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Diagnostic potential of monocyte subsets, TNF-α, and IL-6 in pediatric celiac disease: A case-control study

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ABSTRACT

Celiac disease (CD) is a chronic autoimmune disorder of the small intestine, which is triggered by dietary gluten, especially in individuals with a genetic instinct. Monocytes play an important role in modifying intestinal immunity and inflammation, yet the importance of their subgroups in CD is not clear.

Methods: The case-control study was held at the Pediatric Outpatient Clinic of Minia University Hospital, including 57 CD patients and 29 age- and sex-matched healthy controls. Clinical examination, laboratory check, and history were demonstrated for all participants. The serum levels of the IL-6 and TNF-α cytokines were measured using ELISA, and most analysis was done using flow cytometry. The variable with significant differences was further evaluated for its clinical ability.

Results: Monocytes are more prevalent in CD patients than in controls. In the case group, the average level of monocyte CD14+/CD16+ and CD14–/CD16+ was much higher than in the control group of CD patients (P-value <0.001). Additionally, CD patients who tested positive for antibodies had much higher levels of certain monocyte types compared to those who tested negative (p-values of 0.003, 0.011, and 0.001, respectively). Cytokines were not balanced, as levels of TNF-α and IL-6 were much higher in CD patients than in the control group. There was a significant positive relationship (p-value <0.001) between different types of monocytes and the amounts of autoantibodies, TNF-α, and IL-6.

Conclusion: TNF-α, IL-6, and certain types of monocytes could be useful indicators for diagnosing CD, as we found important differences between the groups we studied.

1. Introduction

Celiac disease (CD) is a chronic, persistent autoimmune disorder that affects the small intestine. It is also a common and complicated inflammatory condition marked by the presence of the autoantigen tissue transglutaminase (tTG) (Giacomo Caio et al., 2019; LópezCasado et al., 2018). The global prevalence of CD ranges between 0.5 % and 1 %, and it is increasing due to the widespread, unregulated consumption of high-gluten diets (Ludvigsson and Murray, 2019). CD is associated with various nonspecific clinical symptoms, some of which may be asymptomatic, while others present with signs of malabsorption such as diarrhea, abdominal pain, and malnutrition, which pose challenges to diagnosis despite the availability of sensitive serological screening tests (Hujoel et al., 2019; Lebwohl et al., 2018; Ludvigsson et al., 2013a). Additionally, non-digestive symptoms, such as anemia, are prevalent in a significant number of patients and may go undiagnosed, especially in the early stages. (Lebwohl et al., 2018; Molberg et al., 2015). Positive celiac serology combined with a normal small intestinal mucosa suggests potential celiac disease. However, the accuracy of the diagnosis heavily depends on the adequacy of the intestinal biopsy. There can be variability in the adequacy of biopsies due to technical factors such as the size of the sample or the site from which it is taken. Individuals with positive serology and inadequate biopsies may be at risk of misdiagnosis or delayed diagnosis, increasing the likelihood of a false-negative result. In a small number of cases, malignancy is a potential complication (Ludvigsson et al., 2013a). Intestinal biopsies, though invasive, are often required to confirm the diagnosis of CD (Catassi and Fasano, 2010). Thus, it is crucial to establish specific diagnostic tests to confirm CD in

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the early stages following initial screening.

Celiac disease (CD) happens when the immune system reacts to gluten and transglutaminase 2 (TG2), which includes T cells and special B cells related to the disease (Kagnoff, 2007). The role of innate immune cells in CD is often overlooked, despite their ability to regulate the adaptive immune response and inflammation (Gianfrani et al., 2005). Innate immune cells, such as monocytes and macrophages, are involved in the pathogenesis of many autoimmune diseases, as well as in tissue repair and immune response regulation during inflammation (Giacomo Caio et al., 2019; Ernst et al., 2014). After entering the lamina propria and turning into macrophages, monocytes either connect directly with intestinal epithelial cells (IECs) or release cytokines, both of which are important for intestinal immunity (Bonnardel et al., 2015). When monocytes from CD patients exposed to gluten are isolated, they produce higher levels of pro-inflammatory cytokines such as TNF-α and IL-6. These cytokines, in turn, can stimulate normal monocytes to produce even more pro-inflammatory cytokines (Harris et al., 2010). In chronic CD, the number of non-classical/intermediate monocytes increases. Therefore, manipulating the abundance and activity of monocyte subsets could help modulate disease pathogenesis (McGettrick et al., 2012). Monocytes from CD patients could be immunophenotyped, and their production of pro- and anti-inflammatory cytokines assessed, to evaluate their contribution to CD pathogenesis, enhancing their diagnostic value. Consequently, this study aims to assess the diagnostic utility of variables that exhibit significant intergroup variation.

2. Materials and methods

From the Pediatrics Department, we gathered 57 patients with celiac disease (42 males and 15 females), along with 29 healthy volunteers matched by age and sex. The patients were identified based on medical checkups, their health history, and positive anti-transglutaminase antibodies, with small intestine biopsies confirming the diagnosis. The patients were chosen based on international guidelines from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) and the American College of Gastroenterology (ACG) for diagnosing celiac disease, which require positive blood tests, symptoms, and a biopsy to confirm the condition. The study was approved by the Ethical Research Committee of Minia University (Approval No. 1048/02/24) and conducted according to ethical guidelines and regulations.

