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Apoptosis in the CD34$^+$ Cells and Glycophorine A$^+$ Cells in Thalassemic Patients

ABDEL-RAHMAN EL-SAIED, M.D.[1]; DOUAA SAYED, M.D.[2]; MEDHAT ESMAIL, Ph.D.[1]; NADIA ABDEL-WAHAB, M.D.[3]; ALZAHRAA ELSAYED, M.D.[4]; SHERIF HELMY, M.D.[3] and AHMAD ALLAM, M.Sc.[2]

The Departments of Clinical Pathology[1], Faculty of Medicine, Sohag University; Oncological Clinical Pathology[2], South Egypt Cancer Institute, Assuit University; Clinical Pathology[3], Faculty of Medicine, Assiut Univesity and Pediatric[4], Faculty of Medicine, Sohag University.

ABSTRACT

ß-thalassemia major is characterized by ineffective erythropoiesis leading to severe anemia and extensive erythroid expansion. The ineffective erythropoiesis is in part due to accelerated apoptosis of the thalassemic erythroid precursors. In a trial to identify the stage of differentiation at which apoptosis occurs, we have investigated the expression and function of Fas, Bcl_2 and Bcl_{XL} on CD34$^+$ cells and glycophorin A$^+$ cells by flow cytometry and serum and BM plasma levels of tumor necrosis factor-alpha (TNF-α) were estimated by ELISA in 15 patients with ß-thalassemia major compared to 10 healthy controls. There was a decrease in the percentage of CD34$^+$ cells, with increased apoptosis in these cells, but not in glycophorin A$^+$ cells in patients. Upregulation of Fas expression occurred in CD34$^+$ cells. However, that did not explain molecular basis for apoptosis in ß-thalassemia patients. There was an increase in the level of TNF-α in patients than controls in both BM plasma (p<0.001) and serum (p=0.037). This suggested that TNF-α may have significant role in Fas upregulation on CD34$^+$ cells. Changes in expression of apoptotic antagonists, suggested that Bcl_{XL} may not play a significant role in regulation of apoptosis in progenitor cells in ß-thalassemia patients also. The present results suggest that apoptosis in ß-thalassemia major is more in the early erythroid precursors than in the more mature cells. An alternative mechanism for apoptosis other than the Fas-induced one is assumed.

Key Words: *Apoptosis – CD34 – Glycophorine A – ß-thalassemia – TNF-α.*

INTRODUCTION

ß-thalassemia is an inherited disorder that arises from mutations in the ß-globin gene that reduces or totally abolish synthesis of ß-globin chain with subsequent development of red cell hypochromia, microcytosis and anemia **[1]**. Several studies have suggested increased apoptosis as a mechanism of ineffective erythropoiesis in the erythroid lineage in the bone marrow (BM) of thalassemia patients **[2-6]**. Despite that many of the molecular abnormalities that led to the disease have been revealed, the underlying mechanisms of increased apoptosis have not yet been clarified **[1]**. There was considerable variation in measurement of apoptosis in each specific form of thalassemia, the variation was at least partially dependent on the extent of erythroid expansion in patient **[2]**. One interpretation of this finding is that under extreme erythropoietic drive leading to greater erythroid expansion, the erythroid precursors are more likely to make errors that turn on apoptotic programs. Morphologic analysis of marrow in severe forms of ß-thalassemia showed a decrease in late orthochromatic normoblasts **[3]**. But in vitro studies have led to the suggestion that apoptosis primarily occurs at the polychromatophilic normoblasts stage **[4]**. Salasaa and Zoumbos **[7]** showed that increased productions of tumor necrosis factor-alpha (TNF-α) in ß-thalassemia, which usually characterize the acute response to infectious agents, have a negative effect on erythropoiesis. Other study showed that addition of TNF-α induced increase fas expression in normal CD34$^+$ cells **[8]**.

Defining the mechanism of increased apoptosis in ß-thalassemia cells is important for understanding the pathophysiology of the disease, as well as for devising new therapeutic strategies.

