Aqueous Levels of Anti-Helicobacter Pylori IgG Antibody in Patients with Primary Open Angle and Pseudoexfoliation Glaucoma

Mohammad Reza Razeghinejad[1], Eskandar Kamali-Sarvestani[2,3], Mohsen Farvardin[1], Arash Pourhabibi[1]

[1]Departments of Ophthalmology and [2]Immunology, [3]Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Background: Glaucoma is a progressive optic neuropathy and is one of the leading causes of blindness worldwide. Different factors have been contributed in the pathogenesis of glaucoma including *H. pylori* infection. **Objective:** To determine the levels of anti-*H. pylori* IgG antibody in the aqueous humor of patients with pseudoexfoliation and primary open angle glaucoma, in comparison with age and sex matched cataract patients. **Methods:** This study was conducted on 41 cases of glaucoma (21 with pseudoexfoliation and 20 with primary open angle glaucoma) and 39 cases of cataract as control group. Aqueous humor was aspirated at the beginning of glaucoma or phacoemulsification cataract surgery in glaucoma and cataract patients, respectively. Anti-*H. pylori* IgG concentration was measured by means of an enzyme-linked immunosorbent assay. **Results:** The aqueous levels of anti-*H. pylori* IgG in primary open angle glaucoma (0.44±0.64 U/ml) had no significant difference with cataract (0.24±0.52U/ml) and pseudoexfoliation glaucoma group (0.63±0.71U/ml) (P=0.18 and 0.44, respectively). However, the concentration of this antibody was higher in the aqueous humor of pseudoexfoliation glaucoma patients compared to the control group (p=0.03). **Conclusion:** The results of this study did not support a relation between *H. pylori* infection and primary open angle glaucoma. The elevated concentration of anti-*H. pylori* IgG in pseudoexfoliation glaucoma compared to cataract patients may be due to the breakdown of blood-aqueous-barrier.

Keywords: Aqueous Humor, Glaucoma, Helicobacter Pylori

*Corresponding author: Dr. Mohammad Reza Razeghinejad, Department of Ophthalmology, Khalili Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Tel. /Fax: (+) 98 711 2304069, e-mail: mrazeghi@sums.ac.ir

INTRODUCTION

Glaucoma is a progressive optic neuropathy and one of the leading causes of blindness in the world (1-2). Optic neuropathy is characterized by retinal ganglion cell death, axon loss and excavated appearance of optic nerve head, and accompanying loss of visual sensitivity (3). Glaucoma is usually associated with elevated intraocular pressure (IOP), but a subset of glaucomatous patients has normal IOP, Normal Tension Glaucoma (3). Beside increased IOP, other risk factors are proposed to play a role in the pathogenesis of open angle glaucoma. While local ischemia, alteration of immune system and oxidative stress have been proposed as possible factors in the development of primary open angle glaucoma (POAG); viral infection is considered as an etiologic factor in the development of pseudoexfoliative glaucoma (XFG) (4,5). Another possible factor introduced by Kountouras and co-workers in the pathogenesis of POAG and XFG is infection with *Helicobacter Pylori* (1). They suggested that *H. pylori* infection could affect the susceptibility to glaucoma by different mechanisms including promoting platelet and platelet-leukocyte aggregation, induction of pro-inflammatory cytokines release and influencing the apoptosis process (6). However, other studies refute this suggestion and bring into question the association between *H. pylori* infection and glaucoma (7). Therefore the present study aimed at investigating the level of anti *H .pylori* IgG in the aqueous humor of POAG, XFG and cataract patients as control participants for any possible relation between *H. pylori* infection and glaucoma.

