عمـــادة البحـــث العلمـــي
DEANSHIP OF SCIENTIFIC RESEARCH

Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Correlation between patient's risk factors and *H. pylori* infection and Seroprevalence of anti- *Helicobacter pylori* IgG and IgA among out patients in Khartoum State, Sudan with different diagnostic tests

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ARTICLE INFO

ARTICLE HISTORY
Received:20/2/2020
Accepted:12/7/2020
Available online:June2020

KEYWORDS:
ELISA, ICT, *Helicobacter pylori*

ABSTRACT

This study was conducted to detect seroprevalence of anti-*Helicobacter pylori* IgG and IgA antibodies by different serological techniques and to evaluate the diagnostic efficiency and reliability of these techniques including Immunochromatography test ICT for rapid *H. pylori* IgG antibodies detection, enzyme linked immunosorbant assay (ELISA) test for *H. pylori* IgA antibodies detection and ELISA for determination of Anti-*Helicobacter pylori* IgG titer. Two hundred and thirty five patients were subjected to blood sampling and data collection in a questionnaire form, consequently the statistical correlation was tested between *H. pylori* infection and patient's risk factors (Age, Gender, Smoking history, Body mass index, Hypertension, Diabetes mellitus, symptoms and *H. pylori* infection history).

The IgG antibody titer mean was 95.21 RU/ml and the maximum was 299.20 RU/ml while every sample above 22 RU/ml was considered positive according to manufacturer, the positive samples for IgG were quite high (88.2%) while for IgA the positives were 30% and the positive cases by ICT were 71.9%. In Sudan and similar developing countries ICT is considered as the most commonly used test for diagnosis of *Helicobacter pylori* infection, this study showed that ICT accuracy was 74.6%, Sensitivity was 75.6% and the Specificity of this test was 88.2%. Hence it is highly recommended that this test should be replaced by other more trusted (invasive or non-invasive) laboratory diagnostic procedure. While the ICT method is not very reliable.

Introduction:

Helicobacter pylori (H. pylori) is a major cause of chronic gastritis and gastric ulcers and considerable evidence supports the notion that infection with this bacterium is also associated with gastric malignancy in addition to various other conditions including pulmonary, vascular and autoimmune disorders (Kariya et al, 2014).

Regarding the role of antibodies in protective immunity, Subsequent experiments have indicated that the relevance of the humoral system for protective immunity is only marginal. Antibodies can effectively prevent infection and reduce colonization in animal models (Marnila *et al*, 2003), (Nomura *et al*, 1994). *H. pylori* infection results in an induction of a Th1-polarized response that does not result, however, in clearance of the infection (Lindholm *et al*, 1998), (Mohammadi *et al*, 1996), (Smythies *et al*, 2000). This is striking, as it is the cellular rather than the humoral immunity that has been reported to play the principal role in sterilizing immunity (Enton *et al*, 2001, Ermak *et al*, 1998, Castriotta *et al*, 1999, Kosaka *et al*,2000). *H. pylori* are thought to downregulate inflammation and control the host's immune response through a wide range of virulence factors that are involved in both provoking and maintaining a proinflammatory immune response (Enton *et al*, 2001).

GERD is a common condition result from the reflux of material (gastric acid, bile, pepsin, and duodenal contents overwhelm normal esophageal protective antireflux barriers,) through the lower eosophageal sphincter into eosophagus or oropharynx causing symptoms and/or injury to the eosophageal tissue (Spechler, 1992). Most people experience normal reflux which are not associated with pathogenic signs and may occur after meal (Szarka and Locke, 1999).

Pathologic reflux can results from variety of clinical presentations that lead to chronic symptoms, inflammation or eosophageal mucosal damage, whenever, GERD is more frequent and has longer duration. The lower eosophageal sphinicter relaxation is the key of etiologic factors (Storr *et al*, 2000).

The pathogenesis of this disorder (GERD) involves an imbalance between acid secretion and gastric mucosal defense. Important eosophageal symptoms include laryngitis and pharyngitis due reflux into the throat (Karilas, 2003). Pathogenesis of GERD is similar to that of other secretary diseases such as duodenal ulcer disease and gastric ulcer disease. *H. pylori* infection is the factor in 85% to 100% of duodenal ulcers and 70% to 90% of gastric ulcers. Eradication of this organism results in a considerable decrease in recurrent ulcer (Sanders, 1996). Various tests have been developed for the detection of *H. pylori*, each with their specific advantages and disadvantages.The available tests are generally divided into invasive tests (endoscopic), based on gastric specimens, and noninvasive tests (nonendoscopic), based on peripheral samples for detection of antibodies, bacterial antigens, or urease activity (Logan, 1998). *Nonendoscopic Tests* include serologic testing for IgG antibodies to *H. pylori* is often used to detect infection. However, a metaanalysis of studies of several commercially available quantitative serologic assays showed an overall sensitivity and specificity of only 85% and 79%, respectively (Loy *et al*, 1996). The appropriate cutoff values vary among populations, and the test results are often reported as positive, negative, or equivocal. Also, this test has little value in confirming eradication of the infection, because the antibodies persist for many months, if not longer, after eradication. The urea breath test involves drinking ^{13}C-labeled or ^{14}C-labeled urea, which is converted to labeled carbon dioxide by the urease in *H. pylori*. The labeled gas is measured in a breath sample. The test has a sensitivity and a specificity of 95% (Vaira and Vakil, 2001). The infection can also be detected by identifying *H. pylori*–specific antigens in a stool sample with the use of polyclonal or monoclonal antibodies (the fecal antigen test). The monoclonal-antibody test is more accurate (Gisbert and Pajares, 2004). For both the breath test and the fecal antigen test, the patient should stop taking proton-pump inhibitors PPI 2 weeks before testing, should stop taking H2 receptor antagonists for 24 hours before testing, and should avoid taking antimicrobial agents for 4 weeks before testing, since these medications may suppress the infection and reduce the sensitivity of

testing (McColl and Kenneth, 2010). *In endoscopicTests: H. pylori* infection can be detected on endoscopic biopsy of the gastric mucosa, by means of several techniques. The biopsy specimens are usually taken from the prepyloric region, but an additional biopsy specimen obtained from the fundic mucosa may increase the test's sensitivity, especially if the patient has recently been treated with a proton-pump inhibitor. The urease-based method involves placement of the endoscopic biopsy specimen in a solution of urea and pH-sensitive dye. If *H. pylori* are present, its urease converts the urea to ammonia, increasing the pH and changing the color of the dye. Recommendations for avoiding proton-pump inhibitors, H2 receptor antagonists, and antimicrobial therapy before testing apply to this test as well, to minimize the chance of false negative results (Midolo and Marshall, 2000) The test has a sensitivity of more than 90% and a specificity of more than 95% (Vaira and Vakil, 2001). Another means of diagnosis involves routine histologic testing of a biopsy specimen; if there is *H. pylori* infection, the organism and associated gastritis are apparent on sections stained with hematoxylin and eosin or Giemsa. Although culturing of the organism is also possible and permits testing for sensitivity to antimicrobial agents, facilities for the culture of *H. pylori* are not widely available and the method is relatively insensitive. (McColl *et al*, 2010).

