

Analysis of Circulating MicroRNA-500a-3p and TNF- α Expression in Children with Celiac Disease

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ABSTRACT

Background: Circulating microRNA-500a-3p (miR-500a-3p) and TNF- α have been proposed as potential biomarkers for celiac disease (CD) in pediatric patients. This study aimed to investigate the expression of these biomarkers and their association with celiac disease pathogenesis. **Methods:** A case-control study was conducted involving 45 pediatric patients clinically diagnosed with CD (aged 8 months to 12 years) and 45 age-matched healthy controls in Al-Samawa City. Demographic and clinical characteristics were recorded for both groups. The expression levels of circulating miR-500a-3p and TNF- α were analyzed using the stem-loop RT-qPCR technique. **Results:** The expression level of miR-500a-3p was significantly elevated in CD patients (7.92 ± 2.76) compared to healthy controls (1.59 ± 0.71 , $p < 0.001$). Similarly, TNF- α expression levels were markedly higher in CD patients (25.67 ± 5.94) than in the control group (2.26 ± 0.91 , $p < 0.001$). **Conclusion:** This study suggests that circulating miR-500a-3p and TNF- α hold promise as non-invasive biomarkers for the diagnosis of celiac disease in pediatric patients. Elevated TNF- α levels highlight its role as a pro-inflammatory cytokine in the pathogenesis of celiac disease and its potential as a diagnostic marker. The findings establish a possible link between these biomarkers and CD-related antibody production, underscoring their relevance in the clinical assessment of pediatric celiac disease.

Keywords: miR-500a-3p, TNF- α , Celiac Disease, Serological Tests, Autoimmune Disease, Immunity

INTRODUCTION

Coeliac disease (CD) is an autoimmune enteropathy triggered by the interaction of environmental and genetic factors. In genetically-susceptible individuals, the ingestion of dietary gluten induces an inflammatory process that results in the damage of the bowel mucosa. This is followed by villous atrophy and crypt hyperplasia in addition to infiltration of a consistent number of lymphocytes and plasma cells in the Lamina propria. In addition to environmental factors, the susceptibility to CD depends also on the genetic background. In fact, CD is associated with specific major histocompatibility complex (MHC) class II alleles that encode for human leukocyte antigen (HLA) HLA-DQ2 or HLA-DQ8 heterodimers (the first occurs in at least 90–95% of patients with CD, whereas HLA-DQ8 characterizes the remaining 5–10% of coeliac individuals). Up to now, the only available treatment for CD is a lifelong adherence to a gluten-free diet. Gluten is the storage protein fraction present in grains of wheat, rye, and barley, and it includes two major protein types, gliadins and glutenins (hordeins and secalins in barley and rye, respectively). Gliadins and glutenins have a high proline and glutamine content and are resistant to the gastrointestinal enzymatic proteolysis. Therefore, the presence of large and potentially immunogenic peptides at the intestinal mucosal surface is quite high [1]. After food ingestion and initial degradation, gliadin peptides cross the epithelium into the lamina propria and undergo deamidation by tissue transglutaminase (tTG). Thus, the peptides acquire negative charges that are able to bind efficiently HLA-DQ2 or HLA-DQ8 molecules on the surface of antigen presenting cells (APCs) that will present them to CD4+ T-lymphocytes. After activation by gliadin peptides, T cells secrete high levels of pro-inflammatory cytokines which, in turn, stimulate natural killer cells and CD8+ intraepithelial T lymphocytes, ultimately leading to the typical coeliac mucosa injury [1, 2]. Primarily, CD affects the gastrointestinal tract and the most common symptoms are malabsorption, diarrhoea,

weight loss, and nutritional deficiency. However, individuals with CD can have extraintestinal symptoms, like anaemia, osteoporosis, or arthritis [3]. Tumor necrosis factor alpha is a proinflammatory cytokine involved in cellular and inflammatory immune reactions. This cytokine has been implicated in the severity of different immune-regulated diseases including autoimmune and inflammatory diseases, lymphoma, and transplantation [4]. The production and/or function of cytokines are at least partially regulated by polymorphisms in their gene sequences [5]. Polymorphisms within the promoter regions of cytokine genes may play a role in the genetically determined production of both T helper cell type 1 and 2 (Th1 and Th2 cells), influencing the susceptibility of a broad spectrum of immune mediated diseases, including CD [6]. In CD patients, exposure of the mucosa to gluten stimulates the expression of IL-6 and TNF- α in vitro [7]. In addition, serum levels of these interleukins significantly increase in patients with active CD compared with healthy individuals, and decrease only after a year on a gluten-free diet [8].

EXPERIMENTAL

Subject

This study was conducted on A total of 45 patients clinically diagnosed as having Celiac disease from aged 8 month to aged 12 years in Al-Samawa City. The non-Celiac participants consisted of 45 individuals from 8 month to aged 12 years. Blood sample were taken; venipuncture using disposable syringes at the Aspiration Unit of the laboratory. Approximately 4 mL of blood were collected in gel tubes, barcoded and sent to the Immunology Unit for onward processing. The samples were centrifuged at 3000 rpm for 20 minutes and 250 mL of supernatant (serum) was aspirated and placed in labeled Eppendorf tubes and add 750 mL of Green Zol reagent (TRIzol), and stored at -

20oC until use. The expression analysis quantification of miRNA 500a, and TNF in patients with Celiac disease and compare the same with those of non-Celiac participants recruited.

STEM-LOOP RT-qPCR

Measured of miRNA 500a, and TNF α expression in serum of patient and control groups by used the stem loop RT-qPCR, and the housekeeping gene (GAPDH) was used as a reference. The TRIzol® reagent kit was used to extraction total RNA from serum samples and following manufacturer guidelines. A Nanodrop spectrophotometer (THERMO, USA) was used to measure the concentration and purity of the extracted RNA through reading the absorbance in at (260/280

nm). DNase I enzyme was used to treat the extracted RNA to eliminate any remaining genomic DNA in the eluted total RNA and done according to the instructions provided by Promega Company (USA). The Sanger Center miRNA database registry was used to choose the miRNA sequence and the miRNA primer design tool was used to design the primers for miRNA 500a, and TNF, table (1).

The M-MLV Reverse Transcriptase kit was used to synthesized cDNA from DNase-I treated RNA samples and done according to company instructions. The housekeeping gene (GAPDH) used in normalization the expression analysis of miRNA 500a, and TNF was quantified using a quantitative Real-Time PCR.

