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Research Article

Phenotypical Analysis of Peripheral T-Cells and γδ-T-Cells in Helicobacter Pylori Seropositive Individuals

Abdullah H Aljohani[1], Abdulelah Aljuaid[1], Mamdouh Allahyani[1], Jood A AL-Kasi[1], Naif D Althuwaybi[1], Renad N AL-Jaiud[1], Osama Abdulaziz[1], Meshari A Alsuwat[1], Sumaya Albazi[2], Ahmed S Alsuwat[3], Thamer A Althobaiti[3], Abeer M Alshehry[3], Wedad almotairi[4] and Mazen M Almehmadi[1]*

[1]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Kingdom of Saudi Arabia*

[2]*Blood bank, Laboratories and blood banks administration, Taif Province, Kingdom of Saudi Arabia*

[3]*Laboratory Department, Children's Hospital, Al-Taif 26735, Saudi Arabia*

[4]*Directorate of health affairs in Taif, TAIF, Makkah Province, Kingdom of Saudi Arabia*

***Corresponding Author:** Mazen M Almehmadi, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Kingdom of Saudi Arabia.

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Abstract

Background: Helicobacter pylori (*H. pylori*) is a gram-negative-bacilli-bacteria that infects humans' stomachs and the duodenum. H. pylori infection is the primary cause of several disorders, including chronic gastritis, gastric ulcers, duodenal ulcers, and gastric adenocarcinomas. In addition, the prevalence of *H. pylori* is common. This study aimed to study the phenotypical analysis of peripheral T-cells and γδ-T-cells in *H. pylori* seropositive individuals.

Methods: A cross-sectional study was conducted on 450 participants at the College of Applied Medical Sciences at Taif University. ELISA was used to screen the participants for *H. pylori*. Then we select seropositive *H. pylori* participants for flow cytometry analysis of peripheral T cells and γδ-T-cells.

Results: 24 out of 450 (5.3%) were IgG-positive for H. pylori. We collected another sample to analyze the peripheral T-cells and γδ-T-cells in *H. pylori*-seropositive participants. The dot plots of CD4 and CD8 showed a slight increase among *H. pylori*-seropositive participants compared to the control group. At the same time, there was a significant increase in γδ-T-cells.

Conclusion: The current research showed a significant rise in γδ-T-cells, with a slight increase in CD4 and CD8 which were insignificant. Furthermore, among participants who tested seropositive for *H. pylori*, an increase in γδ-T- cells was observed in those experiencing H. pylori-related complications. This increase may play a crucial role in the excessive production of cytokines, ultimately leading to gastritis, stomach cancer, and the development of tumors.

Keywords: Helicobacter; cd8 t Helper; cd4 t Helper; γδ-t- Cells; *h pylori*

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative-bacilli-bacteria that infects humans' stomach [1]. *H. pylori* infection is the primary cause of several disorders, including chronic gastritis, gastric ulcers, duodenal ulcers, and gastric adenocarcinomas [2]. Furthermore, it is the most prevalent detected pathogen in gastritis patients [3]. And this bacterium is detected also in asymptomatic peptic ulcer cases [4]. Moreover, peptic ulcer can be developed also by nonsteroidal anti-inflammatory medication (NSAIDs) in combination with *H. pylori* [5]. *H. pylori* transmission occurs by several routes, low hygiene is conditions is the primary route, food and water, person-to-persons in intimate conditions or in the same households where exchanging personal equipment's like toothbrush can carry and transmit this bacterium [6-8]. These transmission routes increase the chance of transmission between family members [9]. According to Hooi et al study, around half of the population is reported to be infected by *H. pylori* during their life [10]. *H. pylori* is commonly found in in all countries world-wide [11]. Abd low socioeconomic conditions are assisting in expanding this bacterium [12].

A study has reported the prevalence of *H. pylori* infection in combination with dyspepsia in Jazan city in Saudi Arabia to be 46.5% [13]. Another recent study published in 2022 reported that the prevalence in the Al-Qasim region to be 24.9% [14]. In Riyadh, the prevalence of *H. pylori* infection was 40% [15].

