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Research article International Journal of Hematology and Blood Disorders Open Access

Erythropoietin Response in Patients with Anaemia of Chronic Disorders

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Received: September 29, 2018; Accepted:October 24, 2018; Published: October 31, 2018

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Abstract

Chronic disease conditions characterized by infection and intense inflammatory response abound in Sub-Saharan Africa and are very often complicated by anemia. Erythropoietin (EPO) is up regulated in response to anemic conditions. In Nigeria there is a dearth of public literature describing Anemia of Chronic Disease (ACD) and Erythropoietin levels in chronic diseases. The aim of the study was to determine the appropriateness of erythropoietin response to anemia observed in chronic disease.

Methods: 215 participants were recruited from the wards and out-patient clinics, 125 cases (i.e. individuals with chronic diseases) and 90 controls consisting of individuals being seen for an annual or physical examination. Venous blood was collected for full blood counts, serum Iron (spectrophotometric), serum Ferritin (ELISA) and serum Erythropoietin (ELISA). Appropriateness of Erythropoietin response was determined by establishing an exponential relationship between erythropoietin levels and Hemoglobin (Hb) levels of controls (log EPO = 2.446 – 0.11 x Hb g/l). Inappropriate Erythropoietin secretion was indicated by an observed/predicted log (serum EPO) ratio of less than 0.8 (O/P<0.8).

Results: The result showed 72% of participants with chronic disease had anemia and 65.5% had anemia of chronic disease. Of the anemic participants, 33% had an Erythropoietin O/P ratio <0.8, which corresponded with inappropriate Erythropoietin secretion. The mean circulating serum Erythropoietin level for control subjects was 13.1 ±10.1mu/l, significantly lower than that of subjects with chronic disease, 32.1±42.1mμ/l (P =0.00). Correlation studies showed a significant negative relationship between Hemoglobin and Packed Cell Volume.

Conclusion: The study showed that about two-thirds (65.5%) of the patients with anemia had Anemia of Chronic Disease, and a third showed evidence of diminished response endogenous Erythropoietin.

Keywords: Anemia; Inappropriate Erythropoietin Response; Erythropoietin; Anemia of Chronic Disease; Chronic Disease; Iron; Ferritin

Introduction

Anemia of Chronic Disease (ACD) is one of the most common anemias worldwide, second only to Iron Deficiency Anemia (IDA). It is also the most frequent anemia among hospitalized patients with an average incidence of 6% between the ages of 18 to 60; and an incidence of 44.4% in men of 85 years and above. Some other studies conclude that as high as a quarter of these hospitalized adults might actually have anemia of chronic disorder. [1, 2, 3, 4].The production of erythropoietin (EPO) in human renal peritubular fibroblasts is regulated by arterial oxygen tensions via a mechanism that invovles oxygen – dependent conformational changes of the haem molecule. An intact EPO response is necessary for the maintainance of adequate produciton and maturation of red cells and a stable haematocrit level in human blood. In healthy individuals and patients with haematological disorders, a drop in haematocrit, (e.g after haemorrhage) leads to a rise in serum EPO levels. [4, 5, 6, 7]. This functional EPO response is the basis of the inverse correlation of haematocrit and the serum EPO level. The normal range of serum EPO depends on the underlying haematocrit level, with a high EPO concentration in individuals with low haematocrit and vise versa. In contrast, an inadequate or diminished EPO response with inappropriate elevation of serum EPO level in response to anaemia is found in ACD.

The mechanism of inhibition of the EPO axis in ACD has been linked to inhibitory effects of proinflamatory cytokines, such as Tumor necrosis factor α (TNF α), Interferon γ (INF γ) and Interleukin 1 (IL1). More recently the protein Hepcidin, has been implicated as the major cytokine. Hepcidin interferes with EPO gene expression, but also has a major impact on Iron metabolism and determines the reticuloendothelial Iron supply for erythropoiesis. Hepcidin is a key regulator for iron metabolism and mediator of anaemia during inflammation. This small peptide produced in the liver, is induced by inflammatory cytokines and its over production results in macrophage iron retention and, consequently, less iron is available for erythropoiesis. This reticuloendothelial iron block cannot be overcome by oral iron administration, whereas intravenous iron may be effective for this purpose. [8, 9, 10, 11].

