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Association of CD24, CD27, and co-stimulatory molecules CD80 immunological marker expression on B-cells of human peripheral blood with development of celiac disease

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Abstract:

BACKGROUND: B-cells express a variety of clusters of differentiation markers during development. These markers determine the basic function of the immune phenotype specific to B-cells. Changes in the expression of these markers are linked to the development of many diseases, including chronic inflammation, autoimmune diseases, and immunodeficiency.

OBJECTIVE: The current study aimed to investigate a change in CD24, CD27, and co-stimulatory molecules CD80 expression on peripheral blood B-cells and the extent of their contribution to celiac disease.

MATERIALS AND METHODS: A total of 60 male children, whose ages ranged between 8 and 14 years, participated in this study. Thirty-five were identified as having celiac disease, while the control group comprised 25 children with anti-tissue transglutaminase (TG) (immunoglobulin G [IgG]), anti-tissue TG (IgA), and deamidated gliadin peptide (DGP) IgG levels normal. The frequency CD24, CD27, and CD80 expression were measured by flow cytometry.

RESULTS: Celiac disease patients showed a substantial decrease in the percentage of CD24, CD27, and CD80 expression on B-cells compared to control groups.

CONCLUSION: These findings suggest that numerical deficiency of CD24, CD27, and CD80 expression on B-cells in the peripheral blood mononuclear cell population, that may involve the loss of auto-tolerance that plays an important role in the immune response associated with inflammation and tissue damage in celiac disease. These immunological markers may be used as diagnostic indicators for this disease.

Keywords:

B cell, CD19, CD24, CD27, CD80, celiac disease

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Introduction

Celiac disease is an immune-mediated systemic disease that is brought on by consuming gluten and related prolamins' in people who are genetically predisposed to it. It is distinguished by the presence of different combinations of small intestinal damage, celiac-specific antibodies, human leukocyte antigen (HLA)-DQ2 or HLA-DQ8, and gluten-dependent clinical manifestations.[1] The only current treatment is to avoid gluten for the remainder of one's life. Unconventional T-cells (T-lymphocytes that express an a γδ cell receptor), such as invariant natural killer T (iNKT) and mucosal-associated invariant T-cells, are important regulators of mucosal barrier function and microbial colonization. iNKT

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cells recognize glycolipids when presented in the context of cluster of differentiation (CD) 1, and play a role in regulating inflammatory responses, autoimmunity, allergy and host defense against infections and recognize cancer cells.[2]

B cell subsets have been shown to reduce inflammation and autoimmune illness in a number of experimental scenarios, suggesting that they may also have a regulatory function in addition to their well-known roles in humoral immunity and cellular immunity.[3] One of the most prevalent autoimmune illnesses is celiac disease. Genetically predisposed individuals have CD when their immune system reacts to several environmental triggers, including gluten use.[4] Recently, it was reported that $CD19^+$ B-cells may play an important role in suppressing the T-lymphocyte effector pathways Th1 and Th17 and boosting Treg cells via FoxP3 gene regulation.[5] The regulation mechanism of Breg cells is yet unknown, despite the fact that interleukin 10 (IL-10) production by specific Breg subsets appears to be important in this network.[6] Indeed, many Breg subsets have been identified, with the primary phenotypes being CD24 h^iCD38 h^i Breg cells and memory Breg cells displaying $CD27^+$ on the cell surface.[7,8] The specific functions of these immune markers, namely CD19, CD24+, $CD27^+$, and CD80+, which are produced by B-cells, have not been given significant attention in studies on celiac disease.[9,10] The present study focused on investigating the role of CD24, CD27, and co-stimulatory molecules CD80 immunological marker expression on B-cells and the extent of its contribution to celiac disease.

Materials and Methods

Topics of research and clinical parameters of celiac disease patients diagnosis

The research was performed between November 2023 and February 2024, a total of 60 male children (from 35 celiac disease patients to 25 controls, blood, and serum samples) were obtained, and the age of all was between 8 and 14 years, patient samples showed positivity for total serum levels of anti-tissue transglutaminase (TG) (immunoglobulin G [IgG]), anti-tissue TG (IgA), deamidated gliadin peptide (DGP) IgG, and deamidated gliadin peptide (DGP) IgA tests.[11]

Statement of ethics

The study was approved by the ethical committees, which include the committee of the Center for Gastrointestinal and Liver Diseases and Surgery (no.1050 on December 31, 2023), Kerbala Health Directorate/Holly Kerbala Governorate, Iraq. Written parental approval was obtained.

