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Research paper

A positive correlation between sickle cell anemia and g6pd deficiency from population of Chhattisgarh, India

Lohit Raj Shivwanshi, Ekta Singh, Anil Kumar*

Department of Biotechnology, Govt. V.Y.T.PG. Autonomous College, Durg, Chhattisgarh Pin – 491001, India

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ABSTRACT

Objective: Present study was undertaken to study the association between sickle cell anemia (SCA) and glucose-6-phosphate dehydrogenase (G6PD) deficiency from Sahu and Kurmi population of Durg and Rajnandgaon district of Chhattisgarh, India.

Method: A random sampling of 1749 individuals was done. SCA and G6PD deficiency was detected by slide test followed by electrophoresis and Enzymatic reaction indicated by change in colour respectively. Further the samples were subjected to analyze glutathione-S-transferase (GST) *i.e.* GSTM1 and GSTT1 gene polymorphism, variance of G6PD among G6PD deficient samples by PCR-RFLP. Oxidative stress and DNA damage by comet assay was also analyzed.

Results: Present finding indicates positive correlation between SCA and G6PD deficiency in Durg and Rajnandgaon district [Durg: ($r = 0.92$; HbAS-G6PDd and $r = 0.56$; HbSS-G6PDd) Rajnandgaon: ($r = 0.63$; HbAS-G6PDd and $r = 0.86$; HbSS-G6PDd)]. Significant changes ($P < 0.05$) in antioxidant enzymatic parameters were observed in HbSS and G6PD with sickle positive individual. Assessment of DNA damage by Comet assay considering Head DNA percent, Tail DNA percent, Tail length and Tail moment also showed significant changes ($P < 0.05$) within all concerned parameters in HbSS and G6PD with sickle positive individual. Analysis of GST gene polymorphism showed that frequency of individuals carrying the GSTM1 null genotype was higher in HbAS (60%) and the frequency of individual carrying the GSTT1 null genotype was found higher in HbSS (66.6%). G6PD variants analysis also confirmed the presence of highest percentage of mutation among G6PD deficient population as compared to control and a positive correlation was observed between G6PD deficiency and mutant variants of G6PD gene [Rajnandgaon: ($r = 0.67$; G6PDd-Mahidol mutated and $r = 0.90$; G6PDd-Union mutated) Durg: ($r = 0.91$; G6PDd-Mahidol mutated and $r = 0.01$; G6PDd-Union mutated)] .

Conclusion: Thus present finding indicates positive correlation between SCA and G6PD deficiency in Chhattisgarh, India.

1. Introduction

Sickle cell anemia (SCA) is a genetic defect that results in abnormal structure of one of the globin chain of hemoglobin molecule. Hemoglobinopathies are group of disease which is characterized by abnormalities both quantitatively and qualitatively in the synthesis of hemoglobin. This is due to genetic inheritance as a result of point mutation.

The sickle cell disease (SCD) was first of all discovered in West Indies in 1910 by Dr. James B. Herrick. The epidemic of Malaria is thought to be cause of the mutation which results in sickle cell. An individual with sickled red blood cells (RBCs) has an increased chance to survive the malaria sickness compared to those without the mutation. G6PD deficiency is prevalent in African, Middle East and South Asian regions of the world where malaria is endemic and sanguinity is also high (Alfadhli et al., 2005; Hagag et al., 2018). Chhattisgarh State is also Malaria prone zone and a large number of Chandrakar, Sahu and Tribal Community are found with sickle cell traits. It seems that the sickling condition in Chhattisgarh is alarming and it was also voiced in state Assembly of Chhattisgath and Parliament of India. The Health Department of Chhattisgarh is doing random sampling for sickling. According to the World Health Organization, out of the total world

Abbreviations: SCA, sickle cell anemia; SCD, sickle cell disease; ROS, reactive oxygen species; G6PD, glucose-6-phosphate dehydrogenase; G6PDd, glucose-6-phosphate dehydrogenase deficiency; GST, glutathione-S-transferase; MDA, malondialdehyde; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, reduced glutathione

* Corresponding author.
E-mail addresses: aimum_aishley@yahoo.co.in, anilkumardurg1996@gmail.com (A. Kumar).

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population 270 million people possess genes determining abnormal hemoglobin (Clegg and Weatherall, 1999; Bandeira et al., 2014). Hb S results due to point mutation from the GAG (glutamic acid) codon to the GTG (Valine) codon at sixth position of the beta-globin chain which leads to alteration in the stability (Rodwell, 2000). SCD occurs when any individual inherits two abnormal copies of hemoglobin gene. Individual with single abnormal copy referred to as carriers.

Sickle cells spontaneously generate approximately double amount of ROS (reactive oxygen species) compared to normal RBCs; this is associated with endothelial dysfunction, inflammation and multiple organ damage and in turn related to the severity of clinical features and also responsible for oxidative stress (Hundekar et al., 2010). Upregulation of GST gene takes place during oxidative stress which is major symptom of SCD. Polymorphism among GST is responsible for several clinical conditions and so far as the state of Chhattisgarh is concerned related literatures are not available.

G6PD deficiency is another important cause of hemolytic anemia. Both HbS and G6PD deficiency is an adaptation against malaria and thus selected in malaria prone zone (Nkhoma et al., 2009). Prevalence is highest in Saharan Africa, Arabian Peninsula and Central India (Beutler, 1994).

So banking on above facts, present study is concerned with the study of the correlation between SCA and G6PD Deficiency.

