
Splenectomy may not influence glutathione metabolism in children with beta-thalassaemia major

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Turk J Haematol 2005;22(1): 25-30

Received: 06.10.2004 **Accepted:** 07.01.2005

ABSTRACT

The present study was undertaken to evaluate glutathione and its related enzymes in beta-thalassaemia major and to elucidate the effect of splenectomy on glutathione metabolism. The study includes three groups, those are: healthy individuals ($n= 35$) taken as control, a group of beta-thalassaemia major children with intact spleen ($n= 29$) and a group of splenectomized beta-thalassaemia major children ($n= 11$). Levels of reduced glutathione, glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenases in erythrocytes were estimated in all groups. Levels of glutathione $p < 0.001$ and glutathione reductase $p < 0.05$ in thalassaemic groups significantly increased, whereas glutathione peroxidase levels $p < 0.005$ were significantly decreased when compared to control. Glutathione and enzymes levels followed the same pattern in splenectomized group when compared to nonsplenectomized one; however the difference between the various parameters was not significant. Glutathione and its redox enzymes were severely disturbed in thalassaemic patients. Splenectomy, however appears to have no role in influencing glutathione metabolism in beta-thalassaemia major children.

Key Words: Thalassaemia major, Splenectomy, Glutathione peroxidase, Glutathione reductase, Glucose 6-phosphate dehydrogenase.

ÖZET

Beta-talasemi majörlü hastalarda splenektomi

Bu çalışma, glutatyon ve ilgili enzimleri beta-talasemi majörde değerlendirmek ve splenektominin glutatyon metabolizmasına etkisini izlemek amacıyla yapılmıştır. Çalışmada üç grup vardır: Kontrol grubu olarak kullanılan sağlıklı bireyler ($n= 35$), dalakları sağlam olan beta-talasemi majörlü çocuklar ($n= 29$) ve splenektomize beta-talasemili çocuklar ($n= 11$). Tüm grplarda eritositlerde redükte glutatyon, glutatyon peroksidaz, glutatyon redüktaz ve glukoz 6-fosfat dehidrogenaz düzeylerine bakıldı. Talasemili grupta glutatyon ($p < 0.001$) ve glutatyon redüktaz ($p < 0.05$) düzeyleri yüksek bulunurken, glutatyon düzeyleri normal kontrole göre ($p < 0.005$) belirgin derecede düşük bulunmuştur. Splenektomize grup ile splenektomi olmayan grup

karşılaştırıldığında aynı değerler bulunurken, aradaki fark anlamlı değildi. Glutatyon ve redoks enzimleri talsemik grupta belirgin derecede bozuktu. Ancak splenektominin beta-talasemi majörlü hastalarda glutatyon metabolizmasını etkilemediği görüldü.

Anahtar Kelimeler: Talasemi majör, Splenektomi, Glutatyon peroksidaz, Glutatyon redüktaz, Glukoz 6-fosfat dehidrogenaz.

INTRODUCTION

The thalassaemia syndromes exhibit considerable heterogeneity both at the clinical and molecular levels. Beta-thalassaemia major is characterized by the deficient or absence of beta-globin chain synthesis resulting in severe anemia and a diverse clinical profile ranging from a mild disorder to a very severe condition with a transfusion dependent survival^[1]. Splenomegaly is a common feature in most of thalassaemic patients, which is associated with a variety of complications including increasing transfusion requirement and physical discomfort splenectomy is undertaken in thalassaemic patients to help improve their clinical performance. Increased membrane lipid peroxidation in erythrocytes of patients with beta-thalassaemia major has been reported, suggesting that superoxide radicals generated in excess following autoxidation of isolated hemoglobin (Hb) chains is an important contributor to the hemolytic process^[2,3]. Recent literature strongly suggested that lipid peroxidation and blood antioxidant system is severely disrupted in beta-thalassaemia major^[4-6]. Glutathione and its redox enzyme system is an essential element of erythrocyte's antioxidant defense mechanism against free radicals accumulations which is generated by iron overload and other sources in thalassemic patients. Splenectomy may reduce iron overload by reducing the number of blood transfusion however little is shown on its role on lipid peroxidation in beta-thalassaemia^[7]. The current study aims to elucidate the status of glutathione and its redox enzyme system in beta-thalassaemia and to study the effect of sple-

nectomy if any in reducing oxidative stress in thalassaemic patients.

