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Association of Glutathione S Transferase M1, T1, P1 (GSTM1, GSTT1, GSTP1) Gene Polymorphisms with Sickle Cell Anaemia Complications in North Kordofan State, Sudan

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Abstract

Sickle cell anaemia (SCA) is an inherited blood disorder that is characterized by chronic haemolysis and episodes of many clinical complications. The number of people living with sickle cell disease globally increased from 5.46 million in 2000 to 7.74 million in 2021. This study aimed to investigate the association of glutathione S transferase M1, T1, P1 (GSTM1, GSTT1, GSTP1) gene polymorphisms with SCA complications. This was a case-control and hospital-based study, conducted in the SCA center, Alkuaiti Hospital, North Kordofan state, Sudan. Following informed consent, one hundred twenty-six participants were recruited to this study, 63 were SCA patients attending Alkuaiti Hospital, and 63 age and gender matched apparently healthy individuals as the control group. The full blood count was done using an automated hematological analyzer, genotyping of the GSTM1 and the GSTT1 polymorphisms were determined using multiplex polymerase chain reaction, while genotyping of the GSTP1 was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Complications data were collected from admission and discharge records. 52.4% (n=33) from the case group were male and 47.6% (n=30) were females. The GSTM1 genotypes in the case group showed that the frequency of the GSTM1Null genotype was 57.1% and the GSTM1 present genotype was 42.9%, the GSTM1 genotypes in the control group showed that the frequency of the GSTM1Null genotype was 52.4% and the GSTM1 present genotype was 47.6%**.** The GSTT1 genotypes in the case group showed that the frequency of the GSTT1 Null genotype was 69.8%, and the GSTT1 present genotype was 30.2%. The GSTT1 genotypes in the control group showed that the frequency of the GSTT1 Null genotype was 49.8%, and the GSTT1 present genotype was 50.2%. The GSTM1 GSTT1 genotypes in the case group showed that the frequency of the GSTM1 GSTT1 Null genotype was 74.6%, and the GSTM1 GSTT1 present genotype was 25.4%. The GSTM1 GSTT1 genotypes in the control group showed that the frequency of the GSTM1 GSTT1 Null genotype was 77.7% and the GSTM1 GSTT1 present genotype was 22.3%**.** The GSTP1 genotype in the case group showed that the wild-type Ile/Ile was (15.9%), the heterozygous Ile/Val was (66.7%), and the homozygous mutant Val/Val was (17.4%). The GSTP1 genotype in the control group showed that the wild-type Ile/Ile was (3.2%), the heterozygous Ile/Val was (84.1%), and the homozygous mutant Val/Val was (12.7%). There were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTM1 genotypes (*P.value* =0.69, 0.47, 0.22) respectively also there were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTT1 genotypes (*P.value* = 0.84, 0.45, 0.48) respectively and the GSTM1 GSTT1 genotypes (*P.value*= 0.53, 0.70, 0.46) respectively. There were no statistically significant differences in the Hb, and TWBCs between the GSTP1 genotypes (*P.value*= 0.15, 0.36) respectively but there was a statistically significant difference in PLTs between the GSTP1 genotypes (*P.value*= 0.07). The study concluded that there were no statistically significant differences in the GSTM1 and the GSTM1 GSTT1 genotypes between the case group and the control group with (*P.value*= 0.36, 0.36) respectively and there were statistically significant differences in the GSTT1 and the GSTP1 genotypes between the case group and the control group with (*P.value* 0.014, 0.02) respectively. The GSTT1 present genotype was significantly associated with acute heart failure (*P.value* 0.02). The GSTP1 (val val) genotype was significantly associated with painful crisis and hepatomegaly as combined complications (*P.value* 0.008). The other GSTT1, other GSTP1, and GSTM1 genotypes revealed no significant associations with SCA complications.

Keywords: Glutathione S transferase, Sickle cell anemia, Sudan.

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INTRODUCTION

Sickle cell disease (SCD) is a set of inherited hemoglobinopathies characterized via way of means of mutations that affect the β-globin chain. The number of people living with sickle cell disease globally increased from 5.46 million in 2000 to 7.74 million in 2021 (Thomson *et al*., 2023). SCD is caused by a variant of the β-globin gene called Hb S. Hb S results from the replacement of glutamic acid by valine in the sixth position of the β-globin chain of hemoglobin (Payne *et al*., 2020). Inherited autosomal recessively, both copies of Hb S are required for disease expression (Ashley-Koch *et al*., 2000). Carrier people have one replica of the sickle variation and one replica of the normal β-globin gene (Hb AS), producing a mixture of sickle hemoglobin and normal hemoglobin. The carrier state for SCD is often referred to as a "sickle cell trait".

