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Association of Sepsis with Iron Overload in Hemodialysis Patients Receiving Intravenous Iron Therapy

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ABSTRACT

Intravenous (IV) iron used to treat anemia of Chronic Kidney Disease (CKD) in Hemodialyzed (HD) patients, may lead to iron overload. The incidence of sepsis in CKD patients with iron overload was studied. Procalcitonin and pro-inflammatory cytokines were used as markers. Serum procalcitonin was estimated semi quantitatively and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were quantified by ELISA. The study groups comprised of CKD patients on hemodialysis, receiving IV iron (n = 33) and CKD patients not on hemodialysis and receiving oral iron therapy (n = 36). Among patients receiving IV iron and on HD, non-survivors had significant ($p<0.05$) iron overload. 36.7% of HD patients had hyperferritinemia (>800 ng mL^{-1}. Among them, those having PCT>10 ng mL^{-1}, had a mortality of 67%. Elevated interleukin-6 and tumor necrosis factor-α were associated with higher rates of mortality. Iron overload is an additional risk factor propagating sepsis in hemodialyzed patients.

Key words: Chronic kidney diseases, hyperferritinemia, hemodialysis, interleukin-6, sepsis, procalcitonin, tumor necrosis factor α, SIRS

INTRODUCTION

Anemia management in dialysis patients remain as a central conflicting discussion matter even today (Coyne, 2008; Spiegel and Chertow, 2009). Anemia is a consistent finding in chronic disease (Baradaran and Nasri, 2005). Deficiency of iron availability for erythropoiesis is very common among the dialyzed as well as predialysis chronic kidney disease patients (Aiello, 2002). Hemodialysis is the main modality of treatment of end stage renal disease (Ahmed *et al*., 2010; Elshamaa *et al*., 2007a) which has made survival possible for more than a million people throughout the world who have end stage renal disease (Himmelfarb and Ikizler, 2010). Maintenance Hemodialysis (HD) patients may lose up to 3 g of iron each year because of frequent blood losses. Intravenous administration is more effective than oral iron supplementation in renal failure patients (Basic-Jukic *et al*., 2006). Administration of intravenous (IV) iron and erythropoietin stimulating agents is the current widely used strategy to treat anemia in hemodialysis patients. The most widely used iron preparations are ferric gluconate, iron dextran and iron sucrose. Intravenous (IV) iron therapy, though it increases the mean hemoglobin levels, may also lead to iron overload (Fishbane *et al*., 2004; Seiler, 2000) in dialysis patients which may

increase the iron deposition in tissues. Intravenous administration of iron in hemodialysis patients increases free radical production, which leads to oxidative stress (Swarnalatha *et al*., 2010). People who undergo hemodialysis are particularly vulnerable to staphylococcal infections, with the vascular access being a major site of entry. Patients with uremia demonstrate deficits in cell-mediated immunity, phagocytosis and antibody production (Marr, 2000) leading to a markedly increased risk for infections which may result in septicemia. Severe illness of almost any etiology is accompanied by a generalized host inflammatory response this has been referred to as the Systemic Inflammatory Response Syndrome (SIRS) (Bone *et al*., 1997). The release of proinflammatory mediators into the systemic circulation is the major reason for SIRS. In maintenance hemodialysis (HDCKD) patients septicemia accounts for more than 70% of the deaths (Basile *et al*., 2003; Foley *et al*., 2004). Release of leptin from adipocytes may be stimulated by cytokines mediating the inflammatory response (Nasri and Baradaran, 2006).

Present hypothesis is that this increased availability of iron puts HD patients at a higher risk for acquiring severe bacterial infections since an essential requirement of pathogens for their continual multiplication in the body is acquisition of sufficient iron for their growth. The aim of present study was to determine whether iron overload was associated with increased incidence of sepsis and mortality in our hemodialysis patient population.

