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Exploration of gastric ulcer in the people of rural area of Peshawar/KPK through enzyme linked immunosorbent assay

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Abstract

Helicobacter pylori reside and grow in digestive tract of human and cause gastric ulcer which in extreme cases leads to gastric adinocarcinoma. People in most of the territories in Peshawar/Pakistan believe that *H. pylori* is due to different life style habits and different acidic food usage. Therefore to clear this contradiction and expose actual cause of this disease and to bring awareness among people, this study has been designed. *H. pylori* antibodies were determined using ELISA (Enzyme Linked Immunosorbent Assay), because this is an easy diagnostic approach and is low cost diagnostic test. This study was started from January 2016 to May 2016 and Blood sample was collected from general population of a rural area of Peshawar KPK. In this aspect we collected blood samples from 80 subjects who had stomach problem and also from 6 normal individuals as control. Gender and age base study was done in this research. Out of 80 patients, 60 patients showed positive results for *H. pylori*, 10 were negative and 10 showed equivocal (nor clearly positive neither negative). Moreover, female have shown more positivity as compared to male and 40-50 age group subjects were more affected. *H. pylori* infection is present in more than 50% of the population and bacteria are involved in causing it not people's life style. Secondly huge population involvement reflects unawareness of the problem concerned.

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Introduction

H. pylori is a gram negative microaerophilic spiral shaped bacteria belongs to division eubacteria. This is motile bacteria which colonizes human stomach lining and causes sores in stomach called ulcer. Infection caused by *H. pylori* is most common infection worldwide (kanbay *et al.*, 2005). This infection is a major cause of gastric carcinoma which is the 2nd most fatal cancer to be diagnosed all around the world (Schistosomes, 1994).

Symptoms of *H. pylori* infection include heart burn, vomiting, weight loss, nausea, and hematomesis. Until 1980, people thought that main causes responsible for causing gastric ulcer are spicy foods, stress, increased secretion of acid in stomach and other life style habits such as alcohol consumption and smoking etc. But in 1983 when Warren and Marshal discovered *H. pylori,* this concept was then changed (Marshal *et al.*, 1997). and all people agreed in the fact that causative agent of stomach ulcer is *H. pylori* not the life style habits, although these habits only increases the severity of the infection. Mode of transmission of *H. pylori* infection is still unknown but most probable routes of transmission are fecal to oral and oral to oral routes (Dowsett *et al.*, 2003). Crowded living areas (Mendall *et al.*, 1992). Closed person to person contact and poor personal and social hygienic conditions and contaminated water are the main factors of transmission of infection. Patients having *H. pylori* infection has six fold increased risk of developing gastric carcinoma (Hartgrink *et al.*, 2009).

Different techniques are there for the detection of *H. pylori* which is divided into two main categories i.e. invasive or direct and noninvasive or indirect diagnostic methods. Invasive techniques were the first to be used in the diagnosis of *H. pylori* (Francis Me graud). Invasive method is conventional method of detection and it involves rapid urease test (RUT), culturing the *H. pylori* and its examination under microscope using biopsy samples (Mégraud F, 1997 or Talley *et al.*, 1991 or Oksanen *et al.*, 1998) and these methods require endoscopic procedure. Noninvasive detection of *H. pylori* involves serum and saliva testing for presence of pathogen, stool antigen test and Urea breath test from breath sample (Reilly *et al.*, 1997), because *H. pylori* has urease enzyme which convert urea to CO_2. Each test has its own advantages and limitations.

This study was carried out to expose the causative agent for gastric ulcer. As no such studies have been done in Khyber Pakhtunkhwa to explore the etiological agents and to eliminate the discrepancies about the causes of gastric ulcer. Ai m of this research study our choice of diagnosing *H.pylori* infection is ELISA because this is a rapid, easy and low cost method (Dhar *et al.*, 1998) and easily accessible to people having low resources. To create awareness among the general population of the rural areas who have misconception about stomach ulcer and it causes, and the primary diagnosis of h pylori through simple diagnostic test which is less harmful and to find out which gender and which age group people are most effected with h pylori.

Materials and methods

Patient population

A total of 80 patients (general population) having gastrointestinal symptoms were selected from a rural area of Peshawar (52 female and 28 male, age ranges from 11-65 years, mean age 40 years). Along with these 6 healthy people were also selected as a control group.

Inclusive Criteria

All these patients have continuous symptoms of gastric problems and they have no awareness of the role of *H. pylori* involved in causing it.

Blood collection

4ml blood was collected from each subject with informed consent in plain sterile tube (red cap). These blood samples were centrifuged at 4000 rpm for 15 minutes to isolate the serum. This serum was stored at – 4˚C for further use.

