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Original article

Helicobater Pylori Infection Among Asymptomatic Children - A Seroprevalence Study

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ABSTRACT

Background: Helicobacter pylori is the most common bacterium colonizing in the parietal cell area of gastric mucosa and chronic infection may cause chronic active gastritis, adenocarcinoma and lymphoma of the Mucosa Associated Lymphoid tissue (MALT) of the stomach. Helicobacter pylori infection is usually acquired in childhood and prevalence of infection increases with age and an early age of acquisition is critical in the development of disease and its complications in the later part of life.

As low socio economic and domestic over crowding predisposes to infection, infections are more prevalent in developing countries like India. Infection results in specific immune response and IgG specific antibodies begin to appear with in 22-23 days of infection. The antibody titre gives indication of infection but not the presence of disease. The children with high titers are also asymptomatic. Estimation of specific IgG antibodies to H. pylori antigens in the serum by ELISA is highly specific.

Material and Methods: 264 children, 150 male children and 114 female children found out as asymptomatic for gastrointestinal tract disease were studied for serum IgG specific Helicobacter pylori antibodies by Medden's Diagnostic B.V.A.A.Vordan, Netherlands, Helicobacter pylori IgG ELISA Quantitative Kit. Concentrations of more than 20 AU/ml indicates positive status for Helicobacter pylori infection and concentrations of $\leq$ 15 AU/ml indicates negative status for Helicobacter pylori infection.

Results: In the study group female children have acquired H.pylori infection at an earlier age of 2 years where as at 2 years of age, all male children were seronegative. The seroprevalence gradually increased with age and by 12 years of age seropositivity among children of both sexes was almost the same. The overall seroprevalence was found to be 44.3% and all seropositive children were from families with low socio economic status.

Conclusion: Screening of children for H.pylori by accurate non-invasive methods helps in early detection, prompt treatment and prevention of late complications.

KEYWORDS: ELISA, Helicobacter pylori (H.pylori), Mucosa associated lymphoid tissue (MALT)

INTRODUCTION

Helicobacter pylori is the most common bacterium colonising the gastric mucosa and chronic infection causes chronic active gastritis, ulcers, adenocarcinoma and lymphoma of the stomach as well as to the more benign tumors of mucosa associated lymphoid tissue (MALT). But most of the patients remain asymptomatic[1,2,3]. Helicobacter pylori colonises parietal cell area of gastric mucosa and it has been cultured from antral biopsy specimens in more than 80% of patients with duodenal ulcer, 50% with gastric ulcer and about 40% to 50% patients with Non ulcer dyspepsia.

Helicobacter pylori infection is usually acquired in childhood and increases with age, and an early age of acquisition seems to be critical for the development of severe complications in the later part of life[4]. Once infection occurs, it results in systemic as well as local immune response characterized by appearance of specific IgG and IgA in serum and secretory IgA and low level gastric IgM[5] . The specific IgG seroconversion occurs within 22 to 23 days of infection. Further there is no correlation between the antibody titre and the onset and severity of the disease. The major risk factors for acquiring Helicobacter pylori infection is low socio economic status and crowded living conditions[6,7]. It is widely present in many developing countries like India. The overall prevalence in children was reported to be 45%. Almost half the children in South India acquire Helicobacter pylori infection early in life which increases slowly and steadily peaking in young adults[8]. As Helicobacter pylori, infection is curable an early diagnosis and treatment in childhood will prevent the late consequences of the disease in future life[9].

The diagnosis of Helicobacter pylori infection can be made by using Invasive diagnostic methods such as culture, histological stains, biopsy urease test and non-invasive techniques like serology and urea breath test (UBT). Serology has proven to be an accurate non-invasive test for detecting H.pylori infection[10]. The enzyme linked immuno sorbent assay (ELISA) is the most commonly used serological test because it is simple, quick and low cost technique suitable for screening large populations. Estimation of specific IgG antibodies to Helicobacter pylori antigen in the serum by ELISA is highly sensitive[11]. The etiologic role of H.pylori in peptic ulcer disease, Type – B gastritis and Lymphoma has been unequivocally proved[12]. However, in the recent unsuspected clinical conditions- especially among paediatric age group-like Sudden Infant Death Syndrome (SIDS), Recurrent Abdominal Pain (RAP), Short Stature, Growth retardation, Increased susceptibility to orally acquired infections like cholera, enteric fever, shigellosis and idiopathic – allergic rashes, association of H.pylori infection has been observed by several investigators[12].

