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## ORIGINAL ARTICLE

# Evaluation of Some Early Inflammatory Markers in Obese Children

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## Abstract

**Background:** Obesity affects both children and adults and is a major burden on world health. Focusing on childhood obesity is crucial to preventing its problems and further development as adults. Around the world, the prevalence of this nutritional condition among children has risen to alarming levels.

**Aim and objectives:** To evaluate interleukin 6 (IL6) as a predictive inflammatory cytokine and its relation to BMI and lipid profile, in obese and overweight children identifying those more likely to develop further complications, and also to assess the correlation between highly sensitive C-reactive protein as an inflammatory marker and obesity. The study also aims to evaluate the usability of neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) in the detection of subclinical inflammation in obese children.

**Patients and methods:** Case–control study best describes this research. Ninety kids of both sexes participated in the study; their ages ranged from 6 to 12 years. The study was carried out on outpatients and inpatients at the Pediatric Department at Al-Zahraa University Hospital.

**Results:** Among the groups that were compared, there was a discernible dissimilarity in anthropometric measurements, triceps skinfold thickness, correlation between BMI and (PLR, NLR, IL6, and high-sensitivity C-reactive protein) and glycated hemoglobin, and lipid profile except for low-density lipoprotein.

**Conclusion:** Children who are overweight or obese tend to have higher NLR and PLR ratios than children who are a healthy weight. Children who are overweight or obese have higher levels of inflammatory mediators including high-sensitivity C-reactive protein and IL6 compared with the healthy control group.

**Keywords:** Inflammatory markers, Neutrophil-to-lymphocyte, Obese children, Platelet-to-lymphocyte

## 1. Introduction

Obesity is a tremendous burden that not only affects adults but also youngsters all over the world and poses a substantial threat to public health. Attention must be paid to the problem of childhood obesity to reduce the risk of the consequences that are linked with it. This nutritional issue has reached epidemic proportions among children around the world. Because of the complicated interplay between genetic predisposition and environmental (or ‘obesogenic’) variables in the development of obesity, the latter provide the foundation for effective therapies.<sup>1</sup>

The CDC defines obesity as having a BMI greater than or equal to the 95th percentile for an individual's age group, while ‘at risk for overweight’ is defined as having a BMI between the 85th and 95th percentiles for one's age.<sup>2</sup>

Diabetes mellitus, high blood pressure, coronary heart disease, and stroke are only a few of the many co-occurring conditions associated with obesity. Each of these co-occurring disorders is facilitated by inflammation to varying degrees. In an obese person, adipocytes trigger inflammatory cascades by undergoing molecular alterations and experiencing intracellular stress.<sup>3</sup>

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Obesity is also linked to problems with the immune system. Adipose tissue is infiltrated by inflammatory cells in obese people, leading to a low-grade, persistent systemic inflammation caused by both fat cells and inflammatory cells. Obesity and its accompanying disorders have been studied in relation to the neutrophil–lymphocyte ratio (NLR) and the platelet–lymphocyte ratio (PLR), both of which are considered indications of subclinical inflammation.<sup>4</sup>

The purpose of this research was to determine whether or not interleukin 6 (IL6), a type of inflammatory cytokine, could be used to predict whether obese and overweight children will go on to develop additional difficulties, based on their BMI and lipid profile.

To assess the correlation between highly sensitive C-reactive protein as an inflammatory marker and obesity.

To evaluate the usability of NLR and PLR in the detection of subclinical inflammation in obese children.

## 2. Patients and methods

A case–control study methodology was used for this research. The study was conducted on 90 children collected consecutively from Al-Zahraa University Hospital (inpatients and outpatients) throughout the period beginning in May 2022 and ending in December 2022. Their ages ranged from 6 to 12 years, males and females, and separated into a couple of groups: group I: a group of patients which was divided into I.a: 30 obese children, chosen according to an excessively high BMI, based on both sex and age without any pathologies (CDC, 2021) and I.b: 30 overweight children with BMI between 85th and 95th percentile for age and sex without any pathology (CDC, 2021) and group II (control group): 30 typically developing children of similar ages and sexes served as a healthy comparison group.

**Inclusion criteria:** children aged 6–12 years, obese children (BMI  $\geq$  95th percentile), overweight children (BMI 85th–95th percentile), both sexes, and all selected children have no signs of puberty.