MATERIALS AND METHODS

This prospective case-control study was approved by local ethics committee and was conducted between August 2003 and May 2005. Informed consent was obtained from all participants who were Iranian, living in Fars province in the south of Iran. All of them underwent a comprehensive ocular examination prior to enrolment. Intraocular pressure was measured by a calibrated Goldman applanation tonometer and the optic nerve head was evaluated by stereoscopic method. The visual field was checked by Humphery 24-2 sequence only in glaucoma patients. The study was conducted on 41 cases of glaucoma (21 with XFG and 20 with POAG) and 39 cases of cataract patients as controls. The criteria for POAG were: IOP > 21 mm-Hg, open iridocorneal angle in gonioscopy, typical glaucomatous optic nerve head and visual field changes (8). In the XFG group, the exfoliative material deposition on the papillary border and lens was added to these criteria (9). In all cataract patients, the IOP was less than or equal to 21 mm-Hg and optic nerve head cup was less than or equal to 0.3 mm without any typical glaucomatous change. In all patient groups, the exclusion criteria included: myopic refractive error exceeding -10.00 diopter, ophthalmic conditions other than glaucoma, history of any intraocular surgery or laser application and use of oral antibiotics in the preceding month.

Aqueous humor samples were obtained prior to entrance to the eye through a limbal paracentesis site using a 27-gauge needle on a tuberculin syringe. The sample was stored at -70^{o}C until analysis for IgG anti-*H. pylori* antibody. A commercial enzyme-linked immunosorbent assay (ELISA) kit (IBL, Germany) was used to detect the level of anti-*H. Pylori* IgG in the aqueous humor of the patients. The sensitivity and

specificity of the test was more than 95%. The procedure was performed following the manufacturer's instructions.

Age, vertical cupping and anti *H. pylori* IgG levels were considered to be continuous variables, while gender and the operated eyes were considered to be discontinuous variables. The discontinuous variables were tested by chi-square and Fisher's exact tests when appropriate. Continuous variables were compared using non-parametric Mann-Whitney U test. P value less than 0.05 considered significant and analysis was carried out with the Statistical Package for the Social Sciences (SPSS) 11.5 for windows.

RESULTS

Demographic data and the level of IgG anti-*H. pylori* antibodies in the aqueous humor are shown in table 1. The glaucoma and control groups were age and sex matched. Table 2 lists glaucoma medications used in the glaucoma group.

Table 1. Demographic data, vertical cupping and the levels of anti *H. pylori* IgG antibodies in the aqueous humor of pseudoexfoliation glaucoma (group1), primary open angle glaucoma (group2), and cataract (group3) patients

Characteristics	Group 1 (n=21)	Group 2 (n=20)	Group 3 (n=39)	P-value		
				1vs2	1vs3	2vs3
Age (years) mean±SD	62.4±8.9	61.1±11.1	65.6±11.3	0.45	0.13	0.07
Female:Male	7:14	7:13	15:24	0.83	0.91	0.98
Operated eye (Rt:Lt)	12:9	14:6	19:20	0.59	0.72	0.20
Vertical cupping mean±SD	0.83±0.25	0.82±0.20	0.24±0.06	0.03	0.0001	0.0001
Anti-H pylori IgG mean±SD	0.63±0.71	0.44±0.06	0.24±0.52	0.44	0.03	0.18

Table 2. Medications used in the treatment of glaucoma patients

Medications	Pseudoexfoliation Glaucoma (n=21)	Primary open angle glaucoma (n=20)
Timolol	1 (4.8%)	2 (10%)
Timolol + Dorozolamide	10 (47.6%)	9 (45%)
Timolol + Dorozolamide + Latanoprost	3 (14.3%)	2 (10%)
Timolol + Acetazolamide	3 (14.3%)	3 (15%)
Other combinations	4 (19.1%)	4 (20%)

The mean concentration of anti *H. pylori* IgG antibodies of patients with POAG and cataract were 0.44 ± 0.64 U/ml and 0.24 ± 0.52 U/ml (P=0.18), respectively. The differences between IgG anti-*H. pylori* antibodies in POAG and XFG also remained insignificant (P=0.44). However, the mean concentration of anti *H. pylori* IgG antibodies of patients with XFG was significantly higher than the control group (0.63 ± 0.71 U/ml vs. 0.24 ± 0.52 U/ml, p=0.03).

