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ASSOCIATION BETWEEN HEMATOLOGICAL PARAMETERS AND METABOLICALLY UNHEALTHY PHENOTYPES IN CHILDREN AND ADOLESCENTS

ASOCIACIÓN ENTRE PARÁMETROS HEMATOLÓGICOS Y FENOTIPOS METABÓLICAMENTE POCO SALUDABLES EN NIÑOS Y ADOLESCENTES

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ABSTRACT

Introduction: Metabolic syndrome has been associated with changes in several hematological parameters, such as red blood cells, platelets, and leucocytes. Therefore, hematologic parameters can be used to identify the subjects at risk of metabolically unhealthy phenotypes (MUP). The current study investigated if hematological parameters can serve as biomarkers to distinguish metabolically healthy phenotype (MHP) from MUP in children and adolescents. **Methods:** Two hundred ninety-two children and adolescents were enrolled in this cross-sectional study. The MUP was diagnosed using consensus-based criteria. Group comparisons were performed using one-way ANOVA. Multiple logistic regression analysis was used to determine if sex, age group, nutritional status, puberty, hematological parameters, and insulin resistance were associated with MUP. **Results:** The subject's age mean was 11 years (SD: 2.61). RDW values were significantly lower in children in the metabolically unhealthy normal weight (MUNW) group compared to children with metabolically unhealthy obesity (MUO) group (12.33 ± 0.90 vs. 13.67 ± 0.52; p = 0.01) and in metabolically healthy obesity (MHO) compared to MUO group (13.15 ± 0.53 vs. 13.67 ± 0.52; p = 0.04). In adolescents, the platelet-to-lymphocyte ratio was higher in the MHNW group, with a mean value of 152.60 (SD 62.97) compared to 111.16 (SD 44.12) for the MHO group. However, after adjusting for age, nutritional status, and puberty, hematological indices were not associated with MUP. **Conclusion:** The study demonstrates that hematologic parameters are not independently associated with the MUP, and it is unlikely that they represent reliable biomarkers for screening for the MUP in the pediatric population.

Keywords: Hematological indices; Neutrophils; Metabolically unhealthy phenotypes; Children; Adolescents. (Source: MESH-NLM)

RESUMEN

Introducción: El síndrome metabólico se ha asociado con cambios en parámetros hematológicos (glóbulos rojos, plaquetas y leucocitos); se pueden utilizar para identificar sujetos en riesgo de fenotipos metabólicamente no saludables (MUP). Se investigó si estos parámetros hematológicos sirven como biomarcadores para distinguir el fenotipo metabólicamente sano (MHP) del MUP en niños y adolescentes. **Métodos.** Estudio transversal, 292 niños y adolescentes. El diagnóstico de MUP fue según consenso. Se utilizó ANOVA unidireccional en las comparaciones, regresión logística múltiple para determinar si el sexo, el grupo etario, el estado nutricional, la pubertad, los parámetros hematológicos y la resistencia a la insulina se asociaron con MUP. **Resultados:** Edad media 11 años (DE: 2,61). Los valores de RDW fueron significativamente más bajos en los niños en el grupo de peso normal metabólicamente insalubre (MUNW) en comparación con los niños con obesidad metabólicamente no saludable (MUO) (12,33 ± 0,90 vs. 13,67 ± 0,52; p = 0,01) y en la obesidad metabólicamente saludable (MHO) en comparación con el grupo MUO (13,15 ± 0,53 vs. 13,67 ± 0,52; p = 0,04). En adolescentes, la relación plaquetas/linfocitos fue mayor en el grupo MHNW (con un valor medio de 152,60 (DE 62,97) vs 111,16 (DE 44,12) para el grupo MHO. Al ajustar por edad, estado nutricional y pubertad, los índices hematológicos no se asociaron con MUP. **Conclusiones:** Los parámetros hematológicos no están asociados independientemente con el MUP, y es poco probable que representen biomarcadores confiables para la detección del MUP en la población pediátrica.