MATERIALS and METHODS

Patients

Forty beta-thalassaemia major confirmed cases attending the Transfusion Unit attached to St. George Hospital's Blood Bank in Mumbai (18 males and 22 females aged 2 to 17 years) were selected for the study. Thalassaemic patients were further divided into two groups: nonsplenectomized group (n= 29) and splenectomized group (n= 11). A third group (n= 35) of healthy control subjects age and sex matched to the thalassaemic group were also included in the study from children visiting the pediatric clinic of J.J. Hospital with a hemoglobin 12.0 g/dL or above and normal blood picture. All participants or their guardians verbally consented to participate in the study.

Methods

A venous blood samples were collected from all patients and controls in 0.1% ethylenediamine tetra acetic acid (EDTA, disodium salt) tubes, experiments were carried out immediately after blood collection. Standard hematological parameters were investigated according to the method recommended by Dacie and Lewis^[8].

Enzymes were studied spectrophotometrically in hemolysate of red blood cells. Erythrocytes were washed three times with cold isotonic saline after every centrifugation and the removal of the buffy coat. Glutathione (GSH) was determined in whole blood following the method of Beutler et al, by using

5,5-dithiobis 2-nitrobenzoic acid (DTNB)^[9]. Glutathione reductase (GSSG-R) was estimated using oxidized glutathione as substrate in presence of nicotinamide adenine dinucleotide phosphate in a reduced form (NADPH), method of Shrier et al^[10]. Glutathione peroxidase (GSH-Px) was measured using the method of Mills and Randall, using hydrogen peroxide as substrate^[11]. Glucose-6-phosphate dehydrogenase (G6PD) assayed by measuring the increase in NADPH concentration at 340 nm, method of Titz^[12].

Results were analyzed statistically, unpaired student's t-test and ANOVA was applied and p value < 0.05 were accepted as significant.

RESULTS

The results of various enzymes and glutathione levels in thalassaemic patients with or without splenectomy compared to the control group are given from Table 1. The levels of

GSH and GSSG-R were significantly increased in thalassaemic patients compared to control group, GSH values were 105.64 ± 12.13 g/dL Hb and 62.60 ± 7.32 g/dL Hb for the thalassaemic and control group respectively $p < 0.001$ whereas GSSG-R values for the respective groups were 5.03 ± 0.814 mol/min/L and 4.61 ± 0.721 mol/min/L $p < 0.05$. The increase in G6PD levels in thalassaemic group was not significant $p > 0.05$ its mean values in the thalassaemic and control group were 15.98 ± 3.61 U/gmHb and 15.18 ± 3.15 U/gmHb respectively. GSH-Px levels in the thalassaemic group significantly decreased when compared to control $p < 0.05$, mean values of 45.27 ± 9.31 U/gmHb/min and 51.06 ± 11.15 U/gmHb/min respectively.

Table 2 depicts the levels of various enzymes and GSH in splenectomized group compared to nonsplenectomized group; there is no significant difference in GSH levels in sple-

Table 1. Levels of GSH and enzymes in healthy controls and thalassemic patients

GSH and enzymes	Healthy subjects (n= 35)	Thalassemic patients (n= 40)	P value
	Mean + SD	Mean + SD	
Reduced glutathione (g/dL Hb)	62.60 ± 7.32	105.64 ± 12.13	$p < 0.001$
Glutathione reductase (mol/min/L)	4.61 ± 0.721	5.03 ± 0.814	$p < 0.05$
Glutathione peroxidase (UgmHb/min)	51.06 ± 11.15	45.27 ± 9.31	$p < 0.05$
Glucose-6-phosphate dehydrogenase (U/gmHb)	15.18 ± 3.15	15.98 ± 3.61	$p > 0.05$

