THE ROLE OF *CAGA*$^+$ STRAINS IN THE IMMUNE RESPONSE IN *HELICOBACTER PYLORI* PATIENTS [+]

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Abstract:

The aim of this study was to elucidate the role of certain host factors (IL-17A and Treg), and bacterial factor (*CagA*) in the immunity against *Helicobacter pylori (Hp)* associated diseases. Sixty dyspeptic patients (as diagnosed by endoscopy and confirmed by histopathology) and thirty apparently healthy individuals were enrolled in this study. All subjects were evaluated for their serum IgG anti-*Hp*, IgG anti-*CagA*, and IL-17A, and blood Treg cells. The results reveal positive IgG anti-*Hp* in 92.5% of *Hp* patients among which 40.8% was *CagA*$^+$. The frequency and the mean titer of IL-17A as well as the Treg cell count were significantly high among the *Hp*-patients. This was true for the Treg: IL-17A ratio. However, no significant difference for such results was noticed between the *CagA*$^+$ and *CagA*$^-$ strains. Finally, the IgG anti-*Hp* titer was significantly higher in *CagA*$^+$ than *CagA*$^-$ patients for cases with gastritis and gastric ulcer. In conclusion, detection of IgG anti-*Hp* in dyspeptic patients is helpful in the diagnosis of *Hp*-associated diseases and avoidance of endoscopy and biopsy. Treg cell count and IL-17A level are both high in the *Hp* patients indicating that both play an impairment role of the host's immune mechanisms in eradication of the bacteria. *CagA*$^+$ strain of *Hp* is not a sufficient indicator for the prediction of the type and severity in *Hp*-associated disease, however, this strain type plays a role in the modulation of the immune response through induction of high titer of IgG anti-*Hp*.

دور سلالة (*CagA*$^+$) في تعديل الاستجابة المناعية في مرضى بكتريا اللولبية البوابية

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المستخلص:

استهدفت هذه الدراسة توضيح دور عوامل المضيف (IL-17A و Treg) والعامل البكتيري (*CagA*) في المناعة الموجهة ضد الأمراض المرتبطة ببكتريا اللولبية البوابية. ادخل في هذه الدراسة 60 مريضا مصابا بعسر الهضم (مشخصة بالناظور والخزعة النسيجية) و30 من الافراد الاصحاء. تم تقييم المستوى المصلي لأضداد اللولبية البوابية من نوع (IgG)، أضداد (*CagA*) من نوع (IgG) و مستوى ال (IL-17A)، وعدد ال (Treg cells). أظهرت الدراسة أن التفشي المصلي لأضداد اللولبية البوابية من نوع (IgG) كان بنسبة 92,5% في مرضى البكتريا البوابية منهم 40,8% اظهروا ايجابية مصلية لاضداد (*CagA*) مع وجود فرقا معنويا لهذه النسب مع مثيلاتها في مجاميع السيطرة. كان عدد مرضى اللولبية البوابية الذين كان مستوى أل (IL-17A) مرتفعا لديهم أكثر ممن كان ذلك

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المستوى منخفضا لديهم وكذلك الحال بالنسبة لمعلم أل (Treg) وهذا ينطبق أيضا على المعدل المعياري لهذين المعلمين عند إجراء المقارنة ما بين مرضى اللولبية البوابية ومجموعتي السيطرة. هذا الارتفاع لم يكن له أثرا ملحوظا عند إجراء المقارنة ما بين مرضى اللولبية البوابية من حملة السلالة الموجبة ($CagA^+$) او السالبة ($CagA^-$). وأخيرا فان الفرق كان ملحوظا عند مقارنة المعدل ألعياري لأضداد اللولبية البوابية نوع (IgG) ما بين السلالتين لهذه البكتريا حيث كان مرتفعا في أفراد أل ($CagA^+$) من أفراد أل($CagA^-$) والأمر كان متشابها بالنسبة لمرضى قرحة ألاثني عشري ولكن بدون فرقا معنويا. الاستنتاجات الخاصة بهذه الدراسة تدل على أن قياس أضداد اللولبية البوابية في مرضى عسر الهضم قد يكون مساعدا في تشخيص الأمراض المتعلقة بهذه البكتريا لتجنب إجراء الناظور أو الخزعة المعدية. ارتفاع معدلات المعلمين (IL-17A) و (Treg cells) في مرضى اللولبية البوابية يشير إلى الدور المعطل لهذين العاملين في ميكانيكية الاجتثاث المناعي لهذه البكتريا. لم يكن لوجود سلالة $CagA^+$ دورا كافيا للتنبؤ بطبيعة المرض وحدته ولكن نوع السلالة الخاصة المتعلقة بال ($CagA$) لهذه البكتريا يلعب دورا في تعديل الاستجابة المناعية من خلال استحداث معيارية عالية لأضداد اللولبية البوابية نوع (IgG).