Palabras clave: Índices hematológicos; Neutrófilos; Fenotipos metabólicamente no saludables; Niños; Adolescentes. (Fuente: DeCS- BIREME)

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INTRODUCTION

The prevalence of overweight and obesity has increased worldwide in children and adults. Being overweight and obese in childhood is strongly associated with an increased risk of metabolic syndrome in adulthood [1-6]. Interestingly, despite the excess fat mass, not all obese children and adolescents present metabolic disorders. Indeed, not all normal-weight individuals are metabolically healthy. Consequently, there are two phenotypes namely "metabolically healthy phenotype (MHP)" and "metabolically unhealthy phenotype (MUP)," which are differentiated by the presence or absence of metabolic disturbances. and that in obese patients they generate more intrigue.

The pathogenesis of the MUP is not fully understood. and is characterized by hyperglycemia, hypertension, low high-density lipoprotein cholesterol (HDL) levels, or hypertriglyceridemia and can be accompanied by insulin resistance or low-grade inflammation [7,8]. MHP subjects commonly has a reduced inflammatory profile compared to MUP subjects and shows higher adiponectin levels than their metabolically unhealthy counterparts[8]. These findings suggest that lower levels of inflammation could play an essential role in the protective profile of the MHP[9].

In adults, metabolic syndrome has been associated with changes in several hematological parameters, such as red blood cells, platelets, and leucocytes[10]. The precise mechanism of hematological disturbances remains unclear but may be related to insulin resistance, changes in cytokine concentration, and chronic low-grade inflammation [5,11,12]. The adipose tissue releases local and systemic bioactive molecules like adipokines (e.g., adiponectin and leptin), and proinflammatory cytokines, mainly tumor necrosis factor-alpha (TNF-a) and interleukin 6 (IL-6). Which are related to the cardiometabolic risk profile [10]. Interestingly, chronic low-grade systemic inflammation associated with obesity has been suggested to play a significant role in developing cardiometabolic complications [8,13,14]. In children, inflammatory markers are strongly associated with adiposity [15]. A strong, significant positive correlation has been described between the body mass index (BMI) and the total number of leukocytes in children, which suggests that the percentage of body fat influences the leukocyte count, especially in neutrophils [7]. Furthermore, obesity in children is related to increased IL-6 and TNF-a, which may contribute to increased circulating leukocytes [7]. An increase in red blood cell distribution width (RDW) positively correlates with BMI and is associated with a higher risk of metabolic syndrome, unfavorable lipid profile, and cardiovascular disease (CVD)[16-19]. Therefore, The clinical utility of assessing metabolic status in childhood lies in identifying those children with worse metabolic profiles and a higher risk of developing CVD [2,20]. In recent years, an increased interest has been focused on biomarkers associated with obesity in children and adolescents. Currently, limited studies are comparing the distributions of hematological parameters between MHP and MUP in the pediatric population. Hematological parameters such as RDW, Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lymphocyte-to-Monocyte Ratio (LMR), and RDW-to-Platelet Ratio (RPR) has been associated with various inflammatory conditions and can be used for the early detection of individuals at risk of CVD[12,14].

Hematologic parameters could constitute an accessible and cost-effective test for screening children and adolescents at risk of presenting the MUP. However, whether they can serve as biomarkers to distinguish MHP from MUP in pediatric populations is unknown. The current study investigated if hematological parameters can differentiate MHP from MUP in children and adolescents. The secondary objective was to explore the correlations of hematological parameters with inflammatory markers, insulin resistance, and adipokines, which could provide insights into the underlying mechanisms of these relationships.

METHODS

A cross-sectional study was conducted from May 2015 to December 2017 using a non-probabilistic sampling of consecutive cases in an outpatient pediatric endocrinology clinic at a secondary care hospital and public schools. The inclusion criteria were age from 5 to 18 years, with presumably good health, without prescription. Participants between 5 and 10 years old were classified into childhood and adolescence between 11 and 18 years[21]. Subjects with an infectious process a month before the study, secondary obesity, insulin-dependent diabetes, congenital metabolic disease, hormonal disorder, or inflammatory

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diseases were excluded. This study was in agreement with the Declaration of Helsinki and approved by the Ethics Committee of the Instituto Mexicano del Seguro Social (R-2013-785086) and Instituto Nacional de Perinatologia (212250-3310-11402011). Participants and their parents signed a free and informed an assent form and consent form.

Power analysis calculations were based on effect size and power using G*Power software. The power calculation for one-way ANOVA assumed a priori alpha error probability of 0.05, a power of 0.8, a medium effect size (F = 0.25), and the number of groups = 6. According to these assumptions, 216 patients would be required for this study.