Materials and Methods

All participants evaluated in this study either had a chronic disorder and were on hospital admission (Cases), or had

presented for an annual physical examination and were recruited from the outpatient clinics (Controls). Inclusion criteria for cases in this study was the presence of ill health for at least 2 months, on hospital admission, consent to the study, drug history of not being on any kind of cytotoxic drugs, cyclosporine A, theophylline, and Zidovudine. [12, 13, 14] Hemoglobin levels were equal or below 11g/L, no evidence of blood loss or hemolysis and were not on any form of micronutrient replacement therapy. All participants that were pregnant or had renal disorders (to avoid the complicated multifactorial senario of anaemia in patients with renal disorders), and to avoid difficulty in evaluating bone marrow activity were excluded. The objective of the study was explained to all participants, their anonymity was protected by coding the samples and permission for this study was obtained from the Hospital's ethical committee. (See Appendix 3)

The patients were enrolled from all outpatient clinics and all Medical and Surgical Wards at the Jos University Teaching Hospital. 5mls of blood were collected from all participants in the morning (to account for the circadian rhythm of iron) to determine full blood count, serum iron, serum ferritin levels, and serum EPO levels (after an overnight fast). Haematological profile was determined with automated counters, while Serum iron was assayed using spectrophotometric method designed by TECO diagnostics, 1268 N. Lakeview Avenue. Anaheim, CA 92807. Ferritin was assayed by a direct sandwich ELISA method, using diagnostic kit of clinotec® and pharmaceuticals, Inc 2101-11871 Horseshoe way Richmond B.C. V7A 5H5 Canada.

Serum EPO assay

Circulating EPO levels were determined by means of a 2-site ELISA (Enzyme-Linked Immuno Sorbent Assay) kit according to manufactures instructions. The test was based on an antibody-antigen-antibody reaction, and utilized 2 different mouse monoclonal antibodies to human EPO and measured biologically active EPO. Detection concentrations derived by standard curve were in the range of (2.5 - 200mU/ml) and a reference range was provided with values ranging from 2.4 – 38U/L.

To define EPO levels as appropriate or inappropriate for a given degree of anemia, an exponential regression of log of serum EPO vs. Hb was determined in 30 control subjects. This resulted in the construction of a regression equation as a reference. For Hb values <11g/l, the regression equation was log (EPO) = 2.446 – (0.11 x Hb g/dL). EPO levels increased exponentially as the Hb level dropped; therefore EPO had to be expressed in relation to the Hb value. Appropriateness of EPO response was estimated by calculating a ratio of log observed EPO level ('O'), against log predicted EPO levels ('P'). An O/P ratio of < 0.8 was accepted as inappropriate response. [9, 14, 18]

Statistical analysis

The data was analyzed using a multipurpose computer statistical program EPI INFO version 3.3. The Student t- test was used to compare the difference of 2 means. ANOVA statistics was used to compare difference of more than 2 means.

Linear regression was performed, using the REGRESS command to test the relationship between variables. E.g. EPO levels and Hb levels. Chi square test was used to test significance of association of discrete variables. P values of <0.05 was considered significant. All our procedures were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1996.

Results

For this study a total of 215 participants were recruited, 90 of which were controls (i.e patients seen at the outpatient clinics for routine/annual physical examination) and the remaining 125 subjects had some form of chronic disease.

Table 1 shows the demographic distribution of cases and controls by age (ranging from 1 – 80 yrs) and gender. There were 47 males (52.2%), 43 (41.8) females amoung cases, and 51 males (56.7%) and 39 females (43.3%) in controls.

Table 1: Age and Sex Distribution by Experimental Group

Age group	Male		Female	
	Case	Control	Case	Control
0 - 15	8	12	4	5
16 - 30	14	12	21	15
31 – 45	18	11	11	11
46 – 60	4	12	6	7
61 – 75	2	4	1	1
≥ 76	1	-	-	-

Aetiology

27.7% of the study group were diagnosed as having Tuberculosis (TB), 15.6% had some form of malignancy, 14.4% had TB/AIDS and 11.1% had AIDS. The next common aetiology were Hepatitis 6, PID+UTI 6 and Rheumatioid anthritis 6. Osteoarthtis and cryptococcal meningitis in AIDS patients were 5 respectively. (Table 2)

