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RESEARCH ARTICLE

Value of hepcidin in diagnosis and monitoring of iron disorders in patients on regular hemodialysis and its relation to hcv infection.

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Abstract

Introdction:-Anemia is a common complication in maintenance Hemodialysis patients and Recombinant erythropoietin (rhEPO) has transformed anemia therapy in those patients,However, rhEPO resistance, often associated with iron deficiency and inflammation, remains a challenging problem. Current available iron indices do not reliably identify iron-restricted erythropoiesis, a sequel of inflammation, or patients who may benefit from parenteral iron therapy .Hepcidin is a peptide hormone produced by the liver and up regulated in inflammatory conditions, including uremia prevents iron absorption from the gut and release from macrophages and hepatocytes. In hemodialysis patients, serum hepcidin level increases resulting in iron restriction. Hepcidin deficiency with hepatic iron overload can complicate chronic hepatitis C virus (HCV) infection and may worsen prognosis, and iron depletion reported to be beneficial. We aimed to measure the relation between hepcidin level and iron deficiency anemia and HCV in hemodialysis patients.

Methods:- Complete blood count , iron, TIBC, Ferritin, Serum creatinine, blood urea, Liver enzymes, CRP, Hepcidin and HCV ab were measured in 30 adult men and women patients on regular hemodialysis having anemia with or without HCV seropositivity and in a control group of 20 healthy adult men and women.

Results:-A significant correlation was found between serum hepcidin and Patients' hemoglobin and iron profile regardless of HCV serology.

Conclusion:-In patients on regular hemodialysis,hepcidin level is higher and can be used in diagnosis and monitoring of iron disorders and hepcidin antagonism can be used in the treatment of iron deficiency anemia and rhEPO resistanceregardless of HCV serology. .

Introduction:-

Anemia is a common complication in maintenance Hemodialysis patients and contributes to reduced quality of life.Recombinant erythropoietin (rhEPO) has transformed anemia therapy in patients with chronic kidney disease (CKD). However, rhEPO resistance, often associated with iron deficiency and inflammation, remains a challenging problem (Eleftheriadiset al., 2009).

Current available iron indices do not reliably identify iron-restricted erythropoiesis, often a sequel of inflammation, or those patients who would likely benefit from parenteral iron therapy (Singh et al., 2007).
To address these issues, it is crucial to understand the molecular mechanisms that link inflammation, iron balance, and erythropoiesis. Hepcidin, an acute phase reactant 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 (Ganz, 2003).

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 rhEPO resistance (Malyszko et al., 2006).

Because of its renal eliminationand regulation by inflammation, it is possible that progressive renal insufficiency leads to altered hepcidin metabolism, subsequently affecting enteric absorption of iron and the availability of iron stores. Thus, hepcidin likely plays a major role in the anemia of CKD as well as erythropoiesis-stimulating agents (ESA) resistance. Thus, in the setting of CKD, increased serum hepcidin and the resulting iron restriction could play a major role in disordered iron homeostasis and resistance to ESA .The removal of hepcidin via hemodialysis (HD) has been demonstrated in adult patients using mass spectrometry (MS)-based assays, with varying degrees of efficacy seen (Brian and Joshua , 2009).

The enzyme-linked immunosorbent assay(ELISA) was used to determine blood hepcidin levels in CKD patients, confirming raised plasma hepcidin levels in renal failure compared with normal healthy controls, which is consistent with the notion of uremia being a chronic inflammatory state (Brian and Joshua , 2009).

Definitive resolution of this issue is needed, because increased removal of hepcidin by intensified HD could provide a much needed therapeutic intervention in cases of functional iron deficiency by relieving reticuloendothelial blockade as a result of inflammation-induced hepcidin overproduction. The improvement in ESA responsiveness reported in prolonged dialysis regimens supports the potential utility of this intervention (Schwartz et al., 2005).

Iron accumulation in the liver, where hepcidin is synthesized, is common in patients with chronic liver disease (CLD), especially in patients with chronic HCV infection.Increased hepatic iron is assumed to potentiate progression towards liver fibrosis in chronic HCV infection (Farid et al., 2004).The strong positive correlations between hepatic oxidative DNA damage and iron overload in CH-C suggest that hepatic iron content is one of the most probable mediators of hepatic oxidative stress in HCV infection, and iron reduction therapy may be beneficial in reducing HCC incidence in CH-C patients (Naoki et al., 2007).

Patients and methods:-

A total of clinically stable 30 adult men and women patients with CKD on regular HD having anemia with or without HCV seropositivity were recruited from the Dialysis Unit in Kasr El-Aini Hospital while the control group consists of 20 healthy adult men and women.

