CHANGES IN TISSUE ERYTHROPOIETIN AND SOME HAEMATOLOGICAL PARAMETERS IN CHRONIC KIDNEY DISEASE SUBJECTS ON BLOOD TRANSFUSION IN PORT-HARCOURT

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ABSTRACT

Chronic kidney disease (CKD) is a progressive loss in renal function over a period of time. The kidney chiefly secretes erythropoietin (EPO) which is a glycoprotein hormone that acts on the bone marrow cells resulting in red blood cell formation. Anaemia is a major complication in CKD. The aim of this study was to assess changes in erythropoietin levels in chronic kidney disease patients on blood transfusion attending health care in Port Harcourt. A total of one hundred and fifty two (152) subjects were recruited for this study. One hundred and twenty two (122) subjects were recruited from those confirmed with renal diseases from the Urology Department of the hospital. Thirty eight (38) subjects were non-transfused with blood and eighty four (84) subjects were multitransfused with blood. Thirty subjects were apparently healthy controls. EPO was determined by sandwich ELISA method while the full blood count was determined using haematologyautoanalyser, Mindray BC-6800..The results were statistically analyzed using GraphPad prism version 5.0 and statistical significance set at $P<0.05$. The result showed a significant decreased ($p<0.0001$) in EPO level in multitransfused and non-transfused subjects with mean values of 4.95±2.95mIU/Land 6.32±2.66mIU/L respectively compared to control 10.51±3.05mIU/L. On assessment of the haematological characteristics, erythropoietin secretions of patients with chronic kidney disease (CKD), the mean haematocrit, haemoglobin, mean cell haemoglobn concentration and red cell count for the multiple transfused CKD were respectively found to be significantly lower compared to that of nontransfused. This could be due to impaired erythropoietin secretion and other factors which suppress marrow erythropoiesis and shortened red cell survival in CKD which was directly associated with a decrease in red cell count and subsequent reduction in the haematocrit level. Transfusion improves anaemia through the increase in haemoglobin and hepcidin and as well suppresses erythropoiesis with an eventual decrease in erythropoietin and growth differentiation.It is therefore concluded that transfusion does not improve anaemia in CKD subjects.
Key words: Erythropoietin, Chronic Kidney Disease, Blood Transfusion, Port-Harcourt

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INTRODUCTION

Anaemia is one of the most consistent and severe haematological complications in chronic kidney disease (CKD). The two most common causes of anaemia with kidney disease are iron deficiency and the lack of haemopoietic growth factors (erythropoietin) secretions which can be managed mainly through whole blood transfusion. This study is targeted to determining the effects of multiple blood transfusion on erythropoietin secretions in chronic kidney disease subjects.Failure of renal erythropoietin secretion in CKD which is the major cause of anaemia**[1]** seems to be neglected as it is not included in any medical request in this part of the

country. Normal renal function is very important for homeostasis, so much so that situations in which renal functions are impaired can be life threatening. Renal impairment are among the most important causes of death and disability in many countries throughout the world **[2].**Chronic kidney disease (CKD) is an increasing global public health problem both in the developed and developing countries which may progress to end stage kidney failure **[3-4]**. Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive and irreversible loss in renal function due to slow destruction of renal parenchyma, over a period of months or years eventually terminating in death when sufficient numbers of nephrons have been damaged **[5]**. CKD exist in five stages, with stage 1 being the mildest and usually causing few symptoms and stage 5 is associated with severe illness with poor life expectancy if untreated. Stage 5 CKD is also called end stage renal disease (ESRD), chronic kidney failure (CKF), end stage renal failure (ESRF) or chronic renal failure (CRF). Severe CKD requires renal replacement therapy, which may involve a form of dialysis or a kidney transplant **[4]**. Chronic kidney disease affects 7.8% of the Nigerian population causing over 50 deaths per year in the affected patient irrespective of the age **[6]**.The principal determinants of anaemia are due to hemodynamic impairment with consequent water and salt retention, kidney dysfunction due to reduced renal flow, tubule-glomerular damage**[7-8]**. The resultant reduction in erythropoietin (EPO) production, deranged bone metabolism, reduced nutritional absorption due to gastrointestinal edema, and systemic pro-inflammatory status **[9]**. In acute status the prevalent mechanism could be recognized in the increase of intra- and extra-cellular plasma content due to hydro-saline retention and fluid overload (false anaemia); whereas, in the chronic state, CKD and renal blood flow redistribution are the most prevalent mechanisms that leads to relative reduction in erythropoietin production **[8][10]**.