**Exclusion criteria:** children with endocrine, hepatic, renal, cardiac, and autoimmune disorders, children on drugs affecting body weight such as corticosteroids, and children with genetic disease and syndromes causing obesity.

### 2.1. Sample size

This study was based on a study carried out by Correia-Costa et al.,<sup>5</sup> which was used to calculate

the sample size by considering the following assumptions: 95% two-sided confidence level, with a power of 80% and an  $\alpha$  error of 5%. The final maximum sample size taken from the output was 82. Thus, the sample size was increased to 90 participants to assume any dropout cases during follow-up.

The following conditions were applied to all groups in the study: full history taken (name, age, and sex).

**Medical history** [any past or current medical history of disease (renal, hepatic, endocrinal), drug use history (steroids and other drugs)], and history of surgical operations.

**Dietetic history:** feeding for the first 6 months, eating fast food, eating nutritious foods like vegetables and fruits, and eating starchy and sweaty food are all part of a healthy diet. Conditions, procedures, and medications previously taken, child's complaints and family history of conditions such as obesity, diabetes, heart disease, eating disorders, gallstones, and high blood pressure.

**Physical examination:** all individuals underwent a thorough physical examination that looked for comorbidities and any underlying causes of their increased weight.

**General examination:** general appearance, vital signs including temperature, blood pressure (using an appropriate sized blood pressure cuff), pulse, and respiratory rate.

A comprehensive clinical examination was performed on all patients, with special attention paid to blood pressure.

Normal blood pressure less than 90th percentile, elevated BP more than or equal to 90th percentile to less than 95th percentile, or 120/80 mmHg to less than 95th percentile (the lowest of the two), stage 1 HTN more than or equal to 95th percentile to less than 95th percentile + 12 mmHg or 130/80–139/89 mmHg (regardless of the lesser) and stage 2 HTN more than or equal to 95th percentile + 12 mmHg, or more than or equal to 140/90 mmHg (whatever value is less).<sup>6</sup>

**Skin examination:** hyperpigmented, papillomatous, velvety thickening of the skin known as acanthosis nigricans can appear anywhere on the body, including the axilla, the sides of the neck, the groin, the antecubital and popliteal surfaces, the umbilical area, and even the mucosal surfaces.

**Systemic examination:** cardiovascular examination, abdominal examination, and musculoskeletal examination.

**Anthropometric measures:** triceps skinfold thickness, weight, height, BMI, waist circumference, hip circumference, and waist-to-hip ratio (Figs. 1 and 2).

