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Association between Methylenetetrahydofolate Reductase C677T Polymorphisms, Plasma Homocysteine Levels, and Primary Open-Angle Glaucoma in Jordanian Patients

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Primary open-angle glaucoma (POAG) is a chronic, progressive optic neuropathy, recognized as the second most common cause of irreversible blindness globally. It is marked by the gradual degeneration of retinal ganglion cells, leading to optic nerve head cupping, optic disc excavation, and progressive visual field loss.The study aimedto investigate the relationship between POAG, methylenetetrahydofolate reductase (MTHFR) C677T polymorphism, and levels of plasma homocysteine,a well-known vascular risk factoramongstPOAG Jordanian patients. This case-control study included 183 participants: 89 POAG patients and 94 non-glaucomatous controls. In total, blood samples from allparticipants were analyzed for assessing plasma homocysteine levels and MTHFR C677T polymorphisms. Homocysteine levels were measured using ELISA, while the MTHFR C677T polymorphismswere identified through polymerase chain reactionrestriction fragment length polymorphism (PCR-RFLP) analysis.Ourfindingsrevealed thatplasma homocysteine levels and hyperhomocysteinemia rate were significantly higher in POAG patients versus the controls ($p<0.05$). In contrast, no significant differences were identified in allelic frequency and MTHFR C677T polymorphismsdistribution between POAG patients and controls.Elevated homocysteine levels and a higher hyperhomocysteinemia prevalence have been observed in POAG patients, suggesting a potential role in the disease's pathogenesis. Furthermore, a statistically significant association and increased risk linked to the CT and TT polymorphisms, as well as T allele, indicate that MTHFR C677T polymorphisms may contribute to POAG development in the Jordanian population.

Keywords: Alleles; Homocysteine; MTHFR C677T; Polymorphisms; POAG.

Glaucoma is an irreversible optic neuropathy that can cause permanent loss of the visual field.[1]In 2010, an estimated 4.5 million and 3.9 million individuals with POAG and primary angle-closure glaucoma (PACG), were bilaterally blind,furthermoreby the year 2020, these figures had increased to 5.9 million and 5.3 million, respectively.[2-4]

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Higher intraocular pressure (IOP) is widely recognized as the most significant modifiable risk factorfor glaucoma onset and itsprogress,[5]particularly POAG. Among patients with primary glaucoma, IOP typically peaks in the morning and gradually decreases through the day in non-clinical settings.[6]

Primary glaucoma is broadly categorized into two main types: POAG,the most common oneand PACG.[2]The main risk factors for primary open-angle glaucoma (POAG) are elevated intraocular pressure (commonly above 21 mmHg), a thin central corneal thickness (under 500 ìm), a family history of the disease, black ethnicity, and high levels of myopia..[7]

Various genetic studies have revealed genetic polymorphismsofdifferent genes withuncertain effects that are linkedtoPOAG includingC677T polymorphisms ofMTHFR gene,[8]some variants of genes encoding formembrane palmitoylated protein [7] (MPP7), tumor protein p53,[9] GRIN2B gene 421C/A, Arg72Pro,[10] and soluble guanylate cyclase sGCá1.[11]

MTHFR enzyme plays vital role in regulating folate,homocysteine (HCY) and methionine metabolism and in maintaining normal plasma HCY levels.[12] HCY is a key intermediate in the metabolism of methionine and cysteine, it is generated through the hydrolysis of S-adenosylhomocysteine in the cycle of methionine. MTHFR catalyzes 5,10- methylenetetrahydofolate reduction to 5 methylenetetrahydrofolate which is the main circulating form of folate and the donor of methyl groups forremethylatingHCY to methionine inareaction catalyzed by a B12-dependent methionine synthaseenzyme.[13]

A 677C/T mutation atposition222inexon 4 of MTHFR gene results in convertingalanine to valine codon. Asa consequence of this mutation, MTHFR enzymatic activityisreducedcomparable to the wild type (CC) enzyme[14]and the enzyme's thermolabilityincreases, [15] thus, mutations ofMTHFR gene are associated with higher levels of HCY.[16]

Elevated plasma levels of HCY have been observed in glaucoma patients.[17]HCY has been implicated in vascular injury[18]alterations of the extracellular matrix,[19] and neuronal cell death due toexcitotoxicity and apoptosis,[20]also, hyperhomocysteinemia has also been associated with structural remodeling of connective tissues[21] and various neurodegenerative disorders.[22]

The aim of this study was to examine the potential link between MTHFR C677T polymorphisms and plasma HCY levels in individuals with POAG, with the objective of enhancing our understanding of the disease's underlying pathogenic mechanisms.