Additionally there are several molecular methods for detecting *H. pylori* include polymerase chain reaction (PCR), real- time PCR, stool PCR, multiplex PCR and fluorescent hybridization to identify *H. pylori* infection. There are several tests available to identify *H. pylori* infection, but none is considered a gold standard. (Malfertheiner *et al*, 2012).

Significant differences in prevalence across the world have been found within and between countries (Figure 1) (Amieva *et al*, 2009). In undeveloped countries, most of the infections seem to be acquired during childhood while in developed countries the incidence increases gradually with age (Brenner *et al*, 2004).

The prevalence of *H. pylori* infection differs among countries and population groups. For middle-aged adults it varies in developed countries between 20 to 50% up to 80% in many developing countries (Dunn *et al*., 1997; Crew *et al*, 2006; Michetti and Suerbaum, 2002). The incidence, in developed countries, is about 1% per year until the age of 50 to 60 years (Bani-Hani *et al*., 2006). Epidemiological studies have shown that, in general, the high incidence of *H. pylori* is correlated with a deprivation in sanitation, hygiene and educational habits. (Axon, 2006; Bani-Hani *et al*., 2006; Dunn *et al*., 1997; Michetti and Suerbaum, 2002).

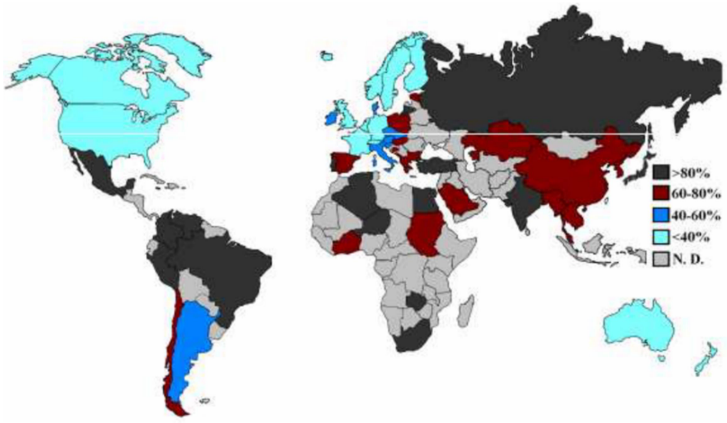

Figure 1: Worldwide prevalence of *H. pylori,* (Amieva *et al*, 2009).

Materials and Methods:

1. Data collection tools:

Questionnaire form was performed about general manifestations and symptoms. Patient's demographic data was retrieved from medical records on enrollment, including: Age, Gender, Smoking history, Body mass index (BMI, calculated as weight in kilograms divided by height in meters squire). Hypertension (divided as a patients on antihypertensive drug for blood pressure over 140/90 mmHg), Diabetes mellitus (DM, divided as fasting glucose ≥ 7.0mmol/L or with past fasting history of diagnosed DM).

2. Sample taking procedure:

Blood Samples were obtained from 235 patients by taking 5 ml of venal blood by the clinical lab's qualified experienced practitioner.

Sample size calculation:

$$n = 1.96 \times \frac{Pexp^2(1 - Pexp)^2}{d^2}$$

n: required sample size

P_{exp}: expected prevalence

d: desired absolute precision = 0.05

Expected prevalence is 70% as recorded by using confidence level 95%.

Enzyme-linked immunoassay (ELISA)

Determination of Anti-*Helicobacter pylori* IgG titer

Anti-*Helicobacter pylori* ELISA (IgG) test kits (EUROIMUM) were used for measurement of an Anti-*Helicobacter pylori* IgG titer in patient's serum. The ELISA was performed according to manufacturer (EUROIMUM). Photometric measurement of color intensity was made in ELISA reader machine (Stat Fax 4200) at a wavelength of 450nm and the reference wavelength between 620 and 650nm, within 30min. of adding the stop solution. Prior to measurement a microplate was shaken slightly to ensure a homogenous distribution of the solution.

Quantitative: results were evaluated quantitavely, the concentration of antibodies was obtained by point-to-point plotting of the extinction values measured for three calibration sera against the corresponding units (linear/linear). Point-to-point plotting for calculation of the standard curve by computer.

Detection of Anti-*Helicobacter pylori* IgA:

Anti-*Helicobacter pylori* ELISA (IgA) test kits (EUROIMMUN) were used**.** The ELISA was performed according to manufacturer (EUROIMMUN).

Semiquantitave: results were evaluated semiquantitatively by calculating a ratio of the extinction value of the control and patient sample over the extinction value of calibrator.

$$\text{Ratio} = \frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}}$$

Screening of seroprevelance of *H. pylori* positive patients by using *H.pylori* Antibody Rapid test cassette (ALL TEST Co.):

The *H. pylori* rapid test cassette (serum/plasma) is a qualitative membrane based immunoassay for the detection of *H. pylori* antibodies in serum or plasma. In this test procedure anti-human IgG is immobilized within the test line region of the test. Specimens react with *H. pylori* antigen coated particles. The mixture

migrates chromatographically along the length of the test and interact with the immobilized anti-human IgG. If the specimen contains *H. pylori* antibodies a colored line will appear in the test line region indicating positive result, if not no colored line will appear in the test region.
Three drops of serum were transferred to specimen well of the test cassette and left in clean area, the test read after 10 min. two red lines indicate positive result.
Proposed Sensitivity, specificity and accuracy of ICT were done according to manufacturer:

Table(1) Proposed sensitivity, specificity and accuracy of ICT according to manufacturer.