The present study was done to know the seroprevalence of Helicobacter pylori infection among children attending paediatric outpatient department, with complaints not related to gastrointestinal tract.

MATERIALS AND METHODS

The present study was carried out at S.V.Medical College and S.V.R.R.G.G.Hospital, Tirupati in the year 2010. A total of 264 blood samples were studied. The sample includes children between 2 to12 years, both males and females.

Criteria for selection of Patients: Children aged from 2 years to 12 years –150 Male children and 114 female children with out any Gastro intestinal tract symptoms were taken for the present study. Children on, immunosuppressive drugs and history of taking any antibiotics, H_2 blockers or proton pump inhibitors in the preceding month were excluded.

Sample Collection: Informed consent was taken and 3 ml of blood was collected under aseptic precautions from antecubital vein. It is allowed to clot and on the same day the sample was centrifuged; serum was separated and stored in a sterile bottle at -20^0c. After collection, the serum samples, were tested for the presence of H. pylori IgG specific antibodies by using Meddens Diagnostics B.V., A.A.VORDON, Netherlands Helicobacter pylori IgG enzyme linked immunosorbent assay(ELISA) Quantitative kit expressed in Arbitrary units per millilitre. (AU/ml).

Procedure: Into each test tube, with 1ml of dilution buffer, 10µl of test serum was added and the test sample was diluted then rinsing buffer was prepared by mixing the entire content of the bottle, with rinsing buffer, (10x) with 540ml of distilled water and it was kept at room temperature for use. Then to 12ml of dilution buffer in a disposable plastic tray, added 120µl of anti IgG –PO conjugate and anti IgG conjugate solution was prepared.Now TMB substrate solution was prepared by adding, 1200µl of TMB (10x) to 10.8ml of TMB diluent and the solution was immediately transferred on to the ELISA plate and it was kept away from sunlight.

Assay Protocol: Three ELISA plates were used for the present study. In each ELISA plate, the wells were H.pylori antigen coated. Then to verify the validity of the test in each ELISA plate, the 1st vertical row, eight ELISA wells labeled vertically from above to below as A1 toH1 were used for the calibrators with values from OAU/ml, 15AU/ml, 75AU/ml, 300 AU/ml and dispensed per calibrator 100µl in duplicate, as shown in the Table1. Later to all the wells labeled from A2 to H2, 100 µl of diluted serum was dispensed. Then the micro plate was covered and incubated at 37°C and at 100% moist atmosphere for one hour. Later, the strips were rinsed for 5 times with the rinsing buffer, in automatic micro titer plate washer. After washing step was over, 100 µl of dilute conjugate was dispensed into each well, starting from A1.The micro titer plate was again covered and incubated in a 100% moist atmosphere for one hour at 37 ^{0}C . Later, a second rinsing of the strips, with rinsing buffer was done for 5 times. Afterwards, 10µl of diluted TMB substrate was dispensed into each well and incubated, for 30 minutes at room temperature in the dark .After 30 minutes the reaction was stopped by adding 100µl of stop solution to each well and the absorbance was read at 450 nm, with reference filter, within 10 minutes. The absorbance results were recorded and detailed tabulations was done.

Interpretation and validation of the Test: Calculated the mean absorbance value of the calibrators and observed that in calibrator 0AU/ml, the mean absorbance remained <0.4 times that of the 15AU/ml calibrator. In calibrator 15AU/ml, the mean absorbance was between 0.2 and 0.6 OD value. In calibrator 75AU/ml, the mean absorbance was between 0.6 and 1.6 OD value.In calibrator 300AU/ml, the mean absorbance was between 1.4 and 2.7 OD value.

Thus the ELISA test done by us was considered validated as the above observed values are in accordance with the criteria given for the validation of the test. Then conversion of absorbance into AU/ml was done by plotting the validated mean absorbance of the standard into a curve and the concentrations expressed in AU/ml of each individual serum sample was read by interpolation, from the standard curve. After calculation of serum IgG concentrations in AU/ml, the results have been interpreted as follows. Concentration of test serum ≥20AU/ml; is evidence for a positive Helicobacter pylori infection and Concentration of ≤15AU/ml; is evidence for a negative Helicobacter pylori infection. Values between 15 AU/ml to 20 AU/ml were considered as grey zone and a possibility of H.pylori infection was suspected.

