NO ASSOCIATION BETWEEN *GSTT1/M1* POLYMORPHISM IN *GLUTATHIONE S-TRANSFERASE (GST)*GENE WITH CATARACT IN HYPERTENSIVE PATIENTS FROM NORTH INDIA

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ABSTRACT
Age-related cataract has globally emerged as the leading cause visual impairment leading to blindness. *Glutathione S-Transferases* and their genetic variantsplay an important role in pathogenesis of cataract. This case-control study was carried out to investigate possible association of *GSTT1/M1* polymorphism with Cataract risk in North Indians. Our study included 221 individuals, 132 as Cataract cases (70 with and 62 without hypertension) and 89 age and ethnicity matched controls. Genetic polymorphism in *GST* gene (*GSTT1/M1* polymorphism) wasevaluated by multiplexPolymerase Chain Reaction (PCR) technique.The frequencies of the *GSTM1-positive* and *GSTT1-positive* in hypertensive cataract cases were 55.71%, 92.86%; while they were 61.29% and 95.16% in cataract cases without hypertension and; 46.07% and 97.75% in healthy controls respectively. The frequencies of *GSTM1-null* and *GSTT1-null* in hypertensive cataract cases were 44.29% and 7.14% %; while they were 38.71% and 4.84% in cataract cases without hypertension and; 53.93% and 2.25% in healthy controls respectively. The frequency of *GSTT1/M1 positive* wild type genotype was 48.57% in hypertensive and 56.45% in normotensive cataract cases while it was 43.82% in control subjects. Our study found no association between *GSTT1/M1* polymorphism with cataract but a nearly significant relationship was observed in *GSTM1 positive* and *GSTM1 null* genotypes (p=0.065) with cataract in subjects without hypertension. The study needs furtherinvestigation due to limited sample size.

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INTRODUCTION
Cataract is one of the most common causes of visual impairment and blindness all over the world. Cataract develops slowly with time and eventually leads to impairment of vision. Age-related cataract makes up80% of all the cataract cases occurring globally. It is characterized by formation of a dense, cloudy area in the lens of the eye due to accumulation of protein clumps that prevents the lens from sending clear images to the retina.Cataract is said to havea multifactorial origin where various environmental and genetic factors contribute to its progression and development.(1)A lot of epidemiologic studies have been conducted globally to understand the pathogenesis of cataract and many of these studies have indicated that hypertension plays an important role in the development of cataract.(2-5) It was reported from a study that hypertension might lead to conformational alteration of the structure of proteins present in lens capsule ,(6) and it may also lead to cataract development through intense systemic inflammation which is causative of cataract. Apart from this, studies have shown that oxidative stress resulting from increase in production of reactive oxygen species and free radicals in the lens also lead to development of cataract.(7) There are many cellular defense mechanisms that protect the human lens from oxidative damage. The glutathione S-transferase (GST) is one of the detoxification enzyme systems and plays important role in inactivating endogenous and exogenous toxic products under oxidative stress. Human GSTs divided into different classes; including mu, theta and omega. Among the various polymorphic variants of *GST* genes, *GSTM1/T1* polymorphisms are the most common and they were found to be significantly associated with various diseases, such as type 2 diabetes mellitus, (8)cataract,(9) rheumatoid arthritis (10), age-related macular degeneration etc.(11)Although several plausible mechanisms have been proposed based on laboratory results, the conclusions from epidemiologic studies remain inconsistent to find out an exact relationship between cataract and *GST* polymorphisms. Thus, this study is aimed to investigate the association of *GSTT1/M1* polymorphism in *GST* gene with cataract among a selected group of hypertensive North Indians.

MATERIALS AND METHODS

Patient's selection

Blood samples of 132cataract patients and 89 healthy controls were collected from the Departmentof Ophthalmology of Era's Lucknow Medical College & Hospital, Lucknow. Data collection was done for each patient on clinical variables including age, alcohol consumption, body mass index, height, weight, cigarettes smoking, family history etc. All subjects with senile cataract had visual disturbance and their corrected visual acuities were under 6/24. A diagnosis of hypertension was based on sustained diastolic blood pressure of >90mmHg and systolic blood pressure of >140 mm Hg. We excluded patients with secondary cataract due to diabetes, trauma, steroid administration, and other causes. Age and ethnicity matched control subjects were collected from unrelated volunteers inthe same clinic. Informed consent was obtained from each subject prior to sample collection and Institutional Ethical clearance was obtained for executing this work, earlier to the recruitment of subjects in this study.