Inclusion criteria:-

1. Patients with CKD on regular HD either hepatitis HCV positive or negative.
2. Patients having iron deficiency anemia.

Exclusion criteria:-

were as follows: Non renal causes of anemia, any causes of renal anemia other than iron deficiency anemia, Patients received iron in the last 4 weeks, Patients received blood transfusion in the last 4 months, serious recent infection or hospitalization that required antibiotics in the last 4 weeksand patients withhigh C-reactive protein (CRP).

All patients were subjected to the following:

Clinical assessment:-

1. Thorough history-taking.
2. Full physical examination

Laboratory assessment:-

1. Complete blood count (CBC).

2. Complete iron profile (iron, TIBC and Ferritin).
3. Kidney function tests (Serum creatinine and blood urea).
4. Liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST)
5. CRP.
6. Hepcidin hormone assessment by ELISA kits.
7. HCV ab by ELISA.

The results were tabulated and statistically analyzed.

Sample collection:-

Blood samples were collected from each participant by venipuncture in Empty centrifuge tubes: incubated in water bath at 37^0C for 15 minutes then centrifuged at 3500 rpm. Sera were separated, divided into aliquots and stored at -80^0C till use. Haemolysed samples were discarded.

Estimation of Hepcidin:-

The quantitative detection of hepcidin in serum levels was performed using a commercially available ELISA kit provided by EIAab following the manufacture recommendations (**www.eiaab.com**).

Statistical analysis:-

Pre-coded data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows. Data was then transferred to the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed.

Data was summarized using range, mean, median and standard deviation for quantitative variables and frequency and percentage for qualitative ones.

Comparison between groups was performed using independent sample t-test (if parametric) or Mann Whitney test (if non-parametric) and one way ANOVA test for quantitative variables and Chi square or Fisher's exact test for qualitative ones.

Pearson or Spearman correlation coefficients were calculated to signify the association between different quantitative variables.

P values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

Results:-

This study was conducted to measure the level of hepcidin in patients on regular haemodialysis to determine its value in diagnosis and monitoring of iron disorders and its relation to HCV infection in those patients.

The study was conducted in 30 maintenance hemodialysis patients having anemia with or without HCV seropositivity [20 men, 10 women; age (mean ± SD) 47.6 ± 18.2 years] and in 20 age-matched healthy control individuals [12 men, eight women; age (mean ± SD) 44.3 ± 7.1 years]. There was no significant difference in age and sex between the two groups.

As regards CBC, Our patients had significantly lower Hb, HCT and MCV with higher TLC.The control group shows normal Hb, RBC, HCT, MCV, TLC and platelets with significant difference than the patients(Table 1). As regards Hb level there is no significant difference between HCV –ve and HCV +ve patients(Table 2).

Table 1:-Comparison between cases & controls regarding CBC findings.

	Case (n=30)			Control (n=20)			P value
HB							
Range	6.9	-	11.4	11.0	-	17.0	**<0.001 S**
Mean ± SD	9.1	±	1.2	14.2	±	1.6	
Median	9.0			14.0			
HCT							
Range	20.7	-	35.9	29.9	-	36.6	**<0.001 S**
Mean ± SD	28.1	±	3.8	33.5	±	1.7	
Median	27.7			33.8			
MCV							
Range	65.0	-	89.7	79.0	-	91.0	**0.001 S**
Mean ± SD	77.9	±	7.1	83.8	±	3.9	
Median	77.3			83.0			
TLC							
Range	3.3	-	11.3	3.9	-	9.4	**0.005 S**
Mean ± SD	7.6	±	2.0	6.0	±	1.5	
Median	8.0			5.7			
PLT							
Range	92.0	-	415.0	151.0	-	325.0	0.6 NS
Mean ± SD	237.1	±	76.2	225.9	±	61.6	
Median	239.5			210.0			

NS= non-significant, S= significant

Table 2:- Cosmparison of Hb level as regards presence of HCV

	HCV						
	+VE (n=11)			-VE (n=19)			P value
Hb							
	7.5	-	11.3	6.9	-	11.4	0.9
	9.2	±	1.0	9.0	±	1.3	
	9.0			9.1			

The control group shows normal iron profile with significantly lower iron and Transferrin saturation in the patients with higher TIBC and ferritin(Table 3).

Table 3:- Comparison between cases & controls regarding iron profile.

