Research Article

Immune and cytokine dysregulation associated with low-grade inflammation in male adolescents

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Abstract

Background: Low-grade inflammation (LGI) is a subclinical inflammatory state characterized by persistent, modest elevations in circulating inflammatory mediators and has been linked to later cardiometabolic risk. Data describing adolescent inflammatory profiles in Middle Eastern populations remain limited. **Purpose:** To evaluate the immune and cytokine profile associated with LGI in Iraqi school-aged male adolescents. **Methods:** This cross-sectional analytical study included 80 male adolescents aged 15-18 years recruited from secondary schools in Baghdad, Iraq, between March and August 2025. Participants were stratified by age and school and were operationally classified into an LGI group (n=15; hs-CRP $\geq$3.0 mg/L) and a control group (n=65). Inflammatory markers were measured using commercial ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China) and an immunoturbidimetric assay. **Findings:** Adolescents in the LGI group had higher hs-CRP (5.13±1.11 vs. 0.57±0.18 mg/L), IL-6 (6.17±1.19 vs. 1.34±0.31 pg/mL), TNF-α (12.84±2.16 vs. 8.37±1.24 pg/mL), and NLR (3.36±0.49 vs. 1.72±0.21), while IL-10 was lower (2.10±0.34 vs. 5.09±0.49 pg/mL; p¡0.001). Standardized effect sizes were large across all markers (Cohen's d: 1.8-2.9). **Conclusion:** In this sample, LGI was associated with a consistent pro-inflammatory profile marked by higher pro-inflammatory mediators, higher NLR, and lower IL-10. These findings support further longitudinal evaluation and targeted prevention strategies in adolescents with cardiometabolic risk factors.

Keywords: low-grade inflammation, adolescents, cytokines, immune dysregulation, hs-CRP, NLR

1 Introduction

Low-grade inflammation (LGI) is a subclinical chronic inflammatory state characterized by modest but persistent elevations in circulating inflammatory mediators without overt clinical symptoms. Unlike acute inflammation, LGI can remain sustained over time and has been implicated in the pathophysiology of chronic disorders including cardiovascular disease, type 2 diabetes mellitus, and metabolic syndrome [1, 2, 3].

The inflammatory response is coordinated by a network of cytokines and acute-phase reactants. High-sensitivity C-reactive protein (hs-CRP) is widely used as a practical marker of systemic inflammation [4]. Among cytokines, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are central pro-inflammatory mediators, whereas interleukin-10 (IL-10) exerts counter-regulatory anti-inflammatory effects [5, 6]. The neutrophil-to-lymphocyte ratio (NLR) has also emerged as an accessible hematologic indicator of systemic inflammatory status [7].

Adolescence is a biologically sensitive developmental period marked by hormonal, metabolic, and immune changes that may influence inflammatory regulation into adulthood [8, 9]. Several factors linked to modern adolescent health, including obesity, physical inactivity, poor sleep, and psychosocial stress, may contribute to LGI during this stage [10, 11]. Prior studies have shown associations between inflammatory markers and cardiometabolic risk factors in young populations [12]; however, data describing immune profiles in adolescents from the Middle East remain limited [13].

Accordingly, the aim of the present study was to characterize the inflammatory profile associated with LGI in an Iraqi school-based sample of male adolescents by comparing pro-inflammatory markers (hs-CRP, IL-6, and TNF-α), the anti-inflammatory cytokine IL-10, and the innate immune indicator NLR.

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2 Materials and methods

2.1 Study design

This was a cross-sectional analytical study conducted between March and August 2025 to examine the immune and cytokine profile associated with LGI among male adolescents.

2.2 Population and sampling

The study enrolled 80 male adolescents aged 15-18 years from Al-Mansour Boys Secondary School and Al-Mansour Mixed School in Baghdad, Iraq. Stratified random sampling was performed according to (1) age group (15-16 vs. 17-18 years) and (2) school in order to maintain proportional representation across strata. Recruitment was restricted to males to reduce variation related to sex-associated hormonal and cytokine differences within this exploratory sample.

2.3 Eligibility criteria

Inclusion criteria were apparently healthy male adolescents with no known history of chronic disease. Exclusion criteria were signs or symptoms of acute or febrile infection during the preceding 2 weeks, current use of anti-inflammatory or immunosuppressive medications, or known autoimmune disease. To reduce misclassification by acute inflammation, participants were screened using a standardized symptom checklist and an axillary temperature measurement on the day of collection (excluding ¿37.5°C).

2.4 Group classification

Participants were operationally classified into the LGI group (n=15) when hs-CRP was ≥3.0 mg/L and into the control group (n=65) when hs-CRP was <3.0 mg/L, using the commonly cited cardiovascular risk stratification threshold as an exploratory cut-point [14].

