1-1 Introduction

Typhoid fever is a serious systemic illness that each year affects over 20 million people, predominantly in developing countries (Crump *et al.*, 2004). Infection with *Salmonella typhi* is transmitted by the faecal–oral route and in several epidemiological studies risk factors were identified that suggested either waterborne transmission or foodborne transmission (Luby *et al.*, 1998). The determination of the relative contribution of distinct environmental risk factors for transmission of disease is essential to focus local control strategies. Also host-related risk factors for infection have been examined, identifying both genetic factors (Dunstan *et al.*, 2001) as well as concurrent *Helicobacter pylori* infection, that was interpreted as a cause of a reduced gastric acid barrier(Bhan *et al.*, 2002).

A high incidence of salmonellosis has been observed in individuals with surgically induced or other types of achlorhydria (pernicious anaemia and chronic atrophic gastritis) according to Kunz and Waddell (1956). Also *H. pylori* infection may exert an effect on the secretion of gastric acid. Approximately 50% of the world's population is infected with *H. pylori* (Torres *et al.*, 2000), and even higher prevalences have been reported in developing countries (Bardhan., 1997), where acquisition occurs at a younger age than in the developed world (Blaser., 1999). Active infection with *H.pylori* is associated with a transient hypochlorhydria that may be present for several months (Harford *et al.*, 2000). Furthermore, *H. pylori*-induced chronic gastritis of the body of the stomach reduces acid secretion and persistent hypochlorhydria constitutes a risk for the development of gastric cancer. In the absence of the acid-mediated inhibition of gastric gastrin release, the serum gastrin concentration increases. In contrast, antral-predominant, body-sparing gastritis due to *H.pylori* increases gastric acid secretion, resulting in duodenal ulcer disease (El Omar *et al.*, 2000).

Consequently, the association between *H. pylori* infection as an indicator of hypochlorhydria and the susceptibility to other gastrointestinal infections is ambiguous. An increased susceptibility to enteric infections in *H. pylori*-infected individuals, as measured by anti-*H. pylori* IgG response, was documented for cholera (Shahinian *et al.*, 2000) and typhoid fever (Bhan *et al.*, 2002). However, the evidence for the association of *H. pylori* infection and diarrhoea is conflicting (Sullivan *et al.*, 1990) and even a protective effect of *H. pylori* infection was demonstrated (Rothenbacher *et al.*, 2000).

1-2 Rationale

Noticed co-infection of *H.pylori* and typhoid fever during duty work in shendi and no previous study about this co-infection and this study aimed to determine association between *H.pylori* and typhoid fever and confounding factor that influence it.

A little information about seroprevalence of *H.pylori* in Shendi City and its surrounding villages.

The usages of tankers for saving water for a long time in surrounding villages which may be a source of infection with both *H.pylori* and typhoid fever.

1-3 Objective

1-3-1General objective:

- To detect anti *H.pylori* antibodies in typhoid patient.

1-3-2Specific objective:

- To determine occurrence of IgG antibodies against *H.pylori* in patient with typhoid fever.
- To identify major risk factors associated with *Helicobacter pylori* (gender, age, smoking, coffee consumption).

2- Literature review

2-1 *Helicobacter pylori*:

This organism was earlier known as *Campylobacter pylori* and believed to be the casual agent of chronic gastritis. Strong evidence has accumulated regarding its close association with gastric and duodenal ulcers. It is also being incriminated as responsible for initiating the process of metaplasia in gastric epithelium which ultimately may lead to carcinoma of stomach (Rajesh and Rattan,2004).

2-1-1Morphology of *Helicobacter pylori:*

In stomach this organism takes form of short spirals or S shaped gram negative bacterium which is about 3µm long, 0.5-1.0µm wide with a wavelength of about 2.5µm. after growth on laboratory media *Helicobacter pylori*(*H.pylori*) is visible under the microscope as curved gram negative (Rajesh and Rattan, 2004).