2. Materials and methods

For the present study two districts from Chhattisgarh state in India were selected (Durg and Rajnandgaon). Total 982 samples (Sahu and Kurmi) from Durg and 767 samples (Sahu and Kurmi) from Rajnandgaon were examined. Sodium metabisulphite slide test and Cellulose acetate paper electrophoresis was performed for screening of SCA and Chemical Enzymatic reaction was performed for analysis of G6PD Deficiency (Pauling et al., 1949). Further two milliliter intravenous blood was collected from 12 control and 15 positive for sickle individuals and 10 samples were collected from each of the group (control, G6PD deficient and G6PD deficient with sickle) by paramedical staff for further analysis following ICMR and Institutional Ethical Committee Guidelines. Oxidative stress and genotoxicity were analyzed using standard protocol. DNA isolation was performed following Sambrook and Russell (2011). Then PCR was performed and results were analyzed after statistical validation of data.

2.1. Determination of oxidative stress and antioxidant parameters

For determination of oxidative stress among sickled and G6PD deficient individuals following parameters were analyzed:

i. Lipid peroxide in terms of malondialdehyde (MDA moles/mg protein) was determined by thiobarbituric acid reaction following Ohkhawa et al. (1979).
ii. Reduced Glutathione (GSH μg/mg protein) was determined following Moron et al. (1979).
iii. Superoxide dismutase (SOD Units/mg protein) was determined following Misra and Fridovich (1972).
iv. Catalase (CAT μ moles of H_2O_2 consumed/min/mg protein) was determined following Bergmeyer et al. (1974).
v. Glutathione peroxide (GPx μg of glutathione utilized/min/mg protein) was determined following Rotruck et al. (1973).

Simultaneously, a parallel set of control was also maintained.

Among all the considered parameters, MDA is an oxidative stress marker as it indicates lipid peroxidation while GSH is a non-enzymatic antioxidant system.

2.2. Alkaline comet assay

Extent of DNA damage was analyzed in sickled and G6PD deficient individual by Alkaline Comet assay or Single cell gel electrophoresis (SCGE) (Singh et al., 1988) from Lymphocyte cells and observation was made using 40× objective on a fluorescent microscope, (Olympus Microscope –CX 21i with Y-FL EPI-Fluorescence attachment), equipped with an excitation filter of 515–560 nm and a barrier filter 590 nm and image was analyzed using Image J 4.18 analysis software Wayane Rasband, NIH, USA.

2.3. Genotyping of GST gene

Identification of polymorphisms of T1 and M1 was performed by multiplex PCR with β globin gene as control. GSTM1 and GSTT1 were amplified using following primers (Rabab and Bothina, 2013): F: 5′-GAACTC CCTGAAAAGCTAAAGC-3′ and R: 5′-GTTGGGCTCAAATATA CGGTGG-3′ for GSTM1 and F: 5′-TCCTTACTGGTCCTCACATCTC-3′ and R: 5′-TCACCGGATCAT GGCCAGCA-3′ for GSTT1. As an internal control, the β-globin gene was co-amplified using the primers F: 5′-ACAC AACTGTGTTCACTAGC-3′ and R: 5′-CAACTTCATCCACGTTC ACC-3′. PCR products were verified by horizontal electrophoresis using 1.5% agarose.

2.4. Genotyping of G6PD gene

Identification of G6PD variant (Mahidol and Union) was performed by PCR-RFLP among individuals suffering from G6PD deficiency along with SCA. G6PD gene was amplified using following primers (Nuchprayoon et al., 2002): G6PD Mahidol gene will be F: 5′- GCGTC TGAATGATGCAGCTCTGAT -3′ and R: 5′- CTCCACGATGATGCGGTTC AAGC -3′ for G6PD Union gene F: 5′-ACGTGAAGCTCCCTGACGC-3′ and R: 5′- GTGAAAATAC GCCAGGCCTTA -3′. After amplification restriction digestion was performed using Hind III for Mahidol mutation and Hha I for Union mutation.

2.5. Statistical validation of data

Test of significance for coexistence of SCA and G6PD deficiency was analyzed by Karl Pearson's correlation coefficient.

3. Results

Among 982 samples collected from Durg, 91 (9.26%) were found sickle positive and out of 767 samples from Rajnandgaon, 52 (6.77%) were found sickle positive. (Table I & Figs. I–II) and 106 individuals were found G6PD deficient among which 66 were sickle positive. G6PD deficient individuals were identified by enzymatic reaction, in which

Table I
Prevalence of sickle cell anemia (HbAS/HbSS) and G6PD deficiency.

S. no.	District	Total sample	Sickle positive samples	Electrophoresis test		G6PD deficient individuals
				Homozygous	Heterozygous	
1.	Durg	982	91	16	75	42
2.	Rajnandgaon	767	52	08	44	24

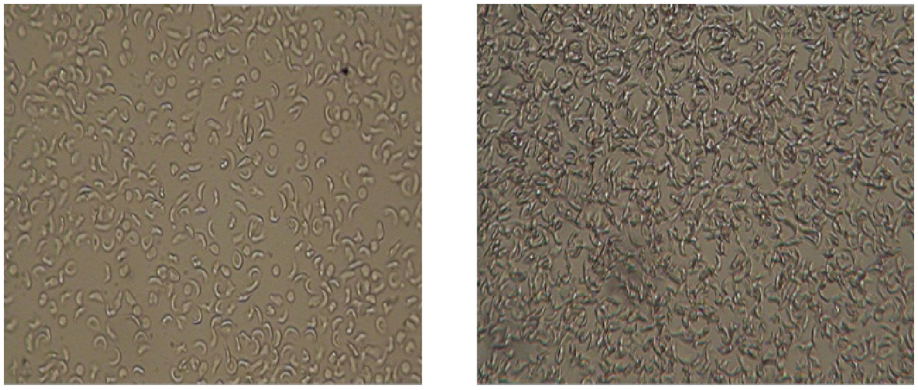


Fig I. Sickle shaped red blood cells.