Introduction:

Helicobacter pylori is one of the most common chronic bacterial infections in humans effecting more than half of the people in whole world, and is recognized as a major etiological factor for chronic active gastritis, gastric ulcers (GU), duodenal ulcers (DU), gastric mucosa-associated lymphoid tissue lymphomas and distal gastric cancer.

The ability of *H pylori* to cause disease is thought to attribute to complex interplay between host genetic factors, environmental and bacterial factors [1]. Elevated expressions of IL-17 have been recently reported in patients with peptic ulcers (PU) [2]. However, the factors involved in the control of IL-17 production in *H pylori*-associated gastritis are yet unclear except in one study which found that serum IL-17 expression was influenced by the cytotoxin associated gene A (*CagA*) factor among *H pylori* DU patients [3].

The role of $CD_4^+CD_{25}^+$ T regulatory cell (Treg) in preventing autoimmune diseases as well as in the suppression of tumor immunity, and induction of dominant transplantation tolerance is well established [4]. However, the role of these Treg cells in *H pylori* infection is poorly defined. In one study, it was concluded that $CD_4^+CD_{25}^+$ cells reduce immunopathology in *H pylori* infection, possibly by reducing the activation of IFN-γ producing CD_4^+ T cells, even at the expense of a higher *H pylori* load in the gastric mucosa [5]. In other study, it had been suggested that gastritis due to *H pylori* is associated with loss of immune-regulation and alteration of several cytokines and cell subsets and cannot be attributed to a single immune pathway [6].

Regarding bacterial factors, *H pylori* strains have been divided into types I and II. Type I strains express *CagA* ($CagA^+$) but type II strains do not express *CagA* ($CagA^-$) [7]. About 60 to 80% of *H. pylori* strains in the world can produce *CagA* [8] which has been identified as a marker of the *Cag* pathogenicity island (Cag PAI), a 40-kilo base pair (kb) deoxyribonucleic acid (DNA) region, which secretes *CagA* genome and *CagA* protein to blood of infected host and considers as a virulence marker of *H pylori* to induce more severe mucosal damages and inflammatory responses [9]. The active *CagA* induces antibodies, and hence the detection of the serum IgG to the *CagA* antigen has been reported to be a reliable marker of carrier of $CagA^+$ *H pylori* strains [10].

This study investigated the seroprevalence of IgG anti-*H pylori* antibodies, the association between the type of *H pylori* strains ($CagA^+$ or $CagA^-$) and the expression level of

IL-17 and cell count level of $CD_4^+CD_{25}^+$ Treg cells as well as the ratio balance between the mean of Treg cell count and the mean of IL-17A level in *H p*ylori-associated gastritis and peptic ulcers.

Materials and Methods:

Subjects: A total of 60 dyspeptic patients (40 males and 20 females with an age range of 18-80 years) attending the endoscopy unit at the Gastroenterology and Hepatology Teaching Hospital-Baghdad during the period from September 2012 to February 2013 were included in this study. Another group of 30 apparently healthy individuals (18 males and 12 females with an age range of 18-65 years) was included in this study as negative control group.

Endoscopy and histopathology: The dyspeptic patients were underwent oesophageo-gastroduodenoscopy (OGD) and gastric biopsy was taken for histopathology from the antrum, incisura, and gastric body. Sections of biopsy were processed and examined for histopathology findings according to the method previously described [11] and hence they were sub classified into *H pylori*-associated patients (53 patients) and non-*H pylori* associated patients (7 patients).

Serology and cytology: From each subject of the patients and controls, 6-8 ml of peripheral blood was collected. Peripheral blood $CD_4^+CD_{25}^+$ Treg cells were counted by flow cytometry (FC) from C4-BE6 Partec, Germany at a private specialist laboratory center using mouse anti-human CD_4 fluorescein isothiocyanate (FITC)-conjugated mab and mouse anti-human CD_{25} phycoerythrin (PE)-conjugated mab from US-Biological, USA. The FC data were analyzed with FCS Four Express software. Serology includes the detection and titration of IgG anti-*H pylori* (*H pylori*-ELISA Demeditec-Germany), IgG anti-*CagA* (*CagA*-ELISA, Cusabio, China), and IL-17 (anti-IL-17A ELISA, Cusabio, China) in the sera of patients and controls.

Statistical analysis: Statistical package for social sciences (SPSS) version 14 was used. Mean and standard deviation (SD) were used to assess difference between groups, chi square test was used to test frequency distribution of categorical variables within groups of the study. Differences were considered statistically significant at *p value* <0.05.

Results:

In this study and for the purpose of simplifying the terms, the following abbreviations were used: HPP (*H pylori*-associated patients), NHPP (non-*H pylori*-associated patients), NC (Negative control or healthy individuals), GS (Gastritis), GU (Gastric ulcer), DU (Duodenal ulcer), *Hp* (*H pylori*), S (significant), and NS (not significant). Table 1 shows that the vast majority of HPP were positive for IgG anti-*Hp* (92.5%) compared to NC (53.3%) and NHPP (28.6%) with a significant difference ($p<0.05$). Within the HPP group, no significant difference in this value was recorded between GS, GU, and DU patients. The Treg cell count exhibited a significant increased level in most subjects of the HPP (73.6%) and NHPP (71.4) groups compared to the NC (46.7%) group (Table 2). For IL17-A (Table 3), most of the HPP and NHPP subjects were with an elevated level (79.2% and 71.4% respectively) compared to the NC (53.3%) with a significant difference ($p<0.05$). However, no significant differences were noticed between GS, GU, and DU groups for this parameter.