Clinical evaluation

Physicians and nutritionists performed physical examinations (height, weight, and waist circumference) standardized according to conventional procedures. Weight was obtained using a SECA portable digital weighing scale, model 803 with 100g accuracy. A SECA portable stadimeter, model 0123, with 0.1 cm accuracy, was used for height. Waist circumference was measured standing at the midpoint between the iliac crest and the last rib with a non-elastic flexible tape to the nearest 0.5 cm. A medical examination assessed the pubertal stage, and participants were classified into two categories: prepubertal (stage I) and pubertal (stages II, III, IV, V) according to Tanner staging[22].

Arterial blood pressure was taken using a mercury sphygmomanometer and a suitable bracelet for each patient's age and complexion, measuring twice with 5 minutes between both and taking the average value. Weight status was assessed using the World Health Organization (WHO) body mass index (BMI) criteria. BMI for age and sex was determined using Anthro Plus software (http://www.who.int/growthref/tools/en/). Children were classified using a BMI z-score as normal (−2 to +1 SD of the population WHO reference), overweight (> +1 to +2 SD), and obesity (> +2SD).

Laboratory analysis

After a 12-hour fast, blood samples were obtained from venous puncture using a vacuum blood collection system. Beckman Coulter model AC-T5 diff blood analyzer (Beckman Coulter, Brea, CA) was used to measure hematological parameters. NLR was calculated as the ratio of peripheral blood neutrophil count to lymphocyte count; PLR was calculated as the ratio of peripheral blood platelet count to lymphocyte count; LMR was calculated as the ratio of peripheral blood lymphocyte counts to monocyte count; RPR was calculated by the ratio of RDW to platelet.

Insulin and ultrasensitive C-reactive protein (uCRP) were determined by chemiluminescence immunoassay method (Inmunolite 1000 Siemens NY, USA). Plasma adiponectin and leptin levels were measured using specific ELISA kits (R&D Systems, Minneapolis, USA). Serum IL-6, IL-1, and TNF-α were measured using enzyme-chemiluminometric assays on the automated Immulite 1000 analyzer. Fasting blood glucose, triglyceride, and HDL cholesterol, were obtained by enzymatic colorimetric assay (DiaSys Diagnostic Systems GmbH, Germany). All analyses of inflammatory parameters were performed twice. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as (fasting plasma glucose - fasting serum insulin)/22.5 [23]. A HOMA-IR value ≥ 3.16 was the criterion for the presence of insulin resistance [23].

Metabolic phenotypes

Participants were classified as having MHP or MUP using consensus-based criteria proposed by Damanhoury et al.[23] Four cardiometabolic risk factors were considered: high-density lipoprotein cholesterol (HDL-c) < 40 mg/dL (< 1.03 mmol/ L), triglycerides > 150 mg/dL (> 1.7 mmol/L), systolic and diastolic blood pressure > 90th percentile, and fasting glucose ≥ 100mg/dL (≥ 5.6 mmol/l). If at least one of the abovementioned risk factors was within the unhealthy range, the subject was classified as MUP. Therefore, each participant was classified into one of the six metabolic phenotypes: metabolically healthy normal weight (MHNW), metabolically unhealthy normal weight (MUNW), metabolically healthy overweight (MHOW), metabolically unhealthy overweight (MUOW), metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO).

Statistical analysis

Categorical variables are presented as frequencies and percentages, and continuous variables are summarized using mean and standard deviation. Missing values were handled using available case analyses. Group comparisons were performed using one-way ANOVA (Bonferroni's test for post-hoc analyses) and Kruskal-Wallis (Dunn's test for post-hoc analyses). Parametric methods gave virtually identical results. For this reason, only ANOVA results are present. Eta-squared was used to measure the effect size; the effect sizes were considered no effect ($0 \leq \eta 2 < 0.01$), minimum effect ($0.01 \leq \eta 2 < 0.06$), a moderate effect ($0.06 \leq \eta 2 < 0.14$), and strong effect ($\eta 2 \geq 0.14$).