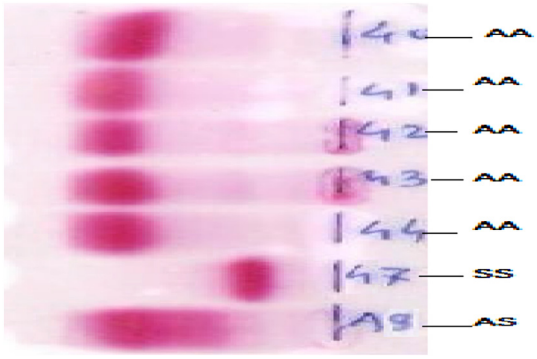
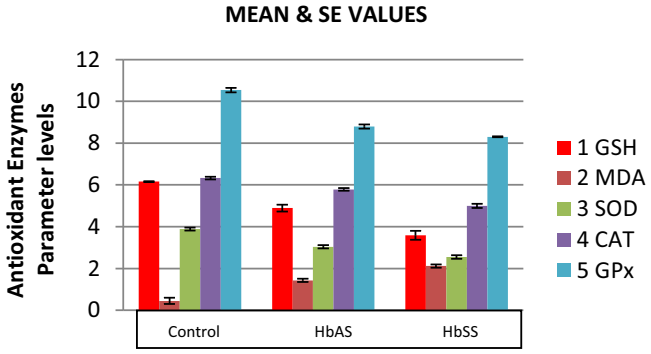


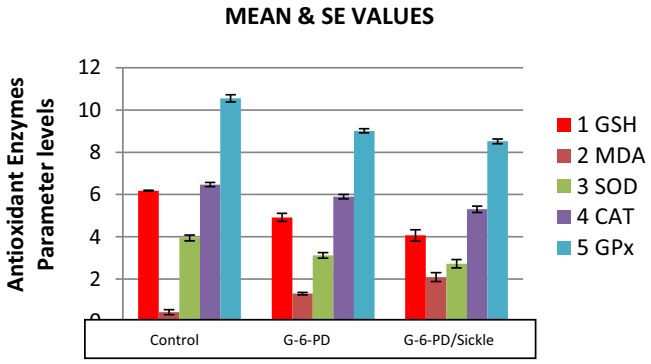
Fig II. Electrophoretic band patterns. (Confirmatory test for sickle cell anemia). AA- normal, AS- heterozygous, SS- homozygous.



Graph I. Variation in MDA (biomarker of oxidative stress), antioxidant enzymatic parameters and GSH (non-enzymatic anti-oxidant) among Control, HbAS and HbSS individuals.

partial or no decolorization of reaction mixture indicates G6PD deficiency while complete decolorization indicates presence of G6PD enzyme (Fig. III). A high and moderate degree of correlation was observed between HbAS-G6PD deficiency ($r = 0.92$) and HbSS-G6PD deficiency ($r = 0.56$) respectively in Durg district while in Rajnandgaon district moderate and high degree of correlation was observed between HbAS-G6PD deficiency ($r = 0.63$) and HbSS-G6PD deficiency ($r = 0.86$) respectively. Sickle cell and G6PD deficient individuals are worst sufferers of oxidative stress and hence oxidative stress was analyzed considering GSH, MDA, SOD, CAT and GPx.

In sickled individuals the level of MDA was found significantly increased in HbAS ($P < 0.05$) and HbSS ($P < 0.05$) as compared to control (HbAA) and the mean values of GSH, CAT, SOD and GPx were found significantly decreased ($P < 0.05$) in HbAS and HbSS as compared to control (Graph I). The oxidative stress among G6PD deficient individuals and G6PD deficient with sickled individuals were also analyzed considering GSH, MDA, SOD, CAT and GPx. Similar results were observed in G6PD deficient individuals. Lipid peroxide in terms of MDA was found significantly increased in G6PD deficient individuals ($P < 0.05$) and G6PD deficiency with sickle individuals ($P < 0.05$) as compared to control and the level of rest of the enzymes (GSH, CAT,



Graph II. Variation in MDA (biomarker of oxidative stress), antioxidant enzymatic parameters and GSH (non-enzymatic anti-oxidant) among Control, G6PD deficient and G6PD with sickle individuals.

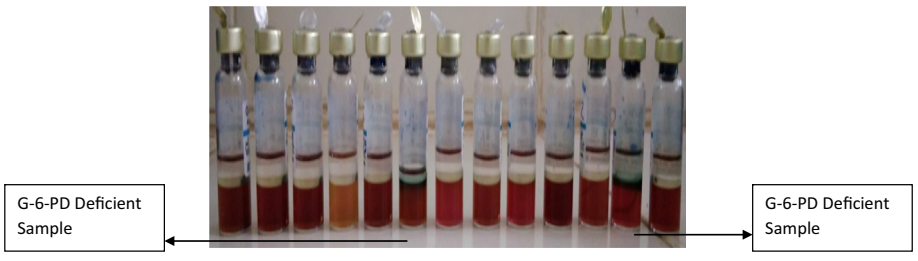


Fig III. G6PD deficiency test (Negative – decolorization observed/Positive – partial/no decolorization.

