EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 11
88-90
Year: 2017

DETECTION OF GLUTATHINE S-TRANSFRASE M1 AND GLUTATHINE S-TRANSFRASE T1 NULL POLYMORPHISM AMONG SUDANESE PATIENT WITH SICKLE CELL ANAEMIA

Abeer Abden Ahmed Mohamed and *Ibrahim K. Ibrahim

Department of Haematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.

***Corresponding Author: Dr. Ibrahim K. Ibrahim**
Department of Haematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.

Article Received on 05/09/2017 **Article Revised on 25/09/2017** **Article Accepted on 15/10/2017**

ABSTRACT

Background: Glutathione S-transferase (GST) enzymes that play a key role in detoxification of activated carcinogens are shown to be one of the potential modifiers of individualized risk for several cancer types. **Objective:** This purpose of this study was to investigate the frequency of the GSTM1 and GSTT1 null polymorphism in sickle cell anemia in Sudan. **Materials and methods:** This study is a case control study, conducted in Khartoum state during the period from May- to July 2017. A total of 40- patients with SCA were enrolled in this study. Two and half milliliter (ml) of EDTA anticoagulated blood was collected from each patient; DNA was extracted by salting out method. The *GSTM1* and *GSTT 1* genotype were determined by multiplex polymerase chain reaction (PCR). **Results:** The rate of GSTM1 null mutation was 77% in children with SCD, while it was 40% in the control group. The frequency of individuals carrying the GSTT1 null mutation was higher in SCD patients (38%) compared to controls (22%). **Conclusion:** The study demonstrated that Egyptian SCD patients have high frequency of GSTT1 and GSTM1 gene polymorphism.

KEYWORD: Sickle Cell Anemia; Glutathione S-transferase Mu 1; null polymorphism.

INTRODUCTION

Sickle cell disease is a group of hemoglobin disorders in which the sickle β-globin gene is inherited. It is inherited as autosomal recessive and accoedingly patient can be either homozygous (sickle cell anemia), heterozygous (sickle cell trait) or doubly heterozygous (e.g HbS/C).[1]

Hb S is originated as a point mutation from the GAG (glutamic acid) codon to the GTG (Valine) codon in the sixth position of the S-globin chain.[2] Hb S is insoluble and forms crystals when exposed to low oxygen tension. Deoxygenated sickle hemoglobin polymerizes into long fibers, each consisting of seven inter wind double strands with cross-linking. The red cells shape change to sickle shape and may block different areas of the microcirculation or large vessels causing infarcts of various organs.[1]

The homozygous state or sickle cell anemia (SS genotype) causes moderate to severe hemolytic anemia. The main clinical disability in those patients arises from repeated episodes of vascular occlusion by sickled red cells resulting in acute crises and eventually in end-organ damage. The clinical severity of sickle cell anemia is extremely variable, this is partly due to the effects of inherited modifying factors, such as interaction with β thalassaemia or increased synthesis of Hb F, and partly to socioeconomic conditions and other factors that influence general health.[3]

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.[4] Glutathione S-transferases (GSTs) constitute multifunctional enzymes that are coded by at least eight distinct loci: α (GSTA); μ (GSTM); θ (GSTT); π (GSTP); σ (GSTS); κ (GSTK); ω (GSTO); and ζ(GSTZ); each one composed of one or more homodimeric or heterodimeric isoforms. These enzymes are involved in the conjugation reactions between glutathione (GSH) and a variety of potentially toxic and carcinogenic compounds. Additionally, GSTs display peroxidase activity and this can protect against oxidative damage.[5,6]

The deficiency in the activity of this enzyme can be derived from the inherited GSTs polymorphisms; GSTT1 (22q11.23), GSTM1 (1q13.3) and GSTP1 (11q13).[7]

Glutathione S-transferase gene deletions are known detoxification agents and cause oxidative damage. In

different studies, variations in null allele frequency have been observed in patients with sickle cell anemia.[8]

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 40 patients with SCA were enrolled in this study. Two and half milliliter (ml) of EDTA anti-coagulated venous blood was collected from each patient; genomic DNA was extracted from EDTA samples by salting out method and The *GSTM1* and *GSTT 1* genotype were determined by multiplex polymerase chain reaction (PCR).

Detection of *GSTM1*and *GSTT1* Null polymorphism

Multiplex polymerase chain reaction was used for the detection of polymorphic deletion of the GSTM1and GSTT1.

The PCR carried out in total volume of 20 µl. It consist of 4 µl of genomic DNA, 1 µl of each primer (Table 1) and 4 µl of "5X FIREPoL" ready to load master mix(Maxime PCR pre mix), 8 µl distilled water.