Table 1: Primer Sequence

Primer		Sequence (5'-3')
miRNA Universal RT Primers		GTCGTATCCAGTGCAGGGTCCGAGGTATTCCGACTGGATACGACTTTTITTTTTTVN
miR-500a	R	AACAAGATGCACCTGGGCA
qPCR Primer	F	GTCGTATCCAGTGCAGGGT
TNF	R	AAGTGCTGGCAACCACTAAG
qPCR Primer	F	TCAAGTCCTGCAGCATTCTG
GAPDH	R	AAATCAAGTGGGCGATGC
qPCR Primer	F	TTCTCCATGGTGGTGAAGACG

Statistical Analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation after performance of Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables. Independent sample t-test was used to study difference in mean between any two groups provided that the variable is normally distributed. Chi-square test was used to study association between any two categorical variables. Odds ratio and 95% confidence interval was estimated to measure risk. In order to detect the cutoff value that predict a positive finding, receiver operator characteristic (ROC) curve analysis was used with its corresponding area under the curve (AUC), accuracy level, sensitivity, specificity and level of significance (P). Odds ratio and 95% confidence interval was estimated to measure risk. The

level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less[9]

Results and Discussion

Gene Expression miRNA-500

Real-time PCR results for miRNA-500, a molecule involved in gene regulation. The y-axis (RFU) shows fluorescence intensity, reflecting miRNA500 levels in patient and healthy control samples. As the PCR cycles progress (x-axis), more miRNA500 is detected, leading to a rising fluorescence signal (Figure1).

Figure 2 displays a melting curve, where fluorescence decreases with increasing temperature as amplified miRNA500 melts. This melting temperature (T_m) helps confirm the specific miRNA500 sequence targeted in the PCR reaction.

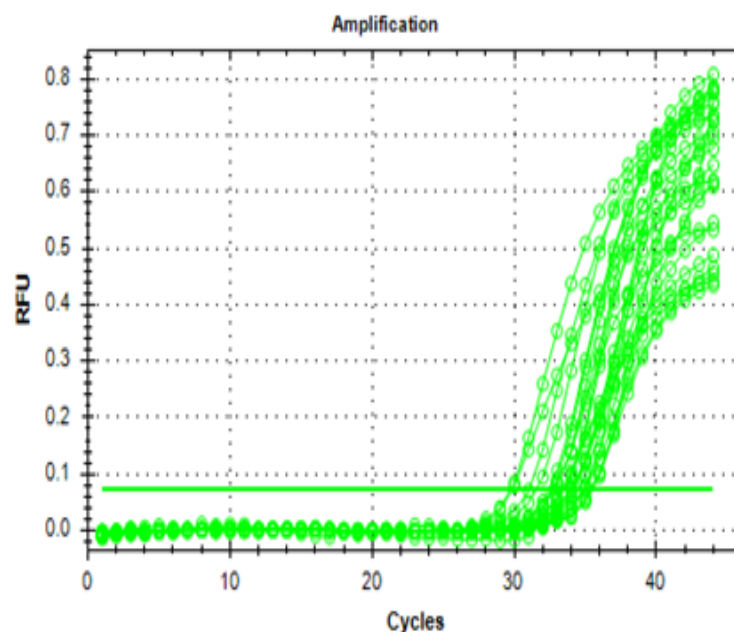


Figure 1: Real Time PCR Amplification Plots for miRNA500a in Patients and Healthy Control Samples

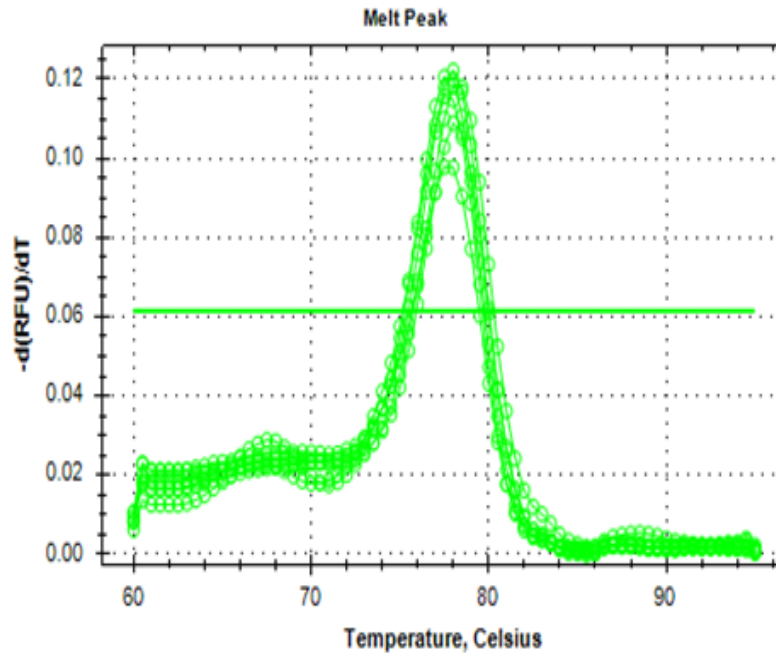


Figure 2: Real Time PCR Melting Curve for miRNA500a in Patients and Healthy Control Samples

miRNA-500a Gene Expression in Pediatric Patients with Celiac Disease (CD) and Healthy Control

The study's findings on miRNA-500a gene expression are consistent with current research on celiac disease biomarkers in children. Similar to studies, it reveals a significant difference in miRNA-500a expression between celiac disease (CD) patients and

healthy controls[10, 11]. The results, with a mean miRNA-500a expression significantly higher in CD patients (7.92 ± 2.76) compared to controls (1.59 ± 0.71) ($p < 0.001$) (Tables 2 and Figure 3), suggest that miRNA-500a may be upregulated in response to celiac disease. This aligns with the growing body of evidence on microRNAs as potential biomarkers for celiac disease diagnosis.

Table 2: miRNA-500a Gene Expression in Patients with CD and Healthy Control.

	Cases –Control Comparison		
	Pediatric Patients with CD n =45	Pediatric Healthy Control n = 45	P
miRNA-500a Gene Expression			
Mean $\pm$ SD	7.92 ± 2.76	1.59 ± 0.71	< 0.001
Range	1.58 – 20.83	0.07-6.68	† HS

n: number of cases; *SD*: standard deviation; $\dagger$: independent samples *t*-test; *HS*: Highly significant at $P \leq 0.001$.