	Case (n=30)			Control (n=20)			P value
Fe							
Range	10.0	-	81.2	55.0	-	227.0	**<0.001 S**
Mean ± SD	38.1	±	19.1	143.7	±	50.7	
Median	36.0			135.0			
TIBC							
Range	100.0	-	504.0	142.0	-	312.0	**<0.001 S**
Mean ± SD	303.5	±	109.5	206.3	±	42.7	
Median	300.0			205.5			
Ferritin							
Range	9.2	-	753.0	50.0	-	235.0	**0.01 S**
Mean ± SD	216.7	±	158.4	118.3	±	58.2	
Median	155.3			95.0			
Transferrin saturation							
Range	5.0	-	19.0	27.0	-	57.0	**<0.001 S**
Mean ± SD	12.7	±	4.8	44.2	±	9.8	
Median	13.9			45.0			

As regards Fe, TIBC and ferritin levels there is no significant difference between HCV –ve and HCV +vepatients (Table 4)

Table 4:-Comparison of iron profile as regards presence of HCV.

	HCV						
	+VE (n=11)			**-VE (n=19)**			**P value**
Fe							
	15.0	-	81.2	10.0	-	68.0	0.3
	35.1	±	24.2	39.8	±	16.0	
	25.0			40.0			
TIBC							
	100.0	-	504.0	150.0	-	498.0	0.5
	329.0	±	145.1	288.7	±	83.6	
	300.0			300.0			
Ferritin							
	9.2	-	358.0	44.0	-	753.0	0.2
	168.8	±	116.4	244.4	±	175.1	
	150.0			200.0			
Transferrin saturation							
	5.8	-	17.6	5.0	-	19.0	**0.005**
	9.5	±	3.7	14.5	±	4.4	
	8.3			15.7			

As regards the liver enzymes our patients show serum ALT ranged between 6.0 and 38.0 with mean 20.6 and serum AST ranged between 9.0 and 49.0 with mean 20.3.

The control group shows normal liver enzymes with significant increase in the patientswith significantly higher levels in HCV +ve than HCV –vepatients.

Serumhepcidin in our patients ranged between 1.5 and 12.8 with mean 9.26.

The control group shows normal serum hepcidin level with significantlyhigher levels in the patients(Table5) and figure (1), but with no significant difference between HCV-ve and HCV +ve patients (Table 6).

Table 5:-Comparison between cases & controls regarding Hepcidin level.

	Case (n=30)			**Control (n=20)**			**P value**
Hepcidin (ngml)							
Range	1.5	-	12.8	0.1	-	1.0	**<0.001**
Mean ± SD	9.26	±	3.16	0.43	±	0.27	**S**
Median	9.75			0.35			

Table 6:-Comparison between Hepcidin levels as regards HCV positivity.

	HCV						
	+VE (n=11)			**-VE (n=19)**			**P value**
Hepcidin (ngml)							
Range	8.5	-	11.0	1.5	-	12.8	0.4
Mean ± SD	9.9	±	0.8	8.9	±	3.9	NS
Median	10.0			9.4			9.75

As regards the HCV positivity our patients show 36.7% are HCV positive and 63.3 are HCV negative.

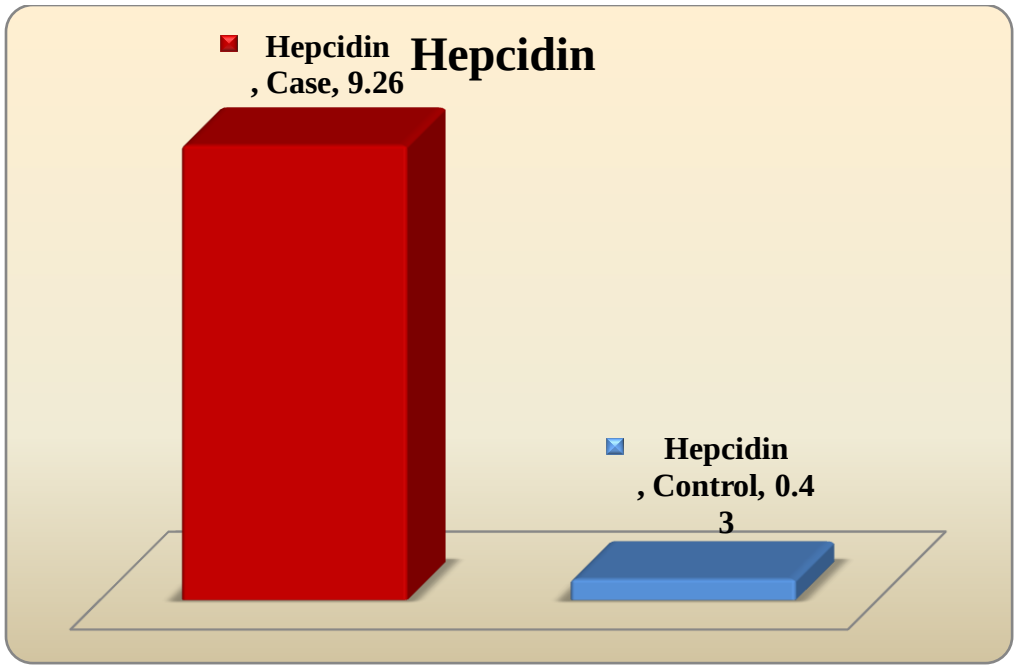

Figure 1:- Comparison between cases & controls regarding Hepcidin level.

