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ORIGINAL ARTICLE

Prevalence of *Helicobacter pylori* Infection in Patients with Primary Open Angle Glaucoma

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ABSTRACT

Background: *Primary Open-Angle Glaucoma (POAG) the most common form of glaucoma is a neurodegenerative disease, which is the third most common cause of blindness worldwide. Among the various factors that have been implicated in the pathophysiology of this disease is infection with Helicobacter pylori (HP), a Gram-negative bacterium that is commonly found in stomach and present in approximately one-half of the world's population. This association has caused a great deal of scientific discussion during the past decades, as the establishment of such a correlation might lead to therapeutic applications for all glaucoma patients.* ***Objective:*** *to determine the prevalence of Helicobacter pylori (H. pylori) infection in the primary open angle glaucoma (POAG) in Egyptian cohort.* ***Methodology:*** *Peripheral venous blood samples were withdrawn from each participant and tested for existence of H. pylori particular immunoglobulin G antibodies by enzyme-linked immunosorbent assay in 63 cases with POAG as well as from aged matched non glaucomatous controls at the glaucoma subspecialty Clinic at the Research Institute of Ophthalmology, Giza, Egypt.* ***Results:*** *63 patients (71.4% (45 cases) of them were females with POAG and 30 healthy controls (70% (11 cases) were females and were recruited in our study. Mean age was 57 for POAG patients and 46 for controls. Helicobacter pylori antibodies G were found reactive using ELISA technique in 52 out of 63 patients for POAG group (82.5%) and 13 out of 30 healthy controls (43.3%).* ***Conclusion:*** *High association could be reported between H. pylori and primary open-angle glaucoma suggesting a potential correlation between both.*

INTRODUCTION

Helicobacter Pylori is a Gram-negative bacterium that infects the upper gastrointestinal tract of about half of the world's population. *H. pylori* has been linked to peptic ulcer disease, gastric lymphoma, and stomach malignancy. Atherosclerosis and cardiovascular disorders, Raynaud's syndrome, autoimmune (idiopathic arrhythmias, thyroiditis, Parkinson's disease, Sjögren syndrome, and skin infections such as urticaria), extra-gastric MALT lymphoma, and even diabetes and Alzheimer's disease have all been linked to extra-intestinal correlations[1].

H. pylori has also been correlated with uveitis, blepharitis, glaucoma, and idiopathic central serous chorioretinopathy in the eyes [2,3].

Glaucoma is a multifactorial disease process which pathogenesis is not yet to be completely understood. One of the most frequent reasons of irreversible blindness worldwide is glaucoma, and its incidence is rising. Glaucoma is associated with progressive degeneration of the optic nerve, which leads to a loss of visual field and, if left untreated, blindness. Glaucoma has a complex etiology with several clinical manifestations. The three kinds of glaucoma include primary glaucoma, secondary glaucoma, and the rarer variants of juvenile and congenital glaucoma. There are two clinical phenotypes of primary glaucoma: open-angle glaucoma (POAG) and angle-closure glaucoma (ACG). Raised intraocular pressure (IOP) is a main risk factor for the development and advancement of glaucoma in all categories and decreasing IOP is presently the only approved method of glaucoma therapy [3-5].

There is a lot of discussion about the mechanisms that could be implicated in the probable correlation between glaucoma and *H. pylori* infection. Numerous authors assume that infection causes glaucomatic neuropathy by releasing proinflammatory and vasoactive chemicals, nitric oxide, endothelin-1, and oxygen free radicals, as well as inducing oxidative stress, mitochondrial DNA degradation, and apoptotic cell death. Systemic H. pylori-stimulated oxidative impairment could represent the link between H. pylori infection, oxidative stress, and glaucoma-causing

destruction to the trabecular meshwork and optic nerve head [6].

Recently, a potential relation between *H. pylori* and POAG has been demonstrated with variable and inconclusive results. Herein, we report the first study of the incidence of *H. Pylori* in a cohort of POAG patients in Egypt in comparison to aged matched healthy non glaucomatous controls.

METHODOLOGY

After study procedure was authorized by the research ethics committee of the Research Institute of Ophthalmology, an overall of 63 instances with POAG and 30 control groups were enrolled from the glaucoma subspecialty Clinic and the Comprehensive Outpatient Clinic of the same institute from April 2021 till November 2021. Patients aged 50-75 years old with confirmed POAG based on evidence of glaucomatous optic neuropathy, visual field changes consistent with POAG and proved intraocular pressure (IOP) measurement of 22 mm Hg or higher were included in this study. Patients with eye diseases other than glaucoma, history of gastric surgery, gastric cancer, alcohol abusers and those with severe cardiac, pulmonary, renal, or liver disorders were excluded. Patients who used H_2-receptor antagonists, proton pump inhibitors, nonsteroidal anti-inflammatory medications and anticoagulant therapy in the preceding four weeks were also excluded.