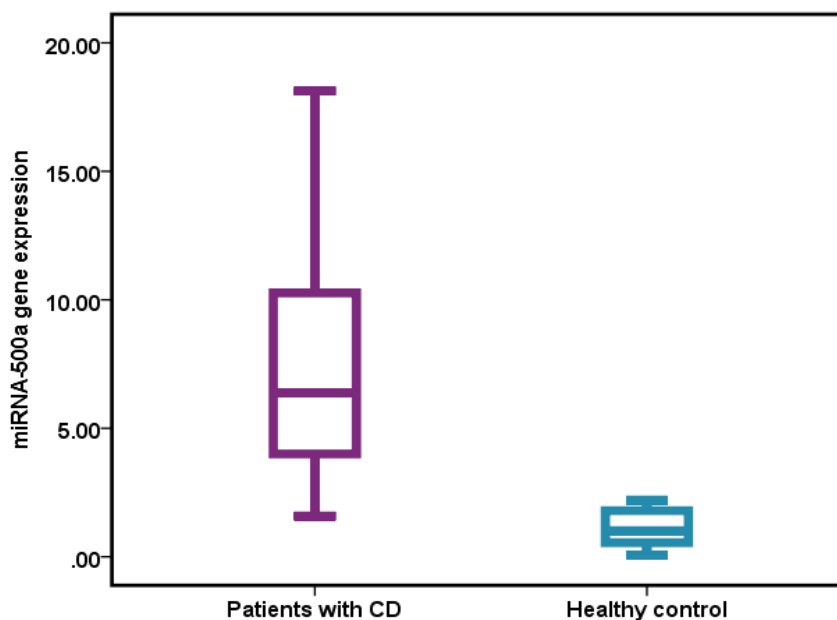


Figure 3: Mean miRNA-500a Gene Expression of Patients and Healthy Controls

Evaluation of miRNA-500a Gene Expression

Almost the totality of researches has focused on miRNA modulation/profiling and examined the miRNAs expression levels in intestinal mucosa of individuals with CD (tissue miRNAs). However, the role of cell-free miRNAs in CD has been underestimated and poorly investigated. Only in last few years, miRNAs have been found circulating in different body fluids, such as serum and plasma, protected by protein binding or enclosed in vesicles and released in the extracellular space [12] and many studies have emphasized their importance as potential biomarkers. Moreover, miRNAs may act as intercellular communication “actors” by conveying their “message” to other distant cells. Since circulating miRNAs are highly stable in circulation and under extreme laboratory conditions (i.e., pH and temperature, repeated freeze-thaw cycles, etc.) and their expression are associated to specific conditions, circulating miRNAs represent indeed reliable and promising diagnostic, prognostic, and therapeutic biomarkers [13]. The study's evaluation of miRNA-500a as a diagnostic marker aligns with similar research efforts to identify non-

invasive diagnostic tools for celiac disease. For instance, studies like those by have explored the potential of microRNAs as diagnostic biomarkers [10, 11]. The high area under the curve (AUC) of 0.983 obtained through ROC analysis (Table 4, Figure 5) indicates excellent diagnostic accuracy for miRNA-500a. This, along with the reported sensitivity, specificity, and positive/negative predictive values of 97.8% all suggest that miRNA-500a has promising potential as a biomarker for distinguishing pediatric celiac disease patients from healthy controls. Although the commonest serological tests are highly accurate to diagnose pediatric CD patients, new biomarkers have to be discovered and used to avoid biopsies, especially in those cases where the antibody levels are lower than the limits suggested by ESPGHAN guidelines [14]. So thinking that circulating miRNAs applied to pediatric diseases might reveal all of their potential as diagnostic and prognostic biomarkers as already demonstrated successfully for other intestinal (i.e., Chron's disease) and non-intestinal diseases and might be used routinely as powerful non-invasive biomarkers in the diagnosis of pediatric coeliac disease [15].

Table 3: Sensitivity and Specificity of miRNA-500a (> 2.28-fold) in CD Disease

miRNA-500a Gene	CD Patients n = 45	Healthy Control n = 45
> 2.28	44	1
< 2.28	1	44
Sensitivity %		97.8 %
Specificity %		97.8 %
PPV %		97.8 %
NPV %		97.8 %
AUC (95% CI)		0.983 (0.963- 1.000)

CI: Confidence Interval, AUC: Area Under Curve.

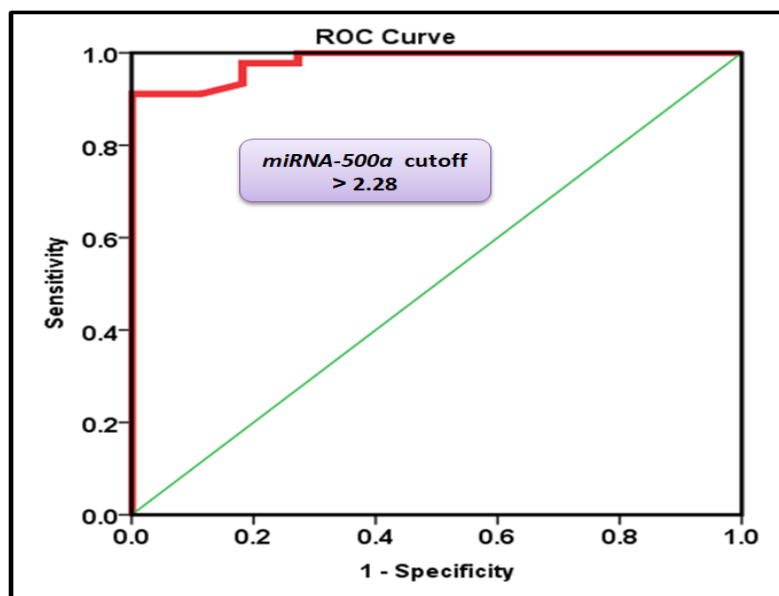


Figure 4: Receiver Operator Characteristic Curve Analysis of *miRNA-500a* for the Calculation of Possible Diagnostic Cutoff Value.

Frequency Distribution of miRNA-500a Gene Expression According to Some Characteristics.

The study's findings on miRNA-500a gene expression add to the understanding of factors potentially influencing its levels in celiac disease (CD). Similar to other research [16], it revealed a significantly higher expression in females compared to males.

This aligns with known sex hormone effects on the immune system. Additionally, the study suggests a possible link between

miRNA-500a and thyroid family history, with higher expression observed in these patients compared to those with a diabetic family history. However, the reasons behind this require further investigation. Interestingly, the study found no significant difference in miRNA-500a expression based on disease duration or presence of other autoimmune diseases. This is in contrast to some studies [11], highlighting the need for further exploration in larger and more diverse populations. Overall, this research contributes to deciphering the complex interplay between miRNA-500a and various factors in pediatric celiac disease.

