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Relationship between Levels of Inflammatory Markers NLR, PLR, and MLR with Bone Mineral Density in Children with Thyroid Disorders

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Abstract

Background: Thyroid disorders affect bone metabolism and reduce bone mineral density (BMD) in children. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) are simple inflammatory markers, but their association with BMD in children with thyroid disorders is unclear. This study assessed the relationship between NLR, PLR, MLR, and BMD in affected children.

Materials and Methods: This retrospective cross-sectional study included 128 children (63 hypothyroid, 30 hyperthyroid, 35 controls) who had undergone DXA scanning. Thyroid function tests, complete blood count, calcium, phosphorus, and alkaline phosphatase were extracted from records. NLR, PLR, and MLR were calculated from complete blood count (CBC). BMD was measured by DXA and reported as Z-scores. Statistical analysis used ANOVA, correlation, and linear regression with adjustment for confounders.

Results: Mean BMD Z-scores in the hypothyroid group were significantly lower than controls at the lumbar spine (-0.70 ± 0.73 vs. -0.27 ± 0.77, P = 0.001) and femoral neck (-0.74 ± 0.74 vs. -0.35 ± 0.77, P = 0.003). In hypothyroid children, significant positive correlations were observed between all three indices and BMD Z-scores (NLR: r = 0.508, PLR: r = 0.575, MLR: r = 0.660; all P < 0.001). No significant correlations were found in hyperthyroid or control groups. In multiple regression adjusted for age, sex, and thyroid hormones, MLR was the strongest independent predictor of lumbar spine BMD (Beta = 0.357, P = 0.002), followed by NLR (Beta = 0.312, P = 0.003) and PLR (Beta = 0.228, P = 0.048). The model explained 41.8% of BMD variance (R^2 = 0.418, P < 0.001).

Conclusion: These findings demonstrate an association between systemic inflammation and reduced BMD in hypothyroid children, though the positive correlation direction requires further investigation. NLR, PLR, and MLR, easily calculated from routine CBC, are inexpensive tools for assessing bone health. Regular BMD screening is recommended for hypothyroid children, especially those with elevated inflammatory markers.

Key Words: Bone mineral density, children, Monocyte-to-lymphocyte ratio, Neutrophil-to-lymphocyte ratio, Platelet-to-lymphocyte ratio, Thyroid disorders.

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1- INTRODUCTION

Bone health during childhood and adolescence is critical because over 90% of peak bone mass is accumulated during this period, and its quality is the primary determinant of the risk of osteoporosis and fractures in adulthood (1). Thyroid hormones are essential for skeletal development and are important regulators of bone maintenance in adults (2). As an endocrine organ, the thyroid acts on the entire body by secreting a series of hormones, and bone is one of the main target organs of the thyroid (3).

In recent years, the field of "osteoimmunology" has addressed the interaction between the immune system and the skeletal system (4). Chronic low-grade inflammation is recognized as one of the key mechanisms reducing bone mineral density (BMD). In this context, inflammatory indices derived from complete blood count (CBC) tests, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), have been proposed as inexpensive and reliable markers of systemic inflammation (5). Inflammatory cytokines such as CRP, IL-1, and TNF-α increase bone resorption by acting on osteoclast precursors (6).

Children with thyroid diseases (e.g., congenital hypothyroidism, Hashimoto's, Graves') are at risk of reduced BMD due to impaired thyroid hormone metabolism and the systemic inflammation resulting from the autoimmune nature of these diseases. Hypothyroidism reduces growth rate and bone mass accumulation, while hyperthyroidism is associated with increased bone resorption (7). A study on 125 children showed that in the hypothyroid group, there were significant positive correlations between BMD Z-score and NLR (r = 0.526), PLR (r = 0.279), and MLR (r = 0.490), but these correlations were not seen in the hyperthyroid or healthy control groups (8). Despite the above evidence, no study in Iran has simultaneously compared the predictive power of all three indices, NLR, PLR, and MLR, for bone density in children and adolescents with thyroid disorders. Since early detection of reduced BMD in these patients is essential for preventing osteoporosis in adulthood, this study aimed to determine the association between levels of NLR, PLR, and MLR with bone mineral density in children with thyroid disorders referred to Imam Khomeini Hospital in Ardabil.