Table 2: Diseases Diagnosed In the study group

Diagnosis	Frequency	%
TB	25	27.8
Malignancies	14	15.6
Osteoarthritis	5	5.5
PID + UTI	6	6.7
Rheumatoid arthritis	6	6.7
AIDS	10	11.1
AIDS + Cryptococcal meningitis	5	5.5
TB/AIDS	13	14.4
Hepatitis	6	6.7

Serum Erythropoietin levels

The serum EPO levels in the cases were significantly higher than those of the controls. Mean serum EPO levels in controls were 13.1± 10mUI/ml, and 32.1± 42.1mUI/mL in the case study group (Table 3). Using the student's t-test and ANOVA, there was no significant difference between serum EPO and gender (F=0.001 DF=1, P= 0.974), serum EPO and Age (F=0.800 DF=5.174, P=0.551) respectively.

Table 3: Haematological parameters of experimental group (i.e. control and study)

Parameter	Control	Case	Reference value	Test of statistical significance
	N=90	N=90		
Hb (g/dL)	13.1± 0.91	9.16± 1.69	13.6 ± 4.9	P=0.000
PCV (L/L)	0.42 ± 0.03	0.29 ± 0.05	0.42 ± 0.78	P=0.000
RBC count ($x10^{12}$/L)	5.0 ± 0.57	3.6 ± 0.8	4.5 ± 1.7	P=0.000
Retic index (%)	1.16± 1.0	0.5±0.3	0.2 – 2.0	P=0.000
Platelets ($x10^{9}$/L)	275.5±98.4	242.5±95.9	135.5±40.11	P=0.024
Iron (µmol/L)	18.98±8.77	6.80±3.76	13-32	P=0.000
Ferritin **(µg/L)**	157.9±165.6	436±242	20-300	P=0.000
EPO (mU/L)	13.1±10.1	32.1±42.1	4.2±27.8	P=0.00

Correlation study of some hematological parameters with serum EPO levels

As expected Red blood cells (rbc) count, Hb levels and Packed cell volume (PCV) showed negative correlations with EPO levels (figure 1), whereas this negative correlation reached significant levels with Hb and PCV (P = 0.025 and P = 0.017 respectively), it failed to reach a significant level with rbc count (P= 0.067). Other hematological parameters Reticulocyte index, mean corpuscle volume (MCV), platelets and White blood cells (WBC) showed no significant correlations (P= 0.485, P= 0.892, P= 0.218 and P= 0.646 respectively). [15, 16, 17]

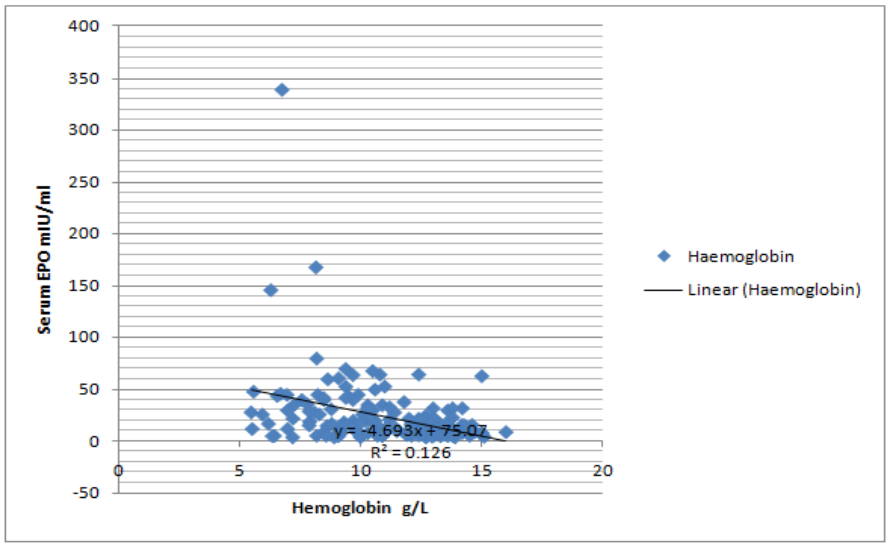

Figure 1: Scatter diagram of serum EPO against Hemoglobin

Appropriateness of EPO Secretion

Of the entire study population with chronic disease (.i.e. 125), 82 participants (65.6%) had ACD, as defined by low serum iron (< 13mmol/l), normal or raised serum feritin >20µg/l and serum EPO (> 4.2u/l). 3 participants (2.4%), were diagnosed with Iron Deficiency Anaemia as defined by low serum iron levels (< 13mmol/l) and low serum ferritin (< 15µg/l).

