Original Research Article

Assessment of the level of erythropoietin in persons living with HIV in Umuahia

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Abstract

The study was done to assess the level of erythropoietin in persons living with HIV in Umuahia. A total number of one hundred and ten (110) subjects were recruited for the study.The subjects were divided into two groups:the first group consists of HIV positive subjects (Test group) and the second group consists of HIV negative subjects (control group).Serum sample was used for the estimation of erythropoietin.Sandwich ELISA method was used to estimate erythropoietin.The results were analysed using t-test with level of significance set at P<0.05.The results showed significant decrease (p<0.05) in erythropoietin level of the HIV patients (6.21±3.46Mu/Ml) compared to HIV negative subjects (13.63±5.19Mu/Ml) respectively.The study showed that there was derangement in the kidney of the HIV patients which decreased erythropoietin synthesis and release and induced anaemia in the patients.

Keywords: Erythropoietin, HIV, Umuahia.

Introduction

Erythropoietin is one of the primary regulators of erythropoiesis (Bunn, 2013; Mastromario *et al., 2011*). In the bone marrow, erythropoietin promotes the proliferation of erythroid progenitor cells and increase the production of erythrocytes.

According to Kertesz *et al.* (2004),erythropoietin is a principal haematopoietic growth factor responsible for the proliferation, survival and differentiation of erythroid progenitor cells. Erythropoietin binds through specific binding to erythropoietin receptor (EpoR).Erythropoietin triggers a chain of intracellular signalling events, including activation of the receptor-associated tyrosine kinase JAK2 and phosphorylation and nuclear translocation of STAT5, leading to progenitor cell proliferation, inhibition of apoptosis and differentiation (D Andrea *et al.,1989;*Watowich *et al.,1996*).

Anaemia is one of the most common blood disorders seen in people living with HIV. The prevalence of anaemia ranges from 10% in people who have no symptoms to 92% in individuals with advanced AIDS according to Levine and Leitz (2003).

The pathogenesis of anaemia during HIV infection is often multifactorial, and contributing factors include iron deficiency, the anaemia of chronic disease associated with HIV, malaria and opportunistic infections (Semba,2001);however, it is responsible to iron and epoietin alpha therapy.

The pathogenesis of HIV infection affects many organs of the body including kidney. This may likely affect the level of erythropoietin which will affect erythropoiesis in the patients(Martinez and Gattell,1999).Erythropoietin level in blood are quite low in the absence of anaemia around 10Mu/Ml. However, in hypoxic stress, erythropoietin synthesis may increase 1000-fold,reaching 10,000Mu/Ml of blood.

Aim

To determine the level of erythropoietin in persons living with HIV in Umuahia.

Materials and Methods

Study Area: The study was done in Umuahia.HIV patients were recruited from those confirmed of HIV infection in Health Services Department of Michael Okpara University of Agriculture,Umudike,Abia State,Nigeria and Daughters of Mary Mother of Mercy Hospital Ahieke,Ihe Ndume,Umuahia.

Ethical consideration: Informed consents were obtained from the subjects prior to sample collection and confidentiality assured to them.

Study population and enrolment: A total number of one hundred and ten (110) subjects were recruited for the study. The subjects were divided into two groups, consisting of fifty (50) HIV positive subjects(test group) and sixty (60) HIV negative subjects (control group).They were recruited from the HIV patients and the apparently healthy subjects attending Health Services Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and Daughters of Mary Mother of Mercy Hospital, Ahieke, Ihe Ndume, Umuahia.

Selection criteria: The test subjects were recruited after been established of having HIV infection using national serial algorithm and not reactive to any other viral infection and without any AIDS indicator conditions. The control group on the other hand was recruited after been established of not having HIV or any other viral infection and were apparently healthy.

Exclusion criteria: The subjects showing any underlying chronic illness other than HIV infection (test group) and reactive to any other viral infections were excluded from the study and also exluded were pregnant women.

Sample collection: Using 5ml syringe,5ml of fresh venous blood was drawn from each study participant by a clean vein puncture from the antecubital vein and delivered into plain tubes and allowed to clot and retract. Serum was separated and transferred into clean test tubes for estimation of serum erythropoietin.

