DISSCUSSION

Thalassemia is a heterogeneous inherited disorder of hemoglobin synthesis due to mutations of the globin gene, leading to various degrees of quantitative defect in globin production and reduced synthesis or complete absence of one or more of the globin chains, resulting in ineffective erythropoiesis and anemia. Beta-thalassemia major (BTM) is usually presented at 4 - 6 months of life, due to the protective effect of high hemoglobin F concentration at birth that slowly declines through the first year of life. Manifestations are those of anemia (pallor, lethargy…etc), failure to thrive and organomegaly. Patients presenting later will have signs of extramedullary hematopoiesis; (frontal bossing of the skull, hepatosplenomegaly, thinning of long bones cortices, widening of medullary and diploic spaces; resulting in bossing of skull, prominence of the upper incisors and wide separation of orbits).

BTM is a significant public health problem in Egypt where over 1–5 million newborns are expected to be affected with this disorder and it is considered the most common genetically determined chronic hemolytic anemia (85.1%) in our locality. A high rate of carriers has been reported in Egypt ranging from 4–10%. This is due to high rate of consanguineous marriage which helps to accumulate deleterious genes in families, reaching 35.3% in our community. Children with untreated or partially treated thalassaemia major die in the first or second decade of life.

The mainstay of treatment is based on adequate, safe blood transfusions and prevention of iron overload. The most widely accepted blood transfusion protocol aims to increase the concentration of hemoglobin to 13-14 g/dl after transfusion, and maintain it at 9-10 g/dl at all times, most patients require transfusion between 2-4 weekly commencing at around 1 year of age. On the other hand, frequent blood transfusion in turn may add to iron overload which may result in hemosiderosis, the later may be a cause of hypogonadism, diabetes mellitus, hypoparathyroidism and other endocrine abnormalities. Several studies reported a higher incidence of growth & endocrine abnormalities in children, adolescents and young adults with BTM. Allogeneic hematopoietic SCT remains the only treatment that can correct the hematological manifestations in patients with thalassemia major. Improving the clinical outcomes of high-risk, heavily transfused patients with liver fibrosis and inadequate iron chelation remains a challenge. Because of the relatively high probability of graft rejection and regimen-related toxicity in many patients receiving SCT for advanced thalassemia major, further development of new treatment regimens is warranted. [285]

The aim of this work is to investigate the association between pre-transplant serum ferritin level and hepatitis C viral (HCV) infection on the outcome of fully matched sibling donor (MSD) peripheral blood stem cell transplantation (PBSCT) in patients with B-thalassemia major (BTM).

Forty-four patients with β-thalassemia major who received allogeneic PBSC transplantation using BU /CY/ATG conditioning regimen during the period from March 2013 to April 2014 at Nasser Institute Hospital were included in this study with a median follow up period of 9.8 months.

The total CD34+ cells/kg infused to the patients involved in this study ranged from 3.2 x 10^6 to 36.5 x 10^6 cells/kg patients' weight with a mean of 14.09 ± 8.725x 10^6 cells/kg patients' weight. The mean numbers of days till TLC reached ≥ 1000/µl was 21days in those patients who received CD 34+ ≤ 7 x 10^6 cells/kg patient□s weight, while the mean numbers of days till TLC reached ≥ 1000/µl was 19.5 in those patients who received CD 34+ > 7 x 10^6 cells/kg patient□s weight. The difference between the two groups of patients was non-significant (p=0.404). Regarding platelets it was noticed that the mean numbers of days till platelets reached 25,000/µl was 41 in those patients who received CD 34+ ≤ 7 x 10^6 while the mean numbers of days till platelets reached 25,000/µl was 26 in those patients who received CD 34 > 7 x 10^6. Also, the difference between the two groups of patients was non-significant (p=0.103). The observed non-significant relation between CD34+ cell dose and engraftment reported in our study, was recently reported by ***Herbert et al (2014).*** They tested the hypothesis that the infused CD34+ stem cells might reliably predict neutrophilic engraftment (NE) in 94 children who received BM from 37 HLA-identical sibling donors (MSD) and 57 matched unrelated donors after myeloablative conditioning. The grafts contained a median of 3.6 × 10^6/kg total CD34+ cells, which consisted of a median of 73% myeloid CD34+ cells and 27% B lymphoid progenitors. Grafts from donors <15 years old yielded significantly lower myeloid fractions compared with grafts from older donors (P < .001). All patients achieved sustained NE after median 20 (range, 11 to 40) days. By multivariate analysis, neither the number of total CD34+ cells (P = .605) nor of myeloid CD34+ cells (P = .981) correlated with NE, whereas transplantation from MSD (hazard ratio [HR] 3.51; P = .019) and the administration of granulocyte colony–stimulating factor (HR 2.24; P = .002) remained independent factors associated with earlier NE. Taking into account that the number of transplanted total CD34+ or myeloid CD34+ cells does not seem to have a relevant impact on time to NE, sepsis rates, or TRM. [(286)]

