Effect of vitamin D on Hepcidin Level and anemia in chronic kidney Disease

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Abstract: Introduction: Hepcidin, a small peptide produced by the liver, is a recently discovered key regulator of iron homeostasis. Via the regulation of ferroportin, hepcidin inhibits intestinal iron absorption and iron release from macrophages and hepatocytes thus prevents the normal recycling of iron needed to support erythropoesis.Hepcidin levels are increased in cases of chronic kidney diseases. Vitamin D insufficiency is highly prevalent in chronic kidney diseases and is associated with erythropoietin hyporesponsiveness. Data suggest that for a proper response to the treatment of anemia, the body must somehow reduce hepcidin in order to increase iron that is needed for red cell production. Many studies suggest vitamin D to affect hepcidin level and thus make iron more available and help to correct anemia in chronic kidney disease. **Objectives**: This study aims to assess the effect of vitamin D on hepcidin level and hence, hemoglobin level, and iron status in chronic kidney disease. **Methods**: -thirty patients were included in this study twenty of which were having ESRD, and were on regular hemodialysis three times per week each session four hours, while the other ten were normal subjects. They were divided into three groups each contained ten persons. Group one: control group. Group two:Renal failure group Group three:Renal failure group treated with calcitriol which is an active form of vitamin D3 at a dose of 15micro gram/day (600 IU/day) for three months At the beginning of the study samples of blood were taken from patients of group two and three to measure urea and creatinine to detect the existence of renal failure. At the end of the study samples of blood were taken from all groups to measure the hemoglobin level, Hepcidin level, serum iron.serum ferritin, serum urea, serum creatinine, and total iron binding capacity. **Results**: The results showed that patients of group two having ESRD have chronic anemia**,** they recorded significantly less iron level and thus hemoglobin level compared to patients of the control group. This may be due to increased level of serum hepcidin in response to the chronic inflammatory state.The study also showed that ESRD patients in group two on regular hemodialysis had non significant increase in hepcidin level compared to control group this might be due to the negative feedback mechanism on hepcidin secretion at the liver, and the efficiency of dialysis which eliminates hepcidin. The study also showed that vitamin D may affect the hepcidin level significantly as group three the ESRD group given vitamin D showed significantly less hepcidin level compared to the ESRD group not given vitamin D.The ESRD group given vitamin D also recorded significant increase in the hemoglobin and iron levels compared to group two. **Conclusion:** it may be of benefit to give the chronic kidney disease patients vitamin D as it may affecthepcidin level and thus make iron more available and help to correct anemia.

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1. Introduction

Hepcidin is a 25-amino acid hormone, synthesized in the liver, secreted in plasma and binds to the cellular iron export channel ferroportin causing its internalization and degradation, thereby decreasing iron efflux from iron exporting enterocytes and macrophages into plasma (1).

Suppression of hepcidin formation occurs during hypoxia, anemia and increased erythropoiesis in bone marrow, where as it is induced in conditions of infection, inflammation and increased iron stores (2).

It is a common clinical observation that many renal failure patients receiving erythropoietin (EPO) require intravenous iron supplementation, despite the fact that they have adequate dietary iron. This strongly suggests an abnormality of iron uptake by the intestine(3).

Hepcidin reduces iron absorption from the gut, beside that its secretion is increased during inflammation & infection (a common finding in renal failure). High blood levels of hepcidin may override the effect of administered EPO in renal failure and

limit the supply of dietary iron for red blood cell production (4).

Hepcidin may mediate the negative effects of inflammation on both disordered iron metabolism and erythropoiesis in hemodialysis patients and that intensification of hemodialysis could be used therapeutically to reduce hepcidin concentrations and thereby improve erythropoiesis-stimulating agent responsiveness (5).

Recent studies have shown an association between the deficiency of the vitamin D with low hemoglobin (Hb) levels and erythropoietin stimulating agent (ESA) resistance suggesting a new pathophysiological co-factor of renal anemia (6).