Patients with inflammatory, infectious, immunodeficiency, malignant, or other autoimmune diseases were excluded from the study. The anti-transglutaminase antibodies of the celiac patients were further divided into two subgroups: the positive antibody group (49 patients) and the negative antibody group (8 patients). Informed consent was obtained from all participants. The Local Research Ethics Committee of Minia Medical College also approved the study.

Blood tests were performed on both patients and healthy people, which included a Complete Blood Count (CBC), checking certain immune cell types (CD14 and CD16), and measuring levels of serum IgA anti-TG2, IL-6, and TNF-α. Approximately 5 ml of blood was collected from each participant. Two ml of blood was anticoagulated for CBC and immunophenotyping, while the remaining 3 ml was used for serum separation, with sera stored at −80 °C until needed. The levels of IgA anti-TG2 in the serum were tested using a method called enzyme-linked immunosorbent assay (ELISA) from AESKULISA, Germany, following the instructions provided by the manufacturer. IL-6 and TNF-α levels were determined using ELISA kits (BioLegend).

Monocyte immunophenotyping was performed using direct immunofluorescence with fluorescent antibodies that focus on CD14 and CD16 antigens (provided by Kemet Medical). The data were collected using a BD-FACS FLOW laser with an Argon source (U.S.A.). Monocytes were divided into three groups based on how they scatter light: classical ($CD14^{+}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$), and non-classical ($CD14^{-}CD16^{+}$), using a standard method. We used flow cytometry with the Argon laser from the BD-FACS FLOW system for this process, and we analyzed the data with Diva software. To find the values of monocyte subsets (shown in Table 2), we calculated the percentage of cells that showed CD14 and CD16 from all the monocytes in the sample. Cells were considered positive if the percentage exceeded 20 % in flow cytometry analysis.

2.1. Immunophenotyping of Monocyte subsets

To analyze the **monocyte subsets**, we employed **flow cytometry**. The following antibodies were used to identify and differentiate the subsets:

- **CD14** (FITC-conjugated, Clone: 63D3, BioLegend)
- **CD16** (PE-conjugated, Clone: 3G8, BioLegend)
- **CD45** (APC-conjugated, Clone: 2D1, BioLegend)
- **Live/Dead stain** (Zombie NIR, BioLegend) was used to exclude dead cells.

2.2. Antibody fluorescence details

Each antibody was conjugated to a specific fluorochrome to allow for distinct detection of cell populations based on their fluorescence emissions:

1. **CD14**:
   - o Fluorochrome: **FITC** (Fluorescein Isothiocyanate)
     oEmission: **520 nm**
     oThis antibody targets the **CD14 antigen** present on monocytes, allowing for the identification of the **$CD14^{+}$** population.
2. **CD16**:
   oFluorochrome: **PE** (Phycoerythrin)
   oEmission: **578 nm**
   oThis antibody targets **CD16**, which is expressed on a subset of monocytes and neutrophils, allowing differentiation between monocyte subsets.
3. **CD45**:
   oFluorochrome: **APC** (Allophycocyanin)
   oEmission: **660 nm**
   oThis antibody is used as a **pan-leukocyte marker** to confirm that the cells being analyzed are part of the leukocyte population, helping to exclude non-immune cells.
4. Zombie NIR (Live/Dead staining):
   oFluorochrome: **Zombie NIR**
   oEmission: **780 nm**
   oThis stain allows for the exclusion of dead cells from the analysis by binding to the dead cell membranes, ensuring only live cells are included in the gating strategy.

Table 1
Demographic data and anthropometric measures of the case and control groups.

Variables	Case Group (N = 57)	Control Group (N = 29)	Test of Significance	P Value
Age (years)	10.35 ± 3.22	11.42 ± 3.19	t = −1.265	0.172
Gender:				
- Male	42 (73.86 %)	15 (51.7 %)	$\chi^2 = 0.271$	0.615
- Female	15 (26.31 %)	14 (49.3 %)		
Weight (cm)	36.34 ± 12.91	35.13 ± 11.57	z = −0.202	0.827
Height (kg)	134.17 ± 13.76	141.72 ± 15.63	t = −0.343	0.721
BMI (kg/m^2)	17.93 ± 3.06	17.48 ± 2.98	t = −0.831	0.399

T-test: Used for comparing the means between two independent groups.
Chi-square (χ^2): Used for categorical data to assess differences in proportions between the groups.
Mann-Whitney *U* test (z): Non-parametric test for comparing two independent groups when data does not follow a normal distribution.

Table 2
Laboratory data of case and control groups.

