Original Research Article

Comparative Assessment of Iron Deficiency Anaemia among Chronic Kidney Disease Subjects in Niger Delta Nigeria

ABSTRACT

Iron Deficiency Anaemia which is reduced red blood cells due to iron deficiency had been reported to be a major challenge among Chronic Kidney Disease patients. The cause of anaemia in these patients is multifactorial, ranging from the inability of the kidneys to excrete hepcidin to even the inability of the kidneys to produce erythropoietin. This study is aimed at comparatively assessing Iron Deficiency Anaemia between Chronic Kidney Disease and Apparently Healthy Subjects in Niger Delta. A total of 88 subjects were recruited, 55(62.50%) Chronic Kidney Disease patients and 33(37.50%) Control subjects. Samples were collected and analysed at the Braithwaite Memorial Specialist Hospital, Port Harcourt, Rivers State. Iron Deficiency Anaemia Indicators such as Serum Hepcidin Levels were measured using commercial DRG Hepcidin-25 kit and other Haematological Indices were determined using Automation (Sysmex KX-21N Automated Haematology Analyzer), Leishman Staining Technique and Supravital Staining Technique; Questionnaire was also used to obtain some data, the data obtained were analysed using SPSS version 21.The mean values for Serum Hepcidin, Haemoglobin(HB), Packed Cell Volume(PCV), Red Blood Cell count(RBC), Mean Cell Volume(MCV), Mean Cell Haemoglobin(MCH), Mean Cell Haemoglobin Concentration(MCHC), Reticulocyte count(Retics) and Red Cell Distribution Width(RDW) were 52.00ng/ml, 10.00 g/dL, 31.00%, 3.74×10^{2}/L, 78.84fL, 26.58pg, 31.79g/dL, 0.64%, and 14.94% respectively in the CKD patients while that for the Apparently Healthy Subjects were 16.00ng/ml, 14.00g/dL, 42.00%, 4.69×10^{12}/L, 89.37fL, 29.59pg, 33.00g/dL, 1.09% and 13.20% respectively. Statistical T-Test of significance revealed that Serum Hepcidin level was elevated significantly in CKD patients (52.00ng/ml) when compared with Apparently Healthy Subjects (16.00ng/ml) $t_{86}= 6.54$, $p<0.05$, Haemoglobin value of 10.00g/dL in CKD patients was significantly lower than 14.00g/dL in Apparently Healthy Subjects ($t_{86} = -8.49$, $p<0.05$), and the values of other haematological indices were lower except RDW that was elevated significantly among CKD patients when compared with the Apparently Healthy Subjects ($p<0.05$) all at significance level of 0.05. The peripheral Blood Film showed microcytic hypochromic red cells among the CKD Subjects while normocytic normochromic red cells in theApparently Healthy Subjects. The study recorded elevated Serum Hepcidin level and decreased levels of the other Haematological indices amongthe Chronic Kidney Disease patients, knowing well that elevated Hepcidin level reduces iron needed for red blood cell production, the estimation of Serum Hepcidin level in CKD patients in addition to the other Haematological indices will improve the diagnosis, treatment and management of Iron Deficiency Anaemia in these patients.

Keywords: *Iron deficiency. hepcidin, anaemia, chronic kidney disease*

1.0 Introduction

Iron is an essential element required by every aerobic organism largely because of its oxygen carrying capacity in, it is also important for the production of red blood cells. A newly discovered 25- amino acid peptide hormone secreted basically by the liver called Hepcidin is the