Unadjusted logistic regression analyses were used to identify explanatory variables of MUP. To examine if sex, age group, nutritional status, hematological parameters, and insulin resistance were associated with MUP, we used multiadjusted logistic regression analysis with metabolic status as the dependent variable. The multiple logistic regression model included parameters showing a p-value < 0.20 in unadjusted logistic regression analyses.

The results are presented as odds ratios (OR) with 95% confidence intervals (CI). A Hosmer–Lemeshow post-estimation test assessed the model's performance. A two-tailed p-value < 0.05 was considered statistically significant. Subgroup analysis by puberty examined differential associations in prepubertal vs. pubertal.The relationships between hematological parameters, inflammatory biomarkers, insulin resistance, and adipokines insulin resistance were investigated using the Spearman correlation coefficient. The strength of correlation was considered weak ($0.1 < rho < 0.20$), moderate ($0.20 < rho < 0.50$), and strong ($rho \geq 0.5$) based on guidelines provided by Cohen et al. [24] The Bonferroni correction was used to adjust the significance threshold for multiple comparisons. Spearman's rank correlation coefficient's 95% confidence intervals were computed using the "ci2" command. Statistical analyses were performed using Stata-14 software (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP)

RESULTS

Participants characteristics

Analysis of 292 children and adolescents included 98 with normal weight (33.56 %), 88 with overweight (30.14 %), and 106 with obesity (36.3 %). The participant's characteristics are summarized in Table 1. The subject's age mean was 11 years (SD: 2.61) and ranged from 6 to 18 years. Sex, nutritional status, and metabolic phenotypes were similar between children and adolescents. Approximately 52 % of the participants were women. Tanner's classification was unavailable in thirty-six participants (12.3 %). Compared with patients in whom Tanner was determined, these patients did not differ in age, sex, nutritional status, or prevalence of the MUP. The percentage of prepubertal patients was 42.2% in the childhood group and 8.28% in the adolescent group.

Table 1. General characteristics of the subjects studied.

Parameters	Childhood (n=135)	Adolescent (n=157)	Total (n=292)	P-value
Women, n(%)	75 (55.5)	77 (49.0)	152 (52.0)	0.26
Nutritional status				
Normal	45 (33.3)	53 (33.7)	98 (33.5)	0.94

Overweight	42 (31.1)	46 (29.3)	88 (30.1)	
Obesity	48 (35.5	58 (36.9)	106 (36.3)	
Tanner stages				
Unkown	16 (11.8)	20 (12.7)	36 (12.3)	
Tanner 1	57 (42.2)	13 (8.28)	70 (23.9)	<0.01
Tanner ≥ 2	62 (45.9)	124 (78.9)	186 (63.7)	
Cardiovascular risk				
HDL-c < 40 mg/dL	26 (19.2)	48 (30.57)	74 (25.3)	0.02
Triglycerides > 150 mg/dL	28 (20.7)	35 (22.2)	63 (21.5)	0.74
Systolic blood pressure > 90th percentile	5 (3.7)	6 (3.8)	11 (3.7)	0.95
Diastolic blood pressure > 90th percentile	19 (14.0)	27 (17.2)	46 (15.7)	0.46
Fasting glucose >= 100mg/dL	17 (12.5)	17 (10.8)	34 (11.6)	0.63
Metabolic phenotypes				
Metabolic Healthy Normal Weight	28 (62.2)	29 (54.7)	57 (58.1)	0.45
Metabolic Unhealthy Normal Weight	17 (37.7)	24 (45.2)	41 (41.9)	
Metabolic Healthy Overweight	22 (52.3)	21 (45.6)	43 (48.3)	0.60
Metabolic Unhealthy Overweight	20 (47.6)	25 (54.3)	45 (51.7)	
Metabolic Healthy Obesity	19 (39.5)	15 (25.8)	34 (32.1)	0.13
Metabolic Unhealthy Obesity	29 (60.4)	43 (74.1)	72 (67.9)	

Values represent frequencies (percentages).
P-values are calculated using the chi-squared test.

