

Original Research Article

Serum Hepcidin level among Chronic Kidney Disease subjects accessing Healthcare in Braithwaite Memorial Specialist Hospital Port Harcourt

ABSTRACT

Hepcidin is the major controller of systemic iron homeostasis and the role of the kidney in regulating hepcidin level is vital in the whole process of iron and hepcidin relationship. This study was aimed at evaluating serum Hepcidin level among Chronic Kidney Disease subjects accessing Healthcare in BMSH Port Harcourt Metropolis. The study was conducted in Port Harcourt at Braithwaite Memorial Specialist Hospital among 55 CKD subjects and 33 normal individuals making up the control group. Subjects were selected randomly and 5mls of blood was collected in plain bottle using venipuncture technique for laboratory assessment of hepcidin. Hepcidin was assayed using competitive ELISA method. T-test was used to compare the mean difference oh hepcidin between both groups. Result showed that there was a significant difference in hepcidin level between CKD and control groups; 52.00 ± 36.00 ng/ml for CKD group and 16.00 ± 13.00 ng/ml for control group, $p < 0.05$. This study has shown that CKD has a significant impact in hepcidin level blood and consequently on iron regulation.

Keywords: *Hepcidin, chronic kidney disease*

1.0 Introduction

A newly discovered 25- amino acid peptide hormone secreted basically by the liver called Hepcidin is the major controller of systemic iron homeostasis. Hepcidin production is reduced when iron stores are not full in physiological condition and this results in increase iron absorption by the enterocytes, recycling by the macrophages and release by the hepatocytes and much iron will be available in the circulation, meaning the ferroportin iron transporting function is increased. Anemia and hypoxia can induce the down-regulation of hepcidin synthesis which will enable the absorption, recycling and releasing of iron from the respective sites for ferroportin exportation to where it is needed for red blood cell production and enable oxygen supply to various parts of the body under normal condition (Papanikolaou *et al.*, 2005; Sandro *et al.*, 2014). When iron stores are full, the liver cells will be sensitized to synthesize hepcidin

through a yet to know mechanism to regulate the concentration of iron in circulation. That is under normal condition, the response to higher than normal level of iron in circulation is the shutting down of iron absorption, recycling and storage in the sites mentioned enterocytes, macrophages and hepatocytes respectively by hepcidin (Weiss and Goodnough, 2005; Jelic *et al.*, 2013). Hepcidin production can also be induced by bacterial lipopolysaccharide and cytokine, especially interleukin-6. Therefore, the gene of hepcidin is seen as an acute phase responsive gene which is over produced in response to inflammation (Jelic *et al.*, 2013).

Iron is an essential and the second most common element on earth, functioning as a building block for steel, nourishing plants and also helps in carrying oxygen the blood (red blood cell). 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.*, 2014; Lia *et al.*, 2015). 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.*, 2013). It occurs in several disease conditions including Chronic Kidney Disease (CKD). Chronic kidney disease can be classified into stages 1 to 5 based on the Estimated Glomerular Filtration Rate (eGFR) and Glomerular Filtration Rate (GFR) $< 60 \text{ ml/min/1.73m}^2$ for 3 months results in chronic kidney disease (Kidney Disease Improving Global Outcomes (KDIGO), 2013) which may eventually result to end stage kidney disease (renal failure), a state of renal insufficiency due to impaired kidney function in which the kidney fails to adequately excrete wastes from the blood (Fyneface *et al.*, 2018; Fyneface *et al.*, 2020). Elevated Hepcidin level had been reported to be one of the causes of Anaemia (Iron Deficiency Anaemia) in Chronic Kidney Disease patients but this had been neglected in the diagnosis, treatment and management of Anaemia in these patients over time. 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. Based on the growing rate of chronic kidney disease, this study is focused on assessing hepcidin level in

chronic kidney disease patients assessing healthcare at Braithwaite Memorial Specialist Hospital Port Harcourt.

Materials and Methods

2.1 Study Area

The study was carried out in Port Harcourt metropolis, capital of Rivers State, southern Nigeria. Port Harcourt is a notable city in the south-south geopolitical zone.

2.2 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.

2.3 Eligibility Criteria

Inclusion Criteria

- Adult chronic kidney disease patients who consented were part of the study
- Adult control subjects whose creatinine levels were within normal range and gave their consent were recruited into the study.

Exclusion Criteria

- Children were excluded from the study
- Adults with acute kidney failure
- Adults with chronic kidney disease and control subjects who did not consent.

2.4 Ethical Consideration/Informed Consent

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

2.5 Sampling method

Subjects were randomly selected using simple random technique to ensure everyone was given equal opportunity for selection.