Variables	Case Group (N = 57)	Control Group (N = 29)	Test of Significance	P Value
WBCs (103/fl)	8.26 ± 1.24	7.96 ± 1.19	t = 0.455	0.455
Platelets (103/fl)	249.38 ± 68.14	252 ± 57.33	t = 0.539	0.539
Monocytes (103/fl)	0.56 ± 0.23	0.66 ± 0.17	z = −0.154	0.154
TTG IgA	15.16 ± 6.54	2.51 ± 0.99	t = −0.001	<0.001*
TTG IgG	53.69 ± 19.43	2.61 ± 1.40	t = −0.001	<0.001*
CD14+/CD16−	93.61 ± 3.01	72.37 ± 6.55	t = −0.001	<0.001*
CD14+/CD16+	7.19 ± 1.08	4.99 ± 0.58	t = −0.001	<0.001*
CD14−/CD16+	14.58 ± 4.92	6.81 ± 0.94	t = −0.001	<0.001*
TNF (pg/ml)	8.3 ± 17.4	0.3 ± 1.1	t = −0.001	<0.001*
IL-6 (pg/ml)	2.9 ± 1.7	0.1 ± 0.6	t = −0.001	<0.001*

2.3. Sample preparation

- Cells were incubated with the above antibodies at a concentration of **1 µg per 100 µL** for 30 min at **4°C** in the dark. After the incubation period, cells were washed with **phosphate-buffered saline (PBS)** to remove excess antibody.
- To identify live cells, **Zombie NIR** was used for **live/dead cell staining** prior to flow cytometry analysis.

2.4. Flow cytometry analysis

The samples were analyzed on a **BD FACSCanto™ II Flow Cytometer**. Gating was performed to identify the monocyte subsets based on the following strategy:

1. Live Cells Selection:
   oUsing the **Zombie NIR stain**, **dead cells** were excluded from the analysis.
2. Monocyte Identification:
   oMonocytes were identified based on the **CD14** and **CD16** markers.
   - **CD14+/CD16−** monocytes were gated as **$CD14^+$** and **$CD16^-$**.
   - **CD14+/CD16+** monocytes were gated as **$CD14^+$** and **$CD16^+$**.
   - **CD14−/CD16+** monocytes were gated as **$CD14^-$** and **$CD16^+$**.

2.5. Representative flow cytometry plot

A representative **flow cytometry plot** showing the **gating strategy** for identifying the monocyte subsets (CD14+/CD16−, CD14+/CD16+, CD14−/CD16+) is presented in Fig. 1.

2.6. Statistics

Given the small sample size and the detailed analysis of predictor factors, Bayesian methods were employed to ensure accuracy. Traditional estimates, such as maximum likelihood estimates (MLE), often have similar prognostic validity to Bayesian estimates based on just 25 % of the sample, as noted in references 15 and 16. Bayesian techniques provide more consistent distributions. A total of 17 Markov Chain Monte Carlo (MCMC) methods were used to model the posterior distribution's random variables. The Markov chain's current value determines the next value for each parameter vector, independent of previous values. Another crucial feature of this approach is the iterative improvement in the approximation of the posterior distribution through multiple simulation runs. This method allows for highly accurate approximations of posterior distributions. To address the issue of convergence in MCMC simulations, we used the autocorrelation function and the Gelman-Rubin diagnostic to estimate autocorrelation for each node.

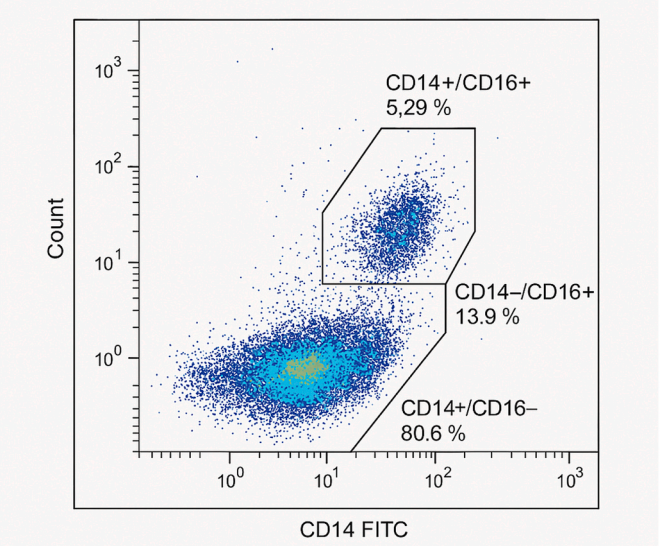

Fig. 1. A representative flow cytometry plot.

3. Results

In this study, the average age of children with celiac disease was 10.35 ± 3.22 years. Regarding the determination of monocyte subgroups, there are no specific age recommendations for this test. However, it is usually recommended to check monocyte subgroups in patients showing signs of celiac disease, especially if their blood tests are positive. While celiac disease diagnosis can take several years as other pathologies are ruled out, once diagnosed, monitoring monocyte subgroups could be performed at regular intervals, such as annually or when there are significant changes in clinical status. Such monitoring would help us understand the disease progression and therapeutic response. Table 1 shows that there were no statistically significant differences between the groups in terms of average age and anthropometric measurements, including weight, height, and body mass index (BMI). The majority of participants in both the patient group (73.7 %) and the control group (51.7 %) were male, demonstrating a high degree of similarity between the two groups in terms of gender distribution.