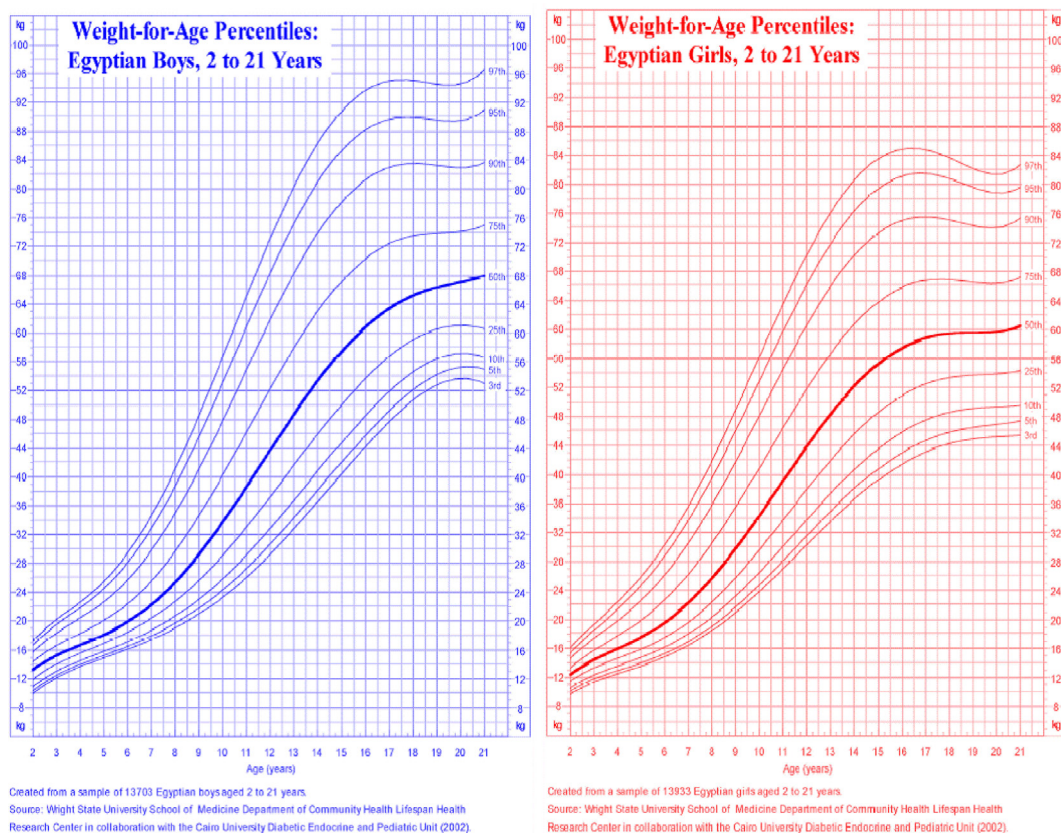


Fig. 1. Weight-for-age percentile (2–21years).<sup>7</sup>

Laboratory investigation: routine investigations and special laboratory investigation.

Special investigation: Special laboratory investigation: high-sensitivity C-reactive protein (hs-CRP): Bios ELISA kit was used for quantitative determination of C-reactive protein concentration in human serum (Chemux Bioscience Inc., South San Francisco, California, USA). Expected normal values: 68–8200 ng/ml.<sup>8</sup>

IL6: TACE ELISA kits were used for quantitative detection of IL6 concentration in serum (Catalog No E1319gh, EIA [WWW.eiaab.com](http://WWW.eiaab.com)). Detection range: 0.3120 ng/ml.<sup>9</sup>

Lipid profile (cholesterol-triglyceride-LDL-HDL): total cholesterol, serum triglyceride, and high-density lipoprotein (HDL) levels were measured using the colorimetric enzymatic method CHOD-PAP, using a fully automated biochemistry device B T 1500 (Biotechnica Instruments, Italy), while low-density lipoprotein (LDL) was calculated by the equation:  $[\text{total cholesterol} - \text{HDL} - (\text{triglyceride}/5)]$ .<sup>10</sup>

Glycated hemoglobin (HbA1c): 2 ml of venous blood specimens was withdrawn under complete aseptic condition and dispensed in a lavender top

vacutainer tube containing EDTA for complete blood count and estimation of HbA1c.<sup>11</sup>

The test was done by highly automated cobas c 311 (Japan).

Ethical considerations: all parent participants (including those in the treatment and comparison groups) provided verbal informed consent. Parents of participants in the studies were informed of the process, the purpose, and the potential advantages and risks. The privacy of every information was protected. Participants in both the patient and control groups could drop out of the trial at any time and for any reason.

## 2.2. Statistical analysis

After data was collected, edited, coded, and double-checked for accuracy, it was entered into SPSS, version 23 by IBM for statistical analysis (IBM SPSS statistics (Statistical Package for Social Sciences) software version 11.0, IBM Corp., Chicago, USA). When the numbers were parametric, they were shown as means and SDs; when they were not, they were shown as medians and interquartile ranges.

