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# Immune Cell Profiling in Peptic Ulcer Disease: Investigation of MAIT Cells and Key Inflammatory Cytokines IL-17 and IFN-γ

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## ABSTRACT

Peptic ulcer disease (PUD) is a common gastrointestinal disorder associated with chronic mucosal inflammation and immune dysregulation. Recent evidence suggests that Mucosal-associated invariant T (MAIT) cells, a subset of T cells expressing CD161 and Vα7.2, are involved in mucosal immunity and inflammatory responses. However, their precise role in PUD remains unclear. Given their dual function in immune defense and inflammation, investigating MAIT cell alterations in PUD could provide novel insights into disease pathogenesis and potential therapeutic targets.

A cross-sectional study was performed, including 25 patients with endoscopically verified PUD and 25 healthy controls. Flow cytometry was used to quantify circulating MAIT cells ($V\alpha7.2^{+}CD161^{++}$) and their subsets ($CD4^{+}$, $CD8^{+}$, and double-negative). Serum interleukin-17 (IL-17) and interferon-γ (IFN-γ) levels were measured using ELISA in 21 PUD patients and 21 healthy controls. Correlations between MAIT cell frequencies and cytokine levels were analyzed using the Spearman correlation coefficient.

PUD patients exhibited a substantially elevated frequency of circulating MAIT cells compared to controls. Among subsets, $CD8^{+}$ MAIT cells showed the most pronounced increase, correlating positively with serum IFN-γ levels (r=0.5819). Furthermore, levels of IL-17 and IFN-γ were substantially elevated in individuals with PUD in comparison to controls.

Our results indicate that MAIT cells, particularly the $CD8^{+}$ subset, may contribute to immune activation in PUD. The observed increase in MAIT cells, along with elevated IL-17 and IFN-γ levels, highlights their potential involvement in disease progression. These findings support the potential role of MAIT cells as biomarkers or therapeutic targets in PUD.

**Keywords:** Helicobacter pylori; Inflammation; Mucosal-associated invariant T (MAIT) cells; Peptic ulcer

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## INTRODUCTION

Peptic ulcer disease (PUD) is recognized as one of the most prevalent causes of mortality worldwide. Approximately 10% of individuals worldwide develop PUD throughout their lifetime.[1] This disease results from an imbalance of invasive components (acid, pepsin, and bacterial toxins) and gastric protective mechanisms. In fact, PUD occurs when the protective mucosal barrier is disrupted by aggressive factors, leading to acid-induced injury. In other words, stomach acid erodes the mucosal lining, creating an ulcer that exposes the submucosa. These ulcers predominantly occur in the stomach and duodenum but can also develop in the esophagus.[2]

Clinical symptoms of PUD vary and are often nonspecific. The most common symptoms include epigastric discomfort, bloating, nausea, heartburn, and back pain in cases of posterior ulcers. Symptoms typically improve with antacids, but can be asymptomatic in some patients. Complications, such as hemorrhage and perforation, are major contributors to mortality and morbidity in patients with PUD.[3]

Numerous risk factors have been found, including chronic stress, obesity, an unhealthy diet, *Helicobacter pylori* (*H pylori*) infection, and nonsteroidal anti-inflammatory drug (NSAID) use. Frequent use of low-dose aspirin and other medications can also contribute to ulcer development.[4] Among these risk factors, *H pylori* infection is the primary etiological factor in PUD.[1] This spiral-shaped, Gram-negative bacterium colonizes the gastric mucosa and infects more than 50% of the global population.[5]

Although most (>80%) infected patients remain asymptomatic, approximately 10% to 20% will progress to develop PUD.[6] The pathogenesis of *H pylori*-induced PUD is linked to its virulence factors, particularly CagA and VacA toxins, which trigger IL-8 secretion, neutrophil infiltration, and inflammatory damage. Recent investigations have elucidated the pivotal role of host immune responses in mucosal inflammation, resulting in PUD and gastric cancer (GC).[7] Among immune cell populations, mucosal-associated invariant T (MAIT) cells have gained attention due to their role in antibacterial immunity and mucosal homeostasis. MAIT cells express a T-cell receptor (TCR) with an invariant Vα7.2-Jα33 α-chain and high levels of CD161, distinguishing them as an innate-like T cell subset.[8]

The predominant population of human MAIT cells exhibits a $CD8^+$ phenotype, while $CD4^-CD8^-$ (double-negative [DN]) and $CD4^+$ subsets are less frequent.[9] MAIT cells recognize bacterial vitamin B precursors (riboflavin derivatives) presented by MHC-related 1 (MR1), which is restricted by major histocompatibility complex (MHC). These cells are found in mucosal surfaces, particularly in the lamina propria of the gut, as well as in lymph nodes, liver, and peripheral blood (where they constitute ~8% of all circulating T cells).[10] Upon activation, MAIT cells quickly generate proinflammatory cytokines, including interleukin-17 (IL-17), interferon-γ (IFN-γ), and tumor necrosis factor (TNF), playing a crucial role in bacterial immunity.[11,12] They may also be triggered in a TCR-independent manner by cytokines, such as IL-12 and IL-18.[13,14] Several studies have highlighted correlations between MAIT cell frequency and disease severity in chronic inflammatory conditions, including inflammatory bowel disease (IBD), multiple sclerosis (MS), celiac disease, and rheumatoid arthritis, suggesting a potential role in immune dysregulation.[15]

