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Research Article

SERUM FERRITIN AND HEPCIDIN LEVELS AS PREDICTORS OF EARLY POST HEMATOPOIETIC STEM CELL TRANSPLANTATION INFECTIONS

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ABSTRACT

Patients undergoing Hematopoietic stem cell transplantation are at risk of infection at different phases of the transplantation. Ferritin and hepcidin are postulated to be major players that linking inflammation or infection with iron homeostasis.In this study, pre-transplant serum ferritin and hepcidin were assessed in 50 patients who underwent HSCT and 50 healthy persons as controls. Median level of serum hepcidin was higher in the patients' group than the control group. Forty patients (80%) had episodes of infections in first 100 days post-transplant. Patients with post-transplant infections had significantly higher mean level of serum hepcidin (p value=0.001) and a significant positive correlation between serum hepcidin and serum ferritin.However the hepcidin specificity (80% at cutoff value of 222ng/ml) was better than ferritin specificity (60% at cutoff value of 205 ng/ml) in predicting early post HSCT infection. Meanwhile, Ferritin specificity (65% at cutoff value of 250ng/ml) was better than hepcidin specificity (52% at cutoff value of 217ng/ml) in predicting survival in first 100 days after transplant.
Conclusion: serum hepcidin was more specific than serum ferritin as a predictor for early post HSCT infection while serum ferritin has higher specificity for overall survival in first 100 days post-transplant. The results encourage pre-transplant assessment of serum hepcidin level as a part of the routine work up and administration of iron chelators to decrease incidence of infections and improve outcome.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) has become a curative treatment for hematologic malignancies with the improvement of outcome in recent decades; this has been achieved by better matching of donor and recipient, prevention of graft versus host disease (GVHD) and supportive care. However, complications due to infections remain an important contributor to transplant-related morbidity and mortality (Bjorklund *et al*, 2007). Infections remain a threat to long-term survivors for many years after HSCT.

Iron is an essential element for all pathological microorganisms (Bjorklund *et al*, 2007). Iron overload is common in patients undergoing HSCT for hematologic disorders as these patients have often been heavily transfused prior to HSCT (Altes *et al*, 2004) and it is associated with adverse clinical outcomes including a propensity to infections (Ales *et al*. 2009). Free iron acts as a free radical catalyser, and may increase mucositis, vasculitis, and hepatic veno-occlusive disease (VOD) (Kataoka *et al*, 2009). These adverse events and the high availability of free iron increase microbial growth and the probability of severe infections. Indeed, High pre-transplant serum ferritin level was strongly associated with lower overall and disease free survival (OS, DFS) in patients with allogeneic HSCT (Armand *et al*, 2007)**.** It is therefore possible that iron overload may significantly increase tansplant-related mortality (TRM) due to toxic death and lethal infections in immunocompromised patients (Altes *et al*, 2004).

Hepcidin, first identified in human blood and urine as an antimicrobial small peptide, is now considered a central regulator of iron metabolism (Park *et al*, 2001).

Hepcidin is strongly induced during inflammation. During infection, hepcidin causes depletion of extracellular iron, which is thought to be a general defense mechanism against many

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infections by withholding iron from the invading pathogens. Conversely, by promoting iron sequestration in macrophages, hepcidin may be detrimental to cellular defense against certain intracellular infections (Michels *et al*, 2015).

Hepcidin decreases iron absorption from the intestine and blocks its release from iron stores by down regulating the expression of the cellular iron exporter, ferroportin. Therefore, it is hypothesized that serum hepcidin level could be a useful predictor of iron overload and inflammatory condition prior to HSCT (Ganz *et al*, 2008).

The purpose of this prospective analysis was to compare between pre-transplant serum ferritin and serum hepcidin levels as predictors of early post HSCT infection.

Patients and Methods

This study included 50 patients, their ages ranged between 21 to 59 years, 36 of them were males and 14 were females, and 50 healthy persons, matched for age and gender were included as controls. All subjects provided written informed consents prior to enrollment. The study was reviewed and approved by Ain Shams University Ethical Committee .The patients were recruited from bone marrow transplantation unit of Ain Shams University hospital and Maadi military hospital over two years from March 2012 - March 2014.