DNA Extraction

Venous blood (4 ml) was collected from all the subjects in 0.5 M vacutainers with EDTA as anticoagulant for DNA extraction and biochemical analysis. Genomic DNA was isolated from whole blood using DNA extraction kit (MACHEREY-NAGE, Germany) following manufacturers protocol. The DNA concentration was determined by aNanodrop 2000 spectrophotometer (Thermo Fisher Scientific) and samples were stored at -20 °C.

Genetic polymorphism Analysis

The *GSTM1* and *GSTT1* genetic polymorphisms were analysed by genomic DNA amplificationusing the multiplex-PCR technique (MJ Mini™Thermal Cycler, Bio Rad Laboratories Inc., Hercules,California, USA). Primers for *GSTM1*were5'AACTCCCTGAAAA GCTAAAGC-3' and 5'-GTTGGGCTCAAATATACG GTGG-3', while primers for *GSTT1* were-5'TTCCTTACTGGTCCTCACATCTC3' and 5'-TCACCGGACTGGCCAGCA-3'.[12] Toavoid false-negative readings, *β-globin* was usedas an internal control. Primers for *β-globin* were5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'. PCR amplificationwas conducted in a total volume of 25 µL using:10 pmol of each primer; 100 ng of genomic DNA;2.5 mmol/L of magnesium chloride; 0.2 mmol/Leach of deoxyribonucleotide triphosphate; and 1 Uof Taq polymerase (Bioline Reagents Ltd). ThePCR amplification was performed using an initialdenaturation at 94 °C for five minutes, followed by 30cycles at 94 °C for one minute, 64 °C for one minute,72 °C for one minute and a final extension of 72 °C forseven minutes. Amplified products were separated ona 1.5% agarose gel stained with 0.5 µg/mL of ethidiumbromide and visualised using a bioimaging and Geldocumentation system (UVP LLC).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version12 (IBM Corp., Chicago, Illinois, USA). Variables were tested for normality using the Kolmogorov-Smirnov test. Variables with a normal distribution were expressed as means ± standard deviation while variables with a non-normal distribution were expressed as medians and inter quartile ranges and were log transformed before statistical analysis. AChi-squared test was used to compare geno typing data between cases and controls. A P value of ≤0.050was considered significant. The odds ratio (OR) and 95% confidence interval (CI) was calculated to test the relative risk for association. Other variables were compared using the Student's t-test for normally distributed variables.

RESULTS

Our study included 221 individuals, 132 as Cataract cases (70 with and 62 without hypertension) and 89 age and ethnicity matched controls. Clinical and biochemical parameters of cases and controls are shown in Table 1.In *GST* genetic polymorphisms, the band sizes observed were 215 bp, 480 bp and 268 bp for *GSTM1, GSTT1* and *β-globin* respectively (Figure 1). Negligible discrepancy in genotyping was observed.

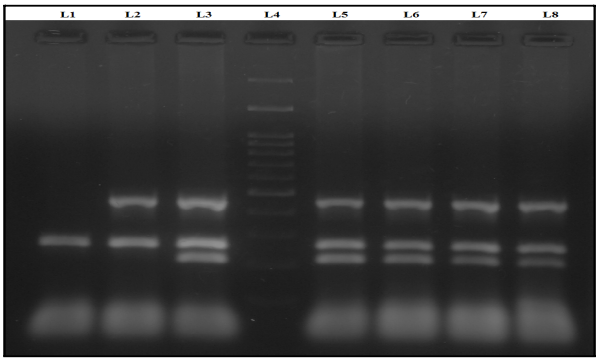

Fig. 1: 2%Agarose gel image showing PCR products of *glutathione S-transferase* (*GST*) gene polymorphisms. The presence or absence of *GSTM1* and *GSTT1*genotype was detected by 480 base pairs (bp) and 210 bp bands, respectively. *β-globin* was considered an internal control at 260 bp. Lane 1 (L1) shows *β-globin*gene, Lane 2 (L2) shows *GSTM1 null* genotype, and Lanes3,5-8 (L3, L5- L8) shows*GSTM1* and *GSTT1* wild-type genotypes. Lane 4 is a 100 bp ladder.