In patients with IBD, including Crohn disease and ulcerative colitis, phenotypic and functional analyses of MAIT cells have revealed a complex pattern of distribution. Peripheral blood MAIT cell frequencies were found to be decreased compared to healthy individuals, whereas MAIT cells were markedly enriched in inflamed intestinal tissues. These cells exhibited increased production of IL-22 and IL-17, but reduced IFN-γ secretion. Subset analysis showed a decreased proportion of $CD8^+$ MAIT and DN MAIT cells in patients compared to controls, while the proportion of $CD4^+$ MAIT cells was elevated.[9]

Similarly, alterations in MAIT cell frequencies have been reported in cancer patients. Eundeag Won et al observed that circulating MAIT cells were significantly reduced in patients with mucosa-associated cancers, such as gastric, hepatic, pulmonary, breast, and thyroid cancers, although their capacity to produce IFN-γ, IL-17, and TNF-α remained intact.[16] Chunyan Shao et al reported a marked decrease in peripheral blood MAIT cells in gastric cancer patients, with most cells being $CD8^+$ or DN and a small fraction being $CD4^+$.[17] Liwian et al demonstrated that circulating MAIT cells were significantly reduced in colorectal cancer patients, with $CD8^+$ and DN subsets predominating, but $CD4^+$ MAIT cells were notably increased compared to healthy controls.[18] Juanfen Mo et al further confirmed that $CD4^+CD8^-$, $CD4^-CD8^+$, and $CD4^-CD8^-$ MAIT cells


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were elevated in the peripheral blood of colorectal cancer patients, and CD161 expression in tumor tissues was higher than in adjacent non-tumor tissues. These findings highlight the dynamic redistribution and functional alterations of MAIT cells in inflammatory and neoplastic conditions, suggesting their potential role in disease pathogenesis. While their role in various inflammatory diseases has been studied, their contribution to PUD remains unclear. We hypothesized that MAIT cell frequencies and inflammatory cytokines would be increased in PUD patients compared to healthy controls. Therefore, we aimed to investigate the involvement of MAIT cells in PUD by analyzing their frequency, subpopulations, and cytokine levels in peripheral blood, which may provide novel insights into disease mechanisms and potential therapeutic targets.

## MATERIALS AND METHODS

### Study Population

A total of 25 patients with endoscopically confirmed PUD were recruited from Rouhani Hospital, Babol, Iran. A control group of 25 age- and sex-matched healthy individuals with no history of gastrointestinal disorders was also included. The average age of PUD patients was 50.92±15.75 years, while the average age of healthy people was 50.52±16.18 years (Table 1). Patients with chronic heart, kidney, lung, or liver diseases, prior gastric surgery were excluded. To eliminate the potential confounding influence of *H pylori* infection on immune cell profiles, all participants were screened for *H pylori* using enzyme-linked immunosorbent assay (ELISA). In both the patient and healthy control groups, only individuals who tested positive for *H pylori* were included. This matching strategy guaranteed that any immunological disparities between groups could not be attributed to variation in *H pylori* infection status. Patients who had taken NSAIDs within the last 2 weeks were excluded to avoid confounding effects on peptic ulcer development.

### Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated and stained with fluorescent-labeled monoclonal antibodies, including anti-TCR Vα7.2 (PE, BioLegend, USA, Cat# 351706, dilution: 5 µL/test), anti-CD161 (PerCP/Cyanin5.5, BioLegend, USA, Cat# 339908, dilution: 5 µL/test), anti-CD8α (FITC, BioLegend, USA, Cat# 301006, dilution: 5 µL/test), and anti-CD4 (APC, BioLegend, USA, Cat# 300514, dilution: 5 µL/test). The stained cells were fixed with 1% paraformaldehyde and analyzed using a FACS Calibur flow cytometer (BD Biosciences, USA). Gating was performed sequentially for lymphocytes (forward scatter [FSC] vs. side scatter [SSC]), single cells (forward scatter height vs. forward scatter area), and MAIT cells ($CD161^+$ TCR $V\alpha7.2^+$). Data were analyzed using FlowJo software (TreeStar, USA).