2- MATERIALS AND METHODS

2-1. Study Design and Setting

This was a retrospective cross-sectional analytical study conducted at the endocrinology clinic of Imam Khomeini Hospital in Ardabil, Iran. Medical records of children and adolescents aged 5 to 18 years with hypothyroidism or hyperthyroidism who were referred between 2021 and 2024 and had undergone BMD measurement by DXA as part of their clinical evaluation were reviewed. The retrospective design involved data extraction from existing medical records, while the cross-sectional component refers to the single time point at which BMD and laboratory data were analyzed.

2-2. Participants

The study included children with a confirmed diagnosis of hypothyroidism or hyperthyroidism based on TSH, FT3, and FT4 levels, who had undergone DXA scanning. Controls were selected from children referred to the clinic for growth monitoring who had no thyroid dysfunction or other chronic conditions. Inclusion criteria included a definite diagnosis based on TSH, FT3, and FT4 levels, the availability of CBC results within a six-month period around the BMD measurement due to the retrospective nature of data collection, and age over 5 years. This 6-month window

was chosen to maximize sample size while acknowledging the temporal limitation; CBC values were assumed to reflect the inflammatory state at the time of BMD measurement. Patients with chronic diseases affecting bone (e.g., chronic kidney disease, IBD, inflammatory arthritis), a history of recent fracture, use of medications affecting BMD or inflammatory indices (corticosteroids, anticonvulsants, methotrexate), or incomplete medical records were excluded from the study.

2-3. Sampling and Sample Size

The sample size was estimated to be approximately 128 based on a correlation coefficient of 0.5, a power of 80%, and α=0.05. This estimate served as the target sample size. However, because all eligible patients meeting inclusion criteria during the study period were included, sampling was performed by the census method. The final sample size of 128 represented all eligible patients with complete records.

Of the total eligible patients initially screened (n=187), 59 were excluded due to incomplete records (n=28), use of exclusionary medications (n=17), or presence of other bone-affecting conditions (n=14), leaving 128 participants for final analysis.

2-4. Control Group Definition and Matching

The control group (n=35) consisted of children referred to the same clinic for growth monitoring or routine check-ups who had normal thyroid function tests and no chronic medical conditions. Controls were not actively matched to cases but were comparable in terms of age and sex distribution. However, pubertal status, vitamin D levels, and physical activity were not systematically assessed or matched, which represents a limitation. Control children were selected from the same source population and time period to minimize selection bias.

2-5. Data Extraction

Demographic data (age, sex, height, weight, BMI), thyroid and biochemical tests (calcium, phosphorus, ALP), and complete blood count were extracted from medical records. Height and weight were measured at the time of clinical visit using standardized equipment; BMI was calculated as weight (kg)/height (m^2). Inflammatory indices NLR, PLR, and MLR were calculated from CBC results using the following formulas: NLR = neutrophil count/lymphocyte count, PLR = platelet count/lymphocyte count, MLR = monocyte count/lymphocyte count. BMD values for the lumbar spine and femoral neck were reported as Z-scores; a Z-score below -2 was defined as "low bone density for age." DXA measurements were performed using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA, USA) with standard pediatric protocols.

2-6. Statistical Analysis

All statistical analyses in this study were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA), and the significance level for all tests was considered 0.05. Initially, to describe the data, quantitative variables were reported as mean ± standard deviation and qualitative variables as frequency (percentage). The normality of data distribution was checked using the Shapiro-Wilk test; variables with normal distribution (age, BMI, calcium, phosphorus, BMD Z-scores) were analyzed using parametric tests, while non-normally distributed variables (TSH, FT4, FT3, NLR, PLR, MLR, alkaline phosphatase, and blood cell counts) were analyzed using non-parametric methods.

One-way ANOVA or the Kruskal-Wallis test as appropriate, followed by Tukey's post-hoc test or Dunn's test for multiple

comparisons, was used to compare quantitative variables among the three study groups (hypothyroid, hyperthyroid, and control), and the Chi-square or Fisher's exact test was used to compare qualitative variables. For correlation analyses, Pearson correlation was used when both variables were normally distributed; otherwise, Spearman's rank correlation was applied. Specifically, Pearson correlation was used for BMD Z-scores with normally distributed variables, while Spearman correlation was used for analyses involving NLR, PLR, and MLR due to their non-normal distribution. To investigate the relationship between inflammatory indices (NLR, PLR, MLR) and BMD Z-score in the lumbar spine and femoral neck, correlation analyses were performed separately for disease groups and by sex.

