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Serologic diagnosis of *Helicobacter pylori* in chidren and its utility – a prospective study

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ABSTRACT

Introduction. *Helicobacter pylori,* an ubiquitary spread bacterium, is one of the main ethiologic factors of gastritis and peptic ulcer in children. Several diagnostic methods of this infection have been developed, both invasive and non-invasive, with the purpose of promoting early identification of the bacterium, therefore preventing its associated complications. Serologic tests, based upon detection of circulating *Helicobacter pylori* antibodies, one of the first diagnostic tests used, are widely available, non-invasive, cost-efficient, but do not possess the sensibility and specificity of histology.

Objectives. Through this study, we intend to identify the usefulness of IgA and IgG antibodies measurement in the diagnosis of *Helicobacter pylori,* by comparison with histhopathology examination. Nevertheless, we wish to establish wheteher there is a correlation between the presence of depictable antibodies and certain changes of the gastric mucosa.

Material and methods. We conducted a retrospective study, which included 84 patients aged between 4 and 17 years, with chronic dyspeptic symptoms, who underwent an upper digestive endoscopy, accompanied by gastric biopsies. Serologic *Helicobacter pylori* tests were performed in each of these patients, their results being compared with the microscopic description of gastric mucosal tissue.

Results. IgA and IgG antibodies had lower sensitivity (50% and 81.25%, respectively) and specificity (88.88% and 81.08%, respectively) than microscopic examination. Positivation of IgA antibodies, as an alone serologic response or in combination with depictable IgG antibodies was significantly corelated with chronic gastritis caused by *Helicobacter pylori* ($p<0.001$).

Conclusions. Serologic tests have a lower diagnostic sensibility and specificity than histhology in the case of *Helicobacter pylori,* according to our study. Presence of this bacteria on microscopy was associated with chronic gastritis in all cases. Therefore, this study cannot provide enough data regarding the anatomopathological modifications associated with positive serum antibodies.

Keywords: *Helicobacter pylori,* peptic ulcer, children, antibodies, serologic diagnosis

INTRODUCTION

Helicobacter pylori (H. pylori) is considered one of the most widespread infections in humans (1). Although its colonization of the antrum usually does not cause symptoms, *H. pylori* still has a high prevalence rate among children with low socio-economical status (2) and is one of the major causative agents of chronic gastic inflammation, peptic ulcer, pre-neoplastic conditions and gastric cancer (3,4). Several tests have been developed for the diagnosis of the infections, both invasive and non-invasive (5). Each of them must be used depending on the clinical settings, with non-invasive tests being more cost-effective, whereas endoscopy accompanied by gastric tissue sampling being the appropriate choice in the context of alarming symptoms (6).

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Antibody-based tests have been one of the first diagnostic tests used (7). These are widely available and provide rapid results, but are not recommended in populations with low prevalences of the infection and cannot provide information regarding the moment of the exposure to the bacterium (8). However, they are preffered in patients with recent proton-pump inhibitor or antibiotic treament, as these normally need to be discontinued at least two weeks before performance of an upper digestive endoscopy due to potential disruption of proper identification of microscopic lesions or bacteria (7).

Serological tests can also be used for the screening of populations, as all circulating antibodies (IgA, IgM, IgG), specifically directed against *H. pylori* can be detected. Most of the tests used in clinical practice are based upon IgG detection (8). Their low costs have produced serological empiric testing at large scales. Immunoglobulin G (IgG) antibodies are positive in the vast majority of patients who have contracted an *H. pylori* infection, according to the literature (9). It is, however acknowledged that these types of antibodies cannot identify an active, chronic or past infection (10). A few past studies seem, however to underline the more important role of identifying IgA specific antibodies, which seem to be associated with active *H. pylori* infection in more than two thirds of peptic ulcer cases (11). However, significant, isolated IgA antibody production has also been reported in healthy populations, with up to 54 % of cases reported after screening of a pediatric, Saudi Arabian population (3). As both types of antibodies (IgA, IgG) seem to arrouse in asymptomatic subjects, it is unsurpringly why the recent "Joint ESPGHAN/NASPGHAN Guidelines for the Management of *Helicobacter pylori* in Children and Adolescents" recommend against performing serological tests for diagnosis of active infection or treatment follow-up (12).

The aim of this study is to identify the utility of serum IgA and IgG antibody detection in the diagnosis of *H. pylori* infection, as compared to the histhopathology examination. Nevertheless, we wish to establish whether there is a correlation between the presence of circulating IgA levels, alone or in combination with depictable IgG antibodies, and certain microcopic changes of the gastric mucosa.

