DISCUSSION

Anemia is a common problem in patients with CKD and its prevalence and severity increases with increasing severity of CKD.[5] Iron deficiency is a main cause of anemia in CKD which may be absolute, functional or reticuloendothelial blockage.[188]

Hepcidin, the hormone responsible for iron homeostasis, has emerged as a key mediator responsible for the disturbed iron metabolism in the anemia of CKD. Hepcidin expression is up-regulated during infections and inflammation, contributing to hypoferremia and limitation of iron supply to the bone marrow (independent of iron status or erythropoietic activity). [189]

The IL-6 inflammatory cytokine, released during inflammatory process, was shown to correlate with increased hepcidin gene expression. [63]

This study was conducted on 30 patients, these patients were divided into 3 groups; group I included 10 normal individuals of average age and sex as a control. group II included 10 patients with end stage renal disease on Continuous Ambulatory Peritoneal Dialysis (CAPD) for more than 6 months treated in the Dialysis Unit in Alex university student hospital. group III include 10 patients with end stage renal disease on on-line hemodiafiltration for more than 6 months treated in the Dialysis Unit in El shefa hospital. All patients underwent regular dialysis for 4 hours 3 times weekly.

In the present study there was statistically significant difference between group I and both groug II and groupIII regarding hs-CRP. It was in its highest levels in group II.

Patients undergoing continuous dialysis either CAPD or OL-HDF are in a chronic inflammatory state. The causes of highly prevalent state of inflammation in dialysis patients are multiple, including decreased renal function, volume overload, comorbidity and intercurrent clinical events according to Stenvinkelet al [190], although Malyszko J et al[191] stated that the level of oxidative stress and inflammatory cytokines tended to be lower in PD patients than HD patients.

Kaysen GA [192] stated that in uremia, pro-inflammatory compounds (such as specific cytokines, acute phase proteins) accumulate, and the activity of defense mechanisms against oxidative injury is reduced; the dialysate purity which used in patient on OL-HDF is a major concern in diminishing the inflamatory state and its potential consequences. Carracedo et al[193] stated that Hemodiafiltration has been associated with a reduced inflammatory state and improved granulocyte function. [175] Several granulocyte-inhibitory proteins retained in uremic patients contributing to the high incidence of infections could be better removed with OL-HDF therapies. [194]

Both patients group had significantly lower Hb level when compared to healthy subject. Observational data suggest that anemia is less common and more sensitive to erythropoietin in peritoneal dialysis patients possibly because blood loss is less marked and residual renal function may be better preserved in patients who receive peritoneal dialysis.[195]

Snyder et al stated that patients who received peritoneal dialysis were substantially less likely to have initiated erythropoietin. Residual renal function can clear larger solutes and produce erythropoietin. This may explain the higher circulating erythropoietin levels reported in peritoneal dialysis patients. (195,196)

The findings of the Peritoneal Dialysis Clinical Performance Measures Project suggest that peritoneal dialysis patients who do not receive erythropoietin are not profoundly anemic. In that project, prevalence data from random national samples of adult peritoneal dialysis patients who participated in the United States End-Stage Renal Disease program have been collected annually since 1995. Between 1995 and 2000, the mean hemoglobin level increased from 10.7 to 11.6 g/dl. In 2000, only 11% of peritoneal dialysis patients had an average hemoglobin level _10 g/dl (195).

The improvement in anemia in patients on CAPD is associated with a much greater clearance of middle molecular weight substances and a less efficient removal of small molecular weight substances in comparison to hemodialysis.(109) These observations have led several investigators to suggest that uremic toxins responsible for inhibition of erythropoiesis are middle molecular weight in size and that it is the difference in relative clearances of these inhibitors of erythropoiesis by RDT and CAPD which is responsible for any difference in effect of the two forms of dialysis on the anemia of renal failure.(110,111)

Salttissi et al (125) showed a significant increase of serum hemoglobin and red cell mass after initiation of CAPD treatment.

The often greater improvement in hematocrit levels during the first 6 months of CAPD treatment in comparison to RDT has been reported to be due to a reduction in plasma volume in addition to an increase in red cell mass.(117)

Among several other factors, the lower extent of iron loss and therefore a lower incidence of iron deficiency during peritoneal dialysis treatment may be one reason for the reported differences.(121,122)

Francisco Maduell et al(134) stated that the Clearance of middle-to-large molecules depends on the type of dialysis membrane and the amount of convection volume and may be increased with OL-HDF treatment.

Van der Weerd et al (197) showed in patients treated with HDF, a rise in hemoglobin levels, improved iron availability and diminished ESA resistance. Three possible explanations for these observations in the HDF patients can be postulated: (1) gastrointestinal iron absorption may be enhanced; (2) iron release from its stores or utilization from intravenous iron therapy may be improved; or (3) red blood cell life span may be prolonged.