Table1. The results of IgG anti-*Hp* in all study groups

Marker	Study groups		+ve № (%)	-ve № (%)	Mean titer	±SD	*p-value*
IgG anti-*Hp*	NC n=30		16(53.3)	14(46.7)	27.95	29.266	NCXNHPP <0.05 (S) HPPXNC <0.05 (S) HPPXNHPP <0.05 (S) Other comparisons are NS
	NHPP n=7		2(28.6)	5(71.4)	9.31	5.805	
	HPP n=53	GS n=28	26(92.9)	2(7.1)	43.12	31.022	
		GU n=8	8(100)	0(0)	63.56	26.946	
		DU n=17	15(88.2)	2(11.8)	57.31	41.943	
		Total n=53	49(92.5)	4(7.5)	50.76	34.777	

Table2. The results of Treg cell count in all study groups

Marker	Study groups		High № (%)	Normal or low № (%)	Mean count	±SD	*p-value*
Treg	NC n=30		14(46.7)	16(53.3)	13.56	8.026	NHPPXNC <0.05 (S) HPPXNHPP >0.05(NS) HPPXNC <0.05 (S) Other comparisons are NS
	NHPP n=7		5(71.4)	2(28.6)	19.72	9.70	
	HPP n=53	GS n=28	20(71.4)	8(28.6)	25.7	17.04	
		GU n=8	6(75)	2(25)	28	19.65	
		DU n=17	13(76.5)	4(23.5)	24.5	16.54	
		Total n=53	39(73.6)	14(26.4)	25.67	16.971	

Table3. The results of IL-17A in all study groups

Marker	Study groups		High № (%)	Normal or low № (%)	Mean conc.	±SD	*p-value*
IL-17A (pg/ml)	NC n=30		16(53.3)	14(46.7)	10.66	2.956	NHPPXNC <0.05 (S), HPPXNC <0.05 (S) HPPXNHPP >0.05(NS) Other comparisons are NS
	NHPP n=7		5(71.4)	2(28.6)	13.4	4.74	
	HPP n=53	GS n=28	23(82.1)	5(17.9)	15.2	9.31	
		GU n=8	7(87.5)	1(12.5)	12	1.82	
		DU n=17	12(70.6)	5(29.4)	12.9	4.00	
		Total n=53	42(79.2)	11(20.8)	13.97	7.225	

The ratio for the mean of Treg cell count: IL-17A serum level in different study groups is shown in Table 4. This ratio was higher for the total subjects of HPP group (1.8: 1) in comparison with NHPP group (1.4: 1) and NC group (1.2: 1). The same mean ratio profile was demonstrated for the subjected with elevated Treg cell count as the highest ratio was noticed within the HPP group compared to other groups.

Table 4. Mean counts of peripheral Treg cells and mean titers of serum IL-17A and their ratio in all study groups

HPP (n=53)								
Total (n=53)			Normal-Low Treg (n=14)			Elevated Treg (n=39)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL17A		Treg	IL17A		Treg	IL17A	
25.6	13.9	1.8: 1	9.9	11.7	0.8: 1	31.5	14.8	2.1: 1
NHPP (n=7)								
Total (n=7)			Normal-Low Treg (n=2)			Elevated Treg (n=5)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL17A		Treg	IL17A		Treg	IL17A	
19.7	13.3	1.4: 1	7.9	15	0.5: 1	24.4	12.7	1.9: 1
NC (n=30)								
Total (n=30)			Normal-Low Treg (n=16)			Elevated Treg (n=14)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL17A		Treg	IL17A		Treg	IL17A	
13.5	10.6	1.2: 1	6.9	9.7	0.7: 1	21.2	11.8	1.7: 1

Comparing the mean count of Treg cells and the mean level of IL-17A between the HPP subgroups (Table 5) reveals that the ratio between the two parameters was in its highest level in the GU group (3: 1) compared to the other two subgroups of HPP; GS (2: 1), and DU (2: 1).