Method		ELISA		Total
H. pylori antibody	result	Positive	Negative	result
rapid test Cassette	Positive	171	8	179
(serum/plasma)	Negative	0	102	102
Total result		171	110	281

Relative sensitivity: > 99.9% (95%CI*:98.3%-100%)

Relative specificity: 92.7% (95%CI*:86.2-96.8%)

Accuracy: 97.2% (95%CI*:94.5%-98.8%)

*Confidence Interval

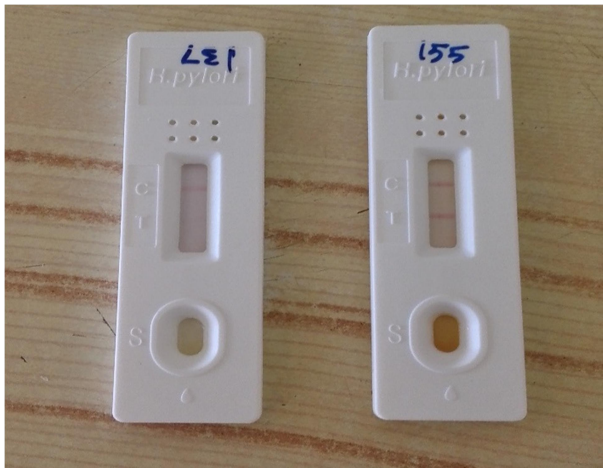

Figure 2: Immunochromatographic test (ICT)

Results:
IgG Antibody titer was found to be significantly (at 99% confident interval, 2 tailed) correlated with ICT (Table2) and IgA ratio (Table3).

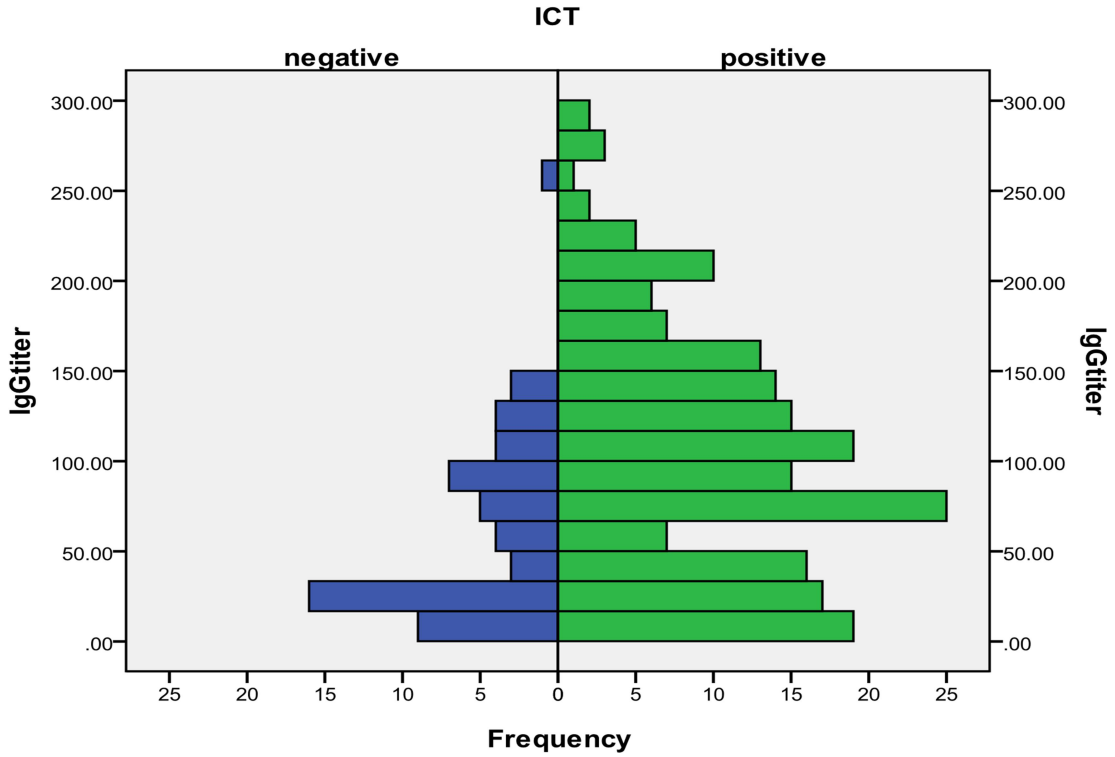

Figure3: The difference in accuracy between two diagnostic tools for *Helicobacter pylori* infection (IgG ELISA and ICT.

H. pylori positive (by ELISA or ICT) cases were found to be significantly (at 99% confident interval, 2 tailed) correlated with upper digestive tract signs (Figure4).

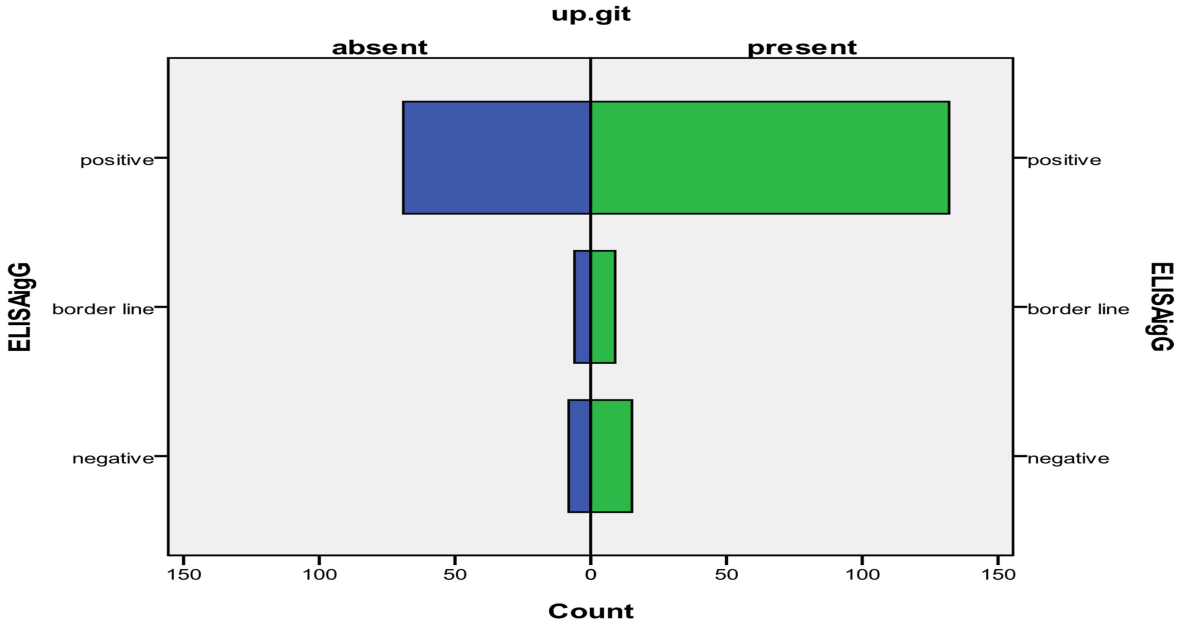

Figure4: The increase of IgG conc. correlating with upper digestive tract signs

Negative correlation was found between IgG titer and fever (at 95% confident interval, 2 tailed). But fever had positive correlation with presence of respiratory symptoms and lower digestive tract illness. A significant correlation was found between ICT method of diagnosis and IgG titer detected by quantitative ELISA. 28.1% were detected positive by ICT and 71.9% were negative.

