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SHORT COMMUNICATION

Methylenetetrahydrofolate Reductase Gene Polymorphism and Homocysteine Level in Patients with Pseudoexfoliation and Pseudoexfoliation Glaucoma in Southern Iran

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Abstract

Background: Pseudoexfoliation (PEX) syndrome leads to elevated intraocular pressure and consequent glaucomatous damage of the optic nerve. This study was performed to investigate the frequency of MTHFR, 677 C-T polymorphism and homocysteine (Hcy) levels in Iranian patients with PEX and PEX glaucoma were compared to that in normal population.

Methods: Thirty four patients with PEX, 27 with PEX and glaucoma, and 32 control subjects were enrolled. Pregnant patients with any systemic disorder such as hypertension and diabetes mellitus, usage of vitamin supplements or any condition affecting homocysteine level were excluded. Fasting total homocysteine (tHCT) levels of all the participants were determined, using an ELISA method and values exceeding 14 micromole /L were considered as an elevation. MTHFR genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotyping of polymorphism was done with polymerase chain reaction.

Results: The patients' mean age was 67 years (range 50-90 years) in all three groups and the male to female ratio was 2:1. The mean plasma homocysteine level was 13.95±8.7 in the PEX, 16.37±8.2 and 14.22±11.32 in the PEX glaucoma and the control group, respectively. The rate of C677T polymorphism was 44% in the PEX, 52% in the PEX glaucoma, and 40% in the control group.

Conclusion: The result of this study implies that neither C677T polymorphism nor hyperhomocysteinemia can be considered as major risk factors for PEX or PEX glaucoma in Iranian population.

Keywords: Pseudoexfoliation; Pseudoexfoliation glaucoma; Gene; Polymorphism

Introduction

Pseudoexfoliation (PEX) syndrome is characterized by accumulation of fibrillar-like material in ocular and also other extraocular tissues. This leads to elevated intraocular pressure (IOP) and consequent glaucomatous damage of the optic nerve (PEX G) in approximately 25-50% of patients.[1-4] In Scandinavian population, for instance, PEX is the most common identifiable cause of secondary open angle glaucoma.[2,3,5] With recent advances in the knowledge of genetics, genetic analysis can be considered as a predictable finding for the diagnosis and prevention of ocular problems.[6] Despite great efforts to show the role of hyperhomocysteinemia and genetic influence of 677 C>T polymorphism of tetrahydrofolate reductase gene (THFR) as was found in primary open angle glaucoma (POAG), the data were unable to confirm this relation in PEX and PEX G.[7-9] Previous studies show conflicting results on this subject.[10-15] However, PEX and hyper homocystinemia were found to have

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common associations with various systemic diseases such as vascular problems and hypertension.[15-17] This study was designed to compare the frequency of 677 C>T polymorphism and plasma homocystine level in patients with PEX and PEX G with a control group in the Iranian population.

Material and Methods

In this cross-sectional study, 93 patients referring to Khalili Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran for cataract surgery including 34 patients with PEX, 27 with PEX G and 32 with cataract were enrolled. The patients gave their written informed consent, allowing analysis of the clinical data and testing for all mutations and polymorphisms mentioned in this article**.** A detailed medical history was obtained to identify any factor that might influence the findings. Exclusion criteria were based on any history of major systemic illness (hypertension, diabetes mellitus, cardiovascular disease, vacuities, gastrointestinal and malabsorption) and also history of substance abuse, hormone replacement therapy, antimicrobial therapy or vitamin supplementation. All the patients underwent complete ophthalmic examination and the IOP was measured, using Goldman applanation tonometry. PEX G was characterized by the presence of an open angle on gonioscopy, characteristic glaucomatous optic damage, and elevated IOP (higher than 21 mmHg). PEX was diagnosed on the basis of typical signs such as deposits of grayish – white material on the anterior lens surface and at the pupillary area and also transillumination defect of the iris, especially at the papillary ruff and margin. The patients with cataract who did not meet the criteria for PEX or PEX G were considered as the control group. Then 5 ml venous blood sample to test for plasma THCY were collected in fasting. The sample was taken to the laboratory immediately, centrifuged within 1 hr, and stored at -80^{o} C. The levels of plasma and total homocysteine level (tHCY) were determined, using ELISA method and levels ≥14 μmol/l were considered as hyperhomocystinemia.