Test Protocol

Serum IgG antibodies of *H. pylori* were detected through commercial ELISA kit (Micro LISA kit Catalog number 10207). Diagnostic test was performed according to the kit manufacturer's protocol.

Briefly, before performing the test all the reagents of the kit and serum samples were taken to the room temperature. Test sample dilutions were prepared and these were coated in the wells of kit, these steps were followed by incubation and washing and finally its absorbance was measured at 450nm in micro titer reader. Absorbance value of each sample is directly proportional to the concentration of *H. pylori*.

Results

In this study ELISA assay was used for the detection of *H. pylori* infection in gastro intestinal patients. A total of 80 Patients from different age groups were selected, Mean age was 40 years, minimum age was 11 years and maximum age was 65 years. Age group and test results are shown in table 1. Out of 80 patients 60 (75%) patients showed positive results for *H. pylori*, 10 (12.5%) were negative and 10 (12.5%) showed equivocal (neither clearly positive nor negative) results (Table 1). This study was based on age factor and gender factor. Out of these 80 patients 52 (65%) were female and 28 (35%) were male. Out of these 52 (65%) female, 41 (51.2%) showed positive result for *H. pylori* 5 (6.2%) showed negative and 6 (7.4%) were equivocal (table 2). In female having age group of 31–40, more seropositive patients of *H. pylori* were seen. In 28 (35%) males, 19 (23.7%) showed seropositivity for *H. pylori*, 5 (6.2%) were negative and 4 (4.9%) were equivocal (table 3). In age group 41–50 there are more individuals infected with *H. pylori* as compare to other age groups (Table 1). Highest concentrations of *H. pylori* was seen in age group of 21-30 and its absorbance values were 34.31nm and 34.21nm at micro titer reader and the patients were 27 years old and 25 years old females respectively. In female age group 31–40 and 41–50 there were more number of seropositive patients as compare to other age groups (Table 2). In male age group 41-50, more patients showed positive result for *H. pylori* (Table 2). It means in age group 41-50 there are more seropositive patients. 6 healthy patients were taken as negative control and their ELISA test results were negative (Table 4).

Table 1. Helicobacter pylori positive and negative subjects.

Age group	Number of patients	positive	negative	Equivocal
11-20	12	9	1	2
21-30	18	13	2	3
31-40	17	12	3	3
41-50	20	18	1	1
51-60	10	6	3	1
61-70	3	2	-	1
Total	80	60	10	10

Values: Below 12 nm = Negative , 12 nm – 20 nm = Equivocal , Above 20 nm = Positive

Table 2. Helicobacter pylori in women.

Age group	Number of patients	positive	negative	Equivocal
11-20	1	4	-	2
21-30	11	9	2	-
31-40	13	10	1	2
41-50	12	10	1	1
51-60	7	5	1	1
61-65	2	3	-	-
Total	52	41	5	6

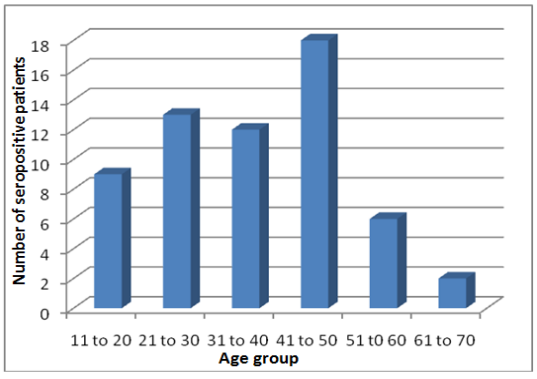
Values: Below 12 nm = Negative , 12 nm – 20 nm = Equivocal , Above 20 nm = Positive

Table 3. Helicobacter pylori in men.

Age group	Number of patients	positive	negative	Equivocal
11-20	9	4	2	-
21-30	7	4	1	2
31-40	4	3	-	1
41-50	8	6	2	-
51-60	3	2	-	1
Total	28	19	5	4

Table 4. Detail of normal Control subjects

Control	Age	Result	Value
Control 1	30	Negative	8 nm
Control 2	35	Negative	7 nm
Control 3	40	Negative	10 nm
Control 4	45	Negative	6 nm
Control 5	52	Negative	6 nm
Control 6	55	Negative	11 nm
Total	6		



Discussion

H. pylori is a human gastric pathogen which colonizes human gastric mucosa of 50% of the world's population and may survive for the life time in the human stomach and cause infectious diseases such as gastric ulcer, peptic ulcer, gastritis and gastric adenocarcinoma. Gastric adenocarcinoma is the 2nd most fatal cancer to be diagnosed worldwide. In 2008, globally, 660,000 patients of gastric cancer were estimated and its main reason was *H. pylori* (De Martel *et al.*, 2008). Studies from different countries have been shown that in developed countries 50% population and in developing countries 90% population is positive for *H. pylori* infection (Kleanthous *et al.*, 1998). In Pakistan *H. pylori* infection is very common, where 90% adult population (Ashraf *et al.*, 2005), 33% children (exposure rate) are infected with *H. pylori* infection (Qureshi *et al.*, 1996).