Therapeutic Regimen: Patients received various conditioning regimens according to their disease status. Fludarabine / busulphan for acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, TBI based conditioning for Acute Lymphoblastic Leukemia, fludarabine/cyclophosphamide and ATG for aplastic anemia, high dose melphalan for multiple myeloma, carboplatin-vepside-cyclophosphamide or melphalan/vepside for all types of lymphoma. Cyclosporin plus methotrexate (MTX) were used as GVHD prophylaxis. Antimicrobial prophylaxis (levofloxacin 500 mg once daily) was used as adjuvant therapy with conditioning according to our institutional protocols. Trimethoprim-sulfamethoxazole was administered as prophylactic therapy for Pneumocystis jiroveci pneumonia. Antifungal prophylaxis was done with fluconazole till day 75 post-transplant and acyclovir was used for viral prophylaxis till the time of revaccination. All patients were followed up for 100 day post-transplant. Exclusion criteria included; 1-patients known to have chronic renal insufficiency (Cr Cl <30 ml/min, 2- patients with chronic liver disease due to various etiologies (chronic hepatitis C or B, autoimmune or metabolic diseases). 3- patients with manifestations of decompensated heart disease. 4- patients with evidence of ongoing infection, inflammation at time of sampling and those taking antimicrobial therapy were excluded.

METHODS

The patient's group underwent Full history taking, clinical examination and routine pre-transplant workups initially. Complete blood picture, Liver and kidney function tests, serum electrolytes, serum LDH level, serum uric acid and ESR were done at regular intervals. Bone marrow aspiration and/or biopsy, flow-cytometric immunophenotyping and cytogenetics studies were performed at presentation and to confirm remission. With each febrile episode, 2 sets of blood samples (peripheral and from central venous catheters), as well as suspicious specimens other than blood were cultured. Necessary imaging examinations were performed to localize the site of infection (Chest- X-ray (CXR) and/or CT scan).

Pre-transplantation (preconditioning) level of serum ferritin using chemiluminescence and serum hepcidin levels (25amino acid) using ELISA were measured in all patients as well as the 50 controls.

Statistical analysis is performed using Statistical Package for Social Sciences (SPSS®) for Windows® version 15.0.

RESULTS

The patients' group included 36 (72%) males and 14 (28%) females. Their ages were between 21 to 59 years with a mean age of 38.16±11.7 years. Eighteen patients suffered from acute leukemia (14 acute myeloid leukemia and 4 Acute Lymphoblastic Leukemia), 14 from multiple myeloma, 6 from bone marrow aplasia, 6 from lymphoma (4 Non Hodgkin Lymphoma, 2 Hodgkin Disease), 4 from myelodysplasia and 2 from Chronic Myeloid Leukemia. Twenty patients underwent autologous transplantation (40%) while 30 underwent allogeneic peripheral blood stem cell transplantation (60%) from non-manipulated fully HLA matched related donner. Seven patients had GVHD following allogeneic BMT (three had gastrointestinal GVHD, two had hepatic GVHD and two had skin GVHD). Two patients had grade III- IV and the others had grade I-II. One patient developed VOD.Patient's characteristics demonstrated in Table 1.

Table 1 Demographic data of the Patients with characteristics features of the disease.

Characteristics	
Mean age (years)	38.16±11.7
Sex, n (%)	
Male	36(72)
Female	14(28)
Diagnosis, n (%)	
Acute myeloid leukemia	14(28)
Acute lymphoblastic leukemia	4(8)
Myelodysplastic syndrome	4(8)
Chronic myelogenous leukemia	2(4)
Non-Hodgkin's lymphoma	4(8)
Hodgkin disease	2(4)
Aplastic anemia	6(12)
Plasma-cellmyeloma	14(28)
Cytogenetic, n (%)	
Normal	32(64)
Abnormal	12(24)
-t(9;22)	4(8)
-t(8;21)	4(8)
-5q	2(4)
-14q	2(4)
Not available	6(12)
Type of BMT, n (%)	
Allogeneic BMT	30(60)
Autologous BMT	20(40)
GVHD, n (%)	
Yes	7(14)
No	43(86)

Serum ferritin and hepcidin levels: median serum ferritin &hepcidin levels were higher in patients group compared to their controls (220 vs 75 ng/ml); (225 vs 180 ng/ml)

respectively, with statistically significant difference for serum ferritin level (p<0.001) (Table 2). Pre-transplant serum ferritin level for all patients ranged from 10 to 525 ng/ml with median level (220 ng/ml) and pre-transplant serum hepcidin level for all patients ranged from160 to 330 with median level (225 ng/ml).

Table 2 Serum ferritin and serumhepcidin levels between patients and controls.

