The Relationship between Neutrophil to Lymphocyte Ratio and Osteoporosis

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Abstract

Introduction: Osteoporosis is a chronic disease which commonly occurs in people after the age of 70 and it is important to identify the contributing factors. Therefore, this study aims to examine the relationship between neutrophil to lymphocyte ratio and osteoporosis.

Materials and methods: This study was conducted on 71 people (59.2%) with osteoporosis and 29 people (40.8%) without osteoporosis. Informed consent was obtained from the subjects and demographic data was recorded; neutrophil to lymphocyte ratio was also recorded. Chi square and Mann–Whitney U test were used to compare the data.

Findings: The findings of the study revealed that the average age of subjects with osteoporosis was significantly higher and the average height and weight of subjects with osteoporosis was significantly lower than subjects without osteoporosis. No statistically significant difference was

found between the two groups of study in terms of the mean neutrophil to lymphocyte ratio (NLR). NLR was not associated with spinal or femoral Osteoporosis severity either.

Conclusion: As there is no association between NLR and osteoporosis and the related severity, it seems that NLR has no effect on Pathophysiology of osteoporosis, though more studies are required to examine this relationship.

Keywords: neutrophil, lymphocyte, osteoporosis

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Introduction

Every year, many people in the world are affected by osteoporosis, for diagnosis of which bone density test is used. In Dual- energy x- ray absorptiometry (DEXA), bones of the spine and hips are scanned using x-ray to determine the bone density in terms of people's age and gender. Because of using x-ray, patients may face the risk of side effects of radiation. Further, DEXA is not an inexpensive method to measure bone density. Therefore, it is necessary to find a faster and cheaper way for diagnosis of this disease. Complete blood count (CBC diff) test is a simple, low-risk, available and inexpensive method. It is very important to find the prevalence of osteoporosis by analyzing the data obtained from (CBC diff) test which neutrophil to lymphocyte ratio was used in this study. Osteoporosis is the most common metabolic bone disease which affects many people in the world. It is an important risk factor for pathologic fractures which imposes mortality, morbidity and extra costs on human society. Several epidemiological studies have been conducted on osteoporosis around the world and it is estimated that around 75 million people in Europe, Japan and US have osteoporosis. For example, 30% of American women in postmenopausal age, 87% of Australian women over 70 years of age and 50% of Taiwan women have osteoporosis. Moreover, bone mineral density is lower in Asians and Caucasians compared with other races. This can be explained by the smaller size of these people which causes most hip fractures in Asia so that 30% of all these fractures occur in Asia. Osteoporosis is a common health issue that mostly threatens postmenopausal women, making them prone to fractures and these fractures are associated with increased morbidity and mortality. It is the most common type of bone disease in humans- a kind of skeletal disease characterized by decreased strength of bones which increases the risk of fractures. Bone strength depends on several bone qualities among which only Bone Mineral Density (BMD) can be measured while other qualities (such as the size of hydroxyapatite crystals, Correlation of trabeculae, degree of mineralization, small bone destructions, …) are not measurable. Bone fractures and the related consequences are the clinical result of osteoporosis. Although the most common sites for bone fractures are proximal femur, vertebrae and distal end of forearm bones (wrist), since osteoporosis is a diffuse bone disease and causes reduced bone mass throughout the skeletal system, almost all fractures in older people result from decrease in bone density. Fractures may be followed by full recovery or result in chronic pain, physical disability or

even death (30-32). The most serious complication of osteoporosis is femoral fractures which may lead to 10-20% increased mortality rate within one year after the fracture. Besides, about 25% of patients with femoral fracture may need long-term care in nursing homes and only 30% of patients may recover their pre-fracture level of ability (33).