CKD is common in congestive heart failure (CHF), and it is associated with reduced production of EPO in the kidney**[11]**. The renal damage is due mainly to haemodynamic and parenchymal derangements. Haemodynamic alteration consists of reduced renal blood flow and intra-renal blood flow redistribution that causes hypoxic renal damage; parenchymal injury is due to tubule-interstitial fibrosis, tubular loss, and glomerulo-sclerosis. The adverse liaison linking heart failure with anemic status is the impaired endogenous EPO synthesis and tissue resistance **[12-13]**. Erythropoietin is a glycoprotein hormone produced primarily in the kidney by specialized peri-tubular fibroblasts**[14]**. It regulates erythroid cell proliferation in the bone marrow in response to tissue hypoxia**[9]**. The primary stimulus for EPO production is reduced oxygen tension that induces the transcription of the EPO gene. This in turn stimulates erythroid cell proliferation and differentiation**[9]**. Oxygen delivery into the kidney is determined by renal blood flow, hematocrit, and the PO_2 of the Hb oxygen-dissociation curve. Conversely, oxygen consumption is determined by proximal tubular sodium reabsorption and the glomerular filtration rate. Both hemodynamic and parenchymal distortion may contribute to a reduction in EPO production **[15]**.

This aim of the study was to assesslevels of erythropoietin and some haematologicalparmetersin subjects with chronic kidney disease (CKD) who previously had or had not received multiple blood transfusion.

MATERIALS AND METHODS

Study Area

The study was carried out in Port Harcourt, the capital city of Rivers State, Nigeria. Port Harcourt situated within geographical coordinates 4°49′27″N 7°2′1″E.Port Harcourt features a tropical wet climate with lengthy and heavy rainy seasons and very short dry seasons. Only the months of December and January truly qualifies as dry season months in the city. The harmattan, which climatically influences many cities in West Africa, is less pronounced in Port Harcourt. Port Harcourt's heaviest precipitation occurs during September with an average of 367 mm of rain. December on average is the driest month of the year, with an average rainfall of 20 mm. Temperatures throughout the year in the city are relatively constant, showing little variation throughout the course of the year. Average temperatures are typically between 25°C-28°C in the city. University of Port-Harcourt Teaching Hospital, Port-Harcourt in Rivers State, Nigeria; was used as the base for the study. University of Port-Harcourt Teaching Hospital, Rivers State, Port Harcourt was established in 1980 with 500 bed spaces.

Study Population

A total study population of one hundred and fifty two (152) subjects were randomly collected. This comprised of 84 CKD subjects (case population) with chronic kidney disease that have received one or more of blood transfusion, 38 CKD subjects (case population) that have not been transfused. The remaining 30 subjects (control) are apparently healthy subjects who were confirmed medically free from kidney disease. Both the case and control subjects were age-matched (18 to 60 years old). The study population were recruited consecutively within a three-month period from the nephrology outpatient clinic, medical outpatient clinics, as well as medical inpatients (with CKD) in UPTH.Consent was obtained from each participant prior to blood collection. Their demographical information was collected from their hospital folder.

Inclusion Criteria for the Cases

Patients with CKD were enrolled into the study if they were eighteen years and above, and had received multiple whole blood transfusions (more than one unit of blood within one month). Also, patients with chronic kidney disease who are eighteen years and above; who have no history of transfusion.

Exclusion Criteria for the Cases

Patients with chronic kidney disease who are HIV positive, and/or had septicaemia or ulcers or other proven causes of anaemia other than primarily CKD, and/or those with a history of kidney transplant.

Criteria for the Controls

Apparently healthy subjects who were confirmed medically free from kidney disease

Sample Size

Purposive sampling and randomized method were used in the selection of subjects, taking into consideration, the total number of patient attending the nephrology clinic in University of Port-Harcourt Teaching Hospital, Rivers State.