Serum hepcidin was significantly correlated to HB level while all other CBC findings were not significantly correlated to serum hepcidin(Table7) and figure (2).

Table 7:- Correlation of hepcidin with CBC findings.

	Hepcidin (ngml)	
	r*	**P value**
HB	**-0.487**	**0.006 S**
HCT	-0.246	0.189 NS
MCV	-0.338	0.068 NS
TLC	-0.332	0.073 NS
PLT	-0.049	0.797 NS

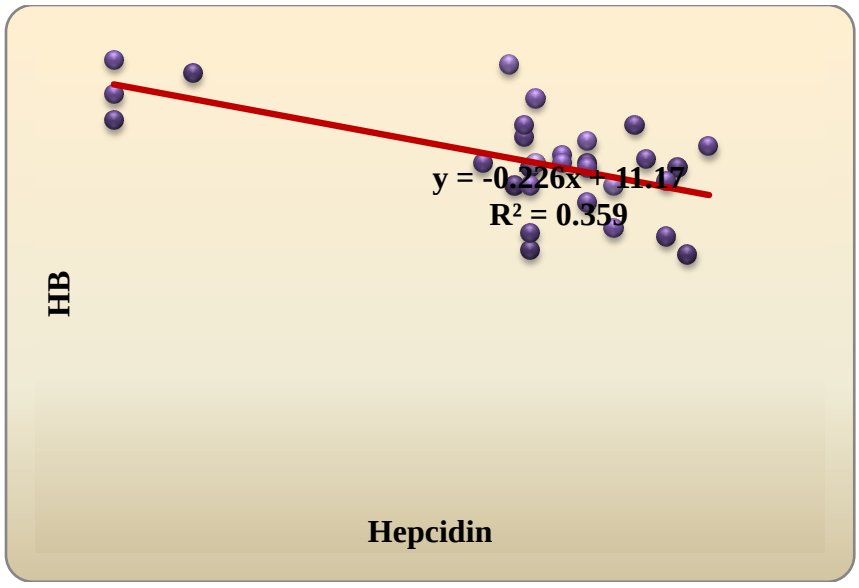

Figure 2:- The relation between serum hepcidin level and Hb level

As shown in table (8) serum hepcidin is significantly correlated to the iron profile.

Table 8:- Correlation of hepcidin with iron profile.

	Hepcidin (ngml)	
	r*	**P value**
Fe	**-0.412**	**0.024 S**
TIBC	**-0.398**	**0.030 S**
Ferritin	**-0.549**	**0.002 S**
Transferrin saturation	-0.058	0.761 NS

The relation between serum hepcidin levels and iron profile is also demonstrated in figures (3), (4) and

