DOI: 10.24996/ ijs.2017.58.4B.9

ISSN: 0067-2904

Evaluation of CD14 expression in *Helicobacter pylori* positive and *Helicobacter pylori* negative gastritis

Manar Mustafa Ibraheem
Pharmacy Department, Institute of Medical Technology AL- Mansur, Baghdad, Iraq.

Abstract

Monocytes are considered a key mediator of inflammatory cytokine secretions during inflammation. This study evaluates CD 14 expression in gastritis tissue biopsies of *H. pylori* and none *H. pylori* gastritis. This cross-sectional study involved 60 gastritis patients that have been classified into *H. pylori* positive (n=30) and *H. pylori* negative (n=30). Formalin fixed paraffin embedded tissue blocks were sectioned and immune-peroxidase staining with anti-CD14, then compared between study groups and clinical parameters. The results showed a marked difference in the percentage of expression in mild and severe intensity of inflammation sub-groups, the results showed a higher percentage of CD14 immunoreactivity (18.29±5.84 vs. 10.2±3.89, p=0.005) and (42.84±19.43 vs. 32.98±9.83, p=0.007) respectively. In conclusion, the percentage of CD14 immunoreactivity may closely related to the inflammatory gastritis induced by *H. pylori* bacterium.

Key words: Monocyte, *H. pylori* and gastritis.

تقيم التعبير البروتيني للمعلم المناعي 14 عند الاشخاص المصابين وغير المصابين ببكتريا الملويات البوابية

منار مصطفى ابراهيم

قسم الصيدلة، المعهد الطبي التقني/ المنصور، الجامعه التقنية الوسطى، بغداد، العراق.

الخلاصه

تعتبر الخلايا وحيدة النواه المفتاح الأساسي لإنتاج النواقل الحركية الالتهابية خلال العملية الالتهابية. تهدف هذه الدراسة الى تقييم التعبير البروتيني للمعلم المناعي ١٤ في المقاطع النسيجية لالتهاب المعدة المأخوذة من اشخاص مصابين وغير مصابين ببكتريا الملويات البوابية. صممت الدراسة بصورة مقطعيه تتضمن ٦٠ شخص مصاب بالتهاب المعدة مقسمين الى مجموعتين احداها تضم ٣٠ شخص مصاب ببكتريا الملويات البوابية والمجموعة الأخرى تضم ٣٠ شخص من غير المصابين بتلك البكتريا. تم اخذ عينات خزعيه من معدة الأشخاص اثناء الفحص الناظوري بعدها تمت معاملتها لتصبح مطموره بشمع البرافين. تم فحص المعلم المناعي ١٤ على المقاطع النيسجية من المرضى بواسطه طريقة التصبيغ المناعي النسيجي الكيميائي وتم مقارنة النتائج للمجموعتين وكذلك المتغيرات السريرية. اشارت النتائج الى وجود فرق في نسبة تعبير بروتين المعلم المناعي ١٤ بين المجموعة الطفيفة والشديدة الالتهاب مما يدل على ان نسبة التعبير البروتيني للمعلم المناعي ١٤ مرتبطة بشدة الالتهاب الناتج عن وجود بكتريا الملويات البوابية.

Email: manar83mostafa@yahoo.com

Introduction

Helicobacter pylori is the most frequent bacteria causing gastritis among population[1], and the presence of these bacteria invasion of *H. pylori* is supported by the presence of the organism in epithelial cells and lamina propria[2]. Infection with this organism induces infiltration of polymorphonuclear (PMN) and mononuclear leukocytes and enhances the production of various proinflammatory cytokines in gastric mucosa [3, 4]. The chronic gastritis characterized by the dominance of phagocytic cells (neutrophils and macrophages CD14) and different lymphocytes subsets (CD4, CD8 and CD19)[2].

Antigen-presenting cells (APCs) are present in the *H. pylori*-infected mucosa and are likely involved in both the induction and maintenance of *H. pylori*- specific immune responses and inflammatory reactions. Originating from a common myeloid progenitor, monocytes can differentiate into tissue macrophages or DCs when crossing the endothelial barrier[5] and have a high capacity to kill *H. pylori*[6]. As classically and alternatively activated macrophages go along with Th1 and Th2 cell induction, these cells have also been classified as M1and M2 macrophages, respectively[7]. Depending on the activating stimulus, M2 macrophages can be further subdivided into at least three distinct subsets that share strong IL-10-secreting properties but are able to react differently upon activation[8–10]. Whereas M1 macrophages are thought to be primarily microbicidal and pro-inflammatory, M2 macrophages exhibit anti-inflammatory and immune-modulating functions and have been reported to be involved in responses against different bacterial and parasitic infections and in tissue remodeling[11, 12].