Estrogen deficiency not only speeds up bone loss in postmenopausal women, it also contributes to bone loss in men. It may lead to excessive bone resorption which is associated with insufficient bone formation. Estrogen deficiency increases the number of osteoclasts and reduces the number of osteoblasts which will result in overall bone mass reduction. It is noteworthy that Estrogen levels are inversely proportional to fracture risk in postmenopausal women (9). Osteoblasts, osteocytes and osteoclasts all express estrogen receptors. Moreover, estrogen affects bones indirectly through cytokines and local growth factors. The estrogen-replete state may enhance osteoclast apoptosis through increased production of transforming growth factor (TGF)–beta.

Estrogen deficiency increases while estrogen therapy reduces the rate of bone remodeling and the rate of bone loss during the remodeling cycle. In the absence of estrogen, T cells promote osteoclast recruitment, differentiation, and long-term survival through interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF)-alpha. A mouse study, in which mice were either ovariectomized or sham-operated, showed that IL-6 and CFU-GM (granulocyte macrophage colony-forming unit) levels were significantly higher in the ovariectomized mice (10). This finding proves that estrogen inhibits the secretion of IL-6, and IL-6 helps the recruitment of osteoclasts from the monocyte cell line, thereby contributing to the development of osteoporosis. The term osteoimmunology is defined as the interplay between the skeletal system and immune system. Osteoclastogenic pro-inflammatory cytokines, especially TNF, IL-1, IL-6 or IL-7, increase in the first ten years in postmenopausal osteoporosis patients. It is noteworthy that T cells play a role in Crohn's and rheumatoid arthritis which are inflammatory conditions that cause osteoporosis; it was also found that they produce more TNF in postmenopausal women with osteoporotic fractures (11).

Recently, a mechanism was proposed whereby loss of estrogen initiates rapid bone loss by activating low-grade inflammation, leading to osteoporosis in the acute phase of bone catabolic activity in ovariectomized rats (11). It was found that ovariectomy in mice increases dendritic cells that express IL-7 and IL-15 and induce antigen-independent production of IL-17A and TNFα in a subset of memory T cells. This study showed that ovariectomy using IL15RA in the mice do not cause lack of expression of IL-17A and TNFα, nor do they increase bone resorption or bone loss (12). Calcium, vitamin D and PTH help maintain bone homeostasis. Dietary calcium deficiency or impaired intestinal absorption of calcium because of aging or disease can lead to secondary hyperparathyroidism. PTH is secreted in response to low serum calcium levels. It increases calcium absorption from bone, decreases calcium excretion, and increases renal production of 1,25-dihydroxyvitamin D (1,25[OH]2D). 1,25[OH]2D is a hormonally active form of vitamin D that optimizes calcium and phosphorus absorption and inhibits PTH (49-51).

It is estimated that osteoporosis will affect 200 million women worldwide (114) and 1 in 3 women over age 50 will experience osteoporosis (115, 116). According to Examination survey (KNHANES) 2008-2011, the prevalence of osteoporosis was 7.0% in men and 40.1% in women (117). Fractures caused by osteoporosis are difficult to treat and may lead to great economic losses (118). By restricting his movement and developing pressure ulcers, a patient may experience cardiopulmonary dysfunction and venous thrombosis which will add to the problem (119). Therefore, Prediction and, if possible, prevention of osteoporosis is of considerable importance.

The inflammatory response protects the injured area and maintains homeostasis (120). However, continuous response and long-term release of inflammatory mediators can cause chronic inflammatory diseases (121-123). Chronic inflammation can be found in vascular diseases as well and is considered as a marker to predict their development (124, 125). Moreover, Osteogenic factors are released during chronic inflammation which may cause the development of osteoporosis (42, 126).

As with inflammatory measures, white blood cell (WBC) count, C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR) are useful in clinical practice, but have low diagnostic specificity (127). Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have recently been recognized as new markers of systemic inflammatory response (1, 128). More particularly, NLRs are predictors of an inflammatory response in the vascular system, and in this sense, it is more accurate than the CRP level, predicting myocardial infarction and mortality (1.2). Further, some researchers have reported that NLR is associated with BMD and osteoporosis (3, 4). However, the relationship between NLR and osteoporosis has yielded conflicting results. All this said, to the best of our knowledge, no studies have been conducted on the relationship between NLR and osteoporosis in the Iranian population. Therefore, we decided to study the relationship between NLR and BMD and examined the relationship between neutrophil to lymphocyte ratio and osteoporosis.