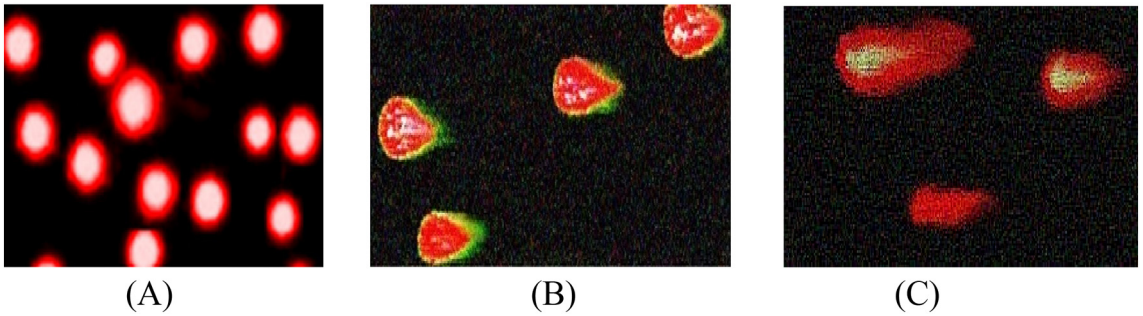


Fig IV. Comet assay of lymphocyte cell DNA (damaged) (B–C) of control (HbAA) individual (A), taken from trianoccular florescence microscope (40×) which is processed to the colour threshold (red).

SOD and GPx) were found significantly decreased ($P < 0.05$) (Graph II). Thus present finding confirms oxidative stress comparatively greater in HbAS/HbSS individuals and G6PD deficient with sickle individuals as compared to control.

DNA damage induced by oxidative stress was also analyzed by Comet assay considering head DNA percent, tail DNA percent, tail length and tail moment. The aberration among HbAS individuals was found significant than control (HbAA) population *viz.* head DNA percent ($P < 0.05$); tail DNA percent ($P < 0.05$); tail length ($P < 0.05$); and tail moment ($P < 0.05$). Similarly the aberration among HbSS population than control population was found significant *viz.* head DNA percent ($P < 0.05$); tail DNA percent ($P < 0.05$); tail Length ($P < 0.05$); and tail moment ($P < 0.05$). The head DNA percent was found decreased in HbAS and significantly decreased in HbSS than control whereas, increase in tail DNA percent, tail length and tail moment in HbAS and significant increase in HbSS than control were reported which confirmed the development of genotoxicity among sickle cell trait (HbAS) and with greater intensity among population with SCD (HbSS) (Fig. IV). The aberration among G6PD deficient population as compared to control population was found significant *viz.* head DNA percent ($P < 0.05$); tail DNA percent ($P < 0.05$); tail length ($P < 0.05$); and tail moment ($P < 0.05$). Similarly the aberration among G6PD deficiency with sickle as compared to control was also found significant *viz.* head DNA Percent ($P < 0.05$); tail DNA Percent ($P < 0.05$); tail length ($P < 0.05$); and tail moment ($P < 0.05$). The head percent was found decreased in G6PD deficient and highly decreased in G6PD deficient with sickle than control whereas, increase in tail DNA percent, tail length and tail moment in G6PD deficient and high increase in G6PD deficient with sickle than control confirmed that genotoxicity also developed among G6PD deficient population (Fig. V).

The null allele frequency has been studied in sickled population compared to the control (HbAA) individuals with respect to GSTM1 and GSTT1 genes by RT-PCR (Fig. VI). The frequency of individuals carrying the GSTM1 gene was found higher in Control (93.33%) in comparison to HbAS (40%) and HbSS (41.67%) and the frequency of individual carrying the GSTT1 gene was found higher in Control (86.6%) (Table II and Figs. VI–IX). In case of G6PD deficiency analysis 10 control samples were analyzed and only two samples were found Mahidol normal (Single band of 104 bp) while remaining 8 control samples were observed as Mahidol mutated (Two bands of 82 and 22 bp); in case of diseased sample only 3 individuals were observed Mahidol normal while others were found Mahidol mutated with presence of two bands. In case of Union variant six samples were found normal with band size of 142, 42 and 27 bp, in some samples two bands of size 187 and 27 bp was obtained *i.e.* Union mutated, while in two samples single band was observed which indicates that sample was not amplified. Detection of mutation in union variant for control samples revealed the presence of mutated variant among 6 samples and 4 samples showed the presence of normal gene with presence of three bands of 142, 42 and 27 bp respectively. Correlation analysis between prevalence of G6PD deficiency and two mutated variants (Mahidol and Union) were calculated as positive between G6PDd and G6PDd mahidol mutant ($r = 0.67$) and between G6PDd and G6PDd union mutant, it was also reported positive ($r = 0.90$) in Rajnandgaon district, while in Durg district almost no correlation was observed between G6PDd and G6PDd union mutant ($r = 0.01$) but a high positive correlation was found between G6PDd and G6PDd mahidol mutant ($r = 0.91$). The existence of poor correlation between G6PDd and G6PDd union mutant is might be due to presence of any other variant while positive correlation indicates that mutated variant is responsible for deficiency of G6PD enzyme which is also responsible for severity of disease (Figs. X and XI).