DCs are specialized in antigen capture, processing, and presentation to naïve T cells after migration to the draining lymph nodes[13]. They are widely distributed in human tissues, including the gastric mucosa, and are capable of penetrating the gut epithelial monolayers to sample, e.g., luminal bacteria[14]. DCs stimulated with *H. pylori* promote Th1 responses in vitro[15]. Furthermore, DCs may also interact with *H. pylori* through CD209 (DC- SIGN) that binds *H. pylori* lipopolysaccharide (LPS) and promotes IL-10 secretion[14]. Moreover, *H. pylori* stimulates DCs to induce IL-17 expression in CD4 lymphocytes[16].

This retrospective study was designed to evaluate the immunohistochemical expression of CD14 cells in tissue biopsies of *H. pylori* and none *H. pylori* gastritis.

Patients, Materials & Methods

Study design, population and setting

This cross-sectional study involved 60 patients presenting various dyspeptic symptoms in the gastroenterology and hepatology teaching hospital in Baghdad, from March 2015 to April 2016. The patients were aged 18-56 years with a male/female ratio of 5:7. *Helicobacter pylori* positivity was defined by rapid urease test for tissue biopsy.

Sample processing and laboratory test

Three gastric tissue biopsies were obtained from each patient. Rapid urease test was performed on one of the biopsies. The other biopsy specimens were paraffin embedded and processed. One section from each block was stained by Hematoxylin & Eosin to study the histopathological features and grading of gastritis was done according to the updated Sydney system[17]. One section was used for immunohistochemical staining for CD14.

Monoclonal Mouse Anti-CD14 (Cat. No. ABIN135747, Supplier: antibodies-online) is intended for laboratory use in immunohistochemistry staining of CD14 positive cells tissue sections. Immunohistochemical staining for CD14 was assessed as positive or negative membranous staining. Technical negative control was obtained by omission of primary antibody.

Statistical analysis

Statistical analysis was performed using SPSS 22 and Microsoft Excel 2016. Continuous numeric variables were expressed as mean + SD. Chi-square test was used to compare between two discrete variables. T-test and ANOVA were used to compare the mean of numeric variables. A P- value of less than 0.05 was considered significant.

Results

Patients characteristics

As shown in Table-1, the age and gender type were not significant between patients from two groups (p>0.05).The age mean was 38.7 in *H. pylori* negative,while the age mean was 41.3 in *H.pylori* positive. Likely, smoking habit, vomiting, antibiotic therapy, H_2blocker and NSAID all of then doesn't reach the statistical significance value. Proton pump inhibitor showed a statistical significance in which *H. pylori* negative cases were 21 (70%) using proton pump inhibitor (PPI) (p<0.05). Also, *H. pylori* positive patients were more using anti-acid treatments 16 (53.33%).

Table 1- summary of demographic and clinical description for study groups.

	***H. pylori* Negative (N=30)**	***H. pylori* Positive (N=30)**	***Total***
Gender (Male) (%)	12 (40)	14 (46.67)	26 (43.33)
Smoking habit (%)	12 (40)	7 (23.33)	19 (31.67)
Vomiting (%)	13 (43.33)	17 (56.67)	30 (50)
Antibiotic therapy (%)	15 (50)	12 (40)	27 (45)
Anti-acid therapy (%)	8 (26.67)	16 (53.33)*	24 (40)
H_2blocker (%)	13 (43.33)	17 (56.67)	30 (50)
NSAID (%)	4 (13.33)	7 (23.33)	11 (18.33)
PPI (%)	21 (70)*	12 (40)	33 (55)
Anti-H. pylori (IgG) (%)	23 (76.67)	27 (90)	50 (83.33)

Data presented as count (%).
*: significant difference (p<0.05).

