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Article

The Relationship between Vitamin D Levels, Insulin Resistance and Monocyte to HDL- -Cholesterol Ratio in Children

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Abstract: Objective: In this study, we investigated vitamin D, inflammatory hematologic ratios such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), MHR and plasma atherogenic index (PAI) and possible relationships with insulin resistance in children. **Methods:** A total of 210 individuals, including 96 children with insulin resistance and 114 children without insulin resistance, aged 6-18 years, who were admitted to the Pediatric Endocrinology Outpatient Clinic at Medicine Hospital, Istanbul Atlas University were included in our study. **Result:** NLR, PLR, SII and MHR were significantly higher in patients with insulin resistance compared to those without. Fasting insulin, PAI, HOMA-IR, HOMA-β were significantly higher and QUICKI was significantly lower in patients with insulin resistance compared to those without insulin resistance. NLR, SII and MHR were lower in normal vitamin D groups than the others were ($p<0.001$). PLR was lower in normal vitamin D than vitamin D<21 groups. **Conclusion:** Vitamin D deficiency in childhood is associated with high levels of circulating inflammatory mediators (NLR, PLR), MHR, PAI), insulin resistance and low insulin sensitivity. Our findings suggest that enhanced inflammatory mediators in 25(OH)D-deficient children may reflect activation of a pro-inflammatory, pro-diabetic and atherogenic pathway, which could be inhibited by vitamin D supplementation.

Keywords: children; vitamin D; insulin resistance; monocyte/HDL ratio; inflammatory index

Introduction

Childhood obesity has an increasing prevalence worldwide. Since adult obesity and obesity-related complications are more common in obese children and adolescents, childhood obesity has become an important health problem (1). Dyslipidemia can be a consequence of obesity in both adults and children. Hyperinsulinism increases TG production from the liver. The most common dyslipidemia seen with obesity is increased triglyceride (TG) levels and decreased high density lipoprotein (HDL) levels. This is called atherogenic dyslipidemia (2,3).

Based on the anti-inflammatory and antioxidant effects of HDL-C as well as the pro-inflammatory effect of monocytes, the monocyte/HDL-C ratio (MHR) reflects inflammation and oxidative stress. This value has been used in many studies to determine whether inflammation and atherosclerosis contribute to the etiopathogenesis of cardiovascular diseases and tip2 diabetes mellitus (T2DM) (3-6). Neutrophil/HDL-C ratio (NHR) is also an easily accessible potential index of inflammation (7).

Vitamin D deficiency has emerged as a widespread public health problem worldwide and is one of the most common undiagnosed nutrient deficiencies in all age groups (8). Vitamin D is inversely

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associated with MHR among young medical staff (9). Vitamin D, which is involved in the function of insulin-sensitive tissues including liver and skeletal muscle and has potential effects on the regulation of insulin secretion and survival of pancreatic beta cells, may play a role in the pathogenesis of insulin resistance (10-12). However, conflicting results of studies (13-15) make it necessary to evaluate the relationship between vitamin D levels and insulin resistance in childhood.

Therefore, the aim of the study was to investigate the mechanisms of obesity-associated insulin resistance, induction of inflammation and vitamin D alteration in the pediatric group. Understanding the impairment of diabetes-related insulin signaling induced by obesity may lead to better pharmacological strategies not only for the treatment but also for the prevention of obesity and diabetes. In this study, we investigated vitamin D, inflammatory hematologic ratios such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), MHR and atherogenic index (AI) and possible relationships with insulin resistance in children.

Materials and Methods

Study Population

This prospective study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Istanbul Atlas University, Medical Faculty Clinical Research Ethics Committee (number of approval E-22686390-050.99-26195; Date: 13 April 2023). Informed consent was obtained from all patients and their parents to perform the original measurements and review their medical records. Also, each participant signed the informed volunteer consent form.

A total of 210 individuals, including 96 children with insulin resistance and 114 children without insulin resistance, aged 6-18 years, who were admitted to the Pediatric Endocrinology Outpatient Clinic at Medicine Hospital, Istanbul Atlas University were included in our study. All measurements were performed by healthcare professionals, who were blinded to the clinical diagnoses of the participants.

Exclusion Criteria

Since we wanted to include only exogenously obese patients (without any other underlying disease) in the patient group, patients with hypothyroidism, Cushing's disease or syndrome that may cause obesity were excluded. In addition, patients with impaired glucose tolerance and diabetes mellitus (DM) were also excluded to create a non-diabetic patient group. Patients with perception and communication problems, thinness, growth retardation, and chronic diseases such as inflammatory diseases, infectious diseases and those taking oral antidiabetic drugs, insulin, antihypertensive drugs and lipid-lowering drugs were excluded. Those who regularly used any nutritional supplements (vitamin D, iron, fish oil, prebiotics, probiotics, etc.) in the last three months,

Clinical and Biochemical Parameters

Height was measured with a Harpender stadiometer with a measurement accuracy of 0.1 cm and weight was measured with a SECA scale with a measurement accuracy of 0.1 kg. participants' weight was assessed after removing all clothing except underwear. The dieticians measured height and body weight, and the body mass index (BMI) of the participants was calculated with the formula, weight (kg)/height^2 (m^2).