This work aimed to study the role of Fas and anti-apoptotic proteins (Bcl_2 and Bcl_{XL}) in CD34$^+$ cells and glycophorin A$^+$ cells in b-thalassaemia major in trial to detect the stage at which apoptosis can occur and the possible role of (TNF-α).

PATIENTS AND METHODS

Patients:

This study was carried on 15 children with *ß*-thalassemia major, 11 boys and 4 girls, 10 of them had been splenectomized, their ages ranged from 6 to 16 years with mean of 9.13±2.7. They were referred to the out patient clinic of Pediatrics Department, Sohag University Hospital for follow-up or blood transfusion. It also included ten apparently healthy controls after having an informed written consent in accordance with Sohag University Hospital ethical committee guide lines. All were subjected to thorough history taking, clinical and laboratory evaluation.

Methods:

1- *Collection of samples:*

- Peripheral blood samples were taken for ELISA assay of (TNF-α).
- BM samples (10ml) collected in preservative free heparin (10ul/ml).

2- *Mononuclear cell separation:*

BM samples were centrifuged at 800rpm for 10min. The supernatant was collected for ELISA assay (TNF-α). The BM sediment was diluted 1:1 in phosphate buffered saline (PBS), pH.7.4 (Biosource international, Camarillo, California, USA). Diluted BM was centrifuged at 1500rpm for 25min on Ficoll-Hypaque (Biochrome AG, USA) and the BM mononuclear cells (BMMCs) were washed twice in PBS supplemented with 1% fetal calf serum (FCS) (Sigma) and 0.05% sodium azide (Winlab, UK).

3- *ELISA assays:*

The TNF-α kit is a solid phase enzyme amplified sensitivity immuno assay (EASIA) performed on microtiter plate (Biosourse International, Camarillo, California, USA). The assay is based on an oligoclonal system in which a blend of MoAbs directed against distinct epitopes of TNF-α are used. Human IFN-γ (hIFN-γ) kit is a solid phase sandwich ELISA (Biosourse International, USA).

4- *Flow cytometry studies:*

BMMCs were subjected to tripple staining followed by acquisition on a minimum of 50000 cells and analysis using FACSCaliber flow cytometry (Becton Dickinson) in South Egypt Cancer Institute, Assiut University. Anti CD34$^+$ monoclonal antibody (Clone 581, Pharmingen, BD, USA) was used as a marker for stem cells and early precursors and anti-glycophorin-A monoclonal antibody was used as a marker for late erythroid series. Every one of them was combined with surface death receptor (CD95, clone DX2, Pharmingen BD) and intracytoplasmic anti-apoptotic markers [Bcl_{XL} (clone 7B 2.5, Chemicon International, USA) and Bcl_2 (clone 3d-21100, Pharmingen BD)]. All were combined with fluorescence dye 7-amino actinomycin D (7AAD) (Pharmingen BD) to study the viability of the cells expressing those markers.

BMMCs were incubated with 40µl of a solution containing 2% human gamma globulin (Sigma), 1% FCS in PBS for 10min at 4ºC, 10µl of PE anti-CD34 or anti-glycophorin A antibody were added and incubated for 30min at 4ºC. For the negative control an isotype matched irrelevant mouse IgG conjugate with PE was used. Cells were washed twice in PBS then 5µl of 7-AAD was added, mixed well and incubated for 10 min at 4oc protected from light then washed twice and resuspended in 50µl of PBS. 10µl of FITC anti-Fas were added and incubated for 20min at 4ºC.

For intracytoplasmic staining, 250µl of cytofix/cytoperm solution was added and incubated for 20min at 4ºC then washed twice in 1ml and resuspended in 50µl of perm/wash buffer then 5µl of FITC Bcl_{XL}, were added in one tube and 10µl of FITC Bcl_2, in another tube, both were incubated for 20min at 4ºC, washed twice and resuspended in 50µl PBS then 0.5ml of parafomaldhyde (Sigma) was added and kept at 4ºC protected from light until analyzed by FCM.