Table 4: Distribution of miRNA-500a Expression According to Some Characteristic

Characteristics		N	miRNA-500a expression Mean $\pm$ SD	P
Gender	Male	15	5.36 $\pm$ 2.11	0.015 † S
	Female	30	8.76 $\pm$ 2.69	
Family History	Diabetics	25	6.32 $\pm$ 1.79	0.027 † S
	Thyroid	20	9.27 $\pm$ 2.86	
Duration of disease	< 3 years	26	5.93 $\pm$ 1.98	0.029 † S
	$\geq$ 3 years	19	8.87 $\pm$ 2.52	
Autoimmune disease	Diabetics	26	6.30 $\pm$ 1.71	0.019 † S
	Thyroid disease	19	9.45 $\pm$ 2.93	
Treatments	Treated	24	7.45 $\pm$ 2.19	0.803 † NS
	Not treated	21	7.79 $\pm$ 2.48	

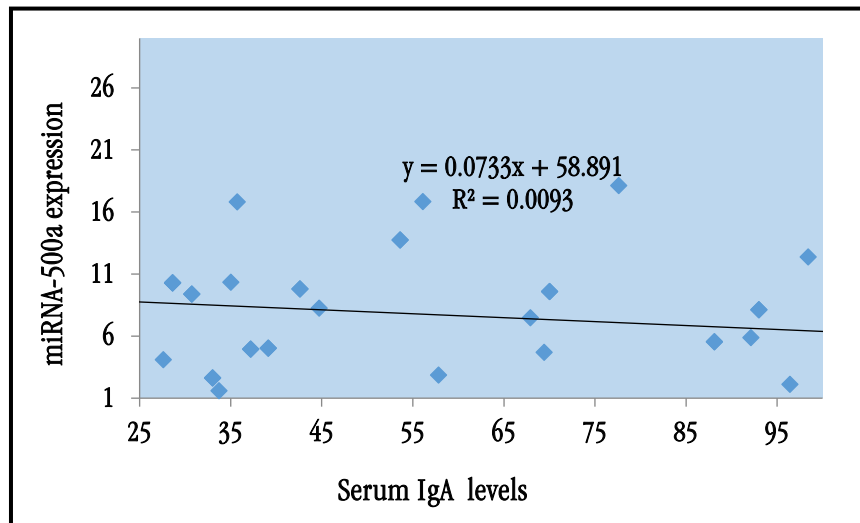
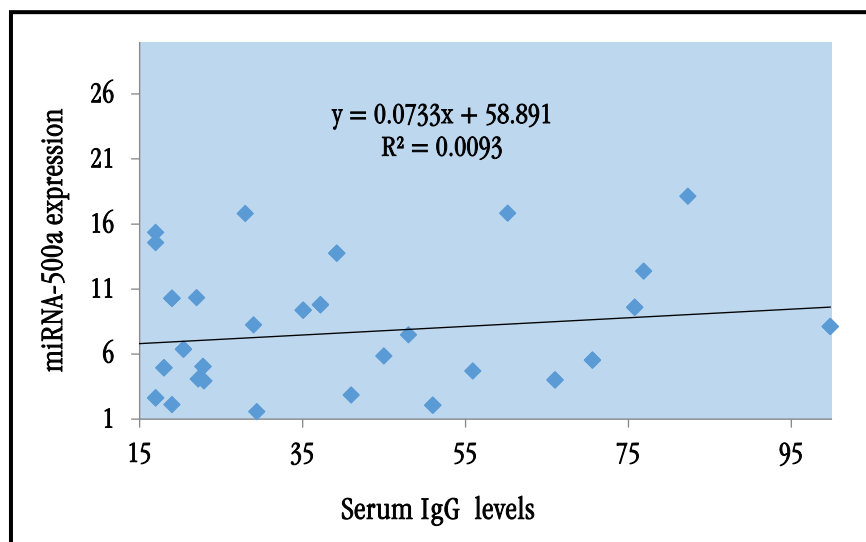
n: Number of Cases; *SD*: Standard Deviation; †: independent Samples t-test; *HS*: Highly Significant at $P \leq 0.001$; *NS*: Not Significant at $P > 0.05$.

Logestic Regression Correlations Between miRNA-500a Gene Expression and Diagnostic Antibody Parameters.

The study's investigation into the correlation between miRNA-500a gene expression and diagnostic antibodies (IgA and IgG) in pediatric celiac disease aligns with current research exploring microRNAs as potential regulators of immune response in the disease.

Similar to studies [10, 11], this research suggests a possible link between miRNA-500a and IgA/IgG levels in CD patients. The logistic

regression model indicating a positive correlation supports the notion that celiac disease may influence the production of these antibodies in relation to miRNA-500a expression. However, as highlighted in [11] further studies are needed to establish the exact direction of this relationship and elucidate the underlying mechanisms by which miRNA-500a might be involved. This research contributes to the growing understanding of microRNAs in celiac disease pathogenesis, potentially opening doors for future investigations into miRNA-based therapeutic strategies.

**Figure 5:** The Logistic Scatter Blot of miRNA-500a Gene Expression and IgA Among Patients**Figure 6:** The Logistic Scatter Blot of miRNA-500a Gene Expression and IgG Among Patients.

Gene expression TNF- α

Real-time PCR results for TNF- α , a molecule involved in gene regulation. The y-axis (RFU) shows fluorescence intensity, reflecting TNF- α levels in patient and healthy control samples. As the PCR cycles progress (x-axis), more TNF- α is detected, leading to a rising

fluorescence signal (Figure 8). Figure 9 displays a melting curve, where fluorescence decreases with increasing temperature as amplified TNF- α melts.

This melting temperature (T_m) helps confirm the specific TNF- α sequence targeted in the PCR reaction.

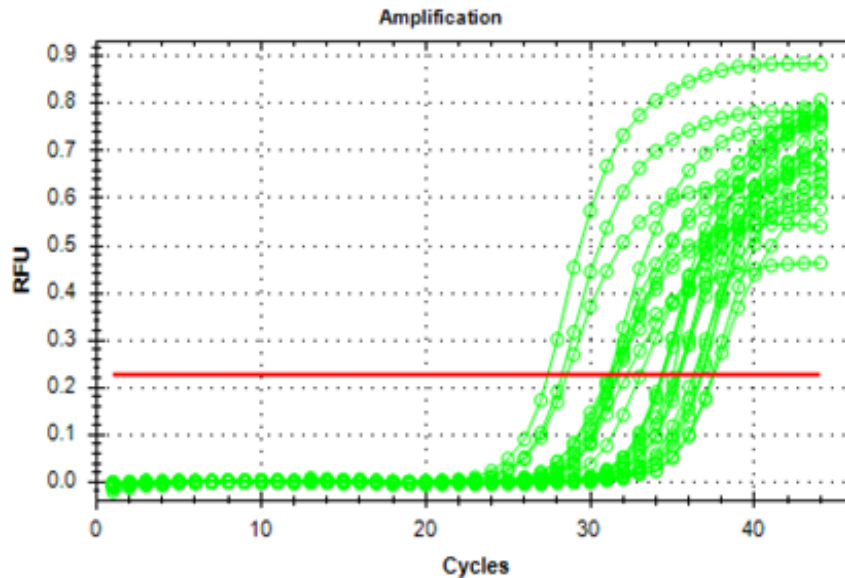


FIGURE 7: Real Time PCR Amplification Plots for TNF in Patients and Healthy Control Samples