**Table 1. Baseline demographic characteristics of the study participants**

| Variable | Controls (n=25) | Patients (n=25) | *p* |
|---|---|---|---|
| Age, y | 50.52±16.18 | 50.92±15.75 | 0.91 |
| Gender (M/F) | 12/13 | 14/11 | 0.78 |

Age is presented as mean±standard deviation and was compared using an independent samples *t*-test.
Gender distribution is shown as count (male/female) and was analyzed using Fisher exact test. No statistically significant differences were observed between the two groups, indicating successful matching. Statistical analyses were performed using GraphPad Prism version 9. F: female; M: male.

### Enzyme-linked Immunosorbent Assay (ELISA)

Serum concentrations of IFN-γ and IL-17 were measured using Karmania Pars Gene ELISA kits (Rafsanjan, Iran,), following the manufacturer's instructions. Briefly, 50 µL of serum was added to wells pre-coated with monoclonal antibodies against human IFN-γ and IL-17 and incubated at 37°C for 60 minutes. The plates were washed 3 times with washing buffer before adding a conjugated detection antibody and incubating at 37°C for another 60 minutes. After 3 additional washes, 50 µL of Avidin-HRP solution was added, followed by a 30-minute incubation at 500 RPM. The reaction was developed using 50 µL of substrate solution in the dark, and stopped using concentrated $H_2SO_4$. Optical density was measured at 450 nm. Values below the limit of detection (LOD) were assigned half of the lowest detectable concentration; this approach, while commonly used, represents a potential limitation of the assay.

**Statistical Analysis**

Data were presented as median (first quartile to third quartile [Q1–Q3]) as applicable. The Shapiro-Wilk test was employed to evaluate the normality of data distribution. Due to the non-normal distribution of the data, group comparisons were conducted utilizing the Mann-Whitney U test. The Spearman rank correlation test was employed to evaluate correlations between variables. A *p* value of less than 0.05 was regarded as statistically significant. To control for multiple comparisons across different MAIT cell subsets and cytokines, Bonferroni correction was applied where appropriate. Statistical analyses were performed utilizing GraphPad Prism 9 (GraphPad Software, USA). The lower limit of detection (LOD) for IFN-γ and IL-17 was 5 pg/mL. For statistical analysis, values that were less than the LOD were reported as <5 and considered as 2.5 pg/mL (half the LOD).

## RESULTS

**MAIT Cell Phenotype and Frequency in the Peripheral Blood of PUD Patients**

Flow cytometry was used to assess the phenotype and frequency of MAIT cells in fresh peripheral blood (PB) from both healthy donors and PUD patients. MAIT cells were identified based on CD161 and TCR Vα7.2 expression and further characterized into subsets using CD4 and CD8 markers; the gating strategy for identifying $CD8^+$ MAIT cells, DN MAIT cells, and $CD4^+$ MAIT cells is shown in Figure 1A–B.

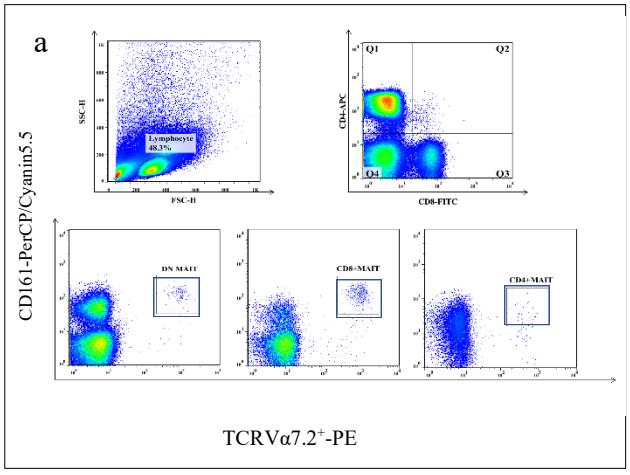
The proportion of $CD8^+$ MAIT cells in the PB of PUD patients (2.7 [1.63–4.3], n=25) was substantially increased in comparison to healthy donors (1.23 [0.75–1.95], n=25, $p<0.0002$). Similarly, the frequency of DN MAIT cells in PUD patients (0.37 [0.23–0.74]) was significantly elevated compared to that in healthy donors (0.15 [0.08–0.36], $p<0.004$). The $CD4^+$ MAIT cell subset was also significantly higher in patients with PUD (0.14 [0.1–0.16]) compared with healthy individuals (0.05 [0.03–0.1], $p<0.05$) (Figure 2).

