Journal of Experimental Biology and Agricultural Sciences, June - 2021; Volume – 9(3) page 388 – 393

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

ROLE OF INTERLEUKIN-2, INTERLEUKIN-4 AND CLUSTER OF DIFFERENTIATION-22 AS AN IMMUNE MARKERS IN INDIVIDUALS INFECTED WITH *Helicobacter pylori*

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Received – May 17, 2021; Revision – June 09, 2021; Accepted – June 22, 2021
Available Online – June 25, 2021

DOI: http://dx.doi.org/10.18006/2021.9(3).388.393

KEYWORDS

Helicobacter pylori

Immune response

IL-2

IL-4

CD22

Iraq

ABSTRACT

Helicobacter pylori is a gram-negative, intracellular, microaerophilic bacteria which causing Peptic ulcer. This bacterium can change its shape which helps the bacteria to survive in the host gastric microenvironment. The Peptic ulcer caused by this bacterium stimulates the humoral and cellular immune response in individuals. The current study was carried out to access the role of interleukin-2, interleukin-4, and cluster differentiation-22 as immune markers in the identification of *H. pylori* infection. The presence of *H. pylori* has been diagnosed by feces test (antigen rapid test). In this study, the presence of three immunological markers viz., IL-2, IL-4, and CD22 were measured in the serum of 60 individuals infected with *H. pylori* and 30 healthy individuals by the Enzyme-Linked Immune-sorbent Assay method. Results of this study indicated a significant increase (P-value=0.0307*) in the concentration of IL-2 (294.27ng/ml), IL-4(151.28ng/ml), and CD22 (492.73ng/ml) in the serum of individuals infected with *H. pylori* while these concentrations were reported 235.98ng/ml, 116.14ng/ml and 369.33ng/ml respectively in the healthy individuals. Results of the study can be concluded that *H.pylori* infection stimulates the Cellular and humoral immune response which resulted in the increased production of IL-2, IL-4, and CD22.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Helicobacter pylori is a spiral, microaerophilic, noninvasive, gram-negative bacterium that colonizes in the human gastrointestinal tract, primarily in the stomach (Mohammadian & Ganji, 2019). It is a well-known gastric pathogen that affects almost half of the world's population, around 4.4 billion individuals globally affected by the infection of this bacterium, and maximum of these are from developing countries (Kusters et al., 2006; Hamzah & Aljanaby, 2020; Reshetnyak et al., 2021). This organism has been identified as an etiological agent for chronic active gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (Hathroubi et al., 2018). The infection of *H. pylori* normally start in infancy and remained throughout life, is leading to serious infections such as gastric carcinoma, duodenum ulcer, gastritis, and gastric ulcer in adults, which are mostly transferred from person to person through saliva, vomit, and fecal matter, very rarely it transferred by the contaminated water and drink (Razavi et al., 2015; Qumar et al., 2021). Various factors such as the vacuolating cytotoxin, the *cagA* and *cag* pathogenicity island (*cag*PAI), motility, adhesins, and the urease enzyme are known to be involved in the virulence of this organism (Dunn et al., 1997). The World Health Organization and the International Agency for Research on Cancer (IARC) have identified *H. pylori* as a type I carcinogen linked to the development of gastric cancer (GC) since 1994 and it is closely linked with the environment, diet, and gene mutations (Hatakeyama, 2014). Furthermore, *H. pylori* may create biofilms to reduce its sensitivity to a variety of antibiotics such as Amoxicillin, Tetracycline, and Ceftriaxone, resulting in multi-drug resistance (MDR), mutations, and further bacterial eradication challenges (Liu et al., 2021a). *H. pylori* can form biofilms on the human gastric mucosa also. Moreover, *H. pylori* could embed in drinking water and formed biofilms in the water or on the surfaces of water distribution systems in developed and developing countries. Therefore, a more thorough understanding of *H. pylori* biofilm could be helpful in the characterization of this microorganism (Guillermo et al., 2020). Early-stage identification of *H. pylori* might help in the proper management of *H. pylori* (Suarez et al., 2006; Xu et al., 2020). Jung et al. (2020) suggested that humans afflicted with *H. pylori* produced specific antibodies, which can be detected in serum as well as gastric aspirates or stomach extracts, this phenomenon can be helpful in the diagnosis of *H. pylori* infection. Further, this bacterium also triggers the immune reactions in the infected organism. The most common test to detect *H. pylori* is the stool antigen test that looks for foreign proteins (antigens) associated with *H. pylori* infection in patients' stool. Furthermore, other tests like a breath test, scope test, and stool PCR can also be used for the identification of *H. pylori* infection (Guillermo et al., 2020). The immune response caused by *H. pylori* is a topic of ongoing research that has prompted a slew of questions (Alipour, 2020). The host's failure to clear *H. pylori* infections might be due to down regulatory processes that limit the subsequent immune responses to suppress dangerous inflammation as a way of protecting the host (Bravo et al., 2018). As a result, the mediated chronic immune response could be insufficient or misdirected, creating a colonization benefit for the bacteria by increasing the availability of adhesion sites (Săsăran et al., 2021). Information regarding the immune response in the *H. pylori* infected patients in Iraq are in scanty. Therefore, the current study was carried out to establish the relationship between humoral and cellular immune responses in *H. pylori* infected individuals.