MATERIALS AND METHODS

183 subjects participated in this study, amongthem 89 POAG patients and 94 non-glaucomatous individuals who served as the control group. All participants were recruited from the Outpatient Clinics of Special Surgeries Department / Ophthalmology Division at Al-Karak Governmental Hospital, Al-Karak, Jordan. The exclusion criteriafrom the study includedpatientswho had any ocular diseases other than glaucoma that could elevate IOP, or if they had cancer, diabetes mellitus, and chronic kidney disease, or patients who receivedimmunosuppressants, antimicrobials, hormonal therapies, lipid-lowering drugs, vitamin supplements,anticonvulsants and antidepressants treatment within six months prior to the study. All participants (both patients and controls) weresubjectedto complete ophthalmic examination, includingtesting of visual acuity with the Humphrey 24-2 protocol, slit-lamp biomicroscopy, measurement ofIOP using a Goldmann applanation tonometer, assessment ofcentral corneal thickness via pachymetry, gonioscopy with a Goldmann 3-mirror lens, optic nerve imaging byOCT (optical coherence tomography).Patients were diagnosed with POAG based on optic nerve damage confirmed by OCT, along with an elevated IOP greater than 21 mmHg, and an open anterior chamber angle on gonioscopy according to Shaffer angle grading. The POAG group included 89 patients (51 males and 38 females), aged between40and74 years, with mean±SD 58.73 ± 9.47 years. Control group consisted of individuals with healthy-appearing optic discs, IOP below 21 mmHg, a cup-to-disc ratio less than 0.4, and normal visual field assessments in both eyes. None of the controls had a history of using IOP-lowering medications or a family history of glaucoma, the group included 94 participants (55 males and 39 females), with ages

between 42 and 76 years with mean±SD 59.26 ± 10.39 years.

Biochemical Analyses

Plasma HCY assay

12 hours fasting blood sample was collected from eachparticipant inthe studyin EDTA-treated tubes for plasma HCYestimation,then,immediately centrifuged at 4°C, plasma fraction was withdrawnforstorage at –20°C until further analysis while,cellular portion was preserved for extraction ofDNA.

Plasma HCY levels were measured using ELISA, according to the method adopted by Engvall et al.[23]. Hyperhomocysteinemia was defined as a HCY concentration exceeding 15 ìmol/L.[24]

MTHFR polymorphisms analysis

Basedon Lahiri and Nurnberger,[25] method,genomic DNA was extracted from leukocytes,then,MTHFR C677T polymorphisms in the gene wereanalyzed using PCR-RFLP technique, as reported in a previous study.[26] The sequences oftheprimers used for amplification ofa 198 bp fragment of the targeted DNA were: Forward: 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' Reverse: 5'-AGG ACG GTG CGG TGA GAG TG-3'.

PCR was initiated with denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was performed at 72°C for 7 minutes.

5 units of *HinfI* enzyme were used for digesting PCR products for 12 hours at 37°C. Electrophoresis was performed on a 2% agarose gel. The digestion yielded a single 198 bp band for CC polymorphism (wild-type), two bands of 175 bp and 23 bp for CTpolymorphism(the heterozygous-type), and a 175 bp and 23 bp for TT polymorphism(homozygous mutant). The 23 bp fragment cannotbeseenbecause of itstoo small size.