The sample size was calculated using Cochran's sample size formula as shown below **[16]**.

N = $\frac{Z^2pq}{d^2}$

Where N =the desired sample size

Z = the Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level

p = the prevalence of target population

q = 1- p

d = degree of accuracy desired set at 0.05

Therefore N = $\frac{(1.96)^2 \times 0.078 \times (1-0.078)}{(0.05)^2}$ p = 7.8% or 0.078

N = 110

By adding 10% of non-respondent, the sample size was be 121.

Sample Collection

Ten milliliters (10) of venous blood sample was drawn from the peripheral vein in the upper limb of subjects; 5mls was transferred into EDTA bottles for the analysis of haematological parameters and the remaining 5mls was transferred into plain plastic bottle containing no additives or anticoagulant for the analysis of erythropoietin and thrombopoietin. The clotted blood was centrifuged at 1500 r.p.m. for 3 mins at room temperature and the obtained serum was stored at -20^{o}C until assayed. Assay was carried out on the serum sample thawed only once.

Experimental Design

This cross sectional study research was carried out in 152subjects of which 84 were CKD subjects who have been transfused, 38 were CKD subjects that have not been transfused, and 30 were apparently healthy subjects (control). These CKD subjects were recruited from the nephrology outpatient clinic, medical outpatient clinic, as well as medical inpatients (with CKD) in the University of Port Harcourt Teaching Hospital after approval from institutional Ethical Clearance Committee from 18th May 2018 to August 10th 2018. Chronic kidney disease subjects were enrolled in the study at first visit irrespective of the disease stage. The demographical data of the subjects were obtained. These included age, sex, and trimester. The women were of varying age ranging from 18 to 36 years and above.

Ethical Consideration

Ethical approval and permission was received from the ethical committee of University of Port-Harcourt Teaching Hospital, Port-Harcourt. Informed consent of the participants involved was obtained. Right of privacy, anonymity & confidentiality was maintained. Any important findings in the study or any specific patient was communicated to the Clinician in the nephrology clinic.

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Laboratory Methods

Estimation of Erythropoietin Using ELISA

The erythropoietin status will be determined according to the method described by **[17]**.The EPO Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay] for the measurement of the biologically active 165 amino acid chain of EPO. It utilizes two different mouse monoclonal antibodies to human EPO specific for well-defined regions on the EPO molecule. One mouse monoclonal antibody to human EPO, is biotinylated and the other mouse monoclonal antibody to human EPO is labeled with horseradish peroxidase [HRP] for detection.

Experimental Procedure

Serum EPO levels were measured by a solid-phase enzyme-linked immunosorbent assay (Quantikine R & D) based on the double-antibody sandwich method. Microtitre wells were precoated with a murine monoclonal antibody against rHu-Epo. Assay diluent was pipetted into each well. Standards and samples were added into wells and incubated for 2 hours at room temperature. Polyclonal antibody against rHu-Epo conjugated to horseradish peroxidase was added to each well after aspiration and incubated for 2 hours. Aspiration and washing were repeated four times and mixture of hydrogen peroxide and tetramethylbenzidine was added to wells and incubated for 20 min at room temperature. To stop the reaction, 2N sulphuric acid was added and optical density was determined within 30 min using a microplate reader set at 450nm. Erythropoietin concentrations were extrapolated from a standard curve. The sensitivity of the test was determined to be 0.6mIU/ml.

Estimation of Haematological Parameters

Haematological parameters: red cell counts (RBC), white blood cell counts(WBC), lymphocyte counts(LYM), granulocyte counts(GRA),mean cell volume(MCV), mean cell haemoglobin(MCH), mean cell haemoglobin concentration(MCHC), Hematocrit (HCT), Haemoglobin concentration and platelet count (PLT) were analysed using an automated system, Mindray BC-6800 Haematologyanalyser**[18]**. It is a five-part machine that analyses 33 parameters. To activate the cell counter, prime button was pressed. The tube of well- mixed EDTA blood was placed under the whole blood aspirator tip (inserted at least 1 inch into the blood), and whole blood button was then pressed. When wipe was displayed on the indicator, the blood sample was removed and aspirator tube was wiped with a piece of gauze moistened in diluents. After which result were then produced and printed out within minutes.