Multiple elements decide the clinical manifestations of SCD. Both intracellular and extracellular factors influence sickling, including the types of hemoglobin in the cell and their concentrations, the level of 2,3-diphosphoglycerate (2,3-DPG), and the hydrogen ion concentration (Ellithy *et al.,* 2015).

Some of these factors are determined predominantly by genetic factors; others are environmentally modified. In addition to physiologic changes such as tissue oxygenation and pH (Ellithy *et al.,* 2015).

The complications of SCD are characterized by chronic hemolytic anemia, severe acute and chronic pain, as well as end-organ damage. SCA patients are generally well-adapted until an episode of decompensation (e.g., a severe infection) occurs (Provan *et al*., 2004).

Severe intermittent acute pain is the most common SCD complication (Brousseau *et al*., 2010). Acute pain is basically associated with vaso-occlusion of sickled red blood cells. Chronic pain may be due to sensitization of the central and/or peripheral nervous system and is often diffuse with neuropathic pain features (Ballas & Darbari., 2013, Sharma & Brandow., 2020).

Priapism is an undesired, persistent, and regularly painful erection that may result in erectile dysfunction.

The vaso-oclusive crisis is an acute episode of pain, additionally typically known as sickle cell pain crises, or vaso-occlusive crises (VOCs). Furthermore, the frequency of VOCs, along with acute chest syndrome (ACS), is the most common predictor of death in patients with SCD (Platt *et al*., 1994).

Bone pain affects long bones and, the spine, and is due to the occlusion of small vessels Triggers: infection, dehydration, alcohol, menstruation, cold and temperature changes – often no cause was found (Provan *et al*., 2004).

Dactylitis specifically in children, metacarpals, metatarsals, backs of hands, and feet became swollen and tender due to small vessel occlusion and infarction (Provan *et al*., 2004).

Acute chest syndrome is a common cause of death. Chest wall pain, sometimes with pleurisy, fever, and shortness of breath. Requires prompt and vigorous treatment (Provan *et al*., 2004).

In visceral sequestration sudden trapping of blood in the spleen or liver causes rapid enlargement of the organ and a drop in hematocrit leading to hypovolemic shock (Nayak & Rai., 2014).

Antioxidants are molecules that quench or inhibit the movement of free radicals in addition to preventing cellular damage. Antioxidants exist as enzymatic and non-enzymatic molecules within side the body (Valko *et al*., 2007). Enzymatic antioxidants act through metabolizing free radicals and removing them from cells. Most of those antioxidant enzymes convert reactive oxidative species (ROS) to hydrogen peroxide and, in the end, to water. Non-enzymatic antioxidants act through interfering with or interrupting the chain reactions of free radicals (Yoshihito., 2012).

Pathological events taking place in sickle cell disease elevate free-radicals production through activation of pro-oxidant enzymes, the release of free hemoglobin, and heme induced by hemolysis, which fosters the Fenton reaction, modification of mitochondrial respiratory chain activity and RBCs auto-oxidation (Chirico & Pialoux., 2012, Ware *et al*., 2017, Schieber & Chandel., 2014).

GSTs are a family of enzymes involved in phase-II detoxification of endogenous and xenobiotic compounds. More than 20 human GSTs have been identified and divided into two subfamilies: the cytosolic and the microsomal forms. The cytosolic GSTs are divided into seven classes termed alpha (GSTA1 and 2), mu (GSTM1 through 5), omega (GSTO1), pi (GSTP1), sigma (GSTS1), theta (GSTT1 and 2), and zeta (GSTZ1). Those in the alpha and mu classes can form heterodimers, allowing the formation of a large number of transferases. The cytosolic forms of GST catalyze conjugation, reduction, and isomerization reactions (Shiba *et al*., 2016).

The most studied one is the GST M1 enzyme in the GST M class with its gene located in Chromosome 1p13.3 and the GST T1 enzyme in the

GST T class with its gene located in Chromosome 22q11.23.

The GSTM1 and the GSTT1 genotypes express a null phenotype; thus, individuals who are polymorphic at these loci are predisposed to toxicities by agents that are selective substrates for these GSTs (Shiba *et al*., 2016).