4. Discussion

Chhattisgarh is the 26th state of India, which is established on 1st November 2000, where high frequency of sickle cell gene has been reported. Balgir (1996) reported 20% of SCA in Madhya Pradesh and in Rajnadgaon as well as in Durg district prevalence of sickle cell gene was found 9.75% and 8.88% respectively. Highest percentage of sickle cell was reported from Sahu community (12.34%). In current investigation 9.26% and 6.77% of SCA was reported from Durg and Rajnandgaon district respectively. In our study a positive correlation was observed

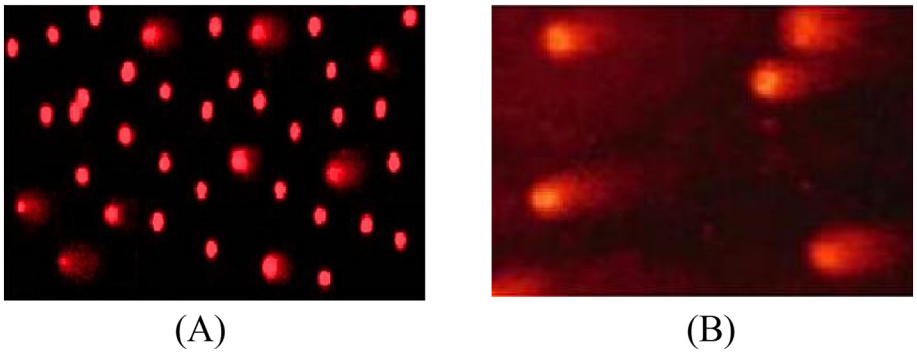


Fig V. Comet assay of lymphocyte cell DNA (damaged) (A) of control individual (B) G-6-PD deficient individuals, taken from trinoccular florescence microscope (40×) which is processed to the colour threshold (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

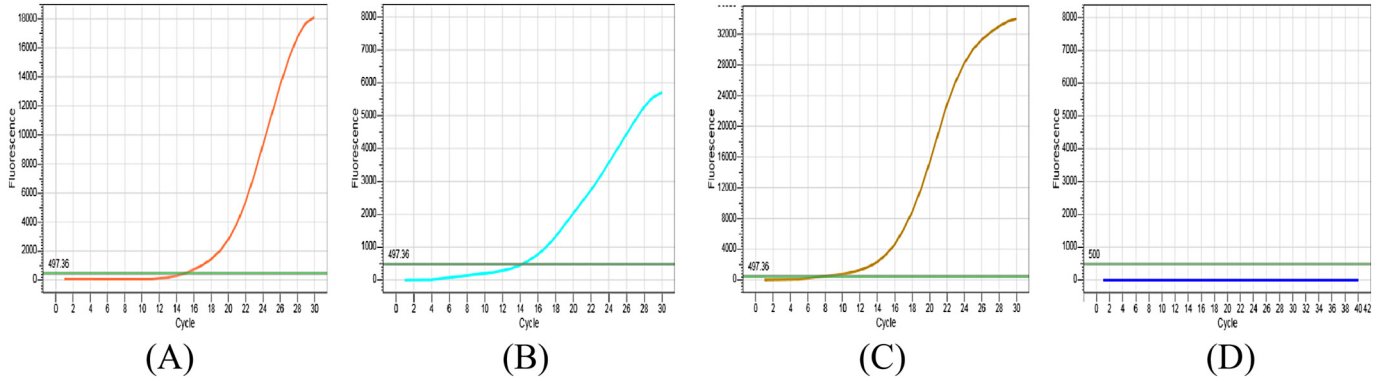


Fig VI. Amplification curve (RT-PCR) for GSTM1 gene generated after completion of PCR cycles (A–B) control samples (C–D) sickled samples.

Table II
Showing frequency of genotypes on the basis of GSTM1 & GSTT1 genes in HbAA (control), HbAS & HbSS subjects.

S. no.	Genotypes	HBAA (n = 15)	HbAS (n = 15)	HbSS (n = 12)
1		GSTM1 frequency		
I	GSTM1 (+)	14	6	5
II	GSTM1 (−)	1	9	7
2		GSTT1 frequency		
I	GSTT1 (+)	13	8	4
II	GSTT1 (−)	2	7	8
3		GSTM1/GSTT1 frequency		
I	GSTM1/GSTT1 (+/−)	2	3	3
II	GSTM1/GSTT1 (+/+)	12	2	1
III	GSTM1/GSTT1 (−/+)	1	6	3
IV	GSTTM1/GSTTI (−/−)	0	4	5

(+) – present; (−) absent.

between sickled and G6PD deficient individuals in both the districts of Chhattisgarh, India irrespective of previous findings of Adu et al. (2016) (Ghana), Lewis (1973) (Ghana) and Piomelli et al. (1972) (New York).

Humans are constantly under the threat of being victimized to many dreadful diseases and stressful life. Various diseases & disorders are developed due to the condition of "Oxidative Stress" which is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. Free radicals are generally formed during normal metabolic reactions but when the body mechanism to detoxify it is decreased, the level of free radicals in the cell is increased and causes oxidative stress (Halliwell and Aruoma, 1991). Sickled patients generate enough amount of free radicals responsible for different clinical manifestations like organ damage, vaso-occlusive crisis, inflammation *etc.* (Hundekar et al., 2010) this free radical is also responsible for damage of protein and lipid constituents of RBC membrane leading to hemolysis. Antioxidants are neutralizing substances that act against free radicals & their harmful effects and they have remarkable property to scavenge or combat free radicals (Moron et al., 1979). Hence, they are known as 'Radical Scavengers' or 'Oxygen Quenchers' (Halliwell and Aruoma, 1991). Low level of antioxidants like vitamin C, glutathione peroxidase, catalase, superoxide dismutase and glutathione S transferase have been reported in sickled patients (Natta et al., 1990; Reid et al., 2006).