RESULTS

The results of the above study are depicted in the following tables. Table 2 depicts Helicobacter pylori seroprevalence as per age and sex. Table 3 depicts details of Age, sex, socio-economic status and family status of Helicobacter pylori seropositive children studied among the study group. Accordingly many Helicobacter pylori seropositive children were from large families and all the Helicobacter pylori seropositive children were from families with low income groups. Table 4 depicts concentrations of serum anti Helicobacter pylori IgG in the study group expressed in Arbitrary Units/ Milliliter of serum (Au/ml). In the present study, the overall seroprevalence of H.pylori infections in between 2years to 12 years of age is 44.3%, and this is corroborating with the reported observations of several otherworkers, as shown in Table5, which depicts the observations of high seropositive prevalence for Helicobacter pylori infection in children from developing countries compared to developed countries.

Table: 1 Calibrators

A1	0	Negative control 0AU/ml yellow capped bottle
B1	0	
C1	15	Calibrator 15AU/ml (cutoff) Green capped bottle
D1	15	
E1	75	Calibrator 75 AU/ml Rose capped bottle
F1	75	
G1	300	Calibrator 300 AU/ml Red capped bottle
H1	300	

Table: 2 Seroprevalence according to Age and Sex.

Age in years	Sex						Total		
	Male (M)			Female (F)			M + F	P	%
	Total Tested	Positive(P)	%	Total Tested	Positive (P)	%			
2-3	18	0	0	30	6	20	48	6	12.5
5- 6	33	15	45.45	24	12	50	57	27	47.36
11-12	99	51	51.51	60	33	51	159	84	52.83
Total	150	66	44.00	114	51	44.73	264	117	44.31

Table: 3 Age, Sex, Family status of Seropositive Children

Age group	Family size		Income Group		
	Large	Small	LIG	MIG	HIG
2-3 years (Toddlers)	6	0	6	0	0
4-6years (Pre-school children)	15	12	27	0	0
7-12yrs (School children)	54	30	84	0	0
Total	75	42	117	0	0

Family size: ≥ 3Large family
≤ 2Small Family

Income group:
LIG: Rs. < 5000 per month
MIG:Rs. 5000-10,000 per month
HIG: Rs. >10,000 per month

Table: 4. Anti H.Plyori IgG Quantity (Arbitary Units/ ml of serum) AU/ml

Anti H.pylori IgG (Au/ml)	2yrs- 3yrs		4yrs -6yrs		7yrs-12 yrs		2 Yrs -12 yrs Total (Male & Female)
	Male	Female	Male	Female	Male	Female	
0 (0-14)	18	21	12	12	36	24	123
15 (>15-19)	-	3	6	-	12	3	24
75 (>20-75)	-	6	15	12	45	24	102
300 (>75-300)	-	-	-	-	6	9	15

H.pylori anti body titres above 75 Au/ml was observed among children above the age of 7 years only and even with such high values, all of them were asymptomatic.

Table 5: Prevalence of H.Pylori infection in children from developing countries compared to developed countries – as reported by other workers.

Author	Year of study	Country	Age Groups	% H.pylori of seropositivity
Mesgrand et al[16]	1989	Algeria	0-10yrs	45. 0%
			11-20 yrs	73.0%
Sullivan et al[17]	1990	Gambia	0-5yrs	31.4%
Graham et al[18]	1991	India	0-9yrs	60.0%
			10-19yrs	69.0%
Prietio et al[19]	1992	Ivory Coast	0-10yrs	55.3%
			11-20 yrs	75.0%
Blecker et al[20]	1994	Belgium	2- 8yrs	5.4%
			9 -14yrs	13.4%
Bujanover et al[21]	1996	France	0-10yrs	3.5%
			11-20yrs	16.3%
V.Kate et al[8]	2001	India	0-5yrs	46.00%
			6-10yrs	44.00%
			11-15 Yrs	44.00%
Present study	2003	India	2-3yrs	12.5%
			4-6yrs	47.36%
			7-12yrs	52.83%

DISCUSSION

ELISA kit of Meddnes Bio-tech, Brummen, has a sensitivity and specificity of 98% and is one of the best methods for primary diagnosis of H.pylori infection. It gives assay results that are proportional to specific antibody concentration which are most convenient for serological follow up on therapy. Analysis by quantitative method, such as, Meddens ELISA as was done in this present study is suitable for fast diagnosis and serological follow up. In present study, as shown in Table 2, of the 150 male children studied, the seropositive prevalence of Helicobacter pylori in the age group of 2 to 3 years is 0% out of 18 samples tested. Similarly, in the age group of 4 to 6 years is 45.45% out of 33 samples tested and, in the age group of 7 to 12 years is 51.51 % out of 99 samples tested.