Blood pressure was measured in a sitting position after at least 5 minutes of rest and as the average of three measurements.

For complete blood count and biochemical tests, blood samples were collected from the antecubital vein between 8-10 hours in the morning at rest after 12 hours of fasting. Blood samples taken for biochemical tests were centrifuged for 10 minutes and serum was obtained. To avoid possible assay variability, all patient blood samples were analyzed together.

Impaired fasting glucose was assessed by internationally defined criteria. Fasting plasma glucose ≤100 mg/dL was defined as normal, 100-125 mg/dL as impaired fasting glucose, and 75 gr. OGTT, 2nd hour plasma glucose 140 mg/dL-199 mg/dL was considered as impaired glucose

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tolerance. OGTT was performed in patients with impaired fasting glucose and severe hyperinsulinemia (16).

Homeostasis model assessment of insulin resistance (HOMA-IR) index was used to evaluate insulin resistance. HOMA-IR calculated by the formula fasting insulin (uIU/mL) × fasting blood glucose (mg/dL) / 405. Different cut-off values for insulin resistance were taken for prepubertal and pubertal periods (prepubertal HOMA-IR >2.5 and pubertal HOMA-IR >4) (17). The status of insulin resistance in participants was also determined using fasting glucose to insulin ratio (FGIR), quantitative insulin sensitivity check index (QUICKI), and HOMA-β (18,19).

Levels of 25(OH)D are interpreted as follows (20): 21-29 ng/mL (52.5-72.5 nmol/L): vitamin D insufficiency; < 20 ng/mL (< 50 nmol/L): vitamin D deficiency.

Routine biochemical parameters such as glucose, cholesterol, and triglyceride were measured with an automated analyzer (Cobas Integra 800; Roche Diagnostics GmbH: Mannheim, Germany).

The levels of fasting insulin were determined using commercial kits and an automatic hormone analyzer (Beckman Coulter; Unicel DXI 600; Access Immunoassay System). The serum 25-hydroxyvitamin D [25(OH)D] levels were measured by enzyme linked fuorescent assay on the Mini Vidas (Biomerieux, Paris, France).

Serum high sensitive C-reactive protein (hs-CRP) was measured with chemiluminescent immunoassay using an ADVIA Centaur XP (Siemens Healthcare Diagnostics, NY, USA).

Neutrophil-lymphocyte ratio (NLR) was calculated by dividing neutrophil count by lymphocyte count and monocyte-HDL ratio (MHR) was calculated by taking the ratio of monocytes to HDL.

Plasma atherogenic index (PAI) was calculated from the logarithm of the ratio of triglyceride to HDL-cholesterol.

Statistical Analyses

Statistical Package for the Social Sciences version 21.0 software package for Windows (IBM Corp., Armonk, NY, USA) and Office 365 were used for data evaluation and analysis. Frequencies (n) and percentages (%) were used to present the descriptive characteristics of the data while numerical variables were represented as mean±standard deviation or median (25. percentile-75. percentile). A chi-square test was used to evaluate the distribution among categorical variables. Whether the data was normally distributed was analyzed through visuals (histograms and Q-Q plots) and descriptive techniques (coefficient of variation, skewness, and kurtosis) and analytical methods (Kolmogorov Smirnov Test). The independent samples t test or Mann-Whitney U test were used to compare continuous variables for two groups. One-way ANOVA or Kruskal-Wallis test were used for comparison of continuous variables between more than two groups; adjusted p values and Tukey-HSD were used for post-hoc significance. The Pearson or Spearman correlation analyses were used for to evaluate relationship between the numerical variables. A p-value <0.05 was considered for statistical significance.

Results

Of the participants, 54.3% (n:114) did not have insulin resistance and 45.7% (n:96) had insulin resistance. There was a statistically significant relationship between insulin resistance and vitamin D deficiency. Among patients without insulin resistance, 61.4% (n:70) had vitamin D in the normal range, 26.3% (n:30) had vitamin D between 21-29, and 12.3% (n:14) had vitamin D <21. None of the patients with insulin resistance had normal vitamin D levels, 41.7% (:40) had vitamin D between 21-29, 58.3% (n:56) <21 (Table 1).

Table 1. The relationship between insulin resistance and vitamin D deficiency.

	Insulin Resistance Status

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	No Insulin Resistance (n:114; 54.3%)		Insulin Resistance (n:96; 45.7%)		
	n	**%**	**n**	**%**	**p value**
Vitamin D					
Normal Vitamin D	70	61.4%	0	0%	
Vitamin D (21-29)	30	26.3%	40	41.7%	<0.001*
Vitamin D <21	14	12.3%	56	58.3%	
Vitamin D					
Normal Vitamin D	70	61.4%	0	0%	<0.001*
Vitamin D <29	44	38.6%	96	100%	

*: Fishers exact test was applied.