Statistical Analysis

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Data collected for this study were subjected to statistical analysis using Graph pad Prism version 5.0. The sample population was grouped among healthy individuals (control), diseased patients who have been transfused with whole blood and those who are naïve to blood transfusion (subject). Quantitative data were analyzed using one-way analysis of variance (ANOVA) and a probability value (p-value) of < 0.05 was considered significant.

RESULTS

The demographical information of the participants are shown in table 4.1.

Table 4.1 Shows the Demographic Information of the Participants

Characteristics	N%	Control N (%)	Non-Transfused N (%)	Multi-Transfused N (%)
Overall	152 (100)	30 (19.7)	38 (25)	84 (55.3)
Age (Mean±SD)	33.17±10.20	30.43±9.24	33.97±11.63	35.11±9.73
Age Group (Years) **Males:** **<21** **21-30** **31-40** **41-50** **51-60** **60+**	 7 (4.6) 12 (7.8) 24 (15.8) 16 (10.5) 18 (11.8) 17 (11.3)	 2 (6.7) 3 (10.0) 6 (20.1) 2 (6.7) 3 (10.0) 2 (6.7)	 2 (5.3) 2 (5.2) 5 (13.2) 6 (15.8) 7 (18.4) 4 (10.5)	 3 (3.6) 7 (8.3) 13 (15.5) 8 (9.5) 8 (9.5) 11 (13.1)
Age Group (Years) **Females:** **<21** **21-30** **31-40** **41-50** **51-60** **60+**	 3 (2.0) 7 (4.6) 10 (6.6) 11 (7.3) 18 (11.8) 9 (5.9)	 1 (3.3) 1 (3.3) 1 (3.3) 4 (13.3) 4 (13.3) 1 (3.3)	 0 (0) 2 (5.2) 2 (5.2) 2 (5.2) 4 (10.5) 2 (5.2)	 2 (2.4) 4 (4.8) 7 (8.3) 5 (6.0) 10 (11.9) 6 (7.1)
Age group of males	94 (61.8)	18 (60)	26 (68.4)	50 (59.5)
Age Group of females	58 (38.2)	12 (40)	12 (31.6)	34 (40.5)

Table 4.2Erythropoietin Values Of Control, Chronic Kidney Disease Subjects Who Have Received Multiple Transfusion (Case 1) and Subjects Who Have Not (Case 2).

Variables	Control n=30	Case 2 n=38	Case 1 n=84	p-value	F-value

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EPO (mIU/L)	10.51 ± 3.05	6.32 ± 2.66	4.95 ± 2.95	<0.0001	40.44
Post hoc: Tukey's Multiple Comparison Test					
Comparison Remarks					

Correlation Coefficient	**PCV (%)**	**Hb (g/dl)**	**MCHC (g/dl)**	**MCH (pg/cell)**	**MCV (fl)**	**PLT ($x10^9$/L)**

EPO Case 1 (mIU/L) vs EPO Case 2(mIU/L)	Significant***
EPO Case 1 (mIU/L) vs EPO Control (mIU/L)	Significant***
EPO Case 2(mIU/L) vs EPO Control (mIU/L)	Significant***

Table 4.3 shows the correlation coefficient between erythropoietin and haematological parameters of chronic kidney disease subjects who have been transfused (case 1). Using Pearson correlation between haemopoietic growth factor parameters and haematological parameters of chronic kidney disease subjects who have been transfused (cases1), erythropoietin in case 1 subjects showed no significant correlation with haematological parameter values: PCV (r = -0.031; p = 0.779), Hb (r = -0.019; p = 0.866), MCHC (r = -0.064; p = 0.564), MCH (r = 0.029; p = 0.790), MCV (r = 0.032; p= 0.770), Platelet (r = -0.099; p = 0.372), RBC (r = -0.026; p= 0.813).

Table 4.3. Correlation Coefficient between Erythropoietin and Haematological Parameters of Chronic Kidney Disease Subjects who have beenTransfused.

