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Seroprevalence of *Helicobacter pylori* Infection in Hyderabad, Sindh

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Abstract: *Helicobacter pylori* is the major causative agent of gastric cancer and peptic ulcer worldwide, but more common in developing countries. The prevalence studies are very important in determining disease burden and devising eradication measures based on the data on rates of infection and affected age groups. Thus, the overarching goal of the current study was to study the prevalence of *H. pylori* in population of Hyderabad city. Blood samples were collected and the serum was separated using gel tubes. Detection of *H. pylori* IgG antibody was performed using enzyme-linked immunosorbent assay. A total of 2835 samples (1,525 males and 1,310 females) were enrolled for detection of *H. pylori* antibodies during January 2015 to December 2015. Individuals yielding anti- *H. pylori* IgG positive with ELISA assay, were reported as positive, while the absence of antibodies were reported as negative for *H. pylori* infection. The seroprevalence of *H. pylori* infection was recorded as 24.87% (*n* = 705 out of 2835). Among the positive samples, males were 13.58% (n=385) and females were 11.29% (n=320). The positivity of *H. pylori* antibodies was found higher (23.67%) in both male and female patients whose age was between 10 and 50 years. The lowest seropositivity was recorded in the age group under 10 years (0.21%) and in the age group of 60 years and above (0.39%). In summary, the findings of present studies demonstrate a high seroprevalence of *H. pylori* in Hyderabad. Moreover, no gender based differences were found and the prevalence appeared lower in younger age of under 10 years.

Keywords: Seroprevalence, *Helicobactor pylori*, Peptic ulcer, Gastric cancer

1. INTRODUCTION

Helicobacter pylori, is a Gram-negative, micro-aerophilic, spiral-shaped bacterium which colonizes the gastric mucosa. *H. pylori* is a common pathogenic bacteria which is carried in the stomachs of more than half of the population throughout the world (Group 1993). It is usually acquired during childhood and if not treated it can survive in the gastric environment throughout the life of an individual. In many countries, the incidence of HP infection has been demonstrated to decrease with improved living standards (Hooi *et al.* 2017). Various factors such as acquisition and the loss rate of infection (Malaty *et al.* 1992), the length of persistence during acquisition and loss period have been reported to contribute to the prevalence of infection including (Pounder andNg 1995). Accordingly, the prevalence rates of *H. pylori* differ from country to country as well as on the basis of difference between ethnic, social, or age groups (Malaty *et al.* 1992; Malaty *et al.* 1999). Based on socioeconomic and hygiene behaviors, the prevalence of *H. pylori* infection has been reported to be higher in developing countries that of the developed countries globally (Megraud *et al.* 1989; Salih 2009). Moreover, in childhood period, the *H. pylori* infection is less prevalent in developed countries (Dooley *et al.* 1989; Rothenbacher and Brenner 2003), accounting less than 5% in under five years age and 10% in adults in USA incidences is higher at (Taylor andBlaser 1991; Dooley *et al.* 1989). While, the rate of *H. pylori* younger age in the developing countries (Sack andGyr 1994; Weaver 1995), occurring 50% in children of five years age (Megraud *et al.* 1989; Sack and Gyr 1994), and the infection rates may reach to 90% or even higher in adults in developing world. The spread of infection has been shown be linked with socioeconomic and hygiene status. Thus, due to less improved socioeconomic status, the developing countries suffering high burden of *H. pylori* infection as compared to the developed countries where hygiene and socioeconomic status is much improved resulting in lower prevalence rate of *H. pylori* infection because of the improved socioeconomic status. Prevalence *of H. pylori* infection has been studied using a number of diagnostic methods such as 'nonendoscopic' (e.g. serological tests and breath tests) or 'endoscopic' (histology, rapid urease test, Culture, PCR). The present study has used ELISA based diagnosis of *H. pylori* for determining the frequency of infection. The aim of the current study was to assess the seroprevalence of anti *H. pylori* antibodies in the population of Hyderabad using ELISA assay.