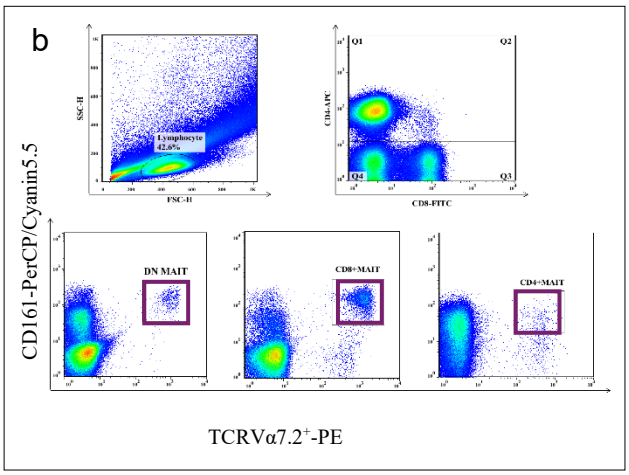
**Correlation Between Circulating MAIT Cells and Serum Cytokines in PUD Patients**

Serum levels of IL-17A and IFN-γ were significantly higher in PUD patients compared to healthy donors (IFN-γ: 14.33 [11.1–18.2] pg/mL vs. 6.21 [2.5–7.3] pg/mL; IL-17A: 28.7 [21.2–35.9] pg/mL vs. 9.8 [2.5–12.8] pg/mL, $p=0.0001$) (Figure 3).

A significant positive correlation was observed between serum IFN-γ levels and $CD8^+$ MAIT cell frequency in PUD patients (r=0.5819, $p=0.005$). Likewise, a significant positive correlation was observed between the frequency of $CD4^-CD8^-$ MAIT cells and serum IFN-γ levels (r=0.472, $p=0.031$), suggesting a potential role of this subset in IFN-γ–mediated inflammation in PUD. In contrast, the frequency of $CD4^+$ MAIT cells showed a trend toward a negative correlation with IFN-γ levels (r=−0.4299, $p=0.0517$), which was not statistically significant (Figure 4).

Additionally, IL-17A levels did not show a significant correlation with any of the MAIT cell subsets ($p>0.05$) (Figure 5). These findings suggest that $CD8^+$ MAIT cells may contribute to immune activation in PUD through IFN-γ production, potentially exacerbating inflammation and mucosal damage. In contrast, the lack of correlation between MAIT cell subsets and IL-17A suggests that other immune pathways may regulate IL-17A levels in PUD, independent of MAIT cell involvement. Further studies are required to clarify the mechanistic link between MAIT cells and cytokine-driven immune responses in PUD.



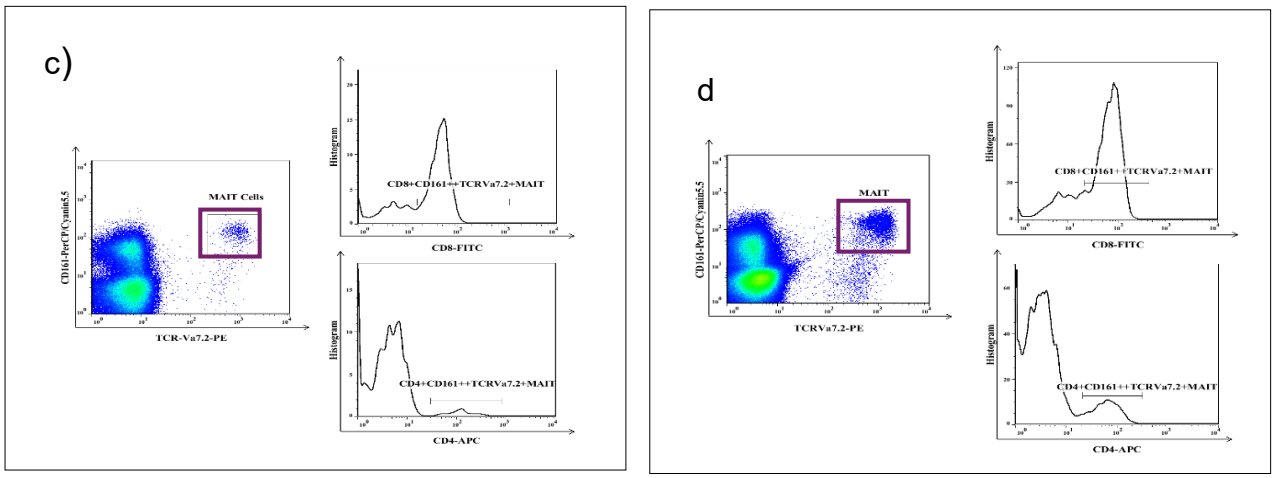



**Figure 1. A. Flow cytometry analysis of mucosal-associated invariant T (MAIT) cell subsets in peripheral blood from a healthy control (A) and a patient with peptic ulcer disease (B). Lymphocytes were gated based on FSC and SSC, then categorized into $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ (double-negative [DN]) populations. MAIT cells were identified as TCR $V\alpha7.2^+CD161^{++}$ within each subset. Data shown represent typical dot plots analyzed using FlowJo software. In the healthy control, the percentage of MAIT cells was: $CD4^+$ MAIT 0.03%, $CD8^+$ MAIT 1.29%, DN MAIT 0.15%. In the patient group, the percentages were: $CD4^+$ MAIT 0.5%, $CD8^+$ MAIT 4.1%, DN MAIT 0.2%. (n=25). B. Histogram plots corresponding to the flow cytometry data shown in Figure 1A, illustrating CD4 and CD8 expression within the gated MAIT (TCR $V\alpha7.2^+CD161^{++}$) cell population in a healthy control (C) and a patient with peptic ulcer disease (D).**