MATERIALS AND METHODS

Subjects: CKD patients admitted by the Nephrology department of Kamineni Hospital's Ltd, Hyderabad, formed the study groups. The study subjects were divided into 2 groups:

- Group A: It had CKD patients on maintenance hemodialysis receiving IV iron (n = 33)
- Group B: It had CKD patients who were not on HD and receiving oral iron (n = 36)

The group B CKD patients receiving oral iron therapy and not on HD served as controls. The 11% of the control group was comprised of the patients with CKD stage 1 and 2 and 14% with stage 3 and 75% with stage 4 CKD. About 89% of this group had advanced CKD stage either 3 or 4. Patients who were on hemodialysis for more than 3 months, receiving IV iron therapy and between the ages of 18 to 70 years were included in the study. This research project was conducted from May 2008 to September 2010 at Kamineni Hospital, Hyderabad. Informed consent was obtained from all participants and the Ethics committee of Kamineni Hospital's Ltd approved this study. Since the underlying pathology of uremia which is an inflammatory state, would be the same in both groups of patients, further changes in the cytokine parameters in IV iron receiving patients may depend chiefly on iron therapy. Here the inevitable drawback of the study design was that CKD patients on HD therapy were already more prone to infections as they have additional risk factors like indwelling catheters etc. We could not use HD patients on oral iron as controls because such patients were very few. Oral iron supplementation could not sufficiently meet the demand of iron required with EPO supplementation due to compromised oral iron absorption in these patients. In chronic renal failure, the limited ability of the kidney to excrete an increased magnesium load may result in toxic concentrations of the ion in serum (Nasri *et al*., 2006).

Iron overload in the patients was defined by NKF/K DOQI guidelines as having serum ferritin greater than 800 ng mL^{-1} (Cooper, 2001). Among the group A and B patients, 5 out of 33 (i.e., 15%) and 3 out of 36 (i.e., 8%) of them, respectively had sepsis at the time of inclusion in the study. The group A and B subjects were further divided into survivors and non-survivors by the end of the study and were also classified according to 1992 ACCP/SCCM definition into those having severe

sepsis, sepsis, or SIRS (Bone *et al.*, 1992). Patients with known malignancy, HIV, HBSAg, CMV infections or any known source of ongoing blood loss or with renal transplant were excluded from the study.

Methods: Blood samples were collected randomly from all patients and analyzed immediately. Procalcitonin was estimated semi quantitatively by using B.R.A.H.M.S. PCT-Q kit (Henningsdorf, Germany) and was reported as per ACCP/SCCM guidelines (Brunkhorst *et al.*, 2000). PCT levels greater than 10 ng mL^{-1} indicated septic shock, PCT 2 to 10 ng mL^{-1} indicated severe systemic infection, PCT >0.5 to 2 ng mL^{-1} indicated sepsis while PCT <0.5 ng mL^{-1} indicated absence of systemic infection. Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were measured by ELISA (Herbelin *et al.*, 1990; Hirano *et al.*, 1990). Iron indices were estimated by Integra-400 (Roche). Tissue iron was calculated by using the mathematical equation (Van Wyck *et al.*, 1989). Survival was followed for 100 days after the episode of sepsis.

Statistical analysis: The normality for the distribution of all parameters were checked by methods like percent difference in mean and median; Mean±SD range; Skewness-Kurtosis, Critical value analysis, Kolmogorov-Smirnov, Shapiro-Wilk tests of normality and by plots (Altman, 1999). Student t-test analysis was performed to compare the means between IV iron receiving and oral iron receiving patient groups and also between non survivors and survivors of IV iron receiving and oral iron receiving patient groups. All parameters were log transformed to achieve normal distribution and multiple comparisons (ANOVA/ Post Hoc tests) and linear regression analysis was performed for the groups. Log ranked Kaplan-Meirs survival analysis (Altman, 1999) was also performed. All statistics were done using SPSS software (SPSS Ver 11.0, Chicago Inc).

RESULTS

All the study subjects were on regular follow up with the Nephrology department of our hospital and they were included randomly in this study. We had observed earlier that the PCT levels were usually found to be raised in HD patients who had increased serum ferritin levels rather than in non-HD patients. This led us to study whether there was any association between the mode of iron supplementation, iron markers and sepsis in these patients. Traditional iron markers like serum iron, serum ferritin and transferrin saturation ratio (TSAT) were analyzed. Procalcitonin and pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α were compared with iron markers in non-survivors and survivors following HD therapy. Table 1 describes the characteristics of non-survivors and survivors of CKD patients receiving IV iron and oral iron, respectively. Group-A patients had higher serum ferritin, tissue iron, IL-6 and TNF-alpha levels than group-B patients. The overall mortality rate was 39% in group-A CKD patients on HD and receiving IV iron. Among group-A patients, non-survivors had significant iron overload as evidenced by mean ferritin of 1079 ng mL^{-1} while survivors had mean ferritin levels of 626 ng mL^{-1} ($p<0.001$). There was significant increase in ferritin levels in IV iron receiving non-survivors when compared to non-survivors of group-B patients ($p<0.0001$) (Table 1).

