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ORIGINAL ARTICLE

The Effect of Iron Deficiency on Immune Parameters, Including Memory B Cells and Infection Presentations: A Cohort Study in Premenopausal Women

Demir Eksikliğinin Bağışıklık Parametreleri, Bellek B Hücreleri ve Enfeksiyon Bulguları Üzerine Etkisi: Premenopozal Kadınlarda Bir Kohort Çalışması

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ABSTRACT

Background: Iron deficiency anemia (IDA) is a condition associated with increased susceptibility to infections in the general population and is more common in premenopausal women (PW). Clinical research focusing on humoral immunity and immune memory is limited in contrast to laboratory research.
Objective: This study aims to explore the effects of iron deficiency (ID) and IDA on immune function in PW by assessing immunoglobulins, lymphocyte subsets, memory B cell (MBC) quantities, and infection presentations.
Methods: A total of 180 PW (34 with IDA, 65 with ID, 81 controls) who presented to the immunology outpatient clinic of a tertiary referral center between 2017 and 2024 were included. Measured parameters included hemoglobin, ferritin, immunoglobulins (IgG, A, M) and IgG subclasses, lymphocyte subsets (CD3+, CD4+, CD8+, CD19+, CD16+56+), and MBCs (CD19+CD27+IgM-IgD-).
Results: Compared to controls, the IDA group showed significantly lower total lymphocyte counts (1961.8±608.9 vs 2275.9±702.4 cells/μL, p=0.008), NK cell counts (167.7±90.3 vs 230.5±109.4 cells/μL, p=0.020), and MBC counts (21.9±10.5 vs 33.8±18.6 cells/μL, p=0.003). The percentage of MBCs among B lymphocytes was also reduced (12.2±4.5% vs 14.4±8.4%, p=0.045). Serum IgG levels were significantly lower in the IDA group (10.2±2.0 vs 11.6±2.9 g/L, p=0.017), with a more pronounced reduction in IgG1 (6.2±1.3 vs 7.8±2.4 g/L, p=0.003). Recurrent pneumonia within 3 years was more frequent in the IDA group compared to controls (29.4% vs 9.9%, p=0.021).
Conclusions: In PW with IDA, reductions in lymphocytes, NK cells, IgG, IgG1, and MBCs, along with the higher frequency of recurrent pneumonia, indicate a diminished capacity of immune responses against pathogens. Given the role of MBCs in maintaining long-term, even lifelong immune responses, the relationship between IDA and MBC levels warrants further investigation regarding the sustainability of long-term immunity.

Keywords: Humoral iImmunity, immunoglobulin, iron deficiency anemia, lymphocyte subset, memory B cell, perimenopause.

ÖZ

Giriş: Demir eksikliği anemisi (DEA), genel toplumda enfeksiyon sıklığındaki artışla ilişkilendirilen bir durumdur ve premenopozal kadınlarda daha sık görülmektedir. Humoral immünite ve immün hafıza üzerine yapılan klinik araştırmalar, laboratuvar temelli çalışmalara kıyasla sınırlıdır.
Amaç: Bu çalışmada, premenopozal kadınlarda demir eksikliği (DE) ve DEA'nın immün yanıt üzerindeki etkileri; immünoglobulin düzeyleri, lenfosit alt grupları, bellek B hücresi (BBH) miktarları ve enfeksiyon öyküsü açısından değerlendirilmiştir.
Yöntem: Bir tersiyer referans merkezinin immünoloji polikliniğine 2017 ile 2024 yılları arasında başvuran 180 premenopozal kadın çalışmaya dahil edildi. Enfeksiyon öyküsü, rutin görüşmelerde uygulanan altı soruluk standart bir form ile kaydedilmiş; elde edilen bilgiler reçeteler, radyolojik görüntüler ve hekim kayıtları üzerinden ulusal sağlık sisteminde doğrulanmıştır. Ölçülen parametreler arasında hemoglobin, ferritin, immünoglobulinler (IgG, A, M), IgG alt grupları, lenfosit alt grupları (CD3+, CD4+, CD8+, CD19+, CD16+56+) ve BBH'ler (CD19+CD27+IgM-IgD-) yer almıştır. Katılımcılar DEA, DE ve hemoglobin ile ferritin düzeyleri normal olan kontrol grubu olmak üzere üç gruba ayrılmıştır.
Bulgular: DEA grubunda toplam lenfosit sayısı (1961.8±608.9 vs 2275.9±702.4 hücre/μL, p=0.008), NK hücre sayısı (167.7±90.3 vs 230.5±109.4 hücre/μL, p=0.020), BBH sayısı (21.9±10.5 vs 33.8±18.6 hücre/μL, p=0.003) ve B hücreleri içindeki BBH yüzdesi (%12.2±4.5 vs %14.4±8.4, p=0.045) kontrol grubuna göre anlamlı düzeyde daha düşüktü. Serum IgG düzeyi (10.2±2.0 vs 11.6±2.9 g/L, p=0.017) ve özellikle IgG1 düzeyi (6.2 ±1.3 vs 7.8±2.4 g/L, p=0.003) de DEA grubunda düşük bulundu. Ayrıca, son 3 yıl içinde iki veya daha fazla radyolojik olarak doğrulanmış pnömoni öyküsü DEA grubunda kontrol grubuna göre daha sık saptandı (%29.4 vs %9.9, p=0.021).
Sonuç: DEA'lı premenopozal kadınlarda lenfosit, IgG, IgG1, NK ve BBH düzeylerindeki azalma ile birlikte tekrarlayan pnömoni sıklığındaki artış, patojenlere karşı bağışıklık yanıt kapasitesinde bir azalmaya işaret ediyor olabilir. Bellek B hücrelerinin enfeksiyonlara veya aşılara karşı uzun süreli immün yanıtların oluşumundaki rolü göz önünde bulundurulduğunda, DEA ile BBH düzeyleri arasındaki olası ilişki, uzun vadeli bağışıklığın sürdürülebilirliği açısından araştırmaya açık bir alan olarak değerlendirilebilir.