Finally, to determine the independent predictive power of each inflammatory index and control for potential confounding variables (e.g., age, sex, thyroid test results), multiple linear regression with the simultaneous entry method was used. Collinearity was assessed using Variance Inflation Factor (VIF) and tolerance values; VIF < 5 and tolerance > 0.2 were considered acceptable, indicating no significant multicollinearity. Model adequacy was assessed based on R^2 and Adjusted R^2 values. No correction for multiple comparisons was applied, as the primary analyses were pre-specified and the number of comparisons was limited; however, this represents a limitation of the study.

Additionally, given the potential for confounding, separate regression models were run including and excluding the three inflammatory indices to assess their incremental predictive value. Sensitivity analyses were performed excluding participants with values more than 3 standard deviations from the mean to ensure results were not driven by outliers.

This study was approved by the ethics committee of Ardabil University of Medical Sciences with ethics code IR.ARUMS.MEDICINE.REC.1403.044. Informed consent was obtained from parents or legal guardians of all participants, and assent was obtained from children aged 7 years and older.

3- RESULTS

In this study, 63 (49.2%) had hypothyroidism, 30 (23.4%) had hyperthyroidism, and 35 (27.3%) were healthy controls. Of the total patients, 64 (50%) were boys and 64 (50%) were girls. The distribution of sex was not significantly different among groups (χ^2=1.24, P=0.538).

There were no significant differences in age and BMI among the three groups. TSH level was significantly higher in the hypothyroid group (10.13 ± 3.87 mU/L) and significantly lower in the hyperthyroid group (0.08 ± 0.04 mU/L) compared to the control group (2.51 ± 0.84 mU/L). Disease duration (mean ± SD) was 3.2 ± 2.1 years in the hypothyroid group and 2.8 ± 1.9 years in the hyperthyroid group, with no significant difference between groups (P=0.342).

FT4 and alkaline phosphatase levels were significantly higher in the hyperthyroid group than in the other two groups. Calcium and phosphorus levels showed no significant difference among the three groups (Table 1).

The results show that all three indices, NLR, PLR, and MLR, differed significantly among the three groups. The highest values of NLR, PLR, and MLR were observed in the hypothyroid group. Neutrophil count was higher in the hypothyroid group than in the other groups (Table 2).

Table-1. Demographic and clinical characteristics by group (mean ± standard deviation).

Variable	Unit	Hypothyroid (n=63)	Hyperthyroid (n=30)	Control (n=35)	P-value
Age	years	12.24 ± 2.67	11.70 ± 2.25	12.38 ± 2.49	0.724
BMI	kg/m²	16.01 ± 5.57	17.45 ± 4.12	15.13 ± 4.23	0.618
TSH	mU/L	10.13 ± 3.87	0.08 ± 0.04	2.51 ± 0.84	<0.001
FT4	ng/dL	0.67 ± 0.08	2.77 ± 0.54	1.21 ± 0.11	<0.001
FT3	pg/mL	2.69 ± 0.65	6.86 ± 1.34	4.05 ± 0.56	<0.001
Alkaline Phosphatase	U/L	202.4 ± 57.94	274.77 ± 72.75	193.69 ± 65.01	0.018
Calcium	mg/dL	9.33 ± 0.47	9.46 ± 0.46	9.48 ± 0.66	0.481
Phosphorus	mg/dL	4.80 ± 0.72	4.77 ± 0.57	4.79 ± 0.81	0.356

Table-2. Comparison of inflammatory indices and blood cell components by group.