MATERIAL AND METHODS

We performed a prospective study on 84 patients with chronic dyspeptic symptoms, with ages between 4 and 17 years, who were admitted in Pediatrics Clinic I, from Tg. Mures Emergency County Hospital, Romania. Subjects with symptoms such as abdominal/epigastric pain, nausea, vomiting, inapetence, pyrosis, bloating and weight defficit were enroled in the study, between February 2018 and November 2019. Serum IgA and IgG *H. pylori* antibodies were determined in each of the patients by enzyme linked immunosorbent assay (ELISA), before undergoing an upper digestive endoscopy (with the help of Olympus gastroscope GIF P30), accompanied by gastric biopsies- at least two pieces, taken from the gastric antrum and corpus. Microscopic examination of the gastric tissue fragments was afterwards conducted, also using Giemsa staining, in order to identify a possible *H. pylori* infection. Results of serum tests were afterwards compared with the microscopical findings.

GraphPad PrismT software was used for statistical analysis. Chi square test helped determine a possible correlation between qualitative variables. Spearman non-parametric test was used to assess correlation between the binary variables (positive/negative *H. pylori* antibodies and *H. pylori* status upon microscopy). The p-value had a significance treshold of 0.05 (for a confidence interval CI of 95%).

Sensitivity was calculated by dividing the number of histologically confirmed cases of *H. pylori* infection in patients with positive IgA antibodies to the total number of *H. pylori* infected patients, whereas specificity resulted from the ratio between the number of subjects with negative IgA antibodies, the lack of *H. pylori* infection being confirmed by histology and the total number of *H. pylori* negative patients.

This research was performed in accordance with the principles of the declaration of Helsinki. The study protocol has been approved by the Ethics Committee of the University of Medicine, Pharmacy, Science and Technology "George Emil Palade" of Tg. Mures (No 64/2018 and No 507/2019). Only patients whose legal guardians signed the informed consent for participation in the study were included.

RESULTS

Mean age of patients included in the study was 12.82 ± 3.13 SD. Out of the 84 patients enroled in the study, *H. pylori* infection was confirmed histhologically in 16 (19.04%) of them. Figure 1 illustrates the gender distribution of the study group, with a female-male ratio of 1.27:1.

A similar, almost equal distribution between the two sexes can be observed when dividing the patients into two groups, depending on the presence of *H. pylori* (figure 2).

Gender does not seem to affect the development of the infection (p = 0.7592, OR = 1.39, CI = 95%). Figure 3 shows the study population's background distri-

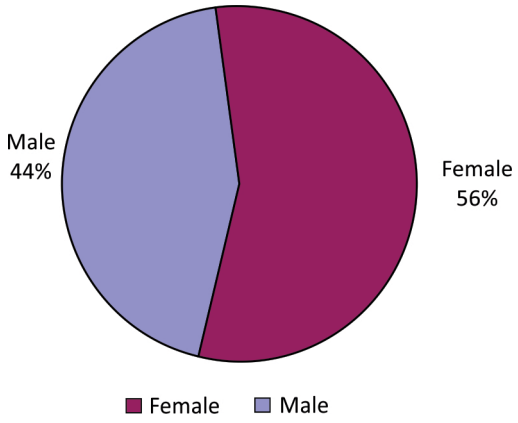

FIGURE 1. *Gender distribution of the study group*

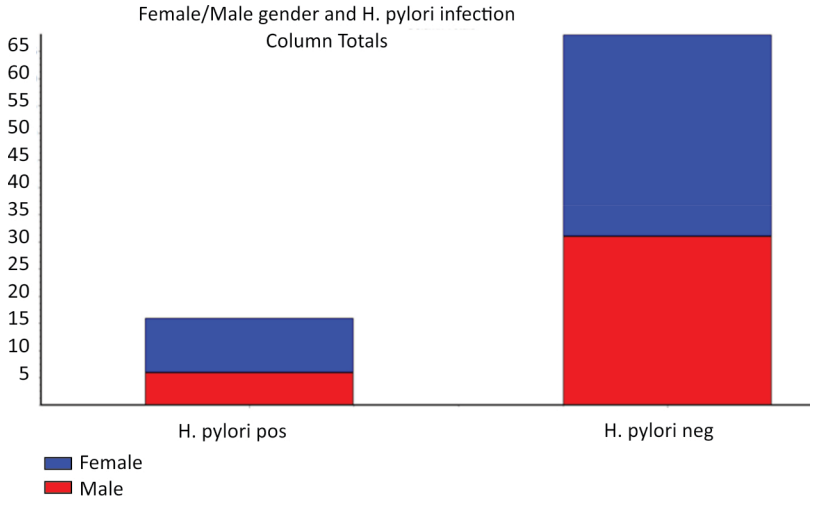

FIGURE 2. *Gender distribution of the study group depending on H. pylori infection status*