The GSTP1 gene is located on the long arm of chromosome 11 and is characterized by a polymorphism in exon 5, at codon 105. Such genetic change results in the substitution of adenine for guanine (A/G) in the DNA coding sequence, triggering a substitution of isoleucine residue for valine (Ile/Val) at the end product of protein, which is linked to the reduction of enzyme activity (Eduardo *et al*., 2016).

Polymorphisms in GST genes have been associated with susceptibility to different diseases (Sanjay *et al*., 2012).

This study was conducted to detect the effect of the antioxidant enzymes GSTs on SCA complications.

MATERIAL AND METHODS

This was a case-control and hospital-based study, conducted at the SCA center, Alkuaiti Hospital, North Kordofan State, Sudan. Following informed consent, one hundred twenty-six participants were recruited for this study: 63 SCA patients attending Alkuaiti Hospital, aged from 1 to 18 years old, with no blood transfusion in the last three months and with no other genetic disorders. Patients under treatment affecting enzyme activities were excluded, and 63 age and gender matched apparently healthy individuals as the control group. Complications data were collected from admission and discharge records, while complications were noted in a complication book and computerized system for storing data. The recorded complications are dactylitis, acute chest syndrome, painful crisis, stroke, leg ulcer, painful crisis with hepatomegaly, dactylitis with hepatomegaly, splenic sequestration, hepatic sequestration, acute heart failure, hepatosplenomegaly, and heart failure with hepatomegaly. The full blood count was done using an automated hematological analyzer (Sysmex KXN 21 Japan) in Alkuaiti Hospital. Genomic DNA was extracted from blood samples using a DNA extraction kit (Qiagen Blood Extraction kit). Genotyping of the GSTM1 and the GSTT1 polymorphisms was determined by multiplex PCR using a housekeeping β-globin gene as an internal control. The program was as follows 95°C for 2 minutes, followed by 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute with a final extension at 72°C for 5 minutes. The GSTM1 and the GSTT1 genotypes were determined by the presence or absence (null) of bands of 219 and 480 bp, respectively, with an internal control of 268 bp (Figure 1). Primers for the GSTM1 and the GSTT1 are in Table 1.

Table 1: Primers sequences used for the GSTM1, the GSTT1 genotyping

Genes	Primers	Fragment size
GSTM1	F: 5′ GAACTCCCTGAAAAGCTAAAGC 3′ R: 5′ GTTGGGCTCAAATATACGGTGG 3′	219 bp
GSTT1	F: 5′ TTCCTTACTGGTCCTCACATCTC 3′ R: 5′ TCACCGGATCATGGCCAGCA 3′	480 bp
β –globin(control)	F: 5′-CAACTTCATCCACGTTCACC-3′ R: 5′-GAAGAGCCAAGGACAGGTAC-3′	268 bp

The GSTP1 (Ile105Val) polymorphism was determined with a PCR-RFLP.

The program was as follows: initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 60 seconds, and extension at 72°C for 60 seconds. completed by one cycle of a final extension step at 72°C for 7 minutes, the product of PCR is 433 bp band (Figure 4.2). The PCR product was digested with the restriction endonuclease Alw26I enzyme CutSmart NEW ENGLAND Biolabs via incubation at 37°C overnight. The amplified fragment after digestion with Alw26I restriction enzyme can give rise to either fragment at 433 bp which indicates the presence of the wild-type (IIe/IIe), or two fragments at 329 and 104 bp which indicates the presence of the homozygous mutant type (Val/Val), or three fragments at 433 bp, 329 bp, and 104 bp, which indicates the presence of heterozygous mutant type (Ile/Val) (Figure 2). Primers for GSTP1 are in Table 2.

Table 2: Primers sequences used for the GSTP1 genotyping

Gene	Primers	Fragment size
GSTP1	F: 5′-GTA GTT TGC CCA AGG TCA AG-3′ R: 5′-AGC CAC CTG AG G GGT AAG-3′	433 bp

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Data Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 23. In dependent T. test and One-way ANOVA test were used. The correlation test was used between quantitative data and study groups. Allele frequency was estimated by counting Hardy- Weinberg equilibrium. The relations between study groups and genotypes were assessed with Chi-square (X^2). The level of statistical significance was set at less than 0.05.

RESULT

Totally one hundred twenty-six participants were recruited to this study, 50% (n= 63) were sickle cell patients considered as the case group and 50% (n=63) were apparently healthy individuals as the control group. 52.4% (n=33) from the case group were male and 47.6% (n=30) were females; their mean age was 8.8±5 years.