1- Correlation between NLR and age
2- Correlation between NLR and gender
3- Correlation between NLR and T score
4- Correlation between NLR and Z score
5- Correlation between NLR and osteoporosis in lumbar vertebrae
6- Correlation between NLR and osteoporosis in femoral neck

The researcher will prove that neutrophil to lymphocyte ratio is associated with osteoporosis and its severity. This association is found by dividing the number of neutrophils by the number of lymphocytes per cubic millimeter of blood using the complete blood cell count test.

Literature Review

In a study conducted by Zeynel AbidinÖztürk et al. (2013) with the aim of investigating NLR in the elderly with osteoporosis, a total of 1635 patients aged 65 years or older were included in this cross-sectional study. BMD was measured using dual energy X-ray absorptiometry (DEXA) at lumbar spine and femur. Complete blood count (CBC), inflammatory biomarkers (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)), glucose/lipid metabolism, and specific risk factors were determined. The osteoporosis group demonstrated higher NLR levels compared to the osteopenic and control groups (respectively 2.54 ± 1.45, 2.37 ± 1.00, and 2.18 ± 0.85). At multivariate analysis, NLR emerged as independent predictor of osteoporosis (OR=1.112; 95%=1.020-1.235, p=0.018) and there was a significant negative correlation between lumbar spine (L2-L4), femoral neck scores and NLR (r=R). (Respectively, r=0.348, p<0.001; r=0.264, p=0.004). Elderly people with osteoporosis have elevated NLR levels, which prove that inflammation may play an important role in bone remodeling (5).

In a study conducted by Francesca Palmacci et al. (2019), with the aim of investigating the relationship between NLR and osteoporosis in patients with celiac disease, adults who were on a strict GFD for at least 6 months were included in the study. Mediterranean diet adherence was measured according to two different scores: The Mediterranean Diet Score (MDS-14), which was assessed through the validated 14-item questionnaire of the PREDIMED study and the MEDScore (score-55), proposed by Panagiotakos. The latter includes the consumption of unrefined cereals. High percentages of osteopenia and osteoporosis were found in subjects who reported bone mineral density (BMD), especially in postmenopausal (post-M) women. NLR was higher in people with osteoporosis compared to subjects with osteopenia and normal BMD. However, retrospective analysis showed both increase and decrease in NLR after GFD, with no significant differences between Marsh grade, anemia, and BMD status. Moreover, previous pregnancy before menopause (Pre-MPP) and post- M showed higher NLR at diagnosis compared to men and premenopausal (Pre-M); but higher differences in recent NLR were observed only between Pre-MPP and men. Chocolate consumption was associated with lower recent NLR. Large prospective studies are needed to clarify the relationship between unrefined cereals and NLR in coeliac patients; in this pilot study, the relationship between NLR, dietary habit, and osteoporosis in coeliac disease was investigated for the first time (6).

In a study conducted by Małgorzata Morawiecka-Pietrzak et al. (2021) with the aim of investigating the relationship between NLR and osteoporosis in patients with anorexia nervosa (AN), electronic records of 73 girls admitted for AN were analyzed retrospectively. The age range of the study group was 12.56-17.67 years. BMD was assessed by dual-energy X-ray absorptiometry (DXA) and expressed as Z-scores according to lumbar spine (s-BMD) and total body (TB-BMD) sites. NLR and PLR were calculated according to complete blood count results. Patients were divided into 2 subgroups with parallel analyses — according to the TB-BMD criterion and the s-BMD criterion: normal (Z-score > –2.0, n = 63) and low s-BMD subgroup (Z-score ≤ –2.0, n =