major controller of systemic iron homeostasis. As important as iron, its excess and reduced states are fatal, so hepcidin helps to maintain a normal level of iron in the circulation for effective erythropoiesis (Conrad and Umbriet, 2000; Evan, 2017). Hepcidin does its function by binding to the iron exporter ferroportin causing its internalization and degradation which results in reduced dietary iron absorption and also reduced iron release from iron storing sites such as the macrophages and liver and this occurs when iron stores are full in a normal condition (Sukru*et al*., 20I4; Lia*et al.,* 20I5). When hepcidin is secreted in excess (up- regulated), iron level falls below normal while when secretion is low (down- regulated), it results in iron overload. The former if not managed properly will eventually result in iron deficiency anaemia(Jeremiah and Koate, 2010; Janz*et al*., 20I3).It occurs in several disease conditions including Chronic Kidney Disease (CKD). In chronic kidney disease patients, hepcidin levels have been reported to be abnormally high due to inability of the kidney to excrete hepcidin and also inflammation. This abnormally high hepcidin level causes reduction in the amount of iron in the circulation and as such iron deficiency, then iron deficiency anaemia(Kemna*et al*., 2007; Jeremiah and Koate, 2010). Following the increasing rate of chronic kidney disease, this study is focused on assessing hepcidin level in chronic kidney disease patients in relation to anaemia (iron deficiency anaemia).

2.0 Materials and Methods

2.1 Study Area

The study was carried out in Port Harcourt, capital of Rivers State and port town in southern Nigeria. It is one of the 23 Local Government Areas of Rivers State. It lies along the Bonny River (an eastern distributary of the Niger), 41 miles (66 km) upstream from the Gulf of Guinea.

2.2 Study Design

This study was designed as a comparative cross-sectional study. Samples were collected at a single point.

2.3 Study Population

55 chronic kidney disease patients attending clinic in Braithwaite Memorial Specialist Hospital, Port Harcourt and 33 apparently healthy individuals as control who are inhabitants of Rivers State making a total of 88 subjects recruited in this study.

2.4 Eligibility Criteria

Inclusion and Exclusion criteria were stated; for the Inclusion Criteria, adult chronic kidney disease patients who consented were part of the study, adult control subjects whose creatinine and haemoglobin levels are within normal ranges who gave their consent were recruited into the study. And the Exclusion Criteria, children were excluded from the study, adults with acute kidney failure and adults with chronic kidney disease and control subjects who did not consent.

2.5 Ethical Consideration/Informed Consent

Ethical approval was obtained from Rivers State Health Ethics Committee. A written informed consent was obtained from the participants.

2.6 Sample Collection, Transportation, Preparation and Storage

A total of ten milliliters (10ml) of venous blood was collected by venipuncture (vacutainer collection), into a plain sample container 5ml was added and the other 5ml was added into an EDTA bottle. The sample in the plain container was allowed to clot, and serum separated by centrifuging at ambient temperature into other sterile plain containers. For hepcidin measurement, the serum obtained from the samples collected in the plain sterile containers were stored at -20^{o}c (frozen at only once) before analysis. The samples in the EDTA containers were analyzed the same day of collection and were not kept on the bench (at room temperature) for more than 6 hours and were used for the full blood count. All the samples were transported from the point of collection to the points of sample preparation, storage and analysis by the help of sample carriers.

2.7 Laboratory Methods

Serum Hepcidin level was measured using the Enzyme Linked Immunosorbent Assay (ELISA) method. The other hemaetological indices (Iron Deficiency Anaemia indicators) Haemoglobin, Packed Cell Volume, Red Blood cell Count, MCV, MCH, MCHC and RDW were determined using the automated analyzer. Reticulocyte count was determined using the New Methylene Blue Staining Technique.Peripheral blood film was made and stained with the Leishman stain for the red cell morphology study.

2.8 Data Analysis

Data obtained were analyzed, descriptively (percentage/frequency, mean, standard deviation) and inferentially (Independent T-Test and Pearson Correlation) at significance level of 0.05 using the Statistical Package for Social Sciences (SPSS) Version 21.