2.6 Sample Collection, Transportation, Preparation and Storage

A total of five milliliters (5ml) of venous blood was collected by venipuncture (vacutainer collection), the 5ml was added into a plain bottle. The sample in the plain container was allowed to clot, and serum separated by centrifuging at ambient temperature into other sterile plain containers. The serum obtained from the samples collected were stored at -20°C (frozen at only

once) before analysis. 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)

Hepcidin Measurement

Competitive Enzyme Linked Immunosorbent Assay Method

Principle: The DRG Hepcidin-25 ELISA kit is a solid phase-enzyme-linked immunosorbent Assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a monoclonal (mouse) antibody directed towards an antigenic site of the hepcidin-25 molecule. The Hepcidin-25 in the sample analysed competes with a Hepcidin-25-biotin conjugate for binding to the coated antibody. The mixture was incubated, after which unbound conjugate is washed off and a streptavidin-peroxidase enzyme complex is added to each well and incubated again. At the end of the incubation, unbound enzyme complex is washed off and substrate solution is added. The blue colour development is stopped after a short incubation time, turning the colour from blue to yellow. The intensity of colour developed is reverse proportional to the concentration of Hepcidin in the sample.

Procedure: The numbers of microtiter wells needed were fixed in a frame holder. 100µL of assay buffer was dispensed into appropriate wells. 20µL of each standard, controls, and samples were added with new disposable tips into appropriate wells. 50µL of enzyme conjugate was added into each well and mixed well for 10 seconds. The mixtures were incubated at room temperature for 60 minutes under agitation (300-700rpm). The mixtures in the wells were poured out and then rinsed with 400µL of diluted wash solution per well for three times using a plate washer. The remaining droplets were removed by shaking the wells on absorbent paper.

100µL of enzyme complex was then added into appropriate wells and incubated at room temperature for 30 minutes without agitation. The contents were also sharply poured out and rinsed with 400µL of diluted wash solution per well for three times too using a plate washer. The remaining droplets were removed by shaking the wells on absorbent paper again. 100µL of substrate solution was added to each well and were incubated the third time for 20 minutes at room temperature. After which 100µL of stop solution was added to each well to stop the

enzymatic reaction. The absorbance (OD) of each well was determined at $450\text{nm} \pm 10\text{nm}$ with A.D Touch ELISA microtiter plate reader (analyzer) within 10 minutes of adding the stop solution.

2.8 Data Analysis

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

3.0 Results

Table 1.0: Socio-Demographic Characteristics

Characteristics	CKD	Control	Total
*Number of Subjects	55 (62.50%)	33 (37.50%)	88 (100.00%)
*Gender			
Male	30 (54.55%)	18 (54.55%)	48 (54.55%)
Female	25 (45.45%)	15 (45.45%)	40 (45.45%)
*Age (years)			
18-37	16 (29.09%)	9 (27.27%)	25 (28.40%)
38-57	24 (43.64%)	17(51.52%)	41 (46.59%)
58-77	15 (27.27%)	7 (21.21%)	22 (25.00%)

Table 2: Comparison of Hepcidin 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			

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

Table 2.0 showed the Mean, Standard Deviation, t and p-values of Serum in both CKD and control subjects. The t-value and p-value for SH were $t_{86} = 6.54$ and $p < 0.05$. There was a significant difference in hepcidin mean values between CKD and control groups.

4.0 Discussion

A total of 88 adults participants were included in this study, 55 Chronic Kidney Disease and 33 Control subjects with age ranging from 18-77 years. The Percentage Frequency Distribution of the Socio-Demographic Characteristics (Number of Subjects, Gender and Age) of the study participants were shown on Table 1.0. A total of 88 subjects were included in this study, out of which 55(62.50%) were CKD subjects and 33(37.50%) were Control subjects. From the study population 48(54.55%) were males while 40(45.45%) were females. In the CKD group 30(54.55%) were males while 25(45.45%) were females and in the Control group 18(54.55%) were males while 15(45.45%) were females. Only adults were used for the study with age ranging from 18-77 years. The age was grouped into 18-37, 38-57 and 58-77 years. The distribution of age among the total study population were 25(28.40%), 41(46.59%) and 22(25.00%) respectively. Among the CKD subjects it was 16(29.09%), 24(43.64%) and 15(27.27%) respectively whereas among the Control subjects was 9(27.27%), 17(51.52%) and 7(21.21%) respectively. Serum hepcidin was measured in the two groups in the study; there was a significant elevation or rise in serum hepcidin level in the CKD group than in the control group. 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.