Anahtar Kelimeler: Bellek B hücresi, demir eksikliği anemisi, humoral immünite, immünoglobulin, lenfosit alt grupları, premenopoz.

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INTRODUCTION

Iron deficiency anemia (IDA) commonly affects women for a significant portion of their lives due to factors such as inadequate diet, menstruation, pregnancy, and childbirth. IDA is one of the leading causes of disability among women worldwide, including the developed countries (1). Depending on the level of development in various geographical regions, prevalence rates range from 4% to 35% (2, 3). Although many countries have implemented support and screening programs aimed at preventing IDA in childhood and pregnancy, and allocate substantial resources for this purpose, unfortunately not the same level of attention is given to women of reproductive age (4-6).

Iron, a critical element for living cells, not only supports the production of red blood cells but also regulates both innate and adaptive immunity (7, 8). In both children and adults, IDA is more frequently associated with infections compared to the general population (9, 10). Consequently, adults with IDA, particularly women of reproductive age where IDA prevalence is high, are often observed to present to hospitals with frequent infections. Immunologists typically conduct certain immunological screening tests for patients presenting with or referred to immunology clinics due to frequent infections, especially those who answer affirmatively to one or more of the questions recommended by the European Society for Immunodeficiencies (ESID) for assessing immunodeficiency (11). Screening generally begins with the measurement of immunoglobulins G, A, and M, as antibody deficiency-related immunodeficiencies are the most commonly observed types in adults. Further testing may include evaluating immunoglobulin G subclasses, vaccine responses, lymphocyte subset measurements, B lymphocyte class switch recombination (CSR), T lymphocyte activation studies, neutrophil oxidative burst tests, measurement of complement levels or genetic testing and more, depending on the patient's medical history. The clinician then applies the tests considered appropriate.

Considering the prevalence of iron deficiency in women, along with the associated decrease in quality of life and the financial burden of hospital admissions, this issue remains highly relevant, and many immunological aspects have yet to be fully elucidated. Recent studies have discussed iron's effects on innate immunity, particularly regarding neutrophil recruitment and oxidative burst, macrophage polarization, and natural killer (NK) cell activity (7, 12). Regarding adaptive immune responses, research has shown that in IDA, the activation and differentiation of Th1 and Th2 cells, as well as the production of immunoglobulins by antigen-stimulated B cells, are affected (7, 13, 14). An important topic related to B cells is the formation of memory cells, which ensure lifelong immunity to the infecting pathogen following a single infection. To our knowledge, no studies have yet examined the impact of iron deficiency (ID) and IDA on the class of switch recombination (CSR) capabilities of B cells, which are crucial for their transformation into memory B cells (MBCs), particularly in humans.

The aim of this study was to investigate whether iron deficiency (ID) and iron deficiency anemia (IDA) affect the circulating levels of memory-type B cells formed through class switch recombination of mature B lymphocytes in premenopausal

women. Additionally, the study evaluates lymphocyte subsets, absolute cell counts, and immunoglobulin levels and subclasses.

