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ORIGINAL RESEARCH

Detection of *Helicobacter pylori* from Saliva using Enzyme-linked Immunosorbent Assay

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ABSTRACT

Aim and objective: To evaluate and correlate the presence of *Helicobacter pylori* antibodies from the saliva of three groups with varied living conditions.

Materials and methods: Unstimulated saliva was collected and assessed for *H. pylori* immunoglobulins from 90 individuals. Among the subjects, 30 were infants, 30 were adolescents, and 30 were elderly individuals. The samples were subjected to enzyme-linked immunosorbent assay (ELISA) testing.

Results: Infants age between 5 to 10 showed 23 positive results, 3 negative results and 4 equivalent. Age group between 20–25 showed 25 positive results, 2 negative results and 3 equivalent and age group 50 and above showed 25 positive results, 1 negative results and 4 equivalent .

Conclusion: The results in this study showed that ELISA can be used as a diagnostic tool in a resource-limited setting for *H. pylori* estimation from saliva.

Keywords: Digestive tract, Gram-negative, Oral fluid, Ulcers.

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INTRODUCTION

Helicobacter pylori is a spiral-shaped curved bacilli frequently found in gastric biopsy specimens of almost all patients with gastric ulcers or gastritis. Duodenal ulcer risk increases with the increase in gastritis. Though histologic identification of the organism is available, noninvasive serologic and salivary diagnostic test for antibodies to *H. pylori* has recently been developed.[1] Serum immunoglobulin reaction to the infection is an important factor indicating mucosal damage. Concomitantly, these immunoglobulins are secreted in the saliva. Studies have proven that levels of circulating immunoglobulins parallel those of salivary immunoglobulins. As stated earlier, confirmatory diagnosis is based on isolation, culture, and identification of the bacteria; these methods are truly expensive, hence a much simpler and a noninvasive techniques is the need of the hour in a resource-limited setting which could be helpful for the rural and the semiurban population. Therefore, this study aims to evaluate the efficiency of RIDASCREEN *Helicobacter* immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) kit for determining the salivary *H. pylori* IgG in three groups of the population, i.e., infants, adolescence, and the elderly.

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MATERIALS AND METHODS

The study included 90 cases of healthy individuals consisting of group I: Children between 5 and 10 years who are not diagnosed with any systemic condition. The children were randomly selected from a home for destitute children. Group II consisted of subjects between 20 and 25 years who are not diagnosed with any systemic condition. The subjects were students pursuing dentistry in various academic years. Group III consisted of subjects aged 50 years and above who are not diagnosed with any systemic condition. All the subjects in this group were from a home for elderly persons.

A brief case history was recorded in a formatted case sheet from the participants. About 2 to 3 mL of unstimulated whole saliva was collected from the 90 individuals in the saliva collecting vials. The collected saliva samples were stored at –10°C till the ELISA test was done. The samples were subjected to ELISA for detection of anti-*H. pylori* IgG using RIDASCREEN *Helicobacter* IgG ELISA kit[2].

Enzyme-linked Immunosorbent Assay

During ELISA, the collected samples were diluted at 1:50 according to the ratio given by the manufacturer. The samples placed in the wells are covered and incubated for 30 minutes at 37°C. The microwell plate is emptied and then washed four times with 300 µL diluted wash buffer. Now 100 µL conjugate is placed in each well including the empty well. The plate is covered again and incubated for 30 minutes at 37°C. The washing of the plate is repeated

four times with the diluted wash buffer. Next 100 µL of substrate is added to all the wells and incubated for 30 minutes at 37°C. After the incubation, 100 µL of stop reagent is added to the well. After the reaction is stopped, the well is subjected to photometric measurement at 450/620 nm for the results.

RESULTS

Thirty saliva samples were collected and analyzed for *H. pylori* from each age group (Graph 1 and Table 1):

- *Group I*: Infants aged between 5 and 10 showed 23 positive results, 3 negative results, and 4 equivalent.
- *Group II*: age 20 to 25 showed 25 positive results, 2 negative results, and 3 equivalent.
- *Group III*: age group 50 and above showed 25 positive results, 1 negative result, and 4 equivalent.