The pathogenesis of renal anemia was dominated for decades by the question of whether primarily due to relative EPO deficiency or uremic inhibition of erythropoiesis,(164) the relative deficiency in EPO is considered the main cause of renal anemia. In addition, factors other than EPO deficiency seem to contribute to an impairment of the erythropoiesis ; several of them have received attention (iron deficiency, inflammatory mediators, parathyroid hormone, etc) but the role of specific uremia-associated inhibitors of erythropoiesis has remained un clear .(165) Although direct proof is

lacking this does not exclude that a variety of different molecules accumulating in the uremic state may contribute to impaired erythropoiesis. Once HDF is started, the patient experience a significant increase in hematocrite in the absence of an improvement of endogenous EPO,[25]Possibly due to removal of these accumulating molecules that suppress erythropoiesis.

OL HDF with a high volume of on-line prepared substitution fluid is the most effective renal replacement therapy for uremic patient; it allows the delivery of a high dialysis dose and enlarges the spectrum of cleared uremic toxins.[162,163]

Our results do seem to go in harmony with those of Bonforte et al.[168] who showed an improvement in anemia in 32 patients with high-volume online replacement fluid. Osawa et al. [169] were able to lower the erythropoietin dose with hemodiafiltration. Maduell et al. [170] reported improved correction of anemia in 37 patients with lower erythropoietin doses when conventional hemodiafiltration (4 liters) was switched to online hemodiafiltration (24liters), This was attributed to the higher convection volumes, although there were also significant differences in urea clearance.

On the other hand, Ward et al.[162] and Wizemann et al. [172] could not confirm these observations in 24 and 23 patients, respectively, treated with online hemodiafiltration compared with 21 patients treated with high-flux hemodialysis and 21 patients treated with low-flux hemodialysis.

In this study there was a statistically significant difference between group II and III as regard the mean serum hepcidin. There was also statistically significant difference between group I and II .There was no statistically significant difference between group I and III. It was in its highest level in group II.

The serum hepcidin level of PD patients may be increased by several factors, including continuous artificial fluid retention, chronic peritoneal irritation by the dialysate, and occult infection or inflammation in the peritoneum .[198]

Regarding the effect of dialysis strategies on hepcidin levels, it has been shown That the hepcidin concentration can be reduced over one dialysis-session, either with low-flux or highf-flux HD, HDF and peritoneal dialysis.[195,199-201]

HDF resulted in superior reduction of hepcidin as compared to (high-f!ux) HD, especially when a double filter was used.[195,200] Hepcidin has been detected in the dialysate and it sticks to the dialyzer membrane.[199] However, hepcidin levels already returned back to baseline within one hour after a dialysis session.[202] this might be explained by removal of unbound, free hepcidin.

In our study there was a statistically negative correlation between Hb and hepcidin in CAPD patients. Supporting our results Aya Eguchi, et al [198] stated that the serum hepcidin levels were negatively correlated with the Hb concentrations in CAPD patients.

It was observed in the present study that there was a statistically positive correlation between hs- CRP and hepcidin in CAPD patients.

However FarukTurgut et al [203] showed different result that dialysis therapy is associated with elevated pro-hepcidin levels and not directly related to CRP, indices of iron

metabolism, or hemoglobin levels. Peritoneal dialysis patients have relatively lower pro-hepcidin levels than HD patients, but larger-scale studies are needed to confirm the possibility of impact on various dialytic modalities

In the present study there was positive correlation between serum ferritine & hepcidin in CAPD group.

In agreement with our results Aya Eguchi, et al[198] demonstrated that in PD patients' serum hepcidin levels were significantly positively correlated with their serum ferritin and TSAT levels and also find iron stores and circulating transferrin bound iron provide distinct signals that affect hepcidin synthesis in hepatocytes[204,205]resulting in the positive correlation between serum iron biomarkers and hepcidin levels.

Supporting our results Jolanta Malyszko et al [195] showed that ferritin was a predictor of hepcidin level in PD patients. Interactions between proinflammatory cytokines and hepcidin, mediators of functional iron deficiency in dialyzed patients, might explain why these patients have high ferritin levels, poor iron absorption, and impaired iron release from macrophages. In multiple regression analysis, residual renal function, ferritin, and hsCRP were predictors of hepcidin in hemodialyzed patients, while residual renal function and ferritin were predictors of hepcidin in CAPD patients.

Also Jack,Ph[206] stated that hepcidin levels were negatively correlated with hemoglobin, and positively correlated with ferritin in CAPD pateint.