MATERIALS and METHODS

Study Design

This study included premenopausal women who reported frequent infections and consequently presented to or were referred to the Immunology Clinic of the Department of Clinical Immunology and Allergy at Medical Faculty of Necmettin Erbakan University between January 1, 2017, and January 1, 2024. All patients were screened using the six ESID Warning Signs questions; those with any positive response, verified through the national health database (radiology, prescriptions, physician records), underwent further immunoglobulin and flow cytometric screening, and additional tests were performed only when clinically indicated. Patients who were not suspected to have primary immunodeficiency after clinician-directed evaluation, and further investigations were concluded, were included in the study.

The subjects were categorized into three groups: those with iron deficiency (ID), those with iron deficiency anemia (IDA), and a control group with normal ferritin and hemoglobin levels. The results of the ESID warning signs and immunological workup data were compared among these groups.

Premenopausal women aged between 18 and 50 years who presented with frequent infections and had findings consistent with at least one of the ESID warning signs were included in the study. All participants underwent clinical and laboratory evaluations, and those in whom primary immunodeficiency was not suspected after clinician-directed workup were considered eligible.

Exclusion criteria comprised of pregnancy or lactation and having received iron therapy within the past three months. Patients receiving steroids, immunosuppressants, cytotoxic agents, or biological agents that could affect immunoglobulin levels for any indication were excluded, as were those with oncological, hematological, or treatment-requiring rheumatological diseases, cancer, or active chronic inflammatory conditions. Other exclusion factors involved alternative causes of anemia such as thalassemia trait or hemolytic anemia, a history of major hemorrhagic events, recent major surgery or blood transfusion within three months, and conditions associated with protein-energy malnutrition such as eating disorders.

Data Collection

Of the 248 women aged 18-50 initially considered for the study, 68 were excluded due to immunodeficiency diagnosis or suspicion, various rheumatological diseases, immunosuppressive treatments, thalassemia trait, malignancy, pregnancy, lactation, data insufficiency, or ongoing iron therapy. The study ultimately included 180 subjects: 34 with IDA, 65 with ID, and 81 with normal hemoglobin and ferritin levels (control).

The patients' positive responses to one or more of the six ESID warning signs were recorded. Before proceeding with advanced immunological screening at the clinic where the study was conducted, the infectious diseases reported by the patients were verified through radiological imaging, prescriptions, or relevant physician reports

obtained from the national health database. Additionally, immunological assessments are routinely performed after recovery, once clinical and laboratory findings are appropriate, for patients with active signs of infection.

The diagnosis of IDA was based on the WHO criteria, which requires a hemoglobin concentration of less than 120 g/L (12 g/dL) and a ferritin level below 30 µg/L.(15) For the ID group, ferritin levels were below 30 µg/L, while hemoglobin levels were 120 g/L or higher.

Laboratory Studies

Simultaneously measured hemoglobin and ferritin levels, leukocyte and lymphocyte counts, IgG, IgA, IgM, and IgG subclasses, as well as lymphocyte subsets (CD3+, CD4+, CD8+, CD16+56+, CD19+), the ratio of T helper to T cytotoxic lymphocytes (CD4/CD8), and class-switched memory B cells (CD27+IgM-IgD-) percentages and absolute counts were recorded using flow cytometry.

Complete blood count was performed using an automatic blood counting device (Beckman Coulter Corporation, Miami, USA). For serum ferritin measurement, an Architect ferritin-assay kit was used by chemiluminescent microparticle-immunoassay technique.

Total serum Ig levels were measured by nephelometry, using commercially available kits (Date Behring Marburg GmbH, Marburg, Germany).

Absolute lymphocyte counts were determined as the products of the white blood cell (WBC) count and the percentage lymphocyte proportion as measured by the Mindray BC-6200 auto hematology analyzer (Nanshan, Shenzhen, China). CD markers of lymphocyte subsets, CD27, IgD, IgM expressions in the CD19 complex were analyzed using flow cytometry (BD Facs Calibur; BD Biosciences, San Jose, USA). Class switched memory B cells defined as CD19+CD27+IgM-IgD-.

Statistical Analysis

Data analyses were performed using the SPSS software (v22; IBM Corp., Armonk, NY, USA). Shapiro-Wilk and Kolmogorov-Smirnov tests were conducted for normality of data distribution. ANOVA tests (with Bonferroni correction) were used to compare hemoglobin (Hg), hematocrit (Htc), mean corpuscular volume (MCV), ferritin, total lymphocyte count, and the percentages and absolute numbers of CD3+, CD4+, CD8+, CD56+, CD19+, and CD27+ lymphocytes, as well as IgG and IgG1 across the three groups. Data are presented as means ± standard deviation. The Kruskal-Wallis H test (with Bonferroni correction) was used to compare age, IgG2, IgG3, IgG4, IgM, and IgA. These variables are presented as medians with interquartile ranges. The Pearson correlation test was employed to assess the relationship between hemoglobin and ferritin levels with total lymphocytes, NK cells, memory B cells (MBCs), IgG, and IgG1, which showed significant differences between-group analyses. The Chi-Square Test was used for comparisons of positive findings in ESID warning signs among the groups. Significance was evaluated at a level of $P < 0.05$.