In this study it was reported that AGVHD occurred in 7 out of the 44 (15.9%) BTM patients included in our study. One/14 (7.14%) of patients, who received in CD 34+ cell count ≤ 7 x 10^6 cells/kg patient□s weight developed AGVHD compared to 6/38 (20%) of patients, who received in CD 34+ cell count > 7 x 10^6 cells/kg patient□s weight group. The difference in the incidences of AGVHD between the groups of patients was statistically non-significant (p=0.288). Also, CGVHD occurred in 4 out of the 44 (9.1%) BTM patients included in this study. 1/14 (7.14%) of patients, who received in CD 34+ cell count ≤ 7 x 10^6 group developed CGVHD while it occurred in 3/30 (10 %) of patients, who received in CD 34+ cell count > 7 x 10^6 group. The difference in the incidences of CGVHD between both groups was also statistically non-significant (P= 0.765). Similar conclusion was reported by ***Martin et al. (2013)*** who assessed 705 adult patients with hematological malignancies who underwent reduced intensity conditioning (RIC) –allogeneic hematopoietic cell transplantation (HCT) at Dana Farber Cancer Institute/ Brigham and Women's Hospital (DFCI/BWH) between 2000 and 2010. The vast majority received a RIC regimen of fludarabine and busulfan (n=698). GVHD prophylaxis was tacrolimus based with or without sirolimus (524 vs. 171, respectively). Recipients of in vivo T-cell depletion (TCD) with antithymocyte globulin or ex-vivo TCD were excluded. Patient's disease risk index (DRI) was categorized as low (n=164), intermediate (n=350), high (n=170) or very high (n=21). Transplants were categorized as matched (MRD n=273, MUD n=374) or mismatched (MMRD n=4, MMUD n=58). Although higher doses of CD34+ cells (> 10 x 10^6/kg vs. < 5 x10^6/kg) resulted in faster engraftment for both

platelets and neutrophils and a decrease in non-relapse mortality (HR 0.53 [0.30-0.93], p=0.027), there was no significant effect on the incidences of acute or chronic GVHD, relapse, PFS or OS. [287]

Another study by ***Storb, et al. (2013),*** the median number of transplanted CD34 stem cells was 12.1×10^6/kg recipient body weight with a wide range of 1 to 54.6×10^6/kg. The median number of co-transplanted CD3 T lymphocytes was 7.6×103/kg (range, 0.5-130×10^3/kg). They did not observe any correlation between the number of transplanted CD34 stem cells and the development of extensive CGVHD. Although 25 of the 76 patients received megadoses of CD34+ cells (at least 20×10^6/kg), no extensive CGVHD was seen in any of the 76 patients. Another study by ***Zaucha et al (2002)***, reported an association between the CD34 cell dose in G-PBMCs collected from HLA–identical siblings and the development of extensive CGVHD is shown. The number of CD3 T lymphocytes or CD14 monocytes in the graft were not significantly associated with extensive CGVHD. Therefore, the authors concluded that increasing the CD34 cell number in G-PBMC products may be counterproductive. [288]