BMMCs were defined on a scatter diagram combining forward and right angle light scatter (FSC & SSC respectively). Region (R1) was drawing to exclude debris. BMMCs were expressed on a scatter diagram combining SSC with antiglycophorin A fluorescence and a region (R2) was drawn around positive population for antiglycophorin A. To quantify viability of

glycophorin A positive cells within R2, region were drawn satisfying 7-AAD-negative (viable), dim (apoptotic) and bright (dead). Glycophorin A positive cells within R1 and R2 were further expressed on a histogram of anti CD95, anti-Bcl_2 and anti-Bcl_{XL} fluorescence. BMMCs were expressed also on a scatter diagram combining SSC with CD34, a region (R8) was drawn around a clear cut population, having low SSC and high CD34 fluorescence. Analysis for $CD34^+$ cells were done as for Glycophorin A^+ one (Fig. 1).

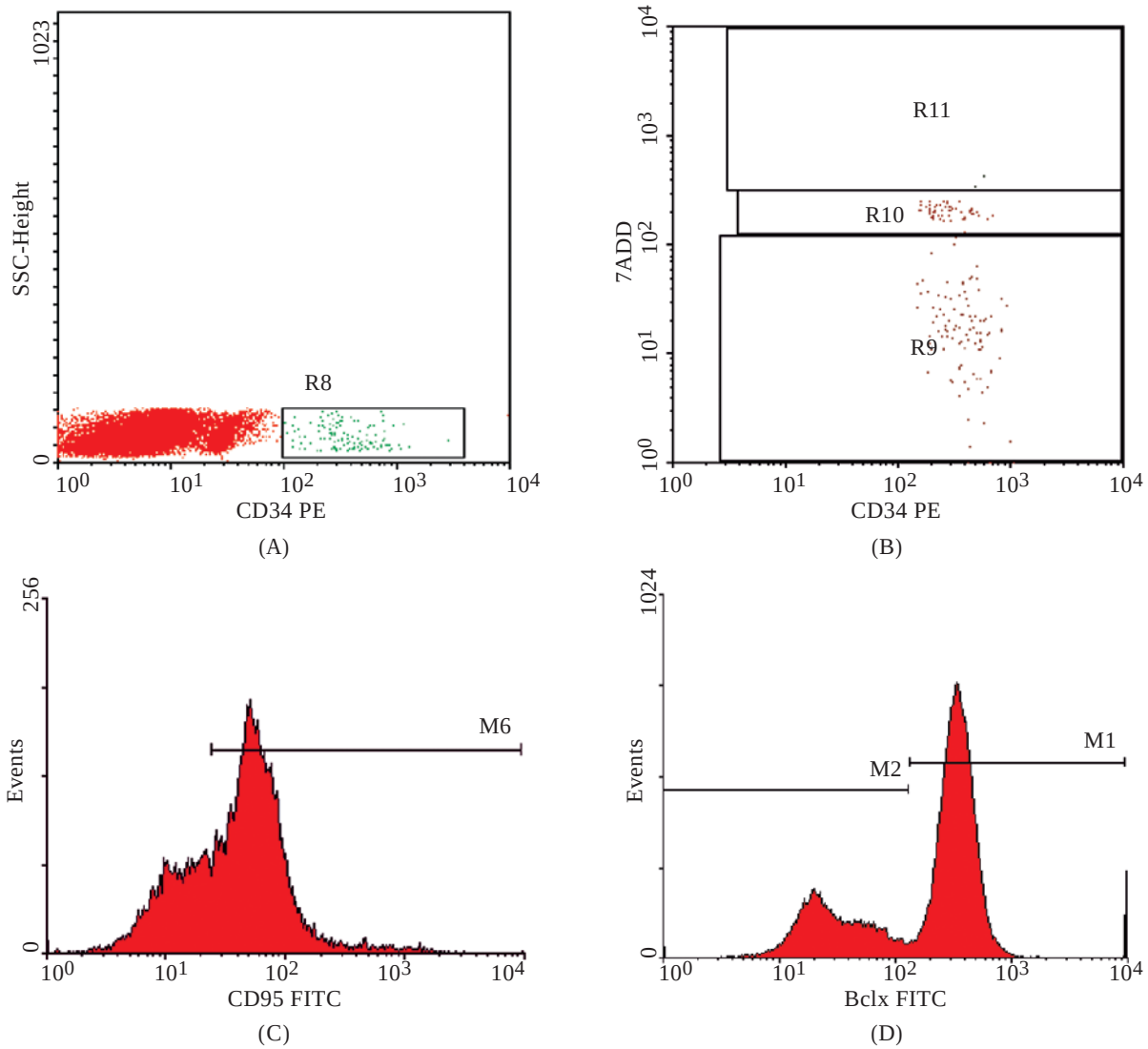

Fig. (1): (A) Dot plot of SSC versus anti-CD34 showing a distinct population of $CD34^+$ cells (R8). (B) Dot plot of FSC of $CD34^+$ cells versus 7AAD fluorescence, divided into viable cells negative for 7AAD (R9), apoptotic cells dim for 7AAD (R10) and dead cells bright for 7AAD (R11). (C) Histogram of CD95 expression of $CD34^+$. (D) Histogram of Bcl_{XL} expression of $CD34^+$ cells divided by median fluorescence into Bcl_{XL}^{hi} (M1) and Bcl_{XL}^{lo} (M2).

RESULTS

There was a decrease in the percentage of the $CD34^+$ cells in ß-thalassemia patients when compared with normal controls, significantly in viable ones (p=0.06) (Table 1).

$CD95$ expression on $CD34^+$ BMMCs and their viability in both patients and controls:

To investigate whether the decreased in $CD34^+$ percentage may be caused by increased apoptosis, we determined the expression of Fas

antigen on CD34+ cells and their viability (Table 2).

Serum and BM plasma levels of TNF-α:

The detection of the levels of TNF-α in both the serum and BM plasma respectively of both patients and normal controls resulted in that there was an increase in the levels of TNF-α in the serum and BM plasma of patients when compared with controls and this increase was statistically significant in the serum and highly significant in the plasma (Table 3).