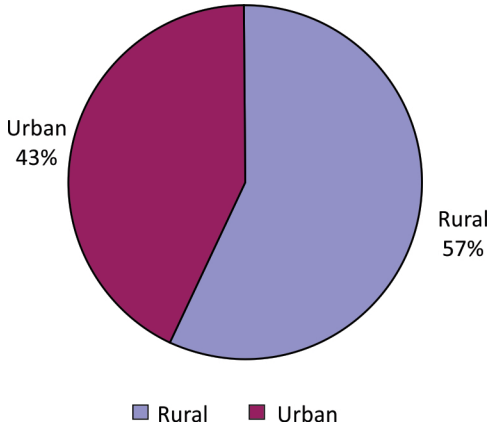

FIGURE 3. *Urban/rural background of the study group*

bution, while figure 4 depicts the relationship between the rural/urban background and the presence of *H. pylori* infection.

An absolute predominance of patients from rural areas can be seen in the category of those with histologically confirmed *H. pylori.* Nevertheless, rural environment has been positively associated with the risk of developing *H. pylori* infection (p = 0.002, OR = 15.909, CI = 95%).

IgA antibodies were positive in only half of patients with *H. pylori* infection, whereas their IgG counterparts were positive in 13 (81.25%) of them. Diagnostic sensitivity of IgA antibodies (as compared with microscopy) was 50%, whereas specificity was 88.88%. The same parameters were calculated for Ig G antibodies, obtaining a higher sensitivity (81.25 %), but a lower specificity (81.08%) as in the case of IgA. An isolated IgA response was found in only 3 (3.57%) patients, *H. pylori* infection being confirmed in only one of them.

In terms of morphopathogy aspects, positive IgA antibodies proved to be significantly associated with chronic gastritis – figure 5 (p < 0.001, OR = 16.36, CI = 95%), but not with acute inflammation of the gastric mucosa (p = 0.93, CI = 95%).

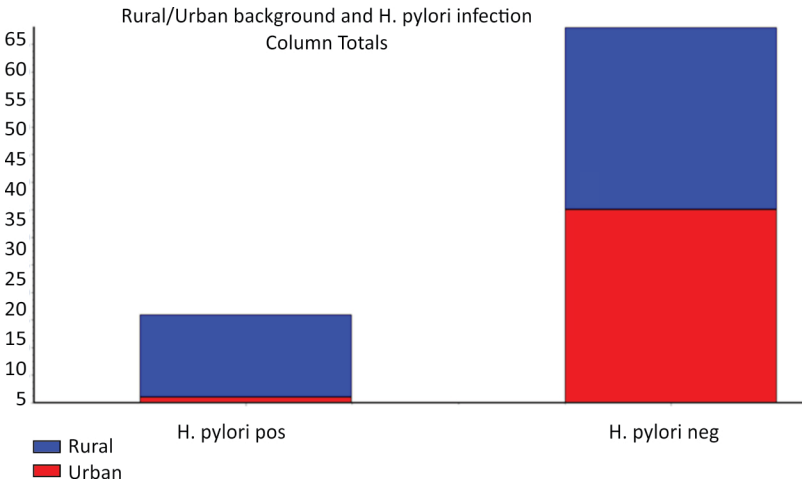

FIGURE 4. *Rural/urban background distribution depending on the presence/ absence of H. pylori infection*

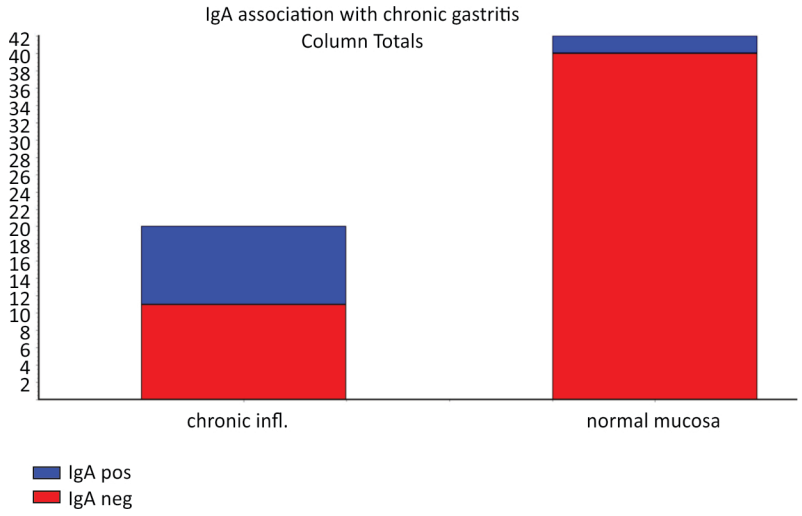

FIGURE 5. *The relationship between positive/negative IgA and chronic inflammatory modifications of the gastric mucosa versus normal histhologic findings*