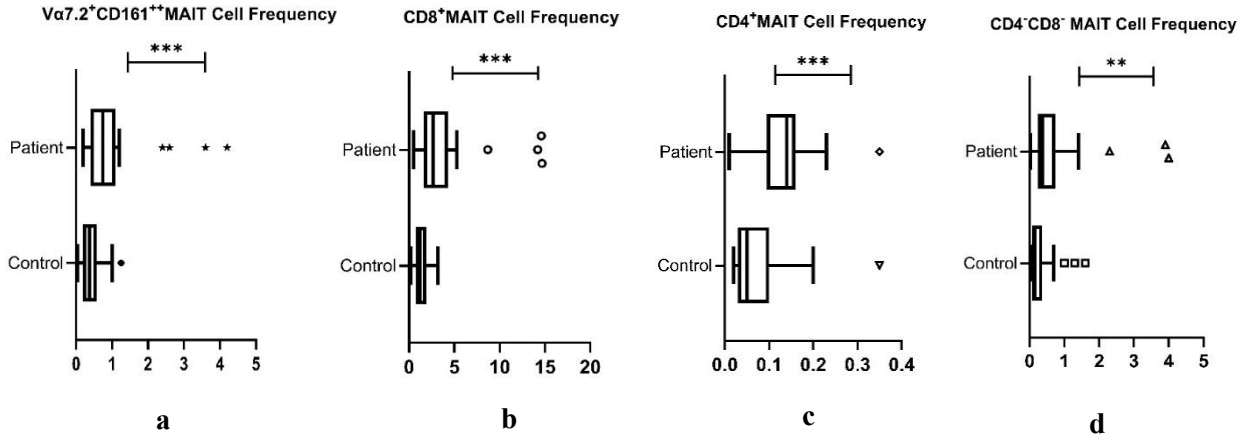



**Figure 2. MAI T c ell frequencies in the peripher al blood of healthy controls and patients with peptic ulcer disease. A. Frequency of total MAIT cells ($V\alpha7.2^+CD161^{++}$). B. $CD8^+$ MAIT cell frequency. C. $CD4^+$ MAIT cell frequency. D. Double-negative ($CD4^-CD8^-$) MAIT cell frequency. Data are presented as median (IQR). Statistical comparisons were performed using the Mann-Whitney U test. *$p$<0.05, **$p$<0.01, ***$p$<0.001, ****$p$<0.0001. Statistical analyses were performed using GraphPad Prism version 9. (n=25)**

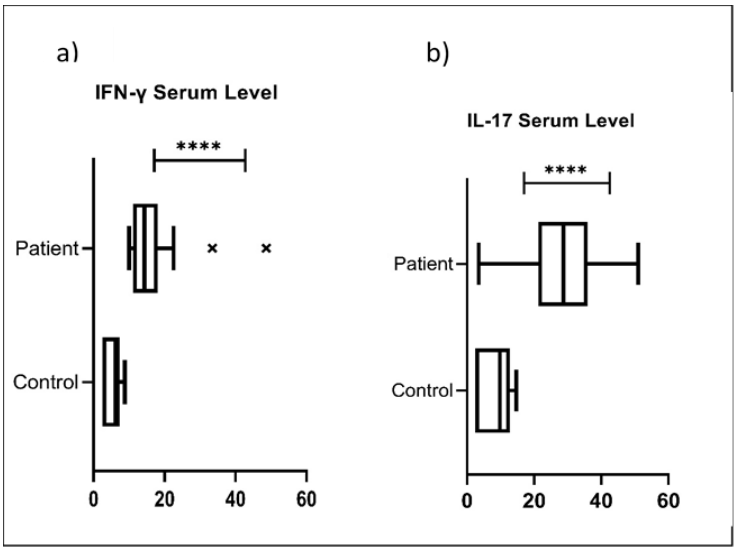



**Figure 3. Comparison of serum cytokine levels between healthy controls and patients with peptic ulcer. A. IFN-γ and B. IL-17 concentrations were significantly higher in the patient group. Results are expressed as median (IQR). Statistical significance was determined using the Mann-Whitney U test. **$p$<0.0001. Statistical analyses were performed using GraphPad Prism version 9. (n=21)**