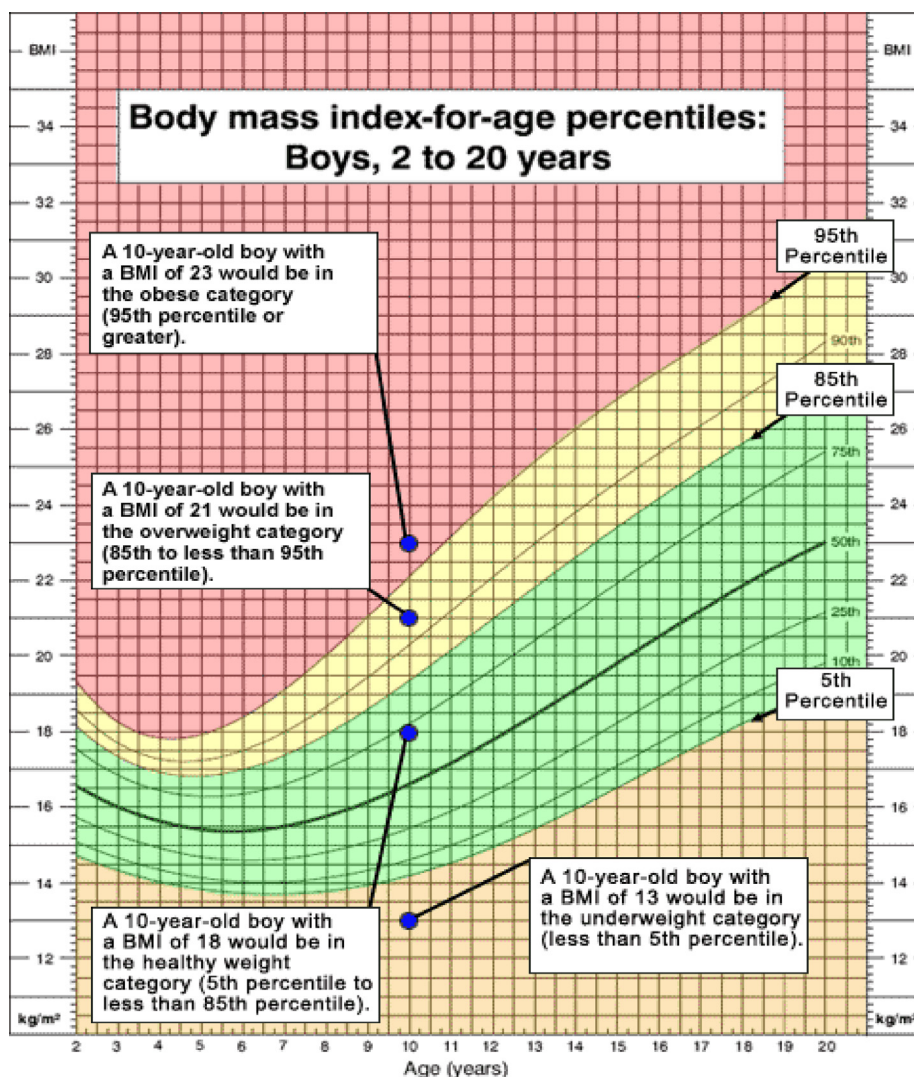


Fig. 2. CDC percentile for BMI for age (2–20) years in boys.<sup>2</sup>

Quantitative and percentage data were also provided for qualitative variables.

The tests were used in the study: one-way analysis of variance, paired *t* test, Mann–Whitney *U* test, Wilcoxon rank-sum test, independent *t* test,  $\chi^2$  test, Fisher's exact test, and analyses using Kruskal–Wallis and Mann–Whitney tests.

Spearman correlation coefficients were used to evaluate the degree to which two quantitative variables from the same set were correlated.

Receiver-operating characteristic curve determined the appropriate marker cutoff using sensitivity, specificity, positive and negative predictive values, and area under the curve.

### 3. Results

#### Table 1.

This showed that concerning anthropometric measurements, there was a statistically substantial rise among the control, overweight, and obese groups with a *P* value of less than 0.001 (Table 2).

Triceps skinfold thickness was substantially distinct across the control, overweight, and obese groups, as shown in Table 3 (Fig. 3).

Table 4 indicated that control, overweight, and obese men had significantly higher Hb and PLR and results indicated extremely noteworthy NLR increases in the examined groups.

HbA1c and lipid profiles, with the exception of LDL, all exhibited statistical significant increases between the control, overweight, and obese groups (Table 5).

No evidence of a statistically significant relationship was found as shown in the previous table among PLR, NLR, IL6, and hs-CRP and BMI in the control group and a high degree of relationship

Table 1. Analyzing anthropometric differences between the control, overweight, and obese groups.