Table 2. Relationship between insulin resistance and clinical and laboratory parameters and indexes.

	No Insulin Resistance	Insulin Resistance	
	mean±std or Median (25p-75p)	**mean±std or Median (25p-75p)**	**p value**
Body Mass Index (BMI) (kg/m^2)	24.33(23.14-26.02)	26.1(23.71-28.28)	0.002¥
Waist circumference (cm)	69.9±8.89	79.22±7.17	<0.001†
Vitamin D	31.1(24-34.8)	18.7(12.55-24)	<0.001¥
Systolic Blood Pressure (mmHg)	108(103-115)	115(108-127.5)	<0.001¥
Diastolic Blood Pressure (mmHg)	65(63-70)	67(63-71)	0.188¥
White blood cell (10^3/µL)	7.54(6.5-8.78)	8.34(7.12-9.75)	0.009¥
Platelet (10^6/µL)	304.01±41.92	310.84±40.07	0.231†
Lymphocytes (10^3/µL)	2.91±0.66	2.6±0.76	0.002†
Lymphocytes %	36.99±8.02	33.84±10.23	0.013†
Neutrophil (10^3/µL)	3.42(2.71-4.41)	4.26(3.36-5.3)	<0.001¥
Neutrophil %	52.1(47.1-58.9)	54(47.55-59.65)	0.182¥
Monocyte (10^3/µL)	5.5(4.9-6.4)	7.3(5.85-8.3)	<0.001¥
Neutrophil lymphocyte ratio (NLR)	1.18(0.99-1.59)	1.6(1.23-2.03)	<0.001¥
Platelet lymphocyte ratio (PLR)	108.4(87.83-120.78)	121.1(96.5-150.85)	0.001¥
Systemic immune-inflammation index (SII)	366.61(296.27-485.16)	510.76(387.23-643.42)	<0.001¥
Monocyte / HDL cholesterol	11.66(9.57-14.22)	18.62(16.02-22.99)	<0.001¥
CRP (mg/L)	0.85(0.5-1.54)	1.96(0.91-2.77)	<0.001¥
Total cholesterol	154.5(147-165)	163(149-180)	0.022¥
HDL cholesterol	48.6(42.4-51.2)	38.5(33.4-43.9)	<0.001¥
LDL cholesterol	89(81-99)	106(95-121.5)	<0.001¥
VLDL cholesterol	17.2(14-18.8)	18.5(16.6-19.8)	<0.001¥
Triglyceride	86(70-94)	92.5(83-99)	<0.001¥

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Glucose (mg/dL)	90.68±8.17	94.22±9.97	0.005†
Glucose (mMol/L)	5.03±0.45	5.23±0.55	0.005†
Fasting insulin ((µIU/ml))	7.25(5.49-9.3)	19.25(16.7-23.2)	<0.001¥
Atherogenic index	0.23±0.12	0.38±0.12	<0.001†
HOMA-IR	1.65(1.26-2.08)	4.56(3.83-5.46)	<0.001¥
FGIR	12.68(9.08-16.92)	5.08(4.04-5.73)	<0.001¥
HOMA-B	95.59(74.63-125)	233.09(189.01-309.18)	<0.001¥
QUICKI	0.36±0.02	0.31±0.01	<0.001†

†: Independent samples t test; ¥: Mann-Whitney U test was applied.

Table 3. Relationship between Vitamin D deficiency and clinical and laboratory parameters and indexes.