Table 4.4 shows the correlation coefficient between haemopoietic growth factor parameters and haematological parameters of chronic kidney disease subjects who have not been transfused (case 2). Using

Correlation Coefficient	**PCV (%)**	**Hb (g/dl)**	**MCHC (g/dl)**	**MCH (pg/cell)**	**MCV (fl)**	**PLT ($x10^9$/L)**	**RBC ($x10^6$/ul)**
EPO (mIU/L)	-0.031	-0.019	-0.064	0.029	0.032	-0.026	-0.026
p-value	0.779	0.866	0.564	0.790	0.770	0.813	0.813
Remark	NS	NS	NS	NS	NS	NS	NS

Pearson correlation between haemopoietic growth factor parameters and haematological parameters of chronic kidney disease subjects who have not been transfused (cases2), erythropoietin in case 2 subjects showed no significant correlation with haematological parameter values: PCV (r = 0.221; p = 0.181), Hb (r = 0.259; p = 0.116), MCHC (r = 0.190; p = 0.252), MCH (r = 0.191; p = 0.250), MCV (r = 0.125; p= 0.454), Platelet (r = -0.017; p = 0.917).

Table 4.4. Correlation Coefficient between ErythropoietinParameters and Haematologicalparametersof chronic kidney disease subjects who have not been transfused.

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EPO (mIU/L)	0.221	0.259	0.190	0.191	0.125	0.017
p-value	0.181	0.116	0.252	0.250	0.454	0.917
Remark	NS	NS	NS	NS	NS	NS

SD= Standard deviation
Percentages may not add up to exactly 100 due to rounding up.
N= total number
All Values: Mean±SD,
*** = highly significant

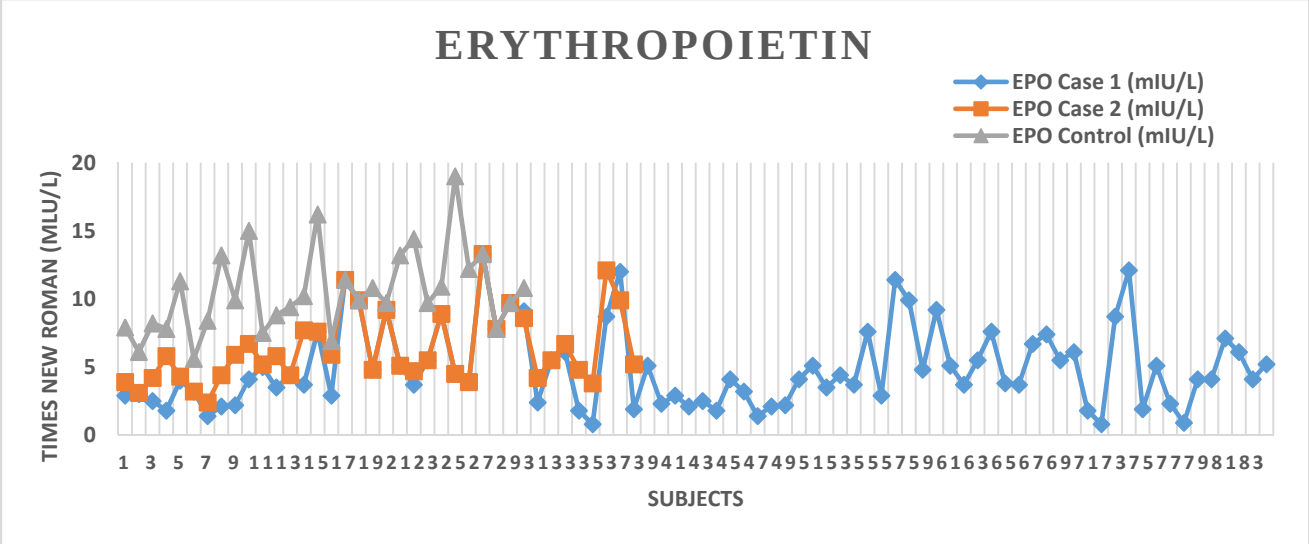

Figure 4.1. Line Graph of Erythropoietin levels of Cases 1, 2 and Control Subjects

DISCUSSION

Chronic kidney disease (CKD) is a condition characterized by a gradual loss of kidney function over time. Anaemia among others is a major complication in CKD. These problems may happen slowly over a long period of time affecting changes in the biological parameters like full blood count (FBC) in the blood.This aim of the study was to assesslevels of erythropoietin and some haematologicalparmetersin subjects with chronic kidney disease (CKD) who previously had or had not received multiple blood transfusion at the University of Port Harcourt Teaching Hospital.