3.0 Results

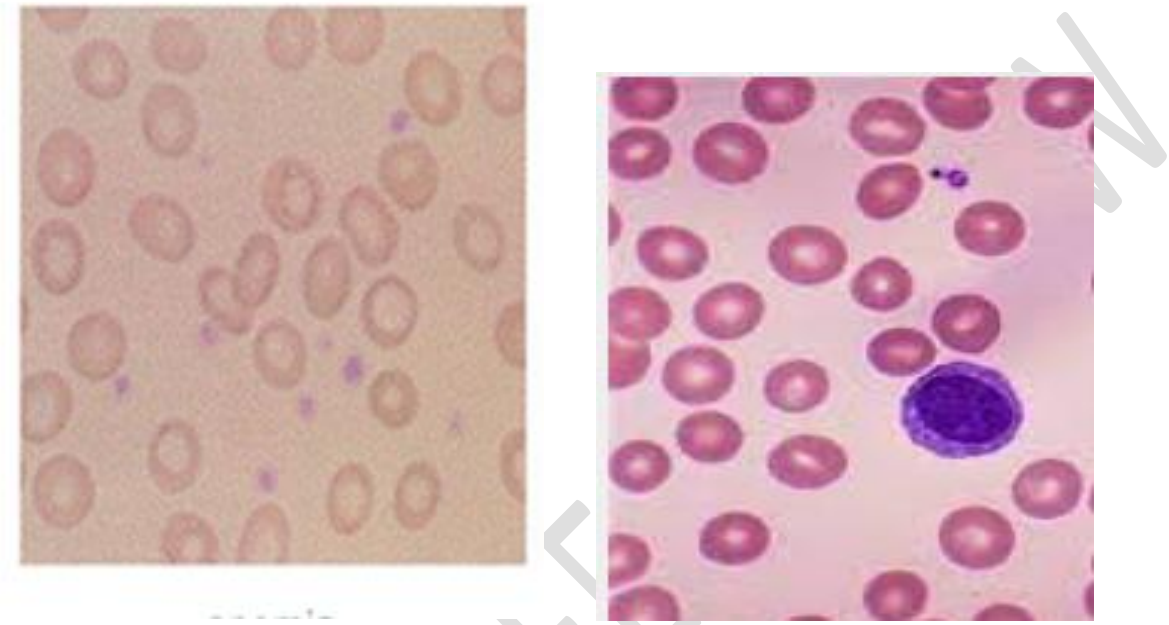
Table 1 showed the Mean, Standard Deviation, t and p-values of Serum Hepcidin and Iron Deficiency Anaemia expressed in some Haematological parameters (Heamoglobin (HB), Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Reticulocyte Count (Retics) and Red Cell Distribution Width (RDW)) of the two groups (CKD and control groups). The t-values and p-values for SH, HB, PCV, RBC, MCV, MCH, MCHC, Retics and RDW were t_{86} = 6.54, -8.49, -8.89, -6.50, -7.55, -5.07, -2.74, -2.80, and 6.28, respectively. The p-values for all the parameters in table were less than 0.05; therefore there were statistically significant differences between the levels of the various parameters between the two groups of the study population.

Table 1: Mean Comparison of Iron Deficiency Anaemia Indices between CKD Patients and Apparently Healthy Subjects

Variables	Groups	N	Mean±SD	t-value	Df	p- value
Hepcidin(ng/ml)	CKD	55.00	52.00±36.00	6.54	86.00	0.00
	Control	33.00	16.00±13.00			
HB(g/dL)	CKD	55.00	10.00± 3.00	-8.49	86.00	0.00
	Control	33.00	14.00±1.00			
PCV(%)	CKD	55.00	31.00± 8.00	-8.89	86.00	0.00
	Control	33.00	42.00±3.00			
RBC($\times 10^2$/L)	CKD	55.00	3.74±0.93	-6.50	86.00	0.00
	Control	33.00	4.69±0.43			
MCV(pg)	CKD	55.00	78.84±9.31	-7.55	86.00	0.00
	Control	33.00	89.37±3.48			
MCH(fL)	CKD	55.00	26.58±3.99	-5.07	86.00	0.00
	Control	33.00	29.59±1.46			
MCHC(g/dL)	CKD	55.00	31.79± 3.02	-2.74	86.00	0.01
	Control	33.00	33.00±1.01			
Retics(%)	CKD	55.00	0.63±0.77	-2.80	86.00	0.01

	Control	33.00	1.09±0.68			
RDW(%)	CKD	55.00	14.94±1.46	6.28	86.00	0.00
	Control	33.00	13.20± 0.80			

P= 0.05, P ≤ 0.05 = Significant, P > 0.05 = Not Significant



CKD **Control**

(microcytic hypochromic RBC) **(normocytic normochromic RBC)**

Plate 1. Peripheral Blood Film for the Study Population

Plate 1. displayed the Peripheral Smear Examination of the Study Population, microcytic (smaller than normal) and hypochromic (pale coloured) red blood cells among the Chronic Kidney Subjects compared to the normocytic and normochromic red blood cells among the Control Subjects from this study. There were also anisocytosis (varying sizes of red blood cells) and poikilocytosis (varying shapes of red blood cells) in the blood films of the Chronic Kidney Disease Subjects though not marked.