Many studies suggest that administration of vitamin D to these patients may be associated with improvement of the anemia and reduction in ESA resistance (7).

Possibly the effect of vitamin D on ESA resistance may be due to the directly stimulating effect of calcitriol on the erythroid progenitors (8).

Studies suggest that immune cells express the vitamin D receptor (VDR) which in turn is involved in the modulation of innate and adaptive immunity. VDR activation inhibits the expression of inflammatory cytokines in stromal and accessory cells and up-regulates the lymphocytic release of interleukin-10 (IL-10) exerting both anti-inflammatory activity and proliferative effects on erythroid progenitors (8).

It is to be suggested that vitamin D may lead to decrease in the prohepcidin cytokines IL-6 and IL-1B release (9).

In Chronic kidney disease (CKD) patients, vitamin D deficiency may stimulate immune cells within the bone marrow to produce cytokines, inducing impaired erythropoiesis. Immune activation involves the reticuloendothelial system, increasing hepcidin synthesis and functional iron deficiency (10).

Studies suggest that the therapeutic use of agents with anti-cytokines properties, such as vitamin D may provide benefit in the prevention and treatment of ESA hypo responsiveness (11).

2. Material and methods

Thirty persons were included in this study; they were matched for age and sex.They were divided into the following groups:

Group I: included 10 healthy subjects as a control group, their age ranged between 24 - 67 yrs.

Group II: included 10 patients with ESRD maintained on regular hemodialysis (HD) 4 hrs. three times weekly, at October 6 university dialysis unit, their age ranged between 23 - 65 yrs.

Group III: included 10 patients with ESRD maintained on regular hemodialysis (HD) 4 hrs. three times weekly, at October 6 University hospital dialysis unit, their age ranged between 23 - 65 yrs and they were given vitamin D calcitriol which is an active form of vitamin D3 at a dose of 15micro gram/day (600 IU/day) orally for three months

All subjects of the study were subjected to the following:

1. Full history taking, including history of iron and EPO intake and complete clinical examination.
2. Laboratory investigation for:

a. B. urea, S. Creatinine, S. iron, total iron binding capacity (TIBC) measured by colorimetric technique (12).

b. Serum ferritin(using spectrophotometer, Gilford Instruments, Inc., Oberlin, OH 44074) ELISA procedure and reagents by EIA-O1-Ferritin).

c. Hemoglobin in gm/dl using automated cell counter sysmex k 1000.

d. Hepcidin level will be measured using immunoenzymometric sequential assay (type 4), ELISA **(**DRG Hepcidin Elisa, EIA-4705/ 2010).

Venous blood samples were collected from patients, in the middle session of the week, before the beginning of hemodialysis, one with EDTA to measure the Hemoglobin (Hb), haematocrit (HCT). Another sample was allowed to clot for 30 minutes and the serum was obtained by centrifugation at 3000 rPM for 15 minutes. Serum was stored at -20 C° until the time of analysis.

Measuring Hepcidin, Principle of the test

The DRG Hepcidin 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 antibody directed towards the antigenic site of the bioactive Hepcidin 25 molecule. Endogenous Hepcidin of a patient sample competes with the added Hepcidin-biotin conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. Incubation with a streptavidin-peroxidase enzyme complex and a second wash step follows.

The addition of substrate solution results in a color development which is stopped after a short incubation. The intensity of color developed is reverse proportional to the concentration of Hepcidin in the patient sample(DRG Hepcidin ELISA (EIA-4705).

Statistical methodology:

Statistical evaluation was done on windows XP, results were analyzed using SPSS version 17.0 released September 2008. Paired T test for analysis of correlation between the values, Pearson correlation analysis were used. Data were expressed as mean, standard deviation, & analysis of variance (ANOVA) tests. ± SD. Differences at the level of probability (P), $P < 0.05$ was considered statistically significant, $P< 0.005$ is considered highly statistically significant, and

P < 0.0005 is considered very high statistically significant.

