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Study of the Association between Helicobacter Pylori Infection and Primary open angle Glaucoma in China

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ABSTRACT

Objective: To assess the relationship between Helicobacter pylori (Hp) infection and primary open-angle glaucoma (POAG); and meantime, to explore the possible mechanism of POAG induced by Hp. Methods: 30 consecutive POAG patients, 30 primary angle-closure glaucoma (PACG) and cataract patients were recruited and divided into three groups according to different diseases. The sera and aqueous humor samples were collected and used to detect Hp-specific IgG antibody (Hp-Ab) with dot immunogold filtration assay (DIGFA). ^{14}C-urea breath test (^{14}C-UBT) was carried out to detect Hp infection of all participants. Results: The Hp-Ab positive rate respectively was 76.7% (23/30) and 66.7% in sera samples and aqueous humor samples for POAG group, which was significantly higher than the corresponding data of the other two groups (all $P<0.05$). In ^{14}C-UBT, the Hp-Ab positive rate was 63.3% in POAG group and it was close to that of serological result detected by DIGFA ($P>0.05$). There were little numbers of positive ANA and ENA in the three groups and no meaning to make statistically analysis. Conclusions: There is positive association between Hp infection and POAG, and the autoimmune is suggested as one of the key mechanisms in our opinions.

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Introduction

Glaucoma is one of the commonest causes for blindness in the world. Generally, glaucoma is divided into primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG).[1] As a leading causes for blindness, the study of POAG causes more and more attention.[2,3]To our understand, POAG is a chronic optic neuropathy characterized by atrophy and increased cupping of optic disk. To date, many aspects of its pathogenesis remain unknown but some significant risk factors are advanced age, African origin, familial history of glaucoma and elevated intraocular pressure.[4,5]

Helicobacter pylori (Hp) is a Gram-negative and microaerophilic bacterium which plays an important role in the development of various upper gastrointestinal diseases. With the development of studies, some researchers reported that Hp was also associated with some extragastric diseases, such as ischemic heart disease,[6] iron-deficient anemia,[7] diabetes mellitus,[8] and so on. In 2001, Kountouras et al[9] established a higher prevalence of Hp infection in the sera of patients with POAG in a Greek population, and suggested a possible causal link between Hp and glaucoma. Subsequently, this finding was evidenced by some scholars in their own studies.[10] But the significance of such an association remains uncertain because of the conflicting findings reported by various studies.[11-13] Aiming to such a discrepancy, further studies are necessary.[14]

In this study, we just do detect Hp-specific IgG antibodies (Hp-Ab) in the sera and aqueous humor of patients with different ocular diseases, including POAG, PACG and cataract, and attempt to further determine the relationship between Hp infection and POAG and to analyze the possible mechanism of POAG induced by Hp.

Abbreviations

ANA, antinuclear antibody; ENA, Extractable nuclear antigen; DIGFA, dot immunogold filtration assay; Hp, Helicobacter pylori; Hp-Ab, Hp-specific IgG antibodies; PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma; ^{14}C-UBT: ^{14}C-urea breath test.

Subjectsand methods

Subjects

30 consecutive POAG patients were enrolled with the average age of 68±7.3 y (ranged from 47 to 78 y). The ratio of the male and the female was 11: 19. Meantime, 30 PACG patients and 30 cataract patients were also recruited, and who were matched by age and sex with the POAG patients. According to different diseases, the participants were divided into POAG, PACG and cataract groups, respectively. All of them were excluded from tumor, immunodeficiency, autoimmune and infectious diseases in clinic, and also had no antibiotics and other medicines related to immunopotentiator or immunosuppressive agents in the six months before the experiment. Written informed consents were obtained from all the participants. The study was approved by the local ethics committee.