Table 2. GSH and enzymes levels in splenectomised and nonsplenectomized subjects

GSH and enzymes	Splenectomized (n= 11)	Nonsplenectomized (n= 29)	P value
	Mean + SD	Mean + SD	
Reduced glutathione (g/dL Hb)	112.23 ± 16.56	103.78 ± 14.37	$p > 0.01$
Glutathione reductase (mol/min/L)	5.12 ± 0.913	5.00 ± 0.871	$p > 0.05$
Glutathione peroxidase (UgmHb/min)	41.71 ± 12.16	46.27 ± 10.47	$p > 0.05$
Glucose-6-phosphate dehydrogenase (U/gmHb)	17.25 ± 4.17	15.62 ± 4.06	$p > 0.05$

nectomized group, 112.23 ± 16.56 g/dL compared to that in nonsplenectomized group 103.78 ± 14.37 g/dL Hb p> 0.01, GSSG-R also increased in splenectomized group 5.12 ± 0.913 mol/min/L compared to nonsplenectomized group 5.00 ± 0.817 mol/min/L however the difference was not significant p> 0.05, G6PD values were 17.25 ± 4.17 U/gmHb and 15.62 ± 4.06 U/gmHb for the two groups respectively the difference between the two groups were not significant p> 0.05. The decrease in the GSH-Px levels in the splenectomized group compared to nonsplenectomized group was not significant p> 0.05, GSH-Px values were 41.71 ± 12.16 U/gmHb/min and 46.27 ± 10.47 U/gmHb/min for the two groups respectively.

DISCUSSION

Glutathione (GSH) is an abundant tripeptide that protects against oxidative stress and damage in nearly all cells and tissues. GSH is the major intracellular antioxidant and functions by scavenging free radicals, detoxifying lipid peroxides via glutathione peroxidase, and conjugating reactive electrophilic toxicants and carcinogens. In addition, GSH is involved in numerous other cellular pathways including protein and DNA synthesis, DNA repair, and immune surveillance. GSH is oxidized to its disulfide form (GSSG), but is subsequently reduced back to GSH by GSH reductase^[13-15].

Inherited red cell enzymopathies causes several types of hemolytic anaemias including congenital nonspherocytic hemolytic anaemia; drug induced hemolytic anaemia and others^[16]. Embden-Meyerhof and pentose-phosphate pathways erythrocytes enzyme defects in beta-thalassaemia are associated with increased membrane lipid peroxidation^[17,18]. The role of GSH and its redox system in beta-thalassamia major is not clearly understood particularly among those with splenectomy. In this study the GSH redox system was severely disturbed. A significant increase in the levels of GSH and GSSG-R and a significant decrease in GSH-Px levels among thalassaemic subjects were observed, whereas G6PD remained stable in these subjects, the pattern was also noticed in the thalassaemic children with or without splenectomy (Figure 1, 2). Increased GSH levels in thalassaemia major have been reported by Swarup et al, Bapat and Baxi reported a similar findings of increased GSH levels in thalassaemia^[19-21]. Higher levels of GSH in thalassaemic patient may be a protective response to an excessive oxidative destruction of erythrocytes; this is also reflected in the GSSG-R increased levels (p< 0.05) among these subjects. Our findings are in good agreement with the previous study of Baxi and Bapat who reported increased GSSG-R levels in beta-thalassaemia major^[21]. The association of GSH levels with increased GSSG-R levels may be interrelated, having bearing on