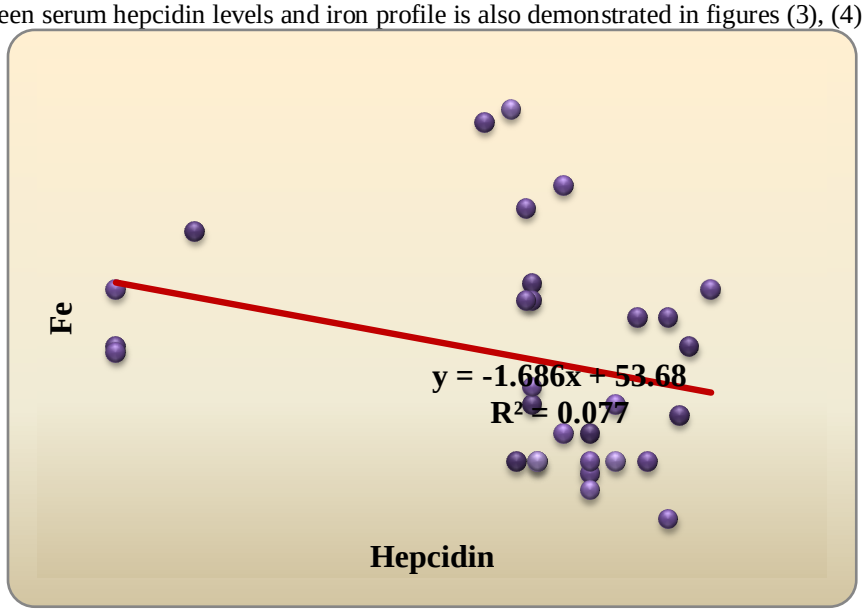

Figure 3:- Relation between serum hepcidin levels and iron

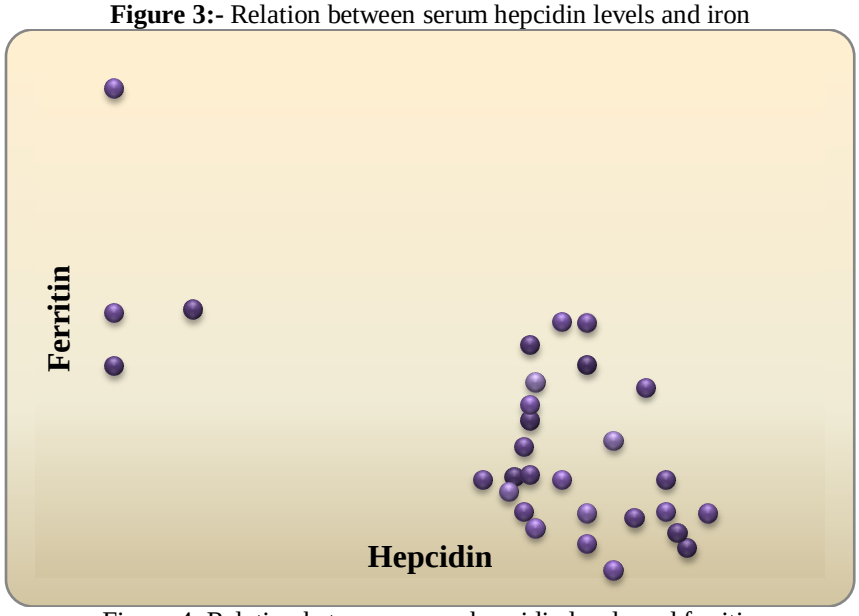

Figure 4: Relation between serum hepcidin levels and ferritin

Serum hepcidin is not significantly correlated to renal functions , liver enzymes, sex or HCV positivity

The correlations between serum hepcidin levels and clinical characteristics in CHC patients were investigated and the results are summarized in Table (9). A significant correlation was found between serum hepcidin and Patients' Hb, Iron, TIBC, Ferritin.

Table 9:-Correlation of Hepcidin with other parameters.

		S. Hepcidin
Age	**R**	0.243
	P value	0.195
Creatinine	**R**	0.027
	P value	0.889
Hemoglobin	**R**	**-0.487**
	P value	**0.006**
Platelet	**R**	-0.049
	P value	0.797
TLC	**R**	-0.332
	P value	0.073
Fe	**R**	**-0.412**
	P value	**0.024**
TIBC	**R**	**-0.398**
	P value	**0.030**
Ferritin	**R**	**-0.549**
	P value	**0.002**
ALT	**R**	-0.342
	P value	0.064
AST	**R**	-0.282
	P value	0.131

r= Correlation coefficient, P = P value

Discussion:-

Hepcidin, an acute phase reactant protein produced in the liver, is a key regulator of iron homeostasis. It inhibits intestinal iron absorption and iron release from macrophages and hepatocytes (Ganz, 2007).To address these issues, it is crucial to understand the molecular mechanisms that link inflammation, iron balance, and erythropoiesis.

High hepcidin levels are expected in ESRD patients and may be correlated with inability to utilize iron and to hypo-responsiveness to ESA therapy (Stenvinkel, 2006). In the future, hepcidin might also become a target of therapy, since lowering hepcidin may aid in improving the gastrointestinal uptake of iron and its release from macrophages, thus limiting the need for I.V. iron, overcoming functional iron deficiency and improving ESA resistance (Fishbane and Maesaka, 1997).Hepcidin concentrations were indeed reported to be increased in patients with CKD , although this could be caused by inflammation which frequently accompanies CKD, even patients without significant inflammation had elevated hepcidin which progressively increased with the increasing severity of CKD, hepcidin gene is an acute-phase responsive gene which is over expressed in response to inflammation (Zaritsky and Young, 2009).

This study was conducted to measure the level of hepcidin in patients on regular HD to determine its value in diagnosis and monitoring of iron disorders and its relation to HCV infection in those patients.

