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SPECIFIC IGE FOR *H. PYLORI* AND SOME IMMUNE FACTORS IN PATIENTS WITH DYSPEPSIA

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ABSTRACT
T helper type 1 (Th1) specific immune responses in protection from *H. pylori* challenge was understood. It is expected that Th2 immune responses are required for protection against extracellular bacteria, such as *H. pylori*. This study aimed to show if *Helicobacter pylori* infection accompanied with IgE or not to reach final recommendation for the necessity for Anti IgE treatment in *H. pylori* infections. One hundred seven (107) adult patients from both genders were attending Gastro Endoscopy Unit at Ramadi Teaching Hospital to undergo selective OGD from December 2012 to May 2013. Multiple mucosal biopsy specimens were taken for rapid urease test (RUT) to detect *Helicobacter pylori* in tissue samples. After endoscopy, blood specimen was taken from each patient to be used for serological tests including; IgG, IgM, Total IgE, & *H. Pylori* Specific IgE antibody by ELISA. Present study showed that patients with serum total IgE positivity had a significant correlation with IgG specific for *H. pylori* and rapid urease test RUT of *H. pylori* infection, especially in younger adults. Findings confirmed that a significant relationship between *H. pylori* infection (RUT) with positive IgE specific *for H. pylori* antigen. It was found that infection with *H. pylori* is accompanied with increased Total IgE and positive IgE specific for *H. pylori* in some patients . So it is necessary to recommend anti IgE treatment for some cases of H. *pylori* infections in future.

KEY WORDS: T helper, *Helicobacter pylori*, OGD, RUT, ELISA, IgE.

INTRODUCTION

Helicobacter pylori is the most important etiological factor responsible for chronic gastritis, duodenal ulcer, gastric ulcer (Sainz *et al*., 1999, Graham *et al*., 2004, Sgouros and Bergele, 2006) and it has an important role in the pathogenesis of gastric cancer (Muler *et al*., 2007). *Helicobacter pylori* colonize the stomach, survive acidic pH of the lumen and burrow into the mucus to reach stomach's epithelial cell layer (Ottemann & Lowenthal, 2002; Sgouros & Bergele, 2006). Bacterial urease activity is clinically important because it forms the basis for several invasive and noninvasive diagnostic tests (Amieva and El-Omar, 2008). *Helicobacter pylorus was* reported to induce vigorous humeral and cellular immune responses. White blood cell, neutrophil or lymphocyte counts were reported to increase in *Helicobacter pylori*-infected patients (Kondo *et al*., 2004). It is expected that Th2 immune responses are required for protection against extracellular bacteria, such as *Helicobacter pylori*. However, (Taylora *et al*. (2008) suggested that Th1 immunity is required for protection. Dendritic cells, in turn, activate T cells in different ways, being capable of inducing either a Th1, Th2/ regulatory T cell or a Th17 response by generation of interleukin IL-12, IL-10, or IL-23, respectively (Banchereau *et al*., 2000). Bacterial infections had been implicated in allergic reactions (Oehiling, 1999; Lafi, 2004; Schaub, 2006). Urticaria patients might develop specific IgE antibodies to *Helicobacter pylori*, an attractive explanation that still requires confirmation (Bruscky *et al*., 2013). This study aimed to show if *Helicobacter pylori* infection accompanied with increased IgE reaction mediators or not to reach final recommendation for the necessity for Anti IgE treatment in *Helicobacter pylori* infections.

PATIENTS & METHODS

Total of (107) adult patients from both genders were attending Gastro Endoscopy Unit at Ramadi Teaching Hospital to undergo selective Esophageal Gastro Duodeno Scopy (OGD) from December 2012 to May 2013. They were suffering from clinical manifestations of gastric dyspepsia. The clinical diagnosis of patients was performed by senior gastroenterologist.

According to the exclusion criteria, a total of 107 patients examined in the Gastro Endoscopy Unit within age range between (18-75 years). Patients were excluded from the study if they were:

1-Taking a proton pump inhibitors. 2-Taking H2-blockers.
2-Taking non-steroidal anti-inflammatory drugs (NSAID).
3- Patients who were under long course of antibacterial therapy.

Specimens

A-Biopsy Specimens

Each biopsy specimen was placed in urea medium for rapid urease test. Plastic slides were incubated at room temperature (15-30˚C) aerobically, and observed for 15-20 minutes and again at one, three, and six hours of incubation for the development of a pink-red or red-violet color. Negative specimens were reincubated for up to 20 hours (Lopez *et al*., 2014).

B-Blood Specimens

After endoscopy, three (3) ml of venous blood specimen was taken from each patient. Serum was pooled from each blood specimen by centrifugation for 3 min at 3000rpm. Serum specimens were kept frozen at (-20 ^{0}C) to be used for serological tests.

EISA Test for IgG, IgM and Total IgE

ELISA test was used for IgG, IgM specific for ***H. pylori*** and total IgE using special DRG (USA) Elisa kit for each test. Methods for ELISA test were followed as described by Manufacturer Company.