In our study, we have reported a significant decrease in all the antioxidant enzyme parameters in sickle cell as well as G6PD deficient subjects, except malondialdehyde (MDA), which was found elevated in affected subjects as compared to the control individuals (Graphs I–II). In our work we have also reported significant DNA damage in sickle cell and G6PD deficient individuals in terms of increased comet tail particularly higher in case of HbSS as compared to HbAS and HbAA and also G6PD deficient individuals (Figs. IV–V).

A study conducted by Fasola et al. (2007), revealed that the mean total antioxidants status (TAS) level was found significantly lowered (TAS < 1.00 mmol/L in 21 *i.e.* 52.2%) among patients with SCA in comparison to the controls ($P < 0.001$) and that the mean value in the SCA group was found less than half of control group. Hierso et al. (2014), compared ROS and GSH content in RBC, along with hemorheological profile of SCA (SS), sickle hemoglobin C (SC) disease patients and healthy subjects at baseline and after *in-vitro* treatment with t-butyl hydroperoxide (TBHP). They reported higher levels of RBC ROS content in SS and SC patients, with highest level seen in SS patients, and also a lower RBC GSH content was observed in sickle syndrome patients, particularly in SS patients.

According to Barberino et al. (2012), for the detection of single-strand breaks as initial DNA damage, the comet assay is an absolute technique which can be used. Saud in 2013 studied the apoptotic behavior and damage to DNA in blood leukocytes of 19 sickle cell patients

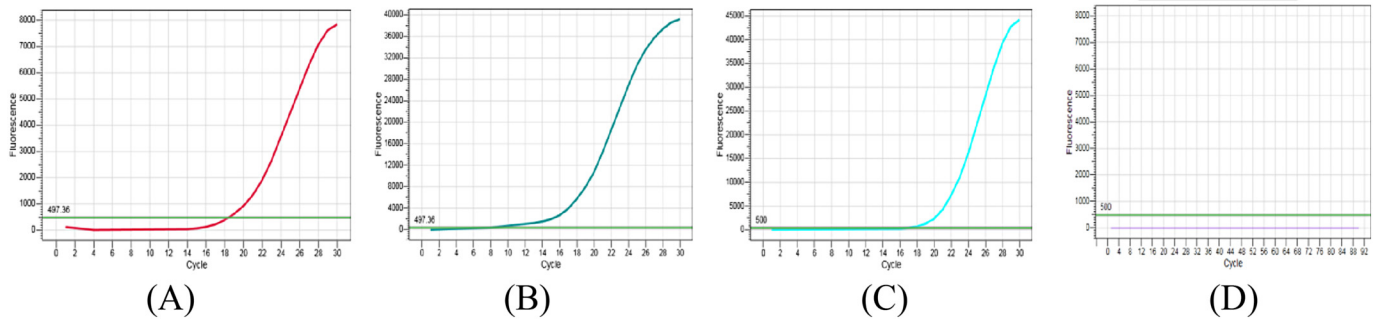


Fig VII. Amplification curve (RT-PCR) of GSTT1 gene generated after completion of PCR cycles (A–B) control samples (C–D) sickled samples.

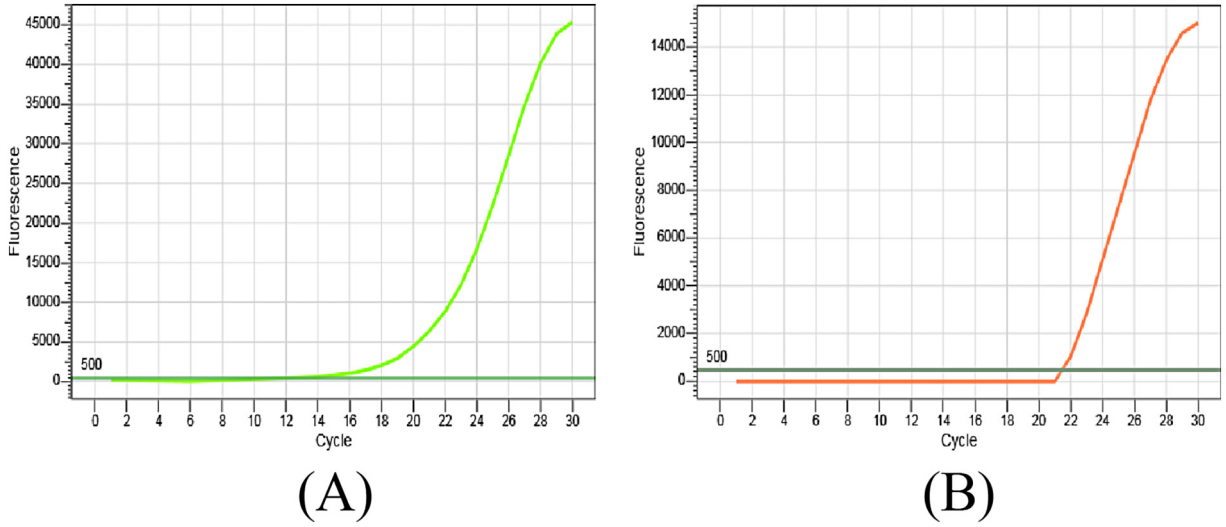


Fig VIII. Amplification curve (RT-PCR) of β-globin gene in DNA samples generated after completion of PCR cycles (A) control sample (B) sickled sample.