Conclusion

This study has shown that in chronic kidney disease which is perhaps due to decline in glomerular filtration rate presented among CKD subjects results in increased level of serum hepcidin which could in turn affect iron homeostasis.

References

- Ashby, D. R., Gale, D. P., Busbridge, M., Murphy, K. G., Duncan, N. D., Cairns, T. D., Taube, D. H., Bloom, S. R., Tam, F. W., Chapman, S. R., Maxwell, P. H. & Choi, P. (2008). Plasma Hepcidin Levels are Elevated but Responsive to Erythropoietin Therapy in Renal Disease. *Kidney International*, 75(9), 976-981.
- Bansal, S. S., Abbate, V. & Bornford, M. (2010). Quantitation of Hepcidin in Serum Using Ultra-high-pressure Liquid Chromatography and a Linear ion trap Mass Spectrometer. *Rapid Communicationn Mass spectrum*, 24(9), 1251-1259.
- Conrad, M. E. & Umbriet, J. N. (2001). Disorder of Iron Metabolism. *The New England Journal of Medicine*, 342(17), 1293 – 1294.
- Evan, M. B. (2017). Merck and the Merck Manual, (5th Edition) United State of America, Kenilworth Press.
- Frazer, D. M., & Anderson, G. J. (2009). Hepcidin Compared with Prohepcidin: An Absorbing Story. *American Journal of Clinical Nutrition*, 89, 475–476.
- Fyneface, C. A., Joel, B. K. and Felix, E. K. (2020). Assessment of creatinine levels in blood and saliva of haemodialysed subjects. *International Journal of Advances in Nephrology Research*, 3(1), 21-25
- Fyneface, C. A., Onengiyeofori, I. and Davies, T. (2018). Evaluation of Saliva for Monitoring Renal Function in Haemodialysis Patients at University of Port Harcourt Teaching Hospital. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 1(2), 1-6
- Ganz, T., Olbina, G., Girelli, D., Nemeth, E. & Westerman, M. (2008). Immunoassay for Human Serum Hepcidin. *Blood*, 112, 4292 – 4297.
- Janz, T. G., Johnson, R. L. & Rubenstein, S. D. (2013). Anaemia in the Emergency Department. Evaluation and Treatment. *Emergency Medicine*, 15(11), 1-15.

- Jelic, M., Cvetkovic, T., Djordjevic, V., Damnjanovic, G., Nahovic, P., Kocic, G., Djindic, N., Jovovic, B. & Antic, A. (2013). Hepcidin and Iron Metabolism Disorders in Patients with Chronic Kidney Disease. *Vojnosanitetski Pregled*, 70(4), 368-373.
- Jeremiah, Z. A. & Koate, B. B. (2010). Anaemia, Iron Deficiency and Iron Deficiency Anaemia among Blood Donors in Port Harcourt, Nigeria. *Blood Transfusion*, 8(2), 113 – 117.
- Kidney Disease Improving Global Outcomes (2013). KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Disease. *Kidney International Supplements*, 3 (1), 1-150.
- Lia, T., Karthym, A. C., Jin, W. K. & Suzy, V. T. (2015). Hepcidin Regulation in Prostate and its Disruption in Prostate Cancer. *Cancer Resources*, 75(11), 2256-2263.
- Papanikolaou, G., Tzilianos, M. & Christakis, J. I. (2005). Hepcidin in Iron Overload Disorders. *Blood*, 105, 4103-4105.
- Peters, H. P., Laarakkers, C. M., Swinkels, D. W. & Wetzels, J. F. (2010). Serum Hepcidin-25 Levels in Patients with Chronic Kidney Disease are Independent of Glomerular Filtration Rate. *Nephrology Dialysis and Transplantation*, 25, 848-853.
- Sandro, A., Regina, K., Grone, H-J., Josef, G., Nobert, G., Matthias, W., Bruno, G. & Martina, U. M. (2014). Resistance of Ferroportin to Hepcidin Binding causes Exocrine Pancreatic Failure and Fatal Iron overload. *Cell Metabolism*, 20(2), 359-367.
- Weiss, G. & Goodnough, L. T. (2005). Anemia of Chronic Disease. *New England Journal of Medicine*, 352, 1011-1023.
- Taheri, N., Roshandel, G. H., Mojerloo, M., Hadad, M., Mirkarimi, H., Nejad, R. K. & Joshaghani, H. R. (2015). Comparison of Serum Levels of Hepcidin and Pro-hepcidin in Hemodialysis Patients and Healthy subjects. *Saudi Journal of Kidney Disease and Transplantation*, 26, 34-38.