The measurement of $CD27+$, $CD24^+$, $CD80^+$, and $CD19^+$ by flow cytometry

To detect intracellular CD19, CD24, CD27, and co-stimulatory molecules CD80 expression on B-cell surface markers by flow cytometry and antihuman monoclonal antibody (all from BD), the following monoclonal antibodies were utilized: anti-CD19-PE-CY7 MAB (HIB-19), anti-CD24/FITC (clone ML5), anti-CD27PE (clone LEU-27), and anti-CD80/APC-H7 (clone L307). The test was conducted in "Imam Zain El-Abidine Hospital." The staining was performed in a darkened room.[12] The samples were examined using the gating technique, as illustrated in Figure 1.

Data analysis

Data studied specimens were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM, Armonk, New York, USA). The independent-sample *t*-tests with 95% confidence intervals were used to find the differences in means between two groups in the same categorical category. The $P \leq 0.05$ was a remarkable statistic, taken into regard. On the other hand, the significance level was specified as * between the groups. The probability level was stated as $^{***}P \leq 0.001$.[13]

Results

Clinical characteristics

The 60 children who participated in this study were a constituent of two groups: the first is a celiac disease patient group of 35 children, while the second is 25 in a control group. The average age was (10.1 ± 4) years in the patients group while being (10 ± 2) years in the other one. The most frequent clinical manifestations at diagnosis were abdominal pain, weight loss, and diarrhea. In comparison to the control group, the levels of anti-tissue TG (IgG), TG (IgA), deamidated gliadin peptide (DGP) IgG, and deaminized gliadin peptide (IgA) were elevated in each patient sample.

Flow cytometry analysis of immunological markers expression

The prevalence CD19+B-cells in B-lymphocyte

According to the presented data explained in Figure 2, a significant decline of the percentage of CD19+B-cells under the level $P \leq 0.001$ for celiac disease patients is detected, in comparison with those ones in the control group. The mean percentage was (5.3545%) for the celiac disease patients group, while it was (1.3583%) for the control group.

The prevalence CD19+CD24+B-cells

As shown in Figure 3, the results revealed a significant decrease in CD19+CD24+B-cells percentage for celiac

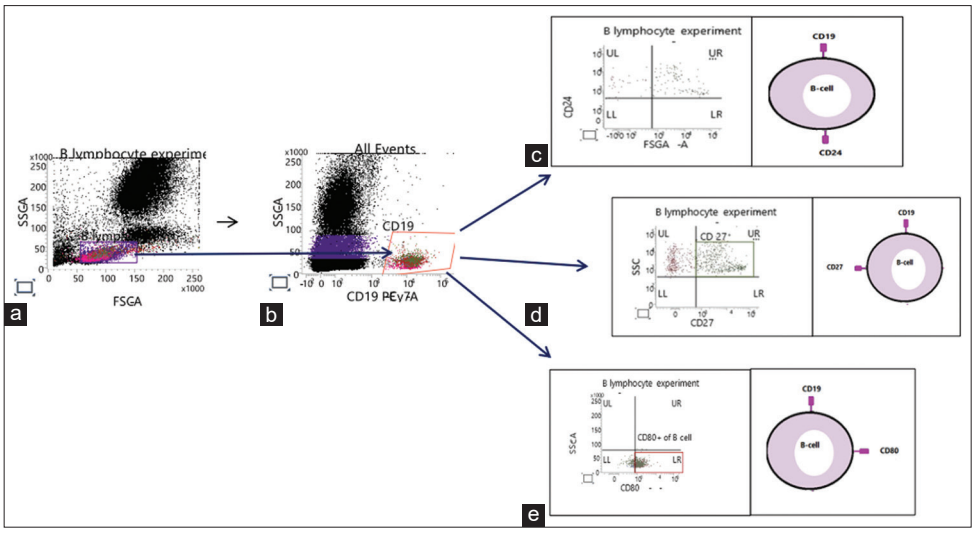

Figure 1: Gating strategy of CD24, CD27, and co-stimulatory molecules CD80 expression on B cell assessment. (a) Within singlets of the examined peripheral blood mononuclear cells population, every lymphocyte was identified based on its forward scatter (FSC) and side scatter (SSC) characteristics, (b) CD19+B-cells were gated, (c) CD19+CD24+B-cells were calculated, (d) CD19+CD27+B-cells were calculated, (e) CD19+CD80+B-cells were calculated. SSGA = Side scatter gating area, FSGA = Forward scatter gating area