	Control group N = 30	Overweight group N = 30	Obese group N = 30	Test value	P value	Significance
Weight (kg)						
Mean $\pm$ SD	25.62 $\pm$ 5.95	42.50 $\pm$ 13.18	62.60 $\pm$ 13.77	77.408●	*<0.001	HS
Range	18.5–38.5	21–66	43–105			
Height (cm)						
Mean $\pm$ SD	122.75 $\pm$ 8.16	138.70 $\pm$ 15.77	143.50 $\pm$ 10.47	24.990●	*<0.001	HS
Range	112–139	108–166	122–165			
BMI (kg/m <sup>2</sup> )						
Mean $\pm$ SD	16.77 $\pm$ 1.92	21.40 $\pm$ 2.32	30.14 $\pm$ 3.58	190.135●	*<0.001	HS
Range	13.7–20.5	18–25.2	25.2–41.4			
BMI percentile						
Median (IQR)	67.5 (41.3–78.5)	94.1 (92.9–94.5)	99.1 (98.3–99.4)	76.681≠	*<0.001	HS
Range	10–88.7	89.1–95	93.8–99.7			
BMI z score						
Median (IQR)	0.5 (0–0.8)	1.6 (1.4–1.6)	2.35 (2.1–2.5)	77.408≠	*<0.001	HS
Range	–1–1.2	1.2–1.6	1.5–2.7			
Waist circumference (cm)						
Mean $\pm$ SD	56.07 $\pm$ 4.15	79.13 $\pm$ 8.90	91.97 $\pm$ 9.00	167.769●	*<0.001	HS
Range	50–66	60–93	75–109			
WC percentile [n (%)]						
10–25th	11 (36.7)	0	0	112.872*	*<0.001	HS
25–50th	13 (43.3)	0	0			
50–75th	6 (20.0)	4 (13.3)	0			
75–90th	0	9 (30.0)	3 (10.0)			
90–95th	0	12 (40.0)	3 (10.0)			
>95th	0	5 (16.7)	24 (80.0)			

P value more than 0.05: nonsignificant, P value less than 0.05: significant, P value less than 0.01: highly significant.

●Independent t-test; ≠Mann–Whitney test.

Table 2. Triceps skinfold comparison between normal weight, overweight, and obese patients.

Triceps skinfold thickness (mm)	Control group N = 30	Overweight group N = 30	Obese group N = 30	Test value	P value	Significance
Mean $\pm$ SD	6.00 $\pm$ 1.26	17.53 $\pm$ 2.49	23.13 $\pm$ 3.86	302.698●	*<0.001	HS
Range	4–9	13.9–23	17–32			
Post-hoc analysis						
Control vs. overweight		Control vs. obese	Overweight vs. obese			
*<0.001		*<0.001	*<0.001			

\* There is highly significant between overweight and obese with control as regard triceps skin fold thickness.

Table 3. Comparison between control, overweight, and obese groups regarding complete blood cells with differential.

	Control group N = 30	Overweight group N = 30	Obese group N = 30	Test value	P value	Significance
CBC						
WBCs (10 <sup>3</sup> /μl)						
Mean $\pm$ SD	8.48 $\pm$ 3.24	8.67 $\pm$ 2.07	8.19 $\pm$ 1.96	0.273●	0.762	NS
Range	3.7–16	5.42–14.3	4.3–11.8			
Hb (g/dl)						
Mean $\pm$ SD	11.24 $\pm$ 1.59	11.80 $\pm$ 0.83	11.92 $\pm$ 0.76	3.146●	*0.048	*S
Range	8.9–14.1	10.3–13.1	9.9–13			
PLT (10 <sup>3</sup> /μl)						
Mean $\pm$ SD	333.13 $\pm$ 125.69	319.97 $\pm$ 77.73	325.37 $\pm$ 67.48	0.149●	0.861	NS
Range	161–612	189–522	186–472			
PLR						
Median (IQR)	110.25 (72.4–139.5)	104.4 (83.4–144.5)	133.5 (108.2–195.3)	7.152≠	*0.028	*S
Range	33.5–306	61–320	67.6–368			
NLR						
Median (IQR)	1.15 (0.5–1.9)	1.79 (1.26–2.1)	1.98 (1.64–2.3)	14.170≠	*0.001	*HS
Range	0.14–7.7	0.71–3.9	1.35–3.6			

\* There is highly significant between overweight and obese with control as regard blood pressure.