Statistical analysis

SPSS software package version 25.0was used for dataanalysiswhich were presented as mean±SD, while, variables in categorieswere expressed as percentages,student's t-test wasappliedforthe assessmentof thedifferences between continuous variables,whereas Chi-square ($\div^2$) test wasused to evaluate the differences between categorical variables and totest Hardy-Weinberg equilibrium by comparingthe observed and expected polymorphisms frequencies. A p-value less than 0.05 was regarded as statistically significant.

RESULTS

Table 1 shows insignificant differences between POAG patients and controls in terms of age, hypertension, diabetes mellitus, or cardiovascular disorders. However, the differences in plasma HCY levels and hyperhomocysteinemia rates were significant.

Table 1. Thebiologicaland clinical characteristics of participants in POAG and control groups

Variables	POAG patients (no. 89)	Controls (no. 94)
Gender	Male/female (51/38)	Male/female (55/39)
Age (range) (mean±SD)	40-74 (58.73±9.47)*	42-76 (59.26±10.39)
Hypertension	53 (60.1%)*	55 (58.6%)
Diabetes mellitus	40 (44.9%)*	45 (47.4%)
Cardiovascular disorder	22 (24.4%)*	13 (13.5%)
Homocysteine (ìmol/l) (mean±SD)	29.59±1.49**	10.14±0.96
Hyperhomocysteinemia	33 (37%) **	19 (20.3%)

* P >0.05 is insignificant versus the controls

** P <0.05 is significant when compared to the controls

Table 2. Distribution of polymorphisms ofMTHFR C677T and allelic frequencies in POAG patientsand control groups

Groups	C677T Polymorphism			Allele	
	**CC (no.) %	**CT (no.) %	**TT (no.) %	**C (no.) %	**T (no.) %
POAG group (no. 89)	43 (48.8%)	28 (31.5%)	18 (19.7%)	114 (64.0 %)	64 (36%)
Controls (no. 94)	54 (57.9%)	25 (26.3%)	15 (15.8%)	189 (70.7%)	55 (29.%)
Odds ratio (95% CI)	1.00	1.38 (0.98-1.95)	1.21(0.87-1.88)	1.00	1.53 (0.97-2.49)
P*	0.461	0.633	0.662	0. 316	0.405

*χ^2 test

**CC (Cytosine-Cytosine), TT (Thymine-Thymine), CT (Cytosine-Thymine), C (Cytosine), T (Thymine).

Table 2 presents C677T polymorphisms'distribution and frequencies of alleles in POAG patients and controls. Among POAG patients, the polymorphisms distribution was 48.8% CC, 31.5% CT, and 19.7% TT. In the control group, the distribution was 57.9% CC, 26.3% CT, and 15.8% TT. Also, the results revealed significant association and positive risk ratio between CT, TT polymorphisms and T allele in POAG patients (OR=1.38, 95%CI=0.98-1.95), (OR=1.21, 95%CI=0.87-1.88) (OR=1.53, 95%CI=0.97-2.49), respectively, while, no significant risk ratio regarding CC polymorphism and C allele was detected.

DISCUSSION

Glaucoma represents a group of chronic eye disorders distinguished by the progressive loss of retinal ganglion cells (RGCs), thinning of the retinal nerve fiber layer, and a gradual decline in visual field. Among these conditions, POAG is the most common type and has been the focus of extensive research.The etiology of POAG is complex and multifactorial, encompassing interactions between environmental influences and significant genetic predisposition. Family linkage analyses have revealed at least 17 genetic loci associated with the disease. Within this framework, MTHFR gene emerges as a compelling candidate due to its critical function in one-carbon metabolism and its regulatory role in maintaining HCY levels.[9, 27]

Our findings demonstrated that plasma homocysteine (HCY) levels were significantly elevated in Jordanian patients with primary open-angle glaucoma (POAG) compared to healthy controls. This observation suggests a potential association between hyperhomocysteinemia and POAG, indicating that elevated HCY may contribute to the disease's pathogenesis. Consistent with this hypothesis, Leibovitch et al. [2x] reported a higher prevalence of hyperhomocysteinemia and increased mean plasma HCY levels in glaucoma patients, accompanied by vascular abnormalities that may play a role in disease progression. Similarly, Bleich et al. [2y] observed a twofold increase in HCY concentrations in the aqueous humor of glaucoma patients, suggesting a possible intraocular contribution of HCY to the disease mechanism. Moreover, Roedl et al. [3p] found elevated HCY levels in tear fluid, providing additional evidence of its pathogenic involvement. Collectively, these observations strengthen the hypothesis that HCY may act as a biochemical mediator in glaucomatous optic nerve damage.Nonetheless, previous studies have yielded divergent outcomes. Wang et al.[31] observed no significant difference in plasma HCY levels between patients with POAG and healthy controls. Likewise, Tongabay et al.[32] found elevated HCY concentrations only in individuals