In the present study we characterized & investigated the hepcidin serum levels and the iron status and liver enzymes in 30 adult males and females Egyptian patients having ESRD on regular hemodialysis with or without HCV seropositivity and normal subjects; subjects of our study were classified into HCV -ve ESRD group (19 patients), HCV +ve ESRD group (11 patients) and control healthy group (20 individuals)

Patients ages ranged from (19-81) years with a mean of 47.6 ± 18.2.The control group ages ranged from (28-58) with a mean of 44.3 ± 7.1.

In the current study 66.7% of the patients were males and 33.3% were females .The control group 60% were males and 40% were females.

In the current study, there was no statistical difference in age (p=0.4) and sex (p=0.8) distribution in the 3 groups.

In another previous study, hepcidin expression in the liver has been reported to differ by gender (Courselaud et al., 2004).Women usually have lower iron stores than men mainly due to the physiological loss of blood. A study utilizing enzyme-linked immune-absorbent assay reported lower serum hepcidin levels in healthy female volunteers compared to those measured in males (Ganz et al., 2008).

In the current study, we employed a second-generation immunoassay, capable of resolving gender differences in serum hepcidin in healthy subjects.

In the current study, Serum hepcidin levels (ng/mL) were significantly higher in both HCV -ve ESRD group 63.3% and HCV +ve ESRD group 36.7%, than in control healthy individuals group, (p=0.002) and these results were in agreement with Zaritsky et al. (2009) & Ashby et al. (2009) both demonstrated an inverse correlation between serum hepcidin and GFR in adults with CKD, with serum hepcidin levels being highest in dialysis-dependent patients.

In our study there is no significant difference in hepcidin level in HCV positive patients ,P value (p=0.4),this result went in agreement with Fujita et al.(2008) who found no relation between HCV RNA load and serum hepcidin.

In the present study, All hematological iron parameters (s.Iron, s. TIBC, TFS and Hb level) were of statistical significance (p=<0.001) among groups comparison .

In the present study Serum Iron levels (µg/dL) were significantly lower in both HCV +ve ESRD group and HCV -ve ESRD group, than in control healthy, P value (p=<0.001).

Serum TIBC levels (µg/dL) were significantly higher in both HCV -ve ESRD group and HCV +ve ESRD group, than in control healthy group, P value (p=<0.001).

Serum ferritin level (μg/dL) was significantly higher in both HCV +ve ESRD group and HCV -ve ESRD group, than in control healthy, P value (p=0.01). We excluded patients with high CRP to avoid its increase as acute phase reactant, Previous studies that used mass spectrometry to measure hepcidin also demonstrated a correlation between ferritin and hepcidin in HD patients (Kato et al., 2008;Tomosugi et al., 2006).

Fujita et al. (2008) demonstrated that the serum ferritin level had a strong positive correlation with the hepatic level of hepcidin mRNA expression. However, it cannot be ruled out that hepcidin primarily regulates the liver iron content, which would in turn regulate serum ferritin levels by hepatic iron content, because hepatocytes and Kupffer cells also express ferroportin(Kumar et al., 2007). More studies are needed to clarify the relationship between hepcidin regulation and iron storage is needed.

Transferrin saturation (TFS %) was significantly lower in both HCV -ve ESRD group and HCV +ve ESRD group, than in control healthy group, P value (p=<0.001).

These results went in agreement with Fishbane and Maesaka(1977), who stated that there are three important mechanisms that have been proposed to explain the high frequency of iron deficiency in dialysis patients; include abnormal iron absorption, external blood loss, and functional iron deficiency.

In our study we found that there is a significant correlation between serum hepcidin levels and iron profile and this was consistent with Valenti et al.,(2009) who stated that serum hepcidin is increased in hemodialysis patients, regulated by iron stores and inflammation. Hepcidin may contribute to the pathogenesis of anemia by decreasing iron availability and also this result was in agreement with Pasricha et al. (2011) who stated that serum hepcidin concentration may be a useful indicator of deficient iron stores.

Hb levels were significantly lower in both HCV -ve ESRD group and HCV +ve ESRD group, than in control healthy group, P value (p=<0.001) this result went in agreement with Kazmi et al. (2001) who stated that nearly 90 percent of patients with a GFR of less than 25 to 30 mL / min have anemia, many with Hb levels below 10 g / dl.

In the current study as regards Hb level there is no significant difference between HCV –ve and HCV +ve patients, P value (p=0.9) and this result is not in agreement with Alaa et al. (2007), who reported higher hemoglobin and hematocrit levels in HCV +ve compared to HCV -ve HD patients in Egyptian HD population and Barril et al.(2008) who demonstrated higher Hb levels in HCV +ve HD compared to HCV -ve patients .