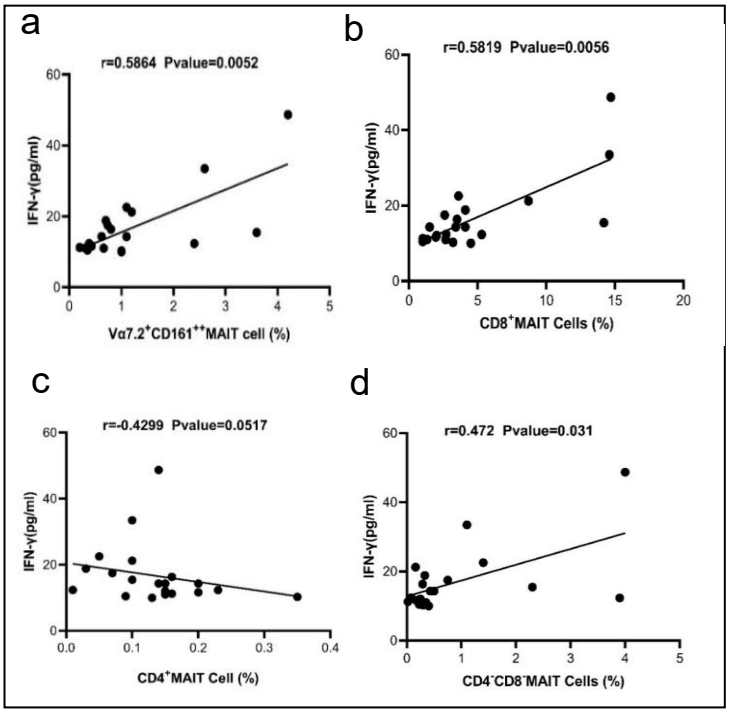



**Figure 4. Spearman correlation analysis between serum IFN-γ concentrations and the frequency of MAIT cell subsets in patients with gastric ulcer. A. A significant positive correlation was observed between IFN-γ levels and the percentage of Vα7.2$^{+}$CD161$^{++}$ MAIT cells (r=0.5864, $p$=0.0052). B. IFN-γ levels showed a strong positive correlation with CD8$^{+}$ MAIT cell frequency (r=0.5819, $p$=0.0056). C. A negative, but not statistically significant, correlation was detected between IFN-γ levels and CD4$^{+}$ MAIT cells (r=−0.4299, $p$=0.0517). D. A statistically significant positive correlation was found between IFN-γ levels and CD4$^{-}$CD8$^{-}$ MAIT cells (r=0.4712, $p$=0.031). *Statistical analyses were performed using GraphPad Prism version 9.**

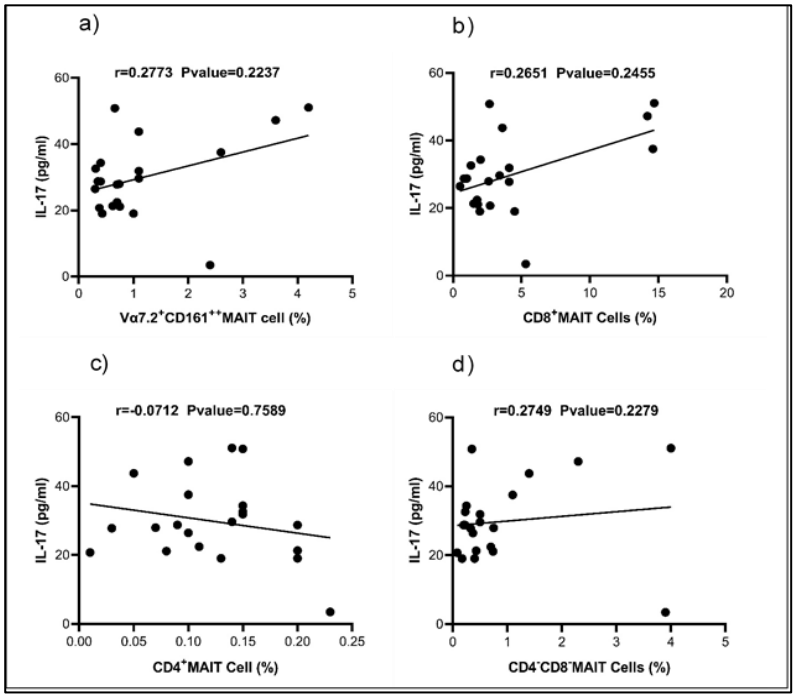



**Figure 5. Correlation between IL-17 serum levels and MAIT cell subsets in patients. Spearman's rank correlation analysis revealed no statistically significant associations between IL-17 levels and the frequency of A. Vα7.2$^{+}$CD161$^{++}$ MAIT cells (r=0.2773, *p*=0.2237), B. CD8$^{+}$ MAIT cells (r=0.2651, *p*=0.2455), C. CD4$^{+}$ MAIT cells (r=−0.0712, *p*=0.7589), or D. CD4$^{-}$CD8$^{-}$ MAIT cells (r=0.2749, *p*=0.2279). Despite the observed trends, none of the correlations reached statistical significance. *Statistical analyses were performed using GraphPad Prism version 9.**

## DISCUSSION

PUD is a prevalent gastrointestinal disorder worldwide, often leading to a reduced health-related quality of life. Chronic and untreated PUD is linked to a heightened risk of GC, which ranks as the fourth most prevalent malignancy worldwide and the second most common cause of cancer-related mortality.[7,19] Genetic predisposition, environmental factors, and persistent mucosal inflammation contribute to the development of PUD and its potential progression to GC. Chronic inflammation in PUD not only increases the risk of GC but also affects immune cell populations involved in mucosal defense.[20] Among these, MAIT cells have emerged as key players in mucosal immunity, yet their role in PUD remains poorly understood.

