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Original Research Article

ASSOCIATION OF METHYLENE TERTAHYDOFOLATE REDUCTASE GENE C677T POLYMORPHISM, SERUM HOMOCYSTEINE, VITAMIN B_{12} AND FOLATE LEVELS IN PATIENTS OF AGE RELATED CATARACT

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ABSTRACT

BACKGROUND:

Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms have been shown to influence serum levels of homocysteine, Vit B_{12} and Folate. Homocysteine has been implicated as a cataractogenic stressor while reduced serum levels of Vit B_{12} and Folate have been found to be associated with cataract cases.

OBJECTIVE:

To investigate the associations of MTHFR C677T gene polymorphisms, serum homocysteine levels, serum Vit B_{12} and serum folate levels in patients with age related cataract.

PATIENTS AND METHODS:

This study was carried out in Eye Department of Swami Dayanand Hospital. Dilshad Garden, Delhi from 1st December 2020 till 30th September 2021 as a case cohort study. 40 cases with cataract and 40 healthy individuals without cataract were enrolled. Informed consent and detialed history were obtained from all participants and a comprehensive examination was done. They were subjected to estimation of MTHFR gene (C667T) polymorphism, Serum Homocysteine, Vitamin B12 and Folic acid and all the biochemical tests were carried out at Institute of Human Behavior and Allied Sciences (IHBAS), Delhi.

RESULTS:

This study included a total of 40 cases with cataract and 40 matched controls with mean age of 57.65±6.52 and 55.45±4.67 years respectively. We observed no significant difference in serum homocysteine, folic acid and vitamin B 12 levels between cases and controls. However, MTHFR C677T gene polymorphism in cataract cases were significantly different from that of controls (p=0.03).

CONCLUSION AND RELEVANCE:

This study is the first to investigate genetic association of MTHFR gene polymorphism with age related cataract in a subset of Indian population. Statistically significant difference of MTHFR C677T gene polymorphism in cataract cases and control was observed. If this finding is supported by future research with larger sample sizes, high quality, and broader ethnicity coverage, then MTHFR C677T gene polymorphism may represent new candidate biomarkers for high-risk ARC population.

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1. INTRODUCTION

Cataract is characterised by opacification of lens or its capsule obscuring the passage of light. It results from destruction of crystalline microstructure of lens owing to long term metabolic disorders.[1] Age Related Cataract (ARC) or Senile cataract remains the leading cause of blindness worldwide. In India it accounts for 50-80% of bilaterally blinds in the country.[2]
Catarectogenesis is a complex process and is considered as the result of the combined effect of congenital genetic factors and acquired environmental factors. Systemic oxidative stress has been considered to play an important role in cataract formation.[3] Recently focus of studies have been shifted to find association of genetic polymorphism and cataract susceptibility.[4]
High plasma level of homocysteine is considered an important oxidative marker and has been found to be associated in the pathogenesis of diabetes, Alzheimer's disease, renal diseases etc.[5] The key enzyme in homocysteine metabolism is Methylene Tetra Hydro Folate Reductase (MTHFR) which catalyzes irreversibly conversion of 5,10-MTHF to 5-MTHF, the main circulatory form of folate. Human MTHFR gene is located on chromosome 1p36.3. C667T polymorphism of MTHFR gene is a C-to-T (Cytosine to Thymine) transition at nucleotide 677 (C677T) in exon 4, which results in an alanine (Ala) to valine (Val) substitution in the MTHFR enzyme and makes the enzyme thermo-labile and less active which leads to hyperhomocysteinemia and hypomethylation in homozygous mutant state and this makes it an important marker for thrombotic events. Functionally, MTHFR enzyme activity is reduced 35% with the heterozygous CT genotype and 70% with the variant TT genotype. Polymorphisms in the MTHFR gene, including mainly C677T and to some extent A1298C A/V, have been shown to be associated with increased homocysteine levels and has been linked to other ophthalmologic disorders such as glaucoma, retinal arterio-venous occlusion, age related macular degeneration (ARMD) and retinal emboli.[6,7,8,9,10,11]
There is however little data available on the role of MTHFR polymorphism in cataract formation. This study was thus conducted to investigate the association of C677T polymorphism in MTHFR gene, serum homocysteine, folate and vitamin B_{12} level in patients of ARC.