There was no correlation between aqueous humor levels of anti-*H. pylori* IgG antibodies and vertical cupping in the POAG and XFG groups (P=0.98 and P=0.78, respectively).

DISCUSSION

Intraocular pressure is an important risk factor for POAG. Other factors such as local ischemia-hypoxia perhaps due to dysfunction of blood-flow autoregulation, abnormalities of axonal or ganglion cell metabolism, disorders of extracellular matrix of the lamina cribrosa, oxidative stress and formation of free radicals, inflammatory cytokines and alterations in immune system may be contributing factors (3). At present, the puzzle of the interplay between multiple causes and glaucoma remain unresolved and the pathophysiology of glaucomatous neurodegeneration is not fully understood. However, Kountouras et al. (1) reported a relationship between *H. pylori* infection and glaucoma. In fact they showed more serum and aqueous levels of IgG anti-*H. pylori* in 26 cases of POAG compared with 31 cases of cataract as control group (2). They also cited positive influence on glaucoma parameters by eradication of *H. pylori* (10). In contrast to Kountouras studies (1-3), the results of the present study show no statistically significant difference in the aqueous humor levels of IgG anti-*H. pylori* in the POAG compared with the cataract group (0.44 U/ml vs. 0.29 U/ml ; p=0.8). Galloway et al. was also not able to show any association between infection with *H. pylori* and POAG by performing ELISA test on the serum of 38 patients with POAG, 19 patients with normal tension glaucoma, 16 patients with XFG, and 24 patients with ocular hypertension (7). Moreover, in another study reported by Jahadi and his colleagues (11) on 60 cases of POAG and 65 non-glaucomatous cases as controls, no statistically significant differences in the prevalence of *H. pylori* infection was detected between groups using ELISA test on the serum and stool (p=0.09 and p=0.43, respectively). Therefore, the result of the present study is compatible with Jahadi's and Galloway's study, indicating that *H. pylori* infection plays no important role in the pathogenesis of POAG.

Pseoudoexfoliative glaucoma is a systemic disorder of the extracellular matrix characterized by deposition of dandrof-like material at the anterior segment of the eye and also in various extracellular sites (12-13). The exact pathogenic mechanism for the development of pseudoexfoliative syndrome remains unclear. However, a role for a number of genetic and environmental factors (such as UV and viral infections) has been taken into consideration (14). In addition, Kountouras and colleagues (2) were the first to demonstrate higher concentrations of anti *H. pylori* IgG in the serum and aqueous humor of XFG cases compared with controls. However, this finding was not confirmed by another study conducted by Galloway et al. (7). They showed that 25% of 16 cases of XFG and 26.3% of 38 cases of POAG were seropositive and no positive correlation was found between open angle glaucoma and seropositivity (7). In the present study, there was also no difference in the levels of anti *H. pylori* IgG antibody between XFG and POAG (0.63 U/ml vs. 0.44 U/ml; p=0.4). However, we found significant higher levels of anti *H. pylori* IgG in the aqueous humor of patients with XFG compared to the cataract control group (0.63 U/ml vs. 0.24 U/ml ; p=0.03). Our findings may be explained by more severe disruption of blood-aqueous barrier in XFG compared to patients with cataract (15-17). In fact, it has been reported that the aqueous humor of these patients contains larger size proteins (immunoglobulins) compared to POAG or cataract patients (18). Therefore, the presence of higher IgG anti-*H. pylori* antibodies in the aqueous humor of XFG than those of cataract may be explained by the degree of breakdown of blood-aqueous barrier compared to controls.

In conclusion, our results did not support the Kontuoras's suggestion (1) that *H. pylori* infection is involved in glaucoma development. Therefore, the findings of the present study do agree with the recent reports (7,11), showing no association between glaucoma and *H. pylori* infection. Regarding the lack of agreement among the results of the present study and those of Kontouras's study (1), further investigation on other populations are recommended.

ACKNOWLEDGEMENTS

The authors would like to thank the technical supports of Hamid-Reza Zare from immunology department of Shiraz Medical School. This study was financially supported by a grant from Shiraz University of Medical Sciences.