Among patients who were not on hemodialysis, there was no significant difference in ferritin levels between non survivors and survivors. There was no statistical difference in mean serum iron levels as well among patients who received IV iron or oral iron.

The correlation analysis showed that serum iron, ferritin, % transferrin saturation and tissue iron were significantly negatively associated with IL-1β among non survivors of the group-A

Table 1: Median and interquartile range values of ferritin, tissue iron, IL-6, TNF-α, IL-1β, Iron, TIBC, %TSAT values between non survivors and survivors of group A (HD patients receiving IV iron) and group B (CKD patients not on HD and receiving oral iron)

	Group A: HD patients on IV iron (n = 33)		Group B: Non-HD patients on oral iron (n = 36)	
Parameter	Non survivors	Survivors	Non survivors	Survivors
N	13	20	8	28
Age	53±10	51±11	60±12	50±13
Ferritin (ng mL^{-1})	1079±697*	627±556	169±152***	186±118
Tissue iron (mg)	344±167**	253±159	38±157**	61±144
IL-6 (pg mL^{-1})	210±807***	20±71	10±9.5***	14±12
TNF-α (pg mL^{-1})	26±32**	14±12	11±11**	17±16
IL-1β (pg mL^{-1})	30±15*	19±13	21±27	13±28
PCT >10 (ng mL^{-1}), n = 15	10	5	0	0
PCT 2to10 ng mL^{-1},n = 9	3	6	0	0
PCT <0.5 ng mL^{-1}, n = 9	0	9	8	28
Serum Iron (μg dL^{-1})	70±79	60±31	52±24	49±42
TIBC (μg dL^{-1})	193±67	165±61	221±121	238±57
% TSAT	26±49	34±32	24±21	20±18

*p<0.01, **p<0.001, ***p<0.0001

Table 2: Correlation between iron indices and pro-inflammatory markers among group-A HDCKD patients

	HDCKD non-survivors				HDCKD survivors	
	Iron (μg dL^{-1})	Ferritin (ng mL^{-1})	%TSAT	Tissue iron (mg)	Ferritin (ng mL^{-1})	Tissue iron(mg)
IL-1β (μpg mL^{-1})	r-0.725*	r-0.673*	r-0.708*	r-0.699*	NS	NS
TNF-α (pg mL^{-1})	NS	NS	NS	NS	r-0.649**	r-0.632$ (pg mL^{-1})

*p<0.01, **p<0.05, NS: Not significant

patients receiving IV iron. Among survivors of group-A HD patients receiving IV iron, only the TNF-α was positively associated with serum ferritin ($p<0.05$) and tissue iron ($p<0.05$). There was also positive association between IL-1β and TNF-α ($p<0.01$) in these patients (Table 2).

Group-A, IV iron receiving HD patients-survival analysis: During the study period the number of group A patients developed sepsis was increased from 15 to 73% (24 out of 33), where as in group-B patients there was no further increase in the number of patients who developed the sepsis. Among Group-A non-survivors with iron overload, 77% (10 out of 13) developed sepsis and had elevated procalcitonin levels (PCT>10 ng mL^{-1}). In group-A patients with PCT>10 ng mL^{-1}, a mortality rate of 67% (10 out of 15), with survival proportion of 0.6 (95% CI 0.296 to 0.9) was observed. The mean survival time for patients with PCT>10 ng mL^{-1} was 5.7 days, where as in patients with PCT 2 to 10 ng mL^{-1}, mean survival time was 43 days (Fig. 1). However, the survival proportion in HD patients with PCT 2 to 10 ng mL^{-1} was 0.33 (95% CI -0.199 to 0.859) and was not significant. Among HD patients with PCT<0.5 ng mL^{-1}, there was no mortality during the study period. This was further supported by log ranked analysis which showed significant difference (static 20.24, $p<0.00001$) in mortality rate.