81.08% of chronic gastritis patients with positive IgA antibodies also had a confirmed *H. pylori* infection. Simultaneous, both positive IgA and IgG antibodies turned out to be predictors of chronic gastritis caused by *H. pylori* in all of the cases. It is important however to mention that all of the patients with identifiable *H. pylori* on microscopic assessment of the gastric tissue also presented chronic inflammatory modifications.

DISCUSSIONS

There are several studies which support the role of serum antibodies measurement, as their titers can be linked to the pathogenesis of *H. pylori* infection. Production of increased levels of IgA and IgG directed against the bacterium is related to its virulence factors, one of which being the CagA antigen, with its well known role in promoting oncogenesis (13). In spite of being related to dyspepsia, presence of the antigen does not necessarily imply that that particular strain has the capacity to secrete the CagA protein, as well (14,15). Data in the literature support the existence of a relationship between the presence of IgG or IgA antibodies directed against the whole cell *H. pylori* antigen and the same type of antibodies produced against the CagA antigen (16). However, a study performed in Finland over a timespan of 21 years strongly supported the use of IgG as an universal antibody against the whole cell antigen, as this type of specific immunoglobuline is more frequently produced than IgA and is also correlated with IgG responses to CagA

antigen (17). In spite of investigating only antibodies directed against the whole cell antigen, our study shows a higher percentage of IgG production in patients with *H. pylori* infection as opposed to IgA, supporting the findings of the previously mentioned study. The use of these IgG antibodies is however limited, as they cannot reflect the status of the infection and remain positive for a long time after erradication of the bacterium (18).

Interestingly, we obtained a lower sensitivity of IgA than most literature data cites. It is, though, well-known that these parameters vary greatly depending on the performance of the detection method used (19). Sensitivity of serological diagnosis was proven to be higher with age increase (20), therefore explaining why sensitivity of serum antibodies tests in our study was far from percentages exceeding 90 %, reported using specific kits, only in adult patients (21).

The importance of IgA production in the defence against *H.pylori,* was investigated by Magen et al., in a study which involved a population with selective IgA deficiency. The authors highlighted the higher incidence of complications related to *H.pylori* in these patients. Nevertheless, they imply that mucosal response is not related to serum IgA or IgG antibody levels, the levels of serum IgA being dependent upon the activity of regulatory T cells. Therefore, they question the sole implications of IgA specific antibodies in the pathogenesis of *H. pylori*- related gastrointestinal conditions, suggesting that a malfunction of the regulatory T cells-IgA pathway may be involved in this process, requiring further investigation (22). This might explain why an isolated IgA serum response has been documented only in low percentages in most of the studies involving children, with 4.9% in a population from Serbia (23). A similar percentage was obtained in our study sample.

Probably the main limitation of this study is the low number of subjects and the even smaller sample of subjects with *H. pylori* infection. Moreover, only antibodies directed against the whole cell antigen were subjects of the current study. An interesting finding is the fact that only chronic modifications of the gastric mucosa were found in relation to *H. pylori.* This explains the positive correlation between IgA secretion and chronic gastritis and the lack of association with acute inflammatory changes in the gastric mucosa, but does not provide any data regarding antibody response in cases of acute gastritis induced by *H. pylori.* Research data support the existence of significant, positive IgA and IgG reaction in the context of both chronic and active gastritis with *H. pylori* (10,13), underlining that these types of serum responses cannot be used to predict the degree of microscopic alterations of the mucosa.

CONCLUSIONS

Serum antibodies directed against *H. pylori* have a lower diagnostic sensitivity and specificity than histology, according to our study. However, they can be used for screening puposes, for diagnosis in conjunction with an upper digestive endoscopy which involves gastric biopsies or alone, as a better option in pre-treated cases. As the infection was associated with chronic gastritis in all cases, the current study cannot provide an insight regarding the predictory value of *H. pylori* serum antibodies in identifying certain degrees of inflammatory alterations of the mucosa.

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Conflict of interest: none declared

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