GSTT1/M1 polymorphism Analysis

The *GSTM1-null* genotype frequency was 53.93% in healthy controls which was higher than the frequency in cataract cases with hypertension (44.29%) (OR = 0.68; 95% CI = 0.36–1.27; x^2 = 1.46; power = 0.227) and nearly significantly higher than cataract cases without hypertension (38.71%)(OR = 0.54; 95% CI = 0.28-1.04; x^2 = 3.39; power = 0.065). The frequency of the *GSTT1-null* genotype was 7.14% (OR = 3.35; 95% CI = 0.63-17.79; x^2 = 2.23; power = 0.135) among hypertensive cataract cases as and 4.84% (OR = 2.21; 95% CI = 0.36–13.64; x^2 = 0.76; power = 0.381) among the normotensive cataract cases as compared to 2.25% in healthy controls, respectively.The frequencies of *GSTM1* and *GSTT1 positive* genotypes were 55.71 % and 92.86% (OR = 1.47 and 0.3; 95% CI = 0.78-2.76 and 0.06-1.59; x^2 = 1.46 and 2.23; power = 0.227 and 0.135 respectively) in hypertensive cataract cases, while they were 61.29% and 95.16% (OR = 1.85 and 0.45; 95% CI = 0.96-3.58 and 0.07-2.79; x^2 = 3.39 and 0.77; power = 0.065 and 0.381) in normotensive cataract caseswhile the frequencuies of *GSTM1* and *GSTT1 positive* genotypes were 46.07% and 97.75% in the control group, respectively.The frequency of *GSTT1/M1* genotype was 43.82% in controls which was somewhat less than the frequency observed in cataract patients with hypertension (48.57%)(OR = 1.21; 95% CI = 0.66-2.27; x^2 = 0.36; power = 0.551). However, the frequency of *GSTT1/M1* genotype in cataract cases without hypertension was comparatively higher (56.45%) (OR = 1.66; 95% CI = 0.86-3.20; x^2 = 2.33; power = 0.127) than controls (43.82%). Data shown in Table 2.

DISCUSSION

Oxidative stress is a major factor involved in various processes resulting in degradation of lens protein involved in cataractogenesis ultimately leading to the development of age-related cataract.This cellular damage was found to be further alleviated by the cellular defense mechanisms involving GST enzymes which play a major role in cell detoxification. Various polymorphisms in *GST* gene have now beenconsidered as risk factors for age-related cataracts, but the results still remain controversial when comparing different ethnic groups. (12)

Sekine and colleagues in 1995 reported for the first time that *GSTM1* null genotype was associated with cataract risk (13). Recently this result was confirmed by a meta-analysis in Asians (14) however, another meta-analysis after including one more study on Asians (15) did not confirm this associationthus the results have been very inconsistent. However a study from north India confirmed significant association between *GSTM1 null* genotype frequency with cataract risk wherethe frequency of *GSTM1 null* genotype was found to be 33.01% (p=0.003) which is similar to results from our finding where we found a nearly significant association of *GSTM1* null genotype in our cataract cases without hypertension (38.71%; p=0.065) however this frequency was lower when compared to Chinese population (52.4%). In our study *GSTM1 positive* genotype was not found to be associated with risk of cataract in hyperetensive cases (p=0.227) which is contradictory to findings from Estonian (16) Iranian (17), Chinese (18) and Egyptianpopulations.(19) However the frequency of *GSTM1 positive* genotype in our cataract cases without hypertension which was found to be nearly significant (61.29%; p=0.065) was similar to its frequency in another study from north India (62.13%) where it was found to be significantly associated with risk of cataract (p=0.025).(9) Similarly the frequency of *GSTT1/M1* wild type genotype was 56.45% in our cataract cases without hypertension which is similar to results from north Indian study where it was found to be 53.39%.(9) Our study showed no significant association between *GSTT1 null* genotype with cataract in both hypertensive and normotensive cases (p=0.135, p=0.38 respectively) which is similar to results from Iranian (17) Turkish (20),Estonian (16) and Chinese populations.(18) The frequency of *GSTT1positive* genotype in our study was found to be 92.86% and 95.16% in hyperetensive and normotensive cataract cases respectively which was higher than the frequencies observed in other studies from north India (86.40%),(9) Iran (73.65%) (17) and Estonian population (85.5%).(16)