2.5 Laboratory investigations

Venous blood samples (5 mL) were collected after an overnight fast (8-12 hours), processed within 2 hours, and stored at -80°C until analysis. The following parameters were measured:

- *hs-CRP:* Immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany), sensitivity = 0.1 mg/L.
- *IL-6:* Sandwich ELISA (Elabscience Biotechnology Co., Ltd., Wuhan, China; Cat# E-EL-H0102), detection range 0.31-20 pg/mL.
- *TNF-α:* Sandwich ELISA (Elabscience; Cat# E-EL-H0109), detection range 7.81-500 pg/mL.
- *IL-10:* Sandwich ELISA (Elabscience; Cat# E-EL-H0103), detection range 1.56-100 pg/mL.
- *NLR:* Calculated from the automated complete blood count (Sysmex XN-1000, Sysmex Corporation, Kobe, Japan).

Quality control: Each sample was measured in duplicate, and intra-assay coefficients of variation did not exceed 10% in accordance with manufacturer specifications. All cytokine values were within the stated detection ranges of the respective kits.

2.6 Ethical considerations

The study protocol was approved by the Ethics Committee of Al-Turath University (Approval: 01/03/2025) in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or guardians, and written assent was obtained from the adolescents.

2.7 Statistical analysis

SPSS v.26.0 (IBM Corp., Armonk, NY) and Python 3.10 (SciPy) were used for the analyses. Continuous variables are presented as mean ± SD. Normality was assessed with the Shapiro-Wilk test, and homogeneity of variances was evaluated with the Levene test. Independent-samples t-tests were used for normally distributed variables with homogeneous variances, whereas Welch's t-test was applied when variances were unequal. Table 2 identifies the specific test used for each biomarker. Box plots and the z ¿ 3.29 rule were used to screen for extreme outliers. Effect sizes were estimated using Cohen's d with 95% confidence intervals (95% CI). Effect sizes were interpreted as small (0.2), medium (0.5), and large (≥0.8); negative d values indicate lower values in the LGI group. Correlations were assessed using Spearman's rank correlation coefficient because hs-CRP was non-normally distributed. Statistical significance was set at $p<0.05$. BMI, diet, physical activity, and sleep quality were not measured and were therefore considered potential sources of residual confounding.

3 Results

3.1 Background sample characteristics

The study included 80 male adolescents, with 15 (18.75%) classified in the LGI group and 65 (81.25%) in the control group. Table 1 summarizes the baseline characteristics. The groups were similar with respect to age (p=0.523), and the distributions across age strata and school were comparable. No extreme outliers were identified for the biomarkers analyzed.

Table 1: Baseline characteristics of the participants

Characteristic	*LGI (n=15)*	*Control (n=65)*	*p*
Age (years), mean ± SD	16.4 ± 1.1	16.2 ± 1.0	0.523
Male sex, n (%)	15 (100%)	65 (100%)	-
Age group 15-16 years, n (%)	8 (53.3%)	36 (55.4%)	0.887
Age group 17-18 years, n (%)	7 (46.7%)	29 (44.6%)	-
Al-Mansour Boys School, n (%)	9 (60.0%)	41 (63.1%)	0.822
Al-Mansour Mixed School, n (%)	6 (40.0%)	24 (36.9%)	-

SD = Standard Deviation. Independent t-test (continuous variables) or chi-square (categorical variables) comparisons.

3.2 Inflammatory and cytokine markers

Table 2 presents the between-group comparison of inflammatory markers. As expected from the operational group definition, hs-CRP was substantially higher in the LGI group. In addition, IL-6 (4.6-fold) and TNF-α (1.5-fold) were higher, IL-10 was 2.4-fold lower, and NLR was approximately two-fold higher in the LGI group. All marker comparisons were statistically significant, and the confidence intervals for the effect sizes did not cross zero.

Table 2: Comparison of inflammatory and cytokine markers between groups

Parameter	*LGI (n=15)*	*Control (n=65)*	*p*	*d (95% CI)*	*Effect*	*Test*
hs-CRP (mg/L)	5.13 ± 1.11	0.57 ± 0.18	*<0.001*	2.89 (2.1-3.7)	Large	Welch
IL-6 (pg/mL)	6.17 ± 1.19	1.34 ± 0.31	*<0.001*	2.65 (1.9-3.4)	Large	Welch
TNF-α (pg/mL)	12.84 ± 2.16	8.37 ± 1.24	*<0.001*	2.53 (1.8-3.3)	Large	t-test
IL-10 (pg/mL)	2.10 ± 0.34	5.09 ± 0.49	*<0.001*	-2.03 (-2.7 to -1.3)	Large	t-test
NLR	3.36 ± 0.49	1.72 ± 0.21	*<0.001*	1.84 (1.1-2.6)	Large	Welch

Data: Mean ± SD. d = Cohen's d. 95% CI = 95% Confidence Interval. NLR = Neutrophil-to-Lymphocyte Ratio. Large effect: d ≥0.8. Negative d values indicate lower values in the LGI group. Welch = Welch's t-test (used when Levene's p<0.05).