4.0 Discussion

Serum Hepcidin was measured in the two groups in this study; there was statistically significant elevated Serum Hepcidin level in the CKD participants as compared with the Control participants. This implies that in chronic kidney disease, the impairment of the kidneys prevents proper hepcidin removal resulting in the accumulation of hepcidin in the circulation, also it has been recorded that inflammation induces over production of hepcidin and this is a very common condition among these patients. The elevated Serum Hepcidin level will contribute to reduced availability of iron in the circulation of the CKD patients.

This report was in line with many studies by various groups (Ashby *et al*., 2008; Ganz*et al*., 2008; Bansal*et al*., 2010; Jelic*et al*., 2013; Taheri*et al*., 2015) with levels of serum hepcidin ranging from 10 folds based on the technique employed, of which many recorded serum hepcidin level as 27.00 – 158.00ng/ml for CKD and normal serum hepcidin to be in the range of 1.00 – 55.00ng/ml, also 1.000 – 130ng/ml (Frazer and Anderson, 2009) and 1.700 – 82.00ng/ml (Peters *et al*., 2010) for CKD subjects. Different from this study Peters *et al*., (2010) recorded decreased Serum Hepcidin levels among dialyzed patients and Yasuhiro and Masafumi, (2010) showed a conflicting results on the cause of the elevated Hepcidin and concluded that the regulation of iron concentration is by many factors not only Hepcidin.

The haematological parameters that were determined to ascertain anaemia (Iron Deficiency Anaemia) in this study were heamoglobin(HB), packed cell volume(PCV), red blood cell count(RBC), mean cell volume(MCV), mean cell haemoglobin(MCH), mean cell haemoglobin concentration(MCHC), reticulocyte count(Retics), red cell distribution width(RDW) and Peripheral Blood Smear. From the results obtained in the present study there were significant reduction in the ranges of these indices in the CKD subjects than in the Control subjects in all the haematological parameters except RDW which was higher in CKD subjects than the Control.

The p-values for all the parameters were less than 0.05; therefore there were statistically significant differences between the levels of the various parameters (HB, PCV, RBC, MCV, MCH, MCHC, Retics and RDW) among the two groups of the study population. The peripheral blood smear morphology pointed to iron deficiency anaemia (microcytic and hypochromic red cells) among the CKD patients compared to the normocytic and normochromic red cells in the Control subjects.

This denotes that anaemia due to lack of iron is prevalent among the Chronic Kidney Disease patients which may be as result of the high Serum Hepcidin recorded among this population and

other factors. Anaemia in these patients is a contributing factor to cardiovascular disease, the major cause of death in this condition, many of which are hospitalized and additional cost of medical care. This study was consistent with that of Shadeda and colleagues (2007) in their study on changes in haematological indices in different stages of chronic renal failure (CRF), they also had significantly lower levels of some haematological indices in CRF compared to normal subjects but disagrees in some ways with the studies of Afshan*et al*., (2013) and Sneha*et al*., (2015) which recorded reduced levels of HB, PCV, RBC, MCH and MCHC, then peripheral blood smear showing normocytic normochromic red cells

Conclusion/Recommendation

Anaemia is one common cause of death in chronic disease patients and this study has shown that iron deficiency anaemia is common among patients with chronic kidney disease and one of the contributing causes is increased hepcidin concentration. . Serum Hepcidin measurement should be included among the panel of tests to be carried out in the treatment and management of anaemia (Iron Deficiency Anaemia) in chronic kidney disease patients.

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