Table 5. Mean counts of peripheral Treg cells and mean titers of serum IL-17A and their ratio in HPP groups

GS (n=28)								
Total (n=28)			Normal-Low Treg (n=8)			Elevated Treg (n=20)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL-17A		Treg	IL-17A		Treg	IL-17A	
25.7	15.2	1.7: 1	8.7	12.8	0.7: 1	32.5	16.2	2: 1
GU (n=8)								
Total (n=8)			Normal-Low Treg (n=2)			Elevated Treg (n=6)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL-17A		Treg	IL-17A		Treg	IL-17A	
27.9	11.9	2.3: 1	10.3	13.5	0.8: 1	33.8	11.4	3: 1
DU (n=17)								
Total (n=17)			Normal-Low Treg (n=4)			Elevated Treg (n=13)		
Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio	Mean		Treg:IL-17A mean ratio
Treg	IL-17A		Treg	IL-17A		Treg	IL-17A	
24.5	12.8	1.9: 1	10	8.1	1.2: 1	29	14.3	2: 1

The frequency of the *H pylori* strains ($CagA^+$ and $CagA^-$) in IgG anti-*Hp* positive subjects is listed in Table 6. The total frequency of $CagA^+$ strains was 37.3% in all study groups, 40.8% in HPP group, 50% in NHPP group, and 25% in NC group. The difference in frequency between $CagA^+$ and $CagA^-$ was significant in HPP and NC groups ($p<0.05$). The level of IL-17A was high for both $CagA^+$ and $CagA^-$ strains with no significant difference.

The difference in the frequency between high and low-normal level of IL-17A within each group for *CagA*$^{+}$ or *CagA*$^{-}$ strains separately was significant in HPP and NHPP groups and not-significant in the NC group. Similar results were noticed for the Treg cell count in all study groups. Table 7 illustrates the IL-17A level and Treg cell count according to *CagA* strain in IgG anti-*Hp* positive subjects within HPP groups. All results in this table were in a similar profile to that in Table 6. The difference in the positivity rate between all groups were not significant as they were ranging from 88.2% in DU group to 100% in GU group. The frequency of *CagA*$^{+}$ strains were always lower than *CagA*$^{-}$ strains in all groups with a total percents of 40.8% and 59.2% for *CagA*$^{+}$ and *CagA*$^{-}$ respectively. Within each group, the difference between *CagA*$^{+}$ and *CagA*$^{-}$ frequency was significant in GS and GU groups and non-significant in DU group. The level of IL-17A was generally high in all groups irrespective of being *CagA*$^{+}$ or *CagA*$^{-}$ and the difference in the frequency between subjects with high and low-normal level IL-17A was significant for all groups of HPP as well as for the total sum of the groups. The same results profile was obtained with the Treg cell count as it is clear in the table.

Table 6. IL-17A level and Treg cell count according to *CagA* strain classification of IgG anti-*Hp* positive subjects in all study groups

Positive IgG anti-*Hp*	*CagA* strains № (%)	IL-17A level № (%)		Treg cell count № (%)	
		High	Low-Normal	High	Low-Normal
HPP n=49/53 (92.5)	*CagA*$^{+}$ n=20/49(40.8)	15/20(75)	5/20(25)	13/20(65)	7/20(35)
	CagA^{-} n=29/49(59.2)	25/29(86.2)	4/29(13.8)	24/29(82.8)	5/29(17.2)
	Total n=49/49(100)	40/49(81.6)	9/49(18.4)	37/49(75.5)	12/49(24.5)
NHPP n=2/7 (28.6)	*CagA*$^{+}$ n=1/2(50)	1/1(100)	0/1(0)	1/1(100)	0/1(0)
	CagA^{-} n=1/2(50)	1/1(100)	0/1(0)	1/1(100)	0/1(0)
	Total n=2/2(100)	2/2(100)	0/2(0)	2/2 (100)	0/2(0)
NC n=16/30 (53.3)	*CagA*$^{+}$ n=4/16(25)	0/4(0)	4/4(100)	0/4(0)	4/4(100)
	CagA^{-} n=12/16(75)	8/12(66.7)	4/12(33.3)	6/12(50)	6/12(50)
	Total n=16/16(100)	8/16(50)	8/16(50)	6/16(37.5)	10/16(62.5)
Total n=67/90 (74.4)	*CagA*$^{+}$ n=25/67(37.3)	16/25(64)	9/25(36)	14/25(56)	11/25 (44)
	CagA^{-} n=42/67(62.7)	34/42(81)	8/42(19)	31/42(73.8)	11/42(26.2)
	Total n=67/67(100)	50/67(74.6)	17/67(25.4)	45/67(67.2)	22/67(22.8)
p-value					
HPPXPC= p<0.05 HPPXNC= p<0.05 PCXNC= p<0.05	**+ve X –ve = p<0.05 for HPP, NC and Total**	**HXL p<0.05 for HPP,PC and total**		**HXL p<0.05 for all groups and total**	

Table7. IL-17A level and Treg cell count according to *CagA* strain classification of IgG anti-*Hp* positive subjects in HPP groups