GSTM1 genotypes

The GSTM1 genotypes in the case group showed that the frequency of the GSTM1Null genotype was 57.1%, and the GSTM1 present genotype was 42.9%, the GSTM1 genotypes in the control group showed that the frequency of the GSTM1Null genotype was 52.4%, and the GSTM1 present genotype was 47.6% (Table 3).

The present study showed that there were no statistically significant differences in the GSTM1 genotypes between the case group and the control group (*P.value*= 0.36) (Table 3).

Table 3: GSTM1 genotypes distribution, differences in the GSTM1 genotypes between the case group and the control group

Genotyps	Case	Control	*P.value*
Null M	57.1% (n=36)	52.4%(n=33)	0.36
Present M	42.9%(n=27)	47.6%(n=30)	

GSTT1 genotypes

The GSTT1 genotypes in the case group showed that the frequency of the GSTT1 Null genotype was 69.8%, and the GSTT1 present genotype was 30.2%. The GSTT1 genotypes in the control group showed that the frequency of the GSTT1 Null genotype was 49.8%, and the GSTT1 present genotype was 50.2% (Table 4).

The present study showed that there were statistically significant differences in the GSTT1 genotypes between the case group and the control group (*P.value* 0.014) (Table 4).

Table 4: GSTT1 genotypes distribution, differences in the GSTT1 genotypes between the case group and the control group

Genotypes	Case	Control	*P.value*
Null T	69.8% (n=44)	49.8%(n=31)	0.014
Present T	30.2%(n=19)	50.2%(n=32)	

GSTM1 GSTT1 genotypes and allele frequency

The GSTM1 GSTT1 genotypes in the case group showed that the frequency of the GSTM1 GSTT1 Null genotype was 74.6%, and the GSTM1 GSTT1 present was 25.4%. The GSTM1 GSTT1 genotypes in the control group showed that the frequency of the GSTM1 GSTT1 Null genotype was 77.7%, and the GSTM1 GSTT1 present genotype was 22.3% (Table 5).

The present study showed that there were no statistically significant differences in the GSTM1 GSTT1 genotypes between the case group and the control group with (*P.value*= 0.36) (Table 5).

Table 5: GSTM1 GSTT1 genotypes distribution, differences in the GSTM1 GSTT1 genotypes between the case group and the control group

Genotypes	Case	Control	*P.value*
Null T,M	74.6%(n=47)	77.7%(n=49)	0.36
Present T,M	25.4%(n=16)	22.3%(n=14)	

GSTP1 genotypes and allele frequency

The GSTP1 genotypes in the case group showed that the wild-type Ile/Ile was (15.9%), the heterozygous Ile/Val was (66.7%), and the homozygous mutant Val/Val was (17.4%). The GSTP1 genotypes in the control group showed that the wild-type Ile/Ile was (3.2%), the heterozygous Ile/Val was (84.1%), and the homozygous mutant Val/Val was (12.7%). Ile allele frequency in the case group was 0.49 and in the control group was 0.45 while Val allele frequency in the case group was 0.51 and in the control-group was 0.55 (Table 6).

The present study showed that there were statistically significant differences in the GSTP1 genotypes between the case group and the control group with (*P.value*= 0.02) (Table 6).

Table 6: GSTP1 genotypes distribution, differences in the GSTP1 genotypes between the case group and the control group

Genotypes	Case	Control	*P .value*
Ile/Ile	15.9%(n=10)	3.2% (n=2)	0.2
Ile/Val	66.7% (n=42)	84.1% (n=53)	
Val/Val	17.4% (n=11)	12.7% (n=8)	
Allele	Case	Control	
Ile	0.49	0.45	
Val	0.51	0.55	

There were no statistically significant differences in the Hb, TWBCs and PLTs between the GSTM1 genotypes (*P.value* =0.69, 0.47, 0.22) respectively (Table 7).

Table 7: Association of the Hb, TWBCs and PLTs, and the GSTM1 genotypes

Genotypes	Haematological findings	Mild	Intermediate	Severe anaemia	*P.value*
Null M	Hb	11	23	2	0.69
Present M		7	17	3	
Genotypes		**Increased**	**Normal**	**Decrease**	***P.value***
Null M	TWBCs	32	4	0	0.47
Present M		23	4	0	
Genotypes		**Increased**	**Normal**	**Decrease**	***p.value***
Null M	PLTs	18	18	0	0.22
Present M		17	10	0	

There were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTT1 genotypes (*P.value* = 0.84, 0.45, 0.48) respectively (Table 8).