10), and normal (Z-score > –2.0, n = 45) and low TB-BMD subgroup (Z-score ≤ –2.0, n = 28). In the low s-BMD subgroup a tendency to an increase of mean NLR, PLR, and WBC values was observed. Respective BMD Z-score values correlated significantly and negatively with PLR in the low s-BMD (R = –0.892, p < 0.001) and normal TB-BMD (R = –0.451, p = 0.002) subgroups, while with NLR only in the normal TB-BMD subgroup (R = –0.685, p < 0.001). In the low s-BMD subgroup the PLR was shown to be a significant and independent predictor of s-BMD (β = –0.881, p < 0.001). The PLR contributed to 77.6% of the s-BMD Z-score variability (R2 = 0.776, p < 0.001). In the normal TB-BMD subgroup, the PLR and NLR levels were significant and independent predictors of TB-BMD (β = –0.352, p = 0.004; β = –0.450, p = 0.001; β = –0.339, p = 0.005, respectively). These results show that there might be a relationship between bone mass loss and inflammation expressed as NLR and PLR in adolescent girls suffering from AN. It seems that NLR and PLR, which are common indicators of morbidity and mortality in many malignancies and inflammatory chronic diseases, can also be useful in the evaluation of bone condition in adolescent females with AN. However, more investigation is required in this field (7).

Discussion

Subjects of the study included patients who referred to Baghiatallah Clinic in Qom during 2022-2023.

To determine the sample size, the formula for determining the sample size for the correlation coefficient was used. Considering the similar article (23), there was an inverse and significant relationship between NLR and BMD (R=0.685, P<0.001); according to the 95% confidence level and 80% study power, at least 60 people were assessed using the following formula and In order to augment the accuracy of the study and the small probability of 15%, this study was conducted on 71 people.

$$\frac{(Z1-\frac{\alpha}{2}+Z1-\beta)^2}{(\frac{1}{2}\log\frac{1+r}{1-r})^2}+3$$

$z1-\alpha/2=1.96$

$z1-\beta=0.84$

$r=0.685$

n=60

Materials and methods

The study is a case-control study. Subjects were divided into two groups: a group of people with osteoporosis and the other group of people without osteoporosis. The blood cell count test was carried out in both groups. Then, neutrophils to lymphocytes ratio was determined and its relationship with osteoporosis was investigated. Simple Probability Sampling was used.

Instruments and determining the validity and reliability

Inclusion criteria for participation in the study

- patients diagnosed with osteoporosis who referred to Baghiatallah Clinic in Qom during 2022-2023
- informed consent to participate in the study
- Patient cooperation in completing the questionnaire

Exclusion criteria for participation in the study

- Incomplete questionnaire data
- History of hematological diseases
- Lack of consent and failing to cooperate at any stage of the study
- Infectious disease in the last 7 days
- Active cancer
- Use of Corticosteroids in the last 14 days
- Receiving chemotherapy or immunosuppressive drugs within the last 29 days
- Liver or kidney failure
- Kidney or liver transplant recipients
- Lack of access to data and sample tests

Data Analysis

For data analysis, first, normality of the data was checked using the one-sample Kolmogorov Smirnov test under the term Lilliefors; upon confirming the normality, proper parametric tests such as Student T Test and variance analysis were used; if the data was not normal, Kruskal-Wallis and Mann-Whitney tests were used. Chi-square test and Fisher's exact test were used for qualitative variables. The software used in this study is IBM SPSS v.20 and the significance level of the tests was considered less than 5%.

Results and Findings

This study was conducted with the aim of examining the relationship between neutrophil to lymphocyte ratio and osteoporosis. 71 people, 42 people (59.2%) with osteoporosis and 29 people (40.8%) without osteoporosis, were examined. The average age of the study subjects was 58.9 ± 11.53 years (23 to 82 years) and it was found that 67 (94.4%) were women and 4 (5.6%) were men. Findings of the study are reported below.