Hp-Ab detection of sera samples

2 ml venous blood was collected from each of the participants. The serum was obtained after centrifugation and used to detect Hp-Ab with dot immunogold filtration assay (DIGFA) according to the manufacturer's instruction of the reagent kit (MP Biomedicals Asia-Pacific Pte. Ltd., Singapore).

Hp-Ab detection of aqueous humor samples

About 50 μl aqueous humor sample was aspirated at the beginning of glaucoma surgery from the each of the patients in the three groups, respectively. Hp-Ab was assayed with DIGFA as same as the detection process of venous blood samples.

Detection of Hp infection with ^{14}C-urea breath test

Referring to Tang's report,[15] ^{14}C-urea breath test (^{14}C-UBT) was carried out in POAG group with Hp detection instrument-YH04 (Yanghe Medical Equipment Co. Ltd., China).

Sera auto-antibodies detection

Serum antinuclear antibody (ANA) was detected with the indirect immunofluorescence assay by a commercialized ANA kit. Extractable nuclear antigen (ENA) was assayed with line immunoassay. All reagents were bought from Jiangsu HOB Biotech Group, China.

Statistic analysis

Using T-test and Chi-square test, all analyses were performed with SPSS 13.0 software. P value less than 0.05 were considered significant.

Results

3.1 Hp infection detection in sera and aqueous humor

Of the sera samples, there were 23 cases exhibited Hp-Ab-positive in POAG group, and the positive rate was 76.7% which was significantly higher than those of PACG and cataract group (43.3% and 36.6% respectively). In the aqueous humor samples, there were 18 patients with positive Hp-Ab in POAG group, and the positive rate was 66.7%. Compared to each data of the other two groups, the difference was statistically significant (Table 1). In POAG group, the mean positive rate of sera samples was similar to that of aqueous humor and no difference existed between them (P = 0.287).

Table 1. The serum and aqueous humor qualitative test results of the patients with glaucoma

Groups	Positive cases	Positive rate (%)	P values
	Sera(n) AH(n)	Sera AH	Sera AH
POAG	23(30) 18(27)	76.7 66.7	0.007[a] 0.011[a]
PACG	13(30) 7(25)	43.3 28.0	0.012[b] 0.009[b]
Cataract	11(30) 8(27)	36.6 29.6	0.211[c] 0.235[c]

AH: aqueous humor; a: POAG group *vs* cataract group; b: POAG group *vs* PACG group; c: PACG group *vs* cataract group.

Hp infection detection with ^{14}C-UBT

In ^{14}C-UBT, there were 19 patients with Hp-Ab-positive, and the positive rate was 63.3%. Compared to the data detected with DIGFA, the difference was not significant (Table 2).

Table 2. Comparison of DIGFA and 14C-UBT for diagnosis of Hp infection in POAG group

Methods	Total cases	Infection cases	Positive rate (%)	P values
DIGFA	30	23	76.7	0.312*
^{14}C-UBT	30	19	63.3	—

* represents comparison of the positive rate detected with the two methods.

ANA and ENA detection

There were 4, 2 and 1 patients with ANA-positive in POAG, PACG and cataract group, respectively. The positive ENA in POAG group were SSA, SSB and Ro-52, and the corresponding numbers were 2, 2 and 1. Only Ro-52 showedpositive in PACG group while there was no positive ENA in cataract group (Table 3).

Table 3. The results for sera ANA, ENA of the patients of each group

Items	POAG	PACG	Cataract
ANA	4	2	1
SSA	2	0	0
SSB	2	0	0
Ro-52	1	1	0
Scl-70	0	0	0
Sm	0	0	0
U1RNP	0	0	0
dsDNA	0	0	0

Discussion

In Greece, a very active research group led by J. Kountouras published several original contributions as well as the reviews concerning the connection between Hp infection and POAG.[14,16] In other counties, there were also several papers containing the similar arguments issued, such as India,[17] Turkey,[18] Korea[19] and so on. In China, Hong et al[20] detected Hp infection and POAG through ^{13}C-UBT, and also found the positive correlation between them. Since then, there was no relative article issued by Chinese could be found in PubMed and other well-known scientific database. In this study, referring to other researchers' reports, we designed and carried out the experiments. In the results, we found that the positive rate of sera Hp-Ab was high to 76.7% in POAG patients, which was significantly higher than those of the other two groups. This finding was close to the data of the previous reports[2,21] and further verified that there was a positive relation between Hp infection and POAG.