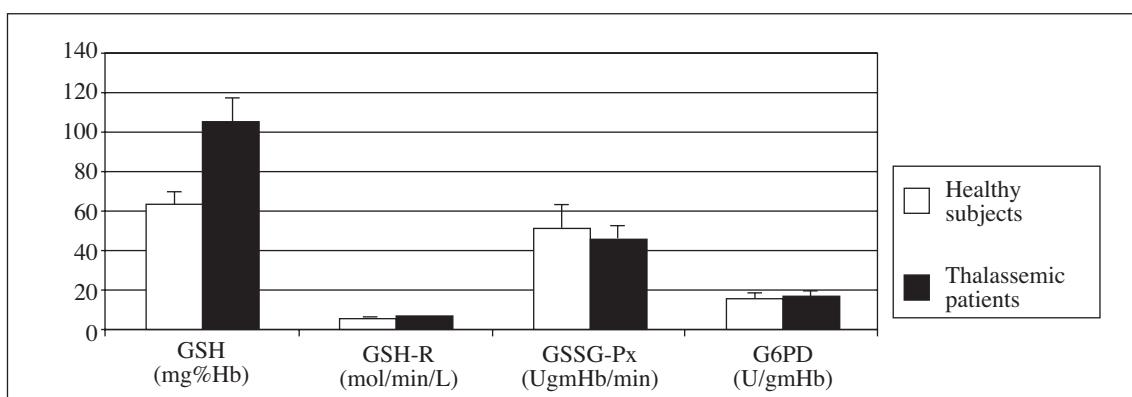


Figure 1. GSH and enzymes levels in healthy and thalassemic subjects.

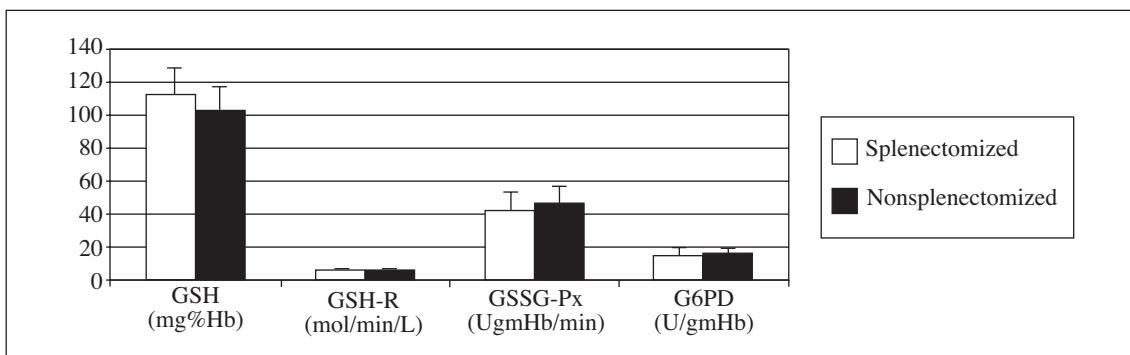


Figure 2. GSH and enzymes levels in splenectomized and nonsplenectomized subjects.

the stability of red cells. On the other hand GSH-Px levels reduced significantly. Our findings are in confirmation with the previous studies of Vaidya et al and Rosan et al, low level of GSH-Px seems to result from the enzyme inhibition or reduced activity due to excessive production of hydrogen peroxide or diminished availability of selenium which could be caused through the use of chelating drugs which are aimed to reduce iron overload. G6PD levels appears unaffected by these alteration its increased levels ($p > 0.05$) in our study among the thalassaemic cohort as well as among splenectomized subjects showed no significant difference^[1,22,23]. The slight increase in G6PD levels could be due to increased number of young red blood cells which are rapidly produced in thalassaemic subjects. G6PD levels in beta-thalassaemia did not seem to be affected by the change in glutathione metabolism and/or its redox system.

Splenectomy was reported to prolong transfusion interval, maintains higher hemoglobin level before each transfusion^[7]. In our study however the difference between the means of various GSH redox system parameters and G6PD in splenectomized subjects and those with intact spleen was not found to be significant. The weakness of the present study is its cross sectional, observational nature as well as the relatively small sample size. Although the current study did not recruit large number of thalassemic indi-

viduals with or without splenectomy, however the study clearly indicate that splenectomy did not show to influence the GSH-redox system. These findings provide compelling evidence that splenectomy may not help in reducing oxidative stress and lipid peroxidation noticed in beta-thalassaemia.

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