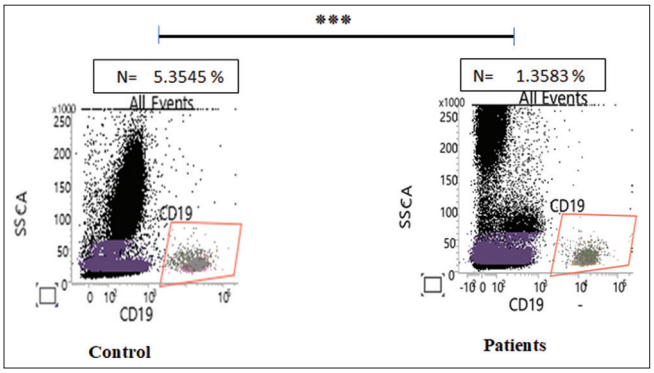

Figure 2: ***$P \leq 0.001$. The percentage of CD19⁺ B-cells in celiac disease patients and control group, *n* = the mean of percentage of B cell (%) from lymphocyte cells found in peripheral blood mononuclear cells population that examined. SScA = Side scatter area

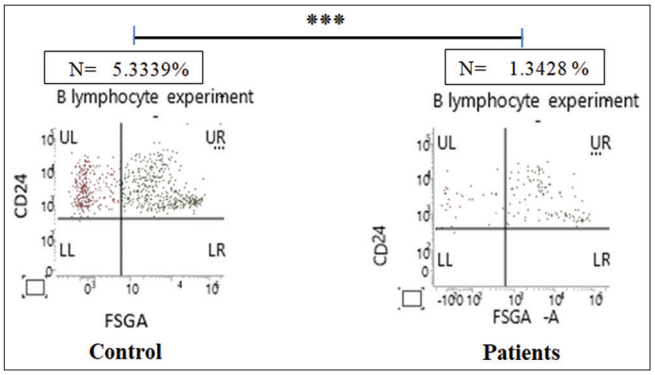

Figure 3: ***$P \leq 0.001$. The percentage expressing of CD24⁺ on B-cells in celiac disease patients and control group, *n* = the mean of the percentage of CD24⁺ (%) from B-cells found in peripheral blood mononuclear cells population that examined. FSGA = Forward scatter gating area

disease patients, in comparison with the ones in the control group. A proximative mean of percentage was (5.3339%) for patients and (1.3428%) for the control.

The prevalence CD19+CD80+B-cells

As shown in Figure 4, the results revealed a slight decrease in CD19+CD80+B-cells percentage for celiac disease patients in comparison with the control group. The mean was (5.3678%) for the celiac disease patients and (1.3159%) for the control one.

The prevalence CD19+CD27+B-cells

According to the presented data explained in Figure 5, a significant decline of the percentage of CD19+CD27+B-cells under the level $P \leq 0.001$ for celiac disease patients groups is detected, in comparison with those in the control group. The mean was (5.1542%) for the celiac disease patients group, while it was (1.7383%) for the control group.

Discussion

The average age of the children with celiac disease participating in this study was (9.5±6) years, while it was (9.7±5) years in the control group. All the subjects included in this study were male. The average disease duration of celiac disease patients was reached (3.8) years. Serum antibody tests play an important role in celiac disease diagnosis and management. The anti-tissue TG and anti-DGP levels are elevated inpatient children. This could be evidence that they have celiac disease compared with healthy individuals.

The results indicate a decrease in CD19⁺B-cell expression on lymphocytes in celiac disease patients compared to control, and these results are consistent with the study of Tompa and Faresjö[14] that indicated a lower prevalence of CD19⁺Bcell. Nevertheless, these B-cells have a significant role in producing autoantibodies,

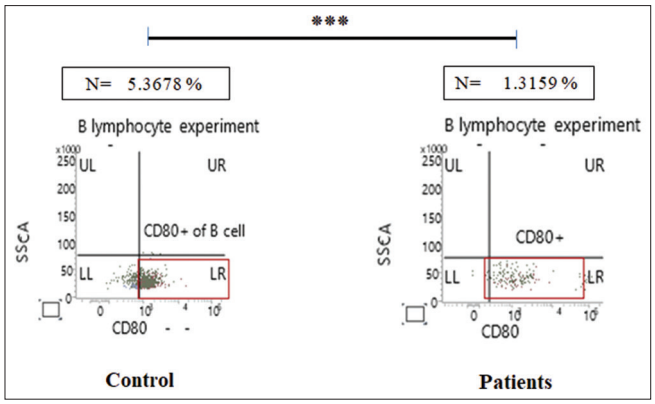

Figure 4: ***$P \leq 0.001$. The percentage expressing of co-stimulatory molecules CD80⁺ on B-cells in celiac disease patients and control group, *n* = the mean of the percentage of CD80⁺ (%) from B-cells found in peripheral blood mononuclear cells population that examined. SScA = Side scatter area