2 Materials and Methods

2.1 Respondent and Study design

This case-control study was carried out at Al-Kufa City, Iraq from September 2020 to February 2021. A total of 60 *H. pylori* infected patients and 30 healthy (as control) male and female age range 20-60 years old were included in this study. *H. pylori* infected patients were admitted to the middle Euphrates hospital in Al-Kufa city. Before starting the study, the written consent and ethical approval were taken from all the selected individuals.

2.2 Detection of *H. pylori* by Feces Test (Antigen rapid test)

The readymade kit was used for immune-chromatographic assay and qualitative determination of *H. pylori* antigen in feces. The test was carried out as per the manufacturer's instruction (EDI, EPITOPE, diagnostic, INC, United States).

2.3 Measurement of IL-2, IL-4, and CD22 concentration by enzyme linked immunosorbent assay (ELISA)

This test was conducted according to the manufacturing company instructions (Bioassay Technology Laboratory. Shanghai, China). Five ml of blood sample were collected from all the individuals and 2 ml of serum for each individual has been obtained by centrifugation at 8000 rpm/10 min. This serum concentration has been used for the measurement of IL-2, IL-4, and CD22 by the enzyme-linked immunosorbent assay (ELISA).

2.4 Statistical analysis

The statistical analysis was carried out by using the computer software (graph pad prism version 6) and a mean value and standard error (SE) have been obtained for each value. Statistically significant P values less than 0.05 were considered for the statistical analysis (Aljanaby & Medhat, 2017; Aljanaby & Aljanaby, 2018; Atif et al., 2021).

3 Results

3.1 Total patients, their gender, and age groups

Out of 60 patients, the results of the present study demonstrated that there were 27 males (45%) and 33 females (55%) were infected by *H.pylori* (Figure 1). Among these, a maximum of 21 patients (35%) infected by *H. pylori* belonged to the age group of 51-60 years, followed by the age group of 41-50 years (15 patients), 31- 40 years (14 patients) and 20-30 years (10 patients) (Table 1).

3.2 Immunological markers

Results related to humeral and cellular immunity revealed that *H.pylori* infection increased the concentration of IL2, IL4, and CD22, and this concentration was significantly higher than the control. Further, the concentration of IL-2, IL4, and CD22 in individuals infected with *H. pylori* was reported 294.27 ng/ml, 151.28 ng/ml, and 492.73 ng/ml, and these concentrations were significantly higher than the healthy individual where these concentrations were reported 235.98ng/ml, 116.14 ng/ml and 369.33 ng/ml respectively (Figure 2, 3 & 4).

4 Discussions

The results of the current investigation suggested that there is a substantial connection between IL-2 and *H. pylori* infection. Further, a cytokine like IL-2 has remarkable beneficial impacts on the immune system and this type of interleukin can be used to treat both metastatic renal cell carcinoma and metastatic melanoma (Buchbinder et al. 2019). The discovery of IL-2 as a "T-cell

Table1 Numbers and percentages of patients infected with *H.pylori* according to age groups

Age groups (years)	Male	Female	Total (%)
20-30	5	5	10 (16.6)
31-40	7	7	14 (23.4)
41-50	6	9	15 (25)
51-60	9	12	21 (35)
Total	27	33	60 (100%)

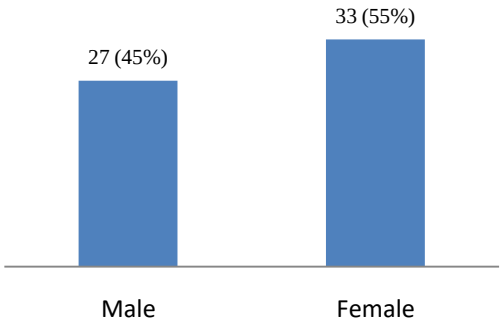

Figure 1 Male and female individuals infected with *H.pylori*

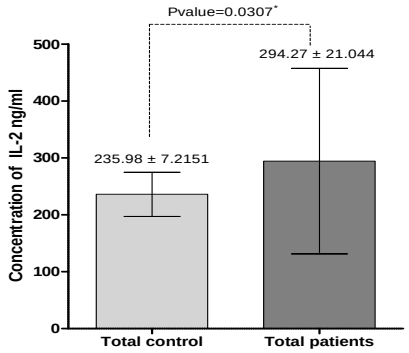

Figure 2 The concentration of IL-2 in *H. Pylori* infected and healthy individuals