Group-B, oral iron receiving CKD patients-Survival analysis: An interesting finding was that patients in group-B had survival proportion of 0.375 (95% CI 0.042 to 0.708) having the mean survival time of 75±5 days and their PCT levels were always less than 0.5 ng mL^{-1} during the study

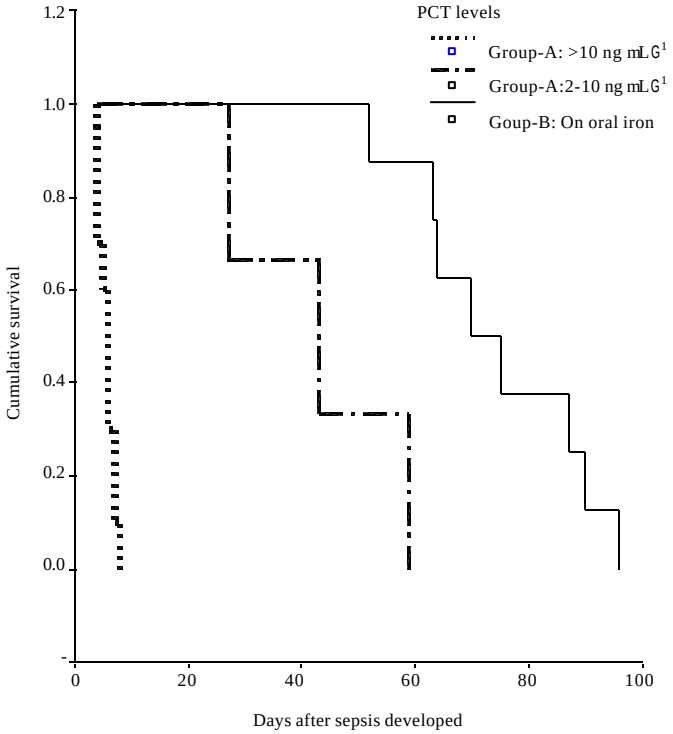

Fig. 1: Survival graph of CKD patients on IV and oral iron therapy

period (Fig. 1). The mortality rate of 22% observed in this group was due to metabolic and cardiac causes. In non-hemodialyzed patients, who were on oral iron, serum iron positively correlated with %TSAT ($r = 0.843$, $p<0.0001$). Serum ferritin did not correlate with other iron markers or with pro-inflammatory cytokines.

Comparison of study parameters in patients on IV iron grouped by PCT levels: Group-A patients on HD were divided into 3 groups based on their serum PCT levels, as having PCT greater than 10 ng mL^{-1}, between 2 to 10 ng mL^{-1} and less than 0.5 ng mL^{-1}. The levels of various iron markers in these groups of patients are shown in Table 3. Lavene's equality of error variance test for null hypothesis was rejected by serum iron, serum ferritin, IL-1β and IL-6 having $p<0.05$. This indicates there was a significant difference in mean levels of these parameters between the groups. Post hoc analysis showed serum ferritin levels were significantly elevated in patients with PCT levels >10 ng mL^{-1}, than those with PCT <0.5 ng mL^{-1} ($p<0.0001$; 95% CI 0.31- 0.85). There was no statistically significant difference in ferritin levels between the patients having PCT levels 2 to 10 ng mL^{-1} and >10 ng mL^{-1}. However, the sepsis was developed when serum iron and ferritin levels were highest (113±63 μg dL^{-1} and 1084±671 ng mL^{-1}) in patients having PCT levels between 2 to 10 ng mL^{-1}. Tissue iron levels were significantly elevated in patients with PCT 2 to 10 ng mL^{-1} and >10 ng mL^{-1} than in those with PCT<0.5 ng mL^{-1} ($p<0.01$ 95% CI 0.67-1.47; $p<0.015$, 95% CI 0.11-0.99, respectively). Serum iron was significantly higher in patients with PCT 2 to 10 ng mL^{-1} than in patients with PCT>10 ng mL^{-1} ($p<0.01$) and PCT<0.5 ng mL^{-1}. There was also significant elevation of IL-6 levels in patients with PCT>10 ng mL^{-1} than in patients with PCT 2 to 10 ng mL^{-1} ($p<0.001$, 95% CI 0.13-0.9). TNF-α levels were significantly increased in

Table 3: Serum markers of iron status in group-A HD patients divided into 3 sub-groups based on their serum PCT levels