About 5ml of peripheral venous blood were obtained from each person. Commercially available enzyme-linked immunosorbent assay kits (PerkinElmer Kit, USA) were utilized to diagnose the IgG antibodies of *H. Pylori* in the serum isolates after centrifugation of blood and using the serum according to the manufacturer's instruction of the reagent kit.

Specimen collection

Principle:

Ophthalmological examination was done, and the medical record of each participant was reviewed by a glaucoma specialist for previous and current intraocular pressure, visual field changes, optic disc examination and the optic nerve head's optical coherence tomography. The patients were also clinically evaluated to confirm the diagnosis of POAG and to rule out secondary causes of glaucoma.

The IgG immunoassay kit for *H. Pylori* is based on solid phase enzyme linked immunoassay utilizing one anti IgG antibody for solid phase (micro-titer well) immobilization and another anti IgG antibody in the antibody enzyme conjugate solution. The serum is added to the IgG antibody coated micro-titer wells and incubated with the zero buffer.

If Human IgG is present, it will combine with the antibody in the well followed by washing and the conjugate reagent is added which will bind immunologically to the IgG in the well, resulting in the IgG molecules being sandwiched between solid and enzyme linked antibodies. Incubation at room temperature then washing to remove unbounded labeled antibodies. A solution of TMB is added and left 20 min incubation, resulting in development of blue color. The color development is stopped with the addition of 2N HCL and the color changed to yellow and then measured spectrophotometrically at 450 nm.

Procedure

20ul standard, specimens and control in each well,100ul zero buffer was added, mixed then incubated at room temperature for 30 min., mixture was removed. Rinse and flick the wells with washing buffer (1X) tem strike the wells to remove all residual water on absorbent paper. 150ul of the enzyme conjugate reagent was dispensed in each well and mixed then incubated at room temp for 30min. Mixture was removed then rinsed and flicked again with washing buffer (1X). All residual water droplets were removed. 100ul TMB substrate were dispensed in each well, mixed then incubated at room temp. for 20 min. By adding the stop reaction solution the reaction was stopped them mixed, followed by reading the optical density at 450nm.

Statistical analysis

Frequencies (n) and percentages were used to represent qualitative data (percent). The relationships between the multiple modalities were investigated using Friedman's test and Wilcoxon signed rank test.

A P-value of less than or equal to 0.05 was established as the significant level. IBM® SPSS® Statistics Version 20 was used for statistical analysis.

RESULTS

Sixty-three patients with confirmed diagnosis of POAG were tested. This study also comprised 30 healthy control subjects.

Among the study subjects, a high prevalence of females was reported; 45 cases (71.4%) to 18 cases (28.6%) males and 11(70%) females to 9 males (30%), in the control group as shown in table 1:

Table 1: Percentage of sex of case and control groups

Sex Ratio	Cases	Controls
Female	45 (71.4%)	11 (70%)
Male	18 (28.6%)	9 (30%)

Table 2: prevalence of *H. Pylori* in cases compared to control group

H. Pylori Ratio	Glaucoma Group	Control Group
	52 (82.5%)	13 (43.3%)

High prevalence of *H. Pylori* was diagnosed in 52 (82.5 %) of glaucoma instances and 13 subjects (43.3 %) of control group.

DISCUSSION

Glaucoma is the world's third most common type of irreversible blindness. It's often followed by an increase in intraocular pressure. Lowering the IOP with ocular hypotensive treatment is the only established preventive method for preventing visual impairment. Glaucoma is treated with medications that either lower the production of aqueous humor or enable fluid to drain from the eye. Nevertheless, only 20-30% of these medicinal procedures are effective. As a result, a logical approach to glaucoma care focuses more on decreasing risk factors linked to high IOP, which may help avoid glaucoma advancement [7].

In the last few years, researchers have looked at the link between a few ocular disorders' *H. pylori* infection and the pathological systems, notably blepharitis, central serous chorioretinopathy, uveitis, and recent research on Glaucoma.

Galloway and Abrishami[8-9] used an enzyme-linked immunosorbent test (ELISA) to identify IgG antibodies in serum against *H. pylori*. The specificity and sensitivity of this sero-diagnostic assay are exceptional (96 percent and 93 percent, respectively).

The current study was carried on the age range group 55 to75 which was in accordance with Raji et al [10] who reported age group 56 years to 65 years. We reported 45 cases (71.4%) females and 18 cases (28.6%) males in patient group while 11(70%) females and 9 males (30%) in control groups which was similar to a study by Ala et al [11] who reported that 19 cases (38%) were females while 11 subjects (22%) in control group were females but was against Raji et al [10] who reported 31 cases (62%) and 39 subjects (78%) in control group were males.