H. pylori can be detected by two methods, invasive and non-invasive procedures. Invasive techniques include urease tests, histological examinations, and bacterial culture tests. Non-invasive techniques include serological testing, urea-breath test, and fecal antigen test [16].

Our study aims to investigate the phenotypical structure of peripheral blood mononuclear cells (PBMCs) T-cells and γδ-T-cells in *H. pylori* seropositive individuals and compare them with seronegative individuals.

Materials and Methods

Sample preparation and survey collection

This study is a cross-sectional study conducted on 450 participants at the College of Applied Medical Sciences at Taif University between May to July 2023. Included participants were asked several questions regarding the common symptoms of *H. Pylori*. Most seropositive individuals are not aware of being infected by *H. Pylori* or peptic ulcer even though they show those common symptoms. Therefore, we read through the literature to collect the most common symptoms and we asked every participant if they have shown any of those symptoms. Serum samples collection was processed according to the following, 3 mL of venous blood was collected (from 250 females and 200 Males) in a serum separator tube (SST tube) and centrifuged at 3500 rpm for 10 minutes. 300µL of the serum was separated from each sample, transferred into an Eppendorf tube, and stored at -80°C refrigerator. The serum was thawed once immediately before the test in a water bath at 37°C for 20 minutes. The participants were asked questions regarding common complications of *H. pylori* infection, which was specified in the survey. The study was performed according to the principles of the Helsinki Declaration and the ethics committee at Taif University IRB HAO-02-T-105 approved ethical approval for this study. In this study, we have included all students in our college between the ages of 18 to 26 years, with no history of *H. pylori* positive diagnosis, no history of gastric cancer, and no history of gastric bypass surgery. The exclusion was any participants who were under antibiotics medication, had a respiratory infection, and who used immunosuppressants.

H. pylori IgG ELISA Kit

The ENZYWELL HELICOBACTER PYLORI IgG kit (lot number D91060 and manufactured by the united diagnostic industry company) is a qualitative-immunoassay test and was for the purpose of detecting *H. pylori* IgG according to manufacture protocol. The Bio-Rad xMarkTM microplate spectrophotometer was used to read and incubate the plate. A 10µL of serum was diluted in 1 ml of sample buffer to give a ratio of 1:101, and 100µL from each of the positive and negative controls, calibrators, and diluted serum were added to the microplate wells. The plate was then incubated at 37°C for 45 minutes. After incubation, the wells were washed four times with 300µL of washing buffer per wash for 30 seconds. All residual wash buffer was removed by tapping the wells on absorbent paper. Enzyme conjugates were loaded into each well and incubated at 37°C for 45 minutes inside the Bio-Rad xMarkTM microplate spectrophotometer. The wells were emptied and thoroughly rewashed. Substrate solution was added to each well, and the plate was then incubated for 15 minutes at room temperature. The stop solution was then added to the wells in the same sequence and at the same speed as the substrate solution, and the microplate was gently shaken to ensure a uniform mixture. Finally, the plate was read at a wavelength of 450 nm.

PBMCs isolation and flow cytometry analysis

A 5 mL of venous blood was collected from participants, in an Ethylene- Diamine-Tetra-Acetic acid tube (EDTA tube) an equal amount of Ficoll-Paque's solution is added to each sample, then centrifuged at 2000 RPM for 20 minutes at 4°C with break-off to collect PBMCs. After that the cells were extracted and washed. Then supernatant layer is eliminated, and the PBMCs were gently resuspended in 1 mL of PBS. After that, different surface markers were selected to stain the PBMCs, as shown in table 1. The stained PBMCs were incubated for 30 minutes at 4°C in the dark, and then the stained polymorphonuclear was resuspended in FACS buffer (PBS, 5-10% FBS) for flow cytometry analysis. All experiments involved a set of single controls for every antibody used, which were simultaneously prepared in the same conditions.