There is no significant difference in the level of TNF-α between patients with spleenectomy and those without in both serum and BM plasma (serum, p=0.44; plasma, p=0.24).

Table (1): Percentage of CD34+ BMMCs and their viability in both patients and controls.

	CD34+ %	Viability of CD34+ cells %		
		Viable	Apoptotic	Dead
Patients	0.878±0.14	86.42±3.4	8.479±2.04	3.86±2.2
Controls	1.17±0.12	94.65±0.61	4.7±0.55	0.699±0.44
p value	0.166	0.06	0.154	0.27

Table (2): CD95 expression on CD34+ cells and their viability in patients and controls.

	CD95+ %	% of Viability of CD95+		
		Viable	Apoptotic	Dead
Patients	1.59±0.92	61.69±9.6	27.11±8.0	4.28±2.02
Controls	0.306±0.07	25.37±13.16	60.96±15.95	3.65±3.12
p value	0.272	0.03	0.04	0.8

Table (3): Levels of TNF-α in the serum and BM plasma of patients and controls.

	Serum TNF-α (Pg/ml)	BM plasma TNF-α (Pg/ml)
Patients	229.4±39.9	656.33±44.88
Controls	114.25±20.58	317.75±20.7
p value	0.037	<0.001

Comparative study between the levels of TNF-α in the serum and plasma:

Comparative study between the levels of TNF-α in the serum and plasma of both patients and controls resulted in that the level of TNF-α in the plasma was more than that of the serum of both patients and controls with highly significant statistical value (p<0.001).

Bcl_{XL} and Bcl_2 expression on CD34+ BMMCs and their viability:

Regarding the anti-apoptotic markers, we found that all the CD34+ cells were expressed BclXL and Bcl2, the median of their expression was used to divide CD34+ cells into CD34+/Bcl_{XL}^{hi} and CD34+/Bcl_{XL}^{lo}. The percentage of expression of Bcl_{XL}^{hi} and Bcl_2^{hi} on CD34+ cells and the percentage of apoptosis in them are shown in Tables (4,5).

Glycophorin-A positive BMMCs and their viability:

The glycophorin A+ cells in patients were more viable when compared with their normal controls (patients: 71.47%±2.85; controls: 47.46%±5.98; p=0.005).

