Prevalence of asymptomatic Helicobacter pylori infection in Kerala, India

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Abstract
Helicobacter pylori is a bacteria found in many untreated water sources in developing countries. They colonize the luminal surface of gastric epithelium in about 50% of population. Though most infections are asymptomatic, they are the major microorganism causing duodenogastric ulcers, carcinoma of the gastric region and gastric mucosa associated lymphoid tissue (MALT) lymphoma. The serologic study to detect prevalence of Helicobacter pylori was done on healthy blood donors. The cross sectional study was done in a medical college hospital in the state of Kerala; India. The sample was collected from the donated blood. The serum was subjected to serological studies to detect Helicobacter pylori specific IgM and IgG antibodies. The results were noteworthy. A seroprevalence of 29.29% to Helicobacter pylori antibodies was detected in the study group. This is less than similar seroprevalence studies done in different parts of the world.

Keywords: Helicobacter pylori, Seroprevalence, Blood donors, Asymptomatic.

Introduction
Helicobacter pylori are pathogenic bacteria present in the luminal surface of gastric epithelium. Around 50% of the population world over is infected with these bacteria.[1] This organism can cause chronic inflammation of the underlying gastric mucosa leading to gastritis and further complications. Robin warren, Barry Marshal cultured and demonstrated Helicobacter pylori from gastric biopsy sample and won Nobel Prize in 2005.[1] This disease has more prevalence in developing countries and less prevalence in developed countries. Helicobacter pylori infection is believed to persist lifelong unless treated with antimicrobials.[4]

Helicobacter pylori are common bacteria but studies on it are relatively less.

Helicobacter pylori are gram negative bacteria exhibiting spiral morphology. It is a motile bacterium with 3-4 polar flagella. It produces urease, catalase and oxidase, making the test to detect them positive. Contaminated water is often the primary mode of transmission where potable water is in short. Helicobacter pylori are capable of altering host physiology and subvert the immune response, which allows the bacteria to survive in the host for life.[1]

Helicobacter pylori have unique properties which contribute to its persistence in the host. Helicobacter pylori produces urease, this urease converts urea to ammonia plus carbon dioxide which raises the pH around the site of growth of the bacteria. This property of the bacteria provides temporary protection against gastric acids produced.[1]

Infected individual are mostly asymptomatic. Helicobacter pylori classically cause functional dyspepsia, which presents as pain in stomach or upper abdomen[1] Helicobacter pylori also causes gastritis. Certain cancers caused by long standing infection even if asymptomatic are colorectal cancer.[1] Helicobacter pylori infection acts as a helping factor in the development of some of the major upper gastro intestinal diseases. They are gastric carcinoma and gastric mucosa associated lymphoid tissue lymphoma and duodenal or gastric ulcer.[4] Other lymphomas like diffuse large B cell lymphoma are also associated to it. Laryngeal and pharyngeal cancer of the squamous cell type is found to have Helicobacter pylori aetiology.[1] In fact Helicobacter Pylori accounts for 5.5% of gastric cancers and 25% of all infection associated cancers.[1] The extra gastric disease associated with Helicobacter pylori are iron deficiency anaemia and idiopathic thrombocytopenia.[1] A skin disease like rosacea is also associated with Helicobacter pylori infection. Helicobacter pylori infection has been connected to the development of pulmonary diseases like bronchiectasis and long standing respiratory diseases like chronic obstructive pulmonary disease (COPD). Certain studies have also linked Helicobacter pylori to neurodegenerative diseases like Alzheimer's and Parkinson's disease.[1] These diseases linked to long standing asymptomatic Helicobacter pylori infection justifies diagnosis of asymptomatic Helicobacter pylori infection. All these reasons justify the study to identify antibodies to Helicobacter pylori in asymptomatic healthy individual by ELISA and treating them essential and useful.

Helicobacter pylori infection can be detected by a number of invasive and non-invasive methods. The invasive methods like endoscopic biopsy specimen collection of gastric mucosa can be done. These endoscopic biopsy samples can be sent to laboratory for rapid urease test, histopathology evaluation and culture in artificial growth media. These tests and procedures can detect the causative Helicobacter pylori.[3]

The non-invasive tests like urea breath test and stool antigen test can also be done to detect Helicobacter pylori.[3]

The detection of the vast majority of asymptomatic cases of Helicobacter pylori infection is in the scope of this study. The voluntary blood donors are considered healthy, routine questionnaires and basic physical examination are done on voluntary blood donors before blood is drawn. Blood of blood donors collected are subjected to other tests

as well, as part of screening. The detection of antibodies in the serum specific to Helicobacter pylori asymptomatic infection in these blood donors seems logical and is less troublesome for the persons.

