Interleukin 3 as an Erythropoietic Marker in Human Immunodeficiency Virus and Hepatitis B Positive Patients in South East Nigeria

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Authors' contributions

Original Research Article

ABSTRACT

Aim: To evaluate the interleukin 3 levels in some Hepatitis B virus and Human immunodeficiency virus (HIV) positive subjects.
Study Design: Cross sectional study.
Place and Duration of Study: Haematology Department, University of Nigeria Teaching Hospital Enugu, Nigeria, between June and September, 2019.
Methodology: A total of 86 subjects were recruited for this study; 40 were positive for the human immunodeficiency virus, 30 were Hepatitis B positive and 16 healthy subjects that served as controls. The controls had tested negative to Hepatitis B Virus, Human immunodeficiency Virus and hepatitis C virus infections. Whole blood samples were collected from the Human immunodeficiency

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Virus positive and control samples. Haemoglobin concentration was analysed using the Orphĕe Mythic 22 automated analyzer. Serum samples collected from all 86 subjects were used to assay Interleukin 3 using the Enzyme Linked Immunosorbent assay based Finetest human interleukin 3 kit.

Results: A highly significant decrease in interleukin 3 levels was observed in Hepatitis B Virus and Human immunodeficiency virus positive subjects when compared with the apparently healthy control subjects, ($P = 0.000 < 0.05$). Average hemoglobin levels were also lower in the Human immunodeficiency virus subjects compared with the controls.

Conclusion: A reduction of the interleukin 3 may be part of the synergistic factors responsible for the anaemia usually seen in the viral infections.

1. INTRODUCTION

Acquired Immune Deficiency (AIDS) is a failure of the immune system. It occurs secondary to infection by the Human Immunodeficiency Virus (HIV). The HIV infection and its associated disease state AIDS remain a major public health problem. This is especially true for resource poor economies [1]. It results to severe damage to the immune responses of the affected individual. It also impairs haematological functions [2,3] Anaemia, thrombocytopenia and leucopenia are usually observed [3,4]. Cluster of differentiation CD4+ T cells depletion is also a characteristic haematological dysfunction associated with HIV infection [5]. These haematological malfunctions serve as indicators of disease severity and prognosis. Cytopenia is generally a major diagnostic indication of disease [6].

Hepatitis B virus (HBV) infection is also a viral infection which results in a complex disease of the liver. It can be acute or chronic [7]. Due to the similarity of mechanism of spread of the two infections, it is not uncommon to find co-infection with HIV and the chronic disease form of Hepatitis B [8]. It has been estimated that more than 380 million people worldwide have HBV worldwide [9].

The two diseases presently have no cure but can be effectively managed (10,11) . One important way of assessing management success is by monitoring the haematological and immunological parameters of the patients undergoing therapy [10,12–14].

Interleukines are a complex group of proteins that function in the body. They mediate immune reactions. They also function as growth factors in haemopoiesis [15]. They have been generally incorporated in the management of haematological disorders [16]. In humans, more than 50 interleukins and related proteins have been identified. A key haemotopoietic interleukin is the interleukin 3 (IL3). It is produced in mast cells and activated T cells. It appears to serve as an bridge to immune responses affecting haemopoiesis [17]. This is by controlling response to infections by increasing immunity effector cells populations [18].

Interleukin-3 (IL-3) is a 28-kiloDalton glycoprotein. It was first identified in mice. Its major function is regulation of haemotocyte production [19]. It was found to induce the expression of 20-α-hydroxysteroid dehydrogenase (a marker of mature T cells) in cultures of athymic mouse spleen cells [20]. This cytokine is expressed on activated T cells, mast cells and natural killer cells. The IL3 gene is expressed on the right lobe of the liver, the testis and in blood.

Activated T helper cells(Th) are a major source of IL 3 [17]. Th2 cells, also called CD4 cells are the major target of the HIV virus. Interleukin 3 can therefore be used to assess HIV prognosis. It also useful in cancer management, as it has been found to promote replenishment of white blood cells and platelets. It also improves the immune response [21]. This improves chemotherapy outcomes. Further work is also ongoing to explore greater use of interleukin 3 as a therapeutic factor in haematologic disorders [21]. Recombinant cytokines have been investigated in improvement of management of these two viral infections [22–26].

Recombinant IL3 has been found to be effective in increasing some haematological parameters in HIV infection [27]. These include total white cell

counts, neutrophil counts and eosinophil counts. Recombinant IL3 has few side effects and stimulates haematologic response in normal bone marrow [28]. It has been used to increase eosinophil count as a way to treat parasitic infections [29]. It improves total neutrophil counts in aplastic bone marrow patients [30]. Haematological parameters are also restored to normal levels faster when IL3 is used in combination with other growth factors [31]. IL 3 has been found to stimulate haemopoietic stem cell expansion which in turn leads to improvement in the haematological profile.