Percentage of CD95 expression on glycophorin A+ and their viability:

The glycophorin A+ cells have shown a reduction in the expression of CD95 in patients and the glycophorin A+/CD95+ were less apoptotic in patients (Table 6).

Table (4): Bcl_{XL} expression on $CD34^+$ cells and their viability in patients and controls.

	Bcl_{XL}^{hi} %	Viability of Bcl_{XL}^{hi} %			Bcl_{XL}^{1o} %	% of the Viability of Bcl_{XL}^{1o}		
		Viable	Apoptotic	Dead		Viable	Apoptotic	Dead
Patients	14.17±3.07	75.79±5.1	22±4.05	2.37±0.67	82.98±3.8	94.69±0.96	7.12±1.9	0.037±0.008
Controls	12.15±3.47	69.42±6.03	27.69±16.1	2.26±1.01	87.85±3.47	94.99±0.66	9.26±4.66	0.02±0.01
p value	0.67	0.365	0.39	0.92	0.38	0.82	0.63	0.25

Table (5): Bcl-2 expression on $CD34^+$ cells and their viability in patients and controls.

	Bcl_2^{hi} %	Viability of Bcl-2^{hi} %			Bcl_2^{1o} %	% of the Viability of Bcl-2^{1o}		
		Viable	Apoptotic	Dead		Viable	Apoptotic	Dead
Patients	8.25±1.82	78.58±7.4	10.43±3.02	10.13±6.9	96.16±0.84	96.16±0.84	8.89±0.84	6.8±0.017
Controls	1.56±0.56	55.75±11.0	33.08±12.6	9.26±4.92	97.45±0.74	97.08±0.47	2.9±0.41	0.025±0.007
p value	0.008	0.087	0.049	0.93	0.298	0.42	0.33	0.064

Table (6): Percentage of $CD95^+$/glycophorin-A^+ and their viability in patients and controls.

	$CD95^+$ %	Viability of $CD95^+$ %		
		Viable	Apoptotic	Dead
Patients	10.15±1.8	77.79±2.1	17.42±1.96	4.02±0.73
Controls	26.43±3.95	33.56±4.89	51.35±4.5	13.42±1.09
p value	<0.001	<0.001	<0.001	<0.001

Bcl_{XL} and Bcl_2 expression in glycophorin A^+ cells and their viability:

As $CD34^+$ cells, all glycophorin A^+ cells were expressed Bcl_{XL} and Bcl_2, their expression was divided into high and low as described previous. The percentage of Bcl_{XL}^{hi} and Bcl_2^{hi} expression on glycophorin A^+ BMMCs and the percentage of apoptotic cells are shown in Tables (7,8).

Table (7): Percentage of Bcl_{XL} expression of glycophorin A^+ cells and their viability in patients and controls.

	Bcl_{XL}^{hi} %	Viability of Bcl_{XL}^{hi} %			Bcl_{XL}^{1o} %	Viability of Bcl_{XL}^{1o} %		
		Viable	Apoptotic	Dead		Viable	Apoptotic	Dead
Patients	45.39±4.66	65.81±6.04	13.7±1.6	26±4.99	53.21±4.7	87.15±1.7	11.68±1.5	0.16±0.17
Controls	52.7±4.7	34.8±8.05	20.6±2.31	49.37±6.25	47.06±4.7	92.08±0.94	6.36±0.79	0.035±0.027
p value	0.229	0.005	0.02	0.008	0.389	0.04	0.014	0.035

Table (8): Percentage of Bcl_2 expression on glycophorin A^+ BMMCs and their viability in patients and controls.

	Bcl_2^{hi} %	Viability of Bcl_2^{hi} %			Bcl_2^{1o} %	Viability of Bcl_2^{1o} %		
		Viable	Apoptotic	Dead		Viable	Apoptotic	Dead
Patients	9.35±1.26	3.0±0.75	19.5±4.02	71±5.36	90.36±1.3	92.73±1.0	6.67±0.91	0.21±0.07
Controls	19.14±2.3	1.17±0.46	19.43±6.6	78.34±6.7	81.85±6.79	96.25±0.46	3.21±0.5	0.04±0.008
p value	0.001	0.08	0.98	0.45	0.003	0.012	0.009	0.066

Correlation studies:

There was highly significant correlation between apoptotic cells and age of the patients (duration of the disease) Fig. (2). However, there was no relation between apoptotic cells and sex, splenectomy, anemia and Hb F.