The most frequent altered metabolic parameters were low high-density lipoprotein cholesterol (n = 74, 25.3 %) and hypertriglyceridemia (n = 63, 21.5 %), see Table 1. By contrast, a low prevalence of systolic hypertension was observed (n =11, 3.7%). The prevalence of insulin resistance was 32.53 % (n = 95), and 54.11 % (n = 158) had MU phenotype. Forty-one (14.04 %) of the participants met the criteria of MUNW, and 34 (11.64 %) of the obese participants met the criteria of MHO. As expected, obese subjects had significantly higher values in RDW (13.5 vs. 13.24, p = 0.04) and NLR (1.32 vs. 1.07, p < 0.01) compared to normal-weight subjects. Contrarily, obese subjects exhibited lower PLR values (124.46 vs. 140.04, p = 0.02).

Comparison of Hematological parameters between MH phenotype and MU phenotype.

As shown in Figure 1, RDW values were significantly higher in children with MUO compared to children with MUNW (13.67 vs. 12.33; p = 0.01), and in MUO compared to MHO (13.67 vs. 13.15; p = 0.04). In adolescents, the PLR was higher for the MHNW group, with a mean value of 152.60 (SD 62.97) compared to 111.16 (SD 44.12) for the MHO group (Figure 1). The size effect was minimum (η2 0.02 and 0.03, respectively). There were no differences between groups regarding NLR, LMR, and RPR values. In the analyses of all participants, there was no significant difference in hematological parameters between the six groups (Table 2).

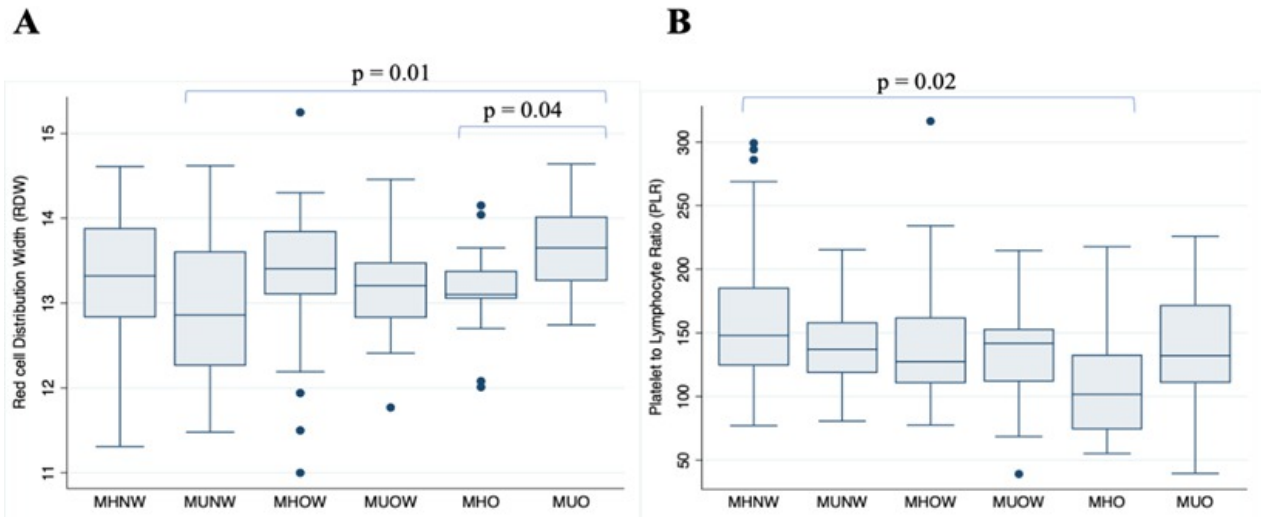

Figure 1. A. Level of Red blood cell Distribution Width (RDW) in children stratified by metabolic phenotype. **B.** Level of Platelet-to-Lymphocyte Ratio (PLR) in adolescents stratified by metabolic phenotype.

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Table 2. Hematologic parameters stratified by metabolic phenotype.

	MHNW (n=57)	MUNW (n=41)	MHOW (n=42)	MUOW (n=45)	MHO (n=34)	MUO (n=72)	P Value	Eta squared [a] (η)^2
RDW	13.25	13.02	13.25	13.05	13.35	13.39	0.38	0.01
	(1.00)	(1.00)	(1.09)	(0.95)	(0.90)	(1.10)		
NLR	1.19	1.35	1.35	1.44	1.52	1.51	0.18	0.02
	(0.69)	(0.71)	(0.75)	(0.75)	(0.72)	(0.76)		
PLR	151.94	140.18	141.30	129.33	131.06	132.23	0.15	0.02
	(54.99)	(37.08)	(57.03)	(39.99)	(46.84)	(49.63)		
LMR	6.57	5.72	8.99	5.54	6.52	5.83	0.14	0.02
	(2.60)	(2.56)	(15.15)	(1.59)	(4.06)	(4.72)		
RPR	42.98	45.35	44.43	45.73	46.84	48.02	0.47	0.03
	(12.35)	(9.61)	(11.00)	(16.33)	(16.94)	(16.44)		

