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RELATIONSHIP BETWEEN LEVELS OF HEPCIDIN, HEMOGLOBIN AND IRON IN THE PATIENTS OF STAGE-4 CHRONIC KIDNEY DISEASE PRESENT WITH ANEMIA

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Abstract:
Objective: *The purpose of this research work was to investigate the possible relationship of levels of hemoglobin and hepcidin with the statuses of iron and inflammation in the patients of Stage-4 CKD (Chronic Kidney Diseases) present with anemia.*
Methodology: *This transverse research work was carried out at DHQ Hospital Narowal, from January 2019 to June 2020. All the patients were suffering from Stage-4 of CKD with anemia. Measurement of serum biochemical factors of the patients as levels of hepcidin, ferritin, interleukin-6, high sensitivity CRP (C - reactive protein) and iron was carried out with the utilization of standard procedures. We established statistical correlations with the use of Pearson's correlation coefficient and regression analysis.*
Results: *30 patients were the participants of this research work in which 37.50% (n: 10) were male patients and 20 (62.50%) were female patients. The average age of the patients was 55.680 ± 14.30 years. We found a significant inverse association between levels of hemoglobin and hepcidin (P<0.050). We discovered a significant relationship between level of serum hepcidin with status of iron, inflammatory & nutritional markers like ferritin, iron binding capacity, levels of albumin and interleukin-6 (P<0.050).*
Conclusion: *There is negative association of hepcidin with the level of hemoglobin in the Stage-4 CKD patients with adequate stores of iron, which can be much efficient for the development of anemia in these patients.*
Keywords: *Anemia, Hepcidin, Chronic Kidney Diseases, Inverse, Coefficient, Regression, Serum, Biochemical, Hemoglobin, Inflammation.*

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INTRODUCTION:

Chronic Kidney Diseases is serious health issue of general public and there is an increase in its prevalence in whole world [1]. One of the main complications in the patients of CKD is anemia [2]. There are many factors as causative elements of anemia in the patients of CKD [3]. Deficiency of relative erythropoietin, decreased survival of erythrocyte and inhibitory effects of erythropoiesis of gathering uremic toxins add to anemia in the patients of CKD [3]. Important fact is that there are many disorders in the iron's systemic homeostasis which is very vital element for the formation of RBC (Red Blood Cells) [3]. Adverse absorption of dietary iron in the patients of CKD and difficulty in the use of stores of iron may lead to anemia [2, 3]. In accordance with the findings of current research works, hepcidin is most important reason of abnormalities in the metabolism of iron in anemia and CKD [4, 5].

It has the ability to regulate the systemic balance of iron by reducing both intestinal absorption of iron and release of iron from enterocytes, macrophages and hepatocytes causing hypoferremia and restricts the availability of iron for erythropoiesis [4]. Concentration of hepcidin increases in the patients of CKD because of inflammation and reduction in the renal clearance [5]. Confrontation to erythropoietin in chronic kidney disease can be resulted by large hepcidin's concentration and limitation of iron [6]. Despite the main role of hepcidin in iron's metabolism, there is much limited data available to provide association between anemia and level of hepcidin in Stage-4 of CKD and this issue is full of controversies [7]. The rationale of this research work was to evaluate the probable associations of levels of hepcidin with levels of hemoglobin, statuses of iron and inflammation in Stage-4 of CKD.

METHODOLOGY:

This transverse research work was carried out at DHQ Hospital Narowal from January 2019 to June 2020. Patients of Stage-4 CKD with anemia were the participants of this research work. Ethical committee of the institute gave the permission to conduct this research work. The size of sample was based on the exclusion and inclusion standard showed in different research works conducted in the past [8-10]. We included the adult patients having greater than 18 years of age suffering from anemia and in Stage-4 CKD which GFR (Glomerular Filtration Rate) 15 to 29 ml/min/1.730 m2 in this research work. We defined the anemia as level of hemoglobin less than 13.0 g/dl for males and post-menopausal females and lower than 12.0 g/dl for pre-menopausal females [11]. All the patients with history of transfusion in last six months, history of MI (Myocardial Infarction) within three months, past history of surgical intervention in last three months, malignancy, DBP (Diastolic Blood Pressure) higher than 100.0 mm/hg, not controllable DM (Diabetes Mellitus), and drug consumers got exclusion from this research work. We obtained the samples of blood from all the patients after overnight fast. We used the Cockcroft Gault formula for measuring the GFR. Levels of hemoglobin (Hb) & hematocrit (Ht) were calculated with the help of blood cell counter. Whole blood & separated serum were placed frozen at -80.0°C until the time of analysis [12]. We analyzed the samples of sera with the utilization of auto-analyzer (BT-3000, made of Italy).

We used the manufacturer's reagent kits for analysis of serum biomarkers like hs-CRP, TIBC (Total Iron Binding Capacity), phosphorus, iron, urea, magnesium, calcium, albumin, creatinine and total protein. Levels of hepcidin & ferritin were assayed with the help of ELISA kits. We followed the instruction of human ELISA kits for the measurement of IL-6 (Interleukin-6). Statistical analysis of the collected data was carried out with the utilization of the scatter plot and Pearson's correlation coefficient. 0.050 was the significant P value.