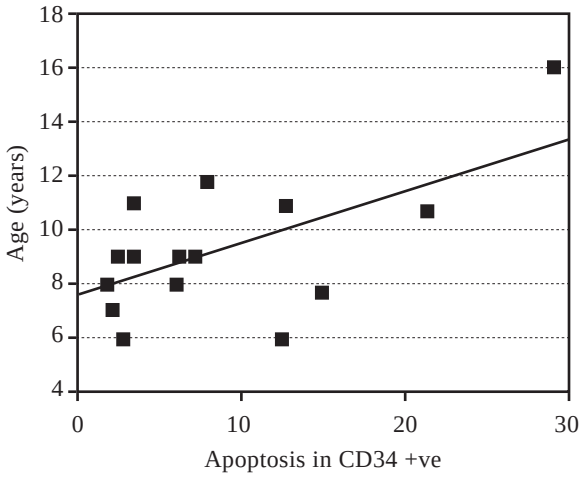

Fig. (2): Correlation between apoptosis in CD34$^+$ cells and age (r=0.633, p=0.01).

There is a positive correlation between the expression of CD95$^+$ and both $Bcl_{XL}{}^{hi}$ and $Bcl_2{}^{hi}$ on CD34$^+$ of patients (r=0.53; p=0.04 and r=0.67; p=0.03 respectively) but there is no correlation among controls (r=–0.117, p=0.74 and r=0.46, p>0.08 respectively). With no correlation between the expression of $Bcl_{XL}{}^{hi}$ and $Bcl_2{}^{hi}$ on CD34$^+$ cells of both patients and controls (patients, r=–0.08; p>0.75: Controls, r=–0.063, p=0.86).

As regard to glycophorin A$^+$ cells, there is no correlation between the expression of both CD95$^+$ and $Bcl_{XL}{}^{hi}$ in both patients and their normal controls (patients, r=–0.41; p=0.12: Controls, r=–0.06, p=0.87). There is no correlation between the expression of $Bcl_{XL}{}^{hi}$ and $Bcl_2{}^{hi}$ in both patients and controls (patients, r=–0.11; p=0.69: Controls, r=–0.5, p=0.14). Conversely there is a positive correlation between CD95$^+$ and $Bcl_2{}^{hi}$ in both patients and controls and it was statistically significant (patients, r=0.5; p=0.05: Controls, r=0.77, p=0.009).

There is no correlation between TNF-α in the serum and BM plasma of patients with CD95$^+$, $Bcl_{XL}{}^{hi}$ and $Bcl_2{}^{hi}$ in both CD34$^+$ and glycophorin A$^+$ cells.

DISCUSSION

ß-thalassemia major is characterized by ineffective erythropoiesis leading to severe anemia and extensive erythroid expansion. Pathophysiology of ß-thalassemia has been the subject of several extensive reviews **[1,9]**.

Ferokinetic studies showed that 60-80% of erythroid precursors die in the marrow or extra medullary sites. However, study of marrow aspirate does not reveal huge numbers of dead and dying erythroid precursors. Angelucci et al. **[10]** explored this apparent discrepancy with the hypothesis that enhanced phagocytosis of thalassemic erythroid precursors was a likely explanation. They concluded that enhanced apoptosis is certainly responsible for part of the increased phagocytosis of thalassemic erythroid precursors. So the ineffective erythropoiesis is in part due to accelerated apoptosis of the thalassemic erythroid precursors; however, the extent of apoptosis is surprisingly variable **[3]**.

In this work, there was a decrease in the percentage of CD34$^+$ cells in ß-thalassemia patients when compared with the normal controls, in which an increase in apoptosis of BM progenitor cells has been observed. This apoptosis was significantly correlated with age of patients which influence the duration of the disease. There are no similar previous studies to compare except the finding of Mathias et al. **[4]** who reported that erythroid cultures initiated from ß-thalassemia major BM CD34$^+$ cells expanded 10 to 20 fold less than from normal BM. They explained that there were less viable cells. But we can suggest that may be due to apoptosis in CD34$^+$ subsets. The studies of Philpott et al. **[11]**, Killick et al. **[12]** and Ismail et al. **[13]** reported that the percentage of CD34$^+$ cells was significantly reduced compared with controls. However, those studies were done on patients with aplastic anemia in which an increase in apoptosis of BM progenitor cells has been observed.