There are different ways for detection of *H. pylori*, which are divided into invasive and noninvasive techniques. These both techniques have their own advantages and limitations. Invasive test includes Rapid urease test, histopathological examination by using biopsy tissue and all these procedures requires endoscopic procedures which is a painful technique for patients and expensive as well.

In endoscopic procedure there are chances of false negative results because in stomach there is Patchy infection (Warren *et al.*, 1983 or Bayerdörffer *et al.*, 1998) and it may be possible that this region have no infection from where biopsy is taken. Noninvasive test include serological examination of blood and Urea breath test is of more importance but its limitation is that its equipments and reagents are very expensive (Bazzoli *et al.*, 1997).

In this study we diagnosed *H. pylori* through serological examination using commercial ELISA kit, which is a rapid, inexpensive and accurate method for detection of *H. pylori*, which supports the previous studies (Evans *et al.*, 1989 or Newell *et al.*, 1998 or Perez-perez *et al.*, 1988) and this is an excellent technique for determining the epidemiology of *H. pylori* (Goodwin *et al.*, 1987 or Hirschl *et al.*, 1998).

Our study consisted of 80 gastro intestinal patients from general population of a rural area of Peshawar having continuous symptoms of gastric problems (52 female and 28 male) in these 80 patients 60 (75%) gave positive results, 10 (12.5%) gave negative and 10 (12.5%) were equivocal. In these 80 patients 52 (65%) were female and out of that 41 (51.2%) were seropositive while out of total 28 (35%) male, only 19 (23.7%) were seropositive for *H. pylori* infection. In female age group of 31–40 and in male age group of 41–50 more seropositive patients of *H. pylori* were seen. However in overall result patients of age group 41-50 were more infected with *H. pylori.* As a common rule Prevalence of *H. pylori* is directly proportional to the age (Hunt *et al.*, 2011) as age increases risk of infection increases. In a previous report from Mexico, it has been shown that the risk of *H. pylori* infection increases with age (Torres *et al.*, 1998) and it is also proved by our study as there are more patients infected with *H. pylori* in the age group of 40- 50 (Table 1). Other studies also highlighted the same factor (Jones *et al.*, 1986). In a previous report from Pakistan (Khyber pakhtunkhwa) it is shown that the prevalence rate of peptic ulcer is more in the age group of 21-30 and 31-40 because patients of these age group were smokers and users of NSAID's for treatment of different diseases and they also mentioned that prevalence of *H. pylori* infection is more in patients of 21-50 years old (Ali *et al.*, 2013).

It is found from our study that there are more females infected with *H pylori* than male as in our study 41 (51%) females are seropositive for *H. pylori* and 19 (23%) male and this is also proved by some previous studies that females are more likely to infect with *H. pylori* (Torres *et al.*, 1998), so we are in agreement with this statement. In some other studies gender is not taken as a factor for infection (Smoak *et al.*, 1994) and another research showed that risk factor of peptic ulcer and gastric ulcer in male is 11–20% and in female is 8-11% (Newton *et al.*, 2008). A study by Arif valliani *et al* (Valliani *et al.*, 2013), suggested that prevalence of *H. pylori* infection is more in male than female but we are in disagreements with it because there may be some.

Other factors involved such as smoking and alcohol consumption in increasing risk factors of infection in male. Some research studies have shown that socioeconomic value and demographic factors has great influence on *H. pylori* infection (Graham *et al*., 1991 or Sitas *et al*., 1991 or Forman *et al*., 1993) and our research is in agreement with it as in our study sample size is selected from rural area of Peshawar where there is low socioeconomic value and low level of literacy and people are living in unhygienic conditions therefore these people have more risk of infection, so people living in such conditions are more prone to infected with *H. pylori* in a previous study from Mexico these factors were seen only in patients younger than 40 years and they mentioned in their study that in Mexico before 1950 (as they started their study in 1987) low socioeconomic value, overcrowding and low education level were not risk factors for causing *H. pylori* infection [28].

Conclusion

It is concluded that population of study has high degree of *H. Pylori* infection 75% and the causative agent is bacteria not the life style habits. It is further confirmed that ELISA is one of the simple, rapid and accurate method for diagnosis of *H. pylori* and risk factors of *H. pylori* increases with age and with low socioeconomic values.

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