In this study, we examined various laboratory data from both the **case group** (celiac disease patients) and the **control group** (healthy individuals) to identify potential biomarkers for celiac disease. The analysis examines key factors such as white blood cell count (WBCs), platelet count, monocyte count, tissue transglutaminase antibody (TTG IGA and TTG IGG), and various types of monocytes, which are important for understanding the immune response linked to the disease. The results indicated that WBC Count and Platelet Count did not show significant differences between the two groups, with P-values of 0.455 and 0.539, respectively. This conclusion shows that these markers may not be useful to separate patients with celiac disease from healthy individuals. However, there were important differences in the levels of Monocyte Most, TTG IGA, TTG IGG, TNF-α, and IL-6, showing that these biomarkers can be very helpful in diagnosing and understanding celiac disease. In particular, the TTG IGA and TTG IGG were highly elevated in the case group, with a P-value of <0.001; their role as a clinical marker for celiac disease was confirmed. Furthermore, monocytes are the most common type, especially the CD14+/CD16, CD14+/CD16+, and CD14−/CD16+ groups, which showed significant changes compared to the control group of patients with celiac disease, with a P-value of less than 0.001, indicating that these specific types of cells in celiac disease could be important to study. The level of TNF-α and IL-6 was also high in

the case group (P-value <0.001), which reflects the inflammatory nature of celiac disease. These findings indicate that the level of monocytes is the highest, and cytokines can serve as a useful marker to understand the immune system in celiac disease and assist in its diagnosis. The detailed laboratory data and statistical analysis are presented in Table 2, which summarizes the results for all the biomarkers and their significance.

- **WBCs (White Blood Cells):** White blood cell count in thousands per microliter.
- **Platelets:** Platelet count in thousands per microliter.
- **Monocytes:** Monocyte count in thousands per microliter.
- **TTG IgA and TTG IgG:** Serum levels of tissue transglutaminase IgA and IgG antibodies.
- **CD14+/CD16−, CD14+/CD16+, CD14−/CD16+:** Percentages of different monocyte subsets.
- **TNF-α (Tumor Necrosis Factor Alpha):** Serum levels of TNF-α, a pro-inflammatory cytokine.
- **IL-6 (Interleukin 6):** Serum levels of IL-6, another cytokine involved in inflammation.

3.1. Statistical tests

- ***t*-test:** Used to compare the means between the two independent groups.
- **Mann-Whitney *U* test (z):** Non-parametric test for comparing two independent groups when the data does not follow a normal distribution.

In this study, we also examined the validity of monocytes as a clinical biomarker for celiac disease. In particular, we evaluated CD14+/CD16–, CD14+/CD16+, and CD14–/CD16+. We found that monocytes were the most effective in distinguishing between cases of celiac disease and healthy controls. The clinical accuracy of each test was assessed using the area under the curve (AUC), and other measures like sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine how useful they are in a clinical setting. The AUC values for the monocyte were found to be high, with CD14+/CD16– 0.749, CD14+/CD16 + 0.991, and CD14–/CD16 + 0.990, indicating strong diagnostic performance. The 95 % confidence interval for each AUC (CI) was also calculated; a high level of accuracy was demonstrated in the clinical capabilities of these highest. Additionally, cut-off points were prescribed for each monocyte of the heroes, with CD14+/CD16+> 80.67, CD14+/CD16+ > 6.98, and CD14–/CD16+ > 6.32, which can be used to separate seal disease cases from control. The sensitivity to monocytes ranges from 75 % to 100 % for CD14+/CD16-, suggesting that these markers can accurately identify a significant proportion of celiac disease cases. The specificity was also high, with 72 % CD14+/CD16+, 97 % CD14+/CD16+, and 92.9 % CD14–/CD16+, showing that these markers can effectively identify healthy individuals. The accuracy of the multitude ranged from 76 % for CD14+/CD16+ to 97 % for CD14+/CD16+, which supports their clinical capacity. Finally, both PPV and NPV showed strong values, further confirming their clinical credibility. These findings highlight that monocytes, the biggest type of white blood cells, are good at finding celiac disease and suggest that these markers can greatly improve the diagnosis of the condition. The results from the ROC analysis and clinical measurements strongly support using these monocytes in identifying celiac disease. Table 3 gives a clear overview of how well different types of monocytes perform in clinical settings, including AUC values, sensitivity, specificity, and other diagnostic measures for each subtype.

- **AUC (Area Under the Curve):** This value represents the diagnostic accuracy of the monocyte subsets. Higher AUC values indicate better diagnostic performance.

Table 3
Validity of monocyte subsets for identifying celiac disease cases among the studied group.

Variables	CD14+/ CD16−	CD14+/ CD16+	CD14−/ CD16+	P Value
AUC	0.749	0.991	0.990	<0.001*
95 % CI of AUC	0.642–0.879	0.980–1	0.984–1	
Cut-off Point	>80.67	>6.98	>6.32	
Sensitivity	75 %	100 %	95.6 %	
Specificity	72 %	97 %	92.9 %	
Accuracy	76 %	97 %	95 %	
PPV (Positive Predictive Value)	71 %	100 %	88 %	
NPV (Negative Predictive Value)	68 %	92 %	91 %	

- **95 % CI of AUC (Confidence Interval of AUC):** The confidence interval for the AUC value, indicating the range within which the true AUC lies.
- **Cut-off Point:** The threshold for each monocyte subset to differentiate between celiac disease cases and control subjects.
- **Sensitivity:** The proportion of true positives correctly identified by the marker.
- **Specificity:** The proportion of true negatives correctly identified by the marker.
- **Accuracy:** The overall diagnostic accuracy of the monocyte subsets.
- **PPV (Positive Predictive Value):** The probability that a positive test result indicates the presence of celiac disease.
- **NPV (Negative Predictive Value):** The probability that a negative test result indicates the absence of celiac disease.
- **AUC:** Area Under the Curve to assess the accuracy of the diagnostic tests.
- **P Value:** The statistical significance of the results, with a value of less than 0.05 considered statistically significant.