REFERENCES

1. Kountouras J, Mylopoulos N, Boura P, Bessas C, Chatzopoulos D, Venizelos J et al. Relationship between Helicobacter pylori infection and glaucoma.Ophthalmology. 2001;108:599-604.
2. Kountouras J, Mylopoulos N, Konstas AG, Zavos C, Chatzopoulos D, Boukla A. Increased levels of Helicobacter pylori IgG antibodies in aqueous humor of patients with primary open-angle and exfoliation glaucoma.Graefes Arch Clin Exp Ophthalmol. 2003;241:884-90.
3. McKinnon SJ. Glaucoma: ocular Alzheimer's disease?Front Biosci. 2003;8:1140-56.
4. Weinreb RN, Khaw PT. Primary open-angle glaucoma.Lancet. 2004;363:1711-20.
5. Ringvold A. Exfoliation syndrome immunological aspects.Acta Ophthalmol Suppl. 1988;184:35-43.
6. Kountouras J, Zavos C, Chatzopoulos D. Primary open-angle glaucoma: pathophysiology and treatment. Lancet. 2004;364:1311-2.
7. Galloway PH, Warner SJ, Morshed MG, Mikelberg FS. Helicobacter pylori infection and the risk for open-angle glaucoma.Ophthalmology. 2003;110:922-5.
8. Spaeth GL. Primary (Idiopathic) open angle glaucoma.In: Eid ,T.E., Spaeth,G.L.,. The Glaucomas. Lippincot Williams & Wilkins. 2000; 89-103.
9. Ritch R. Exfoliation syndrome and exfoliative glaucoma.In : Eid,T.E., Spaeth, G.L.,The Glaucomas.Lippincot Williams & Wilkins.2000:114-120.
10. Kountouras J, Mylopoulos N, Chatzopoulos D, Zavos C, Boura P, Konstas AG et al. Eradication of Helicobacter pylori may be beneficial in the management of chronic open-angle glaucoma.Arch Intern Med. 2002;162:1237-44.
11. Jahadi H, Afarid M, Razeghinejad MR, Alborzi A, Oboodi B, Rasekhi A et al. Relationship between Helicobacter Pylori and open angle glaucoma. J Med Resea 2003; 2: 25-32.
12. Streeten BW, Li ZY, Wallace RN, Eagle RC Jr, Keshgegian AA. Pseudoexfoliative fibrillopathy in visceral organs of a patient with pseudoexfoliation syndrome.Arch Ophthalmol. 1992;110:1757-62.
13. Schlotzer-Schrehardt UM, Koca MR, Naumann GO, Volkholz H. Pseudoexfoliation syndrome. Ocular manifestation of a systemic disorder?Arch Ophthalmol. 1992;110:1752-6.
14. Detorakis ET, Kozobolis VP, Pallikaris IG, Spandidos DA. Detection of herpes simplex virus in pseudoexfoliation syndrome and exfoliation glaucoma.Acta Ophthalmol Scand. 2002;80:612-6.
15. Vannas A. Vascular changes in pseudoexfoliation of the lens capsule and capsular glaucoma. A fluorescein angiographic and electron microscopic study.Albrecht Von Graefes Arch Klin Exp Ophthalmol. 1972;184:248-53.
16. Brooks AM, Gillies WE. Fluorescein angiography and fluorophotometry of the iris in pseudoexfoliation of the lens capsule.Br J Ophthalmol. 1983;67:249-54.
17. Bleich S, Roedl J, Von Ahsen N, Schlotzer-Schrehardt U, Reulbach U, Beck G et al. Elevated homocysteine levels in aqueous humor of patients with pseudoexfoliation glaucoma.Am J Ophthalmol. 2004;138:162-4.
18. Moreno-Montanes J, Lajara Blesa J. IgG, albumin and total IgG index in the aqueous humour of eyes with pseudoexfoliation syndrome.Acta Ophthalmol Scand. 1995;73:249-51.