On comparing the genotype frequencies of *GSTT1/M1* polymorphism among hypertensive and normotensive cataract cases the results were found to be insignificant. However, the frequencies were found to be quite different among both the groups where the frequency of *GSTT1/M1 positive* genotype was 48.57% in hypertensive cases and 56.45% in normotensive cases. Similarly, the frequencies of *GSTM1 positive* and *GSTM1null* genotypes were 55.71% and 44.29% in hypertensive cataract cases respectively which was quite different from the frequencies observed in cataract cases without hypertension (61.29% and 38.71% respectively) There are many factors which might contribute to the disparities in the results of various genetic polymorphism studies and these limitations also inherent to our study like the number of study subjects.(20)

Table 2: Comparison of frequencies of *Glutathione S-Transferase (GST)* genetic polymorphisms among hypertensive and normotensive Cataract cases with healthy controls(n= 221)

	Control (n=89)		Cataract with Hypertension (n=70)						
Genotype/ Allele	**n**	**Frequency (%)**	**n**	**Frequency (%)**	**OR**	**95% CI**	**c^2**	**p values**	**Bonferroni corrected p values**
GSTT1-positive, GSTM1-positive	39	43.82	34	48.57	1.21	0.66-2.27	0.36	0.551	0.551
GSTT1-null	2	2.25	5	7.14	3.35	0.63-17.79	2.23	0.135	0.135
GSTM1-positive	41	46.07	39	55.71	1.47	0.78-2.76	1.46	0.227	0.227
GSTT1 -Positive	87	97.75	65	92.86	0.3	0.06-1.59	2.23	0.135	0.135
GSTM1-null	48	53.93	31	44.29	0.68	0.36-1.27	1.46	0.227	0.227

	Control (n=89)		Cataract without hypertension (n=62)						
Genotype/ Allele	**n**	**Frequency**	**n**	**Frequency**	**OR**	**95% CI**	**c^2**	**p values**	**Bonferroni corrected p values**
GSTT1-positive, GSTM1-positive	39	43.82	35	56.45	1.66	0.86-3.20	2.33	0.127	0.127
GSTT1-null	2	2.25	3	4.84	2.21	0.36-13.64	0.76	0.381	0.381
GSTM1-positive	41	46.07	38	61.29	1.85	0.96-3.58	3.39	**0.065**	0.065
GSTT1 -Positive	87	97.75	59	95.16	0.45	0.07-2.79	0.77	0.381	0.381
GSTM1-null	48	53.93	24	38.71	0.54	0.28-1.04	3.39	**0.065**	0.065

	Cataract without hypertension (n=62)		Cataract with Hypertension (n=70)						
Genotype/Allele	**n**	**Frequency (%)**	**n**	**Frequency (%)**	**OR**	**95% CI**	**c^2**	**p values**	**Bonferroni corrected p values**
GSTT1-positive, GSTM1-positive	35	56.45	34	48.57	0.73	0.37-1.45	*0.82*	0.366	0.366
GSTT1-null	3	4.84	5	7.14	1.51	0.35-6.61	0.31	0.58	0.580
GSTM1-positive	38	61.29	39	55.71	0.79	0.40-1.59	0.42	0.517	0.517
GSTT1 -Positive	59	95.16	65	92.86	0.66	0.15-2.89	0.31	0.58	0.580
GSTM1-null	24	38.71	31	44.29	1.26	0.63-2.52	0.42	0.517	0.517

recruited; the sub categorization of study subjects and inclusion and exclusion of various clinical and lifestyle factors.

CONCLUSION

Our study shows no significant association of GSTT1/M1 polymorphism with hypertensive cataract patients from North India. However, due to small sample size we suggest that further epidemiologic studies on a much larger sample size are needed to test the validity of our study.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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REFERENCES