This current study reported a high correlation between *H. pylori* and POAG documented *H. Pylori* in 52 out of 63 (82.5 %) POAG cases and 13 subjects (43.3 %) of control group. This is in line with the findings of Ala et al [11], who found a (69.2%) association. Also, similar to Raji et al [10], who reported association of (90 %) of cases. In a study conducted in Greece *H. pylori* infection was detected in 87.5 percent of POAG patients [12]. According to this report which was the first to investigate the link between *H. pylori* and glaucoma; *H. pylori* IgG antibody levels were higher in POAG patients' aqueous humors and blood. They also revealed that giving *H. pylori* to POAG patients improved their visual field measurements and IOP. A subsequent study in China demonstrated that people with primary open-angle glaucoma (54.2 percent) had a significantly greater prevalence of *H. pylori* than the control group (20.8 percent), as well as a stronger connection between *H. pylori* and POAG.

Another Iranian study discovered a substantial difference in *H. pylori* infection seroprevalence between individuals with POAG and the control group, with (89.1 percent) and (59.5 percent), respectively, among the control group and individuals with POAG [13]. The frequency of *H. pylori* infection has been documented in Bangladesh. Serologic confirmation of *H. pylori* infection was found in (75%) of instances with POAG and (30%) of individuals without POAG, culminating in a statistically significant difference [14].

Nevertheless, our findings disagree with the findings in a Canadian community that POAG is not induced by *H. pylori* infection[8], and a study that demonstrated non-significant difference in *H. pylori* seropositivity in (50.8 percent) of POAG patients contrasted to (49.2 percent) control groups [15]. This disparity could be attributed to differences in research populations. Potential reasons for this relationship include auto-immune reactions that cause severe or aggravating glaucoma via inducing platelet and platelet leucocyte aggregation and the release of vasoactive and pro-inflammatory cytokines and sharing shared hereditary reasons that regulate apoptosis and inadequate ocular circulation.

The pathophysiology is largely determined by oxidative stress. All the studies seem to behave similarly, comprising a biological reaction in response to an oxidative stress' sublethal dose. These variations appear to be conveyed by both *H. Pylori* infections and ocular ailments, and they all involve: a reduction in mitochondrial dysfunction, a formation of mitochondrial DNA mutations, a higher rate of reactive oxygen species production, a growth in the levels of oxidative DNA, proteins, and lipids damage, and a decrease in the capability to destroy oxidatively damaged proteins and other macromolecules. Regardless of the identity of the injured tissue, this series of events seems to be repeated in various diseases. The trabecular meshwork, conjunctiva, and retina demonstrate how oxidative stress can operate as a shared disease effector as the Helicobacter infection spreads, which is aided by enhanced oxidative injury and inflammation. *H. pylori* can survive in the trabecular meshwork, and alterations in outflow capacity induced by inflammation can result in high ocular pressure and glaucoma [16].

As a result, the pathophysiologic function of *H. pylori* infection must be clarified. Endoscopic biopsy continues to be the gold standard for determining *H. pylori* infection. Nevertheless, this procedure is difficult, necessitates specialized expertise, and is time consuming, making it unsuitable for screening huge populations. Furthermore, it does not confirm the existence of a prior infection. Standardized ELISA experiment, which is simple and quick, can diagnose the existence of IgG antibodies towards *H. pylori*, and ELISA can diagnose *H. pylori* exposure independent of therapy. Furthermore, ELISA is widely regarded as having great specificity and sensitivity at a moderate cost [17].

For the findings of this research, there is a possible link between POAG and *H. pylori* infection and the

Anti-*H.pylori* IgG serologic testing which may be utilized as an alternate diagnostic method for detecting *H. pylori* infection.

CONCLUSION

In ophthalmology, there has recently been a surge of interest in the study of the correlation between the eye and the gut bacteria. Future advancements in this subject may result in a new focus in ophthalmology for understanding and managing ophthalmic illnesses, enabling alternative local or systemic therapeutics to modify the gut microbiota and ocular surface.

We can conclude that anti-*H.pylori* antibodies have a major factor in pathogenesis of POAG in the Egyptian population. Clinicians caring for *H. pylori*-infected patients should be informed that the cause may be both digestive and ophthalmic. Moreover, depending on the research findings, we were unable to determine if the correlation is due to *H. pylori* behavior or to antibodies generated by the host.

Recommendations

More research is needed, as well as adequate proof on a larger scale of instances, to demonstrate that elimination of *H. pylori* infection can positively influences glaucoma parameters.

-There is no conflict of interest

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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