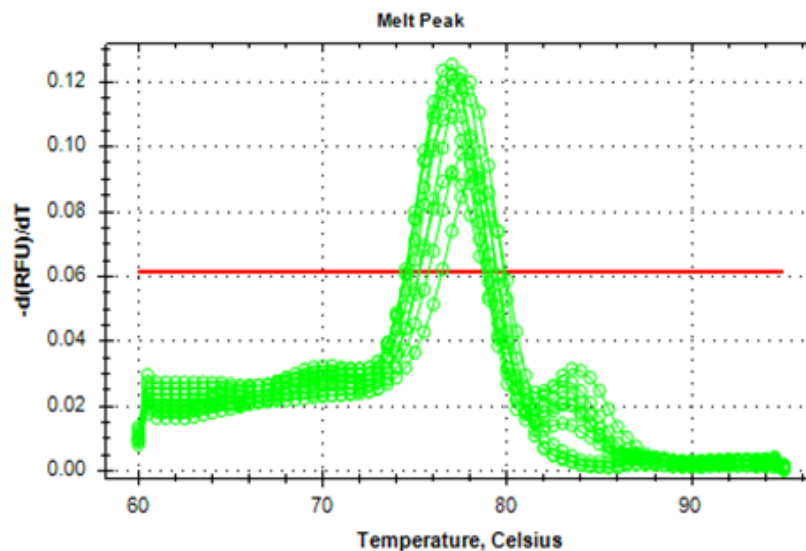


FIGURE 8: Real Time PCR melting curve for TNF in patients and healthy control samples.

Tumor Necrosis Factor- α (TNF- α) Gene Expression in Pediatric Patients with Celiac Disease (CD) And Healthy Control

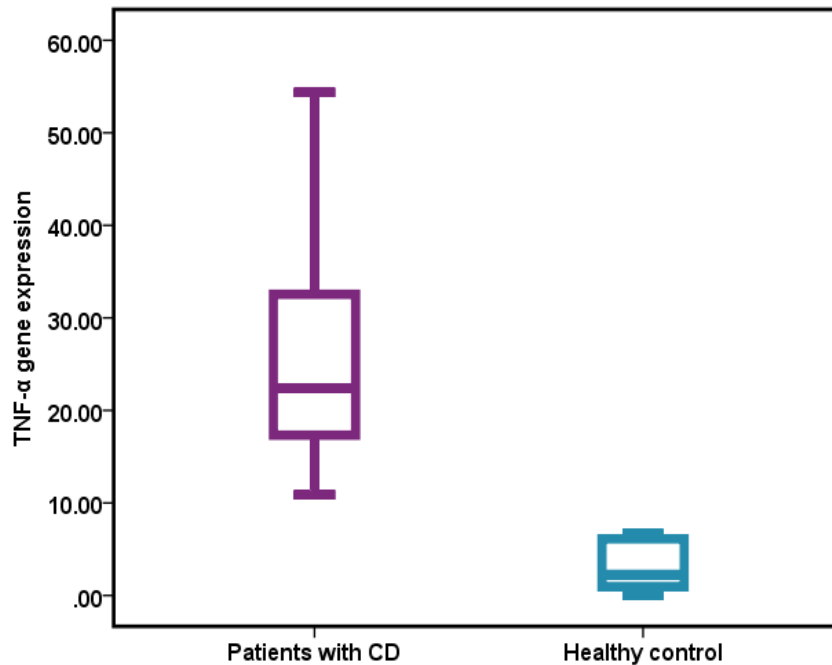
The study's findings on Tumor Necrosis Factor-alpha (TNF- α) gene expression align with research suggesting its potential role in celiac disease pathogenesis in children. Similar to studies by [17, 18] this research found a significantly higher level of TNF- α expression in pediatric CD patients compared to healthy controls. This strengthens the understanding of TNF- α as a potential contributor to the inflammatory response observed in celiac disease. However, as highlighted in [18], TNF- α is pleiotropic cytokine mediates broad range of proinflammatory and inflammatory responses and alter levels

of TNF- α among different individuals can be a major risk for differences in the susceptibility and severity of CD. TNF is a pro-inflammatory cytokine that plays a crucial role in the immune response. In celiac disease, it contributes to the inflammation of the small intestine, leading to tissue damage and the characteristic symptoms of the disease. Also, Gluten exposure, which triggers the immune response in celiac disease, often leads to increased TNF production. This further supports the role of TNF in the pathophysiology of the disease. Overall, this study adds to the current knowledge on TNF- α 's involvement in pediatric celiac disease, potentially paving the way for future therapeutic targets.

Table 5: TNF- α gene expression in patients with CD and healthy control

Cases –control comparison			
	Pediatric patients with CD n =45	Pediatric healthy control n = 45	P
TNF- α gene expression			
Mean $\pm$ SD	25.67 $\pm$ 5.94	2.26 $\pm$ 0.91	< 0.001 †
Range	10.89 – 54.37	0.07–6.66	HS

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

**Figure 9:** Mean TNF- α Gene Expression of Patients and Healthy Controls

Evaluation of TNF- α Gene Expression

The study's evaluation of TNF-alpha gene expression aligns with research highlighting its potential as a prognostic marker for celiac disease in children. Similar to findings by this study found a significant increase in TNF-alpha expression in patients compared to controls. The ROC analysis with a perfect AUC of 1.000 and 100% accuracy across all metrics (sensitivity, specificity, PPV, NPV) suggests excellent differentiation between celiac and healthy children. However, further validation in larger studies is warranted, as noted in TNF, along with other pro-inflammatory cytokines, can provide valuable information about the severity of inflammation and immune dysregulation in celiac disease.

Elevated TNF levels may be associated with more severe mucosal damage, refractory celiac disease, or complications such as

lymphoma. However, TNF alone may not be sufficient for precise prognostication, and its interpretation often requires consideration alongside other clinical and laboratory parameters.

