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Research Article

Association between *Helicobacter pylori* infection and increased risk of typhoid fever in Khartoum- Sudan

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ABSTRACT

Background and objective:

There are some reports on the association between *Helicobacter pylori* infection and typhoid fever in different parts of the world. This study was carried out to detect Association between *Helicobacter pylori* infection and increased risk of typhoid fever in Khartoum- Sudan, and to determine the relationship between them and certain factors such as gender, age, and residence.

Material and method:

It was descriptive cross-sectional study conducted from March- May 2015. A total of 90 febrilepatients who attended different hospitals.(36 males and 54 females) were enrolled. Serum specimens were tested by rapid test for typhoid (50% positive as case and 50% negative as control) then were tested by ELISA IgG of *Helicobacter pylori*. Data was analyzed by chi squared test in SPSS software.

Result:

Seropositivity of Helicobacter IgG was detected among the typhoid patients case group in 84.4% of case subject(38 of 45) and negative was 15.6% of cases(7 of 45). While seropositive of Helicobacter IgGamong control group it was detected in 86.7% (39 of 45) and negative 13.3% (6 of 45).

This study indicated statistically insignificant association between *H.pylori* and increased risk of typhoid fever in Khartoum –Sudan.

Keywords: Association, Helicobacter pylori, typhoid fever, Rapid test, ELISA, Khartoum -Sudan.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the most important factors in the gastro duodenal diseases. The infection is most commonly acquired in early childhood and leads to chronic gastritis in both children and adults and is the leading cause of peptic ulcer disease in humans [(1,2.3.4)]. However, over 80% of individuals infected with the bacterium are asymptomatic. More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries [(5)] . Where acquisition occurs at a younger age that in developing world[(6)].

Typhoid fever is a serious systemic illness that each year affect over 20 million people, predominantly in developing countries [(7)]. Infection with *Salmonella typhi* is transmitted by the fecal-oral route and in several epidemiological studies risk factors were identified that suggested either waterborne transmission[(8)] or foodborne transmission [(8,9)].

A high incidence of salmonellosis has been observed in individuals with surgically induced or other types of achlorhydria[(10)] .Also *H .pylori* infection may exert an effect on the secretion of gastric acid[(11)].In Jakarta Vollaard,*et al* (2006) were evaluated the association between typhoid fever and Helicobacter pylori infection, as the latter microorganism may influence gastric acid secretion and consequently increase susceptibility to *Salmonella typhi* infection [(12)]. Also in Delhi Bhan,*et al*(2002) found an increased risk of typhoid fever in those who had antibodies to *H. pylori*. It may be that established infection, reflected by gastritis and detectable immune response, rather than mere colonization with *H. pylori* is required for the development of hypochlorhydria and increased risk of enteric infections.[(9)]There is no published data are available regarding the association

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between *Helicobacter pylori* and typhoid fever in Sudan. The aim of this study was todetect Association between *Helicobacter pylori* infection and increased risk of typhoid fever in Khartoum- Sudan

MATERIALS AND METHODS:

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from March to May 2015, 90 febrile patients who attended Khartoum hospitalswere enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of Faculty of Graduate studies Al-Neelain University and verbal consent also was obtained from each patient.

Experimental work:

Specimen collection:

Blood samples were collected from patients, under direct medical supervision by vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serum was kept in -20°C till serological study was performed.

Specimens were processed by rapid Immune chromatographic testICT (Acon –USA) for typhoid fever and by using Enzyme linked immune sorbent assay (ELISA)(EUROIMMUN Medizinische Laboradiagnostika AG- Germany), (4th generation ELISA) for detection *Helicobacter pylori* IgG antibodies.

Immune Chromatography Test ICT for typhoid fever:

All specimen and test components were allowed to reach room temperature if refrigerated or frozen. Specimen was mixed well prior to assay once thawed. When ready to test, pouch was opened and notch was removed.Test device was placed on a clean, flat surface.The device was labeled with specimen's ID number.The dropper holded vertically and transferd 1full drop of serum or plasma (approximately 30 µl), one drop of buffer was added (approximately 40 µl).Resultwas read in 10-15 minutes. To avoid confusion, test device was discarded after interpreting the result.

Interpretation of assay result:

Negative result:

If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti-S. typhi antibody is detected. The result is negative.

Positive result:

- In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of anti-S. typhi IgM. The result positive.
- In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of anti-S. typhi IgG. The result positive.
- In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of anti-S. typhi IgM and IgG. The result positive.

Invalid:

If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands. Repeat the assay with a new device.

Enzyme linked immune sorbent assay for detection *Helicobacter pylori* IgG antibodies:

All reagents and samples were allowed to reach room temperature for 15minutes before use washing buffer was prepared 1:20 from buffer concentrate with distilled water. 100µl of sample diluents was added into appropriate wells except the blank well and negative well. 20µl from each sample was added to the appropriate well and mixed by pipette repeatedly until liquids turn blue. 20µl from negative and positive control were dispensed and added to the negative and positive wells separately without dispensing liquid into the blank control well. Micro titer wells was flicked for 30 seconds and mixed well, then plate was covered and incubated for 30 minutes at 37° C. plate was taken out and wash buffer was added to each well (washing 1) and aspirated off after 20 seconds. This step was repeated for 5 times until each well become dry, and 50µl of HRP-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 30 min at 37°C.