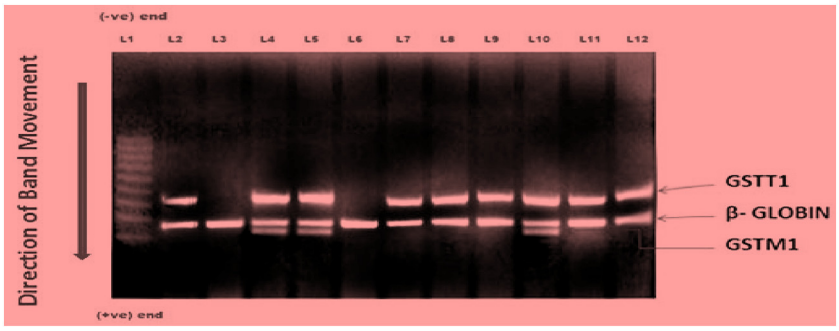


Fig IX. Electrophoretically separated bands of the PCR products for GSTT1 (480bp), GSTM1 (230bp) & β-globin fragments (internal control 268bp).

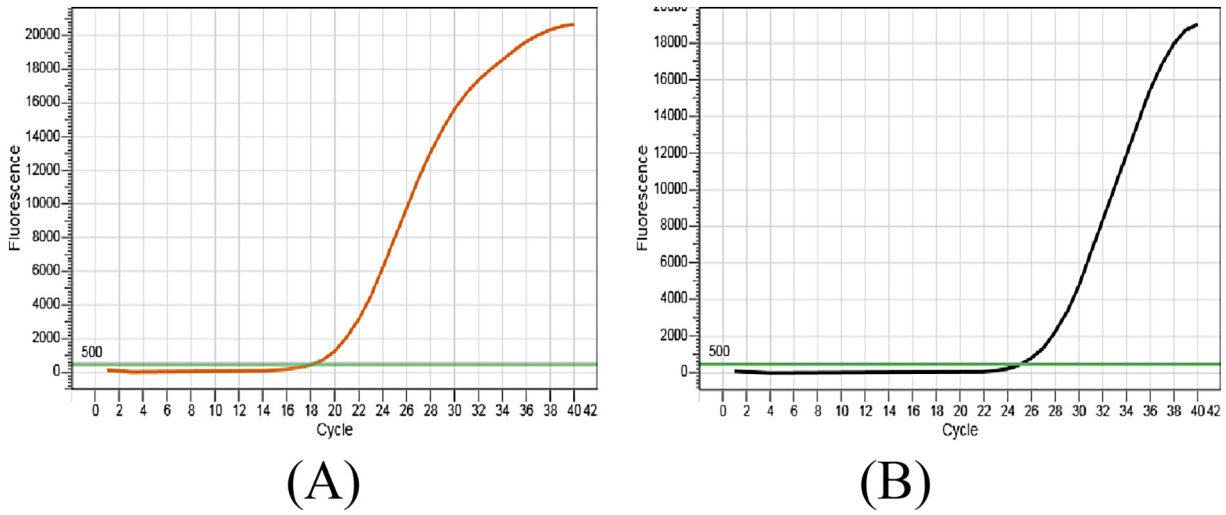


Fig X. Amplification curve (RT-PCR) of G-6-PD control gene in DNA samples generated after completion of PCR cycles.

along with 10 healthy individuals without any hemoglobin disorders by DNA fragmentation electrophoresis assay, Comet assay and Annexin V-FITC1T (1T1TFluorescein isothiocyanate) apoptosis methods. He found severe and higher prevalence of DNA damage in the blood leukocytes in terms of smear shaped gel electrophoretic pattern. Mesbah et al. (2004) reported that the mean level of the DNA strand breakage in mononuclear leukocytes of 36 male G6PD-deficient (Gd−Md) infants was significantly higher ($P < 0.001$) than those observed in the normal lymphocytes.

Glutathione is ubiquitous molecule and water soluble in nature found in animals, plants as well as in micro-organisms. It is found mainly in cytosol and in many organelles *viz.* - mitochondria, nuclear matrix, and peroxisomes (Lomaestro and Malone, 1995). Peroxides, agents that cause harm to cell are also reduced by GSTs (Mannervik, 1985). As GST possess both reductase and peroxidase activity, peroxidase activity of GST requires reduced glutathione, and this reaction in catalyzed in two steps. First step involves reduction of peroxide to alcohol along with the production of hydroxylated glutathione. Second