Parameter	PCT>10 ng mL^{-1} (Severe Sepsis) (n = 15)	PCT 2-10 ng mL^{-1} (Sepsis) (n = 9)	PCT<0.5 ng mL^{-1} (SIRS) (n = 9)
Male: Female Ratio	9:6	6:3	5:4
Age (y)	54±9	47±12	53±11
Iron (µg dL^{-1})	57±41*	113±63*	62±12
TIBC (µg dL^{-1})	191±55	177±32	176±42
Ferritin (µg mL^{-1})	934±606**	1084± 671**	213±93
%TSAT	33±25	63±32	36±10
Tissue iron (mg)	345±138***	366±155 †	116±79

Values are Meant±SD. $p<0.05$ was considered as significant, PCT: Procalcitonin TIBC: Total iron binding capacity *PCT >10 vs. PCT 2-10, $p<0.01$; *PCT>10 Vs PCT<0.5, $p<0.01$;***PCT >10 Vs PCT <0.5, $p<0.0001$; *PCT 2-10 Vs PCT<0.5, $p<0.01$; **PCT 2-10 vs. PCT<0.5, $p<0.001$; **PCT 2-10 Vs PCT<0.5, $p<0.001$

Table 4: Summary of regression analysis between biochemical parameters in HDCKD patients

Predictor	Constant	R^2-value	Adjusted R^2-value
Ferritin	PCT	0.287	0.264**
Ferritin and %TSAT	PCT	0.394	0.354**
IL-6	Ferritin	0.171	0.144*
TNF-α	Ferritin	0.169	0.142*
Ferritin	Tissue iron	0.875	0.871**

*$p<0.05$, **$p< 0.001$

patients with PCT>10 ng mL^{-1} when compared to the patients with PCT levels between 2 to 10 ng mL^{-1} and <0.5 ng mL^{-1} ($p<0.01$, 95% CI 0.02-0.52 and $p<0.005$, 95% CI 0.11-0.62).

Regression analysis between ferritin and PCT levels showed an adjusted R^2 of 0.264 ($F = 12.5$, $p<0.001$) which indicates 26.4% of elevation in PCT levels is predicted by ferritin levels in patients receiving IV iron. In combination with %TSAT and ferritin, the adjusted R^2 is 0.354 which indicates there is 35.4% of PCT elevation, which is predicted by ferritin and %TSAT together. The co linearity statistics between ferritin and %TSAT reflected the VIF (variance inflation factor) of 1.0, indicating the reliability of R and p values (Table 4).

Comparison of iron indices and interleukins between the CKD patients on IV iron and oral iron: Lavene's test for equality of variance was performed. Ferritin, IL-1β, IL-6 had p value of <0.5, hence equal variance was not considered for these parameters. Student t-test analysis showed there was significant mean difference in levels of serum TIBC, ferritin, %TSAT and IL-6 between CKD patients receiving IV iron and CKD patients receiving oral iron ($p<0.001$ for all 4 parameters) (Fig. 2).

Serum ferritin was significantly associated with IL-6 and TNF-α ($r = 0.531$, $p<0.001$ and $r = 0.411$, $p<0.01$, respectively. However, the regression analysis showed adjusted R^2 values for IL-6 and TNF-α on variation of ferritin levels in IV iron receiving patients is 0.144 and 0.142 respectively with p values of 0.05 for both (Table 4). This indicates that only 14% of variation in ferritin levels is due to pro-inflammatory cytokines which also means that changes in serum ferritin are not primarily due to inflammation alone and that they are potentially due also to iron overload. There is significant positive correlation between serum ferritin and tissue iron ($r = 0.935$, $p<0.0001$). In survivors of group A patients receiving IV iron, only the TNF-α was positively associated with