Lymphocytes	Fluorochrome antibody	Concentration
CD3	APC	1 µg/ml
CD8	PE	
CD4	FITC	
γδ-T-cells	PECY7	

Table 1: The applied monoclonal antibodies.

Cluster of Differentiation (CD), Allophycocyanin (APC), Phycoerythrin (PE), Fluorescein isothiocyanate (FITC), Phycoerythrin and Allophycocyanin Cyanine (APC Cy7), Phycoerythrin Cy.7 (PECY7) Microgram per milliliter (µg/ml).

Data analysis

GraphPad Prism software, version 6.04, was used to analyze the data (La Jolla, CA, USA). For comparing seropositive to seronegative regarding levels of lymphocytes, the unpaired T-test was applied, and for comparing the complications Pearson-Chi square test was applied and statistically significant $p \leq 0.05$ was applied. The data were analyzed using FACS Diva software for flow cytometry analysis, and double discrimination was used to remove doublets. Aso, FlowJo software was used (Tree Star, Ashland, and Oregon) to analyze the flow data.

Results and Discussion

Among our study group, we have detected 24 *H. pylori*-seropositive individuals, 15 females and 9 males. Prior collecting the serum, we have asked the participants several questions to study the complications the feel. After running the ELISA test, we have compared

these complications between seropositive and seronegative individuals table 2. All seropositive individuals reported discomfort, burning sensation in their stomach, pain when they are hungry, and nausea.

Survey Questions	*H. pylori*		Chi-square
	Seropositive	Seronegative	
Are you experiencing any discomfort, such as an ache or burning sensation in your stomach?	24 (100%)	9 (2.1%)	0.001
Do you experience an increase in pain when your stomach is empty?	24 (100%)	6 (1.4%)	0.001
Are you experiencing frequent episodes of nausea?	24 (100%)	23 (5.4%)	0.03
Are you experiencing a loss of appetite?	15 (62.5%)	12 (2.8%)	0.03
Do you experience frequent episodes of burping?	18 (75%)	24 (5.6%)	0.001
Do you often experience bloating?	18 (75%)	3 (0.8%)	0.032
Are you experiencing unintentional weight loss?	12 (50%)	9 (2.1%)	0.04

Table 2: Participants common complications.

Participants provided survey responses regarding the common complications of *H. pylori* infection, *H. pylori* seropositive= 24 and *H. pylori* seronegative= 426.

Following that, we compared our study groups in terms of being seropositive and seronegative to evaluate the average levels of T-cells and γδ-T-cells. The dot plots shown in Figure 1A illustrate that the seropositive participant's CD8 and CD4 lymphocyte counts were higher than those of the healthy control, however, this was statical insignificant (figure 1B).

Also, our group have examined the total γδ-T-cells using flow cytometry to identify any differences in γδ-T-cells between the seropositive participants and seronegative controls. As shown in Figure 2A, the mean percentage of γδ-T-cells was higher in the seropositive participants than in the control group, and the variation was statistically significant ($p \leq 0.001$, Figure 2B). Furthermore, this indicates that γδ-T-cells e expression was relatively higher in the *H. pylori*-seropositive participants.

Several studies reported immune cell expansion in response to different infections and this leads to inflammation and in some cases damage to these several organs. Some studies focused on *H. pylori* and reported expansion in those cells. A study by Kondo, et al reported an increase of neutrophils and monocytes in the peripheral blood of *H. pylori*-infected patients [17]. *H. pylori*-specific CD4+ T cells fundamentally regulate host immunity and immunopathologic processes. Th1, Th2, Th9, Th17, Th22, and T regulatory (Treg) cells, can influence how an infection with *H. pylori* is man-

