No Association between CagA- and VacA-Positive Strains of Helicobacter pylori and Primary Open-Angle Glaucoma: A Case–Control Study

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ABSTRACT
INTRODUCTION: Glaucoma is a public health issue worldwide, particularly in Africa. In Cameroon, the prevalence rate of primary open-angle glaucoma (POAG) ranges between 4.5% and 8.2%. Helicobacter pylori (HP) has been implicated in digestive and extra-digestive diseases, including glaucoma. The objective of this work was to evaluate the implication of CagA- and VacA-positive strains of HP in POAG using a case–control design.

METHODS: An analytical study was conducted from October 2013 to December 2013. Participants were recruited in eye care centers in Yaoundé. Enzyme-linked immunosorbent assays (ELISAs) were carried out in the La Grace Laboratory in Yaoundé.

RESULTS: The total sample consisted of 50 POAG patients and 31 controls with a mean age of 58.5 ± 12.2 years and 45.5 ± 14.6 years, respectively. The prevalence rates of HP in the POAG and control groups were 74% (37/50) and 87% (27/31), respectively (P = 0.125). The prevalence rates of CagA-positive HP seropositivity in the POAG and control groups were 26% and 22.58%, respectively (P = 0.47), and the prevalence rates of VacA-positive HP participants were 6% and 0%, respectively (P = 0.22).

CONCLUSION: The HP prevalence rates among POAG patients and controls were 74% and 87%, respectively. There was no significant difference between prevalence rates of HP in the POAG and control groups. There was no association between POAG and CagA- or VacA-positive HP infection.

KEYWORDS: primary open-angle glaucoma, Helicobacter pylori, seroprevalence, CagA, VacA, ELISA test

INTRODUCTION
Glaucoma is a group of diseases that can damage the optic nerve and lead to vision loss. It is known as the second most frequent cause of visual impairment worldwide after cataract. Primary open-angle glaucoma (POAG) is the most common form of glaucoma.1 In Africa, glaucoma incidence and prevalence rates are the highest in the world.2 Moreover, the prevalence rate of African blindness related to glaucoma is the highest in the world.3 In Cameroon, the prevalence rate of glaucoma varies from 5.5% to 8.2%.4,5 Moreover, the prevalence rates of monocular and binocular blindness in the world.2 Furthermore, the prevalence rates of African blindness in Africa, glaucoma incidence and prevalence rates are the highest.3 In Cameroon, the frequency rises as high as 95%.6,7 Moreover, research has been conducted on the CagA-positive strain of HP and its association with glaucoma, none has been done on the VacA-positive strain of HP and its association with glaucoma. Our research hypothesis was that CagA and VacA strains of HP are the risk factors for POAG. Therefore, and/or VacA-positive strains of HP contribute to chronic inflammation and/or atrophy of the gastric or duodenal mucosa with digestive cancers as a complication.10–12 Moreover, HP may be involved in extra-digestive diseases.13–18 It is well established that CagA and VacA are HP virulence proteins.10–12 In recent years, research has suggested the implication of HP in the pathogenesis of glaucoma.19–21 Researchers have concluded that HP, especially CagA- and/or VacA-positive strains, stimulates the production of circulating proinflammatory and vasoactive molecules, which are responsible for the breakdown of the hemato-ocular barrier, leading to retinal ganglion cell apoptosis with optic neuropathy as a complication.22–26

In Cameroon, although there is a need to improve our knowledge about glaucoma, little attention has been paid to infections as potential determinants of glaucoma. Moreover, although research has been conducted on the CagA-positive strain of HP and its association with glaucoma, none has been done on the VacA-positive strain of HP and its association with glaucoma. Our research hypothesis was that CagA and VacA strains of HP are the risk factors for POAG. Therefore,
the main objective of the present work was to evaluate the implication of HP in POAG using a case–control design. A secondary objective was to obtain a demographic profile of those individuals identified with POAG and those who have CagA- and VacA-positive strains of HP.

Materials and Methods
This was a case–control study. Patients were recruited from October 2013 to May 2014. All potential participants were recruited in the eye care unit of the Yaoundé Gyneco/Obstetric and Pediatric Hospital and at the Innel Medical Centre in Yaoundé. Laboratory testing was performed at the La Grace Laboratory in Yaoundé.