Positive IgG anti-*Hp*	*CagA* strains № (%)	IL-17A level № (%)		Treg cell count № (%)	
		High	Low-Normal	High	Low-Normal
GS n=26/28 (92.9)	*CagA*$^+$ n=9/26(34.6)	8/9(88.9)	1/9(11.1)	6/9(66.7)	3/9(33.3)
	CagA$^-$ n=17/26(65.4)	14/17(82.4)	3/17(17.6)	13/17(76.5)	4/17(23.5)
	Total n=26/26(100)	22/26(84.6)	4/26(15.4)	19/26(73.1)	7/26(26.9)
GU n=8/8 (100)	*CagA*$^+$ n=3/8(37.5)	2/3(66.6)	1/3(33.3)	2/3(66.6)	1/3(33.3)
	CagA$^-$ n=5/8(62.5)	5/5(100)	0/5(0)	4/5(80)	1/5(20)
	Total n=8/8(100)	7/8(87.5)	1/8(12.5)	6/8(75)	2/8(25)
DU n=15/17 (88.2)	*CagA*$^+$ n=8/15(53.3)	5/8(62.5)	3/8(37.5)	5/8(62.5)	3/8(37.5)
	CagA$^-$ n=7/15(46.7)	6/7(85.7)	1/7(14.3)	7/7(100)	0/7(0)
	Total n=15/15(100)	11/15(73.3)	4/15(26.7)	12/15(80)	3/15(20)
Total n=49/53 (92.5)	*CagA*$^+$ n=20/49 (40.8)	15/20(75)	5/20(25)	13/20(65)	7/20(35)
	CagA$^-$ n=29/49(59.2)	25/29(86.2)	4/29(13.8)	24/29(82.8)	5/29(17.2)
	Total n=49/49(100)	40/49(81.6)	9/49(18.4)	37/49(75.5)	12/49(24.5)
p-value					
Differences between group p>0.05	**+ve X –ve = p<0.05 for GS, GU and Total**	**HXL p<0.05 for all groups and total in *CagA*$^+$ and *CagA*$^-$**		**HXL p<0.05 for all groups and total in *CagA*$^+$ and *CagA*$^-$**	

Table 8 demonstrates the correlation of the IgG anti-*Hp* positive mean titer with the *CagA*$^+$ and *CagA*$^-$ strains in all study groups. The mean titer of IgG anti-*Hp* positive subjects was higher among the *CagA*$^+$ than *CagA*$^-$ subjects in GS, GU, and NC groups with significant differences (*p<0.05*), whereas it was lower in *CagA*$^+$ than *CagA*$^-$ subjects in DU group with no significant difference (*p>0.05*).

Table 8. Mean titer of IgG anti-Hp positive subjects for *CagA*$^+$ strains in comparison with *CagA*$^-$ strains

№ of IgG anti-*Hp* positive subjects in each study groups		Mean Titer of positive IgG anti-*Hp* (U/ml)		*p-value*
		CagA$^+$	*CagA*$^-$	
HPP	GS n=26/28	61.3	37.8	<0.05 (S)
	GU n=8/8	88.9	48.3	<0.05 (S)
	DU n=15/17	56.2	67.2	>0.05 (NS)
	Total n=49/53	63.4	46.7	<0.05 (S)
NHPP n=2/7		18.2	14.8	Not done
NC n=16/30		56.6	41.9	<0.05 (S)
Total for all groups n=67/90		60.5	44.6	<0.05 (S)

Discussion:

Referring to the histopathology (as a gold standard diagnostic tool), the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the IgG anti-*Hp* serology were 92.5%, 71.4%, 96.1% and 55.6% respectively (data not shown in this study). This level of accuracy would point out that IgG anti-*Hp* serology is an excellent primary non-invasive test with high sensitivity, specificity and PPV for the justification of *H pylori* infection in dyspeptic patients before going to more invasive tests as OGD and gastric biopsy. Serum assaying of IgG and IgA anti-*Hp* could be used for the determination of prevalence of acute and chronic infections [12]. In table 1, the mean titer of IgG anti-*Hp* in

HPP group was 50.76 U/ml compared to 27.95 U/ml and 9.31 U/ml for the NC and NHPP groups respectively with a significant difference *(p<0.05)*. In general, the serum levels of IgG anti-*H pylori* were increased in the presence of infection and could be used as a marker [13]. In this study, the seroprevalence of IgG anti-*Hp* among asymptomatic *H pylori* infection was 53.3%, which in other studies were ranging from 38.9%-67.9% in Indian [14], 46.6% in South Koreans [15], 64.2% in Iranian children [16], 51% in Saudis [17], 70% in American blacks and 34% in American whites [18], and 44.4% in Germans [19]. Generally speaking, the rate of *H pylori* infection tends to be higher in developing countries as South America and Asia (>80%) than developed countries as Europe (25-60%) [20].