Table 8: Association of the Hb, TWBCs and PLTs, and the GSTT1 genotypes

Genotypes	Haematological findings	Mild	Intermediate	Severe anaemia	*P.value*
Null T	Hb	12	28	4	0.84
Present T		6	12	1	
Genotypes		**Increased**	**Normal**	**Decrease**	***P.value***
Null T	TWBCs	39	5	0	0.45
Present T		16	3	0	
Genotypes		**Increased**	**Normal**	**Decrease**	***p.value***
Null T	PLTs	25	19	0	0.48
Present T		10	9	0	

There were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTM1 GSTT1 genotypes (*P.value*= 0.53, 0.70, 0.46) respectively (Table 9).

Table 9: Association of the Hb, TWBCs, and PLTs, and the GSTM1 GSTT1 genotypes

Genotypes	Haematological findings	Mild	Intermediate	Severe anaemia	*P.value*
Null T M	Hb	10	19	2	0.53
Present T M		8	21	3	
Genotypes		**Increased**	**Normal**	**Decrease**	***P.value***
Null T M	TWBCs	28	3	0	0.70
Present T M		27	5	0	
Genotypes		**Increased**	**Normal**	**Decrease**	***p.value***
Null T M	PLTs	15	16	0	0.46
Present T M		20	12	0	

There were no statistically significant differences in the Hb and TWBCs between the GSTP1 genotypes (*P.value*= 0.15, 0.36) respectively, but a statistically significant difference in the PLTs between the GSTP1 genotypes (*P.value*= 0.07) (Table 10).

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Table 10: Association of the Hb, TWBCs and PLTs, and the GSTP1 genotypes

Genotypes	Haematological findings	Mild	Intermediate	Severe anaemia	*P.value*
Ile/Ile	Hb	5	4	1	0.15
Ile/Val		20	18	4	
Val/Val		1	9	1	
Genotypes		**Increased**	**Normal**	**Decrease**	***P.value***
Ile/Ile	TWBCs	7	3	0	0.36
Ile/Val		37	5	0	
Val/Val		9	2	0	
Genotypes		**Increased**	**Normal**	**Decrease**	***p.value***
Ile/Ile	PLTs	5	5	0	0.07
Ile/Val		18	24	0	
Val/Val		9	2	0	

Figure 1: Agarose gel electrophoresis for amplified PCR products of the GSTT1 (480 bp), the GSTM1(219 bp), and B-globin (268 bp) fragments

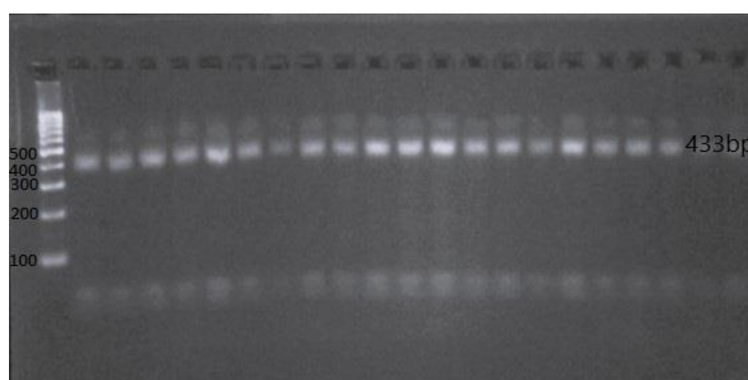

Figure 2: Agarose gel electrophoresis for amplified PCR product of the GSTP1(433 bp)

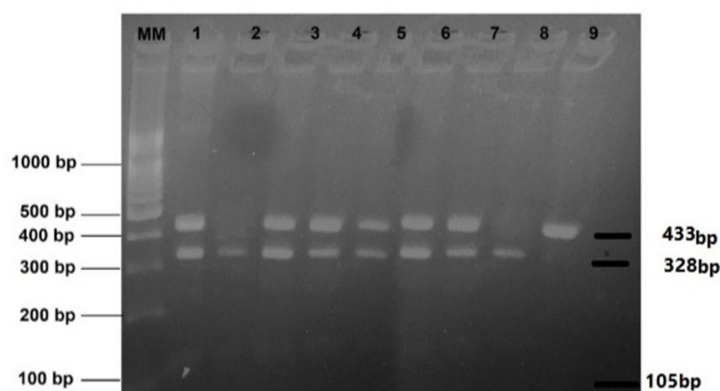

Figure 3: PCR-RFLP analysis of the GSTP1 (Ile105Val) gene polymorphisms using Alw261 restriction enzyme Ile/Ile (433 bp), Ile/Val (433, 328,105 bp), Val/Val (328,105 bp)

Painful crisis was the most frequent complication in SCA patients followed by dactylitis.