Immunoexpression of CD14 among gastritis patients

Our results reported a significantly (p=0.031) higher immunoreactivity of CD14 among *H. pylori* positive group 35.3±14.2 than *H. pylori* negative group 26.9±15.2 Figure-1. Furthermore, we reported that increased CD14 immunoreactivity with the increased intensity of inflammation (according to Updated Sydney System) in both groups (Table-2). In mild and severe intensity of inflammation sub-groups, the results showed a higher percentage of CD14 immunoreactivity (18.29±5.84 vs. 10.2±3.89, p=0.005) and (42.84±19.43 vs. 32.98±9.83, p=0.007) respectively.

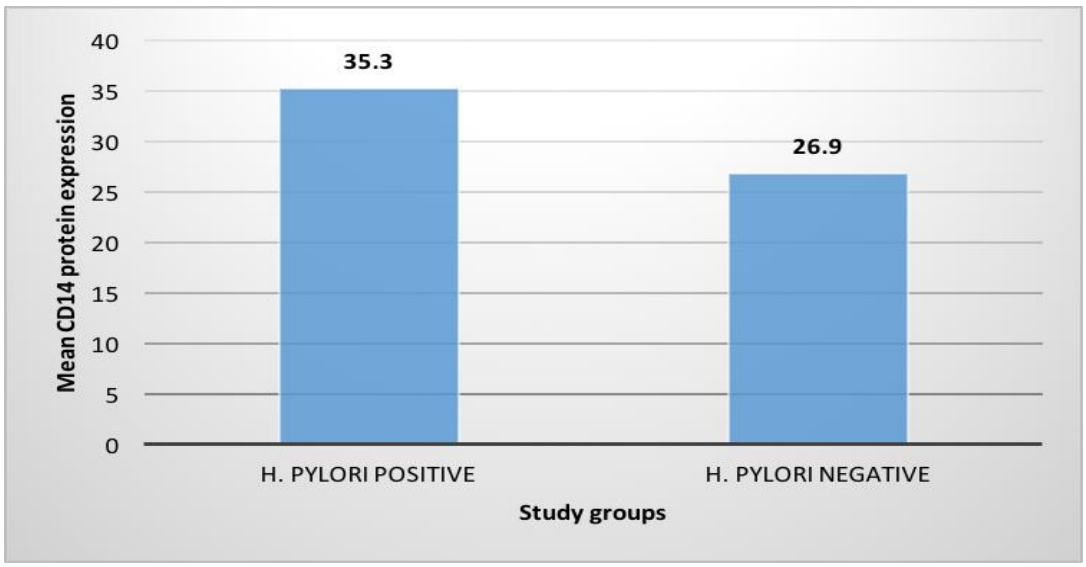

Figure 1-Mean protein expression of CD14 in study groups.

Table 2- immunoreactivity of CD14 according to intensity of inflammation in study groups.

	Absence	Mild	Moderate	Severe	P value
H. pylori positive	4.84±3.22	18.29±5.84	30.55±7.21	42.84±19.43	<0.001**
H. pylori negative	2.77±4.32	10.2±3.89	28.30±8.28	32.98±9.83	<0.001**
P value	0.439 ^{NS}	0.005*	0.591^{NS}	0.007*	

Data presented as mean ± standard deviation.
NS: none statistical significance (p>0.05).
*: Statistical significance (p≤0.05).
**: Highly statistical significance (p≤0.001).