Helicobacter pylori induce a host immune response, but the persistence of the infection suggests that the response is not effective in eliminating the infection. The T cell subset CD_4^+ CD_{25}^+ (Treg) which produce IL-10 and transforming growth factor (TGF)-β is suggested to be one of the reasons of the ineffective response against the bacteria [21]. The presence of Treg in the peripheral blood of *H pylori* infected individuals, could inhibit the response of CD_4^+ T cells to *H pylori* [22]. In the current study, there was an increased Treg cell count in 73.6% subjects of HPP group compared to 46.7% subjects of the NC group with a significant difference *(p<0.05)* (Table 2). The mean of the Treg cell count in the HPP group (25.67±SD 16.971) was significantly higher than NC group (13.56±SD 8.026) but not significantly higher than NHPP group (19.72±SD 9.70). This result would come in consistency with the above mentioned role of Treg cell in *H pylori* infection. In the same table, there was no difference in the level of Treg cell count among the subgroups of HPP (GS, GU, and DU). One study showed that patients with PU had reduced Treg response compared to those without ulcers [23]. Other study showed that Treg cells had increased in all types of *Hp*-associated diseases with a positive association with endoscopic findings of gastroduodenal diseases but negative association with intestinal metaplasia in gastritis and PU groups [24].

IL-17A plays a dual role in the *H pylori* infection as it exerts anti-inflammatory effects on *H pylori*-induced gastritis through suppression of Th1 differentiation in one hand [25] and induces the secretion of IL-8 which attracts neutrophils promoting inflammation in the other hand [26]. In agreement with these studies, higher IL-17A serum levels were observed in *Hp*-infected patients than in NC (Table 3). These findings suggest that IL-17A may play an important role in the pathogenesis of *Hp*-related gastric inflammation and the subsequently developing gastric ulceration. The same table shows no significance different in IL-17A level between gastritis and PU patients, a result which is in consistent with another study [27].

Results shown in Tables 4 and 5 illustrate the relationship between Treg and IL-17A activities. According to these results the response of the Treg was higher than IL-17A in all study groups, and highest in HPP compared to controls. The same results were reported within HPP subgroups (GS, GU, and DU), and these results may predict that the *Hp* infected patients underwent an ineffective immune response due to the hyper-activation of Treg, a result come into full consistency with other previous studies [28]. In the same tables the results showed that when the Treg activity was high, the IL-17A activity was low and vice versa, except in DU patients in which the IL-17A level was lower than Treg in both low and high Treg cases. These results confirm that Th17 and Treg cells are developmentally related and exist in a delicate balance that can dictate the outcome of a bacterial infection.

Cytotoxin-associated gene A (CagA) and other virulent factors as vacuolating cytotoxin A have been proposed as related to more severe gastric diseases in adults [29], although some reports indicate that a high prevalence of *CagA* gene is found irrespective of the disease developed [30]. Other study reveals that the presence of *CagA* is not a reliable marker for prediction of digestive disorders caused by *H pylori* infection [31]. In the current study, the prevalence of $CagA^+$ strains was less than $CagA^-$ strains in HPP (40.8%), NC (25%) groups as

well as in the total subjects enrolled in this study (37.3%) with a significant difference ($p<0.05$) (Table 6). This result was unusual when it was compared with other studies in different places of the world as *CagA* strain prevalence was 78% to 80% in Turkey [32], 82% in Japan [33], 93% in Nigeria [34], and 61.6% in Tunisia [35]. The majority of those studies indicated that in patients with DU, the *CagA* positivity rate is relatively higher than in patients with gastritis or GU which is in agreement with our study. This finding point out that $CagA^+$ and $CagA^-$ strains have an equal chance of developing *H pylori*-associated diseases and the final clinical outcome is weakly associated with type of *H pylori* strain. In some Western countries, *CagA*-positive status has been associated with severe clinical outcomes [36], whereas in Asian countries this association was not observed [37]. This variation would point out the presence of other effective factors; environmental and genetic of both the host and bacteria. One of these possible bacterial genetic factors is the *vacA* virulence factor genotype or alleles. It has been found that s1a, s1b and m1a *vacA* alleles have a higher prevalence in European, African and American strains, whereas the *vacA* s1c and m1b alleles are more prevalent in Asia [38]. In the same tables our results showed no significance different in IL-17A and Treg activities between $CagA^+$ or $CagA^-$ cases and these results agreed with other previous study [28]. There are some evidences to suggest that *H pylori* $CagA^+$ strains could modulate the immune response to *H pylori* [39], which is in consistence with these findings shown in Table 8 in which the IgG anti-*Hp* mean titer is higher in $CagA^+$ strains rather than $CagA^-$ strains with significance difference. These results agreed with other previous study [40]. In the same table, the only exception for this rule was in the DU group in which the mean titer of IgG anti-*Hp* was lower in $CagA^+$ than $CagA^-$ infected subjects with no significant value. This results was in agreement with other study [38] which could be associated with allelic combinations of the *H pylori* that has certain preference to duodenum rather than stomach.

In conclusion, detection of IgG anti-*Hp* in dyspeptic patient's serum is helpful in avoiding a more invasive diagnostic tests as endoscopy and biopsy. Most patients with *Hp*-associated diseases exhibited a high level of IL-17A and Treg cell counts. The Treg: IL-17A ratio is higher in HPP than NHPP as well as in asymptomatic *Hp* infected or non infected people. The presence of $CagA^+$ strain of *Hp* is not enough for the prediction of the type of *Hp*-associated disease as well as the severity of the disease, however, $CagA^+$ strains play a role in the modulation of the immune response through elucidation of high titer of IgG anti-*Hp*. Finally *Hp* strain type has no effect on the IL-17A level as well as the Treg cell counts in patients with *Hp* associated diseases.