2-1-2 Physiology and growth conditions:

The physiological characteristics of *H.pylori* have received relatively little attention. It is a Gram negative spiral-shaped bacteria, although its morphology is not constant. Under adverse conditions it becomes coccoid, but there is controversy about the nature of the coccoid form. Some researchers have stated that this form is either a contaminant or a dead bacterium (Kusters *et al.*,1996) , but others consider it to be a metabolically active form that cannot be cultured in vitro (Bode *et al.*,1993; Nilius *et al.*,1993). It has also been suggested that some cocci can revert to their original spiral shape (Andersen *et al.*,1997). *H.pylori* is microaerophilic; optimal growth occurs in the presence of 5–15% oxygen(Goodwin,1989). Incubation in air results in reduced

survival(West *et al.*,1992) and it grows poorly under anaerobic conditions (Goodwin and Armstrong,1990). The presence of 5% CO2 seems to provide optimal conditions, while 10% CO2 led to a loss in cultivability in one study (Donelli *et al.*,1998).

2-1-2-1Carbon source:

Glucose is not necessary for growth (Albertson *et al.*,1998 ;Reynolds and Penn,1994). Cell yield is not influenced by the presence of glucose, pyruvate, succinate, or citrate, but survival is enhanced by their presence. Prolonged incubation with carbon sources improves the viability of the organism (Albertson *et al.*,1998). *H. pylori* depends on the presence of various amino acids for growth, including arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine. Some strains also need alanine,serine, proline, and tryptophan (Reynolds and Penn,1994).

2-1-2-2 PH and water activity:

H.pylori can be cultured in environments within a pH range of 4.5–9. And at low pH values (e.g. 3.5), the addition of urea increases survival. $NaNO_2$ has no effect if concentrations range from 0 mg/ml to 400 mg/ml; growth is not possible at NaCl concentrations of 52.5 g/l. The pathogen is sensitive to environments with a low water activity (A_w): growth is inhibited at values <0.98. In one study, *Helicobacter pylori* concentrations became undetectable in nutrient-rich laboratory medium within three days when the A_W was 0.96 (Jiang and Doyle,1998).

2-1-2-3 Temprature:

H. pylori only grows at temperatures of 30–37^{o}C. All the required growth conditions are met in the gastrointestinal tract of all warm-blooded animals. At temperatures below 30^{o}C, *H. pylori* could survive in some foods, such as fresh fruit and vegetables, fresh poultry or fish, fresh

meats, and some dairy products (Banwart,1979). *H. pylori* survived at 30^{o}C in laboratory media (Jiang and Doyle,1998), water(West *et al*., 1992), and milk (Fan *et al.*,1998), and survived longer at lower temperatures (Jiang and Doyle, 1998).

2-1-3 Reservoir:

The human stomach appears to be the environment most suitable for the organism's growth; there are no significant animal or environmental reservoirs for strains infecting humans. *H. pylori* has been isolated from domestic, commercially reared cats (Handt *et al*., 1994; Fox, 1995) and it has been suggested that it might be a zoonotic pathogen with transmission occurring from cats to humans. However, there have been no data to support this hypothesis. For example, after adjusting for potential confounders in a study of 447 factory workers in the United Kingdom, there was no association between *H. pylori* seropositivity and cat ownership during childhood (Webb *et al*.,1996). In Ulm, Germany, in 1996–97 among schoolchildren in first grade, neither contact with pets in general nor contact with specific kinds of animals was positively associated with infection (Bode *et al*., 1998).

The possibility that *H. pylori* might be a zoonotic pathogen transmitted from animals other than cats has also been considered (Dunn *et al*., 1997; Fox,1995), but the organism has never been isolated from animals slaughtered for consumption, such as pigs (Fox, 1995) . It has been isolated from some non-human primates, such as macaque monkeys. However, because contact between humans and other primates is rare, it is unlikely that these other animals play an important role in the transmission to humans. It is possible that the inability to isolate the

organism from other animals may be due to the difficulty of detecting the bacterium in materials other than gastric tissue (Fox, 1995).

2-1-4 Route of transmission:

1- Person to person transmission:

Several studies have assessed the relation between *H.pylori* infection and institutionalized populations. Significantly higher rates of *Helicobacter pylori* infection also were found in other institutionalized populations (Lambert *et al*., 1995).

Familial exposures. Most of the selected studies that looked at the relation between *H.pylori* infection and intrafamilial clustering of *Helicobacter pylori* did not use DNA fingerprinting to confirm that relatives had the same strain of *H.pylori* (Mitchell *et al*., 1993).