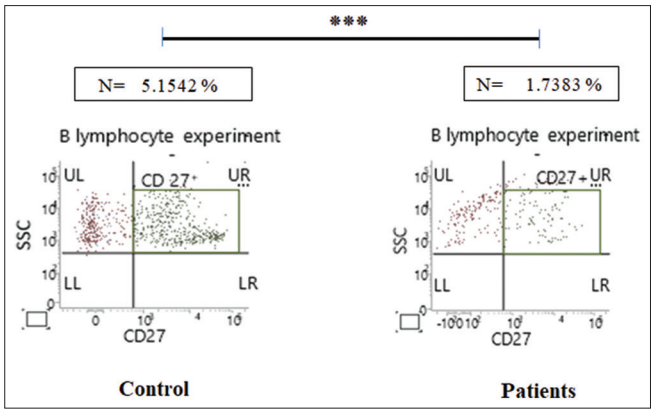

Figure 5: ***$P \leq 0.001$. The percentage expressing of CD27⁺ on B-cells in celiac disease patients and control group, *n* = the mean of the percentage of CD27+ (%) from B-cells found in peripheral blood mononuclear cells population that examined. SSC = Side scatter, UR = Upper right, LR = Lower right

even though disruptions in later phases of B-cell differentiation seem to be more crucial in numerous autoimmune disorders.

The involvement of B cell-expressed CD24, CD27, and co-stimulatory molecules CD80 in celiac disease is well possible. Empirical evidence has shown a substantial reduction in these indicators of immune function in individuals with the illness as compared to those who are in good health. In a prior investigation conducted by Santaguida *et al*.,[15] showed an elevation in CD19+CD24hiCD38negcells in individuals with celiac disease. In contrast, our work yielded unequivocal evidence of a reduction in CD-expressing B-cells. The possible explanation for this might be because this is the first diagnostic for patients, in contrast to the research done over a 12-month period of adhering to a diet that excludes gluten.

CD24 expression on B-cells has been implicated in some recent studies[16,17] CD24 has several immunomodulatory roles, including suppressing the production of self-reactive T-cells in the thymus, regulating the actions of antigen presentation cells, and facilitating the development of autoimmunity.[18] In addition, it plays a part in inhibiting the immune system in cases of autoimmune disorders.[19] Consequently, our findings suggest that reduced CD24 expression may serve as an indicator of the initiation of regulatory cell activation.

CD27 exerts a negative regulatory role on IL-10 production.[20] Therefore, it serves a vital role in regulating the immune response, and its abundant expression in B-cells contributes to the efficacy of regulatory cells and the management of inflammation.[21] The findings suggest a reduced presence of CD27+. CD27⁺ deficiency is a recently identified primary immune deficiency that is characterized by low levels of gamma globulins, poor function of particular antibodies, impaired T cell activity, immunological insufficiency and dysregulation.[22]

These results may apprehend the idea that a numerical shortage percentage of B-lymphocytes co-stimulatory molecules CD80⁺ in comparison to those who are in good health. This discovery aligns with, yet diverges from, the findings of Watanabe *et al*.[23] A higher percentage of B-cells CD80⁺ was seen in patients with advanced illness in comparison to those in the intermediate stage of disease, as indicated by the data. The participants in this research were diagnosed with the condition from the onset and did not adhere to a gluten-free diet. The B-cell expression of these immunological markers was significantly lower in comparison to the control groups. They may have been in the initial stage of the inflammatory phase of the celiac disease, and the immunological markers responsible for inhibition (on B-cells) were not activated.

Conclusion

The percentage of B-cells exhibited a notable reduction in patient samples as compared to those from healthy individuals. Furthermore, there was a significant reduction in the expression levels of CD24, CD27, and CD80 on B-cells across all patient samples in comparison to those from healthy children. These immune markers may have modulatory functions for inflammatory reactions and reducing them, and since the percentage of these immune markers has decreased in patients, this has led to an increase in the activity of cells that help stimulate autoimmune reactions and, thus, to an increase in tissue necrosis and the development of the disease. The results obtained may firstly contribute to selecting these markers as diagnostic markers that help in diagnosing wheat allergy and also targeting them in investigating appropriate treatments for this disease or reducing its severity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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