Variable	Unit	Hypothyroid (n=63)	Hyperthyroid (n=30)	Control (n=35)	P-value
Neutrophil	×10³/μL	5.05 ± 1.42	4.31 ± 0.82	3.86 ± 0.92	0.003
Lymphocyte	×10³/μL	2.09 ± 0.59	2.42 ± 0.72	2.91 ± 0.58	0.002
Monocyte	×10³/μL	0.62 ± 0.18	0.57 ± 0.17	0.50 ± 0.14	0.001
Platelet	×10³/μL	273.63 ± 67.45	271.27 ± 77.95	249.69 ± 65.22	0.042
NLR	---	2.64 ± 1.16	2.00 ± 0.78	1.39 ± 0.45	<0.001
PLR	---	144.37 ± 61.65	119.35 ± 39.40	88.66 ± 27.51	<0.001
MLR	---	0.33 ± 0.18	0.27 ± 0.12	0.18 ± 0.07	<0.001

The results showed that the hypothyroid group had significantly the lowest BMD Z-scores in both the lumbar spine (-0.70) and femoral neck (-0.74). The hyperthyroid group showed the highest mean Z-scores in the lumbar spine (0.14) and femoral neck (0.02). The difference between the hypothyroid and control groups was statistically significant for both sites (P=0.008 for lumbar spine, P=0.015 for femoral neck in post-hoc analysis).

All correlations were calculated using Spearman's rank correlation due to the non-normal distribution of inflammatory indices. In the hypothyroid group, a significant positive correlation was observed between all three indices and BMD Z-score. The highest correlation coefficient belonged to MLR with lumbar spine BMD (r=0.660, P<0.001). The correlation coefficients for PLR with lumbar spine BMD (r=0.575, P<0.001) and NLR with lumbar spine BMD (r=0.508, P<0.001) were also significant. The same pattern was observed in the femoral neck region, with MLR showing the highest correlation (r=0.596, P<0.001).

Table-3. Comparison of bone mineral density Z-scores between groups.

Variable	Hypothyroid (n=63)	Hyperthyroid (n=30)	Control (n=35)	P-value
BMD Z-score Lumbar Spine	-0.70 ± 0.73	0.14 ± 0.82	-0.27 ± 0.77	0.001
BMD Z-score Femoral Neck	-0.74 ± 0.74	0.02 ± 0.90	-0.35 ± 0.77	0.003

Table-4. Pearson correlation coefficients between NLR, PLR, and MLR with BMD Z-score in the hypothyroid group.

Inflammatory Index	BMD Z-score Lumbar Spine (r)	P-value	BMD Z-score Femoral Neck (r)	P-value	Interpretation
NLR	0.508	<0.001	0.439	<0.001	Significant Positive
PLR	0.575	<0.001	0.515	<0.001	Significant Positive
MLR	0.660	<0.001	0.596	<0.001	Significant Positive

Table-5. Pearson correlation coefficients between NLR, PLR, and MLR with BMD Z-score in the hyperthyroid and control groups.

Group	Inflammatory Index	BMD Z-score Lumbar Spine (r)	P-value	BMD Z-score Femoral Neck (r)	P-value	Interpretation
Hyperthyroid	NLR	-0.126	>0.05	-0.094	>0.05	Non-significant
Hyperthyroid	PLR	-0.253	>0.05	-0.304	>0.05	Non-significant
Hyperthyroid	MLR	-0.099	>0.05	0.016	>0.05	Non-significant
Control	NLR	-0.325	>0.05	-0.220	>0.05	Non-significant
Control	PLR	-0.122	>0.05	0.009	>0.05	Non-significant
Control	MLR	-0.166	>0.05	-0.057	>0.05	Non-significant

In the hyperthyroid and control groups, none of the observed correlations between inflammatory indices (NLR, PLR, MLR) and BMD Z-score were statistically significant. This finding indicates that the association between inflammatory indices and bone mineral density is specific to the hypothyroid group.

Stratified analysis by sex in the hypothyroid group showed that the positive correlation between MLR and lumbar spine BMD was significant in both boys (r=0.612, P=0.001) and girls (r=0.641, P<0.001), with no significant sex interaction (P for interaction = 0.782).

The results showed that thyroid grouping (based on TSH and FT4 status) had a significant association with BMD Z-score (P=0.001 for lumbar spine and P=0.003 for femoral neck). Children with abnormal TSH levels (hypothyroid: TSH > 4.5 mU/L) showed significantly lower Z-scores than the control group. Alkaline phosphatase level, as an indicator of bone activity, was significantly higher in the hyperthyroid group than in the other groups (274.77 ± 72.75 U/L vs. 202.4 ± 57.94 U/L in hypothyroid and 193.69 ± 65.01 U/L in controls, P=0.018), indicating increased bone turnover in this group.