RESULTS:

There were 30 patients in this research study. 10 (37.50%) patients were males and 20 (62.50%) were female patients. The average age of the patients was 55.680 ± 14.30 years. We examined an inverse association between concentrations of serum hepcidin and levels of hemoglobin ($P<0.050$). We found no significant association between iron and level of serum hepcidin ($P>0.050$). We found an inverse relationship between serum hepcidin levels and TIBC ($P<0.0010$). There was inverse relation between the levels of albumin & PTH and levels of serum hepcidin ($P<0.050$). We also found a strong positive association of levels of serum hepcidin with levels of IL-6 and serum ferritin ($P<0.050$). We also noted a strong association between hemoglobin and levels of ferritin ($P<0.050$). We were unable to find association of serum hepcidin levels with GFR and hs-CRP.

Table-1: Clinical Characteristics

Parameter	(Mean ± SD)	Parameter	(Mean ± SD)	Parameter	(Mean ± SD)
Hepcidin (ng/ml)	258.86 ±21.94	hs-CRP (mg/l)	10.54 ±1.89	Creatinine (mg/dl)	3.66 ±0.15
	(258.86 ± 138.76)		(12.82 ± 13.74)		(3.66 ± 0.96)
IL-6 (pg/ml) **	0.59 ±3.39	TIBC (pg/dl) *	295.05 ±8.18	Phosphorus (mg/dl)	4.36 ±0.12
	(5.57 ± 9.25)		(295.05 ±51.79)		(4.36 ± 0.77)
Hemoglobin (g/dl) *	10.01 ±0.24	Hematocrit (%)	31.15 ±0.69	Weight (kg)	72.4 ±1.73
	(10.01 ±1.56)		(31.15 ±4.39)		(72.30 ± 10.95)
GFR (ml/min/1.73 m2)	21.51 ±0.74	Urea (mg/dl)	126.38 ±7.12	PTH (pg/ml) *	111.93 ±13.35
	(21.51 ±4.71)		(126.37 ±45.02)		(152.11 ±146.10)
Ferritin (pg/l) **	163.83 ±24.69	Albumin (g/dl) *	4.21 ±0.11	Magnesium (mg/dl)	2.01 ± 0.04
	(241.06 ±276.36)		(4.21 ± 0.74)		(2.01 ± 0.29)
Iron (pg/dl) **	63.5 ±4.64	Total protein(g/dl)	8.11 ±0.17	Calcium (mg/dl)	9.03 ±0.11
	(63.50 ± 29.39)		(8.11 ±1.07)		(9.03 ± 0.75)

TIBC= Total Iron Binding Capacity, IL-6= interleukin-6, PTH= parathyroid hormone, GFR= Glomerular filtration rate. *An inverse significant relationship with hepcidin in Pearson Correlation ($p<0.05$).

**A positive significant relationship with hepcidin in Pearson Correlation ($p<0.05$).

DISCUSSION:

One of the most important complication in Stage-4 CKD is anemia. There is still non-understood pathogenesis for this reason [13]. Inflammation is one of the vital factors related with resistance of erythropoietin resistance and prevalence of anemia in patients suffering from CKD [14]. The expression of hepcidin is part of reaction of inflammation and it may be a part of the pathogenesis of anemia related with inflammation [15]. In this current research work, concentration of serum hepcidin were present with negative association with the levels of hemoglobin and it has a very strong association with the levels of IL-6. The findings of this current research work are not consistent with the results of some research studies conducted on patients of CKD. Research works on non-dialysis patients of CKD could not observe relationship between concentration of serum hepcidin and hemoglobin levels [5, 16]. A research on patients having renal insufficiency was also unable to observe any association between the levels of hemoglobin and concentration of serum prohepcidin [17].

The findings of this current research work are comparable with the results of some other research works on dialysis patients with both heart and kidney failure [15, 18]. The difference in the results may be because of different statuses of iron in different populations and differences in the size of samples. There are many causes of negative association of hepcidin with hemoglobin in the Stage-4 CKD patients with sufficient stores of iron [19]. Hepcidin has the ability to restrict the availability of iron for erythropoiesis [20]. High levels of ferritin in serum generally show overload of iron but it does not mean of sufficient iron stores in bone marrow [21]. One reason is that inflammation causes the induction of hepcidin [2] and there is chronic inflammatory state of the patients suffering from CKD [3]. There are well-understood influences of inflammation on hepcidin' synthesis. Hepcidin also directs the effects of inhibition on the erythropoiesis and influences survival and proliferation of erythroid precursor.