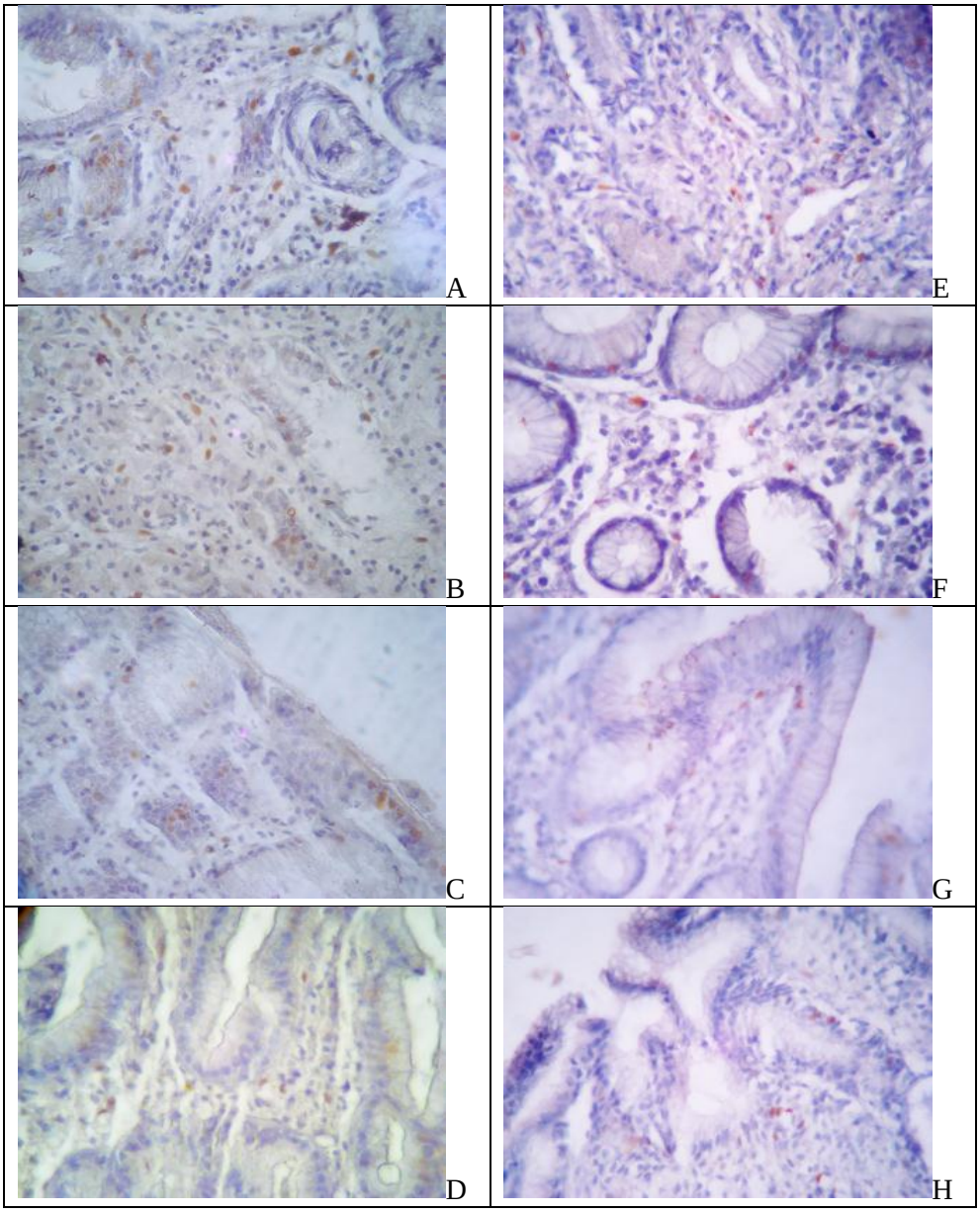

Figure 2-mmunoperoxidase staining of CD-14 in gastritis (A-D) H. pylori positive and (E-H) H. pylori negative. Severe inflammation (A and E), Moderate inflammation(B and F), Mild inflammation (C and G) and absence of inflammation (D and H). 400X magnification.