In another retrospective study performed several years ago, ***Saad et al (2006)*** reported on the correlation of the CD34 and CD3 doses of stem cell transplant with incidence of AGVHD in 67 consecutive patients who were treated between 2003 and 2005. All patients were followed up for at least 100 days following the stem cell transplant. Among the 67 patients, 35 patients developed AGVHD. There was no statistical difference between the mean CD34 doses when comparing the group of patients who developed acute GVHD with the group that did not develop acute GVHD (p= 0.31). However, using a CD3 dose cutoff value of 30 x 10^7/kg IBW, the incidence of AGVHD was statistically significantly less among those who received CD3 dose <30 x 107/kg IBW (p= 0.04). So they concluded that the CD34 dose did not influence the incidence of AGVHD, while CD3 dose < 30 x 10^7/kg IBW was associated with reduced risk of AGVHD. [289]

In this study, 13.9% and 5.6% of patients transplanted in risk class 2 suffered from acute and chronic GVHD respectively, while the incidences of both AGVHD and CGVHD in risk class 3 patients were 25%. The differences in incidences of AGVHD and CGVHD between the two risk groups were statistically non-significant (p= 0.449 and 0.087 respectively.

In this study, VOD was reported in 6 cases (13.6%) in the whole group of patients. Patients transplanted in risk class 2 suffered from VOD in 3/36 (8.3%) of patients compared to 3/8 (37.5%) patients transplanted in risk class 3. The difference in the incidences of VOD between both groups was statistically significant (p=0.030*).

VOD occurred in 2/33 (6.1%) patients in HCV negative group versus 4/11 (36.4%) patients in HCV positive group. The difference in the incidences of VOD between both groups was statistically significant (p=0.01). In literature, the potential role of HCV in the development of SOS was for a long time a matter of debate. [290,291] A 9.6-fold increased risk of fatal VOD or sinusoidal obstruction syndrome (SOS) was demonstrated in HCV-infected patients who received cyclophosphamide and TBI over 12 Gray, related to sinusoidal toxins of those regimens and not directly to HCV [292] , In other words, the increased risk of a fatal outcome from sinusoidal toxicity is related to fibro-inflammatory liver disease and the components of the conditioning regimen, and not to HCV per se. HCV infection is not considered as a risk factor for SOS if the conditioning regimen has

little or no liver toxicity, for example, fludarabine and targeted busulfan or a non-myeloablative regimen of fludarabine plus low-dose TBI. [293] In a study by ***Frickhofen et al. (1994)***, 61 patients from several disease entities treated with BMT were evaluated. HCV genome, as well as antibodies to HCV, was analyzed in sera collected before and serially after BMT. Six patients had been infected with HCV before BMT and three patients acquired the infection during or shortly after BMT. All patients infected before BMT died within 10 weeks after transplantation. Five of these six patients (83%) died of VOD, compared with nine of 52 patients (17%) not infected with HCV ($P < .005$). Risk factors for VOD other than HCV were not more prevalent in these patients compared with uninfected patients. Parallel to the development of VOD, replication of HCV increased, as demonstrated by rising concentrations of viral RNA in serum. HCV infection acquired during or after BMT caused only mild acute hepatitis C, which progressed to chronic hepatitis C in one patient surviving 10 years after BMT. So they suggested that patients with liver disease caused by HCV infection are at high risk of developing lethal VOD after BMT [294], and these results confirms our results regarding the significant correlation between VOD and HCV infection after BMT. In a retrospective analysis of 63 consecutive pediatric HSCT for thalassemia, performed by ***Cappelli et al (2000)***, all of the patients that completed the full course of defibrotide prophylaxis were protected from VOD. This result is even more surprising considering their high risks of developing VOD. All the patients were affected by thalassemia, which is a strong risk factor for VOD, probably because of the pre-existing liver damage caused by iron overload. An association of hepatic VOD with iron overload has been suggested in several reports. [295] Also, ***Riba and colleagues (1997)*** stated that hepatitis-C infection is associated with an increased risk of VOD and GVHD of liver. The reported incidence of VOD was 14% among those with chronic hepatitis C infection while it was only 8% in transplant recipients who were negative for hepatitis C. The chronic inflammation in hepatitis C-infected liver causes endothelial changes in the hepatic sinusoids and this may predispose the patients to VOD during bone marrow transplant. [296]