In this study, we analyzed the relationship between various monocytes and major biomarkers associated with celiac disease. The data was gathered using two main methods: an ROC (receiver operating characteristics) curve to evaluate how well monocyte matrix and monocyte most can identify celiac disease and to measure the strength of the link between monocyte most and biomarkers. We discovered the correspondence between monocytes (CD14+/CD16–, CD14+/CD16+, and CD14– $CD16^{+}$) and Cytokines like TNF-α and IL-6, as well as autoantibodies like TTG IGA and TTG IGG. Correlation matrix provides a comprehensive observation of these relationships, in which each pair is represented by a correlation coefficient (R-Man) with the variables. The matrix also uses the intensity of the color to indicate the strength of the correlation, where positive correlations are represented in warm colors and negative correlations are represented by cooler colors. Statistically significant correlations are highlighted, providing insight into which markers are closely connected to each other.

As shown in Fig. 2, the correlation matrix clearly shows the relationship between monocytes and biomarkers. Important correlations highlight the potential interaction between the immune response markers, which provide useful data to understand the pathophysiology of celiac disease. Next, we looked at how useful monocytes are in distinguishing celiac disease cases from healthy individuals by using the ROC curve. The ROC curve plots the true positive rate (TPR) against the false positive rate (FPR) for various cutoff values. The area under the curve (AUC) is used as a measure of clinical accuracy, indicating better performance in differences between two groups with a high AUC. As shown in Fig. 3, the CD14+/CD16 at monocytes displays its ability as an effective diagnostic marker for Celiac disease in most monocytes. The calculated AUC provides a quantitative measurement of its clinical accuracy and suggests a strong ability to discriminate between high-value cases and controls.

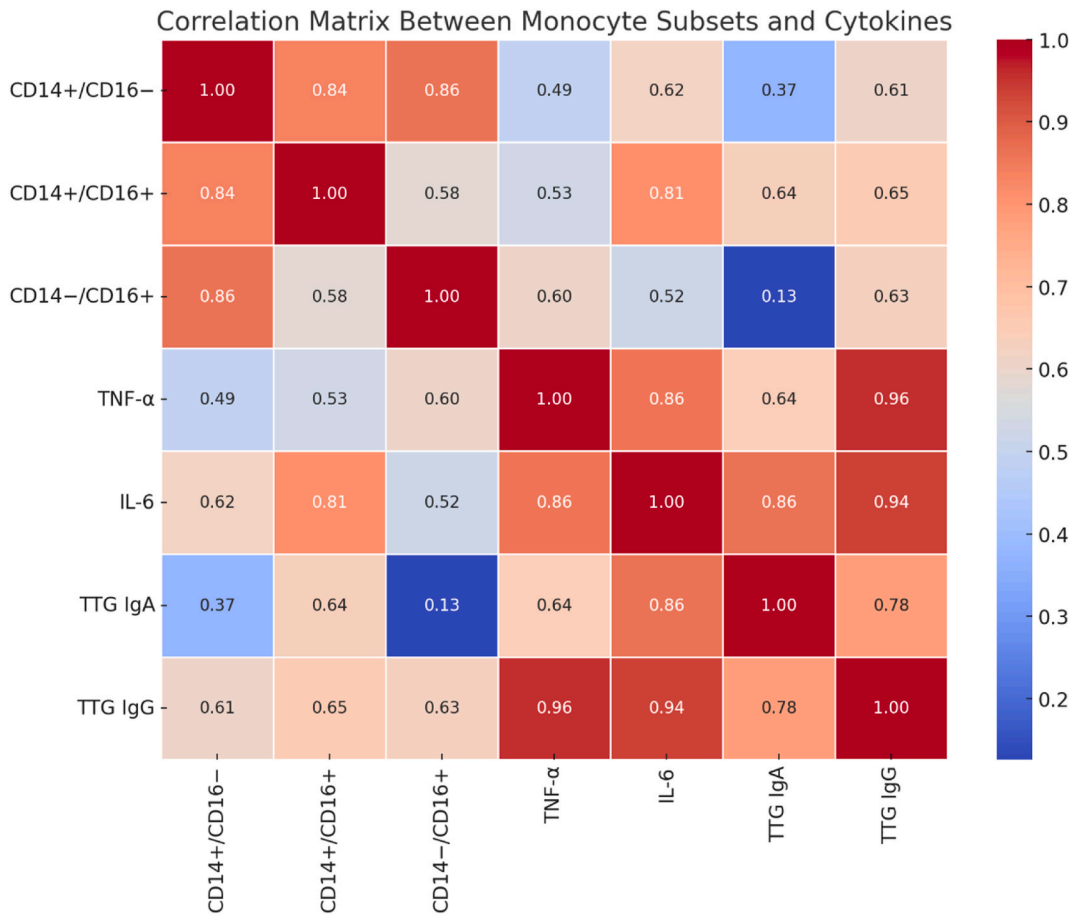

Fig. 2. Correlation Matrix Monocyte Most (CD14+/CD16-, CD14+/CD16+, CD14–/CD16+) and Cytokines (TNF-α, IL-6) as well as Autoantibody (TTG IGA, TTG iGG). The intensity of the color indicates the strength of the correlation.