In present study there was a significant positive correlation between serum hsCRP and serum hepcidin levels in OL-HDF group.

The effects of inflammation on the synthesis of hepcidin are well understood[71,207] and are mediated, at least in part, by IL-6 through induction and binding of signal transducer and activator of transcription 3 (STAT 3) to the hepcidin gene promoter [208].

Previous studies have shown markedly increased serum hepcidin levels in humans with chronic infections and severe inflammatory diseases, suggesting that elevated serum hepcidin levels play a key role in the anemia of inflammation and reticuloendothelial blockade.[63] Correlations between serum hepcidin levels and serum levels of inflammatory markers, including IL-6, IL-1, and high sensitive CRP, have been found in several studies.[209,210]

In agreement with our results Van Der Weerd et al.,[211] in 2012 showed a strong association between hepcidin-25 and hsCRP.

Zaritsky J et al [210] found that the correlation between hs-CRP and hepcidin provides further evidence in support of the relationship between both variables through all stages of CKD.

Although Aya Eguchiet al[198] stated in their study that no significant correlations were found between serum hepcidin levels and either serum CRP levels or IL-6 levels . Since there were no significant correlations with the biomarkers of inflammation despite the fact that hepcidin expression is induced by IL-6, this is likely due to the fact that a certain threshold is needed for stimulation of hepcidin expression by inflammation.

However, several studies have not necessarily shown the relationship between serum hepcidin levels and the levels of these inflammatory markers.

Ashby et al [212] found no significant correlation between serum hepcidin levels and inflammatory markers (hsCRP and L-6) in adults with CKD and hemodialysis patients. Also, Kato et al [213] found no association between hs-CRP and hepcidin in hemodialysis patients, but this may be due to the different assay method.

Costa et al [34] found that the high levels of hepcidin found in HD patients could be related to an underlying chronic inflammation. Moreover, correlations between hepcidin and inflammatory markers (CRP and IL-6) in HD group are similar to observational data derived from the non uremic population.[71,207]

It was observed in the present study that there was a statistically positive correlation between serum ferritine and hepcidin in OL-HDF patients.

The observation that there was a significant and independent correlation between hepcidin and ferritin levels suggests that it plays a major role in regulating iron homeostasis in this patient group. Our results also agreed with Babitt et al [214] who reported that hepcidin levels are likely to be higher in chronic kidney disease patients due to limited hepcidin excretion, tissue iron overload and inflammation.

On the other hand, a significant relation between serum ferritin and hepcidin levels is a consistent finding because serum ferritin is a marker of iron stores in the liver and RES as well as being an acute phase protein. Fujita et al. [215] demonstrated that the serum ferritin level had a strong positive correlation with the hepatic level of hepcidin mRNA expression.

Moreover, Stefánsson BV et al [216] stated That levels of serum hepcidin correlates very well with the level of serum ferritin[199,217], and our results are in line with these reports and also supported by Lin et al.[218] who reported lower ferritin levels and better iron utilization with HDF.

However, it cannot be ruled out that hepcidin primarily regulates the liver iron content, which would in turn regulate serum ferritin levels via hepatic iron content, because hepatocytes and Kupffer cells also express ferroportin. [202]. Further clarification of the relation between hepcidin regulation and iron storage is needed.

Jairam R et al [219] reported use of ferritin levels to define iron overload may be faulty because of the two-to-three fold elevations of ferritin levels with inflammatory activation. However, in the present study an excellent correlation was noted between ferritin and TSAT levels.

Kalantar-Zadeh *et al*.[220] also noted a good correlation of serum ferritin with TSAT and not CRP.

The most interesting finding in Stefánsson BV et al [216] work was the lower level of serum hepcidin with HDF, indicating that more normal iron homeostasis with lower degree of functional iron deficiency could, in theory, be achieved by HDF.

The clinical benefit of lowering hepcidin levels by convective dialysis treatments remains to be investigated. However, patients with chronic renal failure often suffer from functional iron deficiency, which probably is caused by pathologically high hepcidin levels in the circulation. In this condition, transferrin saturation is low even though iron stores are high, and potentially toxic intravenous iron administration is necessary to maintain adequate erythropoiesis. In this way, a vicious circle can arise, leading to further increase in the hepcidin level. Therefore, it seems reasonable that efforts in lowering the level of hepcidin in patients with chronic renal failure will bring the iron homeostasis towards a more normal state. [221]

SUMMARY

Anemia is a common complication among patient with (CKD). Untreated anemia places patient at risk for cardiovascular event, more rapid progression of CKD and significally decrease quality of life. The cause of aneamia is multifactorial in patient with CKD, but inadequate production of erythropoiten (EPO) by the diseased kidney is the common denominator. Aneamia generally becomes more severe as a patients kidney function declines.