Parameters	Patients (n=50)	Controls (n=50)	t	P
Ferritin median ng/ml (IQR)	220 (102.5-300)	75 (26.00-175.00)	-3.543	**<0.001**
Hepcidin median ng/ml (IQR)	225 (212.5-265)	180 (85.00-525.00)	-1.125	0.261

Within transplanted patients, 80% patients (40) suffered from episodes of infections in first 100 days post-transplant (26 post allogeneic BMT patients and 14 post autologous BMT patients) while the remaining 20%(10) patients didn't.

Thirty patients suffered from bacterial infections (60%), 4 patients suffered from fungal infections (8%), and 6 patients suffered from both bacterial and fungal infections (12%).

Pre-Transplant serum ferritin and Hepcidin Levels and post-Transplant Infection Episodes

There is no statistical significant difference between the mean level of pre-transplant serum ferritin (233.7±129 ng/ml) in patients with early post-transplant infection episodes when compared with the patients without infection episodes (158±111.4 ng/ml) (p value = 0. 096).

The mean level of pre-transplant serum hepcidin was significantly higher (244.7±46.47 ng/ml) in patients with early post-transplant infection episodes when compared with its mean level in patients without episodes of infections (218±5.3 ng/ml), (p value = 0.001) (Table 3).There is a significant positive correlation between serum ferritin level and serum hepcidin level among all patients as well as among patients with infections (r=0.476, p<0.001 & r=0.493, p=0.001 respectively) (Fig.1).

Table 3 Serum ferritin and hepcidin levels and post-transplant infection.

Parameter	Patients with Infection n= 40	Patients without infection n=10	t	P value
S. ferritin (ng/ml) Mean± SD	233.7±129	158±111.4	1.698	0.096
S. hepcidin (ng/ml) Mean ± SD	244.7±46.47	218±5.3	3.546	0.001

Measurement of serum ferritin level was clinically relevant with respect to Infection at a cut off of 205 ng/mL by using ROC curve analysis. It showed a sensitivity of 60%, specificity of 60%. (Fig.2); as well as measurement of serum hepcidin level was clinically relevant with respect to infection at a cut off of 222 ng/mL by using ROC curve analysis. It showed a sensitivity of 60 %, specificity of 80 %. (Fig.3)

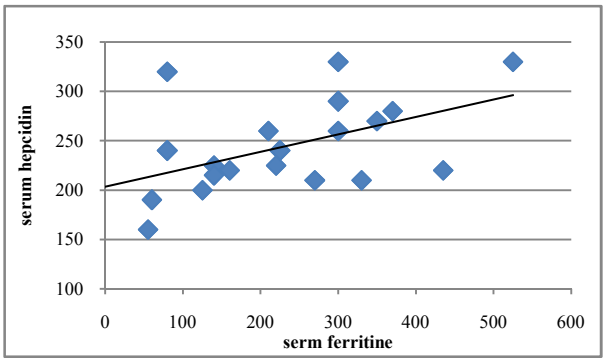

Fig 1 Positive Correlation between pre-transplant serum ferritin and hepcidin in cases with early post-transplant infection

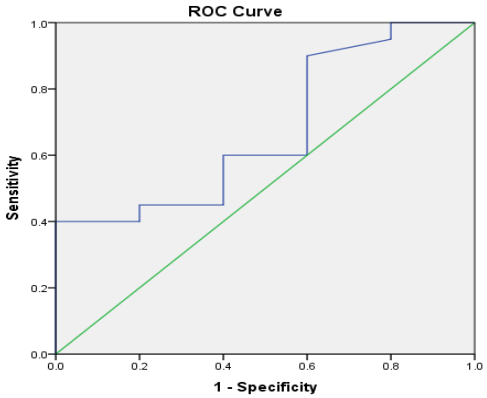

Fig 2 ROC Curve of S. Ferritin (ng/ml) as predictor for early infection post HSCT

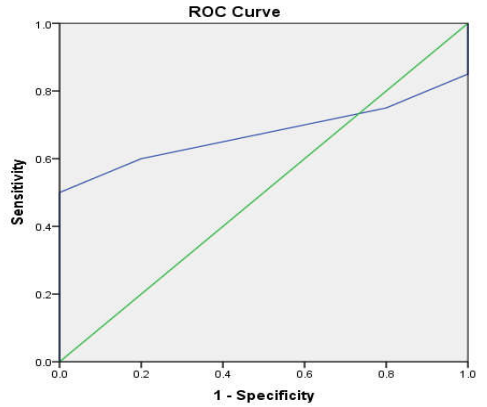

Fig 3 ROC Curve of serum hepcidin (ng/ml) as predictor for early infection post HSCT

The first 100 days post-transplant Overall survival was; 68% (34) and32% (16) patients died early post-transplant. Septicemia & Septic shock accounted for 62.5% (10) deaths, ARDS 25% (4) deaths and 12% (2) of acute bleeding (one of them had also VOD).