In contrast to previously mentioned studies, ***Carlos et al (2009),*** performed a case-control study of the outcomes of 31 patients who were hepatitis-C virus seropositive at the time of allogeneic HSCT and they did not observe any cases of VOD in the anti-HCV-positive patients. [297]

Cheuk et al (2007), confirmed that thalassemia major patients with a pre-existing hepatic damage due to transfusional iron overload and viral hepatitis have a 30% incidence of VOD.[288] Moreover, it is well known that fibrosis progression correlates significantly to the iron concentration both in HCV positive and negative thalassemic patients. [299] Although significant liver dysfunction during or immediately post-transplant is uncommon, bone marrow transplantation recipients with chronic hepatitis C infection have a significantly worse long-term outcome. The estimated incidence of cirrhosis at twenty years post bone marrow transplantation is 24% in those with chronic hepatitis C. [300] Moreover, there is evidence that the annual fibrosis progression rate is significantly higher in post-transplantation patients than those chronic hepatitis C patients without transplantation . [301] In fact, chronic hepatitis C infection ranked the third as a cause of late mortality, after infections and GVHD in post-transplant patients. Once they developed cirrhosis, their survival outcomes are markedly compromised. [300]

The post transplant data published from two British Centers (Westminster and Manchester) in thalassemic patients show 71.0% overall survival. [302] Similar data recently published from Bismillah Taqee Blood Disease Centre Karachi, Pakistan, also showed a 66.6% post-transplant survival in B-Thalassemia patient . [303] These data are comparable to the results of the 44 patients included in our study, where the overall survival of the whole group of patients was 86.4 % while thalassemia-free survival was 77%.

Previously published data from this Pesaro Centre, Italy shows 97% OS with 94% event free survival in Class I patients and overall survival in Class II patients was 89% with 85% disease free survival. Whereas in class III OS was 76% with 70% DFS (364,365). However the recently published 14 years data from the same center indicates 91% thalassemia-free survival in class I (n=124) and 84% thalassemia-free survival in class II (n=297). Overall survival including all groups represents 73% thalassemia-free survival. These data are comparable to the results of patients included in our study where thalassemia-free survival in class II (n= 36) was 83% and for class III (n=8) was 56% taking into consideration that no patient with risk class I was included in our study.