We found a strong association between the levels of ferritin & hepcidin which are much widely utilized markers for the status of iron. Similar to the results of this current research work, it has been stated that IL-6 and inflammatory cytokines may alter the concentrations of serum levels of hepcidin as well as markers of iron statuses. A research on the patients of inflammation-related anemia stated that level of hepcidin associated well with the concentration of serum ferritin. A research on patients under hemodialysis stated that level of hepcidin associated with concentration of ferritin and TIBC. One other research on the patients suffering from chronic liver disease described that there is correlation of level of hepcidin with the level of ferritin. Much similar with these and other results [20], the results of current research work suggest that hepcidin's synthesis is also increased by an enhanced iron store of body. A

strong association between the levels of ferritin and hepcidin shows the relationship between correlation among iron stores and hepcidin in Stage-4 CKD patients. The findings of current research work showed that increases hepcidin in the patients of Stage-4 CKD may be because of increased levels of IL-6 and ferritin in result of inflammation. Low number of patients with Stage-2 CKD and removal of the patients on the basis of inclusion and exclusion criteria are the limitations of this research work.

CONCLUSION:

There is negative association of hepcidin with the level of hemoglobin in the Stage-4 CKD patients with adequate stores of iron, which can be much efficient in the anemia's development in these patients. There is requirement of further research works to investigate the longitudinal alteration in levels of hepcidin, hemoglobin, indicators of iron status and IL-6 in the patients suffering from Stage-4 of CKD.

REFERENCES:

1. Hosseinpanah F, Kasraei F, Nassiri A, Azizi F. High prevalence of chronic kidney disease in Iran: a large population-based study. BMC Public Health. 2009;9:1-8.
2. Babitt JL, Lin HY. Molecular mechanisms of hepcidin regulation: implications for the anemia of CKD. Am J Kidney D is 2010;55:726-41.
3. Malyszko J, Mysliwiec M. Hepcidin in anemia and inflammation in chronic kidney disease. Kidney Blood Press Res 2007;30:15-30.
4. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int 2009;75:976-81.
5. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E, et al. Hepcidin-a potential novel biomarker for iron status in chronic kidney disease. Clin J Am SocNephrol 2009;4:1051-6.
6. El-Khatib MT. The role of inflammation on iron and erythropoietin resistance. J Nephrol Renal Transplant 2009;2:45-54.
7. Nakanishi T, Hasuike Y, Otaki Y, Kida A, Nonoguchi H, Kuragano T. Hepcidin: another culprit for complications in patients with chronic kidney disease? Nephrol Dialysis Transplant 2011;26:3092-100.
8. Goswami S, Bhowmick S, Majumdar A, Sikdar S, Sarkar CN, Chatterjee TK, et al. Appropriateness and Efficacy of the Use of Erythropoietin in Hemodialysis Patients in an Eastern Indian population. Med Sci 2014; 7: 15 -22.
9. Strandhave C, Svensson M, Krarup H, Christensen JH. Haptoglobin genotype and risk markers of cardiovascular disease in patients with chronic kidney disease. Int J Nephrol 2013; 2013: 1-7.
10. Ferrari P, Mallon D, Trinder D, Olynyk JK. Pentoxifylline improves haemoglobin and interleukin-6 levels in chronic kidney disease. Nephrology (Carlton). 2010; 15: 344-9.
11. Daugirdas JT, Ing TS. Handbook of dialysis: Little, Brown; 1988.
12. Chudleigh RA, Dunseath G, Peter R, Harvey JN, Ollerton RL, Luzio S, et al. Influence of body weight on the performance of glomerular filtration rate estimators in subjects with type 2 diabetes. Diabetes care 2008;31:47-9.
13. Nurko S. Anemia in chronic kidney disease: causes, diagnosis, treatment. Cleveland Clin J Med 2006;73:289-97.
14. de Francisco ALM, Stenvinkel P, Vaulont S. Inflammation and its impact on anaemia in chronic kidney disease: from haemoglobin variability to hyporesponsiveness. NDT plus. 2009;2(suppl 1):i18-i26.
15. Van Der Putten K, Braam B, Jie KE, Gaillard CAJM. Mechanisms of Disease: erythropoietin resistance in patients with both heart and kidney failure. Nature Clin Pract Nephrol 2008;4:47-57.
16. Peters HPE, Laarakkers CMM, Swinkels DW, Wetzels JFM. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrol Dialysis Transplant 2010;25:848-53.
17. Taes YEC, Wuyts B, Boelaert JR, De Vriese AS, Delanghe JR. Prohepcidin accumulates in renal insufficiency. Clin Chem Lab Med 2004;42:387-9.
18. Valenti L, Girelli D, Valenti GF, Castagna A, Como G, Campostrini N, et al. HFE mutations modulate the effect of iron on serum hepcidin-25 in chronic hemodialysis patients. Clin J Am Soci Nephrol 2009;4:1331-7.
19. Uehata T, Tomosugi N, Shoji T, Sakaguchi Y, Suzuki A, Kaneko T, et al. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. Nephrol Dialysis Transplant 2012;27:1076-83.
20. van Eijk LT, Kroot JJC, Tromp M, van der Hoeven JG, Swinkels DW, Pickkers P. Inflammation-induced hepcidin-25 is associated with the development of anemia in septic patients: an observational study. Crit Care. 2011;15:1-6.