Measurement of serum ferritin level was clinically relevant with respect to survival at a cut off of 250 ng/mL by using ROC curve analysis. It showed a sensitivity of 50%, specificity of 65% (Fig.4); while measurement of serum hepcidin level was clinically relevant with respect to survival at a cut off of 217 ng/mL by using ROC curve analysis. It showed a sensitivity of 50 %, specificity of 52 %. (Fig.5)

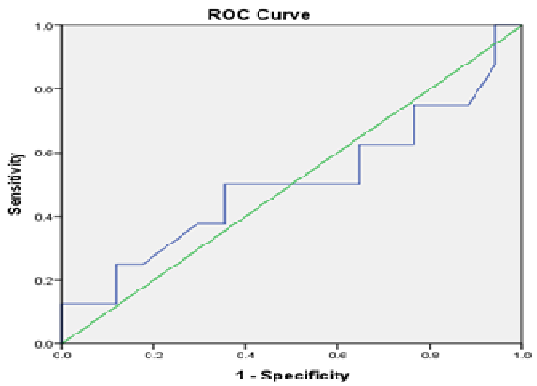

Fig 4 ROC Curve of S. Ferritin (ng/ml) for overall survival post HSCT

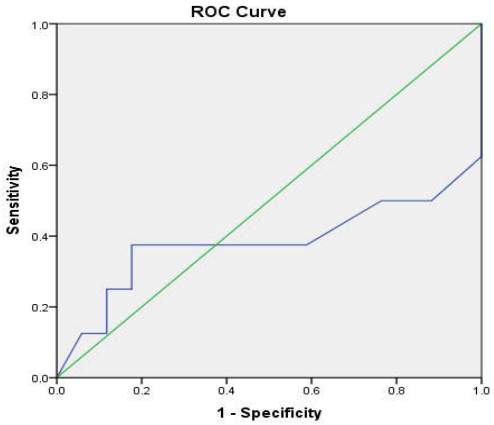

Fig 5 ROC Curve of serumhepcidin (ng/ml) for overall survival post HSCT

DISCUSSION

Patients undergoing HSCT for hematologic disorders commonly have iron over load (Altes *et al*, 2004). There is no reliable serum or plasma marker for body iron burden. Serum ferritin is commonly used as an indirect estimation of body iron stores. Although sensitive, it is not specific for iron overload as it can be elevated in a variety of infectious and inflammatory states Therefore, ferritin is a good screening test and clinically significant iron overload is uncommon in patients with serum ferritin levels less than 1000 ng/ml (Nielsen *et al*, 2000). Elevated Pre-transplantation ferritin levels are associated with increased mortality and worse transplant- related survival following hematopoietic stem cell transplantation. Because of its predictive value, the pre-transplant ferritin level has been incorporated into a prognostic scoring system for patients undergoing myeloablative allogeneic transplantation for acute leukemia and MDS (Armand *et al*, 2008).

Iron overload can be evaluated by pre-transplant serum ferritin and hepcidin. (Park *et al*, 2001; Nielsen *et al*, 2000). Humans do not have any physiological mechanisms to excrete excess iron. Iron homeostasis is primarily regulated at the level of iron absorption by regulatory effectors such as hepcidin (Papanikolaou and Pantopoulose, 2005). In conditions of iron excess, hepcidin levels increase and inhibit intestinal absorption and release of storage iron. Its expression is markedly decreased in iron-deficiency states. The function of hepcidin is thought to be further regulated by the HFE (High iron FE) protein (Hentze *et al*, 2004). Serum hepcidin level may therefore be useful as a predictor of iron overload and inflammatory conditions prior to HSCT (Ganz, 2006).