References:

1. Chatterjee A, Chatterjee S, Bandyopadhyay S. "*H pylori*-induced gastric ulcer: pathophysiology and herbal remedy" Int J Biol Med Res., 3: 1461-5, 2012.
2. Caruso R, Pallone F, Monteleone G. "Emerging role of IL-23/IL-17 axis in *H. pylori*-associated pathology. *World J Gastroenterol*.,13: 5547–51, 2007.
3. Jafarzadeh V, Mirzaee H, Ahmed B, Maryam N, Mohammad R. "Association of the CagA status of *Helicobacter pylori* and serum levels of interleukin (IL)-17 and IL-23 in duodenal ulcer patients" *J Diges Dis*., 10(2): 107–112, 2009.
4. Sakaguchi S, Sakaguchi N, Shimizu J. "Immunologic tolerance maintained by $CD25^+CD4^+$regulatory T cells. their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance" *Immunol Rev*., 182:18–32, 2001.
5. Kaparakis M, Karen L, Odilia W, John P, Pearse M, Ian R., Paul A, Richard A. "$D4^+$ $CD25^+$ Regulatory T Cells Modulate the T-Cell and Antibody Responses in *Helicobacter*-Infected BALB/c MiceInfect" *Immun*., 74(6): 3519-3529, 2006.

6. Brian M, Clinton A, Sara A, Kathryn A. "Complex T Cell Interactions Contribute to *Helicobacter pylori* Gastritis in Mice" *Infect Immun*., 81(3): 740-752, 2013.
7. Umeda M, Murata-Kamiya N, Saito Y. *"Helicobacter pylori* CagA causes mitotic impairment and induces chromosomal instability" *J Biol Chem*., 284: 22166-22172, 2009.
8. Hatakeyama M. "The role of *Helicobacter pylori* CagA in gastric carcinogenesis" *Int J Hematol*., 84: 301-308, 2006.
9. Umit H, Tezel A, Bukavaz S. "The relationship between virulence factors of *Helicobacter pylori* and severity of gastritis in infected patients" *Dig Dis Sci*., 54: 103–10, 2009.
10. Jimenze F, Demaria J, Ahumada C. "Seroprevalence of Helicobacter pylori anti-CagA antibodies and its relationship with epidemiologic factors in Santa Fe" *Acta Gastroenterol Latinoam*., 34: 16–20, 2004.
11. Farmilo A, Ronald H. Fixation (chapter 4) in: Handbook in Immunological Staining Methods, 3rd edition, Dackocytomation, USA, pp. 18-22, 2001.
12. Andersen LP, Rosenstock SJ, Bonnevie O, Jorgensen T. Seroprevalence of immunoglobulin G, M and A antibodies to *Helicobacter pylori* in an unselected Danish population. *Am J Epidemiol,* 143: 1157-1164, 1996.
13. Locatelli A, Catapani W, Gomes Junior C, Paula Silva C, Waisberg J. "Detection of anti-*Helicobacter pylori* antibodies in serum and duodenal fluid in peptic gastroduodenal disease" *World J Gastroenterol*., 10 (20): 2997-3000, 2004.
14. Jais M, Barua S. "Seroprevalence of anti *Helicobacter pylori* IgG/IgA in asymptomatic population from Delhi" *J Commun Dis.*, 36 (2): 132-135, 2004.
15. Kim J, Kim H, Kim Y, Kim S, Kim J. "Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea" *J Gastroenterol Hepato.,* 16 (9): 969-75, 2001.
16. Soltani J, Amirzadeh J, Nahedi S, and Shahsavari S. "Prevalence of *Helicobacter pylori* Infection in Children, a Population-Based Cross-Sectional Study in West Iran" *Iran J Pediatr*., 23(1): 13–18, 2013.
17. Khan M, Ghazi H. "*Helicobacter pylori* infection in asymptomatic subjects in Makah, Saudi Arabia" *JPMA* 57:114-119, 2007.
18. Graham D, Malaty M, Evans D, Evans D, Klein P, Adam E. "Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status" *Gastroenterology*. 100(6): 1495-501, 1991.
19. Wex T, Venerito M, Kreutzer J, Götze T, Kandulski A, and Malfertheiner P. "Serological Prevalence of *Helicobacter pylori* Infection in Saxony-Anhalt, Germany, in 2010" *Clin Vaccine Immunol*., 18(12): 2109–2112, 2011.
20. Goh L, Chan K, Shiota S., Yamaoka Y. "Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter*, 16(1):1–9, 2011.
21. O'Garra A, Vieira P. "Regulatory T cells and mechanisms of immune system control" *Nat Med.,* 10: 801-805, 2004.
22. Lundgren A, Suri-Payer E, Enarsson K, Svennerholm A, Lundin B. "*Helicobacter pylori*-specific CD4+ $CD25^{high}$ regulatory T cells suppress memory T-cell responses to *H pylori* in infected individuals. *Infect Immun* ., 71: 1755-1762, 2003.
23. Robinson K. *"Helicobacter pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses" *Gut*, 57(10): 1357-85, 2008.
24. Hsin-Hung C, Guan-Ying T, Hsiao-Bai Y, Hung-Jung W, Hwai-Jeng L, and Wen-Ching W. "Increased numbers of Foxp3-positive regulatory T cells in gastritis, peptic ulcer and gastric adenocarcinoma" *World J Gastroenterol*., 18(1): 34–43, 2012.

