIV positive subjects and state of hematological & biochemical manifestations need to be understood. It was concluded in the present study that, hematological manifestations of the HIV infection are complication associated with the disease, anaemia being a very common finding. This complications are not related to age or the sex of the patients. The lipid profile of the patients is also seen to be altered by the disease process. Decreased Lipid levels are more pronounced in anemic groups than non-anemic group. Moreover further research that will assess the clinical profile of HIV positive patients in the first visit to the ART centers and their association with hematological profile & biochemical parameters need to be researched as it will provide more important information for the care and management of early stage HIV.

7. Source of Funding

None.

8. Conflict of Interest

None.

Acknowledgments

We extend our gratitude to the staff of the department of biochemistry for providing the support and guidance necessary to complete this project. We are highly grateful to the patients who volunteered to be a part of this study.

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Cite this article: Malapati B, Nadeem SM, Shah MM. Analysis of blood parameters in HIV positive patients. *Int J Clin Biochem Res* 2020;7(3):388-394.