Values represent mean (standard deviation).
MHNW: metabolically healthy normal weight. MUNW: metabolically unhealthy normal weight.
MHOW: metabolically healthy overweight. MHOW: metabolically unhealthy overweight.
MHO: metabolically healthy obese. MUO: metabolically unhealthy obese.
RDW: red blood cell distribution width. NLR: neutrophil to lymphocyte ratio.
PLR: platelet to lymphocyte ratio. LMR: lymphocyte to monocyte ratio. RPR RDW-to-platelet ratio.
[a] No effect ($0 \leq \eta^2 < 0.01$), minimum effect ($0.01 \leq \eta^2 < 0.06$).

Explanatory variables of unhealthy metabolic phenotype

Table 3 shows results from logistic regression analyses, with metabolic phenotype as the dependent variable. In the unadjusted model, obesity, PLR, and insulin resistance were significantly associated with the MUP. In the multivariate logistic regression analysis, obesity, PLR, and LMR were significantly associated with the MUP.

The primary independent factor was obesity (OR 2.45, 95% CI 1.28 to 4.67, p < 0.01). A decrease in PLR was independently associated with a small increased risk of present MUP (OR 0.99; 95% CI, 0.98 to 0.99, p = 0.02). Similarly, a decrease in LMR was associated with a small increased risk of being MUP (OR 0.91; 95% CI 0.84 to 0.99, p = 0.04). Unadjusted and multiadjusted logistic regression analyses suggested no significant association between RDW, NLR, and RPR with MUP.

Table 3. Univariate and multivariate logistic regression analyses of explanatory variables associated with the metabolically unhealthy phenotype (n = 292).

	Univariate OR (95% CI)	P value	Multivariate [a] OR (95% CI)	P value
Age group				
Childhood	1	-		
Adolescent	1.47 (0.93-2.35)	0.09	1.48 (0.88-2.47)	0.13
Sex				
Female	1		-	
Male	0.90 (0.57-1.43)	0.680	-	
Nutritional status				
Normal weight	1		1	
Overweight	1.45 (0.81-2.59)	0.20	1.41 (0.77-2.60)	0.26
Obesity	2.04 (1.66-2.59)	<0.01	2.45 (1.28-4.67)	<0.01
Waist circumference	1.00 (0.99-1.002)	0.51	-	
RDW	0.94 (0.75-1.17)	0.60	-	
NLR	1.15 (0.85-1.55)	0.35	-	
PLR	0.99 (0.99-1.00)	0.05	0.99 (0.98-0.99)	0.02
LMR	0.94 (0.88-1.01)	0.10	0.91 (0.84-0.99)	0.04
RPR	1.01 (0.99-1.02)	0.17	0.99 (0.97-1.01)	0.67
Insulin resistance				
Absent (HOMA-IR < 3.16)	1		1	
Present (HOMA-IR ≥ 3.16)	1.97 (1.19-3.27)	<0.01	1.32 (0.73-2.41)	0.35

PLR: Platelet to lymphocyte ratio. LMR: lymphocyte to monocyte ratio. RPR: Red blood cell distribution width to platelet ratio.
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.
Variable Y = Phenotype (0 = healthy, 1 = unhealthy).
[a]. Hosmer and Lemeshow test, x2 = 11.90, p = 0.15. McFadden's R2= 0.07. Area under ROC curve = 0.67.

Since puberty may play an essential role in the risk to MUP, we performed stratified analysis by pubertal stage (Table 4). The multi-adjusted model showed that obesity was associated with an increased likelihood of having MUP (OR 2.53, 95% CI 1.26 to 5.09, p < 0.01). The associations between MUP and hematological parameters were not statistically significant (p for all ≥ 0.05) after adjusting for puberty.