25. Otani K, Watanabe T, Tanigawa T, Okazaki H, Yamagami H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T. "Anti-inflammatory effects of IL-17A on *Helicobacter pylori*-induced gastritis. *Biochem Biophys Res Commun.*, 382(2): 252-258, 2009.
26. Shahjahan K. "The Role of Interleukin-17 in the Helicobacter pylori Induced Infection and Immunity" *Helicobacter* 16(1): 1–8, 2011.
27. Staples E. "OC-096 TH17 cells are increased in *Helicobacter pylori* infection and may be associated with peptic ulcer disease", *Gut*, 61, 302514a.96, 2012.
28. Fabricio F. "A regulatory instead of an IL-17 T response predominates in *Helicobacter pylori*-associated gastritis in children " *Microbes and Infection*, 14: 341-347, 2012.
29. **Censini S, Lange C, Xiang Z, Crabtree J, Ghiara P, Borodovsky M, Rappuoli R, and Covacci A.** *"cag*A pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc. Natl. Acad. Sci. USA* 93**:**14648-14653, 1996.
30. Domingo D., Alarcón T., Prieto N., Sánchez I., López-Brea M. "cagA and vacA status of Spanish *Helicobacter pylori* clinical isolates" *J. Clin. Microbiol*., 37: 2113–2114, 1999.
31. Bezmin Abadi A, Ghasemzadeh A, Mobarez A. "Low frequency of *cagA*-positive *Helicobacter pylori* strains isolated from Iranian patients with MALT lymphoma. *Internal and Emergency Medicine*, 8(1):49-53, 2013.
32. Erzin, Y., V. Koksal, S. Altun, A. Dobrucali, M. Aslan, S. Erdamar, A. Dirican, and B. Kocazeybek. "Prevalence of *Helicobacter pylori vacA*, cagA, *cagE*, *iceA*, *babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia" *Helicobacter* 11:574-580, 2006.
33. Azuma, T, Yamakawa A, Yamazaki S, Ohtani M, Ito A, Muramatsu A, Suto H, Yamazaki Y, Keida Y, Higashi H, and Hatakeyama M. "Distinct diversity of the *cag* pathogenicity island among *Helicobacter pylori* strains in Japan" *J. Clin. Microbiol*. 42:2508-2517, 2004.
34. Bayerdoffer S, Miehlke S. "Prevalence of *Helicobacter pylori vacA*, *cagA* and *iceA* genotypes in Nigerian patients with duodenal ulcer disease" *J Med Microbiol.,* 51:851-854, 2002.
35. Ben Mansour K, Fendri C. "Prevalence of *Helicobacter pylori* vacA, cagA, iceA and oipA genotypes in Tunisian patients" *Ann Clin Microbiol Antimicrob.,* 9: 10-17, 2010.
36. **Queiroz D, Carvalho A, Rocha G, Oliveira A, Soares T, Santos A, Cabral M, Nogueira A.** "Factors associated with *Helicobacter pylori* infection by a *cagA*-positive strain in children' *J Infect Dis*., 181**:** 626-630, 2000.
37. **Yamaoka Y, Souchek J, Odenbreit S, Haas R, Arnqvist A, Boren T, Kodama T, Osato M, Gutierrez O, Kim J, Graham D.** "Discrimination between cases of duodenal ulcer and gastritis on the basis of putative virulence factors of *Helicobacter pylori*" *J Clin Microbiol.,* 40**:** 2244-2246, 2002.
38. Togrul N, Erkan Y, Bahri A, Fatih K. "Prevalence and Genotypes of *Helicobacter pylori* in Gastric Biopsy Specimens from Patients with Gastroduodenal Pathologies in the Cukurova Region of Turkey. J Clin Microbiol. 47(12): 4150–4153, 2009.
39. Dzierzanowska F, Raeiszadeh M, Crabtree J. "IgG subclass response to *Helicobacter pylori* and CagA antigens in children" *Clin Exp Immunol*., 134(3): 442–446, 2003.
40. Masoumeh D, Hossein G, Mahmoud N, Bahram N. "Immune responses to Helicobacter pylori infection in children with intellectual disabilities", *Elsevier*, 33,: 633-669, 2012.