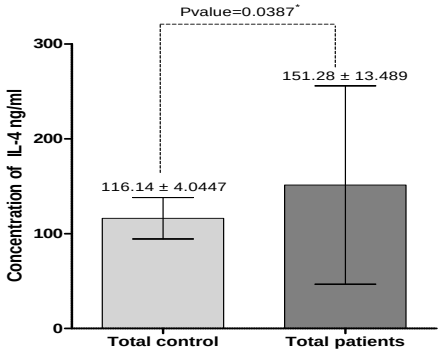

Figure 3 The concentration of IL-2 in *H. Pylori* infected and healthy individuals

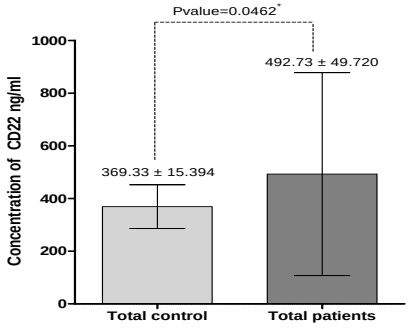

Figure 4 The concentration of CD22 in *H. Pylori* infected and healthy individuals

growth factor" (TCGF) in 1976 was an extremely major milestone in immunology and is the foundation for most of the immunotherapy used in human cancers (Leko & Rosenberg, 2020; Liu et al., 2021b). Further, T cells release interleukin-2, which is a strong immunoregulatory cytokine implicated in cell-mediated immunity, when it triggered by mitogens or by interacting with antigen or major histocompatibility complexes of antigen-presenting cells, and it works as a T-cell growth factor (Davinelli et al., 2019). Lymphocyte cell (T- helper 2) cells produce IL-4, IL-5, IL-6, and IL-10, which activate and develop B cells, while T-helper1 cells produce IL-2 and interferon (IFN-gamma), which boost cell-mediated immune responses (Wong et al., 2015). Interleukin-2 is a strong T cell proliferation factor, and it was swiftly embraced for cancer immunotherapy, especially in the treatment of metastatic melanoma and renal cell carcinoma (Ross & Cantrell, 2018).

The results proved that there was a significant increase in IL-4 level in serum of patients infected with *H.pylori* as compared with healthy individuals. T cell activation, differentiation, proliferation, and survival are well regulated by interleukin-4 in diverse T cell types (Silva-Filho et al., 2014). Further, T-helper 2 also generates IL-4, which has an immune-modulatory impact on B cells, mast cells, macrophages, and a variety of other cell types. Along with this, IL-4 is an important element in the formation of Th2 cells, which release cytokines that cause allergic reactions (Steinke & Borish, 2001). Because of its ability to reduced inflammation and lower serotonin transporter activity, interleukin 4 mainly cytokine type Th2 may protect the cell against bacterial infections (Schneider et al., 2001). The *H. pylori* neutrophil-activating protein (HP-NAP) is one of the numerous bacterial factors that not only drives Th1 inflammation but also inhibits Th2 responses in humans in vivo (Guo et al. 2020). Interleukin-4, therefore, plays a key role in humoral and cell-mediated immunity, demonstrating anti-inflammatory effects through up-regulated cytokine inhibitors and scavenger receptors, as well as improving Th2 mediated immunity (CortesSelva et al., 2019).

B cell response to antigens like *H.pylori* and innate immune signals are influenced by the Cluster of differentiation-22. As a result, interactions between CD22 and CD22L are critical for sustaining self-tolerance (Fan et al., 1994). In the current study, a substantial rise in serum CD22 levels was reported in the individuals infected with *H.pylori*. CD22 capacity to modulate both B-cells and T-cell receptors may be used to manipulate B cell responses to *H.pylori* infection (Fry et al., 2018). Differentiation cluster-22 positive lamina propria lymphocytes have been seen in *H.pylori*-positive people. These data imply that antigenic responses to *H.pylori* are significantly decreased in *H.pylori* positive individuals, perhaps indicating antigenic suppression activation (Salar, 2019).

Conclusions

Results of the study can be concluded that infection of *H. pylori* elevated the level of three immunological markers viz., IL-2, IL-4, and CD22 in human beings, and this will be helpful in the early diagnosis of *H. pylori* infection. Further, it was reported that *H. pylori* infected people have higher activities of B cell and T helper cells.

Conflict of Interest

This study does not have any conflict of interest

Source of Funding

There was no fund in this study, fund by authors themselves

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