Serology testing for Helicobacter pylori is widely done and cost effective. Enzyme assay microtiter plates coated with Helicobacter pylori antigens, with a secondary antibody, are used to detect Helicobacter pylori induced IgM and IgG. Serology results are not affected by acid suppression therapy or recent antibiotic use.

In this study the following are discussed:

1. Helicobacter pylori prevalence in the general population.
2. Usefulness of serological detection of asymptomatic cases of Helicobacter pylori infection.

Persons who come to donate blood voluntarily are considered as healthy individuals, so their blood samples are examined to detect the presence of antibodies in serum to asymptomatic Helicobacter pylori infection in a group of population in Kerala.

Aims and Objectives

To detect the seroprevalence of Helicobacter pylori among healthy blood donors from a group of subjects from Kerala.

Materials and Methods

Type of Study: Cross sectional study
Study Site: Academy of medical sciences (Pariyaram Medical college Hospital)
Period of Study: 1year (October 2016 to September2017)
Study Population: Blood donors
Sample Size: 99 serum samples

Inclusion Criteria

Person in the age group from 18 to 60 years visiting the blood bank of Pariyaram medical college hospital for voluntary blood donation.

Exclusion Criteria

Donors who were previously medically managed for Helicobacter pylori infection. Donors who had received proton pump inhibitors therapy and who received bismuth compounds therapy, four weeks before.

Collection of Specimen and its Processing

Following the universal precaution for asepsis 3ml of blood was collected from the blood bag which was collected directly into a vacutainer. The vacutainer is put into a centrifuge and the centrifuge is run for ten minutes at a speed of 3000 rotations per minute. This separates the serum. The separated serum is stored in serum tubes. This is stored in aliquots at a temperature of -20 °C in a deep freezer. The antibodies produced are then measured with a commercial enzyme immunoassay. The enzyme assay used was manufactured by Cali Biotech El Cajon, CA. This commercial assay kit was used to detect the IgM and IgG antibodies produced in response to Helicobacter pylori infection specifically.

The Principle of Helicobacter Pylori Specific Antibody Enzyme Assay

Into the microtiter plate wells to which the antigen, which is a purified form, is adhered, the test serum is added. If IgG or IgM antibody is present in the test serum it binds to the antigen coated on the microtiter wells. The enzyme conjugate when added will bind to the antigen –antibody complex, if such a complex is formed in the microtiter wells. Then the substrate is added. The wells are incubated to allow for the hydrolysis of the substrate by the action of the enzyme. The intensity of the colour developed depends on the amount of IgG and IgM antibodies present in the serum sample collected.

Procedure of the Enzyme Assay

The required microtiter wells are taken and fixed on the holder. All the reagents and samples are brought to room temperature. The test samples are diluted in the ratio of 1:20. This is done by adding 10µl of the sample to 200µl of the sample diluent provided in the kit. This is mixed well. After the test sample, the negative control, the positive control and after this the calibrator is added into the earmarked enzyme assay microtiter plate well. To this 100µl of serum which is diluted, is dispensed along with controls into the earmarked enzyme assay microtiter wells. A 100µl of diluent is transferred into a marked well. The air bubbles are removed by gentle tapping of the holder. This is again incubated at room temperature for 20 minutes. After the incubation, liquids are removed from the wells with 300µl of wash buffer which is diluted. The microtiter wells are rinsed. To each of the wells 100µl of enzyme conjugate is transferred and mixed gently for duration of 10 seconds. After this incubation is done at room temperature for 20 minutes. After this the enzyme conjugate is removed from the wells with wash buffer which is previously diluted. Then 100µl of TMB reagent is added to each well and mixed gently for 10 seconds. The stop solution of 100µl is added to each well. It is imperative that the blue colour developed earlier changes completely to yellow at this stage. The optical density of the colour change is measured at 450nm using an ELISA reader.

To calculate interpretation values:

Cut off value=Calibrator mean OD* calibrator factor (0.5)

Antibody index = Sample OD /Cut off value

Interpretation

Values less than 0.9 there is no detectable antibody to Helicobacter pylori.