The purpose of this investigation was to quantify the frequency of MAIT cells and their subsets in the PB of PUD patients and to evaluate their functional involvement by assessing cytokine production, particularly IFN-γ and IL-17A. Understanding the distribution and activation of MAIT cells in inflammatory diseases like PUD can provide valuable insights into disease monitoring and potential therapeutic targets.

Our analysis revealed a substantial negative correlation between MAIT cell frequency and age in healthy individuals, while no significant gender-related differences were observed. Given these demographic influences, we next explored the distribution of MAIT cell subsets to determine whether specific populations play distinct roles in PUD pathogenesis. MAIT cells are categorized into three subsets according to the expression of CD4 and CD8 co-receptors: CD4$^{+}$CD8$^{-}$, CD4$^{-}$CD8$^{+}$, and CD4$^{-}$CD8$^{-}$ (double-negative, DN) MAIT cells. While all subsets can produce cytokines, CD8$^{+}$ MAIT cells have been reported to generate slightly higher levels than DN MAIT cells.[17] Our findings demonstrated an increased frequency of MAIT cells in the PB of PUD patients compared to healthy controls (Table 2). Although the overall frequency of MAIT cells was significantly increased in patients, subgroup analysis revealed that CD8$^{+}$ MAIT cells exhibited the most prominent expansion, followed by

$CD4^+$ and double-negative (DN) subsets. This differential expansion pattern may suggest a preferential activation or recruitment of specific MAIT subsets in response to disease-related stimuli. Given that $CD8^+$ MAIT cells are often associated with cytotoxic responses, their predominance might reflect a heightened local immune activity in the context of mucosal damage or inflammation.

**Table 2. Comparison of MAIT cell subpopulations and cytokine levels between control and patient groups**

| Parameter (Unit) | Control | Patient | *p* |
|---|---|---|---|
| TCR Vα7.2$^+$CD161$^{++}$ MAIT Cell (% of PBMC) | 0.37 (0.19–0.57) | 0.74 (0.4–1.1) | 0.0004 |
| $CD8^+$ TCR Vα7.2$^+$CD161$^{++}$ MAIT Cell (% of $CD8^+$ T Cells) | 1.23 (0.75–1.95) | 2.7 (1.63–4.3) | 0.0002 |
| $CD4^+$ TCR Vα7.2$^+$CD161$^{++}$ MAIT Cell (% of $CD4^+$ T Cells) | 0.05 (0.03–0.1) | 0.14 (0.1–0.16) | 0.0006 |
| $CD4^-CD8^-$ TCR Vα7.2$^+$CD161$^{++}$ MAIT Cell (% of DN T Cells) | 0.15 (0.08–0.36) | 0.37 (0.23–0.74) | 0.004 |
| IFN-γ (pg/mL) | 6.21 (2.5–7.3) | 14.33 (11.1–18.2) | <0.0001 |
| IL-17 (pg/mL) | 9.8 (2.5–12.8) | 28.7 (21.2–35.9) | <0.0001 |

Data are presented as median (25th–75th percentile). Differences between groups were analyzed using the Mann-Whitney U test. $p<0.05$ was considered statistically significant. *Statistical analyses were performed using GraphPad Prism version 9. CD4: cluster of differentiation 4; CD8: cluster of differentiation 8; DN: double-negative; IFN-γ: interferon-γ; IL-17: interleukin-17; MAIT: mucosal-associated invariant T; PBMC: peripheral blood mononuclear cell; TCR: T-cell receptor.

One possible explanation for the increased MAIT cell frequency in PUD patients is their activation in response to microbial antigens, particularly those derived from H. pylori. Previous studies have shown that MAIT cells can recognize bacterial metabolites via MR1-restricted antigen presentation, suggesting a potential link between persistent infection and immune activation.[21] Additionally, dendritic cells and macrophages, which are highly active in gastric inflammation, may contribute to MAIT cell activation through cytokine signaling, particularly IL-12 and IL-18.[15] Given this potential confounding role of H. pylori infection, in the present study we matched both the patient and control groups for H. pylori infection status to eliminate its influence as an intervening factor. Therefore, the observed differences in MAIT cell frequency between the two groups are more likely attributable to the presence of ulcerative lesions and the pathological processes associated with peptic ulcer disease itself, rather than to differences in microbial exposure.