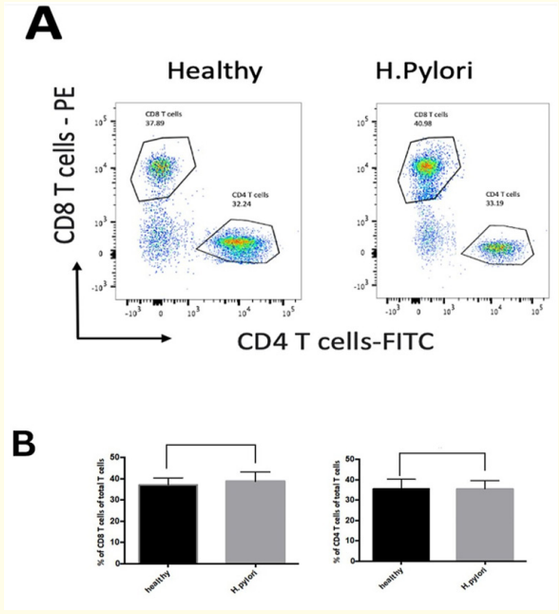

Figure 1: Levels of CD8 and CD4.

CD8 and CD4 T cells were isolated from the peripheral blood of seropositive participants and seronegative control. The T cells were stained with anti-CD8 and CD4 and then analyzed using flow cytometry. A) Representative dot plots showing the percentage of CD8 and CD4 T cells in the samples obtained from seropositive participants and control group. B) The percentage of CD8 and CD4 T cells in the samples obtained from seropositive participants and seronegative control Comparing the mean of seronegative and seropositive revealed an insignificant P value. Cluster of Differentiation (CD 4 and 8).

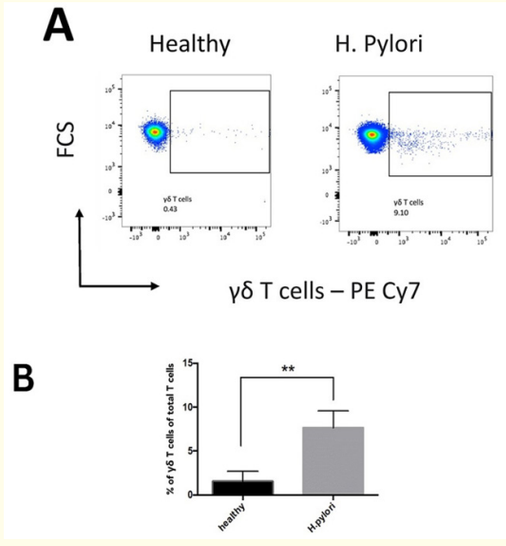

Figure 2: γδ-T-cells evaluation and comparison.

γδ-T-cells were isolated from the peripheral blood of seropositive participants and seronegative control. The cells were stained with anti- γδ-T-cells and analyzed using flow cytometry. A) Representative dot plots showing the percentage of γδ-T-cells in the samples obtained from seropositive participants and control group. B) The percentage of γδ-T-cells in the samples obtained from seropositive participants and seronegative control. Gamma delta T cells (γδ T cells).

aged [18]. A study by Bamford et. al argued that in situ CD4 and CD8 T cell expansion were seen in infected persons' gastric mucosa during *H. pylori* infection [19]. A comparison of the percentage of the CD4 and CD8 T cells showed no significant difference in the rate of CD4 T cells between *H. pylori*- seropositive patients and the control group. While the percentage of CD8 in *H. pylori*-seropositive patients was lower in the control group [20]. A study by Alhasnawi., *et al*. reported that patients with gastric cancer with *H. pylori* infection have significantly higher levels of CD4+ and CD8+ in their blood than healthy people (P-value < 0.05) [21]. However, in contrast to individuals infected with *H. pylori*, the same outcome was seen in stomach cancer patients with *H. pylori* infections. Hence, there were no considerable differences between individuals with stomach cancer who also have *H. pylori* infection and those with *H. pylori* infection alone regarding CD8+ concentration. Regarding early detection of stomach cancer, *H. pylori*, CD4+ and CD8+ can be considered reliable indicators [21]. Another study reported no significant differences in the peripheral lymphocytes, including CD8 and CD4 T cells, between *H. pylori*-infected patients and healthy controls [22]. The effective IFN-γ production by CD8 T cells in response to *H. pylori* stimulation shows that these cells are essential in the *H. pylori*-infected gastric mucosa. However, whether IFN-γ-secreting by CD8 T cells primarily induces inflammatory responses or if they serve any protective purposes is still unclear. CD8 T cells generated more IFN-γ in comparison to CD4 T cells [23].