While TNF measurement can contribute to the overall assessment of disease activity and prognosis in celiac disease, it is typically used in conjunction with other markers such as anti-tissue transglutaminase antibodies (anti-tTG) and histological evaluation of intestinal biopsies. Combining multiple markers and clinical data enhances the sensitivity and specificity of prognostic assessments in celiac disease. While TNF can be sensitive in reflecting inflammation and immune activity in celiac disease, its specificity as a standalone prognostic marker is moderate. Integrating TNF measurements with other clinical and laboratory parameters improves the accuracy of prognostic assessments in celiac disease management [19].

Table 6: Sensitivity and specificity of TNF- α (> 8.77-fold) in CD disease

TNF- α gene	CD Patients n = 45	Healthy Control n = 45
> 8.77	45	0
< 8.77	0	45
Sensitivity %		100.0 %
Specificity %		100.0 %
PPV %		100.0 %
NPV %		100.0 %
AUC (95% CI)		1.000 (1.000- 1.000)

CI: Confidence interval, AUC: Area Under Curve.

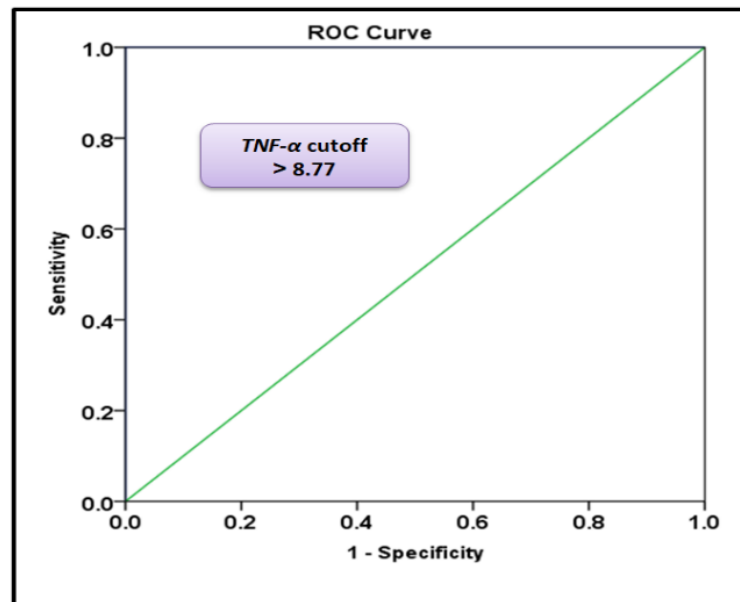


Figure 10: Receiver Operator Characteristic Curve Analysis of *TNF-α* for the Calculation of Possible Prognostic Cutoff Value.

Frequency Distribution of *TNF-α* Gene Expression According to Some Characteristics.

The current study investigation into the frequency distribution of *TNF-α* gene expression according to sex of patients revealed non-significant, but still in female higher than in male (26.22 ± 6.11 vs 24.56 ± 5.23) table, (4-13), this may be due to that males and females can exhibit differences in immune responses, with females often showing stronger immune reactions due to factors like X chromosome-linked genes and hormonal fluctuations. These differences can influence *TNF* expression in response to immune challenges. Sex-related differences in *TNF* expression and immune responses can contribute to variations in disease susceptibility and outcomes. For example, autoimmune diseases like rheumatoid arthritis and lupus are more prevalent in females, partly due to hormonal and immunological factors affecting *TNF* and other cytokines[20]. Again Family history of autoimmune diseases or inflammatory conditions can indicate a genetic predisposition to immune dysregulation and cytokine imbalances, including *TNF*. Certain genetic variants or mutations may influence *TNF* expression and contribute to disease development. In addition to genetic factors, shared environmental influences within families can also impact *TNF* expression. For example, exposure to similar dietary habits, infections, or stressors may contribute to immune activation and *TNF* production in susceptible individuals. Family history can influence epigenetic modifications, such as DNA methylation and histone acetylation, which can regulate *TNF* gene expression. These epigenetic changes

can be influenced by environmental factors and familial patterns [21, 22]. The finding that *TNF-α* expression is significantly higher in untreated patients compared to treated ones (31.98 ± 5.80 vs. 20.15 ± 4.25) corresponds with the known pro-inflammatory nature of *TNF-α* and aligns with the therapeutic effects of celiac disease treatments (Table 10). Interestingly, Studies have shown that adherence to a strict gluten-free diet leads to a decrease in inflammatory markers, including *TNF*, in individuals with celiac disease. This reduction is attributed to the removal of gluten, the primary trigger for immune activation and *TNF* production in celiac disease. A gluten-free diet helps to normalize the inflammatory response in the gut mucosa, leading to reduced *TNF* expression and overall improvement in mucosal healing and immune homeostasis [23]. Patients on a strict gluten-free diet often experience a decrease in symptoms and a normalization of inflammatory markers, including *TNF*, in line with improved disease management and control. *TNF* levels can serve as a marker for disease activity and response to treatment, including adherence to a gluten-free diet. Regular monitoring of *TNF* and other inflammatory markers helps assess the effectiveness of dietary management and disease control [24] Tailoring treatment strategies based on *TNF* expression and dietary adherence allows for personalized approaches in managing celiac disease. This includes optimizing gluten-free diet adherence and exploring adjunct therapies targeting inflammation if needed. Long-term studies are needed to evaluate the sustained effects of a gluten-free diet on *TNF* expression and disease outcomes in celiac disease[25].

Table 7: Distribution of *TNF-α* Expression According to Some Characteristic

Characteristics		N	<i>TNF-α</i> expression Mean $\pm$ SD	P
Gender	Male	15	24.56 ± 5.23	0.665 † NS
	Female	30	26.22 ± 6.11	
Family History	Diabetics	25	23.62 ± 5.40	0.202 † NS
	Thyroid	20	28.23 ± 5.46	
Duration of disease	< 3 years	26	23.71 ± 5.17	0.208 † NS
	≥ 3 years	19	28.34 ± 5.58	
Autoimmune disease	Diabetics	26	23.38 ± 5.27	0.134 † NS
	Thyroid disease	19	28.80 ± 5.57	
Treatments	Treated	24	20.15 ± 4.25	0.001 † S
	Not treated	21	31.98 ± 5.80	

n: Number of Cases; SD: Standard Deviation; †: Independent Samples t-test; HS: Highly Significant at $P \leq 0.001$; NS: Not Significant at $P > 0.05$.

Logestic Regression Correlations between TNF- α Gene Expression and Diagnostic Antibody Parameters

The logistic regression analysis in this study aligns with research investigating immune responses in celiac disease, similar to studies [26] who also used regression models to examine immune markers. The results, indicating a positive correlation between TNF- α gene expression and IgA and IgG antibody levels in children with celiac

disease as current result (Figures 3-15 and 3-16), which support the current understanding of the disease's immunological processes. TNF- α is a known pro-inflammatory cytokine, and its association with increased IgA and IgG levels suggests a potential role for TNF- α in driving antibody production in celiac disease. This finding warrants further investigation into the specific mechanisms by which TNF- α influences antibody production [27].