Patients were evaluated after approval was obtained from the Ethics Committee (Decision No:2024/5084, 5 July 2024). The study was conducted under the principles of the Declaration of Helsinki.

RESULTS

Demographic Characteristics

Age, mean hemoglobin (Hg), hematocrit (Htc), mean corpuscular volume (MCV), and ferritin values are shown in Table 1. As expected, the data for Hg, Htc, and MCV in the IDA group were significantly lower compared to the ID and control groups. For ferritin, however, there were significant differences among all three groups.

ESID Warning Signs Findings

Among the cases presenting with complaints of frequent infections, which are indicative of potential inborn errors of immunity, there were no significant differences between the groups regarding the frequency of infections requiring annual antibiotic use, recurrent infections, and prolonged antibiotic needs. However, there was a significant increase in the incidence of having experienced two or more radiologically proven pneumonias in the past three years in the IDA group compared to the control group (p=0.021, Table 2). When patients with recurrent pneumonia across all groups were analyzed separately, the only significant difference compared to

Table 1: Baseline demografic data

	Ref	Patients with iron deficiency anemia (n=34)	Patients with iron deficiency (n=65)	Control (n=81)	F/H	p
Age (years)		36 (27-42)	29 (25-35)	33 (25-40)	5.21	0.073
Hb (g/dL)	12.1-17.2	11.02 ±1.25	13.5±0.74	13.65 ±.83	**112.69**	**<0.001**
MCV (fL)	82.2-99	78.49 ±7.54	86.45±4,45	87.20 ±5.2	**29.48**	**<0.001**
Htc (%)	36.1-50.3	34.34 ±6.40	40.9±2.2	41.19 ±2.34	**53.94**	**<0.001**
Ferritin (ng/mL)	14.5-290	10.53 ±6.07	18.5±8.8	39.92 ±13.1	**107.73**	**<0.001**

Values are presented **as mean ± standard deviation (SD)** for normally distributed data, and as **median (interquartile range, IQR)** for non-normally distributed data.
ANOVA F-values and Kruskal–Wallis H-values with their corresponding p-values are shown for between-group comparisons. A p-value less than 0.05 is considered statistically significant and is shown in **bold.**

Table 2. Distribution of ESID warning signs among study groups

ESID Warning Signs	Control (n=81)	Iron Deficiency (n=65)	Iron Deficiency Anemia (n=34)	χ^2	p
Four or more infections requiring antibiotics within one year (otitis, bronchitis, sinusitis)	57 (58.9)	50 (47.3)	24 (24.7)	0.88	0,65
Recurring infections or infection requiring prolonged antibiotic therapy	29 (30.2)	22 (24.2)	16 (12.7)	1.79	0,41
Two or more severe bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis)	2	1	1	-	NA
Two or more radiologically proven pneumonia within 3 years	8 (11.7)	8 (9.4)	10 (4.9)	7.77	0,021
Infection with unusual localization or unusual pathogen	1	0	1	-	NA
PID in the family	-	-	-	-	-

PID: Primary immune deficiency, **ESID:** European Society for Immunodeficiencies, expected values according to Chi-Square (χ^2) test are given in parentheses, significance value is $p < 0.05$

those without recurrent pneumonia was in hemoglobin levels (p=0.007). Due to the low incidence of serious infections such as sepsis and meningitis, as well as infections with unusual localization or pathogens, no comparative statistical analysis was performed for these categories.

Lymphocyte and Subgroup Counts and Ratios

According to the results of the ANOVA test, there were significant differences between the three groups in total lymphocyte counts and the number of CD16+56+ natural killer (NK) lymphocytes. The total lymphocyte count and NK cell count were significantly lower in the IDA group compared to the control group (p=0.006 and 0.016, respectively). There were no differences between the groups in terms of the percentages and absolute counts of other lymphocyte subgroups or the ratio of T helper to T cytotoxic lymphocytes (CD4+/CD8+) (Table 3).