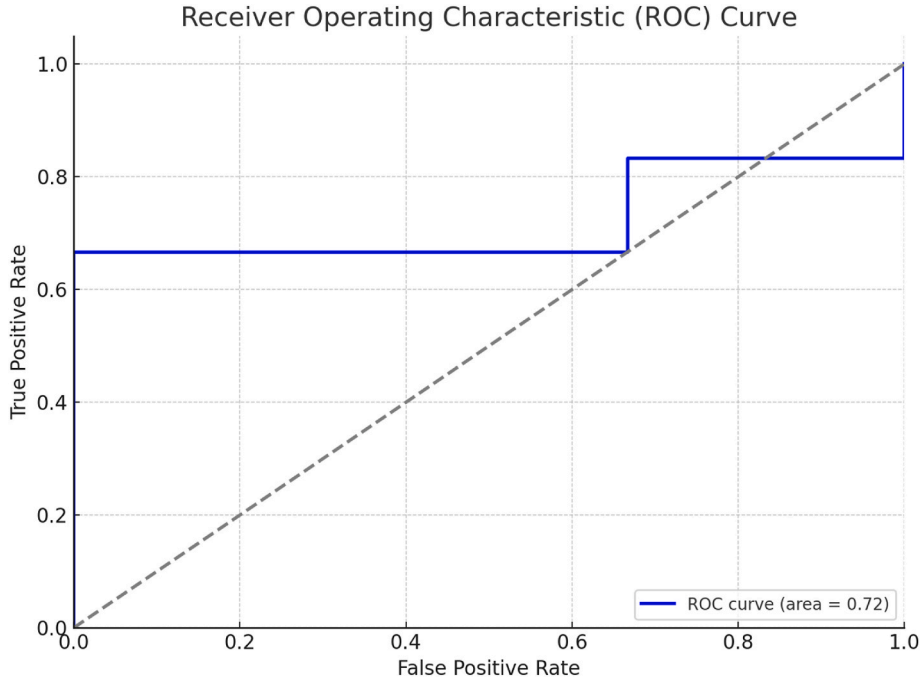

Fig. 3. CD14+/CD16 AC for receiver operating characteristics (ROC), Monocyte Most in separating cases of celiac disease from control subjects. The area under the curve (AUC) represents clinical accuracy.

In this study, we examined the ability of monocytes, the heaviest, to differentiate between celiac disease patients with positive antibodies and negative antibodies. Table 4 presents the monocyte with the highest percentage between these two groups, which was classified based on their serological status (positive vs. negative TTG antibodies). The data shows a significant difference in the percentage of CD14+/CD16, CD14+/CD16+, and CD14–/CD16+ monocytes, with the highest percentage between the two groups. For example, CD14+/CD16-The positive antibody group (92.71 ± 4.22) compared to the negative antibody group (81.17 ± 2.49), with a P-value of 0.003. This means that CD14+/CD16- Celiac can be a useful indicator to find patients with positive autoantibodies, highlighting the importance of this monocyte in the inflammation related to the disease. Similarly, CD14+/CD16+ also showed a significant difference between the two groups, with a p-value of 0.011 compared to the positive antibody group (5.53, 1.30), with a high level in the negative antibody group (5.91, 1.44). This finding may suggest a differential role of CD14+/CD16+ monocytes in the immune response of celiac disease patients with negative antibody results. The group with positive antibodies (18.49, 5.71) had higher levels compared to the group with the highest negative antibodies (15.65, 2.78), with a significant difference of <0.001, indicating that this is closely related to the diagnosis when antibodies are present. The results of Table 4 provide information about how monocytes are associated with serological markers in celiac disease. The important differences found between the two groups suggest that monocytes can be a helpful indicator for identifying patients with celiac disease based on their antibody status. The comparison of monocytes between positive and negative antibody groups is shown in Table 4; the detailed comparison of the highest percentage highlights the clinical ability of these subsets.

- **CD14+/CD16−, CD14+/CD16+, CD14−/CD16+:** These values represent the mean percentages of different monocyte subsets in the antibody-positive and antibody-negative groups.
- **Positive Antibody:** The group of patients who tested positive for antibodies against tissue transglutaminase (TTG).
- **Negative Antibody:** The group of patients who tested negative for antibodies against TTG.
- **Test of Significance:** The *t*-test is used to compare the means between the antibody-positive and antibody-negative groups.
- **P Value:** The statistical significance of the results, with values less than 0.05 considered statistically significant.

3.2. Statistical tests

- ***t*test:** Used to compare the means between the two independent groups (positive and negative antibody groups).