The prevalence of "low bone density for age" (Z-score < -2) was 14.3% (n=9) in the hypothyroid group, 3.3% (n=1) in the hyperthyroid group, and 5.7% (n=2) in the control group, though this difference was not statistically significant (χ^2=4.12, P=0.128).

To determine the independent predictive power of each of the NLR, PLR, and MLR indices, after controlling for confounding variables (age, sex, and thyroid hormones), multiple linear regression analysis was performed in the hypothyroid group.

Table-6. Multiple linear regression analysis for predicting lumbar spine BMD Z-score in the hypothyroid group

Independent Variable	B (Coefficient)	SE	Beta (Standardized)	t	P-value	95% Confidence Interval
Constant	-1.24	0.31	---	-4.00	<0.001	-1.85 to -0.63
NLR	0.18	0.06	0.312	3.00	0.003	0.06 to 0.30
PLR	0.004	0.002	0.228	2.00	0.048	0.000 to 0.008
MLR	1.15	0.35	0.357	3.29	0.002	0.46 to 1.84
Age	0.03	0.03	0.091	1.00	0.320	-0.03 to 0.09
Sex (Male=1)	0.08	0.12	0.063	0.67	0.508	-0.16 to 0.32
TSH	0.012	0.009	0.138	1.33	0.187	-0.006 to 0.030
FT4	0.15	0.42	0.042	0.36	0.722	-0.69 to 0.99

R^2 = 0.418, Adjusted R^2 = 0.371, F(7,55) = 5.63, P < 0.001. All VIF values were < 5 (range: 1.08-2.34), indicating acceptable collinearity.

The multiple regression results showed that the model overall explained 41.8% of the variance in BMD Z-score (R^2=0.418, Adjusted R^2=0.371, P<0.001). After controlling for confounding variables, all three indices, NLR, PLR, and MLR, were significant independent predictors of BMD Z-score. MLR was the strongest independent predictor (Beta=0.357, P=0.002), followed by NLR (Beta=0.312, P=0.003) and PLR (Beta=0.228, P=0.048). Age, sex, TSH, and FT4 were not significant independent predictors in the adjusted model. The VIF values confirmed no significant multicollinearity among the inflammatory indices.

Sensitivity analysis excluding outliers (n=3 with values >3 SD from the mean) did not substantially change the results (MLR Beta=0.341, P=0.004; NLR Beta=0.298, P=0.006; PLR Beta=0.215, P=0.052), confirming the robustness of the findings.

4- DISCUSSION

This retrospective cross-sectional study aimed to investigate the association between levels of inflammatory markers NLR, PLR, and MLR with BMD in children and adolescents with thyroid disorders. The main findings showed that in the group of children with hypothyroidism, there was a significant positive correlation between all three inflammatory indices and BMD Z-scores of the lumbar spine and femoral neck, while no such significant correlation was observed in the group of children with hyperthyroidism or the control group. Notably, the positive direction of these correlations contrasts with findings from adult populations and requires careful interpretation, as discussed below.

In the present study, the sex and age distribution of patients was consistent with other studies conducted in the field of childhood thyroid disorders (1). The higher prevalence of hypothyroidism in girls compared to boys, observed in our findings, aligns with existing epidemiological data, as autoimmune thyroid diseases, including Hashimoto's thyroiditis (the most common cause of acquired hypothyroidism in children), are more prevalent in females (1). However, the sex distribution was balanced overall due to the inclusion of both disease groups.

Comparison of bone density between groups: The findings of the present study showed that Z-score bone mineral density values in the group of children with hypothyroidism were significantly lower compared to the control group, while no significant difference was observed between the hyperthyroid group and the control group. This finding is consistent with the results of the study by Bala and Bala (2022), in which BMD Z-scores in hypothyroid children were significantly lower than in the control group (8). From a pathophysiological perspective, thyroid hormones play a central role in regulating bone remodeling. Deficiency of these hormones is associated with decreased osteoblast activity, slowed bone turnover, and delayed skeletal maturation (1). According to the findings of Lee and Ahn (2023), abnormal TSH levels can serve as an early predictor of bone fragility in children (9). The higher BMD Z-scores in children with hyperthyroidism compared to the control group, observed in the present study, may seem contradictory at first. However, this finding is also consistent with the results of Bala and Bala (2022) (8). One possible explanation is that increased thyroid hormones in the early stages of the disease may be associated with stimulating osteoblast activity, although in the long term and without treatment, hyperthyroidism is associated with increased bone resorption and decreased BMD (10). Additionally, the relatively short disease duration in our hyperthyroid group (mean 2.8 years) may not have been sufficient to demonstrate the

bone-depleting effects of chronic hyperthyroidism.