This study received ethical approval from the Institutional Research Ethics Committee of the Université des Montagnes (CIE-UdM) and the Yaoundé Gyneco/Obstetric and Pediatric Hospital (No. 2013/081/CIE-UOM/Pr 25octobre 2013 and No. 59/CIERSH/DM/2013) and complied with the Declaration of Helsinki.

Potential participants were informed of this study through an informative brochure and gave their written consent to participate in the research.

The inclusion criteria were ≥20 years of age and POAG confirmed by an ophthalmologist on the basis of a comprehensive ocular examination. The exclusion criteria were other forms of glaucoma and <20 years of age.

For controls, the inclusion criteria were ≥20 years of age and the absence of personal or familial history of any form of glaucoma.

Procedure. We used a comprehensive questionnaire to collect demographic and clinical data from the participants.

For all controls, a comprehensive eye exam was performed to exclude any form of glaucoma. It consisted of measurement of best-corrected visual acuity on the Snellen eye chart and of intraocular pressure by Goldmann applanation tonometer and analysis of the optic nerve head with the calculation of the cup/disc ratio. Moreover, a gonioscopic examination and a visual field testing using the Octopus automatic perimeter by Haag-Streit were carried out.

Blood sampling/serology. Blood samples from cases and controls were collected under sterile conditions. All the samples were immediately centrifuged, and the sera obtained were aliquoted and stored at −20° until laboratory testing at La Grace Laboratory in Yaoundé.

Testing was performed with enzyme-linked immunosorbent assay (ELISA) kits according to the instructions of the manufacturer. The method applied was indirect ELISA testing. The following three kits were used:

1. RIDASCREEN® Helicobacter IgG ELISA Kit – 96 tests, from R-Biopharm (item no. 12193) Lot No. 12193;
2. Human Cytotoxin-associated protein (CagA) ELISA Kit – 96 tests, from Cusabio Biotech Co., Ltd. (catalog no. CSB-E09170h) Lot C1025031830;
3. Human Vacuolating cytotoxin A (VacA) ELISA Kit – 96 tests, from Cusabio Biotech Co., Ltd. (catalog no. CSB-E08984h). Lot C1025031835.

Results were obtained after optical reading using an automatic ELISA analyzer, Stat Fax 2100 (450 nm wavelength).

Statistical analysis. Statistical calculations were performed using SPSS Version 18 (Statistical Package for the Social Sciences). Differences between POAG patients and controls were analyzed using t-test for continuous variables and Pearson’s chi-squared test for categorical variables. A P-value of <0.05 was considered statistically significant.

Results
Demography. A sample of 81 participants was obtained. We had 50 (61.7%) POAG patients and 31 (38.3%) controls. There were 43 (53.1%) men and 38 (46.9%) women, with a male/female ratio of 1.13/1 (Table 1).

Seropositivity to HP. The frequency of anti-CagA and anti-VacA antibodies is higher among POAG patients, although the difference is not statistically significant (Table 2).

Discussion
We observed that the average age in the POAG group was >40 years, with a significant proportion of participants aged 51 years and older. Our results are supported by numerous studies on POAG that confirm that age is a risk factor for this disease.27–29 Moreover, Kyari et al, in an African literature review on the prevalence rate of glaucoma in people >40 years of age, noted that prevalence rate increases with age.27

The proportion of men is higher in POAG participants in our study. Although it remains controversial, male gender

Table 1. Sociodemographic characteristics of the study population.

|                         | POAG     | CONTROLS | P-VALUE |
|-------------------------|----------|----------|---------|
| Mean age (SD)           | 58.52 (12.19) | 45.48 (14.67) | 0.000  |
| Age range (years)       | 22–81    | 25–75    |         |
| Sex (Males/Females)     | 30/20    | 13/18    |         |
| Mean duration in years since POAG diagnosis (SD) | 4.92 (4.18) |
| Cup/Disc Ratio (SD)     | 0.8 (0.2) | 0.2 (0.1) | 0.000  |

Table 2. Characteristics of the study population according to seropositivity to HP.

|                       | POAG      | CONTROLS | P-VALUE |
|-----------------------|-----------|----------|---------|
| Seropositivity to HP (%) | 37/50 (74%) | 27/31 (87.00%) | 0.125  |
| Seropositivity to CagA (%) | 13/50 (26%) | 7/31 (22.58%) | 0.471  |
| Seropositivity to VacA (%) | 3/50 (6%)  | 0/31 (0.00%)  | 0.229  |
appears as a risk factor for chronic open-angle glaucoma in the literature.4,27,30