Cytokine levels have been found to vary in some inflammatory and immunologic diseases [32–34] Cytokines like IL6 and tissue necrotic factors have been utilized as markers to determine pregnancy outcome in other viral hepatitis infections [35]. IL 17 which is also produced by T helper cells is found to increase in cases of chronic HBV infection [36]. It has been found that immune responses controlled by cytokines can determine disease prognosis [5,37]. Other interleukines e.g. IL 4 has been shown to inhibit viral replication of HBV [38]. Disease prognosis seemed favourable in a study assessing the effect of recombinant IL4 on chronic HBV infection [39]. IL 6 administration has also been found to reduce the cellular infection by HBV [40].

Improved haemopoiesis remains a need in patients undergoing anti-viral therapy. It has been observed that the immune damage caused by the HIV isn't only attributed or caused by the virus mediated killing [5]. Most of the anti-viral drugs have a depressive effect on haemopoiesis which could be a source of pancytopenia. Viral infections generally have myriad effects on haemopoiesis and the bone marrow [12].

There is a need for improvements in the management therapies of people already infected with HIV and HBV. It is therefore worthwhile to study some of the haemopoietic growth factors in these infections. In addition, a study of the cytokines may be beneficial to understanding better the interplay between these viral infections and the immune system.

Therefore, the aim of the study is assess IL3 levels in HIV and HBV positive Nigerian patients.

2. MATERIALS AND METHODS

2.1 Study Population and Data Collection

A total of Eighty-six (86) participants (males and females) were involved in this study. The inclusion criteria for this study includes; age between 19-64 years, no history of any significant medical conditions including high blood pressure, malignancies or known haematological disorders. Only participants willing and able to give informed consent were included. All were screened for HBV, HIV and HCV. None were on any chemotherapeutic drugs or had signs of malnutrition. The first test group consisted of 40 participants positive for HIV only, and on antiretroviral therapy for a minimum of one year. The second group of 30 participants were positive for HBV only. The HIV and HBV positive groups all had no clinical manifestation of the disease and were asymptomatic (Stage I HIV).The control group consisted of apparently healthy 16 subjects who tested negative for HIV, HBV and HCV. Pregnant and breastfeeding women were excluded. Questionnaires were used to obtain demographic information about the participants.

2.2 Study Area and Design

The study was conducted in Enugu, the capital city of Enugu State Nigeria. The study lasted between June and September 2019. The study adopted a cross-sectional approach.

2.3 Laboratory Analysis

Three (3) ml of blood was collected from the ante-cubital vein of the subjects and transferred into Ethylene Diamine Tetra-acetic Acid (EDTA) containers with the anticoagulant at a concentration of 1.6 mg/ml of blood.

2.3.1 Haemoglobin concentration

Haemoglobin concentration was assayed using the Orphĕe Mythic 22 automated analyzer.

2.3.2 Interleukin 3 assay

This was assayed via ELISA, using the Finetest human interleukin 3 ELISA kit. The tests were run according to the manufacturer's instructions.

2.4 Statistical Analysis

The data obtained from this study was analyzed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Comparison of the plasma IL-3 between HIV-test and control subjects was done using the t test. A P-value of less than (<) 0.05 was chosen as statistically significant.

3. RESULTS AND DISCUSSION

Data obtained from this study shows that there was a decrease in the mean values for IL3 in both HIV and HBV positive individuals when compared with the control group. This work assayed the IL- 3 levels in patients with either HIV or HBV and compared it with controls (Table 1). Furthermore, the total haemoglobin values of the HIV positive people were compared to their total IL3 values. This was to check for the correlation between IL3, a haemopoietic factor, and total haemoglobin values. The difference in IL3 values between the test and control groups was also highly statistically significant, with the P value being = .001 (Table 1).

When the means of the IL3 levels HBV, HIV and controls were compared, it was evident that there

was a marked difference between the test cohort and the control cohort (Fig. 1)

Prior studies investigating the effects of these two viral infections on cytokine levels and the immune response to them have been done [41]. Cytokines are markers of inflammation. HIV and HBV infection are both viral infections with inflammatory properties [10,42,43]. Cytokine levels have been found to vary depending on type and stage of disease, [44,45] and have long been considered as therapeutic agents for the management of HIV and HBV infections [22,25,46–49]. IL- 2 in combination with IL- 16 could help in stimulating the immune response in HIV patients, increasing CD4+ cell populations [22].