3. Results

The results of the present study showed that the ESRD group and the ESRD group given vitamin D showed significantly decreased level of Hb compared to the control group (P <0.0001).

Also the results of the study showed a highly significant increase in Serum Urea and Creatinine levels in the ESRD group and the end stage renal disease group given vitamin D compared to the control group (P <0.0001).

There was a high statistically significant increase in the level of TIBC in the end stage renal disease group compared to the control group (P <0.003).

There was a highly statistically significant decrease in the level of TIBC in the end stage renal disease group given vitamin D compared to the end stage renal disease group (P <0.003).

There was no statistically significant difference between level of Serum Iron in end stage renal disease group and the control group (P =0.1838) while there was a highly statistically significant increase in serum iron in the end stage renal disease given vitamin D compared to the end stage renal disease group not given vitamin D.

There was a very high statistically significant increase in the level of ferritin in end stage renal disease group compared to the control group (P<0.0001) on the other hand there was a highly significant decrease in the level of ferritin in the end stage renal disease given vitamin D compared to end stage renal disease not given vitamin D.

There was no statistically significant difference between levels of hepcidin in end stage renal disease group and the control group (P=0.2057) while there was a statistically significant decrease in level of hepcidin in the end stage renal failure group given vitamin D compared to the end stage renal failure group not given vitamin D.

Table (1): levels for Hb, Urea, Creatinine, Serum iron, Serum ferritin, TIBC, &Hepcidin for the End stage renal failure group compared to the control group and ESRD group given vitamin D (Mean ± SD).

	Control group	End stage renal disease group	End stage renal disease group given vitamin D	*P*. test
Hb. gm/dl	13.5+_1.5	8.94 +_1.68	10.3 +_1.46	<0.0005*
S. CR. mg/dl	0.75 +_ 0.19	7.94 +_ 4.58	8.97 +_ 4.58	<0.0001*
Urea. mg/dl	23.26 +_ 6.84	131.75 +_ 63.75	132.75 +_ 65.65	<0.0001*
S. Iron. µg/dl	214 +_ 38.8	260.4 +_242.9	300.4 +_261.8	<0.0001*
S.Ferritin ng/ml	82.7 +_59.7	683.5 +_83.6	567.5 +_94.6	<0.0001*
TIBC. µg/dl	284+_73.7	534.45 +442.6	364.45 +_352.7	<0.003*
Hepcidin. ng/ml	91 +_37	105.45 +_26.4	99.34 +_26.4	<0.0001*

* Significant (P value < 0.05).