4. Discussion

As the occurrence of celiac disease (CD) is increasing, the debate around the pathological system involved has revealed various principles. A widely discussed principle involves immune-mediated intestinal damage triggered by gluten exposure (Ludvigsson et al., 2013b; Ludvigsson et al., 2013b; Auricchio et al., 1985). The causes of CD have mostly been studied by looking at T cells, the adaptive immune system, and how B lymphocytes help cause inflammation in the intestinal lining (Mazzarella et al., 2008). However, the role of congenital immunity in the CD is not sufficiently defined, and it requires complete re-evaluation. Given the importance of monocytes in the form of congenital immune cells, they can play an important role in CD immunopathogenesis (Theodore et al., 2019). In particular, gliadin peptides are shown to activate blood monocytes in CD patients, which highlights their importance in the pathogenesis of the disease (Cinova et al., 2007). Tissue transglutaminase (TG2), an enzyme that modifies gluten-type peptides, facilitates the activation of cells (Gjertsen et al., 1994; Vader et al., 2002). The role of TG2 in CD was the discovery of Srendipitus, as specific IGA antibodies were found in the CD. Zanoni et al. (2006) showed that autoantibodies can attack toll-like receptor 4 (TLR 4) related to transglutaminase, which leads to the activation of monocytes in celiac disease (CD). The function of serum cytokines in CD pathophysiology is an emerging area of research. To check how well monocytes are working, we looked at the levels of certain supportive and anti-inflammatory substances in the blood of children with CDs. Sami H. According to the findings of et al. (2019), our study raised the TNF-α levels, underlining the important role of this multifunctional cytokine in the CD (Samiei et al., 2019). Khan S et al. (2016) conducted the study. By increasing the level of TNF-α in CD patients, researchers highlight its importance as a mediator of immune responses (Khan et al., 2016). In addition, the ability of gliadin peptides to activate blood monocytes is shown to increase TNF-α expression in CD patients, and TNF-α polymorphism has been linked to increased CD risk (Nasserinejad et al., 2019). Our findings align with the study by Du Pre MF et al. (2015), which increased the level of IL-6 in CD patients compared to controls (Du Pre and Sollid, 2015). Aflatoxin M et al. (2019) found that higher levels of serum IL-6 were linked to a 15 % drop in CD events, showing that IL-6 is involved in the disease (Aflatoonian et al., 2019). Gluten-induced inflammation in the proximal small intestines activates $CD4^+$ T cells, which release pro-inflammatory cytokines, such as IL-6 (Dieterich et al., 1997; Faghih et al., 2018). These T cells then interact with B cells, which leads to the release of autoantibodies for anti-tissue transglutaminase (TTG). [34, 355]. Nonclassical/intermediate monocytes have unique properties, including the ability to reverse and display more efficient migration than classical monocytes. These subsets play a significant role during inflammatory conditions, activating endothelial cells (ECs) more efficiently than classical monocytes, which cannot return to circulation or influence EC activation (Maiti and Pradhan, 2009; Gordóvil Merino et al., 2012; Kordulewska et al., 2019).6] Consequently, nonclassical/intermediate monocytes are capable of rapid and efficient responses to inflammatory stimuli by crossing the endothelium (McGettrick et al., 2012; Tilg et al., 1997). These subsets also have a greater capacity to secrete TNF, facilitating EC activation and neutrophil recruitment via E-selectin expression (Ludvigsson et al., 2013a; Gordóvil Merino et al., 2012; Marafini et al., 2015).8] In contrast, classical monocytes secrete IL-6, which can modulate E-selectin expression and reduce neutrophil recruitment, demonstrating IL-6's dual role as both pro- and anti-inflammatory depending on the context (Wong et al., 2002; Abadie et al., 2011; Kordulewska et al., 2019; Tilg et al., 1997; Marafini et al., 2015; Maiuri et al., 2003). Elevated IL-6 levels in classical monocyte cultures can downregulate the TNF-induced inflammatory response of classical/intermediate monocytes, while nonclassical/intermediate monocyte supernatants rich in TNF are not affected by IL-6's anti-inflammatory properties (McGettrick et al., 2012; Maiti and Pradhan, 2009; Gordóvil Merino et al., 2012; Glickman and Van Dyk, 2007; Gamerman and Lopes, 2006; Ludvigsson et al., 2013b; Auricchio et al., 1985; Mazzarella et al., 2008; Theodore et al., 2019; Cinova et al., 2007; Gjertsen et al., 1994; Vader et al., 2002; Dieterich et al., 1997; Zanoni et al., 2006; Samiei et al., 2019; Khan et al., 2016; Nasserinejad et al., 2019; Du Pre and Sollid, 2015; Aflatoonian et al., 2019; Faghih et al., 2018; Wong et al., 2002; Abadie et al., 2011;

Table 4
Comparison between antibody positive and negative cases regarding monocyte subsets among the studied group.