Table 1: Distribution of Samples

Age (years)	*Total sample size*	*Positive*	*Negative*	*Equivocal*
5–15	30	23 (76.7%)	3 (10.0%)	4 (13.3%)
20–25	30	25 (83.3%)	2 (6.67%)	3 (10.0%)
50 and above	30	25 (83.3%)	1 (3.33%)	4 (13.3%)

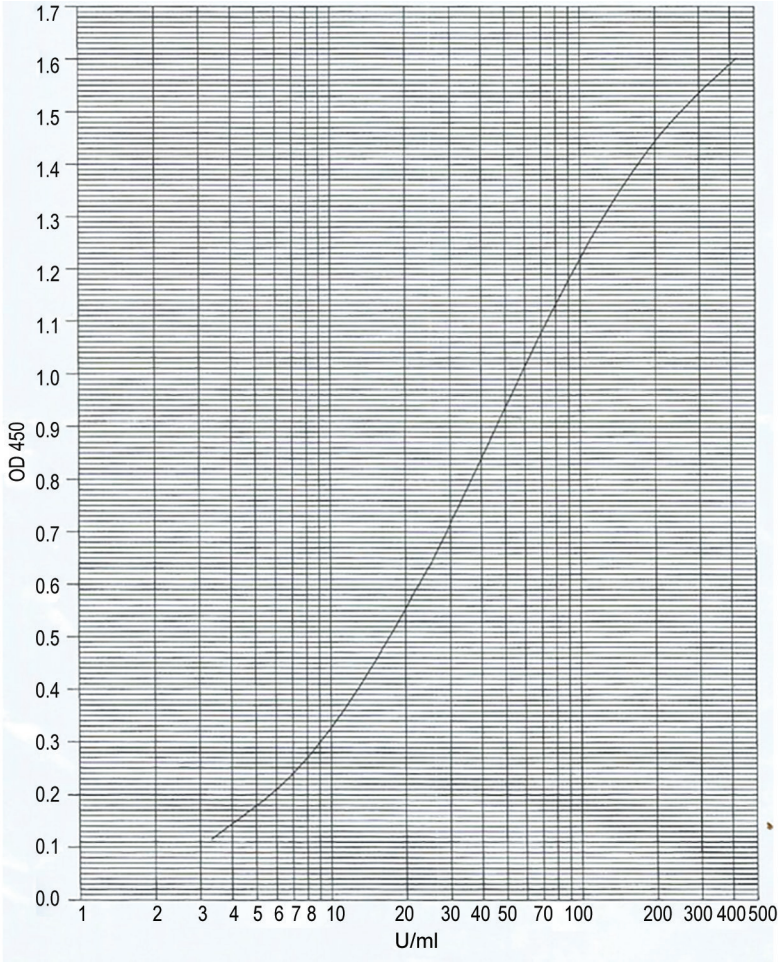
DISCUSSION

A noninvasive test like ELISA is the need of the hour, especially in developing countries where there is a limited health-care setting. Endoscopy is considered a gold standard for the diagnosis of *H. pylori* from gastric ulcers and ulcers of the gastrointestinal tract.

Advancements in the field of research on saliva have opened numerous pathways toward its remarkable potential in diagnosing as well as monitoring diseases like cancer, especially head and neck, lung, breast, and such others, neurological disorders like Alzheimer's and Parkinson's, to monitor the response of antipsychotic drugs, corticosteroids, and drugs of substance abuse. The first reported clinical use of saliva was done to test the acidity (1836) in recovering patients with chronic bronchitis.

Few of the earlier studies done using nested Polymerase chain reaction in 1993 to check the presence of *H. Pylori* showed a 100 % specificity of nested PCR.[3-5]

Helicobacter pylori detection by ELISA was done in serum and the seroconversion rate as 0.49% has been reported ever since 1992 by Parsonnet et al[6] in a cohort of 341 epidemiologists. Drumm et al[7] confirmed the presence



Graph 1: RIDASCREEN *Helicobacter* IgG (Ch.-B./Lot:15096)

of *H. pylori* ELISA in children and their family members with upper gastrointestinal symptoms using biopsy and serum samples. *Helicobacter pylori*-specific antibody was detected more from the parents of children who had colonized bacteria ($p < 0.001$). This could explain the possible presence of bacteria in our child group.[8-10] Seropositivity of H Pylori was confirmed by the prevalence Ig G antibodies from the saliva in adults by Mendall et al in 1992.[11-13] The seropositivity was between 9% to 67% in 215 subjects wheras we found seropositivity between 76% to 83% in 90 subjects. The increase in the prevalence of seropositivity was seen by Mendall et al, Drumm et al.[3,7,14,15]

Detection rate seen in children in our group was 76% and this high prevalence could be because of the crowded and closed living condition of the children.[16-18] The even higher increase in incidence in Group 3 which was 83% also could be co related to the above study.

CONCLUSION

The results showed that the presence of *H. pylori* is quite rampant and prevalent in groups in which there is a close association between humans. These results need to be investigated more in depth in a larger sample size to confirm the effectiveness of saliva as a diagnostic aid and its future use in a resource-limited setting.

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