The overall seropositive prevalence among male children was 44%. Among 114 female children positive studied seropositive prevalence in the age group of 2 to 3 years was 2% out of 30 samples tested. Similarly in the age group of 4 to 6 years was 50% out of 24 samples tested and in the age group of 7 to 12 years was 55% out of 60 samples tested. The overall seropositive prevalence among female children was 44.73%. It was observed

that, female children acquired infection at an early age of 2 to 3 years while male children got infected only after 3 years. There was a steady increase in the age related seropositive prevalence among both the sexes and by the age of 12 years more than 45% children of both sexes have acquired infection. Further it was observed that 12.5% in the age group between 2 yrs to 3 yrs; 47.30% between 4 yrs to 6 yrs and 52.83% between 7 yrs to 12 yrs were seropositive. The overall seroprevalence detected among the 264 children of both sexes was 44.31% and shows increased seroprevalence with age, in accordance with studies of increased Helicobacter pylori positivity with increasing age[4,8,13,14].

Regarding sex related prevalence, in male children between age group of 2 yrs to 3 yrs – there was no seropositive prevalence, but in female children of same age, 25% seropositive prevalence was seen. In the age groups of 4 yrs to 6 yrs – in male children 45.45% seropositive prevalence and in female children 50% seropositive prevalence was seen. In the age group of 7 yrs to 12 yrs – in male children – 51.51% seropositive prevalence and in female children 55% seropositive prevalence was seen. The overall seropositive prevalence in the age groups from 2 yrs to 12 yrs in male children it was 44% and in female children it was 44.73%. This indicates, with increase in age, seropositive prevalence was almost same among both sexes. Similar findings were been observed in a study conducted at Pondichery [8].

Accordingly in Table 3 the following details were observed, in the age group of 2 years to 3 years no seropositive children for H.pylori were from lower income group and 6 children were from large families. In the age group of 4years to 6 years 12 children for H.pylori were from lower income group and 15 children were from large families and 12 children were from small families and in the age group of 7years to 12 years all the 84 children are from lower income group and 54 children were from large families and 30 children from small families. Thus it was observed that out of all 117 seropositive children - 75 seropositive children were from large families and 42 seropositive children were from small families and all the 117 seropositive children were from low income group. These observations are found to be in corroboration with other studies as one of the major risk factors for acquiring H.pylori infection is low socio economic status and larger families[6,7]. Precarious hygiene, crowded living conditions absence or deficiency of sanitation, and a habit of sharing of bed with parents, play a role in interfamilial transmission of H.pylori and these are associated features of families with low socio economic status in India[13-15].

As Concentrations of $\geq$20 Au/ml indicates seropositive status for Helicobacter pylori infection- according to Table 4, the antibody titers were higher among children of 7 to 12 years age group. Further nine out of 60 female children in that age group i.e., 15% showed higher titers of Anti Helicobacter pylori IgG and 6 out of 99 male children of the same age group i.e., 6.1% showed such higher titers. It is a note worthy observation which needs further investigation and it shows no correlation between the anti body titre and the severity of the disease, as children even with very high IgG titres were asymptomatic.

In the present study, the over all seroprevalence of H.pylori infection in between 2 to 12 yrs of age was 44.3% and this is corroborating with the reported observations of several other workers, as shown in Table 5 which depicts the observations of high seropositive prevalence of H.pylori infection in children from developing countries compared to developed countries[8].

CONCLUSION:

It will be ideal method to screen all children from low socio economic status and from large families for H.pylori infection by non-invasive test as it is a curable infection. Early detection and prompt treatment not only eradicates the infection and development of late complications in the affected individuals, but is also useful in breaking the

transmission of certain unsuspected clinical condition. So, efforts should be made to produce and make available an immunogenic and cost effective, H.pylori vaccine. Peroral immunisation with Helicobacter pylori adhesin protein genetically linked to cholera toxin A_2B subunits which is under trail, such vaccination program can be effectively implemented to eradicate and wipe off the H.pylori infection from the community.

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