# Investigation of the association between glaucoma and *Helicobacter pylori* infection using the ¹⁴C-urea breath test

## Investigação de associação entre glaucoma e infecção pelo Helicobacter pylori pelo teste respiratório da ureia ¹⁴C

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## ABSTRACT

**Purpose:** To investigate the association between glaucoma and *Helicobacter pylori* infection by evaluating for the presence of *H. pylori* infection in patients with glaucoma using the ¹⁴C-urea breath test (14C-UBT).

**Methods:** Using 14C-UBT, *H. pylori* infection positivity was compared between a group of patients with primary open-angle glaucoma and a control group with normal intraocular pressure and a normal optic disc or normal perimetry.

**Results:** The 14C-UBT was positive in 18 (51.42%) out of 35 patients in the glaucoma group and in 15 (42.85%) out of 35 patients in the control group. *H. pylori* infection positivity rates were similar between the glaucoma and control groups ($p > 0.05$).

**Conclusion:** According to the 14C-UBT, there is no association between primary open-angle glaucoma and *H. pylori* infection.

**Keywords:** *Helicobacter pylori*/isolation & purification; *Helicobacter* infections/diagnosis; *Helicobacter* infections/complications; Glaucoma; Glaucoma, open-angle; Urea/diagnostic use

## RESUMO

**Objetivo:** *Investigar a associação entre glaucoma e infecção por* H. pylori *por meio do teste para a presença de infecção por* H. pylori *em pacientes com glaucoma usando o teste de respiração da ureia ¹⁴C (14C-UBT).*

**Métodos:** *Foi feita uma comparação em relação a positividade* H. pylori *utilizando 14C-UBT entre um grupo de pacientes com glaucoma primário de ângulo aberto e um grupo controle com pressão intraocular normal e sem verificação de disco óptico glaucomatosa.*

**Resultados:** *O 14C-UBT foi positivo em 18 (51,42%) dos 35 pacientes no grupo de glaucoma e em 15 (42,85%) dos 35 pacientes no grupo de controle. As taxas de positividade foram semelhantes entre os grupos de glaucoma e de controle e não houve diferença significativa entre os grupos ($p > 0,05$).*

**Conclusão:** *De acordo com o 14C-UBT, não há associação entre o glaucoma primário de ângulo aberto e* H. pylori.

**Descritores:** *Helicobacter pylori/isolamento & purificação; Infecções por Helicobacter/diagnóstico; Infecções por Helicobacter/complicações; Glaucoma; Glaucoma primário de ângulo aberto; Uréia/uso diagnóstico*

## INTRODUCTION

Glaucoma is a neurodegenerative disease characterized by retinal ganglion cell degeneration. Although glaucoma is prevalent worldwide, its etiopathogenesis remains incompletely understood. Increased intraocular pressure (IOP) is the most frequently implied cause for retinal ganglion cell degeneration[1]. Recent studies show that cytokines, impaired ocular blood flow, and structural changes in blood vessels are also involved in the pathogenesis of glaucomatous optic neuropathy[2].

*Helicobacter pylori* is a helical, Gram-negative bacterium known to be involved in the pathogenesis of gastritis, gastric ulcer, gastric carcinoma, and Alzheimer's disease[3-5]. Some studies have also suggested that *H. pylori* infection may be involved in nongastrointestinal disorders because of the release of reactive oxygen species[6]. Thus, it is thought that *H. pylori* facilitates glaucoma pathogenesis by causing the release of Nitric Oxide, endothelin-1, and reactive oxygen species[7]. Several recent studies have investigated the association between *H. pylori* infection and glaucoma[8-11]. In most such studies, serum IgG levels have been used to establish the presence of *H. pylori* infection.