The patients' CD34$^+$ cells upregulate fas antigen but this upregulation was statistically insignificant compared with controls. As Krammer's study **[14]** showed that the expression and function of fas in hematopoietic cells directly correlate with the rate of proliferation. This suggests that this upregulation has relation to increased erythroid expansion in patients with

ß-thalassemia and may be influenced by the haematopoietic microenvironment **[15]**.

In this study we found that there is an increase in the level of TNF-α in patients than the controls in both BM plasma and serum and this increase was statistically significant in the serum and highly significant in the BM plasma. This increase may explain the up regulation of fas on $CD34^+$ cells in this study, especially that this increase was more in the plasma than serum ($p<0.001$), although no correlation was observed. Shetty et al., study **[8]** concluded that the addition of TNF-α induced increase fas expression in normal $CD34^+$ cells.

Also in Meliconi et al. **[16]**; Lombardi et al. **[17]** and Chuncharunce et al. **[18]** studies ß-thalassemia patients had high serum TNF-α concentration compared with controls. These were not related also to sex, age (duration of disease), number of blood transfusion or splenectomy.

Wanachiwanawin et al. **[19]** found that serum level of TNF-α above the normal range in 13% of ß-thalassemia major and suggested that elevated serum level could contribute to complications of the disease, such as cachexia and thromboembolic phenomena.

Also Kyriakou et al. **[20]** found that the level of TNF-α in the serum of patients with ß-thalassemia were higher than ß-thalassaemic carrier and normal controls and suggested that these may be related to the vascular complications in these patients and might be useful markers for the follow-up of the vascular disease. This confirmed by Butthep et al., study **[21]** in which increase number of circulating endothelial cells (CEC) was demonstrated in a and ß-thalassemic patients, and ß-thalassemia (both splenectomized and non splenectomized) had higher number of CECs than a thalassemia.

$CD34^+/CD95^+$ were more apoptotic than $CD34^+/CD95^+$ in patients and controls. This might explain molecular basis for exacerbation of apoptosis in marrow cells of ß-thalassemia major. However, we have noticed that $CD34^+/Fas^-$ cells showed apoptosis, and the comparison of apoptosis in $CD34^+/CD95^+$ between patients and controls showed increase of percentage of apoptotic cells in controls than in patients, suggesting an alternative mechanism for apoptosis in addition to fas induced one.

We investigated whether changes in expression of the apoptotic antagonist Bcl_{XL} and Bcl_2 could have a role in the increased apoptosis of stem cells in ß-thalassemia patients and normal controls. We found that all $CD34^+$ cells expressed Bcl_{XL} and Bcl_2 in bimodal distribution of high and low expression in both patients and controls.

Both Bcl_2 and Bcl_{XL} function as suppressors of programmed cell death on growth factor withdrawal in cytokine-dependent hematopoietic cells lines. Peters et al. **[22]** analyzed the expression of Bcl_2 and its related proteins in hematopoietic precursors and progenitors from adult mobilized peripheral blood, cord blood and adult BM. They showed that variable levels of Bcl_2 were expressed by the most primitive hematopoietic cells as well as the most mature ones.

The expression of $Bcl_2{}^{lo}$ & $Bcl_2{}^{hi}$ and $Bcl_{XL}{}^{lo}$ & $Bcl_{XL}{}^{hi}$ within the $CD34^+$ population is unlikely to be related to the permeabilization technique that we adapted for the measurement of intracellular proteins. The fixative and permeabilizing agents we used permeabilizes cells without altering their scatter features and their membrane or cytoplasmic staining.