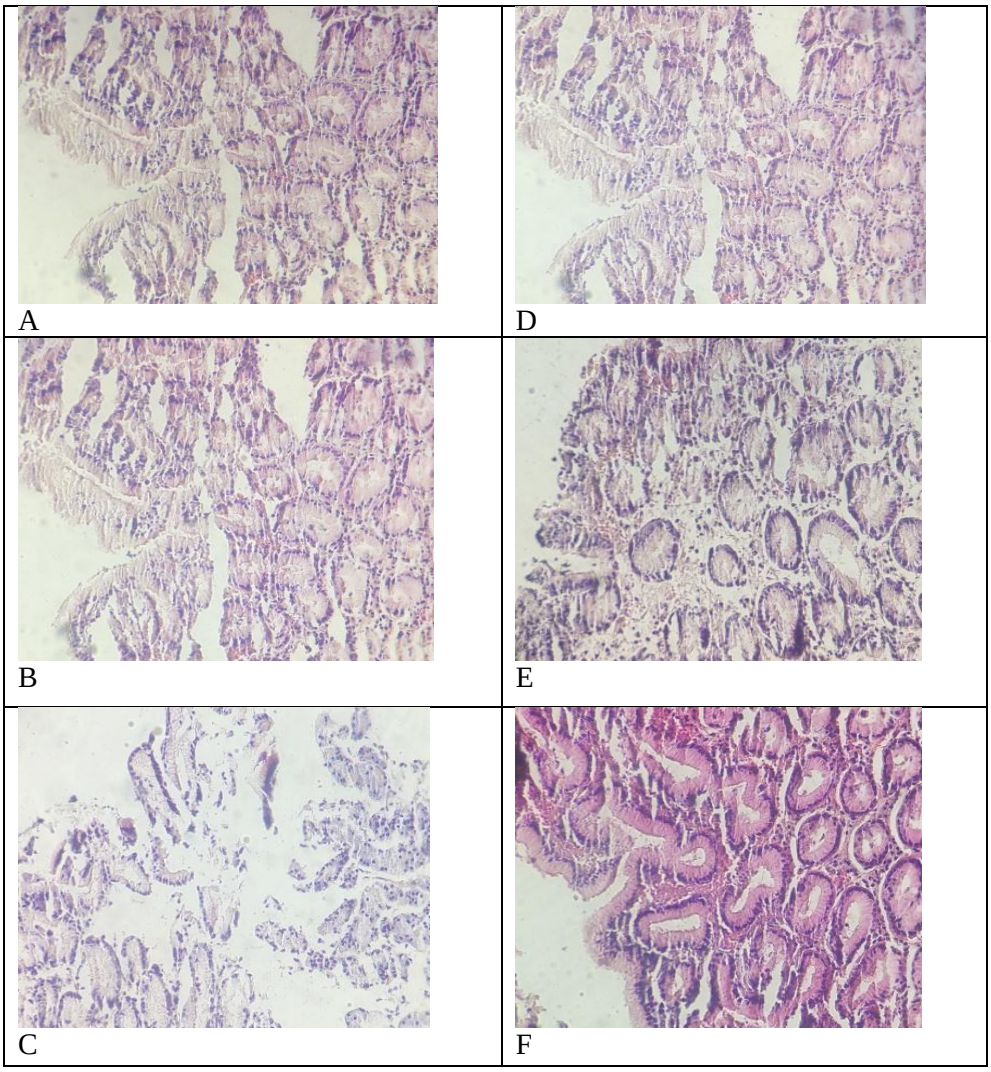

Figure 3-Hematoxylin and Eosin staining of CD-14 in gastritis (A-D) H. pylori positive and (E-F) H. pylori negative. 400X magnification.

Discussion

The majority of previous gastritis studies didn't focus on the relative immunoreactivity of CD14 among *H. pylori* and none-*H. pylori* subgroups[2,18,19] or they provide an evidences for *H. pylori* lipopolysaccharides interaction with monocytes or dendritic cells[15,16]. In this cross-sectional study, a chronic gastritis patients were studied for their detailed intensity of inflammation in relation with percentage of CD14 immunoreactivity.

In agreement with many earlier studies, there was a close association between inflammation and presence of *H. pylori*[15,18]. The higher percentage of CD14 positive cells were indicated in *H. pylori* positive subgroup than *H. pylori* negative subgroup. In all patients with moderate and severe chronic inflammation, a mild or moderate active inflammation was found simultaneously and in addition, all patients harbored H. *pylori*. Active inflammation was not found in any of the patients with mild chronic gastritis and *H. pylori* was present in 10 out of 15 patients in this group. Patients with moderate chronic gastritis without *H. pylori* could not be distinguished from the *H. pylori* positive patients by immunoreactivity of CD14. This suggest that in these patients with moderate inflammation, the density of *H. pylori* was lower than the observed in patients with emphasizing the diagnostic difficulties in patients with moderate inflammation and low density of *H. pylori*.

In vitro studies showed that increased CD14 expression is affected by sensing of bacteria (*H. pylori*) than non-affected cells. Infected macrophages produced predominantly proinflammatory cytokines, i.e., >200-fold more IL-12 than IL-10 compared with DCs, although IL-12p70 was produced only at low levels[14].

In conclusion, the percentage of CD14 immunoreactivity may closely related to the inflammatory gastritis induced by *H. pylori* bacterium.