The amplification conditions were initial denaturation at 94℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 1 min, annealing at 62℃ for 1 min, extension at 72℃ for 1 min, and a final extension step at 72℃ for 10 min.

5µl of PCR product was electrophoresed on 1.5% agarose gel (biotiechnology, korea) containing ethidium bromide, 5 µl of 100 bp DNA ladder (Promega, USA) was applied with each batch of patients' samples. *GSTM1* and *GSTT1* genotype were determined by the presence and absence(null) for each band(215 bp for GSTM1 and 489 bp for GSTT1).

Statistical analysis

All obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) version.

Ethical considerations

This study was approved by scientific research committee, faculty of medical laboratory sciences- Al Neelain University and informed consent was taken from participant parents before sample collection.

Table 1: Oligonucleotide sequence for GSTM1 and GSTT1.

primer	Primer Sequence	Product Size bp	
		GSTM1	GSTM1 Null
GSTM 1 Forward	5'-GAACTCCCTGAAAAGCTAAAGC-3'	215 bp	Absence
GSTM 1 Reverse	5'-GTTGG-GCTCAAATATACGGTGG-3'		
Primer	**Primer Sequence**	**Product Size bp**	
		GSTT1	**GSTT1 Null**
GSTT1 Forward	5-TTCCTTACTGGTCCTCACATCTC-3	489 bp	Absence
GSTT1 Reverse	:5-TCACGGGATCATGGCCAGCA-3		

Data collection and analysis

Patients' data was collected using structured interview questionnaire and analyzed by the statistical package for social sciences (SPSS).

Ethical considerations

This study was approved by scientific research committee, faculty of medical laboratory sciences- Sudan International University and informed consent was taken from each participant before sample collection.

RESULTS AND DISCUSSION

The rate of GSTM1 null mutation was 77% in children with SCD, while it was 40% in the control group. The difference was statistically significant (OR= 5.2, 95% CI= 2.7-9.2, p= 0.001) (Table 2). The frequency of individuals carrying the GSTT1 null mutation was higher in SCD patients (38%) compared to controls (22%) (OR= 2.1, 95% CI= 1.1-4.0, p= 0.0145) This was in agreement with previous study that was performed in Egypt.[8] Therefore both GSTM1 and GSTT1 null genotype may be a risk factor for SCD (Table3).

Table (2): *GSTM1*genotypes in SCA patients and control group.

Group	GSTM1Genotype		Odd Ratio	P.Value	95% CI
	Normal	Null			
Patient	23%	77%	5.2	0.001	2.7-9.2
Control	60%	40%			

Table (3): *GSTT1*genotypes in SCA patients and control group.

Group	GSTT1Genotype		Odd Ratio	P.Value	95% CI
	Normal	Null			
Patient	62%	38%	2.1	0.0145	1.1-4.0
Control	78%	22%			

CONCLUSION

Despite the small sample size, the study demonstrated that Sudanese SCD patients have high frequency of GSTT1 and GSTM1 gene polymorphism.

REFERENCES

1. Hoff brand AV, Cosovsky D, Tuddsnham E. postgraduated haematology 5th Blake well; USA, 2005; 85.
2. Ataga KI, Cappellini MD, Rachmilewitz EA. Beta thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. Br J Haematology, 2007; 139(1): 3–13.
3. Serjeant GR. Sickle Cell Disease. 2nd ed. Oxford University Press Inc., New York, 1992.
4. Kavishe RA, Koenderink JB, McCall MB, et al. Severe plasmodium Falciparum malaria in Cameroon: Associated with the Glutathione S transferase M1 null genotype, Am J Trop Med Hyg, 2006; 75: 827-829.
5. Mo Z, Gao Y, Cao Y, et al. An updating meta-analysis of the GSTM1, GSTT1 and GSTP1 polymorphisms and prostate cancer: a HuGE review. Prostate, 2009; 69: 662-88.
6. Bessa SS, Ali EM, Hamdy SM. The role of glutathione S-transferase M1 and T1 gene polymorphisms and oxidative stress-related parameters in Egyptian patients with essential hypertension. Eur J Intern Med, 2009; 20: 625-30.
7. Sharma V, Kumar B, Saxena R. Glutathione S-transferase gene deletions and their effect on iron status in HbE/beta thalassemia patients. Ann Hematol, 2010; 89: 411-414.
8. M. Aly Rabab, M. Hasaneen, GSTM1 and GSTT1 Polymorphism in Egyptian Sickle Cell Anemia Patients, International Journal of Hematology and Oncology, 2013; 23: 269- 275.