with pseudoexfoliation glaucoma (PEXG), but not in those with POAG. Consistently, Turaçli et al.[33] also reported no significant variation in HCY levels between PEXG patients and control subjects, despite a high overall prevalence of hyperhomocysteinemia in both groups.

These discrepancies may be attributed to methodological variations, differences in study design, limited sample sizes, or the influence of ethnic and dietary factors. Specifically, variations in dietary intake of folate and B vitamins key regulators of HCY metabolism as well as differences in the analytical methods used to measure plasma HCY levels, could account for population-specific differences observed across studies.

Our study revealed no significant differences in the MTHFR C677T polymorphism between POAG patients and controls, in terms of both polymorphisms and allele frequencies. The distributions of CT and TT polymorphisms, as well as the C and T alleles, were nearly identical across the two groups. Consistently, odds ratio analysis showed no significant association between this polymorphism and the risk of developing POAG.

These findings align with several previous studies. George et al.[3]t reported no significant association between MTHFR C677T polymorphisms and either POAG or pseudoexfoliation glaucoma. Similarly, Mabuchi et al.[3]u observed no differences in polymorphism frequencies between patients and controls. A study conducted in a Pakistani population[3]v also found no significant link between MTHFR C677T polymorphisms and POAG; however, they did report a significant association between the TT genotype and primary closed-angle glaucoma (PCAG), suggesting that the influence of this polymorphism may vary depending on the glaucoma subtype.

Our results indicate that MTHFR C677T polymorphisms modulate HCY levels; however, they do not appear to be a significant risk factor for POAG in our population. It is likely that the pathogenic role of hyperhomocysteinemia in POAG arises from a complex interplay of genetic predisposition, environmental factors, and nutritional status, rather than a single genetic mutation.

In our Jordanian cohort, the T allele frequency of the MTHFR C677T polymorphism was 18% among POAG patients. By comparison, previous studies have reported frequencies of 24% in Brazilians, 9% in West Africans, 55% in Mainland Chinese, and 33% in Turks[3]w ¯t p , highlighting significant global variation. These differences may affect statistical power and influence the strength of genetic associations reported across populations.

Moreover, other polymorphisms within the MTHFR gene or in genes involved in the HCY metabolic pathway such as MTR, MTRR, or CBS may exert a greater influence in our population, potentially through gene-gene or gene-environment interactions.

CONCLUSION

This study demonstrates significantly elevated HCY levels and higher rates of hyperhomocysteinemia in Jordanian POAG patients compared to controls, suggesting a contributory role in disease pathogenesis.MTHFR C677T polymorphisms distribution and C allele frequency were higher among individuals with the homozygous CC polymorphisms compared to those with CT or TT polymorphisms and T allele. Furthermore, the results indicate that elevated HCY levels rather than MTHFR polymorphisms are associated with the severity of POAG. This suggests that MTHFR C677T polymorphisms are unlikely to be a significant independent risk factor for POAG. Clinically, this suggests that elevated HCY levels regardless of genetic background warrant further investigation be carried out on a larger number of populations as a potentially modifiable biochemical risk factor in glaucoma management.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable

Author contributions

Mahmoud Kaswal: Conceptualization, visualization; Mohammad Abu-Jeyyab: Data collection, analysis; Khalid Alzubi: Supervision, methodology, editing; Fawaz Alsarireh: Supervision, methodology, editing; Hala Fouad: Project administration, supervision; Samir Mahgoub: Project administration, methodology, writing – original draft, writing – review & editing.

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