The GSTT1 present genotype was significantly associated with acute heart failure (*P.value* 0.02). The GSTP1 (val val) genotype was significantly associated with painful crisis and hepatomegaly as combined complications (*P.value* 0.008). The other GSTT1, other GSTP1, and GSTM1 genotypes revealed no statistically significant associations with SCA complications (Tables 11, 12, 13, 14).

Table 11: Association between the GSTM1 genotypes and SCA complications

Gene	Genotypes	Complications					
GSTM1		Hepatic sequestration		Splenic sequestration		Leg ulcer	
		No	Yes	No	Yes	No	Yes
	Null	32	4	34	2	35	1
	Present	24	3	26	1	27	0
		p.value= 1.0		p.value= 0.73		p.value= 0.38	
Gene	Genotypes	Complications					
GSTM1		Stroke		Painful		Acute chest syndrome	
		No	Yes	No	yes	No	Yes
	Null	31	5	25	11	35	1
	Present	25	2	19	8	26	1
		p.value= 0.41		p.value= 0.93		p.value= 0.83	
Gene	Genotypes	Complications					
GSTM1		Dactylitis		Heart failure+hepatomegaly		Hepatosplenomegaly	
		No	Yes	No	yes	No	Yes
	Null	26	10	36	0	35	1
	Present	20	7	26	1	27	0
		p.value= 0.87		p.value= 0.24		p.value= 0.38	
Gene	Genotypes	Complications					
GSTM1		Acute heart failure		Dactylitis+ hepatomegaly		Painful+ hepatomegaly	
		No	Yes	No	yes	No	Yes
	Null	34	2	36	0	36	0
	Present	27	0	25	2	25	2
		p.value= 0.21		p.value= 0.09		p.value= 0.09	

Table 12: Association between the GSTT1 genotypes and SCA complications

Gene	Genotypes	Complications					
GSTT1		Hepatic sequestration		Splenic sequestration		Leg ulcer	
		No	Yes	No	yes	No	Yes
	Null	38	6	42	2	44	0
	Present	18	1	18	1	18	1
		p.value= 0.33		p.value= 0.90		p.value= 0.12	
Gene	Genotypes						
GSTT1		Stroke		Painful		Acute chest syndrome	
		No	Yes	No	yes	No	Yes
	Null	39	5	32	12	42	2
	Present	17	2	12	7	19	0
		p.value= 0.90		p.value= 0.44		p.value= 0.34	
Gene	Genotypes						
GSTT1		Dactylitis		Heart failure+hepatomegaly		Hepatosplenomegaly	
		No	Yes	No	yes	No	Yes
	Null	31	13	43	1	43	1
	Present	15	4	19	0	19	0
		p.value= 0.48		p.value= 0.50		p.value= 0.50	
Gene	Genotypes						
GSTT1		Acute heart failure		Dactylitis+hepatomegaly		Painful+ hepatomegaly	
		No	Yes	No	yes	No	Yes
	Null	44	0	42	2	43	1
	Present	17	2	19	0	18	1
		p.value= 0.02		p.value= 0.34		p.value= 0.53	

Table 13: Association between the GSTM1 GSTT1 genotypes and SCA complications

Gene	Genotypes	Complications					
GSTM1 GSTT1		Hepatic sequestration		Splenic sequestration		Leg ulcer	
		No	Yes	No	yes	No	Yes
	Null	41	6	45	2	46	1
	Present	15	1	15	1	16	0
		p.value= 0.40		p.value= 0.74		p.value= 0.50	
Gene	Genotypes						
GSTM1 GSTT1		Stroke		Painful		Acute chest syndrome	
		No	Yes	No	yes	No	Yes
	Null	42	5	34	13	45	2
	Present	14	2	10	6	16	0
		p.value= 0.83		p.value= 0.45		p.value= 0.40	
Gene	Genotypes						
GSTM1 GSTT1		Dactylitis		Heart failure+hepatomegaly		Hepatosplenomegaly	
		No	Yes	No	yes	No	Yes
	Null	34	13	46	1	46	1
	Present	12	4	16	0	15	0
		p.value= 0.83		p.value= 0.55		p.value= 0.41	
Gene	Genotypes						
GSTM1GSTT1		Acute heart failure		Dactylitis+hepatomegaly		Painful+ hepatomegaly	
		No	Yes	No	yes	No	Yes
	Null	45	2	45	2	46	1
	Present	16	0	16	0	15	1
		p.value= 0.40		p.value= 0.40		p.value= 0.41	