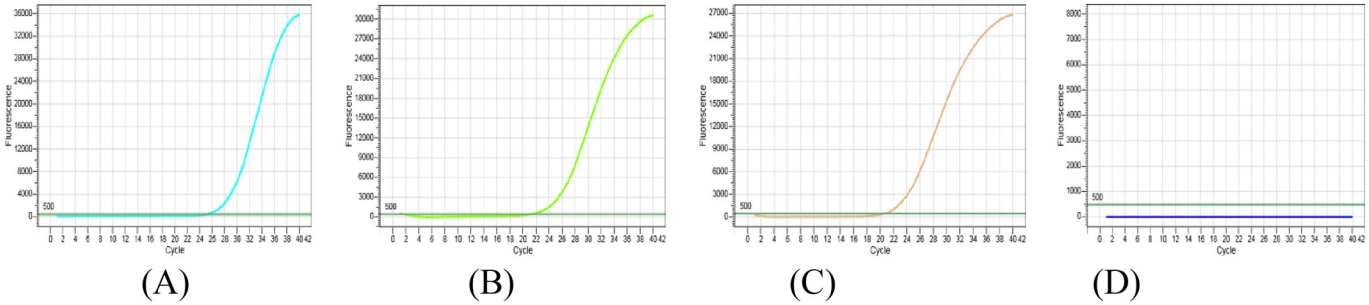
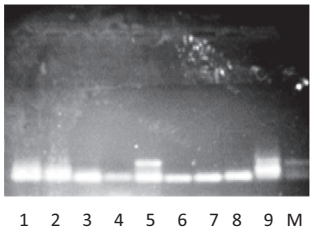


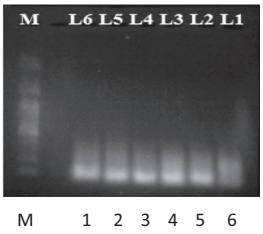
Fig XI. Amplification curve (RT-PCR) of G-6-PD gene in DNA samples generated after completion of PCR cycles (A–B) Mahidol variant (C–D) Union variant.

Table III
Showing frequency of genotypes on the basis of normal and mutated G6PD variants.

S. no.	Sample	Mahidol			Union		
		Normal	Mutated	Unamplified	Normal	Mutated	Unamplified
1.	Control (n = 10)	8	2	0	6	2	2
2.	G6PD deficient samples (n = 10)	3	7	0	4	6	0

M-mutated; N-normal; U-unamplified.





(A) Lane 3,4,6,7 and 8 – Mahidol Normal Lane Lane 1,2 and 5 – Mahidol Mutated	(B) Lane 3 and 4 – Union Mutated Lane 5 and 6 – Unamplified sample Lane 1 and 2– Union Normal

Fig XII. PCR/RFLP band pattern for detection of specific mutation among G-6-PD deficient individuals. (A) Mahidol variant (B) Union variant.

step is a spontaneous reaction in which hydroxylated glutathione (GSOH) with GSH is converted to oxidised glutathione (GSSG) (Jakoby and Habig, 1980). In present study, GSTM1 and GSTT1 gene was found to be involved in anemia, Ozturk et al. (2003) have also reported presence of GSTM1 null genotype in Africans and Indians with 33% and 66% respectively. Kelsey et al. (1997) also reported GSTM1 deletion polymorphism in Africans and Americans and its association with SCD. Elithy et al. (2015) reported that absence of GSTM1 and GSTT1 associated with pulmonary hypertension and non wild type GSTP1 not associated with clinical manifestations of SCD and finally concluded that GST gene polymorphism were associated with worsening of clinical manifestations of SCD. Shiba et al. (2014) reported that GSTM1 null genotype was associated with risk of SCD but no significant association was observed between GST genotypes and frequency of sickle cell disease. Our finding is also affirmative to the previous findings, the frequency of both GSTM1 and GSTT1 gene was found higher in control (Table II and Figs. VI–IX).

G6PD deficiency, widely occurring enzymopathy is known to affect 400 million human population (Mason, 1996). This X-linked inherited disorder is frequently found in regions of African, Asian, Mediterranean, or Middle-Eastern descent. In such condition, RBC becomes unable to counter oxidative stress ultimately leading to hemolysis. The prevalence of G6PD deficiency was observed to be 5%, predominantly in males *i.e.* 9.1% and only 1.79% in female. The prevalence of G6PD deficiency in SC patients (5.26%) was almost similar to that of control group (4.17%) (Memon et al., 2016). Heller et al. (1979) reported that SS may underestimate the true incidence of the genotype for G6PD defect because of the high level of G6PD in hemolytic anemia. The high incidence of G6PD defect in SCD may be the result of a protective influence of the defect on the course of the disease. Praharaj et al. (1977) reported 11% patients with G6PD deficiency, of which 10 were of SC anemia and 2 of SC trait. Kar et al. (1990) screened 60 cases of malaria and observed that sickle Hb was found in 11.5% patients and G6PD deficiency in 5% cases. In present study, mutated as well as normal variant was observed from population, among which mutated variant were observed high in G6PD deficient samples as compared to control (Table III and Fig. XII). This explanation of the findings has important implications on the specific therapy of SCD based on the currently accepted theories of genetic polymorphism.

5. Conclusion

We found that in individuals with SCA and G6PD deficiency, high degree of oxidative stress prevails. Among sickled population HbSS individuals are worst sufferer than HbAS. Similarly individuals with both G6PD deficiency and sickling are worst sufferer than individual with G6PD deficiency only. The population with sickle trait (HbAS) sickle cell disease (HbSS) have also developed genotoxicity but with greater intensity among HbSS, probably due to increased oxidative stress. On the basis of correlation analysis we concluded that there is a positive correlation between SCA and G6PD deficiency among population of Chhattisgarh, India. The novelty of finding is that a very high

degree of positive correlation exists between both conditions which had not been reported in any of previous findings. This study provides base line information for therapeutic application and management for population suffering from SCA and G6PD deficiency.

Declaration of interest

The authors have nothing to declare.

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