***H. Pylori* Specific IgE**

A- Preparation of bacterial antigen discs

Blotting papers (Whatman) number 4 was used for paper discs using paper puncher to get 0.5cm diameter discs. These discs were sterilized by UV light illuminator for overnight. Sterility test was done for sample from these discs. Sixty three (63) sterile discs were impregnated with 1.2ml from Helicobacter *pylori* antigen (Jena Bioscience, Germany) to get antigen concentration 17 microgram for each disc. Discs were dried in incubator at 37 ^{0}C for 30 minutes and kept in sterile test tube at 4 ^{0}C to be used for IgE specific ELISA test within 3 days (Lafi, 2004).

***B-Helicobacter pylori* specific IgE ELISA test**

An enzyme linked immunosorbent assay (ELISA) method was used for the specific IgE determination in sera of patients using *H. pylori* antigen impregnated discs as described by (Lafi, 2004)

Statistical Analysis

All data were analyzed using the SPSS statistical program (Statistical Package for the Social Science) Version 14.0. Statistical significance was taken with P value <0.005.

RESULTS

Rapid Urease Test (RUT)

Positive result of urease test showed pink color in the presence of *Helicobacter pylori*. During one minute to 1 hour, seventy three specimens 73 (68.2) were showing positive result, majority of them within(18-30) and (31-50) years old age groups (Fig- 1).

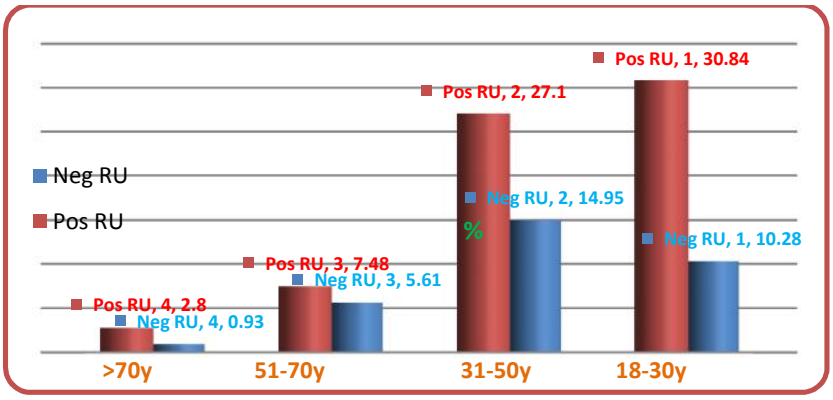

FIGURE 1: Distribution of Helicobacter *pylori* positive and negative rapid urease

***Helicobacter pylori* IgG ELISA Test**

Positive IgG specific for *H. pylori* was detected in 102 (95.33%) serum samples (Fig-2). All IgG positive patients were showing positive urease test. *Helicobacter pylori* positive patients showed significant ($p<0.001$) higher titers of anti -*Helicobacter pylori* IgG (1.840 ± 0.421) than *Helicobacter pylori* negative individuals.

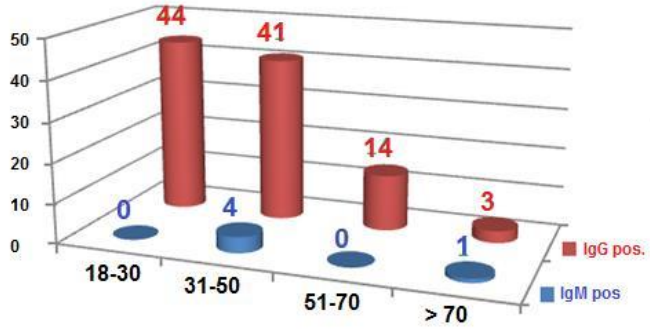

FIGURE 2: Distribution of ELIS A: IgM positive and IgG positive results

***Helicobacter pylori* IgM**: Only five (5) (4.7%) patients were showing positive IgM against Helicobacter *pylori*, four of them were included in 31-50 years old age group (Fig-2).

Total Immunoglobulin IgE: Out of 107 patients cases of dyspepsia, eighteen 18 (23.1%) patients were showing positive total IgE titer in serum (Table -1)**.**

TABLE 1: Total IgE in serum of patients

H. Pylori	age groups									
Total IgE	18-30		31-50		51-70		>70		Total	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Negative	22	28.21%	26	33.33%	9	11.54%	3	3.85%	60	76.92%
	Σ = 48		80%		Σ = 12		20%			
Positive	7	8.97%	8	10.26%	2	2.56%	1	1.28%	18	23.08%
	Σ = 15		83%		Σ = 3		17%			
Total	29	37.18%	34	43.59%	11	14.10%	4	5.13%	78	100.00%

IgE specific for *Helicobacter pylori* in sera of patients

Positive IgE specific for *Helicobacter pylori* was found in sera of thirteen 13 (25.5%) patients. All of them were showing positive IgM and IgG specific for *Helicobacter pylori* and rapid urease test (Table-2, Figure -3).