2. MATERIALS AND METHODS

2. 1. Study location, sample size and detection techniques

In this study, a total of 2835 blood samples were analyzed over a period of one year from January 2015 to

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December (2015) from the Hyderabad city. Antibodies against *H. pylori* were detected by ELISA using commercially available HP IgG Kit (DIA.PRO Italy) according to the instructions of manufacturer. A five ml of blood sample was collected and used for separation of serum using gel tubes. The Gel tubes containing samples were centrifuged at 4000 rpm for 5 minutes. The upper portion representing serum was collected and either analyzed immediately or stored at 4 °C for future use.

2.2 Serodiagnosis of *H. pylori*

Microplates were coated with immunodominant antigens of virulent strains of *H. pylori* grown in tissue culture. During first stage of incubation, diluted samples were added to wells where antigen captured *H. Pylori* IgG antibodies. The microplate was then washed thrice and bound *H. Pylori* IgG antibodies were detected by the addition of anti-HP IgG antibody conjugated with horseradish peroxidase (HRP) enzyme. Finally, the reaction contents were developed using a chromogenic substrate which is utilized by the HRP enzyme and produces color. The intensity of color is proportional to the amount of anti- *H. Pylori* IgG antibodies.

2.3 Enzyme linked immunosorbent assay

Briefly, 100µl of calibrators, control serum and diluted samples were dispensed into each well of microplate, and incubated for 1 hour at 37ºC. The reaction contents were discarded and the microwell plate was washed three times with wash buffer (supplied with kit). Following washing, 100µl of enzyme conjugate solution were pippeted into each well, except blank wells, and the microwell plate was covered with the sealer. The microplate containing reaction contents was then incubated for 1 hour at 37°C. After incubation, wells were washed thrice with wash buffer and 100µl of chromogenic substrate mixture were added to each well containing samples including the blank wells. The microplate was then incubated at 18-24°C (or room temperature) for 15 minutes. The reaction was stopped by adding 100µl sulphuric acid to the wells. The results were recorded by measuring the intensity of the color of solution at 450nm filter (reading) in all wells of microplate.

3. RESULTS AND DISSCUSION

3.1 Distribution of samples collected for diagnosis of *H. pylori*

The present study has investigated the prevalence of anti- *H. pylori* antibodies in population from Hyderabad city. A total of 2835 subjects were studied, out of them 53.79% (n=1525) were males and 46.21% (1310) were females. A huge majority of subjects i.e. 91.11% (2583/2835) were found in the age range of 10–50 years whilst the remaining samples belonged to either under 10 years or above 50 years age.

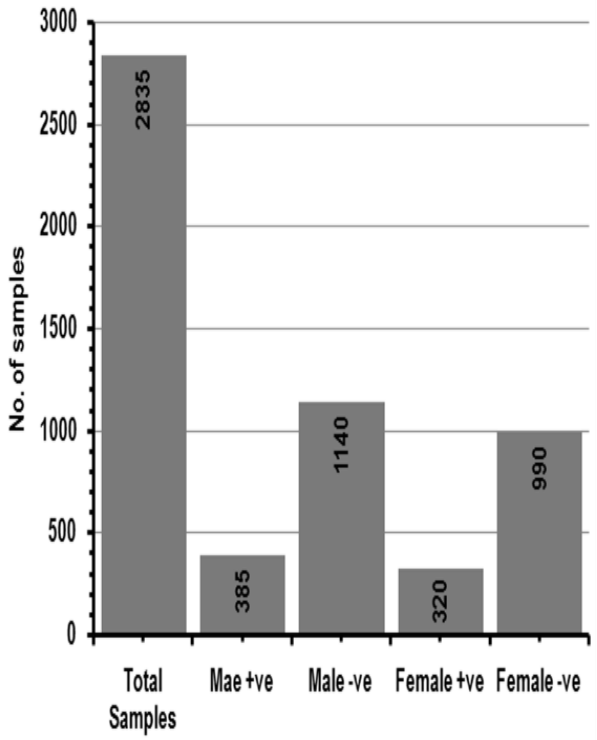


Fig. 1 Graphical representation of age and gender-wise distribution of all samples