Table(2) obtained sensitivity, specificity and accuracy of ICT form this study:

Method		IgG ELISA*		Total results
H. pylori rapid test cassette (serum/ plasma) ICT	result	+	-	
	+	149 [a]	12 [b]	161
	-	48 [c]	15 [d]	63
Total results		197	27	224

- * 11 cases were in border line has been excluded.

$$\text{Accuracy of ICT} = \frac{N\ true\ positive + N\ True\ negative}{N\ true\ positive + N\ true\ negative + N\ false\ positive + \ false\ negative}$$

Accuracy = 74.6% (95% CI*)

* Confidence Interval

$$\text{Sensitivity} = \frac{N\ true\ positive}{N\ true\ positive + N\ false\ negative}$$

Sensitivity = 75.6% (95% CI*)

$$\text{Specificity} = \frac{N\ True\ negative}{N\ true\ negative + N\ false\ positive}$$

Specificity = 88.2% (95% CI*)

By IgG ELISA 84% of the cases were Positive, 11.4% were Negative and 4.6% were border line (Figure6). IgA ELISA showed that 53.2% of individual samples were negative, where as 16.8% were border line and 30% were positive (Figure 5).

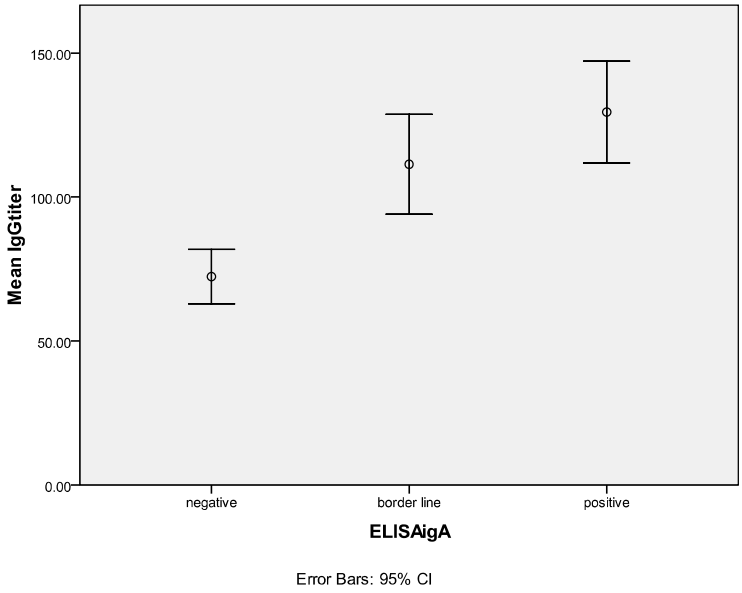

Figure 5: The graph represents the mean of Anti-*Helicobacter pylori* IgG for each categori of Anti-Helicobacter pylori IgA (Positive, border line and Negative cases), this graph showed that even the Positive cases of IgA have low IgG titer; the maximum IgG titer among samples were about 299 Ru/ml.

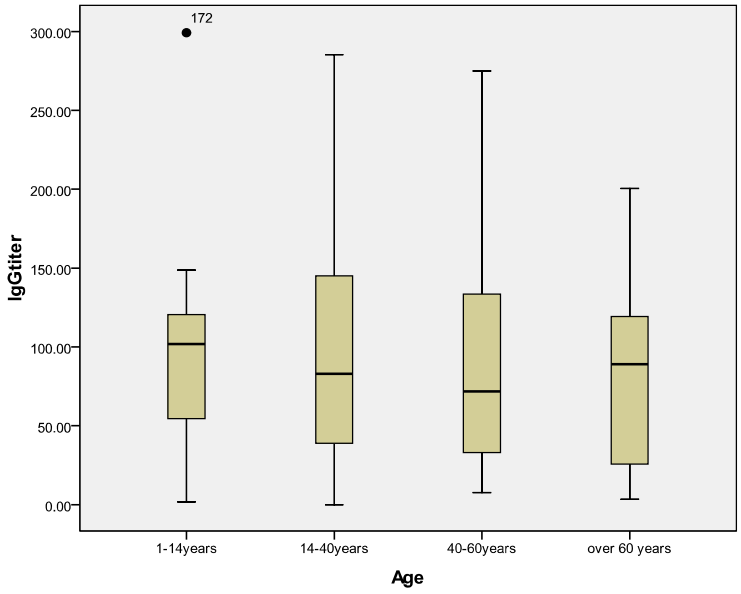

Figure 6: Differences in Anti-*Helicobacter pylori* IgG titer between Age groups. Please notice that the youth group between 14 and 40 years old has highest Anti-*Helicobacter pylori* IgG titer.

Table(3) obtained sensitivity, specificity and accuracy of IgA ELISA form this study:

Method		IgG ELISA*		Total
IgA	Result	+	-	results
ELISA	+	68 $_a$	3 $_b$	71
	-	106 $_c$	21 $_d$	127
Total results		174	24	198

$$\text{Accuracy of IgA ELISA} = \frac{N\ true\ positive + N\ True\ negative}{N\ true\ positive + N\ true\ negative + N\ false\ positive + N\ false\ negative}$$

Accuracy = 45% (95% CI*)

* Confidence Interval

$$\text{Sensitivity} = \frac{N\ true\ positive}{N\ true\ positive + N\ false\ negative}$$

Sensitivity = 39.1% (95% CI*)

$$\text{Specificity} = \frac{N\ True\ negative}{N\ true\ negative + N\ false\ positive}$$

Specificity = 87.5% (95% CI*)

***H. pylori* infection History:**

10.1% of whole collected samples were from individuals with history of previous *H. pylori* infection (Figure 7). Among those with previous infection, about 96% were Positive by IgG ELISA, 80% were positive by ICT and only 8.3% were positive by IgA ELISA. In other word The majority of cases that had at least one *H. pylori* infection before had very low concentrations of anti- *H. pylori* IgA in their body; most of them were diagnosed infected with *H. pylori* in this study; this suggest that developing high level of anti- *H. pylori* IgA is quite essential to protect previously *H. pylori* infected people from being infected again. Also those patients suffering from recurrent *H. pylori* infection are basically have an issue with developing high anti- *H. pylori* IgA titer in their blood and this in turn makes them vulnerable for further *H. pylori* infections in the future (Figure8).