Table (4-1): Mean age and standard deviation in the subjects of the study groups

	group	number	mean	Standard deviation	P-value
Age (year)	Without osteoporosis	29	53.52	12.141	0.001
	With osteoporosis	42	62.62	9.581	

Mean age in patients with osteoporosis was significantly higher than in subjects without osteoporosis (62.62 vs. 53.52 years) (p=0.001).

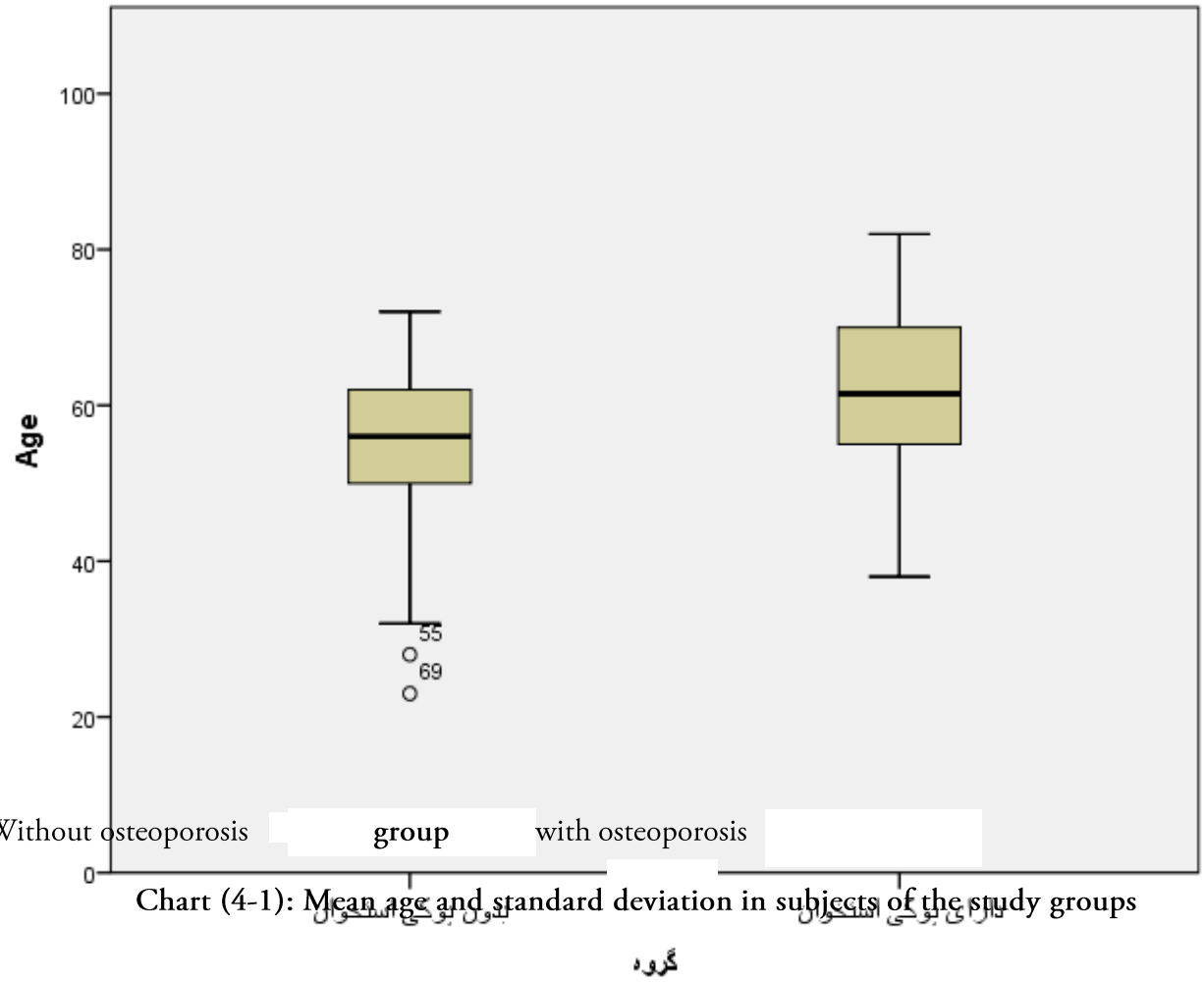


Chart (4-1): Mean age and standard deviation in subjects of the study groups