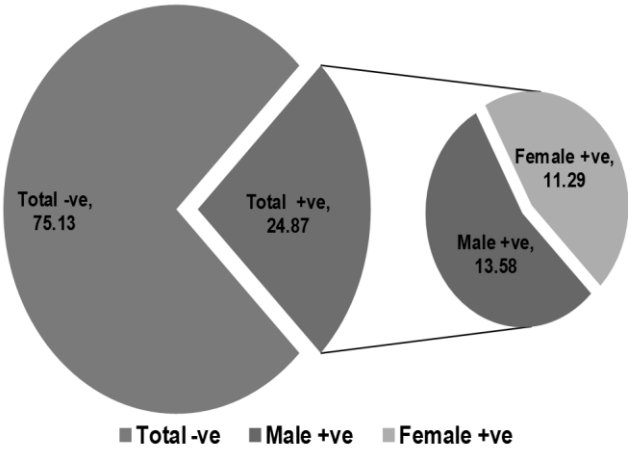


Fig. 2 Graph showing the distribution of all samples and seroprevalence of *H. pylori* infection

3.2 Age and gender-wise prevalence of *H. pylori* infection

The age and sex wise distribution of *H. Pylori* infection demonstrated that 24.87% (n=705) of all subjects were positive for anti- HP positive whilst 75.13% (n=2130) were negative for anti *H. pylori* antibodies. Of all positive samples, 13.58% (n=385) were males and females were11.29% (n=320). It was noted that *H. pylori* infection was considerably higher in both male and female subjects whose age was 10 to 50

years (23.67%). The lowest seropositivity (0.21% (in age group under 10 years and 0.39% was observed in the older age group subjects whose age was above60 years.

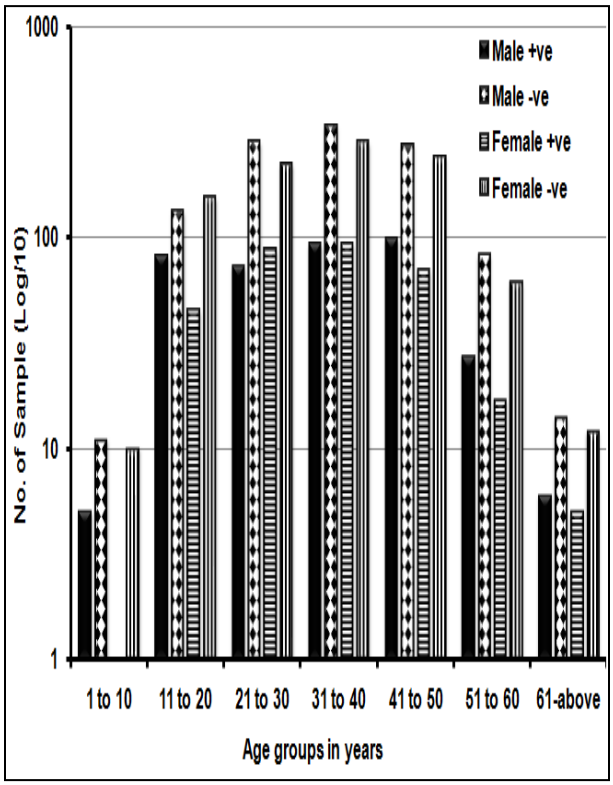


Fig. 3 Age and gender-wise prevalence of *H. pylori* infection

The findings of current study revealed a very low positivity of *H. pylori* infection in age group comprising the subjects under 10years age. The prevalence however has been found to gradually increase with increase in age as can be seen in the age group 11-50 years over all samples and the ratio of anti- *H pylori* appears to be increasing. Highest incidences were recorded in age group 30-40 years and again an obvious decrease was found in the older age group of 61 and above years.

4. CONCLUSION

The present study showed a seroprevalence of 24.87%in Hyderabad which could be considered a high prevalence. There was not a considerable difference is the seroprevalence of male and females. Future studies should be performed focusing on using alternative diagnostic tests such as rapid urease test, histological test and if facilities are available the culture should be performed to investigate the true prevalence of *H. pylori* infection in Hyderabad. Moreover, future studies are also needed to study routes of transmission, source, and risk factors for dissemination of the *H. pylori* in population.

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