Table 14: Association between the GSTP1 genotypes and SCA complications

Gene	Genotypes	Complications					
GSTP1		Hepatic sequestration		Splenic sequestration		Leg ulcer	
		No	Yes	No	yes	No	Yes
	Ile Ile	7	3	9	1	9	1
	Ile Val	38	4	41	1	42	0
	Val Val	11	0	10	1	11	0
		p.value= 0.07		p.value= 0.45		p.value= 0.06	
Gene	Genotypes	Complications					
GSTP1		Stroke		Painful		Acute chest syndrome	
		No	Yes	No	yes	No	Yes
	Ile Ile	10	0	8	2	10	0
	Ile Val	37	5	26	16	40	2
	Val Val	9	2	10	1	11	0
		p.value= 0.40		p.value= 0.13		p.value= 0.57	
Gene	Genotypes	Complications					
GSTP1		Dactylitis		Heart failure+hepatomegaly		Hepatosplenomegaly	
		No	Yes	No	Yes	No	Yes
	Ile Ile	7	3	15	0	10	0
	Ile Val	31	11	31	1	41	1
	Val Val	8	3	16	0	11	0
		p.value= 0.97		p.value= 0.77		p.value= 0.77	
Gene	Genotypes	Complications					
GSTP1		Acute heart failure		Dactylitis+hepatomegaly		Painful+ hepatomegaly	
		No	Yes	No	yes	No	Yes
	Ile Ile	10	0	10	0	10	0
	Ile Val	41	1	41	1	42	0
	Val Val	10	1	10	1	9	2
		p.value= 0.43		p.value= 0.43		p.value= 0.008	

DISCUSSION

Complications of SCD are characterized by chronic hemolytic anemia, severe acute and chronic pain as well as end-organ damage. Anaemia is chronic and patients generally well-adapted until an episode of decompensation (e.g. severe infection) occurs (Provan *et al*., 2004).

Total of one hundred twenty-six participants were recruited to this study, 63 was sickle cell patients considered as the case group, and 63 healthy individuals as the control group, genotypes and haematological findings of the patients were compared with the control group.

This study aimed to investigate the association between GSTs gene polymorphisms and SCA complications.

In this study, in the case group, the GSTM1 Null genotype (57.1%) was the highest frequency, then the GSTM1 present genotype (42.9%), in the control group the GSTM1 Null genotype (52.4%) was highest frequency, then the GSTM1 present genotype (47.6%). There was no statistically significant difference between the case group and the control group.

In the case group, the GSTT1 Null genotype (69.8%) was the highest frequency, then the GSTT1 present genotype (30.2%), in the control group the GSTT1 Null genotype (49.8%) was less than the GSTT1 present (50.2%). There was a statistically significant difference between the case group and the control group.

In the case group the GSTM1 GSTT1 Null genotype (74.6%) was the most common, then GSTM1 GSTT1 present (25.4%), and in the control group the GSTM1 GSTT1 Null genotype (77.7%) was the most common, then GSTM1 GSTT1present genotype (22.3%). There was no statistically significant difference between the case group and the control group.

In this study, in the case group, the heterozygous Ile/Val genotype (66.7%) was the highest percentage then the homozygous Val/Val and Ile/Ile genotypes were (17.4%), (15.9%) respectively, while in the control group Ile/Val high percent (84.1%), then Val/Val (12.7%), and Ile/Ile (3.2%). There was a statistically significant difference between the case group and the control group.

The GSTT1 present genotype was significantly associated with acute heart failure (*P.value* 0.02). The GSTP1 (val val) genotype was significantly associated with painful crisis and hepatomegaly as combined complications (*P.value* 0.008). The other GSTT1, other GSTP1, and GSTM1 genotypes revealed no statistically significant associations with SCA complications.

Like our findings study done by Ali *et al*., there was no statistically significant difference in the distribution of the GSTM1 between the case group and the control group, the GSTT1 was found in 47.6% of SCA patients and 77.8% of the control but the frequency of individuals carrying the GSTT1 null genotype was significantly higher among SCA patients, 52.4% compared to 22.2% of the Control (Ali *et al*., 2021).