It is well recognized that parenteral iron administration is recommended for HD patients treated with r-Hu-EPO, On the other hand, hepatic iron concentration increases in CHC and iron reduction improves serum transaminase levels in these patients (Kohgo et al.,2008).

Serum ALT levels (IU/L) were significantly higher in HCV +ve ESRD group, than in both HCV -ve ESRD group and control healthy group, P value (p=0.02).

In previous studies; ALT and AST activity were upregulated in chronically iron-loaded rats. Rats with chronic iron overload showed higher serum ALT and AST activity (by approximately 2-, 3- and 3.1-fold, respectively) compared with normal control rats ($P < 0.01$) (Li et al., 2009).

Also in previous studies; Serum ALT and AST levels were positively correlated with hepatic hepcidin expression levels(Malyszko et al.,2006), indicating that hepatic inflammatory status also may influence the expression levels of hepcidin in patients with CHC, in another study;Despite the heterogeneity of the patients, hepcidin levels were related to hepatic and body iron stores, hematological parameters, and serum transaminase levels, suggesting that multi regulatory mechanisms act in hepcidin production (Naoki et al,. 2007).

Finally a significant relationship between serum hepcidin levels and seumcreatinine, urea, Hb, Fe, TIBC, ferritin was observed in our patients.

To support their assumption that antagonizing hepcidin mayameliorate the anemic stat as with other inflammatory anemias , a group of scientists generated a monoclonal antibody against hepcidin and have shown that this improves anemia in an inflammatory mouse model (Sasu et al., 2010). An RNAbased antagonist of hepcidin also has been

created. It consists of a 44-nucleotide l-RNA oligonucleotide produced using so-called Spiegelmers technology (RNA molecules in which the ribose component is levorotatory, or the mirror image of the natural righthanded sugar moiety). The Spiegelmer is linked to a40-kDa pegylation chain (NOX-H94), which has been shown to ameliorate anemia of inflammation in cynomolgus monkeys (NoxxonPharma AG., 2011). Rather than antagonizing the hepcidin molecule per se, another strategy could be to inhibit the production of hepcidin. This could be achieved by using antisense oligonucleotides or silencing messenger RNA transcribed from the hepcidingenehepcidin antimicrobial peptide (HAMP). None of the strategies to suppress hepcidin production or antagonize this peptide have been subjected to clinical trials. A theoretical concern could be that inhibition of hepcidin might exacerbate the risk for infections, given its endogenous antimicrobial properties. However, there are counterarguments to this suggestion, and it may be possible to suppress hepcidin to 'safe' levels without obliterating hepcidinactivity completely (Macdougall , 2012).

Further larger studies are required to evaluate the role of hepcidin in the diagnosis of iron deficiency in other groups of patients and also follow up of these patients clearly needed to refine our knowledge on hepcidin regulation in the full clinical spectrum of CHC.

Conclusion:-

Hepcidin level is higher in ESRD patients on regular hemodialysis than in normal patients with no significant difference between HCV +ve and –ve patients. This increase in hepcidin level is contributed to its increased synthesis and decreased elimination by the kidneys. There is a significant relationship between serum hepcidin level and Fe, TIBC, ferritin was observed in our patients. So, hepcidin can be used in diagnosis and monitoring of iron disorders in patients on regular hemodialysis. And hepcidin antagonism can be used in the treatment of iron deficiency anemia and rhEPO resistance.

On behalf of all authors, the corresponding author states that there is no conflict of interest:-

Ethical approval: *"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."*

Informed consent: *"Informed consent was obtained from all individual participants included in the study."*

References:-

1. Alaa, S., Khaled, E. and Khaled, M. (2007): Effect of HCV infection on Hct and Hb levels in Egyptian HD patients. European Journal of General Medicine, 4(1): 9-15.
2. Ashby, D., Gale, D., Busbridge, M. et al.,(2009):Plasma hepcidin levels are elevated but Responsive to erythropoietin therapy in renal disease. Kidney Int. 75: 976-981.
3. Barril, G., Castillo, I. and Arenas, M.D. (2008): Occult hepatitis C virus infection among hemodialysis patients. J Am SocNephrol., 19:2288.
4. Brian Young and Joshua Zaritsky, (2009):Hepcidin for Clinicians.Clin J Am SocNephrol 4: 1384–1387.
5. Courselaud, B., Troadec, M.,Fruchon, S., et al.,(2004): Strain and gender modulate hepatic hepcidin 1 and 2 mRNA expression in mice. Blood CellsMolDis32:283-289.
6. Eleftheriadis, T., Liakopoulos, V., Antoniadi, G., Kartsios, C., Stefanidis, I.(2009) : The role ofhepcidin in iron homeostasis and anemia in hemodialysis patients. Semin Dial; 22:70–77.
7. Farid, A.A., Mohammad, R.F., Osquei. and Mohsen, N.T. (2004): Synergic effect of chronic hepatitis C infection and beta thalassemia major with marked hepatic iron overload on liver fibrosis: a retrospective cross-sectional study.BMC Gastroenterology,4:17.
8. Fishbane, S. and Maesaka, J. (1997): Iron management in end-stage renal disease. Am J Kidney Dis, 29:319-333.
9. Fujita, N., Sugimoto, R., Motonishi, S., Tomosugi, N., Tanaka, H. and Takeo, M. (2008): Patients with chronic hepatitis C achieving a sustained virological response to peginterferon and ribavirin therapy recover from impaired hepcidin secretion. J .Hepatol., 49: 702-710.