On assessment of the haematological characteristics, erythropoietin secretions of patients with chronic kidney disease (CKD), the mean haematocrit, haemoglobin, mean cell haemoglobn concentration and red cell count for the multiple transfused CKD were respectively found to be significantly lower compared to that of nontransfused. This is consistent with **[19][8]**. This could be due to impaired erythropoietin secretion and other factors which suppress marrow erythropoiesis and shortened red cell survival in CKD which was directly associated with a decrease in red cell count and subsequent reduction in the haematocrit level. Transfusion improves anaemia through the increase inhaemoglobin and hepcidin and as well suppresses erythropoiesis

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with an eventual decrease in erythropoietin and growth differentiation **[20]**. But in the blood stream red cells themselves do not express erythropoietin receptor, so cannot respond to erythropoietin in the transfused blood thus resulting in iron overload due to the selective haemolysis of young red blood cells, an indication of suppressed erythropoietin **[14]**. This could also be due to decrease in 2,3-diphoshpoglycerate in stored blood before transfusion.The concentration of erythrocyte 2,3-DPG in preserved RBCs decreases with storage which can lead to anaemia after whole blood transfusion had been done. The decrease is dependent on pH. In whole blood collected in CPDA-1, the pH decreases from 7.16 to 6.73 over 35 days as a result of lactic acid formation, and the 2,3-DPG concentration decreases markedly from 13.2 to 0.7 μmol/g hemoglobin.In the RBC, the glucose intermediate metabolite 1,3-DPG is transformed to 2,3-DPG by a mutase. The 2,3-DPG is then dephosphorylated to 3-phosphoglycerate by a phosphatase. The phosphatase is inactive at pH levels above 7.2 but is more active at the lower pH in older preserved RBCs, contributing to the decreased 2,3-DPG levels.

The function of erythrocyte 2,3-DPG is to bind to deoxy-hemoglobin and facilitate oxygen transport. When 2,3-DPG binds to deoxy-hemoglobin, the deoxy-haemoglobin molecule is stabilized, and the equilibrium between deoxy-haemoglobin and oxy-haemoglobin shifts toward deoxy-haemoglobin. This interaction shifts the oxygen-dissociation curve to the right, decreasing the oxygen affinity of haemoglobin and enhancing oxygen delivery to tissues**[21]**.Therefore, with decreased 2,3-DPG levels, the oxygen dissociation curve is shifted to the left, decreasing oxygen delivery to tissues. It proceeds by blunting EPO and erythroid progenitor cell production at bone level; therefore, it raises tissue EPO resistance by reducing EPO gene expression, perturbing EPO signal transduction, and downregulation of EPO receptors, **[21]**.

EPO is the hormone which is the major homoral regulator of red cell production and helps to maintain the viability of RBC by retarding the cleavage of DNA that occurs normally in CFU-Es. In the absence of EPO, DNA cleavage is rapid and leads cell death.

In comparison of the RBC, PCV and Hb between CKD patient and control groups suggested anemia. This finding is supported by **[22-23]**. This could be due to the adverse connection between heart failure with anaemic status in the impaired endogenous EPO synthesis and tissue resistance **[10]** and **[24]**. EPO is a glycoprotein hormone produced primarily in the kidney by specialized peritubular fibroblasts. It regulates erythroid cell proliferation in the bone marrow in response to tissue hypoxia. The primary stimulus for EPO production is reduced oxygen tension that induces the transcription of the EPO gene. This in turn stimulates erythroid cell proliferation and differentiation. Oxygen delivery into the kidney is determined by renal blood flow, hematocrit, and the PO_2 of the Hb oxygen-dissociation curve. Conversely, oxygen consumption is determined by proximal tubular sodium reabsorption and the glomerular filtration rate. Both hemodynamic and parenchymal distortion may contribute to a reduction in EPO production resulting in anaemia**[25]**.