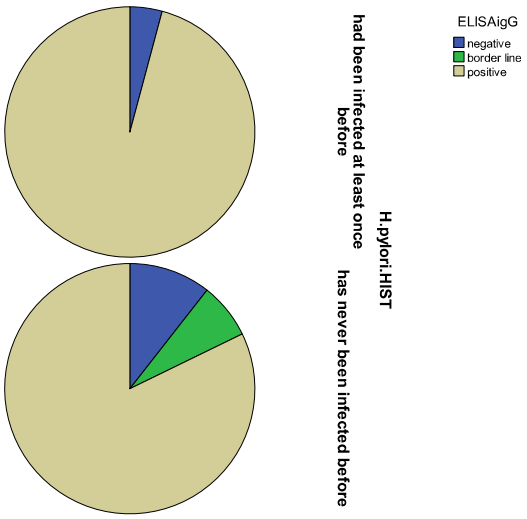

Figure 7: This Pie Chart represent the difference between patients with *H. pylori* history and those with absolutely no previous *H. pylori* infection in IgG categories (Positive, Border line or Positive). Please notice that the majority of patients with previous *H. pylori* history were positive for Anti-*Helicobacter pylori* IgG.

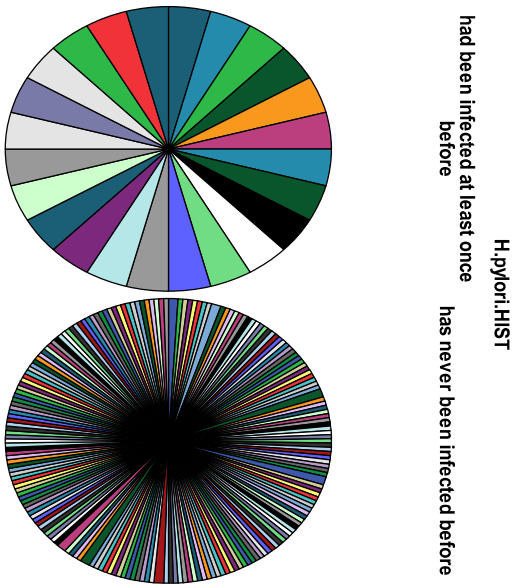

Figure 8: This Pie Chart reflect the huge difference between patients with *H. pylori* infection history and those with no previous *H. pylori* history in Anti-*Helicobacter pylori* IgG titer (each color represent individual Anti-*Helicobacter pylori* IgG titer).

Our samples involved different age groups each had identified Prevalence Rate (PR) 1-14years (PR= 11.9%), 14-40years (PR= 69%), 40-60years (PR= 13.5%) and Over 60 years (PR= 5.6%) (Figure 6). The IgG antibody titer mean was 95.21 RU/ml and the maximum was 299.20 RU/ml while every sample above 22 RU/ml considered positive according to manufacturer, the positive samples for IgG was quite high (88.2%) while for IgA the positives were 30% and the positive cases by ICT were 71.9%. IgA ELISA showed that, 28.7% of males were Positive, while 25.8% of females were affected (Figure 9). On other hand IgG ELISA showed 84% of males were positive and 81.8% of females were Positive. 83% of children between 1 to 14 years were positive, 85% of youth between 14 to 40 years, 80% of older people between 40 and 60 years were positive and 85% of very old people over 60 years old were positive by IgG ELISA.

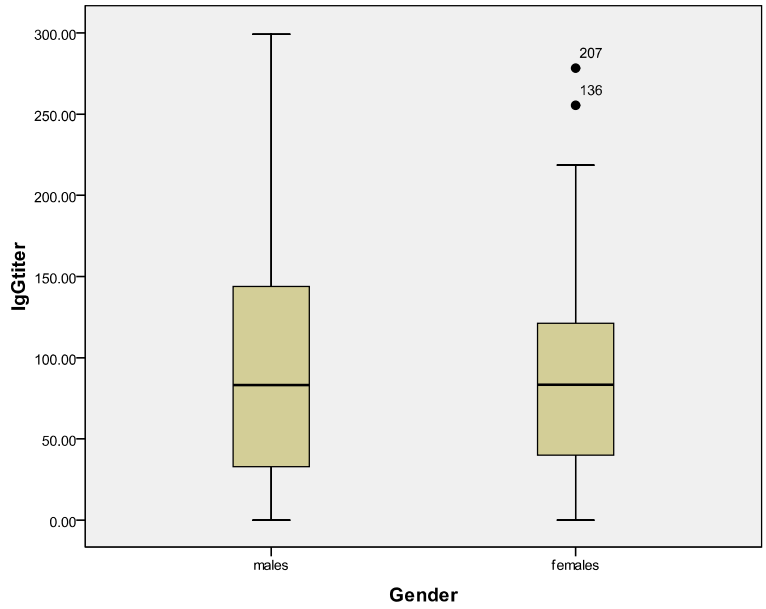

Figure 9: The difference in anti-*Helicobacter pylori* IgG titer between males and females.