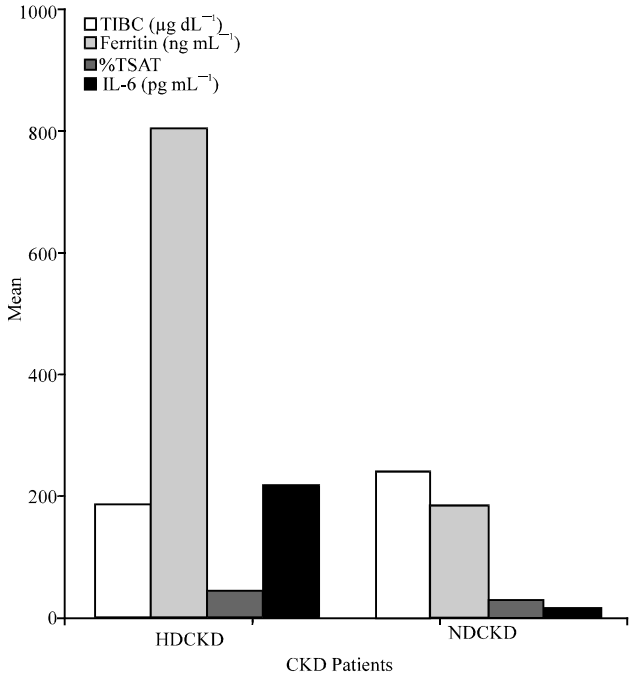

Fig. 2: Mean levels of TIBC, Ferritin, % TSAT and IL-6 among HDCKD and NDCKD patients

serum ferritin ($p<0.01$) and tissue iron ($p<0.01$), where as tissue iron was significantly negatively associated with IL-1β in non-survivors of the group A patients receiving IV iron ($p<0.01$). Regression analysis showed adjusted R^2 of 0.871 which indicates 87% of excess iron might get deposited in tissues in patients receiving IV iron ($p<0.0001$) (Table 4).

DISCUSSION

Iron plays an essential role in immunosurveillance because of its growth promoting and differentiation-inducing properties for immune cells and its interference with cytokine activities (Weiss, 2002). The elevated cytokines in CKD patients may divert the iron from availability to erythroid cells causing anemia, seen in this population. Parenteral iron formulations like iron sucrose and iron gluconate have potent cytotoxic effects which appear to parallel degrees of cell iron uptake (Zager *et al*., 2004a). The iron sucrose administered to studied HD population having highest degree of absorption may increase its cytotoxic effects. Excessive treatment using IV iron to treat anemia in CKD patients may lead to iron overload state, with resultant production of free radicals and cellular damage. The iron overload may compromise the ability of phagocytes to kill micro organisms and often there is increase in susceptibility to infections, one of the major causes of death in hemodialysis patients (Khan *et al*., 2007). In HD patients with PCT >10 ng mL^{-1}, had a mortality rate of 67% and the mean survival time was only 5.7 days. The increased Non Transferrin Bound Iron (NTBI) following IV iron administration in HD patients, forms a toxic labile iron pool when it enters into the liver (Scheiber-Mojdehkar *et al*., 2004). The labile iron pool in this study not estimated. However, it was presumed that the supplemented iron will increase the labile iron pool. Therefore, international treatment guidelines generally recommend that intravenous iron be discontinued when serum ferritin is >800 ng mL^{-1}. Serum ferritin <200 ng mL^{-1} suggests iron deficiency in CKD patients, ferritin levels between 200 and 800 ng mL^{-1} may be related to

inflammation, latent infections and ferritin levels above 800 ng mL^{-1} reflect the iron overload. Both IV iron and oral iron receiving patient groups had advanced CKD stage (stage 3 to 5); therefore, the underlying metabolic state is similar in both groups. Hence, in this study, we explore the relevant issues that show that elevated levels of serum ferritin should be considered as a warning indication of the probability of developing sepsis and associated mortality in these patients.

Serum ferritin is an acute-phase protein and increases two-to fourfold in response to inflammation (Barany, 2001). The uremic patients having inflammation showed elevated serum ferritin levels. The acute-phase response is regulated by a number of pro inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interferon (Barany, 2001; Epstein, 2001; Trey and Kushner, 1995). Elevation of TNF-α and IL-1 cause an increase in ferritin synthesis directly at the transcriptional level (Mascotti *et al*., 1995). Indeed, several studies have shown that plasma TNF-α is elevated among patients on HD (Elshamaa *et al*., 2007b). IL-1 beta and TNF-α level in HDCKD patients were not elevated to significant levels, but increased IL-6 levels might have increased the ferritin levels. Since ferritin plays a dual role as an acute phase protein (in range of 200-2000 ng mL^{-1}) and iron storage protein independent of its serum levels, it is difficult to assess the effect of iron overload during infection. However, as shown by present data the contribution of serum ferritin as an inflammatory indicator is about 14%. Serum ferritin levels in non-survivors of group A patients on HD therapy was higher (1079±697 ng mL^{-1}) than the recommended levels (<800 ng mL^{-})[1] above which iron administration to be discontinued. The regression analysis showed that the variation in serum ferritin levels due to inflammation is only 14% in present study which means that significant increase in its levels may depend mainly on supplemented iron in HD patients. However, the lack of correlation between ferritin and inflammatory markers observed in this study in the face of iron overload warrants further stringent study.