2. PATIENTS AND METHODS

PATIENTS

This study was carried out in Eye Department of Swami Dayanand Hospital. Dilshad Garden, Delhi from 1st December 2020 till 30th September 2021(Ten months). All the biochemical tests were carried out at Institute of Human Behavior and Allied Sciences (IHBAS), Delhi. The study was approved by the Ethical Committee of the hospital.
Informed consent from all participants regarding the study was obtained. A detailed clinical history was taken & comprehensive ophthalmic examination was performed. Distant vision was assessed using Snellen's chart, Near vision was assessed using hand held Jaeger's chart.
Cataract was diagnosed based on the diagnostic criteria for cataract in Ophthalmology:

1. Gradual decline of visual acuity, fixed black spots in front of eyes, monodiplopia.
2. Slit lamp examination of dilated pupil.

INCLUSION CRITERIA

- Patients with unilateral or bilateral cataract with vision loss less than 6/18.
- Age of patients and control subjects equal to and above 50 years.

EXCLUSION CRITERIA

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- Individuals with complicated and traumatic cataract.
- Individuals on anti diabetic treatment.
- Patients with coronary artery disease and any malignancy.
- Individuals taking multivitamin supplements.

GROUPS FOR STUDY

STUDY DESIGN:

This study is a case – control study.

Based upon the presence of cataract, participants were categorised into two groups

1. Group A (Case) - Patients with cataract
2. Group B (Control) - Patients without cataract

About 10 ml of venous blood sample was collected from patients and control subjects for biochemistry profiles namely –

- MTHFR gene (C667T) polymorphism,
- Serum Homocysteine,
- Vitamin B12 and
- Folic acid.

In case of delay, sample were separated and stored at -20degree.

- **SERUM HOMOCYSTEINE ASSESSMENT -** Quantification of homocysteine level analysis was done using the Dialab homocysteine enzymatic cycling reagent kit on CDx 90 mass photometric system of Thermo Fisher. Sensitivity and specificity quality control was maintained by running three level control sera provided along with the thermo scientific kits. The test has been developed to determine homocysteine concentration within a range of 3-50µmol/l while the lower limit of detection is 0.4µmol/L. A concentrations between 12-15 µmol/L was used as the cut-off value for the normal level of homocysteine for adult.

- **SERUM VITAMIN B_{12} ASSESSMENT-**Vitamin $B_{12}$was measured in serum by commercial available kit by Chemiluminicent analyzer, Cobas e611, Roche Pvt.Ltd. method as per given in kit protocol.

- **SERUM FOLATE ASSESSMENT-** Folate levels were measured in serum by commercial available kit by Chemilumoniscent analyser, Cobs e611, Roche Pvt. Ltd. Method as per given in kit protocol.

- **DNA EXTRACTION AND GENETIC POLUMORPHISM STUDIES-** DNA extraction was done by commercially available kit. PCR based allele specific primer or restriction fragments length polymorphism method was used to study the polymorphic variation in these genes leading to change.

- **GENOTYPE ASSESSSMENT-METHYLTETRAHYDROFOLATEREDUCTASE MTHFR-**A PCR based method was used to determine MTHFR C677T polymorphism using the primer 5'TGA AGG AGA AGG TGA ATG AGG GA -3' AND 5'-AGG ACG GTG CGG TGA GAG TG-3'. The 198 bp PCR product was digested At 37 degree Celcius with Hinfl restriction enzyme according to the manufacturer's instructions.

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STATISTICAL ANALYSIS

Data was compiled using MS Excel and analysed using IBM SPSS software version 20. Categorical data was expressed as frequency and proportions whereas continuous data was expressed as mean/median and standard deviation/ interquartile range (IQR). The two groups were assessed for normality distribution. Independent Student t test for normally distributed and Mann Whitney U test (Wilcoxon signed-rank test) for non normally distributed data was used to assess the difference in continuous variables. Chi square test was applied to find out the association of categorical variables across cases and controls. P value less than 0.05 was considered statistically significant.

TABLE 1: Demographic variables

Demographic variables		**GROUP-A Case (n=40)**	**GROUP-B Control (n=40)**	**P-Value**
Sex	Male	13 (32.5)	7 (17.5)	0.12
	Female	27 (67.5)	33 (82.5)	
Age (years)	50–54	13 (32.5)	17 (42.5)	0.33
	55–59	7 (17.5)	10 (25)	
	60–64	15 (37.5)	12 (30)	
	≥65	5 (12.5)	1 (2.5)	

TABLE 2: Biochemical Variables

Biochemical variables [Median (IQR)]	**GROUP-A** Case (n=40)	**GROUP-B** Control (n=40)	P-Value
Serum Homocysteine	13.50(9.00)	17.00(11.50)	0.16
Serum Folic Acid	6.50(5.30)	7.15(6.80)	0.50
Serum Vitamin B12	325.50(262)	354.50(313)	0.97
No Statistical significant difference was observed in Biochemical variables were observed between Case & Control groups.			