References

1. Shiota, S., Suzuki, R., Yamaoka, Y. **2013**. The significance of virulence factors in Helicobacter pylori. *J Dig Dis*,**14**(7): 341–9.
2. Lopes, AI., Victorino, RMM, Palha AM., Ruivo, J., Fernandes, A. **2006.** Mucosal lymphocyte subsets and HLA-DR antigen expression in paediatric Helicobacter pylori-associated gastritis. *Clin Exp Immuno*, l; **145**(1): 13–20.
3. Michalkiewicz, J., Helmin-Basa, A., Grzywa, R., Czerwionka-Szaflarska, M., Szaflarska-Poplawska, A., Mierzwa, G, *et al.* **2015**. Innate Immunity Components and Cytokines in Gastric Mucosa in Children with *Helicobacter pylori* Infection. *Mediators Inflamm*, **2015**:1–7.
4. Futagami, S., Hiratsuka, T., Tatsuguchi, a., Suzuki, K., Kusunoki, M., Shinji, Y., *et al.* **2003**. Monocyte chemoattractant protein 1 (MCP-1) released from Helicobacter pylori stimulated gastric epithelial cells induces cyclooxygenase 2 expression and activation in T cells. *Gut*, **52**(9): 1257–64.
5. Randolph, GJ., Jakubzick, C., Qu, C. **2008**. Antigen presentation by monocytes and monocyte-derived cells. *Curr. Opin. Immunol*, **20**(1): 52–60.
6. Borlace, GN., Butler, RN., Brooks, DA. **2008**. Monocyte and macrophage killing of *Helicobacter pylori*: Relationship to bacterial virulence factors. Helicobacter, **13**(5): 380–7.
7. Hill, Charles D Mills AM., Kincaid, K., Alt, JM., Mills, CD., Heilman, MJ., Hill, AM. **2000.** Paradigm M-1/M-2 Macrophages and the Th1/Th2 M-1/M-2 Macrophages and the Th1/Th2 Paradigm. *J Immunol Ref J Immunol UB Marbg*, **164**: 6166–73.
8. Benoit, M., Desnues, B., Mege, J-L. **2008**. Macrophage Polarization in Bacterial Infections. *J Immunol*, **181**(6): 3733–9.
9. Mantovani, A., Sica, A., Locati, M. **2007**. New vistas on macrophage differentiation and activation. *Eur J Immunol*, **37**(1): 14–6.
10. Murray, PJ., Allen, JE., Biswas, SK., Fisher, EA., Gilroy, DW., Goerdt, S., *et al.* **2014**. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity*, **41**(1): 14–20.
11. Gordon, S. **2003**. Alternative activation of macrophages. *Nat.Rev.Immunol*, **3(**1474–1733 (Print)): 23–35.
12. Gordon, S. and Martinez, FO. **2010**. Alternative activation of macrophages: Mechanism and functions. *Immunity*, **32**(5): 593–604.
13. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, YJ., *et al.* **2000**. Immunobiology of dendritic cells. *Annu Rev Immunol*, **18**: 767–811.
14. Fehlings, M., Drobbe, L., Moos, V., Viveros, PR., Hagen, J., Beigier-Bompadre, M., *et al.* **2012**. Comparative analysis of the interaction of Helicobacter pylori with human dendritic cells, macrophages, and monocytes. *Infect Immun*, **80**(8): 2724–34.
15. Bimczok, D., Clements, RH., Waites, KB., Novak, L., Eckhoff, DE., Mannon, PJ., *et al*. **2010**. Human primary gastric dendritic cells induce a Th1 response to H. pylori. *Mucosal Immunol*, **3**(3): 260–9.
16. Khamri, W., Walker, MM., Clark, P., Atherton, JC., Thursz, MR., Bamford, KB., *et al*. **2010**. *Helicobacter pylori* stimulates dendritic cells to induce interleukin-17 expression from CD4+ T lymphocytes. *Infect Immun*, **78**(2): 845–53.
17. Rugge, M., Genta, RM. **2005**. Staging and grading of chronic gastritis. *Hum Pathol*, **36**(3): 228–33.
18. Andersen, LP., Holck, S., Janulaityte-Günther, D., Kupcinskas, L., Kiudelis, G., Jonaitis, L., *et al*. **2005**. Gastric inflammatory markers and interleukins in patients with functional dyspepsia, with and without Helicobacter pylori infection. *FEMS Immunol Med Microbiol*, **44**(2): 233–8.
19. De Paulis, A., Prevete, N., Fiorentino, I., Walls, AF., Curto, M., Petraroli, A., *et al.* **2004**. Basophils infiltrate human gastric mucosa at sites of *Helicobacter pylori* infection, and exhibit chemotaxis in response to H. pylori-derived peptide Hp(2-20). *J Immunol*, **172**(12): 7734–43.