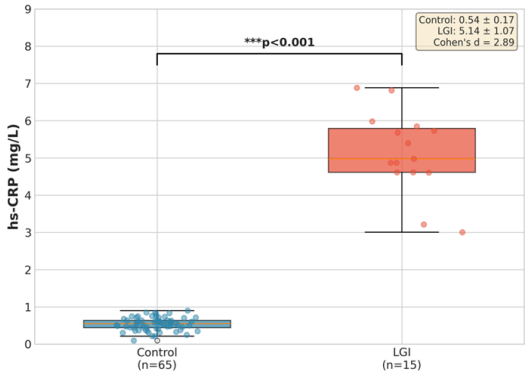

Figure 1: High-sensitivity C-reactive protein (hs-CRP) levels in the low-grade inflammation (LGI) and control groups. Box plot with individual data points. ***p<0.001 (Welch's t-test)

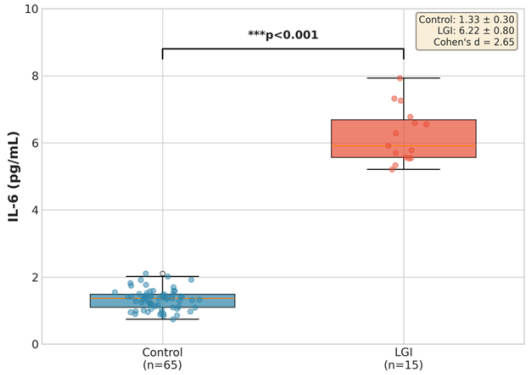

Figure 2: Interleukin-6 (IL-6) levels in the LGI and control groups. Box plot with individual data points. ***p<0.001 (Welch's t-test)

The distributions of the inflammatory markers are shown in Figures 1-4. Figure 1 illustrates the expected elevation of hs-CRP in the LGI group based on the operational classification criterion. Figure 2 shows higher

IL-6 concentrations, Figure 3 compares TNF-α and IL-10 across groups, and Figure 4 demonstrates a strong positive association between hs-CRP and NLR (Spearman r=0.87; p<0.001).

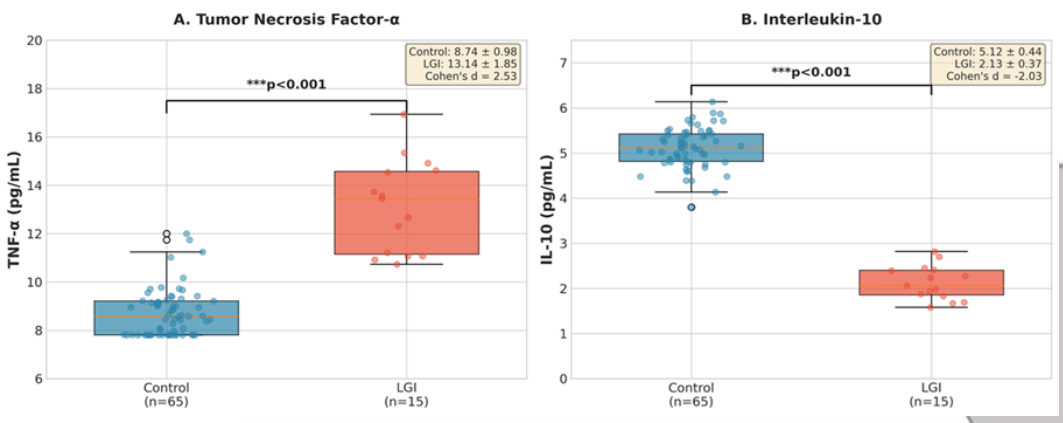

Figure 3: Pro-inflammatory TNF-α and anti-inflammatory IL-10 levels in the LGI and control groups. (A) TNF-α was higher in the LGI group. (B) IL-10 was lower in the LGI group. ***p<0.001 (t-test)