	Vitamin D			
	Normal Vitamin D	**Vitamin D (21-29)**	**Vitamin D (<21)**	
	mean±std or Median(25p-75p)	**mean±std or Median(25p-75p)**	**mean±std or Median(25p-75p)**	**p value**
Body Mass Index (BMI) (kg/m²)	23.73(22.64-24.46)[a]	27.11(25.62-28.25)[b]	25.36(22.66-29.04)[c]	<0.001¥
Waist circumference (cm)	65.01±6.21[a]	75.09±5.29[b]	82.39±6.71[c]	<0.001†
Systolic Blood Pressure (mmHg)	105(102-108)[a]	120(110-125)[b]	110(108-130)[b]	<0.001¥
Diastolic Blood Pressure (mmHg)	65(63-70)	65(60-75)	67(65-70)	0.146¥
White blood cell (10³/µL)	7.49(6.63-8.63)[a]	8.02(6.29-9.1)[a,b]	8.39(7.25-11.11)[b]	0.016¥
Platelet (10⁶/µL)	309.3±42.84	300.9±52.26	311.2±22.27	0.290†
Lymphocytes (10³/µL)	3.03±0.56[a]	2.71±0.67[b]	2.57±0.84[b]	<0.001†
Lymphocytes %	38.62±6.98[a]	34.54±7.39[b]	33.46±11.79[b]	0.002†
Neutrophil (10³/µL)	3.15(2.53-3.71)[a]	4.27(3.06-5.2)[b]	4.26(3.44-5.27)[b]	<0.001¥
Neutrophil %	51.2(46-56.4)	53.95(48.9-60.8)	53.6(47.5-58.9)	0.158¥
Monocyte (10³/µL)	5.1(4.6-6.1)[a]	7.35(6.3-8.5)[b]	6.3(5.3-7.5)[c]	<0.001¥
Monocyte (%)	510(460-610)[a]	735(630-850)[b]	630(530-750)[c]	<0.001¥
Neutrophil lymphocyte ratio (NLR)	1.06(0.87-1.29)[a]	1.59(1.23-2.06)[b]	1.57(1.21-2.22)[b]	<0.001¥
Platelet lymphocyte ratio (PLR)	107.91(86.3-117.86)[a]	112.01(90.78-131.73)[a,b]	121.1(102.85-145.24)[b]	0.002¥
Systemic immune-inflammation index (SII)	328.44(264-404.76)[a]	486(355.01-630.97)[b]	498.72(381.33-648.41)[b]	<0.001¥
Monocyte / HDL cholesterol	10.41(8.94-12.2)[a]	18.4(14.69-22.7)[b]	17.06(14.01-19.79)[b]	<0.001¥
CRP (mg/L)	0.7(0.4-1.1)[a]	1.55(0.95-2.35)[b]	1.99(0.88-2.89)[b]	<0.001¥
Total cholesterol	150(145-157.5)[a]	165(152-181)[b]	166.5(149-186)[b]	<0.001¥
HDL cholesterol	49.8(46.6-52.6)[a]	41.1(35.4-48.4)[b]	39.5(33.2-43.2)[b]	<0.001¥
LDL cholesterol	84.5(78-90)[a]	102.5(95-121)[b]	109.5(97-124)[b]	<0.001¥
VLDL cholesterol	17(14-18.4)[a]	16.6(14-19)[a]	19(17.8-19.8)[b]	<0.001¥
Triglyceride	85(70-92)[a]	83(70-95)[a]	95(89-99)[b]	<0.001¥
Glucose (mg/dL)	88.67±6.4[a]	93.44±9.66[b]	94.79±10.03[b]	<0.001†
Glucose (mMol/L)	4.92±0.36[a]	5.19±0.54[b]	5.26±0.56[b]	<0.001†
Fasting insulin ((µIU/ml))	5.75(5.05-7.3)[a]	14.2(9.7-19.8)[b]	18.15(15.3-22.2)[c]	<0.001¥

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Atherogenic index	0.2±0.11 [a]	0.3±0.14 [b]	0.41±0.1 [c]	<0.001[†]
HOMA-IR	1.28(1.13-1.63) [a]	3.39(2.13-4.83) [b]	4.08(3.54-5.23) [b]	<0.001[¥]
FGIR	14.83(12.68-18.02) [a]	6.58(5.12-8.84) [b]	5.19(4.14-6.35) [c]	<0.001[¥]
HOMA-β (%)	84.63(65.15-105.6) [a]	170.44(122.78-234.98) [b]	206.44(153.81-276.46) [b]	<0.001[¥]
QUICKI	0.37±0.01 [a]	0.32±0.02 [b]	0.31±0.02 [b]	<0.001[†]

[†]: one-way ANOVA test; [¥]: Kruskall-Wallis test were applied.

Table 4. Relationship between indexes and Insulin resistance indicators.

		HOMA-IR	FGIR	HOMA-B	QUICKI	Monocyte / HDL-C	NLR	PLR	SII
Atherogenic index	r	0.682	-0.641	0.518	-0.639	0.574	0.256	0.174	0.269
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001
HOMA-IR	r		-0.952	0.781	-1.000	0.739	0.447	0.231	0.434
	p		<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
FGIR	r			-0.926	0.952	-0.709	-0.438	-0.212	-0.421
	p			<0.001	<0.001	<0.001	<0.001	0.002	<0.001
HOMA-β:	r				-0.781	0.591	0.358	0.139	0.333
	p				<0.001	<0.001	<0.001	0.044	<0.001
QUICKI	r					-0.739	-0.447	-0.231	-0.434
	p					<0.001	<0.001	0.001	<0.001
Monocyte / HDL-C	r						0.351	0.222	0.341
	p						<0.001	0.001	<0.001
NLR	r							0.402	0.946
	p							<0.001	<0.001
PLR	r								0.529
	p								<0.001

Pearson correlation was used for the atherogenic index- QUCKI and Spearman correlation for the others.

BMI and waist circumference were significantly higher, and vitamin D was significantly lower in the group with insulin resistance than without insulin resistance. Platelet and neutrophil were similar between insulin resistance groups. WBC, neutrophil, monocyte, CRP, total cholesterol, LDL cholesterol, VLDL cholesterol, triglyceride, glucose levels were higher in insulin resistance group than without insulin resistance; lymphocyte, lymphocyte percentage, HDL cholesterol levels were lower.