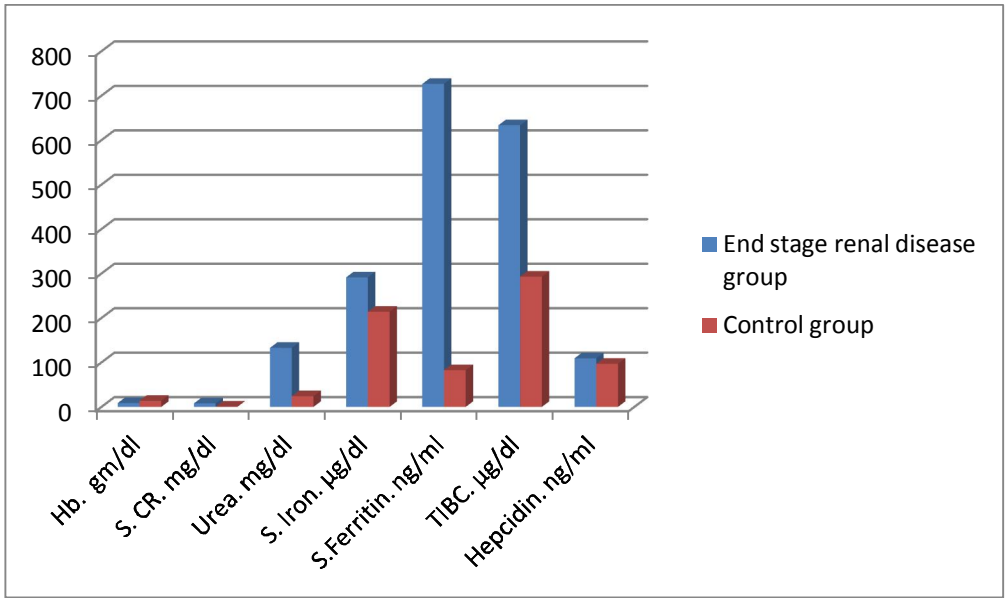

Figure (1): levels for Hb, Urea, Creatinine, Serum iron, Serum ferritin, TIBC, &Hepcidin for the End stage renal failure group compared to the control group.

4. Discussion

Anemia is an important contributor to morbidity and mortality in ESRD. However, despite the wide spread of the use of erythropoietin and iron therapy, the modest target of hemoglobin is 11 to 12 g/dL is difficult to be achieved (13).

Hepcidin, an acute phase protein produced in the liver, is a recently discovered key regulator of iron homeostasis. It inhibits intestinal iron absorption and iron release from macrophages and hepatocytes. Because hepcidin production is increased by inflammation, and high hepcidin concentrations limit iron availability for erythropoiesis, hepcidin likely plays a major role in the anemia of inflammation and Recombinant human erythropoietin hormone resistance (14).

Vitamin D is synthesized in the skin or derived from nutritional sources and is transported to the liver, where it is metabolized to 25-hydroxivitamin D [25(OH)D_3], which is the main circulating form of vitamin D. The second hydroxylation takes place mainly in the kidney, where the 1α-hydroxylase enzyme converts 25(OH)D_3 to the active hormone 1,25-dihydroxyvitamin D [1,25$(OH)_2D_3$], the hormone that acts on mineral and bone metabolism. In the course of CKD, vitamin D abnormalities not only include the progressive loss of kidney function to form 1,25(OH) $_2D_3$, but also the capacity to maintain serum 25(OH)D_3 levels. This latter deficiency is due to insufficient sunlight exposure in chronically ill patients, malnutrition with dietary exclusion of vitamin D-rich food, urine loss in proteinuric nephropathies and effluent loss in peritoneal dialysis. In addition, with the progressive reduction in GFR renal megalin expression decreases and in turn reduces the renal uptake of 25(OH)D_3.(15)

Previous studies have shown that vitamin D has pleiotropic effects which affect many organ systems, including the haemopoietic system. 1,25$(0H)_2D_3$ exerts its action by binding to the vitamin D receptor (VDR), a member of a nuclear receptor superfamily present in several tissues, such as bone marrow, stromal and accessory cells. In blood samples drawn for DNA analysis, VDR genotype was found to be linked to Hb values and EPO dose requirements in dialysis patients(16). More recent analysis of the Third National Health and Nutrition Examination Survey (NHANES III) has provided evidence of a linear association between 25(OH)D_3 concentration and the degree of anaemia in CKD patients not requiring dialysis(17). An inverse association between 25(OH)D_3 levels and EPO resistance index was shown in HD patients. In a large cohort of early CKD subjects, 25(OH)D_3 and 1.25$(OH)_2D_3$ deficiency were independently associated with decreased Hb values and anaemia. Finally, patients with severe deficiency of both native and active vitamin D have a greater prevalence of anaemia than subjects with low serum levels of a single form of vitamin D. It is intriguing that lower 1.25$(OH)_2D_3$ levels were associated with higher hepcidin, thus suggesting a link between vitamin D deficiency and anaemia in CKD patients (18).