Based on the calculated O/P ratio (see appendix A), 30 (33.3%) of the 90 cases with anemia showed evidence of inappropriate or diminished response to EPO. TB was the commonest disease amongst patients with ACD followed by malignancy (table 2). This was also observed when considering table 5, with 28% (7) of the patients with TB exhibiting an inappropriate EPO response. Participants with a variety of malignancies had the second lowest EPO response (6), while those with TB and AIDS co-infection (5) had the third lowest response to EPO (See Table 4). [15, 16, 17, 18, 19, 20]

Table 4: Distribution of appropriateness of EPO response with type of disease

Disease	O/P ratio<0.8 (inappropriate/ diminished EPO response)	O/P ratio>0.8
TB	7	18
Malignancy	6	8
TB+AIDS	5	8
AIDS	4	6
AIDS +Meningitis	2	3
PID+UTI	3	3
Osteoarthritis	1	4
Rheumatoid arthritis	1	5
Hepatitis	1	5

A chi-square statistics showed there was no significant relationship between the type of chronic disease and the likelihood of inappropriate EPO secretion. (Chi-square = 3.99, P = 0.88).

Of all hematological parameters estimated, only serum ferritin appeared to be discriminatory between subjects with ACD, who had appropriate EPO response to anemia and those who had inappropriate EPO response (i.e. a diminished response) to their anemia. Mean serum ferritin levels for the appropriate responders, 395 ± 220µg/L was significantly lower than that for the inappropriate responders (516 ± 268.01 µg/L) P=0.025 (Table 5). On observation (although not of significant levels P=0.11) the inappropriate responders demonstrated lower Hb concentration 8.76 ± 1.77, than anemic but appropriate responders with a higher mean Hb concentration of 9.37 ± 1.63. (Table 5)

Table 5: Comparison of Haematological parameters between appropriate and inappropriate responders to EPO

Parameters	Inapp. Response N=30				App. Response N=60				P value
	O/P ratio <0.8				O/P ratio ≥0.8				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max	
Hb	8.76	1.77	5.49	11	9.37	1.63	5.59	13.5	=0.111
PCV	28.4	5.73	19	38	29.92	5.45	15	39	=0.225
MCV	82.67	10.24	56	112	81.43	9.71	55	100	=0.579
MCH	25.5	3.65	20	36	25.47	2.73	18	34	=0.961
Platelet	220.1	80.32	98	375	253.73	101.63	112	545	=0.117
Serum Ferritin	516.5	268.01	15	870	395.83	220.20	10	820	=0.025
WBC	56.06	38.88	20	187	77.5	92.8	9	550	=0.229

Discussion

ACD remains the second commonest form of anemia worldwide. ACD usually presents as a mild to moderate anemia with an underlying co-morbidity like infectious diseases in sub saharan Africa, where appropriate and timely intervention would reduce the deletarious sequealae of anemia on the cardiovascular system through adequate tissue perfussion, improving the prognosis of the chronic disease and ultimately increase and improve the quality of life of these patients. [9, 18, 19, 24, 25, 26, 27]

The result of our study confirms the prevalence of anemia in the 125 participants with chronic disease as 72%, the prevalence of ACD at 65.5%, based on the criteria serum iron (<13mmol/L), ferritin (>20µg/L) and erythropoietin(>4.2U/L), and establishing a 33.3% prevalence of inappropriate EPO response to anaemia in these individuals. When compared to the different studies on the prevalence of ACD, ours' was closeset to figures quoted by Opasich et al 57%73 and 52.32% by Cazzola. [9, 19, 20] Other figures were 6% by the American Family Physician Journal, 25% by Cash and Sears and 20% by Guralink et al. [1, 8, 18, 19, 20, 21, 22]

This study confirms the inverse relationship between Hb and serum EPO levels in healthy individuals and some of the cases. Serum ferritin levels were reported to be significantly high in our study population, with that of cases being twice as high as in the controls (Table 3), while the mean serum ferritin levels for the inappropriate responders was shown to be about 1.5 times higher than that for appropriate responders (Table 5). However serum ferritin levels are not a very reliable indicator for the diagnosis of ACD, because of the role proinflamatory cytokines (such as TNF α, INF γ, IL-1 and Hepcidin) play in Iron homeostasis, but in Sub-Saharan Africa, it may serve to increase the index of suspicion of ACD cases.