Recently, noninvasive and invasive methods have been used to evaluate the presence of *H. pylori*. Invasive methods include histopathological examination gastric mucosal samples, polymerase chain reaction, culture methods, and the rapid urease test. Noninvasive methods include serological tests, ¹⁴C-urea breath test (14C-UBT), and *H. pylori* antigen level measurements in stools[12]. Accurate diagnostic rates of 80%-84% have been reported for noninvasive tests by the serological method; however, tests can be positive for 6-12 months after *H. pylori* eradication; thus, positivity does not necessarily confirm active infection. Because samples need to be stored at -20°C, the use of the *H. pylori* stool antigen method is restricted. The other noninvasive test is the 14C-UBT, which is the most common test reported in the literature. Many studies have reported a sensitivity of 95%-100% and a specificity of 97%-100% for 14C-UBT[13,14]. Thus, in recent years, it has become the preferred method for detecting *H. pylori* infections[15,16]. To the best of our knowledge, this is the first study to investigate the relationship between *H. pylori* and glaucoma using 14C-UBT.

## METHODS

The Ethical Committee Gaziantep University Hospitals, Gaziantep, Turkey provided the ethical approval for this study. The study group included 35 patients (mean age: 59.08 ± 9.82 years; range: 28-80 years; male/female: 20/15) diagnosed with primary open-angle glaucoma

(POAG) presenting at the Department of Ophthalmology, School of Medicine, Mustafa Kemal University. All patients were evaluated by the same ophthalmologist (E.A.T., N.P.). The control group comprised 35 patients (mean age: 58.77 ± 9.44 years, range: 29-74 years; male/female: 17/18) with normal IOP (<21 mmHg) and normal perimetry or without glaucomatous optic neuropathy. Both groups underwent ophthalmological examination consisting of angle evaluation by Goldmann three-mirror gonioscopy, IOP measurement by Goldmann tonometry, and optic disc examination using a +90 lens. In addition, the study group underwent visual field evaluations using the 24-2 program of Humphrey's automated perimeter. POAG is defined as an IOP of >21 mmHg, open angle, typical glaucomatous visual field changes in the perimetry, and typical glaucomatous cupping of the optic disc. All patients provided written informed consent. Exclusion criteria included previous gastric surgery or treatment for *H. pylori* eradication, history of angle-closure glaucoma or other kinds of glaucoma, corneal opacity, diabetes mellitus, uveitis, and central serous chorioretinopathy.

Patients brushed their teeth before the test to prevent contamination from urease-producing resident flora in the mouth. After overnight fasting (≥4 h), the patients swallowed 37 kBq (1 µCi) of an encapsulated form of $^{14}C$ (Helicap™, Kibion, Uppsala, Sweden) with 50 mL of water. The breath samples were collected using a dry cartridge system (Heliprobe BreathCard™, Kibion) 10 min afterward. The patients exhaled into the cartridge until the indicator membrane changed color from orange to yellow. The breath card was then inserted into a Geiger-Müller counter (Heliprobe™ analyser, Kibion) and the activity counted for 250 s. The results were expressed both as counts per minute (CPM) and as a grade (0: not infected, CPM <25; 1: equivocal, CPM: 25-50; 2: infected, CPM >50).

All analyses were performed using SPSS 15.0 software (IBM Corporation, Armonk, NY, USA). 14C-UBT positivity was compared using Pearson's chi-square test. Continuous variables between groups were compared by *t* tests for normally distributed values (age). *p* values of <0.05 were considered significant.

## RESULTS

Table 1 summarizes the demographic characteristics of both groups. The mean age in the glaucoma and control groups were 59.08 ± 9.82 and 58.77 ± 9.44 years, respectively. The mean age and gender were similar between the two groups. In addition, the incidence of dyspeptic symptoms was similar between the two groups. No adverse effects related to the 14C-UBT were observed.

Table 1 also presents the *H. pylori* and 14C-UBT statuses. No significant difference regarding the rates of *H. pylori* infection and 14C-UBT positivity were observed between the study and control groups (*p*=0.473). *H. pylori* was detected in 18 (52.9%) patients with glaucoma compared with 15 (47.1%) controls (Table 1).