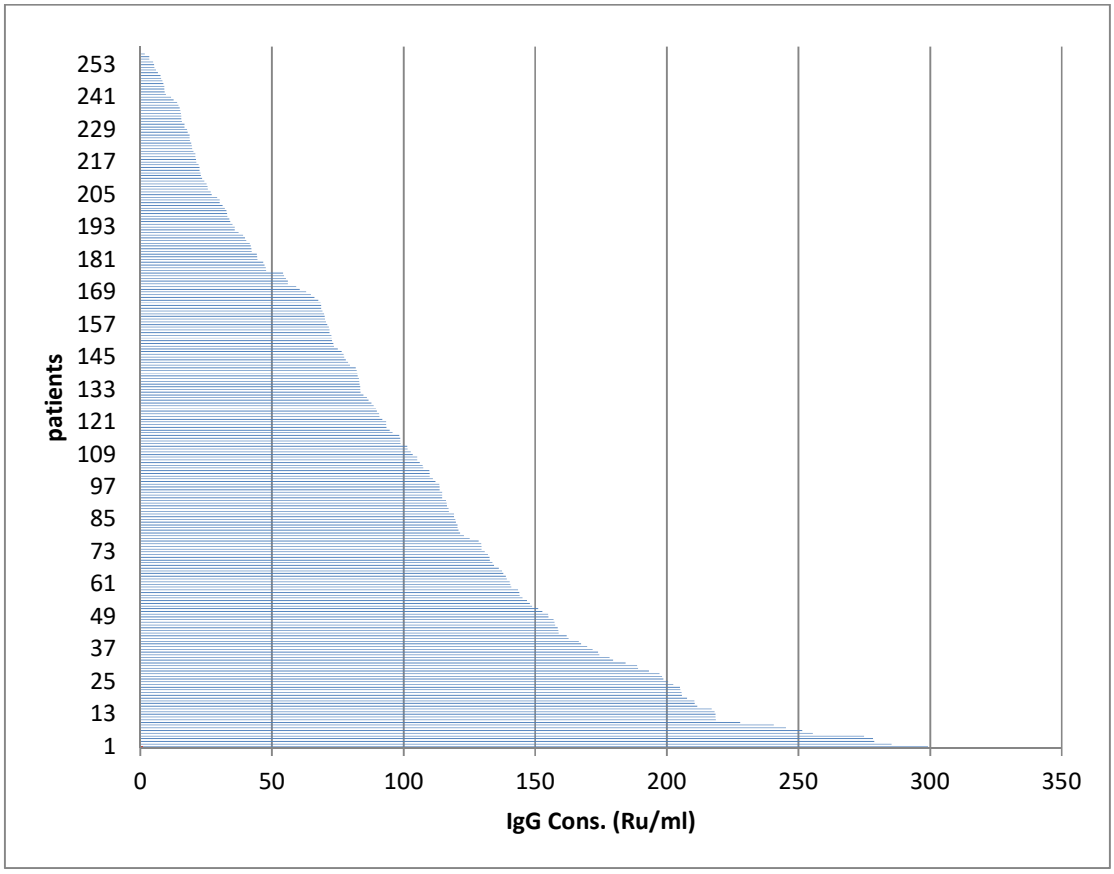

Figure 10: IgG Concentration against *H. pylori* infection (Ru/ml) with the frequency among patients.

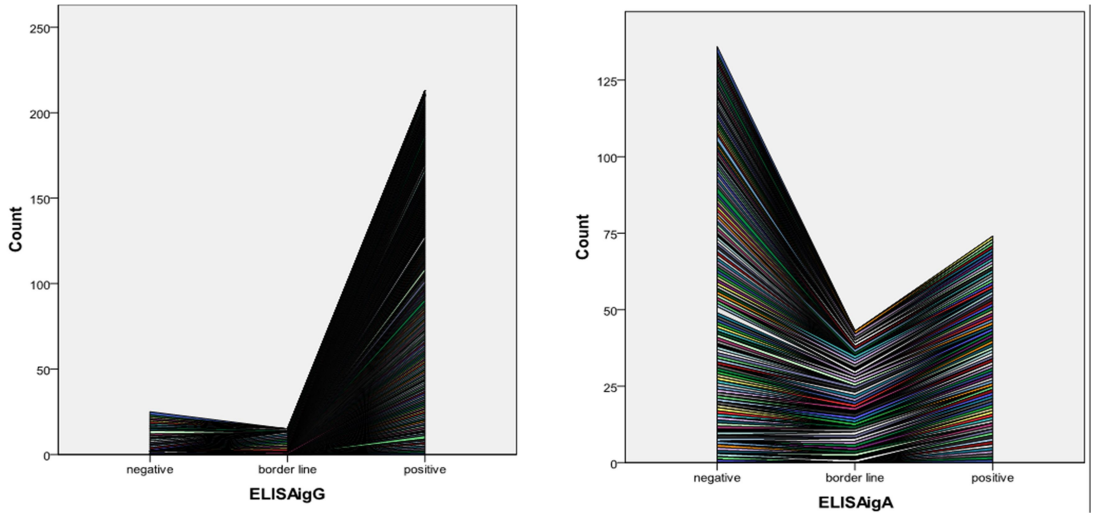

Figure 11: This chart reflects the big difference in patient frequencies between Anti-*Helicobacter pylori* IgG and IgA category.

Discussion:

The result showed that 84.2% of patterns were positive for IgG, only 29.5% were positive for IgA, while 26.7% were positive for both. In contrast to a previous study that 63.3% of samples in Khartoum, Sudan were positive for IgG (Elhag and Omer, 2014), we suggest that means the prevalence of *H. pylori* infection is increasing. In Kenya Siekmann and his colleagues (2003), found 70.2% were seropositive for IgG. But in Saudi Arabia the seropositive IgG was quite different, only 22% were seropositive for IgG (Mubashir and Hani, 2007).

Males were more affected than females (Figure9), (among positive IgG samples76.6 % were males and 38.3% were females) in contrary to Elhag and Omer study in (2014) where Females were more affected than males.

The present study results showed insignificant correlation between age and *H. pylori* antibodies, this was similar to study done in Iran by Alavi *et al*, (2010) and also same as what found 5 years ago in Sudan by Elhag and Omer study in (2014). But it differ from Kabir, (2007) in Sweden who reported that the percentage of infected people increase with age. Also Forman and Burley in (2006), stated that the prevalence of *H. pylori* infections increase with age.

In this study the higher percentage of infection observed among age group 14-40 years (70%), this high percent may be due to the vast majority of individuals acquire this infection during childhood (Cherian *et al*, 2008).

Although our results differ from Elhag and Omer, (2014); because we found that males were more affected and had more IgG and IgA antibodies than females but we still the present agree with Elhag and Omer, (2014) along with Mirghani and his colleagues (Mirghani *et al*, 2002), as there was insignificant correlation between *H. pylori* antibodies and gender (P >0.05), also it was in agreement with that obtained in Egypt by Manal *et al*, (2007), and other studies by Huang *et al*, (2004) in Malaysia, (Kikuchi, *et al* 2005) in Iran, and Mukherjee *et al*, (2005) in Netherlands. On other hand, Leandro *et al*., (2005) found that the prevalence was significantly higher in boys, also Versalovic and Fox, (2003) reported that *H. pylori* is more prevalent among the elderly and more frequent in males than females.

Anti – *Helicobacter pylori* IgG antibody is more antibody type that affected by presence or absence of the pathogen and is the more trustful than IgA, although there were significant positive (p= 0.05) correlation between IgG and IgA levels, but high number of positive IgG were negative by IgA (Figure 11).

On the other hand IgA was found to be more effective in protection and immunogenicity against *H. pylori* infection and its absence or low level may be the cause of active recurrent *H. pylori* infection. So we suggest that, failure of *H. pylori* infection treatment could be, beside antibiotic resistance (particularly clarithromycin), attributed to failure of immune system to raise effective IgA level. These reasons justify the necessity of a novel vaccine to prevent *H. pylori* infection.

Among all individuals complaining from respiratory signs, 89% of them were confirmed with *H. pylori* infection. Some authors identified *H. pylori* VacA toxin in human lungs, possibly influencing the course of some respiratory diseases by promoting inflammation (Nakashima *et al,* 2015).