Genomic DNA was extracted from a peripheral blood sample by a commercial extraction kit (DNG plus DNA extraction Kit, Sina Gene Company, Tehran, Iran) and was stored at -20°C until the analysis was performed. DNA fragments containing the C677T MTHFR variant were amplified by PCR. [11] Chi-Square test was used to detect the differences between the three groups. Also, logistic regression analysis was used to compute odd ratios and a 95% confidence interval (CI) was considered for the comparison of PEX G and PEX with the normal population. A P< 0.05 was considered significant. Statistical analysis was performed, using SPSS software (version 12.0, Chicago, IL, USA).

Results

There were 34 patients with PEX, 32 with cataract and 27 with PEX G. The mean age of the patients was 67 years with approximately 2:1 male to female distribution. The means of THCY level were 13.9±8.7, 16.0±8.2 and 14.2±11.3 in PEX, PEX G and control group, respectively. The mean plasma tHCY had no significant difference between the three groups (p=0.250). Similarly, hyperhomocysteinemia was detected in 44.1% of the patients with PEX and 59.3% of PEX G while in the control group it was 41.9% (Table 1). Multiple regression analysis was performed to show the relation of 677C>T polymorphism and hyperhomocysteinemia with either PEX or PEXG. The results were in favor of odds ratio of about 2.4 [OR=2.4; 95% CI (0.81-7)] for 677C>T polymorphism in PEX G while that of PEX was about OR=0.84 [95% CI (0.3-2.3)]. Subsequently, OR=1.8 [95% CI (0.61-5.3)] and OR=0.86 [95% CI(0.32-2.3)] in relation to hyperhomocysteinemia in patients with PEX and PEX G, respectively. Although odds ratio for 677C>T polymorphism was high enough to claim that this gene may be a risk factor for the development of PEX G, the frequency of this gene was not statistically different in the three studied groups (p=0.360) (Table 1). Also the rate of hyperhomocysteinemia was not higher in either PEX or PEX G in

Table 1: The genotype distribution in three studied groups.

Genotype	Patients with PEX N0. (%)	Patients with PEXG N0. (%)	Control subjects N0. (%)
677 CC	13 (38.23)	15 (55.5)	11 (35.48)
677 CT	20 (58.82)	11 (40.74)	20 (64.51)
677 TT	1 (2.95)	1 (3.76)	1 (0.1)

Results were in favor of no statistically significant difference between groups (p=0.360).

comparison with the control group (p=0.510). tHCY<14 was 55.9% (19 cases) in PEX and 40.7% (11 cases) in P EXG groups while for the control group was 58.1% (18 cases). These figures for tHCY>/ 14 were 44.1% (15 cases), 59.3% (16 cases) and 41.9% (13 cases) respectively. Figure 1 shows the distribution of values of tHCY in the three diagnostic groups, using a box plot chart.