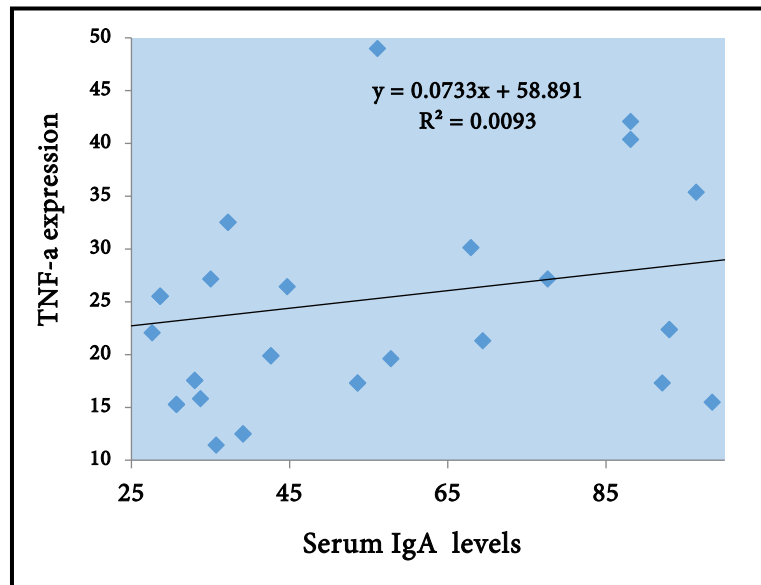


FIGURE 11: The Logestic Scatter Blot of TNF- α Expression and IgA Among Patients.

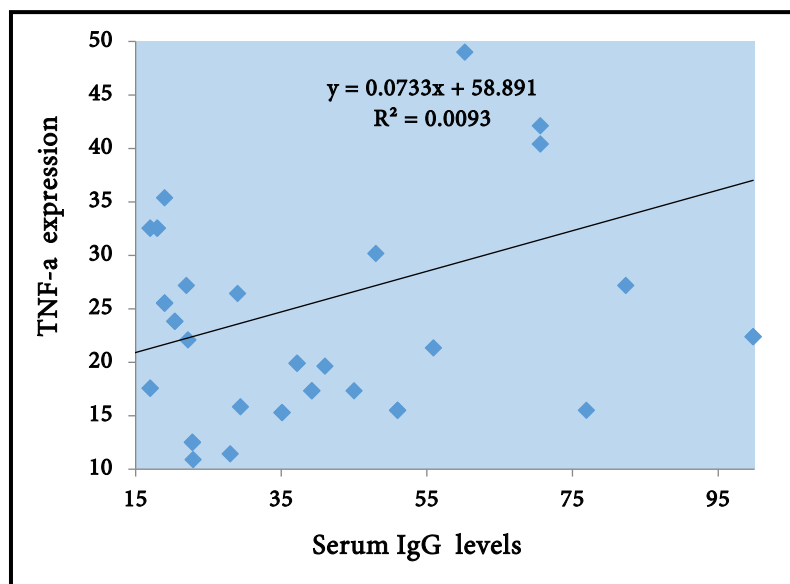


FIGURE 12: The Logestic Scatter Blot of *TNF- α Gene Expression* and IgG Among Patients.

Correlation between Gene Expression Parameters and Other Different Characteristics.

The positive correlation between miRNA-500a and TNF- α expression with disease duration (Tables 11) suggests that these factors may become more pronounced as celiac disease progresses. In the context of celiac disease, the duration of exposure to gluten and the chronicity of immune responses may lead to elevated miR-500 expression over time. miR-500 regulates multiple targets involved in inflammatory pathways, including cytokines, chemokines, and signaling molecules. Celiac disease is characterized by chronic

inflammation in the small intestine, triggered by gluten ingestion in genetically susceptible individuals. Prolonged exposure to gluten and ongoing immune activation contribute to persistent inflammation and immune dysregulation in celiac disease. Studies have shown that miR-500 expression levels can increase in response to chronic inflammation and sustained immune activation [28]. Its upregulation can enhance pro-inflammatory cytokine production (such as TNF- α , IL-6) and promote immune cell activation and differentiation, contributing to the maintenance of chronic inflammation in celiac disease. The positive correlation between miR-

500 expression and celiac disease duration suggests that miR-500 levels could serve as a biomarker of disease progression and chronicity. Monitoring miR-500 expression may provide insights into the ongoing inflammatory processes, immune dysregulation, and potential therapeutic targets for managing celiac disease and its complications. Investigating the therapeutic potential of targeting miR-500 or its downstream effectors could offer novel strategies for modulating inflammation and improving outcomes in celiac disease. Overall, the positive correlation between miR-500 expression and the duration of celiac disease reflects the dynamic interplay between immune regulation, inflammation, and disease progression in this autoimmune condition [26].

MiRNA-500 is a microRNA that plays a regulatory role in gene expression by targeting specific messenger RNAs (mRNAs) for degradation or translational inhibition. Its exact functions and targets may vary depending on the context, and research on miRNA-500 is

ongoing to understand its role in different biological processes, including immune regulation and inflammation. Hypothetically, if miRNA-500 is involved in modulating immune responses or inflammatory pathways relevant to celiac disease, its expression levels might change over time in response to disease progression.

For example, increased miRNA-500 expression could be linked to the chronic inflammatory state characteristic of long-standing celiac disease, reflecting ongoing immune dysregulation and tissue damage. Establishing a positive correlation between miRNA-500 expression and the duration of celiac disease would have clinical implications. It could suggest miRNA-500 as a potential biomarker for disease progression or severity, aiding in the assessment of long-term outcomes and the development of targeted therapies. Further studies are needed to investigate the specific role of miRNA-500 in celiac disease pathogenesis, especially regarding its interaction with immune pathways and inflammatory mediators [29].

Table 8: Correlation Between Gene Expression and other Different Characteristics

Parameters	miRNA-500a		TNF- α	
	r	P	r	P
Age	0.094	0.537	0.215	0.156
Gender	0.160	0.401	0.066	0.665
Duration of disease	0.355	0.029*	0.194	0.202
IgA	0.279	0.063	0.277	0.065
IgG	0.180	0.238	0.388	0.008*

r: correlation coefficient.