2- Fecal-oral route:

Also another possible method of *H.pylori* transmission is the fecal-oral route. *H. pylori* DNA has been detected in feces of infected subjects by some researchers (Namavar *et al*., 1995; Shimada *et al*., 1994; Gramley *et al*., 1999) but not others (Van Zwet *et al*., 1994). Recently, Gramley *et al*. found detectable *H. pylori* DNA in the feces of 73 percent of infected subjects. Isolation of *H.pylori* by fecal culture has been performed by a number of investigators from around the world. However, isolation of *H. pylori* from feces has been problematic for some researchers, especially for those unable to obtain fresh feces. Delay in processing could have resulted in the small number of *H. pylori* organisms present being overgrown by other fecal bacteria. Recently, Parsonnet et al. were able to culture *H.pylori* from cathartic-induced diarrheal stools in 7 of 14 *H. pylori* infected subjects but not from normal stools (Parsonnet *et al*.,1999).

3-Vector borne or zoonotic transmission:

H. pylori has been isolated from nonhuman primates and domestic cat. (Handt *et al*., 1994)

4- Iatrogenic transmission:

Risk factor for iatrogenic transmission of *H.pylori* is endoscopy Because of the complex structure of the endoscope and difficulty in disinfecting it (Fantry *et al*., 1995).

2-1-5 diseases caused by *Helicobacter pylori:*

Most infected persons are never suffering from any symptoms related to infection, however, *H. pylori* causes chronic active, chronic persistent, and atrophic gastritis in adults and children. Infection with *H. pylori* also causes duodenal and gastric ulcers. Infected persons have a 2- to 6-fold increased risk of developing gastric cancer and mucosal associated-lymphoid-type lymphoma compared with their uninfected counterparts. The role of *H. pylori* in non-ulcer dyspepsia remains unclear.

Active infection with *H. pylori* is associated with a transient hypochlorhydria that may be present for several months (Harford *et al.*, 2000). Furthermore, *H. pylori*-induced chronic gastritis of the body of the stomach reduces acid secretion and persistent hypochlorhydria constitutes a risk for the development of gastric cancer (Suerbaum and Michetti, 2002; El Omar *et al*., 2000).

2-1-5-1 Symptoms of ulcer:

The most common ulcer symptom is gnawing or burning pain in the epigastrium. This pain typically occurs when the stomach is empty, between meals and in the early morning hours, but it can also occur at other times. It may last from minutes to hours and may be relieved by eating or by taking antacids. Less common ulcer symptoms include nausea, vomiting, and loss of appetite. Bleeding can also occur; prolonged bleeding may cause anemia leading to weakness and fatigue. If

bleeding is heavy, hematemesis, hematochezia, or melena may occur (Center for disease Control and Prevention., 1998).

2-1-5-2 Risk factor of *H.pylori* infection:

The major factors investigated for their possible association with *H. pylori* positivity are The following topics included: smoking, alcohol consumption, diet, occupational exposures, waterborne exposures, hygiene practices, density/crowding, social factors, and family history of gastric disease.

Smoking: Studies have assessed the possible association between *H. pylori* infection and smoking. Whereas some found that *H.* pylori seropositive subjects were overall more likely than seronegative subjects to be current smokers, results were often not consistent by race or gender (Lin *et al.*, 1998; Fontham *et al.*, 1995).

Alcohol consumption: None of several recent epidemiologic studies of the relation between alcohol consumption and *H. pylori* infection found a positive association, but many noted a nonstatistically significant reduction in risk, (Brenner *et al.*, 1997; Brenner *et al.*, 1999(a,b)) who incorporated a quantitative measure of alcohol consumption while controlling for potential confounding factors, found a significant negative association with alcohol consumption, especially at moderate to high levels. In two of these studies, the association was stronger for wine than for beer.

Several studies did not adequately control for potential confounding variables or did not present the actual risk estimate or prevalence; thus, it is difficult to evaluate whether alcohol consumption has a "protective" effect on the prevalence of *H. pylori. H.pylori* is better able to survive in the acid environment of the stomach than other bacteria are because of its production of urease. Therefore, it is not surprising that the reduction in pH that may accompany alcohol consumption would have little effect on

the prevalence of *H. pylori* (Jenkins, 1997). However, alcohol is known to have direct antimicrobial effects that appear to be more pronounced for wine than for other types of alcoholic beverages (Klontz, 1999). The differing results may be due to the different methodologies used or to real differences in either the type or amount of alcohol consumed and its effect on *H. pylori* in different populations.

