Systemic Humoral Anti Helicobacter pylori Immune Response in Dyspeptic Patients

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ABSTRACT:

BACKGROUND:

Helicobacter pylori (HP) cause a continuous gastric inflammation in virtually all infected persons. It induces a vigorous systemic and mucosal humoral and cellular immune response. In spite of these responses, the vast majority of infected hosts are unable to clear the infection and it persists for decades.

OBJECTIVE:

Determination the humoral immune response to HP infection in dyspeptic patients.

PATIENTS AND METHODS:

The immunological serological tests were evaluated in 80 dyspeptic patients divided into two groups: (HP +) and (HP-). Levels of specific HP IgG antibodies were determined through a specific immunological non invasive Enzyme Linked Immuno Sorbent Assay (ELISA) test from Biohit PIC, Helsinki, Finland. Immunoglobulin levels and complement were done (IgG, IgA, IgM, C3 and C4) using single radial immune diffusion (BioMaghreb-Tunis).

RESULT:

About 62.5 % of dyspeptic patients had HP + infection. There was no significant differences between two groups in the levels of (IgG, IgA, IgM, C3 and C4) and most of them were within normal values.

CONCLUSION:

Humoral immune response had an important role in the control and limits the inflammation of gastric mucosa.

KEY WARDS: helicobacter pylori, immunoglobulin, complement.

INTRODUCTION:

Helicobacter pylori (HP) is a gram negative bacteria which colonize gastric epithelium [(1)]. It inhabits about half world population and persists in the human stomach for decades or for entire life time [(2)]. HP induced gastric inflammation without any symptoms [(3)]. It is also a recognized cause of dyspepsia , chronic gastritis , peptic ulcer, gastric carcinoma and mucosa associated lymphoid tissue(MALT) [(4)].

Once individual acquired the infection, HP elicits a strong local and systemic humoral and cellular immune response, but it is not able to eliminate this bacteria [(5)].It is well known that abnormal immune response may play an important role in HP related gastropathy.

The immune response to HP include many factors produced by gastric mucosa that limit the proliferation of bacteria like gastric acidity, lactoferrin, beta – defensin [(6)] and Toll-like receptors-2 that presents on the surface of gastric epithelial cells and can recognize pathogen[(7)]. If binding of HP to epithelial cells takes place andinvasion of gastric epithelial barrier occur, the alternative pathway of the complement was activated, so measurement of complement components like C3 and C4 is important to detect if there is any abnormality in this pathway. Then invasion bacteria encounter macrophages and neutrophils which is the first defense mechanism. Since most HP localize within the gastric mucosal and do not invade gastric tissues, so contact with these phagocytic cells occur due to disruption in the gastric epithelial barrier.

The enemy HP possesses many mechanisms to over come these first innate host defense mechanisms like urease , flagella, Cag A, Vac, outer membrane proteins (BabA, SabA, AlpA and Hop2) [(8)]. Once Hp attaches to epithelial cells, it influences the development of gastric mucosal inflammation and infiltration of granulocytes, lymphocytes (T-cells CD3+CD4+) that bears TCR(αβ+), increased of CD4/CD8 ratio, B-cells and plasma cells[(9)]. Lastly production of antibodies

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to bacterial components were produced specific to HP GagA strain like IgG that had been detected at high titers both in the mucosa and in the sera of infected patients [10]. A humoral immune response to HP like IgG, IgM and IgA were elicited in nearly all HP infected humans [5]. In addition to that, production of autoantibodies to host cells and finally parietal cells will be lost (11).

So this study try to investigate the effect of HP on the humoral immune response by measuring serum levels of IgG, IgA, IgM, C3 and C4 in the sera of dyspeptic patients infected with HP bacteria.

PATIENTS AND METHODS:

A total of eighty dyspeptic patients were integrated in this study who consulted private clinic in the period between May 2005 to August 2008. Those with alarm symptoms were excluded like anaemia, melaena, haematemesis and dysphagia. Among them fourty were female and the rest were male. All of them were resided in Baghdad province were investigated by the Central Public Health Laboratory – Immunological Department in Baghdad.

Those patients were subjected to investigate the presence of antibody to Gag A HP strain IgG Ab.

The serum of those patients were tested for IgG, gA, IgM, C3 and C4.

At the beginning blood was sampled from ten hours fasting patients. Serum was separated and used for (Enzyme Linked Immuno Sorbent Assay) –ELISA- from Biohit PIC diagnostic, Helsinki, Finland for anti HP IgG antibodies. Then serum was used for single radial immune diffusion for detection the levels of IgG, IgM, IgA, C3 and C4 (BioMaghreb-Tunis).

STATESTICAL ANALYSIS:

Data were presented as percentages and statistical analysis was evaluated using student t-test.

RESULT:

A total of eighty dyspeptic patients. Forty of them were female and the rest were male. Their age ranged from eighteen to sixty-two years old with a mean age was (34.45).

Their age distribution was shown in table-1-. The highest percentage of dyspepsia was in (20-29 years).

Table 1: Age distribution of dyspeptic patients

Age distribution in years	Dyspeptic patients No.	%
(10-19)	4	5
(20-29)	28	35
(30-39)	20	25
(40-49)	14	17.5
(50-59)	10	12.5
(60-69)	4	5
Total	80	100

Those patients were subjected to anti HP CagA IgG antibodies which is specific for diagnosis HP infection because it was specific to CagA antigen, as shown in (table-2-) 62.5 % were HP + which is significantly difference from HP negative group (<0.05).