Though there was no direct evidence to support that ferritin levels more than 800 ng mL^{-1} leads to deposition of tissue iron, the calculated tissue iron levels were increased in proportion to increase in serum ferritin in these patients (Table 1). Since the calculated tissue iron levels are directly proportional to serum ferritin, tissue iron levels show the same significant correlation with infection rate and mortality as serum ferritin. Iron overload, according to the K/DOQI guidelines, may occur in patients who have more than 800 ng mL^{-1} serum ferritin levels but this is extremely variable. The serum ferritin level of 800 ng mL^{-1} which the K/DOQI guidelines propose as an upper limit for intravenous iron therapy, was an opinion-based cutoff; it was not evidence based. In contradiction to previous study which have shown that about 38% of HD patients receiving iron doses of >1000 mg over 6 months duration, had ferritin levels >800 ng mL^{-1} (Van Wyck *et al*., 1989). In this study 39% had ferritin levels >800 ng mL^{-1} in HD patients, who were receiving 1000 mg iron sucrose in 5 sessions of dialysis (One and half month). The high cellular uptake of iron sucrose might increase the % of HD patients with ferritin >800 ng mL^{-1} and iron overload.

Procalcitonin is a marker of bacterial infection in critically ill patients (Muller *et al*., 2000; Ugarte *et al*., 1999). The CKD patients having susceptibility to invading pathogens, were more prone to infections. Procalcitonin is a 116 amino acid protein with a sequence identical to that of the prohormone of calcitonin (32 amino acids) (Neiland, 1981). The bacterial endotoxins (Lipo polysaccharides) are the most potent stimulator for PCT induction (Dandona *et al*., 1994). SIRS has been associated with elevation in PCT levels.

The overall mortality rate among CKD patients receiving IV iron therapy was 33%. The mortality was due to septicemia as evidenced clinically by ACCP/SCCM criteria and supported by

microbiological studies with associated increased serum procalcitonin levels. Those having PCT >10 ng mL^{-1}, had a mortality of 67%, with survival proportion of 0.6 (95% CI 0.296 to 0.9) and their mean survival time was 5.7 days. Regression analysis showed there was a significant elevation of PCT levels as serum ferritin increased ($R^2 = 0.264$, $p<0.001$). This observation supports the experimental study by Zager *et al.* (2004b) which showed that parenteral iron therapy exacerbates experimental sepsis in mice. It was observed that the incidence of sepsis was developed in patients having highest serum iron and ferritin levels (113±63 μg dL^{-1} and 1084±671 ng mL^{-1}) in patients having PCT levels between 2 to 10 ng mL^{-1}. Parenteral iron administration can induce modest TNF- release. The increased levels of TNF-α and IL-6 in patients with PCT >10 ng mL^{-1}, may contribute to significant high mortality in HD patients along with other co-morbid conditions.

Mortality was markedly elevated in Hemodialysis (HD) patients. Between 30 to 50% of dialysis patients have elevated serum levels of inflammatory markers (Stenvinkel, 2001). Around 40% of HDCKD patients in this study had elevated IL-6 levels. The presence of chronic or episodic inflammation has been found to be associated with increased mortality risk. The frequent vascular infections in HD patients were caused by *Staphylococcus* species. In a recent study, Barton *et al.* (2006) showed that the non transferrin bound iron was present in HD patients receiving 100mg of iron sucrose and this fraction of iron was associated with enhanced *S. aureus* growth. NTBI was more frequently found in patients with base line %TSAT more than 30%. The average %TSAT in HD patients of our study was 30±40.5 and therefore these patients may have had high NTBI levels which may lead to higher rate of infection in these patients.

CONCLUSION

We conclude that the use of PCT, IL-6 and TNF-α as a markers for sepsis helps to identify patients at risk of sepsis and death. Monitoring of serum procalcitonin levels during a SIRS episode may provide early warning indication of impending sepsis in HD patients with iron overload. In those patients when both PCT (>10 ng mL^{-1}) and serum ferritin (>800 ng mL^{-1}) were elevated, the individual mortality was increased. Hence serum ferritin should be monitored regularly to prevent iron overload and elevated levels of serum ferritin should be considered as a warning indication of the probability of developing sepsis and associated mortality in these patients.

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