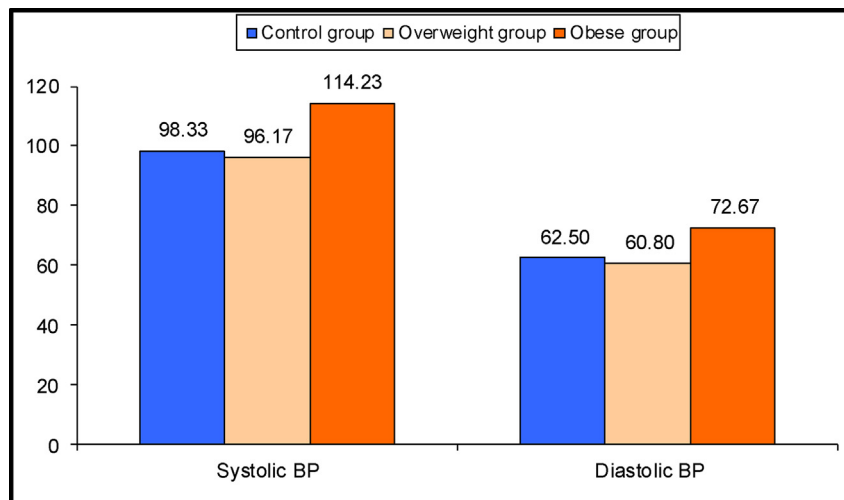


Fig. 3. Comparison between control, overweight, and obese groups regarding blood pressure (systolic and diastolic).

Table 4. Comparison between control, overweight, and obese groups regarding glycated hemoglobin and lipid profile.

	Control group N = 30	Overweight group N = 30	Obese group N = 30	Test value	P value	Significance
<b>Investigation</b>						
<b>HbA1c</b>						
Mean ± SD	5.02 ± 0.41	5.72 ± 0.28	5.73 ± 0.43	33.565•	*<0.001	*HS
Range	4–5.7	5–6.3	5–6.7			
<b>Lipid profile</b>						
<b>Cholesterol (mg/dl)</b>						
Mean ± SD	143.45 ± 16.50	169.23 ± 28.47	172.33 ± 37.53	8.814•	*<0.001	*HS
Range	119–184	101–225	110–301			
<b>TG (mg/dl)</b>						
Mean ± SD	80.03 ± 20.10	104.50 ± 41.92	117.37 ± 37.09	8.900•	*<0.001	*HS
Range	42–125	35–211	72–208			
<b>HDL (mg/dl)</b>						
Mean ± SD	34.33 ± 4.85	47.48 ± 13.00	43.20 ± 11.13	12.797•	*<0.001	*HS
Range	25–44	18–74.4	28–70			
<b>LDL (mg/dl)</b>						
Mean ± SD	95.40 ± 15.78	97.43 ± 21.49	102.67 ± 32.88	0.706•	0.496	NS
Range	67–132	52–160	68–260			

\* There is highly significant between overweight and obese with control as regard glycated hemoglobin and lipid profile.

Table 5. Correlation between BMI and (platelet-to-lymphocyte, neutrophil-to-lymphocyte, interleukin 6, and high-sensitivity C-reactive protein) in the studied groups.

	BMI (kg/m <sup>2</sup> )					
	Control group		Overweight group		Obese group	
	r	P value	r	P value	r	P value
PLR	-0.233	0.215	0.689**	0.000	0.652**	0.000
NLR	-0.140	0.461	0.618**	0.000	0.687**	0.000
IL6 (ng/ml)	-0.240	0.202	0.784**	0.000	0.817**	0.000
hs-CRP (mg/ml)	0.164	0.395	0.816**	0.000	0.797**	0.000

\*\* There is positive correlation between BMI and (PLR, NLR, IL-6, hs-CRP) in obese and overweight and no significant correlation between them in control group.

between the studied parameters and BMI regarding overweight and obese groups.

#### 4. Discussion

In this study, the obese group had significantly higher mean values for all anthropometric measurements compared with the control group. This included weight, height, BMI, waist circumference, hip circumference, waist-to-hip ratio, and waist-to-height ratio ( $P < 0.001$ ).

This result corroborated the findings of Fayed et al.,<sup>12</sup> who discovered a vastly different rise in weight between the obese and control groups.