NLR, PLR, SII and MHR were significantly higher in patients with insulin resistance compared to those without. Fasting insulin (19.25(16.7-23.2) vs 7.25(5.49-9.3); p<0.001), PAI (0.38±0.12 vs 0.23±0.12; p<0.001), HOMA-IR (4.56(3.83-5.46) vs 1.65(1.26-2.08); p<0.001), HOMA-β (233.09(189.01-309.18) vs 95.59(74.63-125); p<0.001) were significantly higher and QUICKI (0.31±0.01 vs 0.36±0.02; p<0.001) was significantly lower in patients with insulin resistance compared to those without insulin resistance.

NLR, SII and MHR were lower in normal vitamin D groups than the others were (p<0.001). PLR was lower in normal vitamin D than vitamin D<21 groups. FGIR levels were significantly different between the three groups. FGIR was 14.83 (12.68-18.02) in the normal vitamin D group, 6.58 (5.12-8.84) in the vitamin D (21-29) group and 5.19 (4.14-6.35) in the vitamin D<21 group. Fasting insulin and PAI levels were significantly different between the three groups. It is found that fasting insulin and PAI were lower in the normal vitamin D group compared to the others and higher in the vitamin D<21 group compared to the others.

QUICKI was significantly higher in the normal vitamin D group than the others (p<0.001); HOMA-IR and HOMA-β were significantly lower (p<0.001).

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PAI was strongly correlated with HOMA-IR (r:0.682; p<0.001), FGIR (r:-0.641; p<0.001), QUICKI (r:-0.639; p<0.001), moderately correlated with HOMA-β (r:0.518, p<0.001), MHR (r:0.574; p<0.001), weakly correlated with NLR (r:0.256; p<0.001) and SII (r:0.269; p<0.001), very weakly correlated with PLR (r:0.174; p:0.012).

HOMA-IR was very strongly correlated with FGIR (r:-0.952; p<0.001), QUICKI (r:1.0; p<0.001), strongly correlated with HOMA-β (r:0.781; p<0.001) and MHR (r:0.739; p<0.001), moderately correlated with NLR (r:0.447; p<0.001) and SII (r:0.434; p<0.001), weakly correlated with PLR (r:0.231; p<0.001).

FGIR was very strongly correlated with HOMA-β (r: -0.926; p<0.001), QUICKI (r:0.952; p<0.001), strongly correlated with MHR (r:-0.709;p<0.001), moderately correlated with NLR (r:-0.438; p<0.001) and SII (r:-0.421; p<0.001), weakly correlated with PLR (r:-0.212; p<0.001).

HOMA-β was strongly correlated with QUICKI (r: -0.781; p<0.001), moderately correlated with MHR (r:0.591; p<0.001), weakly correlated with NLR (r:0.358; p<0.001) and SII (r:0.333; p<0.001), very weakly correlated with PLR (r:0.139; p<0.001).

MHR was weakly correlated with NLR (r:0.351; p<0.001), SII (r:0.341; p<0.001) and PLR (r:0.222; p<0.001).

Discussion

Insulin resistance is a complex cellular disorder that affects multiple organ systems and leads to severe metabolic defects. In current study, vitamin D was significantly lower in insulin resistance group than the group without insulin resistance. NLR, PLR, SII and MHR were also higher in patients with insulin resistance compared to those without. NLR, SII and MHR were lower in normal vitamin D groups than the others. PAI was strongly correlated with HOMA-IR. The importance of vitamin D levels should not be forgotten when monitoring and treating insulin resistance and related pathologies. Keeping vitamin D at optimal levels will be an inexpensive and easy preventive approach to metabolic control. Vitamin D functions as an immune modulator via monocytes and macrophages. Our findings suggest the supplementation of vitamin D may be helpful in metabolic control, prevention of insulin resistance and related pathologies.

Vitamin D deficiency has recently become very common and has been associated with the pathogenesis of many diseases including metabolic abnormalities (21,22). The relationship between vitamin D deficiency and insulin resistance is also gaining importance (23). The results of our study showed that insulin resistance children had higher levels of vitamin D deficiency and insufficiency than non-insulin resistance children. Sharifi et al. (24) found that serum 25(OH)D levels are inversely associated with insulin resistance. Their results suggest that in metabolic syndrome (MetS) patients it may benefit to determine cutoff value of 25(OH)D levels based on HOMA-IR. Moschonis et al. (25) showed that there was a negative correlation between serum 25 (OH) vitamin D concentrations and HOMA-IR levels and that insulin resistance children had a higher prevalence of vitamin D deficiency and insufficiency compared to healthy age groups. Although it is not known exactly how vitamin D reduces the risk of developing metabolic disorders, vitamin D receptors and metabolizing enzymes have been detected in most insulin-sensitive cell types such as pancreatic cells and adipocytes (26). Evidence suggests that vitamin D has a regulatory effect on pancreatic insulin secretion and blood glucose control.