The OS rate at 5 years was found to be significantly better in patients with ferritin levels < 1000 ng/ml as reported in a retrospective cohort of 114 AML and MDS patients.[304] ***Tanaka et al (2012)*** evaluated the outcome of 47 patients with acute leukemia or MDS who underwent reduced intensity HSCT. High ferritin level, defined as >1000 ng/ml, was associated with worse 2-year OS on multivariate analysis. [305] Another study by the same group demonstrated the adverse impact of elevated ferritin levels on 5 year OS in a cohort of 143 patients with acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) who received allo–HSCT with myeloablative and nonmyeloablative conditioning regimens. [306] In another study, transfusion dependency, predicted by serum ferritin levels, was found to be independently associated with reduced OS and increased NRM in a retrospective cohort of 357 MDS patients undergoing allo–HSCT . [307] The transplant iron score which included serum ferritin level above 1000 ng/ml was tested in 78 patients who received allo or auto–HSCT. Also, the independent impact of iron overload (IO) on transplant survival was indicated with the most pronounced predictive power of the iron score restricted to allo–HSCT recipients. A high iron score (≥2) was associated with 50% absolute decrease in OS at 1 year. [308] ***Lim et al (2010)*** reported the adverse impact of elevated serum ferritin on OS in 99 MDS patients who underwent reduced intensity HSCT.[309] ***Altes et al (2002)*** demonstrated that serum ferritin levels ≥3000 µg/l and TS ≥100% were associated with a decreased OS and increased TRM, which was attributed to a high infectious mortality. [310] ***Mahindra et al (2008)*** demonstrated a pre–transplant serum ferritin > 685 ng/ml was associated with lower OS and relapse-free survival in 315 patients with Hodgkin and non-Hodgkin lymphoma who received auto–HSCT, whereas the same ferritin level exhibited a higher incidence of relapse and relapse mortality. They identified the baseline ferritin level was best correlated with poor survival and they concluded that elevated iron stores may also increase tumor growth, as tumor cells require more iron for DNA synthesis due to rapid proliferation. [311] The same group confirmed their results in a study of 222 allo–HSCT recipients, in which a serum ferritin level >1910 µg/l was associated with lower OS, lower relapse-free survival and higher NRM rates. [312] Furthermore, they also demonstrated inferior survival rates related to higher rates of TRM and relapse mortality in patients with elevated ferritin levels who received non myeloablative conditioning. [313] ***Sucak et al (2010)*** demonstrated an adverse impact of a pre–transplant serum ferritin level >500 ng/ml on OS and TRM in 250 patients who

received auto- and allo–HSCT, underscoring the prognostic effect of IO in auto transplants.(314)

In a large retrospective study by ***Armand et al (2007)***, an elevated pre–transplant serum ferritin level was significantly associated with lower OS and DFS. This association was particularly restricted to patients with thalassemia and MDS which was particularly attributed to transfusion load. They suggested a possible role of iron chelation therapy in the pre- and post–transplant setting, as they showed an absolute difference of 37% in 5–year OS for patients between the highest and lowest ferritin quartiles. (315)

Our results are comparable with the above mentioned studies, as we found that OS and DFS were 72%, 61% respectively for patients with ferritin level >2000 µg/l, statistically significantly lower than those patients with ferritin level < 2000 µg/l (OS and DFS were 96%, 88% respectively) (p=0.02), demonstrating the impact of pre-transplant ferritin level on the OS and DFS on successful transplants. Regarding the impact of HCV positivity on the OS and DFS of cases included in our study, it was reported that cases with HCV PCR positivity had OS and DFS of 73 % and 64% ,respectively, while those with negative HCV PCR had OS and DFS of 90 % and 82% respectively (p= 0.13). Our results are comparable to the results of the previously mentioned study by ***Carlos et al (2009),*** in which the OS survival post-allogeneic HSCT was significantly inferior in the anti-HCV antibody-positive group. (316)

Ramos and co-workers (2009) report a case-control study of the outcomes of 31 patients who were HCV seropositive at the time of allogeneic HCT.14 HCV patients were considered candidates for stem cell transplantation only if they had no significant evidence of hepatic dysfunction. Matched controls (n = 31) were seronegative for viral hepatitis and were paired on age, diagnosis, disease stage, conditioning regimen and donor type. Authors also compared the HCV patients to all seronegative patients (all controls, n = 1,800) transplanted during the same period, to adjust for other confounding effects. The median OS was 3, 18 and 20 months, and The one-year survival was 29%, 56% and 56% in the HCV, matched and all controls groups respectively (p<0.001 in multivariate analysis). Non-relapse mortality at one year was 43%, 24% and 23% respectively (p<0.01). Disease progression and GVHD rates were comparable. Thus, they concluded that HCV seropositivity remained a significant risk factor for non-relapse mortality after allogeneic hematopoietic stem cell transplantation even with normal or minimally abnormal liver function tests. (317)