Like earlier mentioned, depending on disease stage and severity, cytokines tend to have different disease responses. IL 35 has been found to decrease in chronic HBV infection [50], while the same IL 35 also has been found to increase in acute HBV patients [51]. This probably leads to an establishment of the infection. IL 35 is an anti-inflammatory cytokine, it suppresses inflammatory response. Other studies investigating the role of IL 35 as a

Table 1. Demographic characteristics, Haemoglobin in HIV and interlekin 3 level in HIV and HBV positive cases and controls

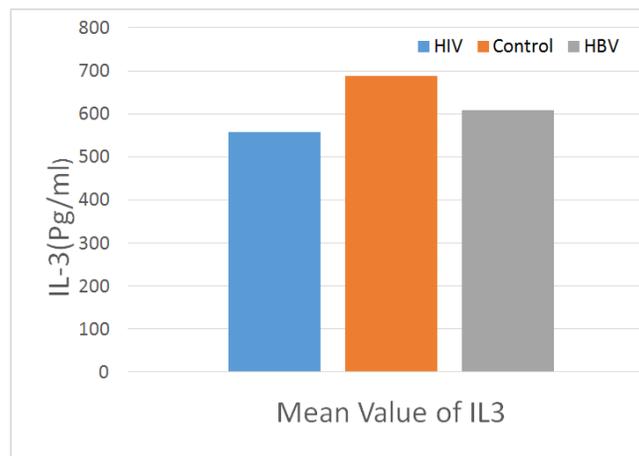| Parameters	Control (n=16)	HIV (n=40)	HBV (n=30)	
M	15	13	-	
F	1	27	-	
Age (years0	18 - 65	18 - 65	18 - 65	
	(Mean ±SD)			P value
Haemoglobin(g/dl)	14.6± 1.0	11.3± 1.5	-	
Interleukin 3 (pg/ml)	687.8±191.4	557.3±127.5	608.6±143	= .001

Fig. 1. Comparing IL3 means of HIV, HBV and controls

therapeutic agent in chronic HBV are ongoing [52]. Though, due to time constraints, the present study was not able to separate the subjects into chronic and acute infections. Acute and chronic infections have different cytokine patterns. However, cytokine depression has been found to enhance infective capacity of HBV [53].

Pro - inflammatory cytokines levels are usually elevated in hepatitis. IL-6, a pro inflammatory cytokine has been found to increase in HBV infection. This is to be expected as HBV has inflammatory effects on the liver.

Simple assays similar to that in this study have also been done just to assess levels of different cytokines in these diseases. IL- 1 was found to be increased while IL- 2 was found to be decreased compared to controls in patients with chronic HBV [54]. Other studies showed that IL-6 and IL-10 levels were higher in ART naïve HIV subjects when compared to ART experienced subjects [10]. This is to be expected because both IL6 and IL10 are both inflammatory markers.

Antiretroviral therapy tends to suppress cytokines. Though inflammatory cytokines appear to rise in untreated HIV, treatment tends to reduce the levels [55]. One limitation of this study was the inability to get naïve HIV subjects.

Of interest is the fact that recombinant inlerleukin 3 has already been studied as a therapeutic agent to treat cytopenia. Red blood cell indices and haematocrit values were generally unchanged. Though some patients showed improvements in hemoglobin values with IL 3 injection.

Anaemia is a common finding in HIV infection, with rates of its occurrence increasing with disease progression [56]. The aetiology of this anaemia is heterogenous [57]. It ranges from suppression of erythropoiesis by lymphoma cells, bacterial co-infections e.g. tuberculosis, nutritional deficiencies, anti-viral therapy etc. [13,58,59]. Results from this study also seem to inform that interleukin 3 which is a haemopoietic growth factor seems to be depressed in these 2 viral infections. Its decrease is also directly proportional to haemoglobin concentration. This is complementary to earlier studies in HIV patients on erythropoietin levels, another haemopoietic growth factor [56]. Levels appeared to be lower than expected in response to anaemia in HIV patients. The erythropoietic

response was also depressed. This could be due to the effects of antiviral drugs.

Further work is still necessary. This may be helpful to determine if all the decreases vary with acute or chronic infections. Other haemopoietic growth factors for instance erythropoietin, granulocyte- macrophage colony stimulating factor (GM-CSF), interleukin 11, granulocyte colony stimulating factor (G CSF) can also be assayed as a profile to fully understand the effects of these viral infections on haemopoiesis. Already, recombinant erythropoietin is standardly used in treatment of anaemia [24]. Interleukin 3 has potential as a therapeutic agent for use in improving haemoglobin concentration in HIV and HBV patients with cytopenia. As IL 3 has been studied for use in treating bone marrow failure [28], and in management of cancers in combination with other agents [60], it could also be incorporated into the HIV treatment cocktail.

4. CONCLUSION

In conclusion, IL3 levels are decreased in people with HIV and HBV infections compared with controls. Supplementation with external sources of this cytokine could help in improving disease management outcomes.

CONSENT AND ETHICAL APPROVAL

This study was conducted after obtaining ethical clearance from Health Research and Ethics committee of the University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu, with approval number NHREC/05/01/2008B-FWA00002458-1RB00002323. Prior to recruitment, details of the procedures involved in the study were explained to the participants, those who gave their consent by signing the informed consent form were recruited for the study. Anonymity and confidentiality of participants' data were maintained throughout the study. Participants were also informed of their right to withdraw from the study at any time. The study was designed with minimal attendant risk to participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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