There are studies showing that Vit D has immunomodulatory and anti-inflammatory properties (27). Vitamin D deficiency has been suggested to impair the immune system and cause infections. Markers of systemic inflammation are observed to increase in Vit D deficiency (28). Vit D is known to have benefits in immune initiation, mucosal protection and endothelial function. Vit D deficiency has also been associated with increased markers of systemic inflammation associated with multiple organ failure (29). Reyman et al. (30) found that the relationship between 25(OH)D deficiency, enhanced systemic inflammation and reduced insulin sensitivity in childhood obesity. In current study, NLR, PLR, SII and MHR were higher in patients with insulin resistance compared to those without. NLR, SII and MHR were also lower in normal vitamin D groups than the vitamin D deficiency and insufficiency. HOMA-IR was strongly correlated with MHR, moderately correlated

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with NLR and SII, weakly correlated with PLR. When we look at the biological basis of NLR increase, lymphocytes increase first in the immune response after hypertrophy of adipose tissues (31) and then lymphocytes produce cytokines such as TNF-alpha, IL-6, IL-1, IL-8, and adipokines (leptin, resistin, and visfatin) and mediate the recruitment of monocytes into adipose tissue and increase the number of neutrophils (32). Thus, both neutrophils and lymphocytes increase in the early phase of inflammation with insulin resistance. In our study, CRP, an indicator of acute inflammation, increased in children with insulin resistance and vitamin D deficiency and insufficiency. In low-grade inflammation, monocytes are activated and some of them transform into lipid- loaded macrophages (33). Thus, monocytes and macrophages trigger the formation or progression of cardiovascular diseases. In the study by Johnsen et al. (34), it was shown that increased monocyte levels were predictive of plaque development in arteries without prior plaque. In addition, there are many recent studies showing that HDL is effective on monocyte activation and inflammation in the development of atherosclerosis (35,36). HDL has been found to be an anti-inflammatory molecule. Monocyte and HDL parameters may be indirect indicators of inflammation (37). In our study, total cholesterol, LDL, VLDL, triglyceride (TG) levels were higher in insulin resistance group than the group without insulin resistance, HDL were lower. MHR increased correlatively as HOMA-IR increased. Increased MHR is an expected result in proinflammatory backgrounds such as insulin resistance and vitamin D deficiency.

The PAI is a newly introduced index that reflects cardiovascular disease risk and dyslipidemia well (38). In current study, PAI were lower in the normal vitamin D group compared to the others and higher in the vitamin D<21 group compared to the others. Also, PAI was positive correlated with HOMA-IR, HOMA-β, MHR, NLR and SII, while PAI was negative correlated with FGIR and QUICKI. Vitamin D deficiency and insulin resistance are associated with waist circumference and BMI. This may be because large amounts of triglycerides and free cholesterol stored in body adipose tissue next to adipocytes are added to the circulation with increasing obesity. Thus, free circulating blood TG level suppresses hepatic lipoprotein lipase activity. As this enzyme is suppressed, circulating HDL begins to decrease. Low 25(OH)D levels are associated with increased insulin resistance and impaired lipid profile (39). This may cause a tendency towards atherogenesis as well as an increase in PAI. The relationship between dyslipidemia and obesity may be explained by this mechanism (40). The PAI value may contribute to the determination of cardiovascular risk in children with vitamin D deficiency, insulin resistance and obesity by primary care physicians by being included in the laboratory result evaluation form without additional testing and cost.

It is possible to assess insulin sensitivity easily, quickly and inexpensively in daily practice. Various tests have been described for this purpose and these methods have been found to show strong correlation in the assessment of insulin resistance. Although the hyperinsulinemia euglycemic glucose clamp (HEGC) is considered the "gold standard" for determining peripheral insulin resistance, it is not used in routine clinical practice (41). Several studies have found that it correlates with HOMA-IR (41-43). Roth et al. (44) found that higher insulin, HOMA-IR, and HbA1c as well as lower QUICKI values were found in obese children with lower 25(OH)D concentrations even after adjustment for gender, age, and body mass index. Hypovitaminosis D is a risk factor for developing insulin resistance independent of adiposity. In a study conducted in Turkey (45), the HOMA-IR level and vitamin D deficiency were identified as effective secondary factors in the formation of dyslipidemia in obese children. In current study, fasting insulin, HOMA-IR, HOMA-β were significantly higher and QUICKI was significantly lower in patients with insulin resistance compared to those without insulin resistance. HOMA-IR was negative correlated with FGIR, while HOMA-IR was positive correlated with QUICKI and HOMA-β. The HOMA-IR, QUICKI, HOMA-β and FGIR tests are tests that can evaluate insulin resistance and sensitivity in children in a practical way. Children with low 25(OH)D concentrations have lower insulin sensitivity (QUICKI). Globally, serum vitamin D deficiency is a major public health problem in all age groups, even in populations living in countries close to the equator, where sun exposure is generally assumed to be sufficient to prevent this deficiency, and in industrialized countries where vitamin D supplementation has been practiced for years. Identifying metabolic markers related to vitamin D and determining the treatment

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possibilities for these markers is one of the interesting topics that may be useful in the fight against insulin resistance in the future.

Limitations of the Study

Factors affecting vitamin D levels such as climate, season, lifestyle (dressing, sunbathing, diet) are not considered.