Table 3: Total lymphocyte counts, subgroup percentages and absolute numbers, memory B Cell counts, and percentages within B Cells

	Ref	Patients with iron deficiency anemia (n= 34)	Patients with iron deficiency (n=65)	Control (n=81)	F	p
		Mean ± Std deviation	Mean ± Std deviation	Mean ± Std deviation		
Lymphocyte count (cells/µL)	800-5500	1961.81 ±608.95	2189.06±653.7	2275.88 ±702.38	4.98	**0.008**
CD3+ T lymphocyte (%)	62-88	77.11 ±6.97	75.8±7.82	76.17 ±**5**.79	0.43	0.435
CD3+ T lymphocyte count (cells/µL)	678-2504	1501.63 ±509.80	1737.3±715	1709.70 ±508.24	1.99	0.067
CD19+ B lymphocyte (%)	6.3-20	10.00 ±3.92	10.54±4.26	10.39 ±**3**.82	0.21	0.409
CD19+ B lymphocyte count (cells/µL)	96-515	203.16 ±111.23	242.14±131.3	235.05 ±114.2	1.23	0.170
CD16+56+ NK cells (%)	3.2-23.2	8.96 ±4.26	9.58±4.35	10.27 ±4.10	1.26	0.317
CD16+56+ NK cells count (cells/µL)	45-523	167.71 ±90.29	216.27±116.04	230.49 ±109.37	4.00	**0.020**
CD4+(helper) T lymphocyte (%)	35.3-61.1	46.51 ±9.31	43.50±7.63	44.43 ±7.21	2.14	0.24
CD4+ (helper) T lymphocyte count (cells/µL)	414-1679	920.96 ±369.34	1008.90±451.82	1004.23 ±355.86	0.69	0.39
CD8+(cytotoxic) T lymphocytes (%)	11.2-37.3	30.28 ±7.85	29.29±6.48	28.9 ±6.81	0.46	0.254
CD8+ (cytotoxic) T lymphocytes (cells/µL)	162-1038	572.96 ±176.13	673.99±290.63	649.68 ±232.42	1.90	0.164
CD4+/CD8+ ratio		1.65 ±0.53	1.61±0.64	1.67 ±.64	0.13	0.607
CD19+CD27+IgD- (memory) B lymphocytes (%)	9.2-18.3	12.19 ±**4**.51	13.96±6.17	14.40 ±8.44	3.15	**0.045**
CD19+CD27+IgD- (memory) B lymphocytes (cells/µL)	18-40	21.91 ±10.52	30.97±14.96	33.807 ±**18**.62	5.91	**0.003**

The ANOVA test results with F and p values are presented. A p-value less than 0.05 is considered statistically significant and is shown in **bold. Abbreviations:** NK: Natural killer; $CD3^+$: Total T lymphocytes; $CD4^+$: Helper T lymphocytes; $CD8^+$: Cytotoxic T lymphocytes; $CD19^+$: B lymphocytes; MBC: Memory B cells; %: Percentage within the parent lymphocyte population.

Memory B Cell Percentages and Counts

Compared to the control group, a significant decrease in the percentage of isotypic switching memory B cells expressing CD19+CD27+IgM-IgD- was observed in the IDA group (p=0.042). Additionally, there were significant differences in memory B cell counts between the IDA and control groups as well as between the IDA and ID groups, with lower counts observed in the IDA group (p=0.02 and 0.025, respectively; Table 3).

Immunoglobulins

ANOVA testing revealed significant differences in IgG and IgG1 levels between the groups. Notably, IgG levels differed between the control and IDA groups (p=0.014). For IgG1, significant disparities were observed between the IDA and control groups, as well as between the IDA and ID groups (p=0.002 and 0.011, respectively, Figure 1). In contrast, for immunoglobulins IgG3, IgG4, IgM, and IgA, which do not follow a normal distribution, the Kruskal-Wallis H test showed no significant group differences (Table 4).

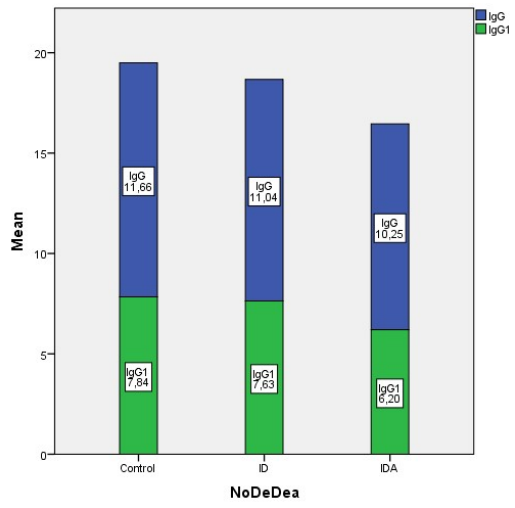

Figure 1. Bar graph showing mean serum IgG and IgG1 levels in the control, ID, and IDA groups.