Interpretation of the positive correlation requires consideration of several factors. First, in pediatric populations, the relationship between inflammation and bone may differ fundamentally from that in adults due to ongoing skeletal growth and maturation. Second, in the hypothyroid state, reduced thyroid hormones may cause relative lymphopenia, artificially elevating NLR without necessarily indicating severe systemic inflammation. Third, the inflammatory milieu in autoimmune thyroiditis (Hashimoto's) may be qualitatively different from other inflammatory conditions, potentially involving regulatory immune responses that affect bone differently. Recent research has shown that neutrophils, through the production of mediators such as IFN-γ, IL-6, and RANKL, can affect the balance between osteoblasts and osteoclasts, and in different clinical conditions, this effect can be diverse and even dual (11). Therefore, the positive correlation may reflect a compensatory or regulatory immune response rather than inflammation causing increased BMD.

The positive correlation of MLR with BMD Z-score found in hypothyroid children ($r=0.660$, $P<0.001$) is another significant finding of this study. Studies have shown that monocytes play an essential role in osteoclastogenesis, and the main precursors of osteoclasts originate from peripheral blood monocytes (11). A study by Fu et al. (2024) showed that MLR acts as an independent risk factor for bone fractures (12). Also, Zhang et al. (2024) demonstrated in a study on postmenopausal women that high MLR levels are associated with a higher risk of osteoporosis (13). The study by Bala and Bala (2022) also reported a significant positive correlation between MLR and BMD Z-score in hypothyroid children, confirming the findings of the present study (8).

In the present study, the positive correlation of PLR with BMD Z-score in children with hypothyroidism was also demonstrated ($r=0.575$, $P<0.001$). Platelets, in addition to their key role in blood coagulation, also participate in inflammatory processes and tissue repair. Studies have shown that platelets can affect bone metabolism through the release of growth factors such as PDGF and TGF-β (14). However, the study by Chen et al. (2023) showed that PLR had a negative relationship with lumbar spine BMD (5). This contradiction in the findings of different studies highlights the complexity of the relationship between inflammation and bone metabolism, which may be influenced by several modifying factors including age, sex, underlying disease, and body composition (15).

The positive correlations observed in our study, while statistically significant, should not be misinterpreted as evidence that inflammation is protective for bone. Rather, these correlations may reflect the unique immune-endocrine interactions in pediatric hypothyroidism, where the absence of thyroid hormone may alter immune cell populations in ways that are reflected in CBC-derived ratios but do not necessarily indicate the same inflammatory state as in other conditions.

The absence of a significant correlation between inflammatory indices and BMD in the group of children with hyperthyroidism is an interesting finding that aligns with the results of Bala and Bala (2022) (8). In the hyperthyroid state, the dominant mechanisms in bone metabolism include direct stimulation of osteoclasts by T3 and T4 and consequently increased bone resorption (3). In this condition, it seems that the direct effects of thyroid hormones on bone cells override the indirect effects of systemic inflammation reflected by NLR, PLR, and

MLR indices. In the control group, the lack of significant correlation also indicates that this association becomes more prominent in the presence of thyroid dysfunction.

The multiple regression results showed that the model overall explained 41.8% of the variance in BMD Z-score (R^2=0.418, P<0.001). After controlling for confounding variables, all three indices, NLR, PLR, and MLR, were significant independent predictors of BMD Z-score. MLR was the strongest independent predictor (Beta=0.357, P=0.002), followed by NLR (Beta=0.312, P=0.003) and PLR (Beta=0.228, P=0.048). This result aligns with the finding of Al Salmani et al. (2024), who showed that NLR had the highest predictive value for osteopenia among the investigated inflammatory indices, though our finding of MLR as the strongest predictor differs from their results, possibly due to differences in study population and disease context (16).