## DISCUSSION

Glaucoma is a multifactorial, progressive ocular condition with an unknown etiology. Previous studies have demonstrated that many risk factors could be involved in glaucoma pathogenesis. However,

increased IOP is known to be a major risk factor[1]. *H. pylori* infection is also associated with gastrointestinal and other conditions[17]. In recent years, several studies have investigated the relationship between *H. pylori* infection and the pathogenesis of some ocular disorders, such as central serous chorioretinopathy[18,19], glaucoma[8,9,10,11,20], blepharitis, and uveitis. However, the present study failed to find an association between H. pylori infection and glaucoma. In the previous studies, a serological method (ELISA) was used to detect *H. pylori* infection; although ELISA is a simple and practical test, it shows high false-positive rates in the elderly and in those not having received *H. pylori* eradication treatment[21,22]. In addition, because elderly individuals comprise the majority glaucoma cases, ELISA was considered inappropriate. Furthermore, in a recent study, the sensitivity and specificity of the urea breath test were 91.4% and 93.8%, respectively, which was comparable with the values for histopathological examination in the elderly[23]. Therefore, we preferred the 14C-UBT method, which demonstrates high sensitivity (92%-93%), specificity (88%-93%), and accuracy (90%-93%), in addition to being noninvasive and reliable in the elderly[15,16].

Deshpande et al. examined *H. pylori* antibodies in the aqueous humor and serum of patients with POAG, those with pseudoexfoliation glaucoma (PXFG), and controls[11]. Serum IgG positivity was significantly higher in the POAG group than in the control and PXFG groups; however, no significant differences in the antibody levels of the aqueous humor were found between the POAG and control groups. However, significantly higher antibody levels were found in the aqueous humor of the PXFG group than in the other groups[11]. Deshpande et al.'s study was limited by the older age of patients in the glaucoma group than in the control group. The higher positivity rate in glaucoma patients was expected because the serological method used has a high false-positive rate. In another study using the same method, Galloway et al. found no relationship between *H. pylori* infection positivity and glaucoma[24]. Similarly, no relationship was found between glaucoma and *H. pylori* infection positivity in a study by Kurtz et al., in which serology and cytotoxin-associated geneA (CagA) seropositivity were evaluated[25]. Handa et al. reported that the *CagA* gene may be associated with apoptosis, irregular gastric cell activity, and carcinogenesis[26]. In addition, *H. pylori* strains harboring CagA were reported to produce more interleukin-8 than those without CagA, causing higher rates of gastric inflammation and atrophy, by Israel et al.[27]. Kurtz et al. suggested that *H. pylori* has no effect on apoptosis that plays a role in the pathogenesis of glaucoma, because the CagA levels in patients with glaucoma were similar to those in the control group. However, Kountouras et al. advocated that *H. pylori* infection induces apoptosis and causes the development and progression of glaucomatous optic neuropathy by releasing several vasoactive and pro-inflammatory substances[28].

In Turkey, the rates of serological *H. pylori* positivity in the general population are high, with rates of up to 68% in symptomatic cases[29]. In the present study, the rates of *H. pylori* positivity detected using the 14C-UBT method were in agreement with the previously reported levels. The finding of high rates of *H. pylori* positivity is expected in countries with higher rates of *H. pylori* positivity, such as Turkey.

## CONCLUSION

Our study has some limitations. First, we did not compare our method with other *H. pylori* evaluation methods, such as the serological and stool *H. pylori* antigen methods, in patients with glaucoma and healthy subjects. Although the reliability of the tests could be compared when used in combination, the use of additional tests would have increased the cost. Second, our study included a limited number of subjects. Further comprehensive studies are needed to verify these results and investigate the association between glaucoma and *H. pylori* using multiple methods.

**Table 1. The demographic characteristics and 14C-UBT positivity of the study groups**

| | POAG | Controls | |
| --- | --- | --- | --- |
| | n=35 | n=35 | *p* |
| Mean age (range) | 59.08 ± 9.82 (28-80) | 58.77 ± 9.44 (29-74) | 0.892 |
| Gender (female/male) | 18/17 | 15/20 | 1.000 |
| 14C-UBT positivity (%) | 18 (51.42) | 15 (42.87) | 0.473 |

14C-UBT= 14C-urea breath test; POAG= primary open-angle glaucoma.

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