Table 4. Multivariate logistic regression analyses of explanatory variables associated with the metabolically unhealthy phenotype (n = 251).

	OR (95% CI)	P-value
Age group		
Childhood	1	
Adolescent	1.48 (0.80-2.71)	0.20
Puberty		
Tanner = 1	1	
Tanner ≥ 2	0.86 (0.45 - 1.65)	0.66
Nutritional status		
Normal weight	1	
Overweight	1.20 (0.62-2.29)	0.57
Obesity	2.53 (1.26-5.09)	<0.01
PLR	0.99 (0.98-1.002)	0.19
LMR	0.92 (0.85-1.007)	0.07
RPR	0.99 (0.97-1.02)	0.96
Insulin resistance		
Absent (HOMA-IR < 3.16)	1	
Present (HOMA-IR ≥ 3.16)	1.23 (0.65-2.34)	0.51

PLR: Platelet-to-Lymphocyte ratio. LMR: lymphocyte to monocyte ratio. RPR: Red blood cell distribution width to platelet ratio. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.
Variable Y = Phenotype (0 = healthy, 1 = unhealthy).
Hosmer and Lemeshow test, x2 = 9.25, p = 0.32. McFadden's R2= 0.06. Area under ROC curve = 0.66.

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Correlates of hematological indices and inflammatory biomarkers, cytokines, and insulin resistance

Among the hematologic indices and inflammatory biomarkers, NLR showed positive but low correlations with IL-6 (rho = 0.24, 95% CI: 0.12 to 0.34) and uCRP (rho= 0.23, 95% CI: 0.11 to 0.33). Similarly, the LMR showed negative and low correlations with the uCRP (rho = 0.22, 95% CI: -0.32 to -0.10). Positive and low correlations were found between NLR and leptin (rho = 0.26, 95% CI: 0.16 to 0.37) and HOMA-IR (rho = 0.22, 95% CI: 0.12 to 0.34). As shown in Table 5, correlation coefficients revealed no correlation between RDW, RPR, PLR, inflammatory biomarkers, adipokines, and HOMA-IR.

Table 5. Spearman's rank correlation coefficients between hematologic parameters, inflammatory biomarkers, adipokines, and insulin resistance.

	RDW	NLR	PLR	LMR	PRP
Ιντερλευχινα−1β	0.06	-0.02	-0.02	-0.10	-0.01
Interleucina-6	-0.13	0.24*	-0.01	-0.13	0.01

Ultrasensitive C-reactive protein	0.05	0.23*	0.02	-0.22*	-0.01
TNF-α	0.12	-0.12	-0.05	-0.03	0.04
Adiponectin	-0.16	-0.04	0.03	0.02	-0.05
Leptin	0.05	0.26*	-0.09	-0.10	0.01
HOMA-IR	0.02	0.22*	-0.12	-0.09	0.10

TNF-α: tumor necrosis factor-alpha. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.
* $p < 0.05$ after Bonferroni correction.

DISCUSSION

Although RDW was increased in MUO in childhood and PLR was reduced in MHO in adolescence, our results did not support the usefulness of hematologic parameters as tests for screening of MUP. After adjusting for age, nutritional status, and puberty, hematologic parameters were not associated with MUP. Therefore, hematologic indices are unreliable biomarkers of MUP in children and adolescents. The confounding effect of obesity and insulin resistance may partly explain this lack of association.

Barazzoni et al. suggested that the association between BMI and hematological parameters is mediated by their associations with insulin resistance markers and abdominal fat [25]. In this study, Waist Circumference (WC) was not an independent variable for MUP. However, It is essential to acknowledge that WC may not always reflect visceral fat as it cannot differentiate between subcutaneous fat in the abdominal area and visceral fat accumulation[26].

Hematologic parameters in obese children are often disturbed. The high RDW and NLR values in obese subjects in this study are consistent with those reported by other researchers [5,11,18]. Although RDW is seemingly increased in obese subjects, our results showed that this parameter does not explain the presence of MUP. The mechanisms linking RDW and obesity remain to be elucidated. Several mechanisms have been proposed to increase RDW in obesity, including inflammation, oxidative stress, or iron deficiency[18,27,28]. Although it has been proposed that proinflammatory cytokines may produce an increase in RDW by inhibiting erythrocyte maturation[15], our results do not support an association of RDW with three proinflammatory cytokines (IL-1b, IL-6, TNF-a).

