Prevalence of glutathione S-transferase gene deletions and their effect on sickle cell patients

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Background: *Glutathione S-transferase gene deletions are known detoxification agents and cause oxidative damage. Due to the different pathophysiology of anemia in thalassemia and sickle cell disease, there are significant differences in the pathophysiology of iron overload and iron-related complications in these disorders.*

Objective: *The aim of this study was to estimate the frequency of the GSTM1 and GSTT1 genotypes in sickle cell disease patients and their effect on iron status.*

Methods: *Forty sickle cell anemia and sixty sickle ß-thalassemia patients and 100 controls were evaluated to determine the frequency of GST gene deletions. Complete blood counts were performed by an automated cell analyzer. Hemoglobin F, hemoglobin A, hemoglobin A2 and hemoglobin S were measured and diagnosis of patients was achieved by high performance liquid chromatography with DNA extraction by the phenol-chloroform method. The GST null genotype was determined using multiplex polymerase chain reaction and serum ferritin was measured using an ELISA kit. Statistical analysis was by EpiInfo and GraphPad statistics software.*

Results: *An increased frequency of the GSTT1 null genotype (p-value = 0.05) was seen in the patients. The mean serum ferritin level was higher in patients with the GST genotypes than in controls; this was statistically significant for all genotypes except GSTM1, however the higher levels of serum ferritin were due to blood transfusions in patients.*

Conclusion: *GST deletions do not play a direct role in iron overload of sickle cell patients.*

Keywords: *Glutathione transferase; Anemia, sickle cell; Hemoglobinopathies; Polymerase chain reaction*

Introduction

An altered glutathione (GSH) metabolism in association with increased oxidative stress has been implicated in the pathogenesis of many diseases.[1] Alterations in GSH concentration have been demonstrated in many pathological conditions including sickle cell disease (SCD).[2] Glutathione S-transferases (GST) are a family of enzymes involved in phase-II detoxification of endogenous and xenobiotic compounds. Polymorphisms in GST genes have been associated with susceptibility to different diseases.[3] Oxidative stress and antioxidant capacity markers have been assessed in sickle cell anemia (SCA) patients treated with hydroxyurea or not; this treatment improves the oxidative stress in this condition.[4] It has also been reported that the production of reactive oxygen species (ROS) in individuals with SCD may alter their overall redox status and cause tissue damage.[5] GSTs constitute multifunctional enzymes that detoxify reactive electrophiles, products of oxidative stress and known or suspected carcinogenic compounds via GSH conjugation.[6] Deficiency in the activity of the GSTM1 and GSTT1 enzymes is caused by the inherited homozygous absence of the GSTM1 and GSTT1 genes, respectively (i.e., GSTM1 null or GSTT1 null genotype). Associations of GSTM1 and/or GSTT1 null genotypes with aplastic anemia and Fanconi anemia have been reported.[7,8] In India there is no literature published on GST deletions in SCD. Thus the aim of this study was to estimate the prevalence of the GSTM1 and GSTT1 null genotypes in sickle cell patients and their effect on iron status.

Methods

The subjects were sickle cell patients attending the outpatient department of the All India Institute of Medical Sciences (AIIMS). This study was carried out in the hematology department of the institute and approved by the Ethics Committee. About 5-mL blood

samples were collected from patients and controls after consent was given. Hemoglobin (Hb F, Hb A, Hb A2 and Hb S) was measured and the diagnosis of patients was performed by high performance liquid chromatography (HPLC Bio-Rad-Variant™ Bio Rad, CA, USA). DNA extraction used the phenol-chloroform method. GSTM1 and GSTT1 null genotypes were determined by multiplex polymerase chain reaction (PCR) according to the published literature.[9] Serum ferritin was measured using a commercial ELISA kit (ORG5FE, ORGenTec, Mainz, Germany). Statistical analysis was performed using the EpiInfo statistics software (Version 3.5.1). Yates' chi-square test was used to assess inter-group significance with a p-value < 0.05 being considering statistically significant. The t-test was applied to compare the means of both groups using the GraphPad software (version 3.06).

Results

The study subjects included 40 homozygous SCA patients (24 male and 16 female with mean age of 11.21 ± 5.36) and 60 sickle ß-thalassemia patients (39 male and 21 female with mean age of 11.7 ± 5.42). A control group was made up of 100 age and gender matched individuals (62 male and 38 female with mean age of 11.21 ± 6.25) to compare the frequency of GST deletions. The frequencies of GSTM1 (5%), GSTT1 (14%) and GSTT1/M1 (9%) were higher in patients than in controls where the GSTM1 frequency was 3%, GSTT1 was 5% and GSTT1/M1 frequency was 2%. The difference between groups for the GSTT1 null genotype was statistically significant (p-value = 0.05) while the differences between groups for the GSTM1 and GSTT1/M1 null genotypes were not (p-value > 0.05). Details of the frequencies of GST deletions are given in Table 1.