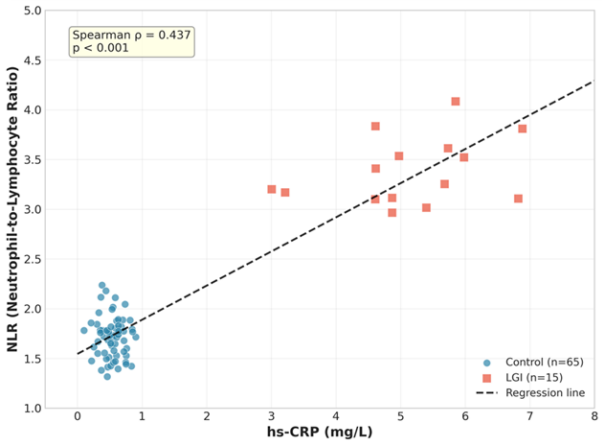

Figure 4: Spearman correlation between hs-CRP and the neutrophil-to-lymphocyte ratio (NLR). A strong positive rank correlation (r=0.87; p<0.001) is shown between the two inflammatory markers.

4 Discussion

This study provides preliminary evidence that Iraqi male adolescents classified as having LGI also exhibit a broader pro-inflammatory profile. Beyond the expected difference in hs-CRP resulting from the operational grouping criterion, the LGI group showed higher IL-6, higher TNF-α, higher NLR, and lower IL-10, with large standardized effect sizes. The concordant direction of these markers strengthens the internal consistency of the observed inflammatory pattern.

The hs-CRP level in the LGI group (5.13 mg/L) was above the 3.0 mg/L threshold used to define the group, so this result primarily confirms the classification framework rather than representing an independent discovery. Nevertheless, the observed pattern is clinically relevant because prior data in youth suggest that higher CRP is associated with adverse cardiometabolic risk profiles [15, 16]. The concomitant elevation of IL-6 and TNF-α is also biologically consistent with previous reports linking inflammatory activation to adolescent metabolic risk [17, 18].

The lower IL-10 concentration observed in the LGI group is notable because IL-10 functions as a central anti-inflammatory regulator [19]. Reduced IL-10, alongside higher pro-inflammatory mediators, suggests weaker counter-regulatory balance within the inflammatory milieu. Given the cross-sectional design, however, these findings should be interpreted as associations rather than evidence of a causal mechanism or fixed immune dysregulation.

The higher NLR further supports the presence of systemic immune activation using a routinely available hematologic marker [20]. Although the effect sizes were large (d=1.8-2.9), the modest LGI subgroup and the absence of key covariates such as BMI, diet, physical activity, and sleep require cautious interpretation of the magnitude and generalizability of these differences.

4.1 Public health implications

These findings may inform adolescent health programs, but they should be interpreted cautiously. The proportion of participants classified as LGI (18.75%) suggests that low-grade inflammatory profiles can be detected even among apparently healthy adolescents; however, the present data are not sufficient to support routine population-wide school screening. Instead, the results support targeted risk assessment and preventive lifestyle counseling for adolescents with established cardiometabolic risk factors, while larger longitudinal studies clarify the predictive value of hs-CRP and related biomarkers [21].

4.2 Strengths and limitations

Strengths of the study include its school-based design, community sampling of adolescents, systematic exclusion of probable acute infection through symptom screening and temperature assessment, inclusion of both pro-inflammatory and anti-inflammatory biomarkers, and duplicate laboratory measurements with predefined quality-control thresholds. The study also contributes data from an underrepresented regional population.

Important limitations should also be acknowledged. The cross-sectional design precludes causal inference, the LGI subgroup was small (n=15), and cytokines were measured at a single time point without repeated sampling. In addition, BMI, waist circumference, dietary patterns, physical activity, sleep quality, and pubertal maturation were not assessed, leaving substantial potential for residual confounding. Because only males were included, the findings should be generalized cautiously. Future longitudinal studies with larger, more diverse samples and multivariable adjustment are needed to confirm these associations.

5 Recommendations

Based on the findings of this study, we suggest:

- Consideration of targeted clinical risk assessment for adolescents with established cardiometabolic risk factors (obesity, sedentary lifestyle, or family history of cardiometabolic disease), rather than immediate population-wide screening;
- Development of lifestyle intervention programs, including dietary education and promotion of regular physical activity;

- Longitudinal studies to clarify inflammatory trajectories and determinants in Middle Eastern adolescents of both sexes, with measurement of major confounding variables.

6 Conclusion

This study showed that Iraqi male adolescents operationally classified as having LGI had a consistent pro-inflammatory biomarker pattern characterized by higher IL-6, higher TNF-α, higher NLR, and lower IL-10. Although the findings support the relevance of early risk-oriented preventive strategies in adolescents with cardiometabolic vulnerability, they should be interpreted as preliminary associations that require confirmation in larger longitudinal studies before broad screening recommendations are made.

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