The study also demonstrates 33.3% (O/P ratio <0.8) of cases as inappropriate responders to EPO. Serum EPO and SF levels were significantly higher in cases than controls and when comparisim was made between appropriate and Inappropriate responders, EPO levels although higher in the Inapproriate group did not show a corresponding and appropriate increase in thier Hb levels, therfore giving raise to the expression diminished or blunted response to EPO. This observations is of clinical importance, as a combination of intravenous iron and subcutaneous administration of recombinant human EPO, in selected cases has been proven to be of benefit as a rational theraputic approach to managing the anaemia in these patients. [9, 22, 23, 24, 25, 26, 27]

The predominant red blood cell morphological type observed was normocytic normochronic. 60% of our cases exhibited the expected normocytic normochronic picture, while the remaining 40% represented a spectrum of the other morphological subtypes. This further enforces the point that in as much as one subtype is termed predominant (i.e. normocytic normochromic); this does not invalidate the fact that other morphological types may be a form of presentation in ACD. The morphological picture of patients with ACD is also dependent on the length of the illness, severity and the presence of other co-morbid states. [3, 4, 7, 11, 13, 18, 20, 21, 22, 24, 25, 27] Figure 2 shows the Red cell morphology distribution of study group.

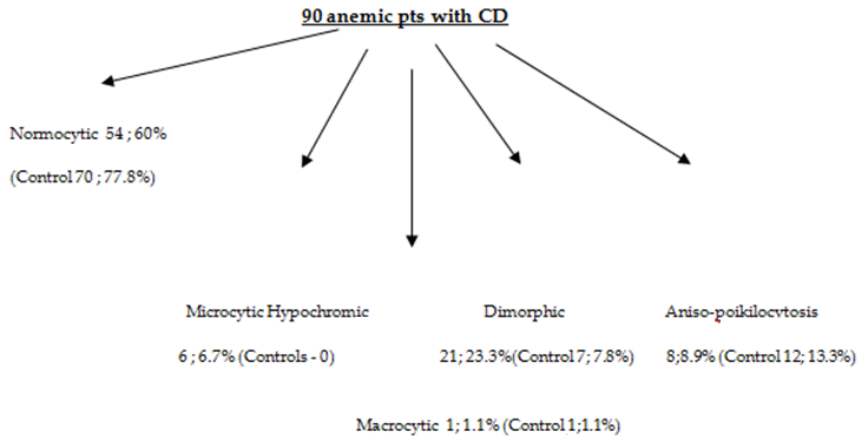

Figure 2: Red cell morphology of study group

Based on rbc morphology from blood films made, normocytic normochronic anemia was the commonest morphologic type while macrocytic anaemia was the least common in both controls and study group. Our study findings are in agreement with previous reports that patients with chronic disease who have chronic anaemia tend to have raised plasma EPO levels in response to the chronic anaemia but is usually inadequate for the degree of anaemia. A relative deficiency of EPO, despite normal renal function, may contribute to the development of chronic anaemia in patients with chronic disease.

Conclusion

Anemia is a common multifactorial complication in patients with co-morbidities, chronic Infections and inflammatory diseases, with a resultant increase in the morbidity and mortality of these individuals. The present study revealed reduced EPO levels in patients with chronic disease, which can be interpreted to mean a blunted or diminished response to endogenous Erythropoietin and or defective Iron supply. Therefore enforcing the theory that chronic disease with anemia, results in an Erythropoietin response, although inappropriate for the degree of anemia

Acknowledgment

The authors thank the study participants for their cooperation. We are also grateful to Mr. Joseph Awari, Mr Sani, and Mr. Badung for all their assistance while performing the experiments and the laboratory staff of Hematology and Chemical pathology laboratories of the Jos University Teaching Hospital.

Author Contributions

Tinuade O. Piwuna conceived, designed the experiments,

contributed reagents/materials/wrote part of the paper, and performed the experiments; Alani S. Akanmu contributed to designing the experiments, analyzing the data and writing the paper, while Christiana O. Ukoli contributed to writing and reviewing the paper.

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