Diet: Studies have also looked at dietary associations with *H. pylori.* Although the studies cover many different types of populations and include both adults and children, some consistent associations suggest that nutritional status may be related to *H. pylori* infection (Goodman *et al.*, 1997).

Occupational exposures: Occupational exposures have been studied by several researchers to determine whether people working in certain occupations with potentially greater exposure to *H. pylori* had an increased prevalence of infection.(Bohmer *et al.*, 1997; Friis *et al.*, 1996) Waterborne exposures. Water has been suggested as a possible source of *H. pylori* infection (Goodman *et al.*, 1996).

Hygiene practices: Studies also have assessed the relation between H. pylori infection and various hygiene practice indicators in a number of countries. Overall, poor hygiene practices, especially during childhood, appear to be related to a higher seroprevalence of *H. pylori* (Goodman *et al.*, 1996).

Family history *of gastric disease.* Studies have also evaluated the relation between *H.pylori* infection and family history of gastric disease (Brenner *et al.*, 2000).

2-1-6 Diagnosis of *H.pylori:*

Several methods may be used to diagnose *H. pylori* infection. Serological tests that measure specific *H. pylori* IgG antibodies can determine if a

person has been infected. The sensitivity and specificity of these assays range from 80% to 95% depending upon the assay used.

Another diagnostic method is the breath test. In this test, the patient is given either 13C- or 1 4C-labeled urea to drink. *H. pylori* metabolizes the urea rapidly, and the labeled carbon is absorbed. This labeled carbon can then be measured as CO2 in the patient's expired breath to determine whether *H. pylori* is present. The sensitivity and specificity of the breath test ranges from 94% to 98%. Upper esophagogastroduodenal endoscopy is considered the reference method of diagnosis. During endoscopy, biopsy specimens of the stomach and duodenum are obtained and the diagnosis of *H. pylori* can be made by several methods:

• The biopsy urease test - a colorimetric test based on the ability of *H. pylori* to produce urease; it provides rapid testing at the time of biopsy.

• Histologic identification of organisms - considered the gold standard of diagnostic tests.

• Culture of biopsy specimens for *H. pylori*, which requires an experienced laboratory and is necessary when antimicrobial susceptibility testing is desired (Center for Disease Control and Prevention., 1998).

2-1-7Treatment of *H.pylori*:

H.pylori sensitive to penicillin, cephalosporins, tetracycline, erythromycin, rifampicin, aminoglycosides and nitrofurans. (Rajesh and Rattan, 2004).

2-2 Typhoid fever:

Typhoid fever is caused by *Salmonella typhi*, a Gram-negative bacterium. A very similar but often less severe disease is caused by *Salmonella* serotype *paratyphi* A. The nomenclature for these bacteria is confused because the criteria for designating bacteria as individual species are not clear. Two main views on the nomenclature of the genus *Salmonella* have been discussed. Le Minor and Popoff suggested that two species should be recognized: *Salmonella bongori* and *Salmonella enterica*. *S. enterica* included six subspecies, of which subspecies I (one) contained all the pathogens of warm-blooded animals. *S. typhi* was a serotype within subspecies I: *Salmonella enterica* subspecies I serotype *typhi*. This proposal was rejected by the International Judicial Commission because the name was not well known to clinicians and its use might cause accidents endangering health or life. The original rules therefore remain in force. Ezaki and colleagues have noted in the International Journal of Systematic and Evolutionary Microbiology that the correct nomenclature for the causal agent of typhoid fever is *Salmonella typhi* and have requested that the current subspecific status of serotype *paratyphi* A should be raised to specific status, i.e. *Salmonella paratyphi A* (WHO, 2003).

S. typhi has several unique features, the genetic basis of many of which is known as a result of early genetic studies and the recent sequencing of the whole genome. Although many genes are shared with *E. coli* and at least 90% with *S. typhimurium*, there are several unique clusters of genes known as pathogenicity islands and many more single genes that seem to have been acquired by *S. typhi* during evolution. *S. typhi* can be identified in the laboratory by several biochemical and serological tests. One of the

most specific is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated *S. typhi* and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (*Citrobacter freundii*, *Salmonella paratyphi C* and *Salmonella dublin*) but not in exactly the same genetic context. The ratio of disease caused by *S. typhi* to that caused by *S. paratyphi* is about 10 to 1 in most of the countries where this matter has been studied (WHO., 2003).