Correlation Analyses

Correlation analyses revealed significant relationships between hemoglobin levels and total lymphocyte count (r=0.177, p=0.018), MBC count (r=0.240, p=0.001), and NK cell count (r=0.186, p=0.013). No significant correlations were observed between hemoglobin levels and MBC percentages, NK percentages, or IgG and IgG1 levels.

Ferritin levels were positively correlated with total lymphocyte count and absolute MBC count (r=0.21, p=0.023, and r=0.21, p=0.04, respectively), but no correlation was found with NK cell count (p=0.247). Additionally, no significant correlations were observed between ferritin levels and immunoglobulins.

No correlation was observed between MBC counts and IgG1 levels, the two parameters that showed a strong significancy against IDA in the intergroup comparisons (r = -0.034, p = 0.65, Table 5).

DISCUSSION

In the study, total lymphocyte counts and NK cell counts were significantly lower in premenopausal women with IDA compared to those without IDA. Regarding immunoglobulins, women with IDA had reduced levels of IgG compared to the control group, and their IgG1 levels were lower than those in both other groups. Although no significant differences were found between the groups in terms of the percentage and absolute number of B cells, the percentage and absolute number of memory B cells that had undergone isotypic class switch were significantly lower in the IDA group compared to the control group.

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Table 4: Immunoglobulins (IgG, IgA, IgM) and IgG Subclasses

	Ref	Patients with iron deficiency anemia (n= 34)	Patients with iron deficiency (n=65)	Control (n=81)	F/H	p
IgG (g/L)	7-16	10.19 ±2.03	11.03±1.99	11.62 ±2.90	4.18	0.017
IgM (g/L)	0.46-3.04	1.31 (0.83-1.95)	1.24 (0.85-1.62)	1.14 (0.93-1.50)	0.23	0.739
IgA (g/L)	0.7-4	1.92 (1.24-2.39)	1.62 (1.06-2.19)	1.43 (1.05-1.97)	0.69	0.510
IgG1 (g/L)	4.05-10.11	6.20 ±1.28	7.63±1.83	7.83 ±2.44	6.18	0.003
IgG2 (g/L)	1.69-7.86	4.01 (2.95-4.56)	3.94 (3.22-5.03)	3.97 (3.04-5.04)	0.23	0.66
IgG3 (g/L)	0.11-0.85	0.34 (0.25-0.57)	0.36 (0.26-0.49)	0.37 (0.29-0.52)	0.67	0.84
IgG4 (g/L)	0.03-2.01	0.33 (0.14-0.60)	0.39 (0.15-0.90)	0.32 (0.15-0.78)	1.44	0.77

Values are presented as mean±standart deviations for normally distributed data and median (IQR) for non-normally distributed data. ANOVA F-values and Kruskal-Wallis H-values with corresponding p-values are shown for between-group comparisons. A p-value less than 0.05 is considered statistically significant and is presented in **bold** text.

Table 5: Pearson correlation coefficients (r) and p-values among hematologic, immunologic, and cellular parameters

	Hg	Lymphocyte	Ferritin	IgG	IgG1	NK (% of lymphocytes)	NK number	MBC (% of B cells)
Lymphocyte	0.177** p=0.018							
Ferritin	0.381** p<0.001	0.211** p=0.004						
IgG	0.114ns p=0.128	-0.030ns p=0.687	0.173* p=0.021					
IgG1	0.128ns p=0.096	-0.047ns p=0.539	0.166** p=0.030	0.796** p<0.001				
NK (% of lymphocytes)	0.104ns p=0.167	-0.061ns p=0.413	-0.023ns p=0.762	0.136ns p=0.069	0.127ns p=0.097			
NK number	0.186* p=0.013	0.496** p<0.001	0.087ns p=0.247	0.132ns p=0.078	0.062ns p=0.419	0.778** p<0.001		
MBC (% of B cells)	0.090ns p=0.229	-0.093ns p=0.215	0.014ns p=0.849	-0.008ns p=0.919	0.097ns p=0.996	0.079ns p=0.079	0.131** p=0.043	
MBC number	0.240** p=0.001	0.430** p<0.001	0.211** p=0.004	-0.011ns p=0.878	-0.034ns p=0.655	-0.014ns p=0.848	0.259** p<0.001	0.426** p<0.001