Table (4-2): frequency of gender in the subjects of the study groups

Group / Gender		Without osteoporosis	With osteoporosis	total	P-value
male	number	2	2	4	0.701
	percentage	6.9%	4.8%	5.6%	
female	number	27	40	67	
	percentage	93.1%	95.2%	94.4%	
total	number	29	42	71	
	percentage	100%	100%	100%	

There was no statistically significant difference in the frequency of gender in the subjects of the two study groups (P>0.05).

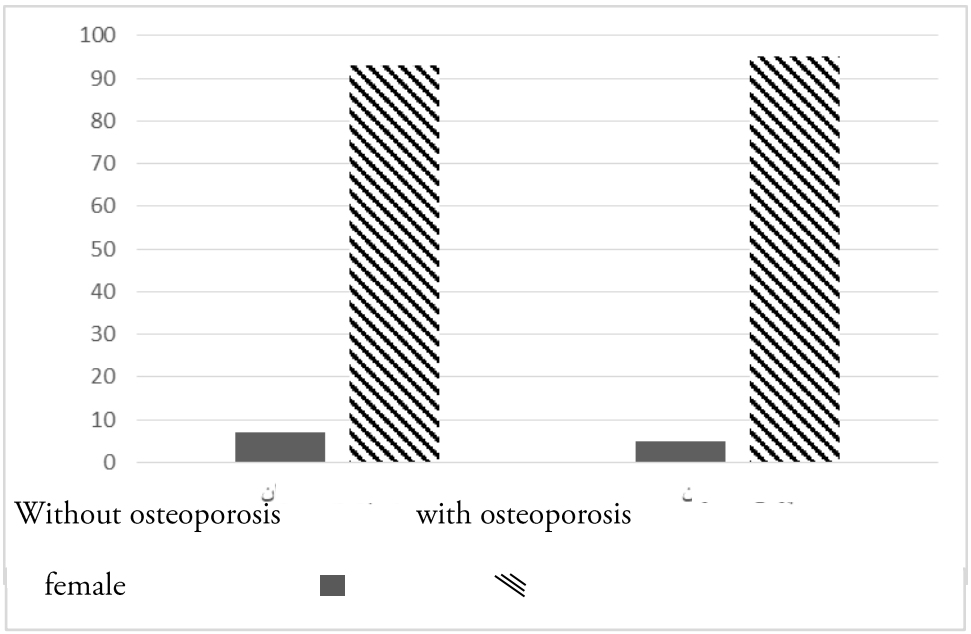
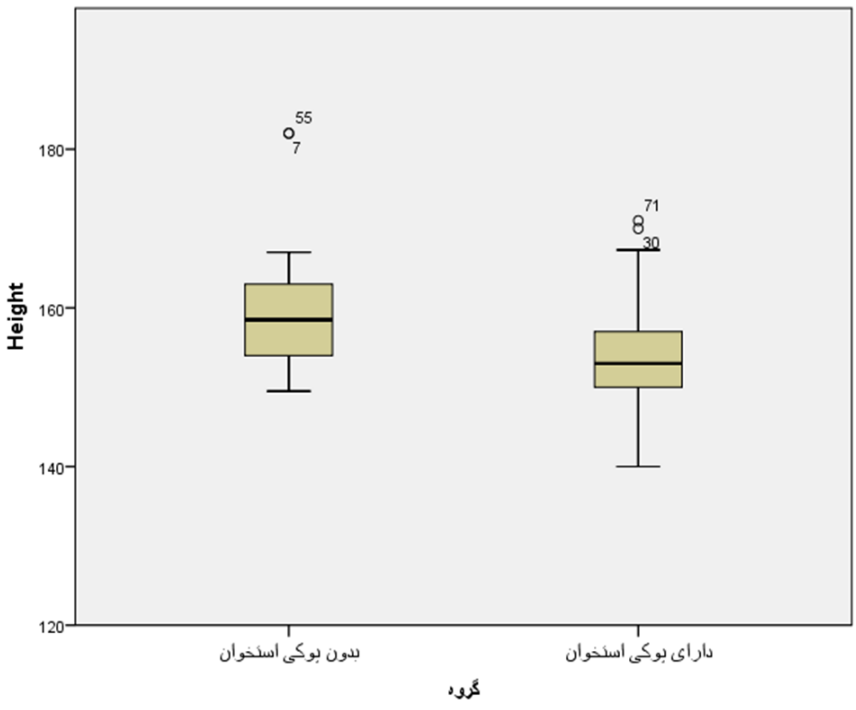