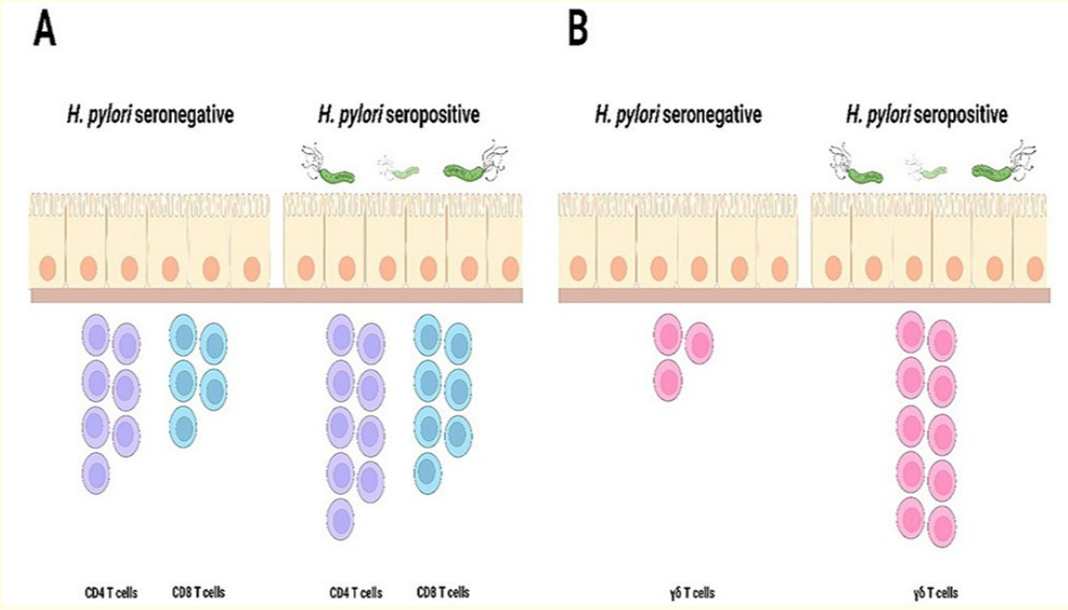

Figure 3: A schematic representation of what is occurring to the T cells population between the seropositive and seronegative Pylori participants.

There was a slight increase in CD4 and CD8 cells and a significant increase in γδ T cell populations among individuals who tested seropositive for H. pylori compared to the control group. Gamma delta T cells (γδ T cells), and Cluster of Differentiation (CD 4 and 8).

While our study showed a slight increase in CD8 and CD4 T cells (Figure 3A), we may detect similar findings if our sample size was more prominent.

γδ-T-cells accumulation in the gastric mucosa of *H. pylori*-infected individuals appears to be correlated with the degree of gastritis and infiltration of inflammatory cytokines. γδ-T-cells count significantly elevated in infected *H. pylori* patients with grade III gastritis, decreasing effectively after the eradication therapy.

Moreover, in the gastrointestinal mucosa, γδ-T-cells count was substantially linked with IL-1beta and IL-7 levels. While in peripheral γδ T lymphocytes, *H. pylori* urease stimulated the production of IFN-γ and IL-10 [24]. Our study revealed a significant rise in γδ-T-cells count (Figure 3B). This may suggest that γδ-T-cells count may differ according to the infected *H. pylori* patient's status and production of bacterial enzymes and cytokines. In addition, our study exhibited a significant increase in γδ-T-cells among participants who tested seropositive for *H. pylori* while also experiencing *H. pylori*-related complications, as demonstrated in table 2.