Laboratory methods and procedures

The reagents were commercially purchased from a reputable supplier and manufacturers' standard operating procedures were duelly followed. Erythropoietin was estimated using ab119522 Erythropoietin Human ELISA kit (AbcamPlc, Science Park, Cambridge,UK.).

HIV Screening: Two different HIV screening kits were used (determine and Unigold test kits) for the detection of HIV-seropositivity following serial algorithm.

Serum Erythropoietin Assay (Sandwich ELISA Method)

Principle: Erythropoietin specific antibodies have been precoated out in the well plates. Standards and test samples were added to the wells along with a biotinylated erythropoietin detection antibody and the microplate is then incubated at room temperature. Following washing with wash buffer a streptavidin-HRP conjugate is added to each well, incubated at room temperature and unbound conjugates are then washed away using wash buffer.TMB is then added and catalysed by HRP to produce a blue colour product that changes to yellow after addition of an acidic stop solution. The density of yellow colouration is

directly proportional to the amount of erythropoietin captured in plate.

Procedure

Microplate was washed twice with 400µl wash buffer per well with thorough aspiration of microplate contents between washes. Wash buffer was added and allowed remain in the wells for 10seconds before aspiration. After the last wash step, wells were emptied and microplate tapped on absorbent pad to remove excess wash buffer.100µl of prepared standard was added to the appropriate wells.50µl of sample diluent was added to appropriate wells. 50µl of Biotin conjugated antibody was added to all wells. The wells were covered with adhesive film and incubated at room temperature for 1 hour. After incubation, the adhesive film was removed and the wells were emptied. The microplate strips were washed 6 times with 400µl wash buffer per well with thorough aspiration of microplate contents

between washes.100µl of sreptavidin-HRP was added immediately to all wells, including the blanks wells. Microplate was covered with an adhesiv film and incubated at room temperature for 15 minutes. After incubation, microplate was removed and wells were emptied. Microplate was washed 6 times with 400µl wash buffer per well with thorough aspiration of microplate contents between washes.100µl of TMB substrate solution was added to all wells immediately after washing. Then the microplate was incubated at room temperature for 10 minutes. Enzyme reaction was stopped by adding 100 µl of stop solution into each well. Absorbance of each microplate was read on a spectrophotometer using 450nm wavelength. The plate reader was blanked with blank wells. The absorbance of both the samples and the standard were determined.

Statistical Analysis: The analysis was done using t-test and the values were expressed as mean ± standard deviation (SD) and statistical significance set at $P<0.05$.

Results

Status	Erythropoietin(mu/ml)	p-level
HIV Positive	6.2±3.46	p<0.05
HIV Negative	13.63±5.19	

Discussion

The study showed that there was significant decrease (P<O.05) in serum erythropoietin level of HIV positive subjects when compared with HIV negative subjects. The result agreed with the study done by Adebola *et al*. (2003), which reported reduced EPO response among HIV anaemic subjects and also agreed with the work of Kanyeki *et al*. (2013), which showed decreased serum erythropoietin level in HIV positive subjects. These decrease in serum erythropoitin level in HIV patients could be due to derangement of the kidney caused by the oxidative effect of HIV with release of free radicals on the organs in the body (Martinez and Gattel, 1999, Salman and Berrula, 2012).

The pathogenisis and pathophysiology of anaemic in HIV infection is multifactorial as this ranges from direct infection of the erythroid progenitors (Krenzer and Rockstroh, 1997). There are three major mechanisms involved in the pathogenesis of anaemia in HIV infection, these are : decreased red blood cell production which is consequent to

infiltration of the bone marrow by neoplastic cells (Sipas *et al.*,1999), or infection (Hambleton, 1996), decreased production of endogenous erythropoietin, a blunted response to erythropoietin (Spivak *et al.*, 1989) and hypogonadism. The second mechanism is increased red blood cell destruction (Levine, 1999). Thirdly, ineffective deficiencies most commonly in iron, folic acid, vitamin B12 (Levine, 1999).

Conclusion

The study showed a significant decrease in both serum erythropoietin level in the persons living with HIV in Umuahia. This shows that the kidney of these patients are deranged and should be taken care of because that will hinder survival of some of the patients due to anaemia that was seen in them. Proper care and attention should be given to them both by the society and the health workers who are involved in the management of the patients.

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