Values are presented with their corresponding p-values. *p < 0.05, **p < 0.01, **p < 0.001; ns: not significant. Abbreviations: Hg: Hemoglobin; MBC: Memory B cells; IgG: Immunoglobulin G; IgG1: Immunoglobulin G subclass 1; NK: $CD16^{+}CD56^{+}$ natural killer cells; % of lymphocytes: Proportion among total lymphocytes; % of B cells: Proportion among $CD19^{+}$ B lymphocytes.

types and severity of frequent infections revealed that the IDA group had a higher incidence of recurrent pneumonia (more than once in the last three years) compared to the other two groups. The incidence of serious infections, such as meningitis and sepsis, was extremely low across all groups. In contrast, upper respiratory tract infections and sinusitis—relatively milder conditions—were the most common types of infections observed, with no significant differences between the groups. Lower respiratory tract infections, a major cause of mortality worldwide, are increasingly observed in children due to factors such as malnutrition, overcrowding, and exposure to smoke (16). In adults, who got immunodeficiency primarily due to HIV infection, is a major risk factor, the incidence of which is known to increase during pregnancy (17). Regarding IDA, while data indicate that in children, IDA is associated with an increased incidence of community-acquired pneumonia, such data are lacking for adults, warranting further extensive investigation (18, 19).

Analysis of laboratory parameters revealing significant differences between groups showed that total lymphocyte counts were lower in the IDA group compared to both the control and ID groups. Although weak correlations were observed between lymphocyte counts and both hemoglobin and ferritin levels, it is our belief that the presence of anemia was the primary factor determining the significant differences between groups, especially considering the broader correlations of hemoglobin with other parameters and the associated p-values. Optimal levels of iron and associated reactive oxygen species (ROS) are essential for the production and maturation of lymphocytes, as well as for the development and differentiation of other hematopoietic stem cells (HSCs). Low ROS levels are essential for maintaining bone marrow quiescence (imperturbability), while an increase in ROS is required for HSC differentiation. (20) Similar studies supporting the negative impact of IDA on lymphocyte counts through clinical findings exist (21, 22). These studies particularly attribute the decrease to reductions in CD3 T lymphocyte counts, and in one study, additionally in CD4 T lymphocytes (22). However, in the present study, the observed intergroup differences in total lymphocyte counts were due to nonsignificant reductions across all lymphocyte subgroups. The only subgroup with a significant decrease, albeit the least impactful on the total count, was the Natural Killer (NK) cells expressing CD16 and CD56.

The decrease in the number of NK cells in the IDA group found in this study does not directly indicate a decrease in their activity. Therefore, no conclusions can be drawn regarding IDA-induced NK cell functions. However, Salomon et al. demonstrated that NK activity in IDA mice infected with a virus was lower compared to non-IDA mice (12). This critical lymphocyte group, which plays a role in the innate immune response, is particularly important in defending against large DNA viruses such as the Epstein-Barr virus and Human Papillomavirus. While marker changes in stressed cells are sufficient for their activation, NK cells also play a significant role in guiding adaptive immune responses (23). NK cells are known to have a multifaceted interaction with B cells, involving IFNγ-mediated viral killing and subsequently influencing the isotype-switching ability of B lymphocytes, thereby impacting humoral immunity (24).

Recently, NK cells have been targeted in the development of drugs for viral and cancer immunotherapy due to their multifaceted crosstalk on adaptive immunity as well as being strong killer innate cells (25, 26). The iron balance in the organism is critically important for these essential cells.

The question of whether the number and function of B lymphocytes, the key cells of humoral immunity, are affected by IDA remains a topic of debate. Similar studies support the findings of this study, demonstrating that peripheral B cell counts are not affected by IDA (21, 22). While some publications conclude that IDA does not influence immunoglobulin levels and their subgroups, there are also studies that have detected IDA-induced decreases in IgG levels (8, 27, 28). In the current study, IgG levels were found to be lower in the IDA group compared to other groups, with this difference being primarily driven by a decrease in IgG1. However, for a comprehensive assessment of humoral immunity, it seems insufficient to measure only the number of mature B lymphocytes and immunoglobulin levels in the blood. The activity of humoral immunity can primarily be assessed by the ability of B lymphocytes to differentiate into plasma cells capable of producing specific immunoglobulins in response to pathogen stimulation. In addition, the formation of memory B cells (MBCs), some of which can rapidly differentiate into plasma cells upon re-exposure to the same pathogen and produce a specific immunoglobulin response, while others migrate into the germinal centers of lymphoid structures, is a crucial component of the humoral immune response. The MBCs that rapidly differentiate into plasma cells are predominantly of the IgG1 subtype, and once differentiated, they retain the capacity to produce large quantities of IgG1 antibodies.(29) The success of nearly all vaccines currently in use is evaluated based on their ability to induce B cell memory, specifically IgG1 type MBCs (30). Although it can be hypothesized that there may be a relationship between the amount of IgG1 and the number of MBCs, which showed a significant difference against IDA in the intergroup comparison, there is insufficient evidence to support this hypothesis, and no correlation was found between the two.