Table (4-3): Mean height, weight and BMI and standard deviation in the subjects of the study groups

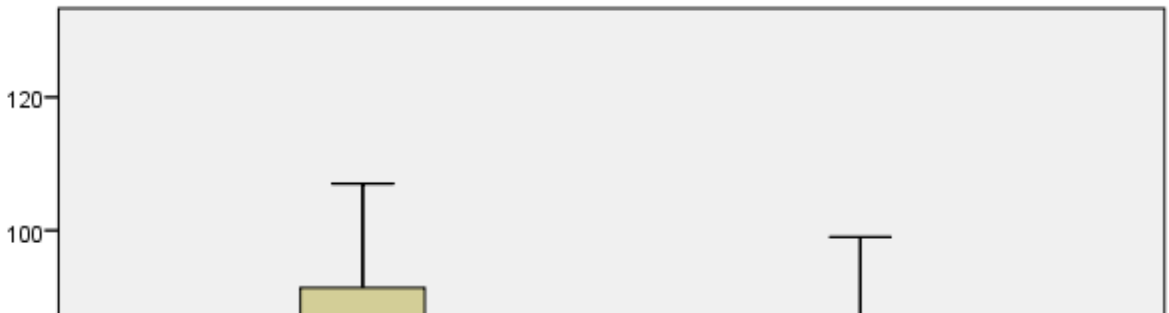
	group	number	mean	SD	P-value
height (cm)	Without osteoporosis	29	159.87	7.621	0.001
	with osteoporosis	42	154.05	6.475	
weight (kg)	Without osteoporosis	29	79.33	13.917	0.002
	with osteoporosis	42	68.78	12.830	
BMI	Without osteoporosis	29	31.10	5.362	0.092
	with osteoporosis	42	28.95	4.982	

The mean height in subjects with osteoporosis was significantly lower than in patients without osteoporosis (159.87 versus 154.05 cm) (P=0.001). it was also found that the mean weight in people with osteoporosis was significantly lower than in patients without osteoporosis (79.33 vs. 68.78 kg) (P=0.002). However, no statistically significant difference was observed based on BMI (P=0.092).