10. Ganz T(2003): Hepcidin, a key regulator of iron metabolism and mediator Of anemia of inflammation. Blood; 102:783–788.
11. Ganz, T., Olbina, G., Girelli, D., et al.,(2008): Immunoassay for human serum hepcidin. Blood112: 4292–4297.
12. Ganz, T.(2007): Molecular control of iron transport. J Am SocNephrol 18: 394– 400.
13. Kato, A., Tsuji, T., Luo, J., et al.(2008): Association of prohepcidin and hepcidin-25 with erythropoietin response and ferritin in hemodialysis patients. Am J Nephrol; 28:115–121.
14. Kazmi, W., Kausz, A. and Khan, S. (2001): Anemia: an early complication of chronic renal insufficiency. Am J Kidney Dis, 38:803.
15. Kohgo Y., Ikuta K., Ohtake T., Torimoto Y. and Kato J.(2008): Body iron metabolism. Int J Hematol, 88(1):7-15.
16. Kumar, S., Raftery, M., Yaqoob, M., et al.(2007): Anti-inflammatory effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors (statins) in peritoneal dialysis patients.Perit Dial Int; 27:283287.
17. Li, H., Rose, M.J. and Tran, L. (2009): Development of a method for the sensitive and quantitative determination of hepcidin in human serum using LC-MS / MS. Journal of Pharmacological and Toxicological Methods, 59(3): 171-180
18. Macdougall IC. (2012): New anemia therapies: translating novel strategies from bench to bedside .Am J Kidney Dis ; 59:444–451.
19. Malyszko, J., Pawlak, K. and Mysliwiec, M. (2006): Hepcidin, iron status, and renal function in chronic renal failure, kidney transplantation, and hemodialysis. Am J Hematol 81:832–837.
20. Naoki, F., Ryosuke, S. and Masaki, T. (2007): Hepcidin Expression in the Liver: Relatively Low Level in Patients with Chronic Hepatitis C. Mol Med, 13(1-2): 97-104.
21. NoxxonPharma AG. (2011): Pipeline. NOX-H94, 44-nucleotide l-RNA oligonucleotide linked to 40 kDa PEG. Available at: http://www.noxxon.com/index.php?option_com_content&view_article&id_88&Itemid_100.
22. Pasricha, McQuilten, Westerman, Keller, Nemeth, Ganz, Wood (2011): Serum hepcidin as a diagnostic test of iron deficiency in premenopausal female blood donors.Haematologica ., 96: 1099-1105.
23. Sasu,BJ., Cooke, KS., Arvedson, TL., et al.(2010):Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. Blood; 115:3616–3624
24. Schwartz,DI.,Pierratos, A., Richardson, RM., Fenton, SS. and Chan, CT. (2005): Impact of nocturnal home hemodialysis on anemia management in patients with end-stage renal disease. ClinNephrol 63: 202–208.
25. Singh,AK., Coyne, DW.and Shapiro, W. et al., (2007): Predictors of the response to treatment in anemic hemodialysis patients with high serum ferritin and low transferrin saturation. Kidney Int 71: 1163–1171.
26. Stenvinkel, P. (2006): Inflammation in end-stage renal disease: the hidden enemy. Nephrology, 11(1):36-41.);
27. Tomosugi, N., Kawabata, H., Wakatabe, R., et al.(2006): Detection of serum hepcidin in renal failure and inflammation by using ProteinChip system. Blood ; 108:1381–1387.
28. Valenti ,Girelli, Castagna, Como (2009) : HFE Mutations Modulate the Effect of Iron on Serum Hepcidin-25 in Chronic Hemodialysis Patients. CJASN ., vol. 4 no. 8: 1331-1337.
29. Zaritsky, JJ.and Young, BY. (2009): The utility of multivariate analysis in the study of hepcidin. Kidney Int 76: 912–913.