Patients undergoing HSCT are at risk of granulocytopenia, impairment of barrier defenses, impairment of cell-mediated immunity (CMI) and of humoral immunity. This impairment leads to an immunocompromized state, allowing microorganisms (even those with limited pathogenicity) to cause infection more easily; at different phases of the transplantation process. Infections are among the most common conditions associated with inflammatory process. The predominant infections during the pre-engraftment phase of transplantation are bacterial infections, occurring in 15%-50% of recipients of BMT (Woo *et al*, 2001).

In this study, the pre-transplant serum level of ferritin was significantly higher in BMT patients compared to normal controls. The increased ferritin level in our patients group in comparison to control group may be attributed to an iron overload mainly due to multiple blood transfusions given to BMT patients as part of their supportive care for chronic anemia. Patients may also require further transfusion therapy following conditioning and prior to engraftment. As each unit of transfused packed red cells contain approximately 200–250 mg of iron, patient who are administered regular transfusions can receive a daily iron excess of up 0.5 mg /kg (Andrews, 1999). With no physiologic mechanism for clearing excess iron taken in as a result of transfusions, iron accumulation is an inevitable sequel, and patients can become iron overloaded after as few as 10–20 transfusions (Porter, 2001).

In the present study the increase in serum hepcidin level in our patients was not statistically significant compared to controls. However, pre-transplant level of serum hepcidin was significantly higher in patients who developed early post-transplant infection (p value= 0.001) compared to patients who did not have infections. A level of 222 ng /ml for serum hepcidin was used to predict early post-transplant infection with specificity of 80% and sensitivity of 60%. A significant association was found in previous studies between the pre-transplant serum hepcidin levels and the cumulative incidence of documented bacterial infection suggesting that it can be used as a good pre-transplant biomarker to predict bacterial infection in a patient scheduled for HSCT (Nemeth *et al*, 2004).

Previous studies showed that pre-transplantation increased serum ferritin level was a risk factor for the occurrence of blood stream infection (BSI) within 100 days after allo-HSCT (Tachibana *et al*, 2010). An increase in plasma non-transferrin-bound iron (NTBI) is considered to have an important role in the adverse effect of iron overload on bacterial infection. Under normal conditions, toxic reactions due to the production of NTBI are prevented by circulating transferrin, which forms a compound with Fe^{3+}. However, plasma NTBI increases to a measurable level in patients with iron overload because transferrin is almost saturated with Fe^{3+}. The inhibition of iron utilization in erythrocytes by chemotherapeutic agents and irradiation further increases NTBI levels. Hydroxyl radical reactions by NTBI exacerbate mucosal damage caused by chemotherapeutic agents and irradiation, which allows bacterial organisms to enter through circulation. In addition, iron is an important nutrient for the proliferation of bacteria and fungi (Weinberg, 2009).

In this study a significant positive correlation between serum ferritin and serum hepcidin levels were found among all patients as well as among patients with infection. This could be attributed to a similar hepcidin and ferritin response to changes in iron status. Similarly, Nemeth *et al*., showed a strong correlation between hepcidin and ferritin in response to inflammation and changes in iron stores (Nemeth *et al*, 2003). During follow up of the studied group, 16 (34%) patients died in first 100 days post transplants and septicemia is cause of death in 10 of them.

A cut off value of 250 ng/ml for serum ferritin and 217 ng /ml for serum hepcidin were used to predict overall survival in first 100 days after transplantation with specificity of 65% for serum ferritin and 52 % for serum hepcidin. This is in concordance with studies that reported adverse impact of high ferritin on overall survival (Armand *et al*, 2007). Patients with elevated pre-transplantation serum ferritin level had inferior survival because of increased NRM (non-relapse mortality) mainly from infections and organ failure (Altes *et al*, 2007). On the other hand studies didn't detect any adverse impact of high hepcidin level on overall survival in the first 100 days after transplantation (Kanda *et al*, 2009).

An association between iron chelation therapy and longer overall survival was shown in patients with MDS or severe anemia requiring multiple blood transfusions,where adequate iron chelation therapy was recommended for such patients (Raptis *et al*, 2010).

Conclusion: Pre-transplant high level of serum hepcidin is probably related to increased incidence of infections (in first 100 days after transplant) while serum ferritin was related to overall survival in first 100 days post-transplant.

This may encourage assessment of serum hepcidin levels as a part of the routine pre-transplant work up for prediction of patient's liability to infection and administration of iron chelators prior to conditioning regimens for management of iron overload may reduce the mortality & morbidity due to infection risk.

Further studies are warranted in this context to establish & consolidate suitable biomarkers to minimize infection rate in patients with iron overload undergoing stem cell transplantation.

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