Variables	Positive Antibody (N = 49)	Negative Antibody (N = 8)	Test of Significance	P Value
CD14+/CD16−	92.71 ± 4.22	81.17 ± 2.49	t = 3.098	0.003*
CD14+/CD16+	5.53 ± 1.30	5.91 ± 1.44	t = −0.896	0.011*
CD14−/CD16+	18.49 ± 5.71	15.65 ± 2.78	t = 4.467	<0.001*

Kordulewska et al., 2019; Tilg et al., 1997). Regarding the need to identify monocyte subgroups, TNF-α, and IL-6 in all patients with celiac disease, these tests are generally recommended for cases where there is uncertainty in the diagnosis or when additional markers are needed to assess disease severity or progression. Routine testing of these markers for all celiac disease patients may not be necessary unless the clinical presentation or serology warrants further investigation. As for the cost of this study, while the techniques used, such as flow cytometry and cytokine analysis, can be pricier than traditional diagnostic methods (such as serology and biopsy), they may offer valuable insights into disease mechanisms and help in identifying patients at higher risk for complications. Thus, the added expense should be considered the potential benefits of early diagnosis and personalized treatment plans.

Our study evaluated the diagnostic value of various markers, revealing significant differences between CD patients and healthy controls. The initial diagnostic phase for CD typically involves measuring anti-transglutaminase (IgA or IgG) antibodies (KellyCP et al., 2015). A duodenal biopsy is then used to confirm the diagnosis, although this procedure can be invasive and costly, particularly in pediatric cases with positive serological tests (Pelkowski and Viera, 2014).

Our research found the best cut-off points to tell apart CD cases from controls (>80.67, >6.98, >6.32), showing very high specificity (72 %, 97 %, 92.9 %) and sensitivity (75 %, 100 %, 95.6 %). The area under the curve values was highly significant ($p < 0.001$), consistent with previous studies such as Giacomo Caio UV et al. (2019) (Giacomo Caio et al., 2019). This is the first study we know of that shows levels of CD14+/CD16+, CD14+/CD16−, and CD14−/CD16+ are much higher in patients with positive tissue transglutaminase antibodies than in those with negative antibodies, indicating they might play a role in the disease's development or seriousness.

Even though the inflammatory response is important in many diseases, like Celiac Disease, it also shows a strong link between inflammatory markers, such as cytokines and immune cells, in forensic medicine when looking at injuries and toxicology. In this context, forensic medicine plays a crucial role in analyzing the inflammatory response to identify potential causes of death, which enhances our understanding of the role of inflammatory responses in other diseases like Celiac Disease.

Forensic medicine plays a vital role in healthcare systems by examining trauma injuries and determining the causes of death. After death, blood, urine, or tissue samples can be analyzed for various factors, including the presence of harmful substances. However, a comprehensive assessment often involves checking a range of indicators to exclude para-anatomical alterations in tissues (A. Naser et al., 2011).

Inflammation, a critical adaptive response to harmful stimuli such as microbes and toxins, can affect both local tissues and distant organs. If inflammation spreads, it may disrupt homeostasis and lead to organ failure. In forensic medicine, inflammation and its effects on wounds are of particular interest, as postmortem analyses can reveal macroscopic and microscopic changes linked to persistent inflammation, providing insights into trauma lesions and the nature of injuries (V Radzyukevich et al., 2021).

Inflammatory markers are extensively studied in forensic medicine for their ability to provide valuable insights into clinical and toxicological findings. These markers are useful for diagnosing conditions, prioritizing patients based on injury severity, and determining timeframes for exposure to toxins (Italiani et al., 2020; Franze et al., 2013). Forensic pathologists use these markers to distinguish between different causes of death and assess potential environmental or accidental factors.

In forensic toxicology, inflammation is often part of a pathogenic cascade. Defining stable endophenotypes—patterns of inflammation considered normal—can help distinguish between abnormal, illegal, or excessive conditions in forensic investigations (Italiani et al., 2020). While inflammation is often associated with the overproduction of cytokines and immune cell alterations, understanding the individual's initial "state" (e.g., behavior, history) is essential for predicting disease progression or the effects of toxins (V Radzyukevich et al., 2021).

In conclusion, this study provides strong evidence that the monocyte subsets CD14+/CD16−, CD14+/CD16+, and CD14−/CD16+, along with elevated levels of TNF-α and IL-6, are significantly associated with Celiac Disease (CD) in juvenile patients. These markers, together with the presence of anti-tissue transglutaminase antibodies, highlight the substantial differences between CD patients and healthy controls. Our findings contribute to the growing body of research suggesting that specific cytokine levels and monocyte subsets could serve as non-invasive alternatives to current diagnostic methods, such as serological testing and invasive intestinal biopsies. While obtaining blood for examination is a minimally invasive procedure, it still involves an intervention compared to non-invasive methods. However, it should be noted that these biomarkers may also be elevated in other inflammatory diseases, and therefore, they cannot be used as exclusive diagnostic tools for celiac disease without further studies to establish their specificity. Moreover, the study's limitations, including a small sample size and lack of detailed nutritional data, should be addressed in future investigations.

CRediT authorship contribution statement

Naglaa Makram Farag: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Noura Elbakry:** Visualization, Writing – original draft, Writing – review & editing. **Mahmoud Mousa:** Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mohamed S. Hemeda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Zamzam Hassan Mohamed:** Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Data availability

Data will be available on request.

Consent for publication

The authors give consent to the publishers to produce and publish the work.

Finding

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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N/A.

List of abbreviations

CD14 (Cluster of differentiation), IL-6 (interleukin 6), EC (E cell), TNF (tissue necrotizing factor), CD (celiac disease), TTG (tissue

transglutaminase).

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