Given the central role of IFNγ in modulating immune responses, its elevated levels in PUD patients suggest a potential contribution of MAIT cells to the inflammatory milieu of the gastric mucosa, potentially exacerbating ulcer formation and tissue damage.[22,23] Previous studies have indicated that peripheral blood MAIT cells predominantly produce IFNγ and TNFα, while IL-17A is often undetectable in healthy individuals.[24] However, in inflammatory conditions such as PUD, IL-17A production is significantly increased, making its detection feasible.[17] Since MAIT cells can secrete IL-17A, a cytokine also associated with Th17 cells, understanding the interplay between these two immune populations is crucial in the inflammatory response of PUD. Th17 cells, a subset of effector $CD4^+$ T helper (Th) cells, play a critical role in inducing tissue destruction and inflammation and are considered key markers of various immune-inflammatory diseases.[25] The balance between their pathogenic and regulatory functions is influenced by the presence or absence of IL-17A. In the absence of IL-17A, IL-22 can exert a protective function, whereas the synergistic action of these two cytokines can exacerbate inflammation.[26] A study by Shamsdin also demonstrated that IL-17A levels, similar to IL-22, were elevated in patients with PUD.[27] This suggests that the combined effect of IL-17A and IL-22 may contribute to heightened inflammation, leading to more severe tissue damage and degradation. This mechanism could explain why individuals with PUD experience more extensive inflammation and tissue injury compared to those with gastritis.[28] In line with previous studies indicating elevated levels of IL-17A and IFN-γ in inflammatory conditions such as PUD, our results demonstrated significantly increased concentrations of both cytokines in PUD patients compared to controls. Specifically, IL-17A and IFN-γ levels were approximately threefold higher in the patient group, reflecting a heightened pro-inflammatory state in the gastric mucosa (Table 2). Notably, we observed a

positive correlation between IFN-γ levels and the frequency of $CD8^+$ and DN MAIT cells, suggesting their active contribution to the local inflammatory response, possibly through cytotoxic or IFN-γ–mediated mechanisms. Conversely, a negative correlation was detected between IFN-γ levels and the $CD4^+$ MAIT subset, which may reflect the regulatory or immunosuppressive role previously attributed to $CD4^+$ MAIT cells in certain inflammatory contexts. Interestingly, no significant correlation was observed between IL-17 levels and any MAIT cell subset. This dissociation may reflect the biological characteristics and cellular sources of IL-17. IL-17 is produced by multiple immune cell populations, including Th17 cells, γδ T cells, and innate lymphoid cells, rather than exclusively by MAIT cells. Among these, Th17 cells are a key source of IL-17 in mucosal tissues and contribute significantly to systemic IL-17 levels in inflammatory conditions such as PUD, highlighting that circulating IL-17 may reflect combined input from several immune populations. Consequently, circulating IL-17 concentrations are influenced by diverse contributors and may not directly parallel MAIT cell frequencies. In addition, IL-17 primarily functions as a mucosa-associated cytokine and is predominantly generated locally at sites of tissue inflammation, where it mediates neutrophil recruitment and epithelial immune responses. Therefore, its systemic levels may not necessarily mirror local immune activity. Functional heterogeneity within MAIT subsets should also be considered, as $CD8^+$ MAIT cells predominantly produce IFN-γ, whereas IL-17 secretion is restricted to specific MAIT17 subsets. Collectively, these factors may explain the absence of a detectable association between MAIT cells and serum IL-17 in PUD.

These findings support the hypothesis that differential activation and function of MAIT cell subsets may modulate the cytokine milieu in PUD, and that the imbalance between pro-inflammatory and regulatory MAIT cells could play a role in disease severity and mucosal damage.

This study has some limitations. First, it is a cross-sectional study, which limits our ability to determine causal relationships between MAIT cell alterations and PUD progression. Second, our analysis was restricted to peripheral blood samples, and the role of MAIT cells within the gastric mucosa remains unexplored. Future studies should include gastric biopsy samples to better understand MAIT cell dynamics at the site of ulceration.

Another limitation of this investigation was the employment of the LOD or half the LOD (½ LOD) values to estimate the quantities of IFN-γ and IL-17 in samples with cytokine levels below the assay's detection threshold. This method, frequently utilized for quantities beneath the detection threshold, may compromise the accuracy of cytokine measurement and create potential bias. Consequently, the findings related to these markers must be approached with caution, and subsequent research is advised to employ more sensitive measuring techniques. Finally, more functional tests are required to determine whether MAIT cells are a direct cause of tissue damage or merely a consequence of persistent inflammation.

Moreover, future studies should explore whether targeting these cells could offer novel therapeutic approaches to mitigate ulcer-related tissue damage and improve disease outcomes.

## STATEMENT OF ETHICS

The Ethics Committee of Aja University of Medical Sciences approved the study (No: IR.AJAUMS.REC.1402.098). Written informed consent was obtained from all participants.

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This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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## DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## AI ASSISTANCE DISCLOSURE

The authors used Grammarly (Grammarly Inc.) and QuillBot for language editing and paraphrasing assistance.

## REFERENCES


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