Inflammatory cytokines elaborated in CHF due to CKD, such as tumor necrosis factor alpha and interleukin-6, can cause anemia by reducing EPO production in the kidney and its activity in the bone marrow and by eliciting EPO resistance at the peripheral tissue level **[26]**. All these occurrences are mediated by a specific peptide produced by the liver acting as in the erythroid metabolism as in the iron absorption and reuptake: hepcidin. It proceeds by blunting EPO and erythroid progenitor cell production at bone level; therefore, it raises

tissue EPO resistance by reducing EPO gene expression, perturbing EPO signal transduction, and downregulation of EPO receptors **[27]**. As previously reported, hepcidine inhibits the protein ferroportin-1, leading to a reduction in gastrointestinal iron absorption as in iron release from its storage (macrophages and reticuloendothelial cells) **[28]**. This results in low serum iron and decreased delivery of iron to the bone marrow, and thus iron deficiency anemia (the so called functional iron deficiency anemia). This inflammatory-induced anemia is probably the main cause of anemia in CKD **[27][29][26][28]**.

Also, anemia in CKD could be as a result of hemodilution**[7]**. This is consistent with the fact that as the GFR declines, there is a corresponding decline in the Hb concentration, which is mostly due to the reduced synthesis of erythropoietin **[7][30]**.

Furthermore, the haemolytic factor implicated in decreased red blood cells survival is presumed to be a toxic substance normally excreted or metabolized by the kidney, one such substance is guanidine and its derivatives which appear to be a subset of the of the many retained metabolites, adversely affect erythtrocyte survival. The haemoglobin concentration and haematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced. In CKD because of the impaired EPO secretion, increased destruction of red blood cell leads to a fall in red blood cell count, which reduced the Hb concentration and haematocrit. There was a significant decrease between the platelet count of CKD and control groups. This is similar to the report by**[31-32][13]**. This could be due to bleeding and shear stress of glomerular **[9]**.Platelets circulate in close contact with the endothelium, continually monitoring its integrity. When the vessel wall is damaged, platelets bind to sub-endothelial proteins, initiating the process of primary hemostasis. At sites of blood loss, the platelets aggregate to form a vessel sealing plug to halt bleeding. Activated platelets at sites of injury also provide a surface for assembly of coagulation reactions, resulting in the production of fibrin and consolidation of the thrombus resulting in platelet consumption that accounts for a progressively larger fraction of the reduced total daily production as the platelet count drops.

A negative correlation between erythropoietin (EPO) and PCV, Hb, MCHC, RBC, and PLT in non-transfused and multiple transfused was observed in this study supported by **[22].**This is an inverse relationship that could be a reflection of the severity of chronic kidney disease.Thrombopoietin levels in blood are inversely related to the number of platelets in the blood and megakaryocytes in the bone marrow; unlike erythropoietin production **[9][33][20]**. A known direct relationship normally exist between plasma erythropoietin levels and haemoglobin (Hb) concentration and haematocrit (PCV). As erythropoietin is increasing, the haemoglobin and haematocrit will also increase get to it maximum with an eventual suppression of erythropoiesis pending on when stimulated again by anaemic condition. This showed the blood transfusion has no good effect on the repair of the damaged renal tissues by decreasing the levels of EPO which induces apoptosis of the cell. The presence of anaemia is an indication of dyregulation of iron homeostasis and inflammatory processes act as the main mediators **[34]** due to the impaired nature of EPO that can increase iron absorption by suppressing the hormone hepcidine**[35].**

Erythropoietin levels can affect thrombopoietin secretions due to the extensivehomology between erythropoietin and thrombopoietin, erythropoietin acts as the major humoral regulator of platelet mass.

CONCLUSION

In conclusion, a negative correlation was observed between EPO and PCV, Hb, MCHC and PLT, between the non-tranfused patients and multiple transfused patients. This showed that the blood transfusion in deranged kidney has no effect on improving the anaemic state of the patient. Chronic kidney disease subjects show abnormal haematological parameters due to impairment of EPO levels in CKD that resulted in the decreased production of haematological parameters.

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