The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer. This step was repeated for 5 times until each well become dry (washing 2).

50µl of substrate A and 50µl substrate B solution were added in to each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 15 min. 50 µl Stop solution was added into each well and mixed gently.

Measuring the absorbance: The plate reader was calibrated with blank well and the absorbance was read

at 450nm. The results were calculated by relating each sample optical density
(OD) value to the Cut off value of plate. Calculation of cut off (C.O) value.
C.O = *Nc*2.1
*Nc= the mean absorbance value for the three negative controls.
The absorbance was read with micro well reader at 450nm.

Interpretation of Results:
Negative results: samples giving absorbance less than Cut-off value are negative for this assay.
Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive.
Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended.

Data analysis: Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16.

RESULT:
A total of 90 febrile patients who attended Khartoum hospitalsduring the period from March-May 2015, consented to the study were included, of them 36(40.0%) were males and 54(60.0%) were females .The mean age of patients was 30.5 years (range from 6 to 85 years) ,most of patients 51 (56.7%) were belonged to the age group (21-40)(fig1).In this case – control study, 50 of them were test group positive for typhoid fever. For each patients, one subject was matched with age, sex and residence characteristic as control. All of the case and control were tested for*Helicobacter pylori* IgG by ELISA method. The overall result showed that seropositive *Helicobacter pylori* IgG antibodies was detected in 84.4% of case subject(38 of 45) and negative was 15.6 % of cases(7 of 45). While sero positive of Helicobacter IgG from control was detected in 86.7% (39 of 45) and negative 13.3% (6 of 45).table (1).

The statistical analysis showed insignificant association between *Helicobacter pylori* and increased risk of typhoid fever in Khartoum-Sudan,p.value = 0.764

DISCUSSION:
More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract and typhoid fever caused an estimated 21.7 million illnesses and 217,000 deaths [5].

The present study result revealed 84.4% (38 of 45) was positive for typhoid and *H.pylori* and 15.6% (7 of 45) was positive for typhoid but negative for *H.pylori***.** How ever among control group it was 86.7% (39 of 45) positive and 13.3% (6 of45) was negative. When compared with other studies in Sudan, there was no similar study. The present study give higher result than the previous study in Delhi 2002 obtained by Maharaj *et al* found an increased risk of typhoid fever in those who had antibodies to *H. pylori*. It may be that established infection, reflected by gastritis and detectable immune response, rather than mere colonization with *H. pylori* is required for the development of hypochlorhydria and increased risk of enteric infections, Serum anti–*H. pylori* IgG antibodies**,**was detected in 64% of case subjects (53 of 83) and 50% of neighborhood control subjects.However, the obtained seropositivity also was higher than the result of 67% obtained by Vollaard*et al* (2006) in Jakarta, Indonesia. They evaluated the association between typhoid fever and Helicobacter pylori infection, as the latter microorganism may influence gastric acid secretion and consequently increase susceptibility to Salmonella typhi infection.

Conclusion:
This study indicated the insignificant association between *H.pylori* and typhoid fever in Khartoum –Sudan. The discrepancies of this result may be due to small sample size and differences in the used techniques, for this large scale screening is recommended.

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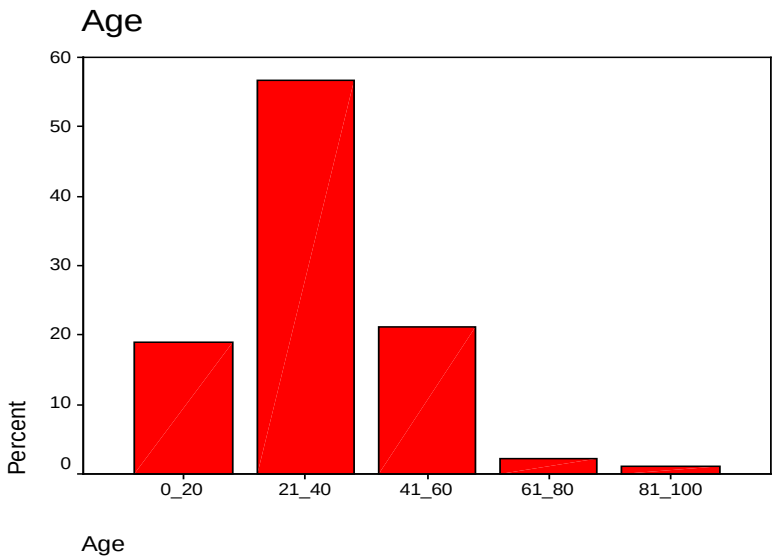

Figure: 1distribution of study population (n=90) according to their age.

Table 1: Association between Typhoid fever and *H.pylori* infection among test and control group

Test / Group	No	H.pylori IgG		Total
		positive	Negative	
Test group	45	38(84.4%)	7(15.6%)	45
Control group	45	39(86.7%)	6(13.3%)	45
Total	90	77	13	90

P.value = 0.764

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