Long-term protection against infection is achieved through the two specific B cell types mentioned above: long-lived plasma cells that produce protective antibodies, and memory B cells that can respond to reinfection by pathogens and even their variants (29). The differentiation into both cell types occurs in the germinal centers of lymphoid organs, mediated by T cells. The activity of these two specialized cell types can be observed in clinical studies through various methods. The formation and activity of short- and long-lived plasma cells can be indirectly monitored through post-vaccination antibody levels. The effect of IDA on these cells was clearly demonstrated by Jiang et al. in mice. Specific short-term antibody production, as well as long-term antibody levels observed after including booster doses, were found to be significantly lower in iron-deficient mice compared to the control group. Following iron replacement, the formation of CD138+ plasma cells normalized. Furthermore, the analysis of the spleens of vaccinated mice revealed that those with IDA had fewer germinal center regions (14). Considering that memory B cells are derived from the

same group of activated B cells in the germinal center and can rapidly differentiate into plasma cells upon stimulation by the same pathogen, it is plausible to conclude that MBCs are affected by IDA similarly to plasma cells. Consistent with this, Lehrke et al. demonstrated in mice that an increase in ROS is necessary for the proliferation and class-switching capabilities of B cells, a process mediated by iron-sulfur clusters and their transporters (31). In this study, the amount of circulating B cells that had previously been exposed to antigen and class switched into memory B cells (MBCs) was evaluated using surface markers, along with their percentage in non-activated mature B lymphocytes. It is noteworthy that anemia causes a decrease in total lymphocyte counts; however, its negative effect on memory B cell (MBC) counts occurs without a significant reduction in overall B cell levels. To the best of our knowledge, this study is the first clinical research to specifically investigate the impact of IDA on the quantity of circulating MBCs. An additional strength of our study is the use of a well-defined premenopausal cohort, the combined assessment of cellular, humoral, and clinical parameters, and the verification of infection history through the national health database, which together enhance the reliability and novelty of the findings.

This study has a couple of limitations. First, it was conducted in a single center with a relatively small sample size. Clinical case-control studies in this field usually involve similar or smaller sample sizes.21, 22, 27 An additional limitation of our study is that all groups consisted of women who presented with frequent infections, and therefore, a truly healthy control group without recurrent infections was lacking. This restricts the generalizability of our findings to the broader population. Future studies should include infection-free healthy controls in order to better delineate the specific immunological impact of iron deficiency and iron deficiency anemia. Third, the study assessed the number of MBCs and their proportion in mature B cells in blood samples. However, MBCs were evaluated only by surface phenotyping (CD19+CD27+IgM-IgD-), without performing functional validation. While this approach allows the identification of class-switched memory-type B cells, it does not provide information on their antigen specificity or functional responsiveness, such as the ability to differentiate into plasma cells upon re-exposure to antigens. Finally, although infection history was verified through the national health database, thereby reducing the risk of misreporting, some degree of recall bias may still be present due to the reliance on patient-reported data. In this study, the observed relative reduction in MBCs was presented together with alterations in immunoglobulins and NK cell counts, alongside the clinical findings, in order to provide an integrated perspective. Such an approach is rare in adult cohorts and was intended to contribute to the literature. Nevertheless, definitive conclusions can only be drawn in future studies with larger multicenter cohorts, ideally including a truly healthy control group. In addition, the inclusion of functional tests such as cell activation or vaccine response assessments may clarify the interpretation of long-term immune competence.

CONCLUSION

In conclusion, premenopausal women with IDA showed reductions in lymphocyte counts, NK cells, IgG and IgG1 levels, and memory B cells, together with a higher frequency of recurrent pneumonia, which may indicate a diminished capacity to mount effective immune responses. While our study aimed to provide an integrated perspective by combining cellular, humoral, and clinical findings, the absence of a truly healthy control group and the lack of functional validation limit definitive interpretations regarding long-term protective immunity. Future studies with larger, multicenter cohorts and prospective follow-up designs will be essential to clarify the temporal course of infections and immune parameters and to validate these findings through functional assays such as vaccine response evaluations. Premenopausal women as a priority target group for health interventions, as they play a vital role in the well-being of society.

Conflict of Interest

The authors declare no conflicts of interest.

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