TABLE 3: Gene Polymorphism

GENE POLYMORPHISM	**GROUP-A CASE (n= 40)**	**GROUP-A CONTROL (n=40)**	**P- VALUE**
CC	55.0%	77.5%	0.03
CT	45.0%	22.5%	
GENE POLYMORPHISM WAS STATISTICALLY SIGNIFICANT BETWEEN CASE AND CONTROL GROUPS			

3. DISCUSSION:

Blindness and MSVI has been has been found to impose immediate productivity losses on the Indian economy and is considered major barrier toward reaching the country's growth goals such as becoming a $5 trillion economy by 2024–2025.[12] Cataract being the leading cause of blindness, has always been the part of research for obvios reasons.

Environmental and genetic factors have been found to be confirmed contributing to the pathogenesis of ARC.[13.14] Gene polymorphism has been recognized as a significant factor in the development of cataract and recently the focus of research has shifted correlate association of incidence of cataract with various gene polymorphism.[4] Researchers have successfully documented risk of developing catarct with polymorphism of DNA repair genes

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XPD and XRCC1, 8-oxoguanine DNA glycosylase gene Ser326Cys polymorphism and MMP-2 gene polymorphism.[15,16,17,18]

Systemic oxidative stress has been considered to play an important role in cataract formation and High plasma level of homocysteine is considered an important oxidative marker.[3,5] MTHFR (Methylene Tetra Hydro Folate Reductase) is the key enzyme of Homocystein Metabolism. Polymorphisms in the MTHFR gene, including C677T have been shown to be associated with increased homocysteine levels. Functionally, MTHFR enzyme activity is reduced 35% with the heterozygous CT genotype and 70% with the variant TT genotype.[8]

Sen S K et al observed a significantly high serum level level of homocystein while significantly low level of serum folate in ARC as compared to control group.[19] Tan A G et al in also found that higher prevalence of PSC (Posterior Subcapsular Cataract) was associated with higher serum homocystein and lower serum folate.[20] Ava Grace Tan et al were also of the opinion that MTHFR polymorphism and elevated homocysteine levels contributed separately and jointly to increased risk of cortical cataract.[21]

Current study was conducted as a case control study on a total of 40 cases with age related cataract and sex matched controls. with a hypothesis that MTHFR gene polymorphism could be one of the plausible factor for development of age related cataract. We also compared serum level of homocysteine, folate and Vitamin B_{12} in patients of age related cataract with the reference group. The study found a statistically significant genotype distribution of MTHFR C677T in cataract cases and control group. However, difference in serum levels of homocysteine, folate and Vitamin B_{12} between ARC and control group were not of found to be of significance as observed in other studies. This may be explained by the observations made by Ava Grace Tan et al as they could attribute only 33% incidence of cortical cataract to indirect pathway of MTHFR polymorphism via elevated Homocysteine levels while rest 67% to the direct pathway.[21] Wang, Xue-bin et al observed that variants in MTHFR gene might individually and jointly influence susceptibility to ARC by affecting MTHFR enzyme activity and tHcy levels.[22]

Although our study found that MTHFR C677T gene polymorphism might be related ARC, this stiudy has several limitations. The population was hetrogenous and did not take consideration of socioeconomic status, dietary habits and environmental factors. Lastly, other restriction sites of MTHF gene may be involved in development of cataract.

In summary, we conclude that MTHFR C677T gene polymorphism may represent new candidate biomarkers for high-risk ARC population, however additional original research with larger sample sizes, high quality, and broader ethnicity coverage remain anticipated.

4. CONCLUSION:

Our study is the first to investigate genetic association of MTHFR gene polymorphism with age related cataract in a subset of Indian population. We observed a statisically significant association between MTHFR C677T gene polymorphism and ARC cases as compared to the reference group. Thus it may represent a new candidate biomarkers for high-risk ARC population. C677T mutation is irreversible, thus steps should be taken how to mitigate the reduced activity of C677T gene encoding enzyme or adopt a replcement therapy in order to protect high - risk population from developing ARC.

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