Conclusions

In 25(OH)D-deficient children, PAI value can be used as a marker to predict insulin resistance and inflammation. The PAI may be a potential therapeutic target in the treatment and prevention of insulin resistance in children. The high circulating inflammatory mediators (NLR, PLR, SII, MHR) and PAI value may reflect activation of a pro-inflammatory, pro-diabetic and atherogenic pathway, which could be inhibited by vitamin D supplementation. The HOMA-IR, QUICKI, HOMA-β and FGIR tests can be used to practically evaluate insulin resistance and sensitivity in children. Keeping vitamin D at optimal levels will be an appropriate approach in the diagnosis, follow-up and treatment of chronic diseases and in the management of metabolic processes that start with insulin resistance. Vitamin D deficiency in childhood is associated with high levels of circulating inflammatory mediators, insulin resistance and low insulin sensitivity. Our findings suggest that the supplementation of vitamin D may be helpful in preventing insulin resistance and enhanced systemic inflammation. Therefore, IR should be considered as a cluster of abnormalities that impair various physiological functions, rather than as a metabolic disorder alone.

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References

1. NCD Risk Factor Collaboration (NCD-RisC) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet. 2017;390:2627–2642.
2. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. Obesity reviews. 2012;13 (3):275-86.
3. You S, Zhong C, Zheng D, Xu J, Zhang X, Liu H et al. Monocyte to HDL cholesterol ratio is associated with discharge and 3-month outcome in patients with acute intracerebral hemorrhage. J Neurol Sci 2017; 372: 157-61.
4. Karataş MB, Çanga Y, Özcan KS, Ipek G, Güngör B, Onuk T; et al. Monocyte to high-density lipoprotein ratio as a new prognostic marker in patients with STEMI undergoing primary percutaneous coronary intervention. Am J Emerg Med 2016; 34(2): 240-4.
5. Canpolat U, Çetin EH, Cetin S, Aydin S, Akboga MK, Yayla C; et al. Association of Monocyte-to-HDL Cholesterol Ratio with Slow Coronary Flow is Linked to Systemic Inflammation. Clin Appl Thromb Hemost 2016; 22(5): 476-82.

 doi:10.20944/preprints202408.1041.v1

6. Sivgin H, Çetin S. Effect of empagliflozin use on monocyte high-density lipoprotein ratio and plasma atherogenic index in obese and non-obese type 2 diabetic patients. Eur Rev Med Pharmacol Sci. 2023;27(17):8090-8100.
7. Chen T, Chen H, Xiao H, Tang H, Xiang Z, Wang X; et al. Comparison of the value of neutrophil to high-density lipoprotein cholesterol ratio and lymphocyte to high-density lipoprotein cholesterol ratio for predicting metabolic syndrome among a population in the southern coast of China. Diabetes Metab Syndr Obes 2020; 13: 597-605.
8. De Cosmi V, Mazzocchi A, D'Oria V; et al. Effect of Vitamin D and Docosahexaenoic Acid Co-Supplementation on Vitamin D Status, Body Composition, and Metabolic Markers in Obese Children: A Randomized, Double Blind, Controlled Study. Nutrients. 2022;14(7):1397.
9. Tang L, Zeng H, Yang B; et al. Vitamin D is inversely associated with Monocyte to HDL-C ratio among medical staff in Chengdu, China. BMC Endocr Disord. 2023;23(1):149.
10. Altieri B, Grant WB, Della Casa S, Orio F, Pontecorvi A, Colao A; et al. Vitamin D and pancreas: The role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. Crit Rev Food Sci Nutr. 2017;57(16):3472-88.
11. Al-Shoumer KA, Al-Essa TM. Is there a relationship between vitamin D with insulin resistance and diabetes mellitus? World J Diabetes. 2015;6(8):1057-64.
12. Szymczak-Pajor I, Śliwińska A. Analysis of association between vitamin D deficiency and insulin resistance. Nutrients. 2019;11(4):794.
13. Saglam D, Samur G, Turan S. Assessment of vitamin D status in Turkish adolescents: Its relation to obesity, cardiometabolic risk factors and nutritional status. Prog Nutr. 2019;21(4):762-8.
14. Torun E, Gönüllü E, Ozgen IT, Cindemir E, Oktem F. Vitamin D deficiency and insufficiency in obese children and adolescents and its relationship with insulin resistance. Int J Endocrinol. 2013;2013:631845.
15. Wang L, Wang H, Wen H, Tao H, Zhao X. Relationship between HOMA-IR and serum vitamin D in Chinese children and adolescents. J Pediatr Endocrinol Metab. 2016;29(7):777-81.
16. American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2021. Diabetes Care. 2021;44(1): 15–33.
17. Valerio G, Licenziati MR, Iannuzzi A, Franzese A, Siani P, Riccardi G et al. Insulin resistance and impaired glucose tolerance in obese children and adolescents from Southern Italy. Nutr Metab Cardiovasc Dis 2006;16:279-84.
18. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics. 2005;115(4):e500-e503.
19. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index:a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000; 85: 2402-10.
20. Hollis BW, Wagner CL. Normal serum vitamin D levels. *N Engl J Med*. 2005;352:515-6.
21. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, Zheng SG. Vitamin D and Chronic Diseases. Aging Dis. 2017; 8: 346-53.
22. Szymczak-Pajor I, Śliwińska A. Analysis of Association between Vitamin D Deficiency and Insulin Resistance. Nutrients. 2019;11(4):794.
23. Tao S, Yuan Q, Mao L, Chen F-L, Ji F, Cui Z-H. Vitamin D deficiency causes insulin resistance by provoking oxidative stress in hepatocytes. Oncotarget 2017; 8:67605-13.
24. Sharifi F, Mousavinasab N, Mellati AA. Defining a cutoff point for vitamin D deficiency based on insulin resistance in children. Diabetes Metab Syndr. 2013;7:210-213. .
25. Moschonis G, Androutsos O, Hulshof T, Dracopoulou M, Chrousos GP, Manios Y. Vitamin D insufficiency is associated with insulin resistance independently of obesity in primary school children. The healthy growth study. Pediatr Diabetes 2018;19:866-73.
26. Abbas MA. Physiological functions of Vitamin D in adipose tissue. J. Steroid Biochem. Mol. Biol. 2017; 165: 369-81.
27. Peterson CA, Heffernan ME. Serum tumor necrosis factor alpha concentrations are negatively correlated with serum 25(OH) D concentrations in healthy women. J Inflamm 2008; 5: 10.
28. Hewison M, Zehnder D, Chakraverty R, and Adams JS: Vitamin D and barrier function: A novel role for extra-renal 1 alpha-hydroxylase. Mol Cell Endocrinol 2004; 215: 31-8.
29. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR; et al. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. J Transl Med 2009; 7:28.
30. Reyman M, Verrijn Stuart AA, van Summeren M; et al. Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. Int J Obes (Lond). 2014;38(1):46-52.