We discovered that the average weight of obese children was significantly greater than that of the control group, which is consistent with the findings of Eldin et al.<sup>13</sup> The same results are detected in Mohammed et al.<sup>14</sup>

While the BMI is a useful tool for gauging the likelihood that a child is overweight and at risk of developing metabolic syndrome, it is important to keep in mind that BMI is not a direct measure of adiposity and may slightly overestimate fatness in children who are particularly tall for their age or have unusually high muscle mass.<sup>15</sup>

In contrast to the control group, the obese had considerably higher systolic and diastolic blood pressure ( $P > 0.001$ ).

Children who are overweight are more likely to develop hypertension, as shown in the research by Fayed et al.<sup>12</sup>

Hb (g/dl) values were significantly different between patients and controls.

Meanwhile, Aloufi et al.<sup>16</sup> aimed to raise awareness of the dangers of obesity by assessing the correlation between overweight kids and anemia in Saudi Arabia's Al-Taif region. Blood-mass index was found to have a statistically significant inverse relationship with hemoglobin levels ( $r = 0.429$ ,  $P = 0.017$ ).

Considering PLR differences between the three groups were significant in statistical terms.

Erdim et al.<sup>17</sup> reported that PLR was considerably higher in 127 overweight children than in children of normal weight.

Platelet and lymphocyte counts were shown to be higher in children who were overweight or obese in a separate study by Mărginean et al.,<sup>18</sup> but there was no change in PLR.

WBCs and PLT were not statistically different between patients and controls.

When HbA1c was evaluated, cases had a highly statistically significant higher level than controls ( $P < 0.001$ ).

The HbA1c test – is a simple blood test that measures average blood sugar levels over the past 3 months. It is one of the commonly used tests to diagnose prediabetes and diabetes, and is also the main test to help the healthcare team to manage diabetes.

This study agreed with Gnanaselvam et al.<sup>19</sup> who showed that in comparison to nonobese individuals, the average HbA1c levels of the obese were much higher.

The results of this investigation showed that, with the exception of LDL, the lipid profile increased dramatically in cases compared with controls.

Dyslipidemia is a cardiovascular risk factor related to obesity. It is estimated that about 42% of

obese children have lipid abnormalities, particularly those with visceral obesity, the most common lipid abnormality pattern consists of elevated triglycerides, decreased HDL-C, and normal to mildly elevated LDL-C.

Our findings were in agreement with Martin et al.<sup>20</sup> who showed in a cross-sectional study in 8–17-year-old children that there is a higher triglyceride, LDL-C, TC, and HDL-C in obese children and adolescents.

Increased inflammatory markers in children with obesity probably affect their health. CRP is linked to insulin resistance and carotid intima-media thickness in children who are obese ( $8 \pm 2$  years). In children who are overweight and have atherosclerotic risk factors, inflammatory cytokines IL6 and TNF- $\alpha$  are also higher. Increased insulin, insulin resistance, BMI, and waist circumference were all linked to IL6 even in models that controlled for age and sex.<sup>21</sup>

The study revealed that significantly higher rates of growth were observed regarding hs-CRP (mg/ml) between cases and control with  $P$  value less than 0.001.

This corroborated the findings of Fayed et al.,<sup>12</sup> who found that hs-CRP levels in obese children were considerably greater than in the control group.

We found evidence of a statistically significant rise regarding IL6 (ng/ml) between case and control.

Also, this study coincides with the De Filippo et al.<sup>22</sup> study which detected that high levels of IL6 are related to obesity and insulin resistance in children and adolescents.

Our results showed a positive correlation between BMI and NLR, PLR, IL6, and hs-CRP in obese and overweight children.

PLR is a measurement of how well inflammation and thrombosis are controlled in the body. Megakaryocytes proliferate more quickly and cause thrombocytosis as a result of the inflammatory state. Increased platelet counts and decreased lymphocyte numbers are other markers of risk as they have been linked to aggregation and inflammation.<sup>23</sup>

Among 600 obese patients, Yu et al.<sup>24</sup> found a stronger association between white blood cell count and hs-CRP and visceral adipose tissue than between PLR and NLR, and these measures of inflammation in the blood.

#### 4.1. Conclusion

Obese and overweight children have higher NLR and PLR ratios than the healthy control group. Obese and inflammatory indicators like hs-CRP are more prevalent in children who are overweight and IL6 than the healthy ones.



## Conflicts of interest

There are no conflicts of interest.

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