Hepcidine is a low molecular weight hepatic peptide that plays an important role in iron metabolism .lately hepcidin has been recognized as the main hormone behind the pathogenesis of anemia of chronic disease.

The net effect of hepcidin is the diminished absorption of dietary iron, sequestration of iron in macrophages and in hepatic stores.

Low hepcidin levels were associated with anemia, depleted iron stores, increased iron needs, and an active erythropoiesis, whereas high hepcidin levels were found in case of increased iron stores resulting from low epoetin, ineffective erythropoiesis, or overzealous iron therapy, or in case of inflammation .

The clinical benefit of lowering hepcidin levels by dialysis treatments either CAPD or HDF remains to be investigated.

So, this work was conducted with the aim to evaluate the influence of peritoneal dialysis and hemodiafiltration on anemia and serum hepcidin levels in chronic kidney disease patients.

This cross-sectional study was performed in a cohort of 30 subjects who were divided into three groups: Group I:10 healthy subjects served as a control subjects, Group II: 10 patients with end stage renal disease on Continuous Ambulatory Peritoneal Dialysis (CAPD)for more than 6 months, Group III: 10 patients with end stage renal disease on ON-LINE hemodiafiltration (OL- HDF) more than 6 months.

previously diagnosed non renal causes of anaemia, patients with chronic infection or inflammation , patients with occult bleeding, patients with history of blood transfusion , patients with history of malignancy and end stage liver disease or chronic hypoxia. was excluded.

To all studied subjects, Laboratory investigations were done including Complete blood picture, Serum iron and other iron indices including serum ferritin, transferritin saturation (TSAT) & total iron binding capacity (TIBC),C-reacive protein ,Serum hepcidin level by enzyme linked immunosorbent assay (ELISA) were estimated.

The study showed the following results:

Plasma level of Hepcidin-25 was significantly higher ($p=0.003$) in CAPD patients (125.80 ± 39.63) than in controls (61.40 ± 19.30) and it was also statistically significantly higher ($P=0.010$) in CAPD patients (125.80 ± 39.63) than in OL- HDF patients (69.15 ± 23.55).

In simple correlation analysis, In group II , plasma hepcidin correlated positively withCRP (p =0.041), TSAT (p = 0.018), ferritin (p <0.001), iron (p = 0.013) & it correlated negatively with serum,Hb (p = 0.022) . In group III , plasma hepcidin correlated positively with CRP (p =0.004) , ferritin (p = 0.001) , serum iron (p <0.001) and it was correlated negatively with Hb (p = 0.192).

From this work we can conclude that:

OL-HDF is the best modality to decrease level of serum hepcidin and improvement of iron hemostasis.

Serum hepcidin levels is important factor in the iron metabolism and its high level may be a cause of anemia.

A close interaction between haematological data, inflammation, iron status and hepcidin serum levels, which ultimately regulate intracellular iron availability.

Hepcidin and its regulatory pathways are potential therapeutic targets, which could lead to effective treatment of anemia of chronic dialysis and the availability of the ELISA assay for serum hepcidin will facilitate the routine measurement of hepcidin in clinical practice.

CONCLUSION

- Serum hepcidin level is associated with iron status and inflammation in both CAPD & OL HDF patients.
- The correlation between hs- CRP and hepcidin in CAPD & in OL-HDF group indicating the presence of certain magnitude of the inflammatory process .
- A close interaction between haemoglobin , iron status and hepcidin serum levels, which ultimately regulate intracellular iron availability is present in both CAPD & OL HDF patients.
- Hepcidin seems to play a significant role in anaemia of CAPD & OL HDF patients; however, it is difficult to be used as a clinical marker due to the many influences and interrelations.
- The lower level of serum hepcidin with OL HDF, indicating that more normal iron homeostasis with lower degree of functional iron deficiency could be achieved by OL HDF.
- Hepcidin is an important factor in the iron metabolism and its higher level may be acause of anemia.

RECOMMENDATION

- Larger-scale studies are needed to confirm the possibility of impact of various dialytic modalities on serum hepcidin level.
- The usage of OL-HDF in patient with CKD to improve anemia through better clearance of hepcidin.
- Availability of the CELISA assay for serum hepcidin which will facilitate the routine measurement of hepcidin in clinical practice.
- Hepcidin and its regulatory pathways are potential therapeutic targets, which could lead to effective treatment of anemia of chronic dialysis patient.
- The clinical benefit of lowering hepcidin levels by convective dialysis treatments remains to be investigated.
- Study to develop certain hepcidin antagonists that could be used as a theraputic tool to lower or antagonize hepcidin action allowing mobilization of stored iron and improvement of intestinal iron absorption.