In the present study, we also assayed Hp infection with ^{14}C-UBT. Encouragingly, the positive rate of Hp infection was 63.3%, which was very close to 76.7% detected with DIGFA. This result further indicated the existence of the relation between Hp infection and POAG. However, Bagnis et al[22] thought that the studies based on Hp serological assessment might be misleading, since serum antibodies were not the sensitive markers of active Hp infection; while ^{13}C-UBT could clarify the actual prevalence of POAG among patients infected by Hp. In fact, there were still deficiencies for ^{13}C- or ^{14}C -UBT, because it was more suitable for the detection of gastrointestinal Hp infection, and to an extent, there were false-negatives in the test.[23] This probably was the just reason for what the positive rate in DIGFA was little higher than that in ^{14}C-UBT in this study. As to the cresyl fast violet staining on the histology preparations of tissue samples of trabeculum and iris introduced by Zavos et al,[24] although it could provide the direct and strong evidence for Hp infection in the pathophysiology of POAG, the difficult harvest of the sample limited its application. Therefore, in our opinions, the serological assay is suitable to detect Hp infectionand used to assess the relationship between Hp prevalence and POAG.

Except for detecting sera Hp-Ab, we also detected Hp-Ab in the aqueous humor collected from the majority of participants. As the results shown, the positive rate of the POAG group was statistically higher than each of the other groups, respectively. This result was consistent with that of the serological assessment and again showed the positive relation between Hp infection and POAG. However, in another similar study, Deshpande et al[17] also found a statistically significant difference between the POAG patients and the controls in the concentration of serum Hp-Ab, but they did not find any significant correlations between the Hp concentrations of the aqueous humor of the different patient groups. This disagreement probably associated with the damage degree of blood-brain barrier (BBB), because the sera Hp-Ab could reach the trabeculum and iris under the condition of the BBB disruption.[25] According to the results of the present study, we supported the hypothesis related to POAG onset that Hp-Ab in circulation might get through the blood-aqueous humor barrier, further condensed in aqueous humor and finally induced or aggravated glaucomatous damage.[2]

As to the occurrence of POAG, we thought another autoimmune mechanism was most probable and should not be ignored: Hp infection initiated autoimmune response because of the common genetic components shared in Hp and human nerve tissue; and then, cell destruction which mediated by apoptosis direct caused glaucoma.[26] Just based on the theory, we designed and detected sera ANA and ENA of the POAG patients and the control participants, and hoped to find any evidences related to autoimmune. As a result, we found that the positive rate of every group was rather low and there was no difference between them. However, this seronegative result can't deny the hypothesis of autoimmune mechanism in POAG; and the auto-antibodies specific to eyes, such as trabeculum and iris, were suggested to be detected in future study in our opinions.

Conclusion

The positive association between Hp infection and POAG not only using serum sample but also aqueous humor sample is found in this study. And further, through the experimental data, it is suggested that the autoimmune induced by Hp infection probably is the key mechanism for POAG onset, and Hp detection should be taken as a routinized index applied to the prevention and therapy of POAG in clinic. However, we cannot sufficiently investigate the possible mechanism of POAG relates to Hp infection. Is it true that Hp infection only relative to POAG but not a causative factor for POAG?[18] What are the initial mechanisms of Hp in POAG if the pathogen takes part in the onset of the disease? Such questions will be the study topics to the medical researchers worldwide in future.

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Conflicts of interest

There is no any conflict of interest between all of the authors.

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