 doi:10.20944/preprints202408.1041.v1

31. Ghigliotti G, Barisione C, Garibaldi S; et al. Adipose tissue immune response: Novel triggers and consequences for chronic inflammatory conditions. Inflammation. 2014;37(4):1337-53.
32. Vieira-Potter VJ. Inflammation and macrophage modulation in adipose tissues. Cellular microbiology. 2014;16(10):1484-92.
33. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. Journal of Endocrinology. 2014;222(3):R113-R27.
34. Johnsen SH, Fosse E, Joakimsen O; et al. Monocyte count is a predictor of novel plaque formation: A 7-year follow-up study of 2610 persons without carotid plaque at baseline the Tromsø Study. Stroke. 2005;36(4):715-9.
35. Murphy A, Chin-Dusting J, Sviridov D; et al. KJ. The anti inflammatory effects of high density lipoproteins. Current Medicinal Chemistry. 2009;16(6):667-75.
36. Usta A, Avci E, Bulbul CB; et al. The monocyte counts to HDL cholesterol ratio in obese and lean patients with polycystic ovary syndrome. Reproductive Biology and Endocrinology. 2018;16(1):34.
37. Akboga MK, Balci KG, Maden O; et al. Usefulness of monocyte to HDL-cholesterol ratio to predict high SYNTAX score in patients with stable coronary artery disease. Biomarkers in Medicine. 2016;10(4):375-83.
38. Onat A, Can G, Kaya H; et al. "Atherogenic index of plasma"(log10 triglyceride/high-density lipoprotein–cholesterol) predicts high blood pressure, diabetes, and vascular events. Journal of Clinical Lipidology. 2010;4(2):89-98.
39. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. Prog Lipid Res 2011;50(4):303-12.
40. Zhu X, Yu L, Zhou H; et al. Atherogenic index of plasma is a novel and better biomarker associated with obesity: A population-based cross-sectional study in China. Lipids in Health and Disease. 2018;17(1):1-6.
41. King-Morris KR, Deger SM, Hung AM; et al. Measurement and Correlation of Indices of Insulin Resistance in Patients on Peritoneal Dialysis. *Perit Dial Int*. 2016;36(4):433-44.
42. So A, Sakaguchi K, Okada Y; et al. Relation between HOMA-IR and insulin sensitivity index determined by hyperinsulinemic-euglycemic clamp analysis during treatment with a sodium-glucose cotransporter 2 inhibitor. *Endocr J*. 2020;67(5):501-507.
43. Anoop S S, Dasgupta R, Rebekah G; et al. Lipid accumulation product (LAP) as a potential index to predict risk of insulin resistance in young, non-obese Asian Indian males from Southern India: Observations from hyperinsulinemic-euglycemic clamp studies. *BMJ Open Diabetes Res Care*. 2021;9(1):e002414.
44. Roth CL, Elfers C, Kratz M, Hoofnagle AN. Vitamin d deficiency in obese children and its relationship to insulin resistance and adipokines. J Obes. 2011;2011:495101.
45. Erol M, Bostan Gayret Ö, Hamilçıkan Ş; et al. Vitamin D deficiency and insulin resistance as risk factors for dyslipidemia in obese children. Arch Argent Pediatr 2017;115(2):133-139.