Although the use of Erythropoesis Stimulating Agents (ESAs) has revolutionized the management of renal anemia, and significantly improved patient quality of life by avoiding the need for blood transfusion nevertheless, ~5–10% of patients exhibits an inadequate response to ESAs. Having an inability to achieve or maintain target haemoglobin (Hb) levels leading to poor clinical outcomes, with increased cardiovascular morbidity, and higher ESA requirements may lead to an increased risk for adverse outcomes due to the underlying factors affecting EPO response, such as inflammation, and the potential non-erythropoietic effects of greater administered ESA doses (19).

Many studies suggest that deficiency in the vitamin D may be an additional pathophysiological co-factor of specific anemia subtypes, such as renal anemia and anemia due to inflammation. and that increasing vitamin D may affect anemia positively through improving the biomarkers of inflammation (20). In addition, data are available showing an inverse association between vitamin D levels and EPO requirements in CKD patients. The wide array of biological actions exerted by vitamin D and its analogues includes modulation of the immune system mediated through anti-inflammatory effects (21).

It is to be documented that vitamin D may suppress hepcidin mRNA expression in the lipopolysaccharides cells in a dose dependent manner. Also it may lead to induced NRAMP1 mRNA expression which is an endosomal iron transporter playing a significant role in iron homeostasis and that this increase is dose dependant (21).

Under pathological conditions, such as chronic disease anaemia and inflammation, suppressive cytokines derived from accessory cells as [interleukin-6 (IL-6), interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α)], negatively influence differentiation and proliferation activities. In the late phase of erythropoiesis (22). Erythropoietin is the essential stimulus to erythroid maturation and lack of it is the major cause of CKD anaemia. In addition to EPO, iron availability for erythroid precursors is also needed. Hepcidin, a small polypeptide produced and secreted by the liver, is a key mediator of systemic iron homoeostasis-regulating absorption and utilization. In CKD patients, multiple forms of interference on iron metabolism as well as inhibition of iron release from the reticuloendothelial system

occur. Excessive hepcidin production by pro-inflammatory cytokines encoding gene expression contributes to the functional iron deficiency and associated renal anaemia and EPO resistance (23).

The results of the present study showed an increase in serum hepcidinin the ESRD group compared to the control group as well as serum creatinine, urea, TIBC and ferritin. These results are in agreement with the results of (24) that have found that in patients with chronic renal failure, hepcidin correlated significantly with creatinine, urea, & TIBC. This is because the mechanism that control hepcidin secretion is affected by the iron status and inflammatory status**.**

This observation is consistent with (25) results**,** which found that, increased plasma and stored iron stimulate hepcidin production, which in turn blocks dietary iron absorption and further iron loading. Hepcidin is suppressed in iron deficiency, allowing increased absorption of dietary iron and replenishment of iron stores. The feedback loop between iron and hepcidin ensures the stability of plasma iron concentrations**.**

In ESRD, hepcidin levels are elevated probably in part because hepcidin is normally cleared by the kidneys, and perhaps also because of increased hepcidin expression in the presence of certain inflammatory cytokines. As a result, iron uptake from gut is diminished (26).

Routine measuring of serum hepcidin in ESRD patients on regular hemodialysis is not recommended, as hepcidin is being eliminated if they are receiving efficient hemodialysis.

The results of the present study agree with the results of (27) where they stated that administration of vitamin D and analogues has been associated with an improvement of anemia and/or a reduction in EPO requirements. The results also agree with (28) where they stated that high-dose oral alfacalcidol showed a positive impact on anemia in a small group of HD patients with a good iron status and efficacious dialysis parameters.

(29)stated that A significant increase in Hb and haematocrit was obtained after three months of treatment with calcitriol in a group of CKD patients undergoing HD and on conservative management. In this study, active vitamin D administration increased BFU-e proliferation. Responders to calcitriol therapy showed a maintenance in EPO dose needed to achieve the target hemoglobin level.

Further study for serum hepcidin in renal transplantation patients is recommended.

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