Discussion

Plasma homocysteine concentrations are affected by both environmental and genetic factors such as the methylenetetrahydrofolate reductase (MTHFR) 677 C>T polymorphism, the most common genetic factor of plasma homocysteine level.[9,17] MTHFR catalyzes the conversion of 5, 10 methyltetrahydrofolate to 5 methyl tetra hydrofolate which is essential for remethylation of homocysteine to methionine.[17,18] Some researchers reported high frequency of the 677 C>T polymorphism of MTHFR in patients with POAG.[7-9] While PEX is the most common known cause of secondary open angle glaucoma, the presence of this genetic association in patients with PEX and PEX G has been a subject of interest till now. However, neither hyperhomocysteinemia nor the frequency of 677 C>T polymorphism was detected to be different in PEX, PEX G compared with the control group in the multivariant studies in Turkish population, Austria and Iowan.[9-12] Only Leibovitch *et al*. reported that hyperhomocysteinemia might be associated with PEX G, which may clear the increased risk of vascular diseases among patients with pseudoexfoliation syndrome in Israelii population.[13] This finding may be due to the presence of a especial race (Ashkenazi a) in the Israeli population. In other words, since polymorphism was a population dependant variant, studies in other populations cannot be attributed to the other groups.[6] Moreover, as it was mentioned, the plasma levels of homocysteine are influenced by various factors. Deficiencies of folate, vitamin B12 and B6 account for the majority of the cases of hyperhomocystinemia.[10,12,15,16]

In addition, lifestyle factors (substance abuse) and some kinds of drugs are known to increase plasma homocysteine levels.[17-20] Thus, we excluded all the patients with manifest risk factors of malnutrition or vitamin deficiency; however, as we did not check their serum levels, the possibility of this confounding effects is still present. In the present study, the rate of hyperhomocysteinemia (defined as plasma level ≥14 µm/l) was not higher either in PEX or PEX G patients. On the other hand, despite the odds ratio of this gene polymorphism in relation to PEX G (i.e. OR=2.5), this gene could not be related to the presence of PEXG as its frequency was not statistically higher in these patients (p=0.36). The lack of signifi-

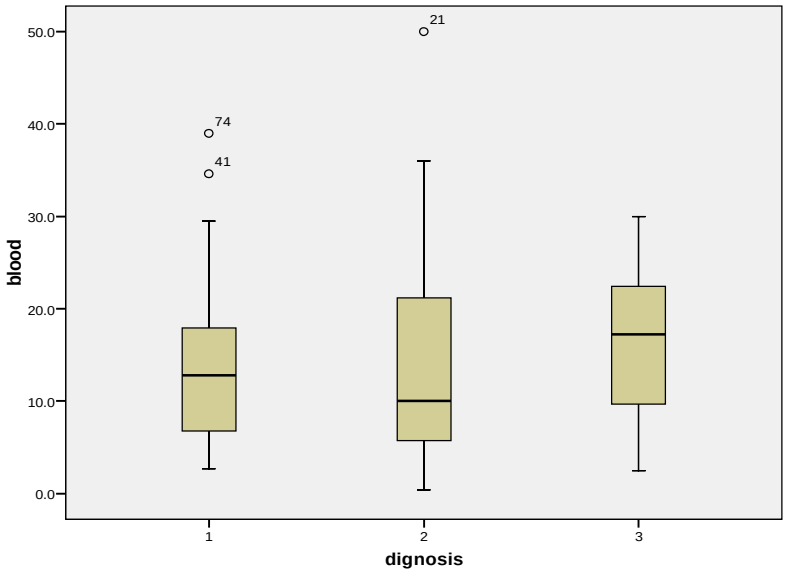

Fig. 1: The distribution of tHCY level in three diagnostic groups

cant differences between three groups may, of course, be related to small sample size and further studies with larger sample size will be desired. The frequency of MTHFR polymorphism of 677 C>T was not different in the PEX and PEX G.

As shown in Table 1, the frequency of MTHFR 677 C>T polymorphism was about 44.1%, 59.3% and 41.9% in the PEX, PEXG and control groups, respectively. The difference was not statistically significant among the three groups. Therefore, the results of this study was in agreement with previous researches, indicating the absence of any considerable correlation between homocysteine blood level or 677C>T gene polymorphism and the presence of PEX or PEX G.[9-11] In conclusion, no statically significant difference in genotype distribution of the MTHFR 677 C>T polymorphism was found between patients with PEX or PEX G and the control group. This finding suggests that this polymorphism may not be a major risk factor for the investigated disease.

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Conflict of interest: None declared.

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