If value in the range of 0.9-1.1, it is borderline positive to antibody to Helicobacter pylori. Follow up testing is recommended if clinically indicated.

Values more than 1.1-Dectectable antibody to Helicobacter pylori.

The test was performed according to instructions provided in the test kit by the manufacturer.

Results

A total of 99 samples from the blood donor blood specimen were included in the study. All 99 serum samples were

tested for the presence of Helicobacter pylori antibodies of the IgG and IgM classes. Of the tested samples out of 99 serum samples from healthy blood donors 8 was positive for IgM antibodies produced against Helicobacter pylori, which is 8.08% of samples were positive for IgM antibodies against Helicobacter pylori. That means they are currently infected with Helicobacter pylori. Out of the 99 specimen collected for study 21 of the tested samples where positive for IgG antibodies against Helicobacter pylori. This shows that 21.2% of the subjects were positive for IgG antibodies, which were produced as a result of immune stimulation against Helicobacter pylori infection. That means, they had been infected by a previous Helicobacter pylori infection and developed antibody as part of normal immune respose. The total number of seropositive persons of the samples analysed is 29 out of the 99 samples. This is 29.29% of the study group.

Discussion

The information revealed by the study will contribute to a better understanding of the subgroups at higher risk of Helicobacter pylori infection in Kerala and should serve as a guide for future research, as well as to tailor better gastric cancer prevention and control strategies for this population.

The result of this study is different from other similar studies done to detect seroprevalence of Helicobacter pylori.

In the present study the total number of samples positive for Helicobacter pylori antibody is 29 out of the 99 specimen tested. The samples positive for IgM antibody to Helicobacter pylori out the tested 99 specimen is 8. The samples tested positive for IgG antibodies to Helicobacter pylori is 21 out of the total of 99 specimen tested. The percentage of positive specimen to antibodies against Helicobacter pylori in the study is 29.9%. The percentage of samples positive for IgM antibody against Helicobacter pylori is 8.08%. The percentage of specimen positive for IgG antibody against Helicobacter pylori is 21.2%.

In a study conducted in Ethiopia, Africa, the seroprevalence was 65.7% of the study group.[5] According to the researchers Mathewos B et al the high prevalence of Helicobacter pylori is due to poor environmental and sanitation situation prevalent in the study group in the city Addis Ababa of African nation, Ethiopia, This is way more than the 29% seroprevalence shown in the present study. The seroprevalence with studies conducted in some countries of Asia and Europe, like the Gulf country of Kuwait and other countries like USA, Canada, Hong Kong, Iran, the percentage of the prevalence was 49%, 9.4%, 23.1%, 42.8% and 43% in respective order of reference. This all showed lower than the Ethiopian study. This might be best explained by association between social and economic status and Helicobacter pylori infection, since in countries with low socioeconomic status, there is low level of hygiene. The sanitation problem and lack of provision for potable water which are known factors which can be a predisposing factor for the infection.[5] In another study in Puerto Rico the prevalence rate was 33%.[6] This study discussed aspects like living condition of the study group, the betterment of provision for better sanitation and potable drinking water influences prevalence of Helicobacter pylori infection. The comparison in this study was with other states of united states and other Latin American countries.[6] This is somewhat similar to the present study done here in Kerala. In a similar study in South Korea the seroprevalence was 41.5%.[7] This study took in to consideration the socio economic situation in comparison with other developed countries. This is higher than the present study which is only 29.29%. In the present study the prevalence of Helicobacter pylori infection is less compared to different studies conducted among a wide range of population from different socio economic backgrounds. The present study shows low prevalence of Helicobacter pylori infection in Kerala, which is reason to cheer considering the good health indices and parameters validated by National census survey report,[8] from among states in India.

Conclusion

The present study has shown that in the group from Kerala, India the seroprevalence detected are less than similar studies done in some developing as well as developed countries. The reason for the reduction in number of seropositive cases of Helicobacter pylori in the present study group may be due to better availability of potable drinking water and overall general health of population studied. The health indices of Kerala are comparable to developed countries in different parts globally. To throw light on the causes of reduction in seroprevalence in cases who are asymptomatic to Helicobacter pylori infection in this study group from Kerala, India, further studies may be undertaken.

Conflict of Interest: None.

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