With regard to the average duration of glaucoma since diagnosis and the age of glaucomatous participants, we ensured that the diagnosis of glaucoma has been mainly confirmed in patients >40 years of age. Indeed, although glaucoma screening is recommended in melanoderm populations >40 years old, it seems to be already late when we consider the size of papillary excavations in our POAG group.27 The average cup/disk ratio obtained in our series confirms the advanced stage of glaucoma of our patients. In a Cameroonian study, the late detection of glaucoma was found to be a leading cause of visual impairment.4,30 Moreover, according to a hospital-based study by Ellong et al.,4 the prevalence rates of monocular and binocular blindness due to glaucoma were 8% and 32.9%, respectively, and Eballe et al reported that POAG was the second cause of blindness (monocular: 14.1% and binocular: 19.7%) behind the cataract.30

The prevalence rate of HP infection in our series is comparable to that found in a Cameroonian hospital-based study (72.5%).9 Considering African studies, it appears that the prevalence rate of HP infection in the general population ranges between 60% and 94% and also corroborates our findings.6,7,31

In our series, seropositivity to HP was much more frequent than that found by Galloway et al.32 (anti-HP immunoglobulin by ELISA test in the glaucoma and the control group, 26% and 20.2%, respectively). However, our results, like those of their study, showed a lack of association between HP infection and glaucoma.

Kurtz et al, in an analytical study in Israel involving 51 glaucomatous participants and 36 controls (anti-HP immunoglobulin G by ELISA test in the glaucoma and the control group, 26% and 20.2%, respectively). However, our results, like those of their study, showed a lack of association between HP infection and glaucoma.9

In 2008, Kurtz et al reported a lack of difference between seropositivity to HP in a case–control study (anti-CagA immunoglobulin in glaucomatous cases: 51% and controls: 52%, P = 0.67).33 Our results are comparable to those of Kurtz et al, although the frequency of anti-CagA immunoglobulin is higher in their series than in ours. Our results suggest that strains responsible for the immunologic reaction in our participants are probably different.

Considering anti-VacA immunoglobulin, instances are very few in our sample. This shows that strains carrying the VacA virulence factor (VacA-positive strains) are less frequent than those carrying the CagA factor in our series.

The proportion of participants carrying anti-VacA immunoglobulins is greater in glaucoma participants, although there is no significant difference between the two groups (POAG group: 6%; controls: nil).

In addition, we noticed a lack of difference between the two groups for these two virulence proteins of HP. Although CagA and VacA toxins are known as activators in apoptotic mechanism (caspase activation by intracellular signal transduction transmembrane) and have been described as potential factors in the pathogenesis of glaucoma,22–26 our results did not add to support of this point.

Limitations of the study. Despite the limited size of our sample and the difficulty of perfectly matching participants with controls, our results contribute to the research on HP...
strains and its implication in the pathogenesis of glaucoma in our environment. Further studies should be encouraged on the risk factors of glaucoma in our context. Moreover, genomic studies on HP strains in Cameroon should be conducted because many studies have highlighted the genetic variability of circulating strains worldwide.36–40 Thus, further investigations could help to determine the genetic profile of circulating strains in Cameroon.

Conclusion

In summary, the prevalence rates of HP in POAG patients and controls were 74% and 87%, respectively. The prevalence rates of HP in POAG group and controls were not significantly different. The seroprevalence rates of CagA-positive HP in POAG group and controls were 26% and 22.58%, respectively (P = 0.47), and the seroprevalence rates of VacA-positive HP were 6% and 0%, respectively. There was no association between POAG and various (CagA, VacA) HP strains that are responsible for an immune reaction in our participants. Further studies should be encouraged on POAG to improve our knowledge on its risk factors in this environment.

Authors Contribution

Conceived and designed the study: CDN. Analyzed the data: CDN, ON. Contributed to the writing of the manuscript: CDN, ON, FXE. Agreed with manuscript results and conclusions: CDN, ON, FXE. Jointly developed the structure and arguments for the paper: CDN, ON, FXE. Made critical revisions and approved the final version: CDN, ON, FXE. All the authors reviewed and approved the final manuscript.

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