The association between NLR and metabolic syndrome in adults presents conflicting results. Two studies showed that the NLR was significantly associated with presence and severity of metabolic syndrome [10,29]. Opposite, Wang et al.[19] showed no significant differences in the counts of neutrophils between MHO and MUO phenotypes. Similarly, Bahadir et al. reported that the NLR was not associated with metabolic syndrome[30].

In children, a study performed in Turkey with 187 children and adolescents (aged 6 to 15 years) showed that NLR was significantly higher in obese adolescents compared with healthy controls [5]. In contrast, three studies showed that NLR did not differ significantly between overweight/obesity and normal weight children and adolescents [14,31,32]. Our results revealed that NLR values were similar in subjects with MHP and MUP. Similarly, a study conducted in Italy with 552 obese children and adolescents has shown that the average NLR value did not differ significantly between the obese group with metabolic syndrome compared to the obese group without metabolic syndrome[10].

PLR has been identified as a potential biomarker of inflammation, insulin resistance, the severity of obesity, and a predictor of metabolic syndrome (MetS) [33-35]. In our study, PLR was significantly lower than the normal weight group. These findings are different and contradictory to previous studies. In adults, PLR was significantly higher in morbidly obese adult patients than normal-weight patients. In another study, PLR could not predict newly diagnosed MetS[33].

In pediatric populations, there was no difference in PLR in obese subjects compared to healthy control groups [5,14]. Additionally, the association between PLR and MUP was blurred after adjusting for the age group, puberty, HOMA-IR, and nutritional status in our study. Taking together, these findings indicate that PLR cannot be suggested as a biomarker of MUP in the pediatric population.

In our study, NLR levels correlated positively, although weak, with IL-6, leptin, CRP, and HOMA-IR. This finding might indicate that the increase in NLR in obese children could be an expression of low-grade inflammation and an insulin-resistant state [19]. A study in healthy young adults showed that the correlation between NLR, CRP, and fibrinogen was highly significant in obese individuals, reflecting the subclinical inflammation in this group [13]. In addition, Zaldivar et al.[7] reported increased neutrophils in children with obesity. Interestingly, there was a significant positive correlation between NLR and leptin, which can be related to the chemotaxis of neutrophils induced by leptin[3]. Adipose tissue neutrophils produce chemokines and cytokines, facilitating macrophage infiltration, which could contribute to the development of insulin resistance[3].

An unexpected result of this study was the high prevalence of the MUNW phenotype (41.9 %). Our results show that risk factors for cardiovascular disease are present even in childhood and adolescence, regardless of the nutritional status of these subjects. Currently, there is limited data on the prevalence of MUNOW in pediatric populations. Previous studies reported that 14 to 22 % of normal-weight children and adolescents were metabolically unhealthy[36].

Damanhoury et al.[26] reported that approximately 30% of children with overweight/obesity were classified as having the MUP. Marín-Echeverri et al.[37] evaluated the association between abdominal obesity (AO) and cardiovascular risk factors in 232 children (2–5 years), of whom 50 % had AO, and 88 % of these were overweight or obese. The authors reported that 75.8 % of children with AO had ≥ 1 risk factors for metabolic syndrome. Shashaj et al.[38] reported at least one metabolic abnormality in 86 (39.3 %) of children who manifested new-onset overweight or obesity, of which dyslipidemia (25.1 %) and hypertension (13.2 %) were the most prevalent alterations [38]. Guzzetti et al.[39] showed that dyslipidemia is frequent in children and adolescents with obesity, while hypertension is rare [39]. These findings agree with our results.

Limitations in this study need to be noted. First, the cross-sectional design does not allow for establishing causal relationships between the variables studied, and the duration of obesity could not be determined. Second, a direct measurement of body fat distribution was not performed (for example, using dual-energy X-ray absorptiometry). Although confounding variables are considered in the study, there are other confounders (e.g., diet and exercise) that were not included, which could influence the results. Finally, the findings in this study could not be generalized to all pediatric patients since most of the participants included in this study were recruited from one reference hospital.

In conclusion, the study demonstrates that hematological parameters are not independently associated with the MUP, and they are unlikely to represent reliable biomarkers of MUP in the pediatric population.

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