Serological methods in diagnosis of *H. pylori* become increasingly important last year's and we are persuaded that it will be in the future even more trustworthy corner stone for diagnosis of *H. pylori* infection specially in developing countries like Sudan; because of the growing rates of disease prevalence and also due to its efficiency, availability and simplicity. More than twenty years ago Cutler *et al,* (1995) have speculated that serology probably will be the test of choice for patients not previously treated for *H. pylori.* Serological testing may be accomplished either by enzyme-linked immunosorbent assay performed in a reference laboratory (IgG or IgA serology) or by one of the newly available in-office immunoassay kits (i.e. ICT), (Figure 3) for all the above mentioned reasons we used these three serological method and

measure their specificity, sensitivity and accuracy in Sudanese patients in order to report the pros and cons of each method and hence help the researchers and physicians to appoint the suitable diagnostic tool for each case accordingly.

IgA ELISA had low specificity and sensitivity For *H. pylori* infection comparing to IgG ELISA; this finding has many agreements since Cutler *et al,* (1995).

On the other hand among all individuals with upper digestive tract illness signs (signs of laryngeopharyngeal reflux) (LPR), 85% of them were confirmed with *H. pylori* infection.

Among diabetic patients 83.3% of them were infected with *Helicobacter pylori*, while the majority of them were negative for Anti- *H. pylori* IgA. Other previous studies found a significant association between Diabetes Mellitus (DM) and *H. pylori* infection, Hsieh *et al,* 2013 and Yang *et al,* (2014). We proposed that this finding could be attributed to the Immune system debilitation accompanied with DM.

Among all individuals complaining from respiratory signs, 89% of them were confirmed with *H. pylori* infection.Sore throat cases were found to be significantly (at 95% confident interval, 2 tailed) correlated with ICT results, and also significantly correlated with symptoms of general body fatigue and respiratory signs. IgG Antibody titer was found to be significantly (at 99% confident interval, 2 tailed) correlated with ICT and IgA ratio.

After data analysis we found is that ICT accuracy was 74.6%, while ICT Sensitivity was 75.6% and ICT Specificity was 88.2%, this were different from ICT manufacturer provided data, while they mentioned that sensitivity is greater than 99.9%, relative specificity is 92.7% and accuracy is 97.2%. We speculated that the difference could be attributed to the use of fresh samples in most of our samples, the reduction in Accuracy, specificity and sensitivity rates while using fresh samples instead of stored samples had been mentioned before (Sharma *et al*, 1997), another possible justification is the use of high-molecular-mass cell-associated protein (HMCAP), an antigen highly specific to *H. pylori,* as the only Ag in ICT kit (Sharma *et al*, 1997) and (Evans *et al*, 1989). On the other hand the antigen used in IgG ELISA was the whole bacterial cell lysate derived from *H. pylori* strain "ATCC43504".

Conclusion:

In conclusion it would appear that, the prevalence of *H. pylori* infection is increasing in Sudan. We found that males were more affected by *H. pylori* infection than females, and the higher percentage observed among (14-40) years age group. Among people complaining from respiratory signs most of them were confirmed with *H. pylori* infection. Among diabetic patients the majority of them were confirmed with *H. pylori* infection. Among individuals with upper digestive tract signs (LPR) most of them were also confirmed with *H. pylori* infection. IgG antibody was found to be correlated with ICT and IgA ratio. IgA ELISA had low sensitivity, specificity and accuracy comparing to IgG ELISA. The low level of IgA may be the cause of active recurrent *H. pylori* infection. ICT Antibody detection method for *H. pylori* infection diagnosis is not very reliable.

References:

Alavi SM, Adel SMH, Raja AR. (2010). Seroprevalence study of *Helicobacter pylori* infection among visitors of cardiac patients in Razi Hospital in Ahvaz, Iran. *Jundishapur Journal of Microbiology*, **3**(1): 28-31.

Amieva M.R., Tan, S., and Tompkins L.S. (2009). *Helicobacter pylori* usurps cell polarity to turn the cell surface into a replicative niche. *Journal of Public Library of Science Pathogens*; **5**(5): 1-41.

Axon A. (2006). Helicobacter pylori what do we still need to know? *Journal of Clinical Gastroenterology*; **40** (1):15–9.

Bani-Hani KE, Nawaf JS, Qaderi SE, Khader YS and Bani-Hani BK. (2006). Prevalence and risk factors of Helicobacter pylori infection in healthy schoolchildren. *Chinese Journal of Diagnostic Diseases*; **7**:55–60.

Brenner H., Perez-Perez, G.I., and Rothenbacher D. (2004). Epidemiology of Helicobacter pylori Infection. *Helicobacter*; **9** Suppl 1: p. 1-6.

Castriotta L., Pappo, J., Torrey D., Savinainen A., Kabok Z., and Ibraghimov A. (1999). Helicobacter pylori infection in immunized mice lacking major histocompatibility complex class I and class II functions. *Infection and Immunology;* **67**:337–341.

Cherian S., Forbes D., Sanfilippo F., Cook A., Burgner D., (2008). Serodiagnosis of *Helicobacter pylori* evolution of rapid miniapurized immunochromatographic test. *Medical Journals,* **8**: 438-415.

Crew KD and Neugut AI. (2006). Epidemiology of gastric cancer. *World Journal of Gastroenterology*; **12**(3): 354–62.

Cutler A. F., Havstad S., Ma T C. K., Blaser M. J., Perez-Perez J. G. I., And Schubert T. T. (1995). Accuracy of Invasive and Noninvasive Tests to Diagnose *Helicobacter pylori* Infection; *Gastroenterology*; **109**:136-141.

Dunn BE, Cohen H. and Blaser MJ. (1997). *Helicobacter pylori*, clinical microbiology reviews. *American Society of Microbiology*; **10**(4):720–41.

Elhag WI. and Omer Ali LE. (2014). Frequency of *H. pylori* Antibodies among Patients with Gastrointestinal Symptoms Attending Khartoum Teaching Hospital- Sudan. *SOJ Microbiology and Infectious Diseases,* **2**(1)*:* 5.

Enton, K. A., Mefford M., and Thevenot T. (2001). The role of T cell subsets and cytokines in the pathogenesis of Helicobacter pylori gastritis in mice. *Journal of Immunology;* **166**:7456–7461.

Ermak, T. H., Giannasca P.J., Nichols R., Myers G.A., Nedrud J., Weltzin R., Lee C.K., Kleanthous H., and Monath T.P., (1998). Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *Journal of Experimental Medicine;* **188**:2277–2288.

Evans DJ, Evans DG, Graham DY, Klein PD. (1989). A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology*; **96**:1004–8.

Forman D. and Burley VJ. (2006). Gastric cancer: global pattern of the disease and an overview of environmental risk factors. *Best Practice and Research Clinical Gastroenterology,* **20**(4): 633-649.