TABLE 2: IgE Specific for *H. Pylori* in patients

H. Pylori	Age groups									
Specific IgE	18-30		31-50		51-70		>70		Total	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Negative	21	41.18%	13	25.49%	4	7.84%	0	0.00%	38	74.51%
	Σ = 34		90%		Σ = 4		10%			
Positive	6	11.76%	4	7.84%	2	3.92%	1	1.96%	13	25.49%
	Σ = 10		77%		Σ = 3		23%			
Total	27	52.94%	17	33.33%	6	11.76%	1	1.96%	51	100.00%

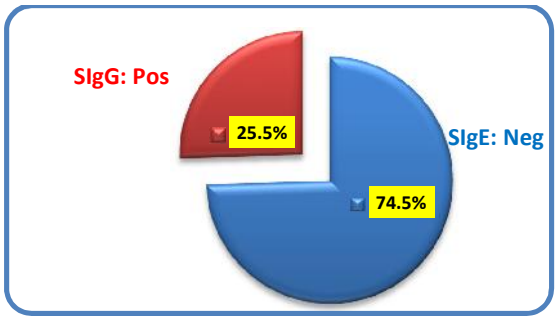

FIGURE 3: Distribution of *H. pylori* Specific IgE

In this study, patients with serum total IgE positivity had a significant correlation with IgG (*p*=0.001) and RUT (0.006) of *H. pylori* infection, especially in younger adults (83% from all positive 18 patients). A significant correlation was fond between Specific *H. pylori* IgE and RUT (p=0.008), in younger adults (77% from all positive 13 patients (Fig-4).

								Smk
							SIgE	**0.132**
						TIgE	**0.609**	**0.331**
					IgG	**0.001***	**0.126**	**0.720**
				IgM	**-**	**0.370**	**0.712**	**0.893**
				0.346	**0.003***	**0.002***	**0.757**	**0.481**
		RU		**0.165**	**0.159**	**0.006***	**0.008***	**0.107**
	Sex	**0.727**		**0.163**	**0.588**	**0.324**	**0.300**	**-**
Age	**0.163**	**0.551**		**0.039***	**0.776**	**0.789**	**0.241**	**-**

**Correlation is significant at the 0.05 level*

FIGURE 4: Correlation coefficient between parameters which used in present study

DISCUSSION

The presence of specific Low IgM positive ratio in this study was ought to delay of patients to consult physicians, this delay shifts majority of cases to secondary immune response stage through which titers of IgG increase while IgM titers decrease (Male *et al*., 2006). *H. pylori*- directed IgG antibodies have shown excellent correlation with the presence of *H. pylori* enteric infection, this was also indicated by (Megraud & Lehours, 2007). A negative value in RUT depends on non homogeneous distribution of the microorganism in the stomach and this situation is overcome by use of (3-5) specimens for the same patient (Lim *et al*., 2004). So we minimize the specimen error and this explains the 2 (1.9%) patients which gave negative result. Increased positive results of *H. pylori* IgG in patients within age groups (18-30) (31-50) years were also mentioned by Al-Marsoumiy & Jabbo (2013), they found that (79.2%) of patients in Baghdad area were

under 50 years , while Hasan (2011) from Erbil detected that increase of infection in age less than 50 years (76%). These findings agreed with the findings of Vilaichone *et al.* (2013) in Thiland who found the more infection in patients below 50 years old (76%) than in older patients (24%). this can be explained that prevalence of *H. pylori* infection increases with psychosomatic in young patients which affected with socio-economic status and habits of individual. In spite of above mentioned results, not all positive total IgE patients were showing positive IgE specific for *H. pylori.* This can be explained due to hypersensitivity of some patients to other allergens like environmental and food allergens. Exposure to chronic infections, such as *H. pylori*, in early life is necessary for the normal of immune response, so as to achieve a balance between T-helper type 1 (Th1; protective immunity) and T-helper type 2 (Th2; immunity for allergic diseases) cytokine responses. Particular allergic conditions have been increasing because of Th1- and Th2-type immune response imbalances due to modern lifestyles (Schaub *et al*., 2006). Using an ELISA technique (Aceti *et al*., 1991) detected specific *Helicobacter pylori* IgE in 69% of the patients with infection, and histamine release with *H. pylori* surface components in 84% of them. Other studies suggested a relationship between *H. pylori* infection and allergic disease (Imamura *et al*., 2010; Arnold *et al*., 2011 and Zhou *et al*., 2013). In conclusion in this study findings indicated that;

1- There was a significant relationship between results of tests for *H pylori* infection, (IgG & RUT).
2- Detection of Th2 related mediators, Total IgE and IgE specific for *H. pylori* antigen.
3- A significant relationship between (RUT) with IgE specific *H. pylori* antigen in *H. pylori* infection, so we recommend necessity for anti IgE treatment in case of *H. pylori* infections.

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