H. pylori-infected patients have higher levels of cytokines like IFN-γ, TNF-α, IL-1, IL-6, IL-7, IL-8, IL-10, IL- 17A, IL-18, IL-21, and IL-22 in their stomachs compared to healthy individuals [25-26]. The production of inflammatory and anti-inflammatory cytokines from several immune cell types, such as Th1, Th2, Th17, macrophages, monocytes, mast cells, and neutrophils, is often linked to the immunological response to the *H. pylori* infection in the stomach mucosa. Many of these cytokines, including TGF-β, IFN-γ, IFN-, CXCL12, CXCL4, IL1, IL2, IL6, IL10, IL17, and IL23 are released both at the site of infection and in the general blood circulation [27]. Moreover, the activation of signaling pathways linked to inflammation, gastric carcinogenesis, or tumor progression may be significantly influenced by TNF-α 26 The elevation in cytokine secretion may be associated with the overproduction of γδ-T-cells (Figure 4).

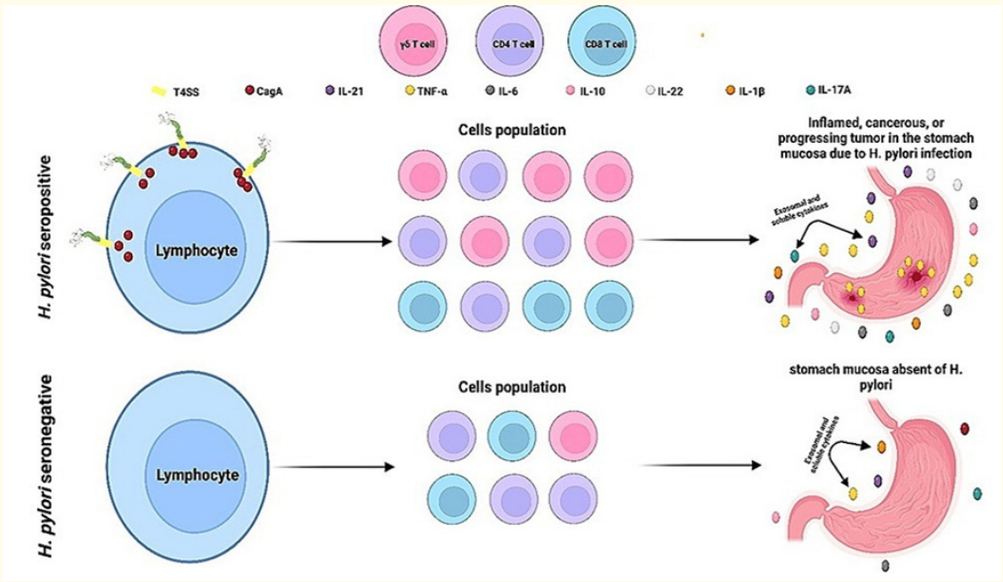

Figure 4: A schematic representation of the type of immune cells and their cytokine production in population between the seropositive and seronegative Pylori participants.

A suggestion in the relationship between γδ-T-cells and cytokines overproduction. Type IV secretion systems (T4SSs), Cytotoxin-associated gene-A (CagA), Interleukin-21 (IL-21), Tumour Necrosis Factor alpha (TNF alpha), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin-22 (IL-22), Interleukin-1 beta (IL-1 β), Interleukin-17A (IL- 17A).

Conclusion

The current research showed a significant rise in γδ-T-cells, with a slight increase in CD4 and CD8. Furthermore, among participants who tested seropositive for *H. pylori*, an increase in γδ-T-cells was observed in those experiencing *H. pylori*-related complications, as indicated in this study. This increase may play a crucial role in the excessive production of cytokines, ultimately leading to gastritis, peptic cancer, and the development of tumors. For this reason, it is necessary to conduct further studies with more participants and analyze the lymphocyte phenotype in co-culture with *H. pylori* and the cytokines detection to validate our findings.

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Conflict of Interest

No- financial interest or any conflict of interest exists.

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