Gisbert JP. and Pajares JM. (2004). Stool antigen test for the diagnosis of *Helicobacter pylori* infection: a systematic review. *Helicobacter*; **9**:347-368.
Hsieh MC, Wang SS, Hsieh YT, Kuo FC, Soon MS and Wu DC. (2013). *Helicobacter pylori* infection associated with high HbA1c and type 2 diabetes. *Eurpian Journal of Clinical Investigation;* **43**:949–56.
Huang SS, Hassan AK, Choo KE, Ibrahim MI. and Davis TM. (2004). Prevalence and predictors of *Helicobacter pylori* infection in children and adults from the Penan ethnic minority of Malaysian Borneo. *American Journal of Tropical Medicine and Hygiene,* **71**(4): 444-450.
Kabir S. (2007). The current status of *Helicobacter* vaccines. A review. *Helicobacter,* **12**(2): 89-102.
Karilas P. (2003). GERD pathogenesis, pathophusiology and clinical manifestation, *Cleve Clinical journal of medicine*; **5(**70**)**:4019.
Kariya; S., Nishizaki; K., and Okano; M. (2014). Association between Helicobacter pylori and upper respiratory tract disease: Fact or fiction. *World J Gasteroenterol*; 20(6):1470 – 1484
Kikuchi S., Dore MP. (2005). Epidemiology of *Helicobacter pylori* infection. *Gastroenterology & Hepatology* **10**: 1-10.
Kosaka T., Sutton, P., Wilson J., Wolowczuk I., and Lee A. (2000). Therapeutic immunization against Helicobacter pylori infection in the absence of antibodies. *Immunology and Cell Biology*; **78**:28–30.
Leandro Liberato SV, Hernandez Galindo M, Torroba Alvarez L, Sanchez Miramon F and Leandro Ciriza SE. (2005). *Helicobacter pylori* infection in the child population in Spain: Prevalence, related factors and influence on growth. *Anales de Pediatria (Barc),* **63**(6): 489-494.
Lindholm, C., Quiding-Jarbrink M., Lonroth H., Hamlet A., and Svennerholm A.M. (1998). Local cytokine response in *Helicobacter pylori*-infected subjects. *Infection and Immunology;* **66**:5964–5971.
Logan, R. P. (1998). Urea breath tests in the management of *Helicobacter pylori* infection. *Gut*; **43**(Suppl. 1)**:**S47–S50.
Loy CT, Irwig LM, Katelaris PH, Talley NJ. (1996). Do commercial serological kits for Helicobacter pylori infection differ in accuracy? A meta-analysis. *American Journal of Gastroenterology*; **91**:1138-1144.
Malfertheiner P., Megraud F. and O'morain C.A. (2012). Management of *Helicobacter pylori* infection. *Gut;* **61**(5): 646-664.
Manal E. Kandil, Azza El Hamshary and Nahed AR (2007). Seroprevalence of *Helicobacter pylori* in juvenile rheumatoid arthritis and its relation to disease severity. *Journal of Medical Sciences,* **7**(5): 716-723.
Marnila, P., Rokka S., Rehnberg-Laiho L., Karkkainen P., Kosunen T.U., Rautelin H., Hanninen M.L., Syvaoja E.L., and Korhonen H. (2003). Prevention and suppression of Helicobacter felis infection in mice using colostral preparation with specific antibodies. *Helicobacter;* **8**:192–201.
McColl and Kenneth E.L. (2010). *Helicobacter pylori* infection. *New England Journal of Medicine*; **362**: 1597-1604.
Michetti P. and Suerbaum S. (2002). Helicobacter pylori infection, medical progress. *New England Journal of Medicine*; **347**(15):1175–86.

Midolo P and Marshall BJ. (2000). Accurate diagnosis of *Helicobacter pylori*: urease tests. *Gastroenterology Clinics of North America*; **29**: 871-878.
Mirghani YA, Salah AM and Fedial SS., (2002). *Detection biochemical and immunological characterization of H. pylori in Sudanese patients with gastro duodenal inflammation*. M.Sc. University of Khartoum, Sudan.
Mohammadi, M., Czinn S., Redline R., and Nedrud J. (1996). *Helicobacter* specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. *Journal of Immunology;* **156**:4729–4738.
Mubashir AK and Hani OG. (2007). *Helicobacter pylori* infection in asymptomatic subjects in Makkah, Saudi Arabia. *Journal of Pakistan Medical Association,* **57**(3): 114-116.
Mukherjee P, Chacko B, Singh T, Pawar G and Kaur H. (2005). Prevalence of *Helicobacter pylori* infection in children with recurrent abdominal pain. *Tropical Gastroenterology,* **26**(2): 102-104.
Nakashima S, Kakugawa T and Yura H. (2015). Identification of Helicobacter pylori VacA in human lung and its effects on lung cells. *Biochemical Biophysical Research Community;* **460**:721–6.
Nomura, A., Stemmermann G.N., Chyou P.H., Perez-Perez G.I., and Blaser M.J. (1994). *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. *Annals of Internal Medicine,* **120**:977–981.
Sanders SW. (1996) .Pathogenesis and treatment of acid peptic disorders: Comparison of proton pump inhibitors with other antiulcer agents. *Clinical Therapeutics*; **18**:2–35.
Siekmann, J.H., Allen, L.H., Watnik, M.R., Nestel, P., Neumann, C.G., Shoenfeld, Y., Peter, J.B., Patnik, M., Ansari, A.A., Coppel, R.L. and Gershwin, M.E. (2003). Titers of antibody to common pathogens: relation to food-based interventions in rural Kenyan schoolchildren. *The American Journal of Clinical Nutrition*, **77**(1):242-249.
Smythies, L. E., Waites K.B., Lindsey J.R., Harris P.R., Ghiara P., and Smith P.D. (2000). *Helicobacter pylori*-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN-gamma, gene-deficient mice. *Journal of Immunology;* **165**:1022–1029.
Spechler SJ. (1992). Epidemiology and natural history of gastroesophageal reflux disease. *Digestion*; **51**(suppl 1):24–29.
Szarka LA, Locke GR. (1999). Practical pointers for grappling with GERD. *Postgraduate Medical Journal*; **105**:88–106.
Storr M, Meining A, Allescher HD. (2000). Pathophysiology and pharmacological treatment of gastroesophageal reflux disease. *Digestive Diseases and Science*; **18**:93–102.
Vaira D and Vakil N. (2001). Blood, urine, stool, breath, money, and Helicobacter pylori. *Gut*; **48**:287-289.
Versalovic J and Fox JG. (2003). Manual of Clinical Microbiology. (8th edn), Washington DC (1): 915-928.