2-2-1 pathogenisity:

During an acute infection, *S. typhi* multiplies in mononuclear phagocytic cells before being released into the bloodstream. After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages (Mp) that ingest the bacilli but do not generally kill them. Some bacilli remain within Mp of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by Mp. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors. Incubation periods ranging from 3 days to more than 60 days

have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (~1_10 bacteria per ml of blood) (WHO.,2003).

2-2-2 Symptoms:

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications. Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculums ingested, host factors (e.g. HLA type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S. typhi* and *S. paratyphi* (Gotuzzo *et al*., 1991). Evidence of *Helicobacter pylori* infection also represents an increased risk of acquiring typhoid fever (WHO, 2003).

Acute non-complicated disease: Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever, up to 25% of patients show exanthem (rose spots), on the chest, abdomen and back (WHO, 2003).

Complicated disease: Acute typhoid fever may be severe. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients, and

up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases. Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen (WHO, 2003).

Altered mental status in typhoid patients has been associated with a high case-fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic symptoms, although rare, have been reported. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uraemic syndrome. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic carrier state may develop (WHO, 2003).

Carrier state: 1_5% of patients, depending on age, becomes chronic carriers harboring *S.typhi* in the gallbladder (Edelman and Levine Myron ,1986).

2-2-3 Contamination and transmission:

Humans are the only natural host and reservoir. The infection is transmitted by ingestion of food or water contaminated with faeces. Ice

cream is recognized as a significant risk factor for the transmission of typhoid fever. Shellfish taken from contaminated water, and raw fruit and vegetables fertilized with sewage, have been sources of past outbreaks.

The highest incidence occurs where water supplies serving large populations are contaminated with faeces. Epidemiological data suggest that waterborne transmission of *S. typhi* usually involves small inocula, whereas foodborne transmission is associated with large inocula and high attack rates over short periods. The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period. In volunteers who ingested 109 and 108 pathogenic *S. typhi* in 45 ml of skimmed milk, clinical illness appeared in 98% and 89% respectively. Doses of 105 caused typhoid fever in 28% to 55% of volunteers, whereas none of 14 persons who ingested 103 organisms developed clinical illness. Although it is widely believed that *Salmonella* is transmitted via the oral route, the transmission of *S. typhimurium* via the respiratory route has been demonstrated in a mouse model (Ivanoff *et al*., 1980).

2-2-4Diagnosis of typhoid fever:

Laboratory diagnosis of typhoid fever depends upon following parameter:

- Isolation of causative agent.
- Detection of microbial antigen.
- Titration of antibody against causative agent.

2-2-4-1Cultural method:

The method of choice for isolation of causative agents of typhoid fever is blood culture.

- Blood culture: with all aseptic precautions, about 10 ml of blood should withdrawn. This large quantity of blood is required because in many cases number of bacteria in blood is too little (just one bacteria

per ml). as far as possible sample should be collected prior the administration of any antibacterial therapeutic agent to patient.

- Bone marrow culture: it may give positive result when blood culture fail, particularly in patients admitted to hospital after prolonged antibiotic therapy.
- Stool culture: a spoonful of feaces should be collected in clean container. Numerous selective media are available.
- Urine culture: it can cultured either as such or the deposit obtained after centrifugation, duodenal fluid, and bile can be processed in the same way as fecal specimen is processed (Rajesh and Rattan, 2004).

2-2-4-2Slide agglutination test:

If on bases of biochemical reaction the organism has been identified as *Salmonella*, its identify can be confirmed with slide agglutination test and serotype ascertained. The identify of genus can be confirmed by observing for agglutination with polyvalent O antisera and polyvalent H antisera against *salmonellae*. For identification of serotype the isolate is reacted with group specific antisera followed by monovalent O specific antiserum. Similarly H antigen in phase 1 or phase 2 can be determined to reach at final antigenic structure of organism (Rajesh and Rattan, 2004).

2-2-4-3Detection of microbial antigen:

The circulating *Salmonella* antigen may be detected in blood of patients with typhoid fever by coagulation method. ELISA has also been attempted by some workers to detect Vi antigen in the urine of patients.

2-2-4-4Titration of antibody:

Large numbers of serological tests have been devised to detect and titrate the antibody against common agents of typhoid fever:

- Widal tube agglutination test.