Without osteoporosis **group** with osteoporosis

Chart (4-3): Mean height and SD in the subjects of the study groups

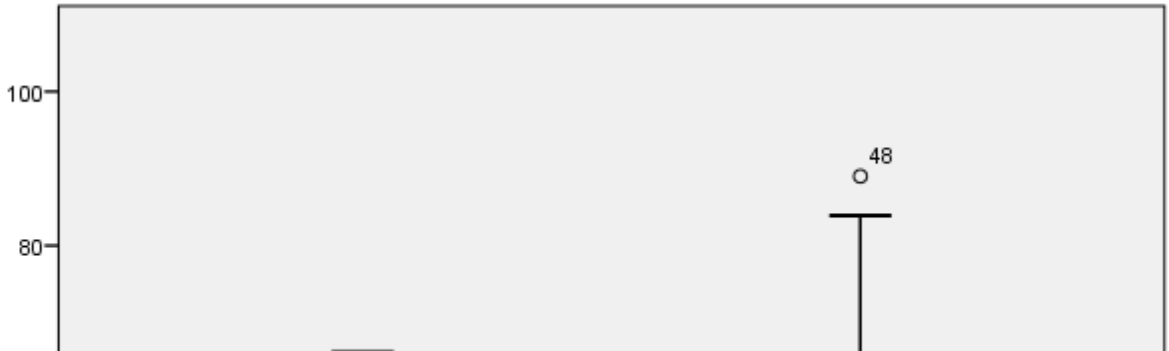


Without osteoporosis **group** with osteoporosis

Table (4-4): Mean neutrophil, lymphocyte and NLR ratio and SD in the subjects of the study groups

	group	Number	mean	SD	P-value
neutrophil	Without osteoporosis	29	51.05	8.930	0.068
	with osteoporosis	42	55.65	11.938	
lymphocyte	Without osteoporosis	29	36.68	9.689	0.291
	with osteoporosis	42	33.81	10.372	
NLR	Without osteoporosis	29	1.58	0.785	0.531
	with osteoporosis	42	2.10	1.724	

There was no statistically significant difference in the mean neutrophil, lymphocyte and NLR ratio in the subjects of the study groups (P>0.05).



Without osteoporosis **group** with osteoporosis

Table (4-7): Mean and SD of NLR ratio in the subjects of the study groups based on vertebral column T-Score

	group	number	mean	SD	P-value
NLR	normal	27	1.55	0.808	0.111
	osteopenia	11	2.63	2.887	
	osteoporosis	33	1.91	1.037	

There was no statistically significant difference in the mean NLR ratio of the subjects based on vertebral column T-Score (P>0.05).

Conclusion

This study aimed to examine the relationship between neutrophil to lymphocyte ratio and osteoporosis. 71 subjects, 42 people (59.2%) with osteoporosis and 29 people (40.8%) without

osteoporosis, were examined. The mean age in the subjects with osteoporosis was significantly higher than in the patients without osteoporosis (62/62 vs. 52/53). It was also found that the mean height in the subjects with osteoporosis was significantly lower than in patients without osteoporosis (159.87 vs. 154.05 cm). Similarly, the mean weight in the subjects with osteoporosis was significantly lower than in patients without osteoporosis (79.33 vs. 68.78 kg). However, no significant statistical difference was found based on BMI. It was finally determined that there was no statistically significant difference in the mean neutrophil, lymphocyte and NLR ratio in the subjects of the study groups; there was no association with spinal and femoral osteoporosis severity either.

The findings of the study showed that the mean age in subjects with osteoporosis was significantly higher and the mean height and weight in subjects with osteoporosis was significantly lower than in patients without osteoporosis; however, no statistically significant difference was found in the mean neutrophil, lymphocyte and NLR ratio in the subjects of the study groups; there was no association with spinal and femoral osteoporosis severity either. Therefore, as there is no relationship between NLR and osteoporosis and its severity, it seems that NLR has no effect on the Pathophysiology of osteoporosis, although more studies are required to investigate the relationship.

Pedagogical Implication

Lack of sample size, which requires a more detailed investigation with further expansion in subsequent studies, as well as the impossibility of investigating other important variables, including other laboratory findings, clinical findings, etc., together with osteoporosis, which requires further studies with a higher sample size in future.

Ethical Code

This study was presented in the Research Ethics Committee at the Faculty of Medicine and was registered with a particular code. The patient information is kept confidential and the checklist has a code which was delivered to them if needed. This study was conducted based on the clarifying statement included in the Declaration of Helsinki and no coercion was imposed on individuals. After receiving an informed consent, patients participated in the study and they were ensured that their personal information would not be disclosed. The Declaration of Helsinki and ethical principles were followed all through this study.

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