Our results agree with the result of Peters et al. **[22]** and Ismail et al. **[13]**, but contrast with the result of Park et al. **[23]** who reported that most primitive BM haemopoietic cells ($CD34^+/Lin^-/CD38^-$) express Bcl_{XL} but not Bcl_2. However, it should be noted that the later used immunocytochemistry to detect Bcl_{XL} & Bcl_2 expression in contrast to the flow cytometry used in this study and those of Peters et al. **[22]** and Ismail et al. **[13]**.

Maurillo et al. **[24]** study suggested that in an early phase progenitor cells are protected from apoptosis mostly by Bcl_X subsequently by Bcl_2 which in turn, is lost with differentiation.

The percentage of high expression was higher in patients than controls, it was significant in Bcl_2 but insignificant in Bcl_{XL}. Although there was a positive correlation between the expression of $CD95^+$ and both $Bcl_{XL}{}^{hi}$ and Bcl_2 on $CD34^+$ in patients, with no correlation

between the expression of Bcl_{XL}^{hi} and $Bcl2^{hi}$. These results suggest that upregulation of fas affects the upregulation of antiapoptotic members of Bcl_2 family. Unfortunately, we could not find similar previous studies to compare with except that of Maurillo et al. **[24]** which done on normal hematopoietic precursors not thalassemic patients. They observed that Fas and Bcl_2 in early progenitor cells are not inversely balanced. They suggested that these proteins expressed independently of one another, as we found in our controls. That may be as a consequence of positive and negative stimuli induced by cytokines and adhesion molecules.

This result implies that the regulation of fas antigen in the CD34$^+$ cells in ß-thalassemia patients may be influenced more by antiapoptotic effect of Bcl_2 than Bcl_{XL}. As well as previous studies have shown that fas-induced apoptosis may or may not be inhibited by expression of Bcl_{XL} or Bcl_2 depending on the cell type **[13,25-29]**.

The glycophorin A$^+$ cells from the patients were less apoptotic in this study than the controls but without statistical significance and this suggests that apoptosis in ß-thalassemia major is more in the early erythroid precursors than in the more mature cells, where the percentage of apoptosis in glycophorin A$^+$/CD95$^+$ were more in controls than patients. This agrees with the result of De Maria **[30]** who reported that Fas is rapidly upregulated in early erythroblasts and expressed at high levels through terminal maturation. However, Fas cross linking was effective only in the less mature erythroblasts, particularly at basophilic level, where it induced apoptosis antagonized by high levels of erythropoietin. Also, in the most recent study for Liu et al. **[31]** which was done in the mouse spleen, an erythropoietic reserve organ, early erythroblasts were present at lower frequencies and were undergoing higher rates of apoptosis than equivalent cells in BM. A high proportion of splenic early erythroblasts coexpressed the death receptor Fas, and FasL. Fas-positive early erythroblasts were significantly more likely to coexpress annexin V than equivalent, Fas-negative cells, suggesting that Fas mediates early erythroblast apoptosis in vivo. They examined several mouse models of erythropoietic stress, including ß-thalassemia, but didn't compare them with normal.

On comparison of apoptosis in glycophorin A$^+$/Bcl_{XL}^{hi} and $Bcl2^{hi}$ between patients and controls. We have shown that Bcl_{XL} and Bcl_2 expression don't influence the effect of Fas, as we found that although there was a decrease in the percentage of apoptosis in the glycophorin A$^+$/Bcl_{XL}^{hi} cells in the patients than the controls with statistical significance. There was no correlation between CD95 and Bcl_{XL}^{hi}, and the percentage of apoptosis in glycophorin A$^+$/Bcl_2^{hi} cells was similar in patients and controls although there was correlation between CD95$^+$ and Bcl_2^{hi}.

In conclusion: Apoptosis occurs in CD34$^+$ more than mature cells. Although, Fas antigen is upregulated on CD34$^+$ cells, its role in the pathophysiology of apoptosis in ß-thalassemia is still unclear, indicating that many factors positively or negatively interfere with the Fas-mediated pathway. TNF-α may have role in that and may influence the susceptibility to the precursor cells to Fas mediated killing. Furthermore Bcl_{XL} may not play a significant role in regulation of apoptosis in hematopoietic progenitor cells in ß-thalassemia.

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