Unlike our findings, Ali *et al*., found the distribution of the homozygous (Val/Val) of the GSTP1, the heterozygous (Ile/Val), and the wild-type genotype of the GSTP1 (Ile/Ile) forms were found in 9.7%, 35.5% and 54.8% of SCA cases, respectively. In the Control, the homozygous (Val/Val) of the GSTP1 Ile105Val, heterozygous (Ile/Val), and the wild-type genotype of GSTP1 (Ile/Ile) forms were 1.6%, 39.7%, and 58.7%, respectively. There were no statistically significant differences in the distribution of the GSTP1 and the GSTT1 gene polymorphisms between SCA patients and the controls and no association between the GSTP1gene polymorphisms and clinical manifestation of SCD (Ali *et al*., 2021).

Like our result the GSTT1 null genotype showed no statistically significant difference with ACS, VOC, dactylitis and splenomegaly, the GSTM1 GSTT1 null genotype showed no statistically significant difference with splenomegaly, VOC, ACS, and Stroke (Ali *et al*., 2021).

Similar to our observation, Abu Duhier & Mir observed that the GSTM1 null genotype had a statistically non-significant difference also observed that patients with SCD possess higher frequency of the GSTT1 null genotype and there was a statistically significant difference when compared to the control (Abu Duhier & Mir., 2017).

Like our study, Ellithy *et al*., observed that the highest prevalence was for the GSTM1 null genotypes however, there was no statistically significant difference when compared to the control (Ellithy *et al.,* 2015).

Similar findings, Sanjay *et al*., observed that the difference between groups for the GSTT1 null genotype was statistically significant, while the differences between groups for the GSTM1 present and the GSTT1/M1 null genotypes were not (Sanjay *et al.,* 2012).

Like our study, RABAB & BOTHINA observed no find any significant association between both the GSTT1 and the GSTMT1 null genotypes and clinical severity of the disease in SCD patients (RABAB & BOTHINA, 2013).

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Unlike our study, Ellithy *et al*., observed no significant difference in the frequency distribution of the GSTT1 and the GSTP1 polymorphisms between the case and the control. ACS was the most frequent complication of SCA and the GSTT1, the GSTM1 null genotypes associated with ACS and VOC. (Ellithy *et al.,* 2015).

Like our study, Ellithy *et al*., reported no statistically difference in the frequency distribution of the GSTM1 between the case and the control groups, the GSTM1 and the GSTT1 null genotypes were a non-significantly for ACS and the GSTP1 polymorphisms (I/V or V/V) were not significantly for ACS or VOC (Ellithy *et al.,* 2015).

Like our study, in meta-analysis study by Verma *et al*., shown the GSTP1 associated with significantly increased risk of SCA. Unlike our study the GSTT1 associated with significantly increased risk of SCA except in this study the GSTT1 present increases the risk of acute heart failure (Verma *et al*., 2020).

The result showed a statistically significant difference association between some hematological findings in the case and the control group (decreased of Hb and increased TWBCS and PLTs in the case group (*P*.value 0.00,0.00, 0.00) respectively.

In the case group showed that no significant difference in the TWBCs and PLTs count. The hemoglobin level was a statistically significant higher in females in comparison with male *P.value* (0.01).

In this study, there were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTM1, the GSTT1and the GSTM1 GSTT1 genotypes but there was a statistically significant difference in the PLTs between the GSTP1 genotypes.

Similar findings, Ali *et al.,* reported there was no statistically significant difference in the PLTs between the GSTM1 genotypes and also there were no statistically significant differences in the Hb, TWBCs, and PLTs between the GSTT1, the GSTM1, and the GSTT1 genotypes and no statistically significant differences in the Hb, TWBCs between the GSTP1 genotypes.

Unlike our findings Ali *et al*., 2021 there were statistically significant differences in the TWBCs, Hb between the GSTM1 genotypes and no statistically significant difference in the PLTs between the GSTP1 genotypes (Ali *et al*., 2021).

CONCLUSSION

The study concluded that there were no statistically significant differences in the GSTM1 and the GSTM1 GSTT1 genotypes between the case group and the control group (*P.value*= 0.36, 0.36) respectively and there were statistically significant differences in the GSTT1 and the GSTP1 genotypes between the case group and the control group (*P.value* 0.014, 0.02) respectively. The GSTT1 present genotype was significantly associated with acute heart failure (*P.value* 0.02). The GSTP1 (val val) genotype was significantly associated with painful crisis and hepatomegaly as combined complications (*P.value* 0.008). The other GSTT1, other GSTP1, and GSTM1 genotypes revealed no statistically significant associations with SCA complications.

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