In a study done by ***Erer B, et al (1994)*** where ninety-eight patients with homozygous-β thalassemia who had undergone allogeneic bone marrow transplantation (BMT) between May 1990 and March 1992 were tested for hepatitis C antibodies (anti-HCV) before and after BMT. Anti-HCV positivity was detected in 50 of the 98 patients (51%) before BMT. Seroconversion was demonstrated in seven of the 40 evaluable seronegative patients. In four cases it was probably due to the different sensitivity of first and second generation ELISA. Of the 46 evaluable seropositive patients 4 had transient and 5 persistent negativity for HCV antibodies after BMT. The high prevalence of anti-HCV positivity in thalassemic patients is related to the continuous requirement for blood transfusions. They found a strong correlation between biochemical and histological evidence of liver damage and anti-HCV positive status in multi-transfused patients. They reported that HCV hepatitis does not influence the outcome of BMT.(318)

SUMMARY

Thalassemia major (TM) originated in Mediterranean, Middle Eastern, and Asian regions, in Egypt it is considered the most common chronic hemolytic anemia (85.1%). However, because of migration, TM now occur globally and represent a growing health problem in many countries. Despite the remarkable improvements in medical therapy for hemoglobinopathies. Hematopoietic stem cell transplantation (HSCT) still remains the only available curative approach.

Thirty years have elapsed since the first HSCT was performed for patients with TM, and allogeneic transplantation in TM is now accepted as standard clinical practice. Recent results show that, with modern transplantation approaches, and careful patient selection, even better results could be obtained. At the same time, however, survival without transplantation of TM patients has improved as the result both of a better understanding of the pathophysiology of iron overload and improvements in the medical therapy of TM; survival into the fourth or fifth decade of life is now possible for well-treated patients.

The aim of this work is to investigate the association between pre-transplant serum ferritin level and hepatitis C viral (HCV) infection on the outcome of fully matched sibling donor (MSD) peripheral blood stem cell transplantation (PBSCT) in patients with B-thalassemia major (BTM).

The study included forty-four patients with β-thalassemia major who received allogeneic PBSC transplantation using BU /CY/ATG conditioning regimen during the period from March 2013 to April 2014 at Nasser Institute Hospital were included in this study with a mean follow up period of 9.8 months.

The age ranged between 2-18 years with mean age of 5.6± 3.587. The male/female ratio was 2.38. Thirty-six Patients were transplanted at risk class 2 and eight patients at risk class 3.6 patients had HCV Ab + and 11 were HVC PCR +,regarding serum ferritin 18 patients had serum ferritin > 2000 ng/dl while patients with ferritin below 2000 ng/dl were 26 cases.

The study results show that OS and DFS was 86.4 % and 77% respectively, in all patients included in this study, while subgroup analysis of patients with serum ferritin below 2000 ng/dl were 96% and 88% for OS and DFS respectively, and for those with levels above 2000 were 72% and 61% for OS and DFS respectively (p value 0.02*) . Cases with HCV PCR positivity had OS and DFS of 73 % and 64% respectively, while those with negative HCV PCR had OS and DFS of 90 % and 82% respectively (p value 0.13). Regarding OS and DFS in different risk class groups, we reported 91% and 83% for OS and DFS, respectively in risk class 2 patient, while for cases with risk class 3 the OS and DFS was 62.5% and 56% respectively (p value 0.04*)

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CONCLUSIONS

In conclusion, this study shows that:

- There is a significant impact of pre-transplant ferritin level on the outcome of SCT of cases with BTM cases transplanted using PBSCs transplantation from a matched sibling donor regarding OS and DFS.
- As regarding the impact of HCV status on the OS and DFS it shows no significant impact on both OS and DFS.
- There was an increased incidence of VOD in the HCV positive group.
- There is a significant impact of risk class status on the OS and DFS as both were higher in the low risk group.

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