Conclusion

Study is needed to comprehend miR-500a-3p's diagnostic value properly. Further investigation is needed to elucidate the precise mechanisms by which TNF- α influences disease development and progression

References

- [1] B. Meresse, J. Ripoche, M. Heyman, and N. Cerf-Bensussan, "Celiac disease: from oral tolerance to intestinal inflammation, autoimmunity and lymphomagenesis," *Mucosal immunology*, vol. 2, no. 1, pp. 8-23, 2009.
- [2] D. Schuppan, Y. Junker, and D. Barisani, "Celiac disease: from pathogenesis to novel therapies," *Gastroenterology*, vol. 137, no. 6, pp. 1912-1933, 2009.
- [3] S. Guandalini and A. Assiri, "Celiac disease: a review," *JAMA pediatrics*, vol. 168, no. 3, pp. 272-278, 2014.
- [4] E. Rossi *et al.*, "TNFA Haplotype genetic testing improves HLA in estimating the risk of celiac disease in children," *PLoS One*, vol. 10, no. 4, p. e0123244, 2015.
- [5] Y. M. Mosaad, H. Fathy, Z. Fawzy, and M. A. El-Saied, "Tumor necrosis factor- α -308 G> A and interleukin-6-174 G> C promoter polymorphisms and pemphigus," *Human Immunology*, vol. 73, no. 5, pp. 560-565, 2012.
- [6] H. J. Galipeau and E. F. Verdu, "Gut microbes and adverse food reactions: Focus on gluten related disorders," *Gut Microbes*, vol. 5, no. 5, pp. 594-605, 2014.
- [7] V. Kumar, C. Wijmenga, and S. Withoff, "From genome-wide association studies to disease mechanisms: celiac disease as a model for autoimmune diseases," in *Seminars in immunopathology*, 2012, vol. 34: Springer, pp. 567-580.
- [8] R. de Albuquerque Maranhão, F. Martins Esteves, S. Crovella, L. Segat, and P. Eleutério Souza, "Tumor necrosis factor- α and interleukin-6 gene polymorphism association with susceptibility to celiac disease in Italian patients," *Genetics and Molecular Research*, vol. 14, no. 4, pp. 16343-16352, 2015.
- [9] W. W. Daniel and C. L. Cross, *Biostatistics: a foundation for analysis in the health sciences*. Wiley, 2018.
- [10] G. Buoli Comani *et al.*, "miRNA-regulated gene expression differs in celiac disease patients according to the age of presentation," *Genes & nutrition*, vol. 10, pp. 1-12, 2015.
- [11] I. L. Tan *et al.*, "Circulating miRNAs as potential biomarkers for celiac disease development," *Frontiers in Immunology*, vol. 12, p. 734763, 2021.
- [12] A. Baldassarre, C. Felli, G. Prantera, and A. Masotti, "Circulating microRNAs and bioinformatics tools to discover novel diagnostic biomarkers of pediatric diseases," *Genes*, vol. 8, no. 9, p. 234, 2017.
- [13] E. Bell and M. A. Taylor, "Functional roles for exosomal microRNAs in the tumour microenvironment," *Computational and structural biotechnology journal*, vol. 15, pp. 8-13, 2017.
- [14] K. Giersiepen *et al.*, "Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report," *Journal of pediatric gastroenterology and nutrition*, vol. 54, no. 2, pp. 229-241, 2012.
- [15] A. Masotti *et al.*, "Oral glucose tolerance test unravels circulating miRNAs associated with insulin resistance in obese preschoolers," *Pediatric obesity*, vol. 12, no. 3, pp. 229-238, 2017.
- [16] M. Sangineto *et al.*, "Identification of peculiar gene expression profile in peripheral blood mononuclear cells (PBMC) of celiac patients on gluten free diet," *PLoS One*, vol. 13, no. 5, p. e0197915, 2018.
- [17] E. M. Domsa *et al.*, "Expression of selected genes and circulating microRNAs in patients with celiac disease," *Medicina*, vol. 58, no. 2, p. 180, 2022.
- [18] R. Przemioslo, M. Kontakou, V. Nobili, and P. Ciclitira, "Raised pro-inflammatory cytokines interleukin 6 and tumour necrosis factor alpha in coeliac disease mucosa detected by immunohistochemistry," *Gut*, vol. 35, no. 10, pp. 1398-1403, 1994.

- [19] K. Lindfors *et al.*, "Coeliac disease," *Nature reviews Disease primers*, vol. 5, no. 1, p. 3, 2019.
- [20] S. Sanchez *et al.*, "TNF-alpha stimulated immune response: are there sex differences?," *Physiology*, vol. 38, no. S1, p. 5733842, 2023.
- [21] A. Rubio-Tapia and J. A. Murray, "Classification and management of refractory coeliac disease," *Gut*, vol. 59, no. 4, pp. 547-557, 2010.
- [22] E. Khalkhal, M. Rezaei-Tavirani, M. R. Zali, and Z. Akbari, "The evaluation of laser application in surgery: a review article," *Journal of lasers in medical sciences*, vol. 10, no. Suppl 1, p. S104, 2019.
- [23] A. Lerner and T. Matthias, "Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease," *Autoimmunity reviews*, vol. 14, no. 6, pp. 479-489, 2015.
- [24] D. A. Leffler, P. H. Green, and A. Fasano, "Extraintestinal manifestations of coeliac disease," *Nature Reviews Gastroenterology & Hepatology*, vol. 12, no. 10, pp. 561-571, 2015.
- [25] C. Maaser *et al.*, "ECCO-ESGAR Guideline for Diagnostic Assessment in Inflammatory Bowel Disease Part 1. Initial diagnosis, monitoring of known IBD, detections of complications," *Journal of Crohn's and Colitis*, pp. 144-164, 2019.
- [26] M.-L. Lähdeaho, M. Mäki, K. Laurila, H. Huhtala, and K. Kaukinen, "Small-bowel mucosal changes and antibody responses after low-and moderate-dose gluten challenge in celiac disease," *BMC gastroenterology*, vol. 11, pp. 1-9, 2011.
- [27] D. Delbue *et al.*, "Celiac disease monocytes induce a barrier defect in intestinal epithelial cells," *International journal of molecular sciences*, vol. 20, no. 22, p. 5597, 2019.
- [28] X. q. Wu *et al.*, "Emerging role of micro RNA s in regulating macrophage activation and polarization in immune response and inflammation," *Immunology*, vol. 148, no. 3, pp. 237-248, 2016.
- [29] K. Chandan, M. Gupta, and M. Sarwat, "Role of host and pathogen-derived microRNAs in immune regulation during infectious and inflammatory diseases," *Frontiers in immunology*, vol. 10, p. 3081, 2020.



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