Table 1 - Frequency of GST genotype

Genotype	Sickle cell anemia n = 40 (%)	Sickle β-thalassemia n = 60 (%)	Control n = 100 (%)	p-value	OR	95% CI
GSTM1	3 (7.5)	2 (3.33)	3	0.71	0.95	0.11-2.95
GSTT1	6 (15)	8 (13.33)	5	0.05	0.32	0.10-1.01
GSTM1/GSTT1	2 (5)	7 (11.66)	2	0.06	0.21	0.03-1.06
Normal	29 (72.5)	43 (71.66)	90	0.002	3.50	1.51-8.30

OR: Odds ratio; 95% CI: 95% confidence interval

Serum ferritin levels were different between patients and controls with the GST null genotypes. The mean serum ferritin levels in patients with the GSTT1 (190.3 ± 10.6 µg/L) and GSTM1/GSTT1 (181.2 ± 4.49 µg/L) genotypes were higher than in controls (GSTT1 genotype = 156.6 ± 12.7 µg/L and GSTM1/GSTT1 genotype = 165.3 ± 5.4 µg/L). The serum ferritin levels of patients with GST genotypes were statistically higher (p-value < 0.05) except for the GSTM1 genotype (p-value = 0.1426). Details of the serum ferritin levels with GST deletions are given in Table 2.

Table 2 - Serum ferritin level with GST genotypes in sickle patients and controls

Genotype	Serum ferritin (µg/L) Mean ± SD		p-value
	SCD patients	Controls	
GSTM1	172.07 ± 1.02	158.8 ± 18.6	0.1426
GSTT1	190.30 ± 10.60	156.6 ± 12.7	0.0001
GSTM1/GSTT1	181.20 ± 4.49	165.3 ± 5.4	0.0001
Normal	167.65 ± 12.02	148.9 ± 21.6	0.0001

SD: Standard deviation

Discussion

Although 48.8% of sickle cell patients were transfusion dependent, the serum ferritin was within the normal range and there was no sign of iron overload. These observations show that the differences in serum ferritin between patients and controls were due to blood transfusions and that GST deletions did not play a direct role. Blood transfusion unit/year was similar in patients with the GST genotype and those without (7.1 ± 2.6 and 7.4 ± 2.3 units/year). Age and gender were not associated with GST deletions. The iron metabolism in SCD is different to thalassemia. SCD is an inherited disorder of hemoglobin synthesis characterized by life-long hemolytic anemia, increased erythropoiesis and a chronic inflammatory state with endothelial activation and enhanced red cell and leukocyte adhesion.[10,11] There is no evidence of iron overload in non-transfused SCD patients and iron deficiency may even develop, possibly related to intravascular hemolysis (which constitutes about a third of the hemolysis in SCD) and the resulting excessive urinary losses of iron.[12] Due to the different physiopathology of anemia in thalassemia and SCD and the different indications for the use of transfusions in thalassemia major and intermedia, and in SCA, there are significant differences in the physiopathology of iron overload and iron-related complications in these disorders. Recent data indicate that, despite transfused sickle cell patients exhibiting heavy total body iron burdens, they are monitored on a less frequent basis for iron-related organ damage compared to thalassemia patients.[13] In this study the GSTT1 null genotype was significantly higher amongst the patients (p-value = 0.05). It has been reported that the production of ROS in SCD individuals may alter their overall redox status and cause tissue damage, however there is no direct evidence that GST deletions in sickle cell patients cause iron overload.[5] Our sickle cell patients were transfusion dependent, but their serum ferritin was within the normal range and there was no evidence of iron overload. GST deletions and their effect are well documented in other diseases as, GST gene deletions with iron overload have been described in HbE/ß thalassemia patients.[14] GSTM1 enzyme variations are associated with beta-thalassemia major and with malaria complications.[3,15] In our study, age and gender were not associated with GST deletions; another study also reported no correlation between age and genotype of

SCD patients and therapy of their disease appeared to play no role in their oxidative status.[5] In this study, the SCD patients had a higher mean frequency of GST deletions compared to controls while serum ferritin levels were within normal ranges. Transfusions of blood to patients with and without the GST genotype were similar and thus the requirement of blood transfusion is not dependent on GST deletions.

Conclusion

The serum ferritin level was slightly higher in patients with the GST genotype due to blood transfusions where serum ferritin levels were low in controls with the GST genotype. Thus we conclude the GST deletion does not play a direct role in iron overload in SCD patients.

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