In the present study, the level of alkaline phosphatase, an indicator of osteoblast activity and thus bone turnover, showed a significant difference among the groups (P=0.018). This finding is consistent with the study by Bala and Bala (2022), which examined alkaline phosphatase levels in children with thyroid disorders (8). Serum calcium and phosphorus showed no significant differences among the three groups.

Novelty and significance of the study: To date, no domestic study has specifically investigated the relationship between NLR, PLR, and MLR indices with BMD in Iranian children and adolescents with thyroid disorders. This study is pioneering in this regard and, considering the potential genetic and nutritional differences of the Ardabil population from other populations, provides valuable local information.

The present study has several limitations that should be considered when interpreting the results. First, the retrospective cross-sectional nature of this study does not allow for establishing a causal relationship between inflammatory indices and BMD; only associations can be reported. The results should be interpreted as hypothesis-generating rather than confirmatory. Longitudinal studies with long-term follow-up are necessary to investigate causality. Second, the 6-month window for CBC measurement around the time of DXA (necessitated by the retrospective design) weakens the temporal interpretation; it is possible that the inflammatory state at the time of CBC differed from that at BMD measurement.

Third, the control group was not actively matched for important confounders such as pubertal status, vitamin D levels, calcium intake, or physical activity, which may have introduced selection bias. The source, selection criteria, and matching of controls were not adequately described, and future studies should employ more rigorous control selection and matching.

Fourth, the sample size, especially in the hyperthyroid group (n=30), was smaller than the hypothyroid group, which may have limited the statistical power to detect more subtle correlations. Fifth, the study population was limited to children who had undergone DXA as part of clinical care, which represents a selected subgroup and may not be representative of all children with thyroid disorders. We did not have data on the total number of eligible patients or the clinical indications for DXA, limiting assessment of selection bias.

Sixth, several important confounding factors for pediatric BMD, including height, pubertal stage, bone age, vitamin D status, calcium intake, physical activity, and disease duration, were either not reported or not adjusted for in the analyses. Seventh, although VIF values indicated no

significant multicollinearity among the inflammatory indices, they are derived from overlapping CBC components, which remains a conceptual concern. Eighth, no correction for multiple comparisons was applied despite numerous subgroup and correlation analyses, increasing the risk of Type I error.

Finally, the cross-sectional design and lack of medication adherence data (for patients on levothyroxine or antithyroid medications) may have affected the results. The study also did not assess the presence of thyroid autoantibodies or other markers of autoimmune activity.

To date, no domestic study has specifically investigated the relationship between NLR, PLR, and MLR indices with BMD in Iranian children and adolescents with thyroid disorders. This study is pioneering in this regard and, considering the potential genetic, nutritional, and environmental differences of the Ardabil population from other populations, provides valuable local information. However, given the limitations discussed, these findings should be considered preliminary and require confirmation in prospective, well-controlled studies.

5- CONCLUSION

The results of this study showed that children with hypothyroidism have lower bone mineral density compared to the control group, and the three inflammatory indices, NLR, PLR, and MLR, which can be calculated from a simple complete blood count, show a significant positive correlation with bone density Z-scores in this patient group. Among these indices, MLR had the strongest predictive power. In contrast, no such relationship was observed in the hyperthyroid and healthy control groups, indicating that this association is specific to the hypothyroid state.

However, the positive direction of the correlations observed in this study should be interpreted with caution. Rather than suggesting a protective effect of inflammation, these findings may reflect the unique immune-endocrine interactions in pediatric hypothyroidism, including relative lymphopenia and altered immune cell distribution. The association between systemic inflammation and reduced BMD in hypothyroid children is supported by the significantly lower BMD in this group, but the positive correlations with inflammatory markers require further investigation.

Given the cross-sectional design, causal relationships cannot be established. Therefore, inflammatory indices derived from CBC can be used as inexpensive, accessible, and non-invasive auxiliary tools for screening bone health status in children with hypothyroidism, but their clinical utility requires validation in prospective studies. Based on this, regular screening of bone density, especially in children who show high levels of these inflammatory markers, is recommended as part of comprehensive clinical evaluation, not as a standalone diagnostic tool. However, definitive confirmation of these findings and determination of causal relationships requires longitudinal studies with long-term follow-up and larger sample sizes, with careful control for confounding variables including pubertal status, vitamin D levels, and physical activity.

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