1. Shiels A, Hejtmancik JF. Genetic origins of cataract. Arch Ophthalmol. 2007; (125),165–73.
2. Tsai SY, Hsu WM, Cheng CY, Liu JH, Chou P.Epidemiologic study of age-related cataracts among an elderly Chinese population in Shih-Pai, Taiwan. Ophthalmology. 2003;(110);1089–1095.
3. Shah SP, Dineen B, Jadoon Z, Bourne R, Khan MA.Lens Opacities in Adults in Pakistan: prevalence and risk factors. Ophthalmic Epidemiology. 2007;(14);381–389.
4. Rim TH, Kim MH, Kim WC, Kim TI, Kim EK.Cataract subtype risk factors identified from the Korea National Health and Nutrition Examination survey 2008–2010. BMC Ophthalmol. 2014;(14);4.
5. Mukesh BN, Le A, Dimitrov PN, Ahmed S, Taylor HR, et al. Development of cataract and associated risk factors. Arch Ophthalmol. 2006;(12);4 79-85.
6. Lee SM, Lin SY, Li MJ, Liang RC.Possible mechanism of exacerbating cataract formation in cataractous human lens capsules induced by systemic hypertension or glaucoma. Ophthalmic Res. 1997;(29);83–90.
7. Ottonello S, Foroni C, Carta A, Petrucco S, Maraini G. Oxidative stress and age-related cataract.Ophthalmologica. 2000;(214),78–85.
8. Raza ST, Abbas S, Ahmad F, Ahmed ZH Zaidi and Mahdi F .Association of Glutathione-S-Transferase (GSTM1 and GSTT1) and FTO Gene Polymorphisms with Type 2 Diabetes Mellitus Cases in Northern India. Balkan J Med Genet. 2014;17(1);47–54
9. Chandra A, Raza ST, Abbas S, Singh L, Rizvi S, Ahmed F, Eba A et al. Polymorphism of GST and FTO Genes in Risk Prediction of Cataract among a North Indian Population. Ophthalmic Genet2016;37(1);19-24.
10. Ji JD and LeeWJ. Association between the polymorphisms of glutathione S-transferase genes and rheumatoid arthritis: a meta-analysis. Gene. 2013;521(1);155–159.
11. Güven M, Görgün E, Ünal M et al. Glutathione S-transferase M1, GSTT1 and GSTP1 genetic polymorphisms and the risk of age-related macular degeneration. Ophthalmic Research. 2011;46(1); 31–37.
12. Rizvi S, Raza ST, Siddiqi Z, Abbas S and Mahdi F. Association of Angiotensin-Converting Enzyme and Glutathione S-Transferase Gene Polymorphisms with Body Mass Index among Hypertensive North Indians. Sultan Qaboos Univ Med J. 2015;15(4);e477–e485.
13. Sekine Y, Hommura S, Harada S. Frequency of glutathione-S-transferase 1 gene deletion and its possible correlation with cataract formation. Exp Eye Res. 1995;(60);159–163.
14. Sun L, Xi B, Yu L, Gao XC, Shi DJ, Yan YK, et al. Association of glutathione S-transferases polymorphisms (GSTM1 and GSTT1) with senile cataract: a meta-analysis. Invest Ophthalmol Vis Sci. 2010;(51);6381–6386.
15. Sun W, Su L, Sheng Y, Shen Y and Chen G. Is there association between Glutathione S Transferases polymorphisms and cataract risk: a meta-analysis? BMC Ophthalmol. 2015;(15);84.
16. Juronen E, Tasa G, Veromann S, et al. Polymorphic glutathione S-transferases as genetic risk factors for senile cortical cataract in Estonians. Invest Ophthalmol Vis Sci. 2000;(41);2262–2267.
17. Saadat I, Ahmadi Z, Farvardin-Jahromi M, Saadat M. Association between cataract and genetic polymorphisms of GSTM1, GSTT1, and GSTO2 with respect of work place. Mol Vis. 2012;(18); 1996–2000.
18. Jiang Z, Liang K, Zhang Q, Tao L.Glutathione S-transferases polymorphisms confer susceptibility to senile cortical cataract in the Han Chinese population. Mol Vis. 2012;(18); 1247–1252.
19. Abdel Azeem AA, Mahmoud AA, Salaheldine MM, Amr K. Implication of glutathione S-transferase M1 and T1 polymorphisms in the development of senile cataract among Egyptians. .Bratisl Lek Listy. 2009;110(11); 678-83.
20. Gűven M, Unal M, Sarici A, et al. Glutathione-S-transferase M1 and T1 genetic polymorphisms and the risk of cataract development: a study in the Turkish population. Curr Eye Res. 2007; (32);447–454.