Table 2: Prevalence of HP IgG Abs in dyspeptic patients.

Title	HP+ IgG >38EIU	HP- IgG <38EIU	Total
Number of patients	50 *	30	80
% of patients	62.5 *	37.5	100

*p<0.05 (Normal value < 38 EIU)

When testing immunoglobulins levels IgG, IgM and IgA in the sera of HP + patients , it had been found that most of them in the normal levels which is not significantly different from HP- group (table-3-4-5-) .

While C3 and C4 were also in the normal range and significantly not difference from HP- group (table-6-7-).

Table 3: Numbers and Percentages of serum IgG level in the blood.

s.IgG level mg/dl	HP+		HP-	
	No.	%	No.	%
Normal (600-1650)	47(1)	94	23	76.6
More than 1650	3 (1)	6	7	23.3
Less than 600	0	0	0	0
Total	50		30	

(1) not significance

Table 4 :Numbers and Percentages of serum IgA level in the blood.

s.IgA level mg/dl	HP+		HP-	
	No.	%	No.	%
Normal (90-400)	50 (1)	100	30	100
More than 400	0	0	0	0
Less than 90	0	0	0	0
Total	50		30	

(1) not significance

Table 5: Numbers and Percentages of serum IgM level in the blood.

s.IgM level mg/dl	HP+		HP-	
	No.	%	No.	%
Normal (75-300)	49 (1)	98	27	90
More than 300	1 (1)	2	3	10
Less than 75	0	0	0	0
Total	50		30	

(1): Not significance

Table 6: Numbers and Percentages of serum C3 level in the blood.

s.C3 level mg/dl	HP+		HP-	
	No.	%	No.	%
Normal (80-160)	38 (1)	76	24	80
More than 160	9 (1)	18	3	10
Less than 80	3 (1)	6	3	10
Total	50		30	

(1)not significance

Table 7: Numbers and Percentages of serum C4 level in the blood.

s.C4 level mg/dl	HP+		HP-	
	No.	%	No.	%
Normal (20-40)	29 (1)	58	21	70
More than 40	16 (1)	32	6	20
Less than 20	5 (1)	10	3	10
Total	50		30	

(1)not significance

DISCUSSION:

Helicobacter pylori is a microorganism able to stimulate robust inflammatory systemic and mucosal humoral immune responses [12].

This immune response may be an important factor for the course of infection and many immunoglobulins may be developed like IgG antibodies (AB) specific to cytotoxic –associated protein (CagA) using whole cell antigen, these Abs were increased in HP infected patients[13]. It had been found that isocitrate dehydrogenase of HP induces humoral immune response in infected patients [14].This study showed 62.5% of dyspeptic patients were infected with HP. Other reports mentioned 36% [15], 45% [16], 71.4% [17] of dyspeptic patients had HP infection.

Other Abs (IgG, IgA and IgM) that directed against different HP antigens had been detected at high titers both in inflamed gastric mucosa and sera of infected patients [18]. Patients included in this study, showed normal levels of these parameters which are not significantly difference from HP negative group . Other reports showed systemic increased in serum IgG, IgA and IgM [19.20]. This increase in Abs titers may be due to the reminiscence of HP infection resulting in a certain arrearage between serum antibodies and HP [21]. So decreasing in immunoglobulins levels indicating eradication of HP infection [22]. Thus, estimation of IgG, IgM and IgA levels are cheap and reliable means for monitoring this bacterial infection[23]. Patients in this study had levels within normal values because most of individuals might have life long exposed to this bacteria in the childhood period especially peak age distribution in the age between 20-29 years. So they acquired infection in childhood age between 2-16 years [24] that induce immunological tolerance which is a state of unresponsiveness to microbial antigen mediated by early exposure to antigen by oral ingestion of bacteria and persistence of this antigen in the host ending in a state of anergy [25]. Also, the possible mechanism for developing this tolerance ranging from (the deletion of Ag specific T-cells) to(immune devation), inducing a state of anergy by suppressing (T_{reg}) .This tolerance seems to be acquired during childhood period and mucosal immune system learned to tolerate HP colonization[26]. Other report demonstrated that 30-60% of children in developing countries acquired HP infection [27].

HP is an extracellular bacteria , once they are within the host , constantly exposed to humoral host defense mechanisms (complement and antibodies) to become prey for phagocytes[28].

Several lines of evidence suggests that complement system may have an important role in HP induced inflammation [29]. Mechanisms of complement activation via classical pathway by antiendotoxin Abs and alternative pathway activation by Abs directed to conserved proteins such as heat shock protein -70 [30]. In addition , HP produced urease enzyme resulting in production of ammonia that directly activate complement[31]. Also HP by its self directly activate the complement [32]. Results in this work demonstrated normal levels of C3 and C4 in most patients that is not significantly different from HP negative group while other study showed decreased in its levels [20] and others showed increased [33]. This differences in the results may be due to the effect of IL-10 and its level in the circulation because absence of IL-10 may lead to increased in complement production due to increased in the production of inflammatory cytokines (IL-1, Il-6, TNF-α and IFN-γ [34].So complement system had a pivotal role in the inflammatory response of gastric mucosa .

CONCLUSION:

Humoral immune response had an important role in the control and limits the inflammation of gastric mucosa.

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