- Widal slide agglutination test.
- Indirect haemagglutiation test.
- Counterimmunoelectrophoresis.
- ELISA.

(Rajesh and Rattan, 2004).

2-2-5Previous study:

A case control study conducted in urban slum community in south Delhi from November 1995 to October 1996 to determine the association between typhoid fever and *Helicobacter pylori* , 83 case subjects of culture-proven typhoid fever were identified through one year surveillance of subject age 0-40 years, two age-sex matched neighborhood control subjects selected for each case subject. Serum anti H.pylori immunoglobulin G antibodies were measured in case and neighborhood control subjects, the result was a significant association between typhoid fever and *H.pylori* that anti *H.pylori* IgG detected in 64% of case subjects (53 of 83) and 50% (83 of 166) neighborhood control subjects (Maharaj, 2002).

3- Materials and Methods

3-1 Study design:

This was a cross-sectional hospital based study conducted in Shendi Teaching Hospital to detect *H.pylori* in typhoid patients. The study was performed during period from 10 July to 20 august at laboratory of research in Sudan University.

3-2 Study area:

Shendi Town is a town located in River Nile State away from Khartoum about 150 kilometers to the north-east direction, between latitude 17 and 18 degrees north and longitude 23 and 24 degrees east.

3-3 Study population:

The target people in this study were those with typhoid fever with exclusion of patients that have been infected with typhoid fever during the last six month.

3-4 Sampling:

Ninety two serum samples were collected randomly from diagnosed typhoid patients with symptoms of illness and twenty control samples of non typhoid subject with no symptoms of illness and no history of typhoid fever using sterile syringe.

The subject's veins were sterilized with 70% alcohol using impregnated cotton then puncture was made with needle smoothly and whole blood was collected from the vein in sterile plain container. Then after blood was clotted it was centrifuged at 3000 for 5 minutes. Patients were grouped into males and females and according to ages 15-30 , 31-45 and more than 45 years.

3-5 Data collection:

3-5-1 Data collection tool:

The data collected from the patient using closed answer questionnaire.

3-5-2 Data analysis:

The collected data were analyzed with SPSS program using chi square.

3-6 Ethical consideration:

All patient under study were informed about the objective of research , the verbal consent was taken from them before enrolled under study.

3-7 anti *H.pylori* ELISA:

ELISA kit purchased from EUROIMMUN company containing microplate wells 12×8 , three calibrator 1,2 and 3, positive and negative control, enzyme conjugate, sample buffer, wash buffer, chromogen/substrate solution and stop solution.

3-7-1 Principle of test:

The ELISA test kit provides a semi-quantitative in vitro assay for human antibodies of the IgG class against *H.pylori* in serum or plasma. The kit contains microtiter strips each with 8 break-off reagent wells coated with *H.pylori* antigens. In the first reaction step, diluted patients samples are incubated in the well. In case of positive samples, specific IgG antibodies will bind to antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a colour reaction.

3-7-2 Preparation of sample and washing buffer:

Patients samples were diluted 1:101 in sample buffer 10 µl serum in 1.0 ml sample buffer and mixed by vortexing.

Concentrated wash buffer diluted from 10 x to 1.0x by adding 100ml of of washing buffer to 900 ml of distilled water.

3-7-3 Procedure of semi-quantitative analysis:

For semi-quantitative method calibrator 2 along with positive , negative controls and patients samples.

First step, sample incubation: 100 µl was pipetted per well and incubated for 30 minutes at room temperature, then the well were manually washed by empting the well and subsequently washed three times using 300 µl of working wash buffer.

Second step, conjugate incubation: 100 µl of enzyme conjugate (peroxidaselabled anti human IgG) was pipetted into each microplate wells and incubated for 30 minutes at room temperature, then the well were manually washed by emptying the well and subsequently washed three times using 300 µl of working wash buffer.

Third step, substrate incubation: 100µl of chromogen/substrate (TMB/H_2O) solution was pipetted into each microplate wells and incubated for 15 minutes at room temperature.

Stop the reaction 100µl of stop solution(0.5 M Sulphuric acid) was pipetted into each microplate wells in the same order and at the same speed as chromogen/substrate solution was introduced.

Last step: Photometric measurement was done by using ELISA reader which performed as absorbance in filter 492 nm.

3-7-4 interpretation of result:

Results had been interpretated by calculation a ratio of the extinction value of control or patient sample over the extinction value of the calibrator 2.

Formula of the ratio:

$$\frac{\text{extinction of control or patient sample}}{\text{extinction of calibrator 2}}$$

-ratio < 0.8 negative

-ratio ≥ 0.8 to < 1.1 borderline

-ratio ≥ 1.1 positive

4-Results

Table (4-1) prevalence of *H.pylori* in typhoid patients and non typhoid subjects:

Sample	Positive	negative	Total	Frequency
Typhoid patients	64	8	72	88.8%
Non typhoid subjects	10	10	20	50%

P value =0.000

Out of 72 typhoid patients 64 of them were positive (88.8%) and out of 20 non typhoid subjects 10 of them were positive (10%).

Table (4-2) Frequency of *H.pylori* according to gender:

Gender	Total number	positive	Frequency
Male	37	33	89%
Female	35	31	88%

P value =0.934

Out of 37 of males 33 of them were positive (89%) and out of 35 females 31 of them were positive (88%). There is no significant differences between male and female in prevalence of *H.pylori*.

Table (4-3) show frequency of *Helicobacter pylori* according to age:

Age group	Total number	positive	Frequency
15-30	30	23	77%
30-45	36	35	97%
More than 45	6	6	100%

P value = 0.02

All age group were have positive for IgG increasing with age and all age groups more than 45 years old were positive.

Table (4-5) frequency of *Helicobacter pylori* according to smoking behavior:

Smoking behavior	Total number	positive	frequency
Smokers	25	23	92%
Non smokers	47	41	88%

P value = 0.540

There is no variation between smokers and non smokers in the prevalence of *H.pylori* .

Table (4-6) show frequency of *Helicobacter pylori* according to coffee drinking:

coffee drinking	Total number	positive	Frequency
Yes	32	29	91%
No	40	35	88%

P value =0.675

There no significant difference between coffee drinkers and non coffee drinkers in the prevalence of *H.pylori* .

5-1 Discussion

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status (SES). Because it is not possible to ascertain when infection occurs clinically (Parsonnet, 1995), most of the information on the rates of *H. pylori* in geographically and demographically diverse populations comes from seroprevalence studies. The main finding in this cross-sectional study that the prevalence of *H.pylori* among typhoid patients was 64 (88.8%) out 72 and 10 (50%) among 20 controls samples of non typhoid subjects. The prevalence was higher in patients with typhoid more than control non typhoid subjects and this agree Maharaj, (2002), and this more likely to be that *H.pylori* change in acid barrier of stomach by causing transient hypochlorhydria (Harford *et al*., 2000) which facilitate infection with *Salmonella*.

The study revealed that the prevalence of *H.pylori* was higher in both male and female and there is no statistically difference (*p* value=0.934) in prevalence between male and female and this agree with (Yvonne *and Rob*., 2001).

Also the study revealed that prevalence of *H.pylori* in older age (more than 45) group was higher than in other group. It was noticed that the disease prevalence was high in all group irrespective of their ages and gender. However, high prevalence in older age group may be attributed to long time exposure to the causative agent during their early childhood. (Mitchell *et al*., 1992)

Also the study revealed that there is no statistically difference in prevalence of *H.pylori* is among smoker group and non smokers (p value= 0.540) and that agree with an most of recent research (Zhang *et al.*, 1996; Fraser *et al*., 1996 ; Brenner *et al.*, 1997) and this may be due to uncontrolled confounding by social class .

Also the study revealed that statistically there is no difference in prevalence of *H.pylori* in between coffee drinkers and non drinkers also this may be due to uncontrolled confounding by social class.

5-2 Conclusion:

Cross-sectional hospital base study conducted in Shendi City with 72 case subject and 20 control for detection of *Helicobacter pylori* in typhoid patients and the result was significantly high prevalence. Also results showed that the prevalence was significantly increased with age as a risk factor.

5-3Recmondatin:

1. Create an effort by the government for eradication of *Helicobacter pylori* which increase risk of infection with typhoid fever.
2. Health education program should be taken place especially on personal hygiene.
3. In general, it is always wise for persons to wash hands thoroughly, to eat food that has been properly prepared, and to drink water from a safe, clean source.
4. Research with large sample size will powered by estimation gastric acid for better result.

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