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Blood Analysis of Patients with Rheumatoid Disease from Kosovo Using Non-Steroidal Anti-Inflammatory Drug – Aspirin

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BALKAN JOURNALS

Abstract

Bone tissue, one of the body's most intricate structures, has a unique capacity for spontaneous regeneration and repair under optimal physiological conditions. Aspirin, a widely used non-steroidal anti-inflammatory drug (NSAID) recognized for its anticoagulant properties, plays a role in bone regeneration by activating cytokines and mediators within osteoclasts, osteoblasts, and progenitor cells at sites of damage. This activation promotes bone repair and stimulates angiogenesis by enhancing endothelial cell migration, which is essential for the formation of new blood vessels necessary for fracture healing. This study analyzed blood samples from 100 Kosovo patients (57 female, 43 male; ages 42–89) with rheumatic osteoporosis who regularly use aspirin. Key biomarkers measured included vitamin D, serum calcium, serum iron, sedimentation rate, glucose, triglycerides, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Findings indicated that 37% of these osteoporosis patients exhibited elevated triglyceride levels (>1.7 mmol/L), while nearly 40% had cholesterol levels exceeding 5 mmol/L. Additionally, AST and ALT levels were elevated (>40 IU/L) in 30% of patients, with aspirin users generally showing statistically significant increases across these blood parameters. Body mass index showed no statistically significant correlation with any blood parameter values. However, increasing patient age correlated with reduced vitamin D levels and elevated triglycerides, cholesterol, sedimentation rate, and glucose levels. Understanding the link between diet and osteoporosis is critical for healthcare professionals, including doctors, nutritionists, and researchers, as it supports efforts to prevent and manage osteoporosis. Given this, synthesizing current evidence and conducting further studies on the diet-osteoporosis interaction is essential.

Keywords: Aspirin, Blood samples, Determination, Bone tissue, Rheumatoid disease

Introduction

Bone Tissue

Bone tissue's composition, comprising diverse cell types, proteins, blood, nerve vessels, and a mineral matrix, enables a process called bone metabolism. Bone remodeling, an ongoing metabolic process, involves osteoclasts and osteoblasts working in tandem at the same site: osteoclasts resorb aged bone while osteoblasts deposit new bone. During periods like puberty and adolescence, bone formation surpasses resorption. Around age 30, these processes equalize, and by age 50, resorption typically begins to outpace formation, a shift that is part of the normal biological process. Any imbalance between these activities can lead to bone metabolic disorders (Apostolova, 2014).

Structure of Bone Tissue

Bone tissue is a complex, dynamic, and mineralized structure with three primary functions: providing structural support, protecting internal organs, and maintaining mineral homeostasis. It consists of three cell types—osteoblasts, osteocytes, and osteoclasts—and an extracellular matrix divided into an organic component (mainly collagen fibers) and an inorganic component. This inorganic matrix, constituting 65% of the total matrix, is predominantly composed of calcium and phosphorus complexes in the form of apatite crystals, with a chemical formula of $[Ca_{10}(PO_4)_6(OH)_2]$ (Figure 1) (Hadjidakis & Androulakis, 2006; Abrahamsen, 2000). Calcium phosphate apatite is a hard, water-insoluble salt essential for bone and teeth formation (Kini, 2012).

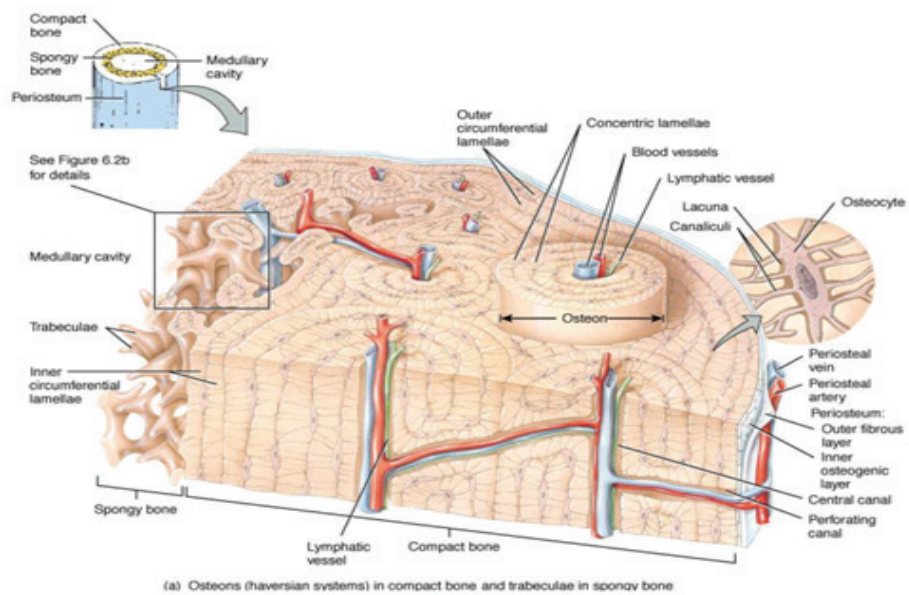
Bone formation occurs through two processes: intramembranous ossification, which forms the facial skeleton and parts of the mandible, and endochondral ossification, responsible for the cranial base and other mandible areas (Sandor, 2003; Ide, 2007).

Osteoblasts are mononucleated, metabolically active cells responsible for synthesizing osteoid, a non-mineralized organic matrix comprised of 90% type 1 collagen and 10% non-collagenous proteins, including osteocalcin, osteopontin, fibronectin, and alkaline phosphatase. Osteoid mineralization with inorganic salts provides bone with its characteristic strength and hardness (Kalfas, 2001). Osteocytes, terminally differentiated osteoblasts, are embedded in lacunae within the bone matrix and help regulate extracellular calcium and phosphorus levels. Both osteoblasts and osteocytes derive from mesenchymal stem cells known as osteoprogenitor cells, found in the periosteum and bone marrow (Downey, 2006).

Osteoclasts, large multinucleated cells that originate from hematopoietic stem cells rather than osteoprogenitors, are responsible for bone resorption. Located in Howship's lacunae, these cells produce hydrolytic enzymes that demineralize the bone matrix by breaking down proteins and releasing minerals, which reduces bone density and strength. Bone resorption can be physiological (age-related) or pathological (from trauma or infection) (Prolo, 1990).

Figure 1

Structure of Bone



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Source: Ashammakhi & Ferretti, 2003.

Bone tissue consists of various cell types, proteins, blood and nerve vessels, and a mineralized matrix that collectively support bone metabolism, a process governed by multiple factors. Bone cells play a fundamental role in metabolism, with their activity levels influenced by age, circulating bone-regulating hormones, calcium and vitamin intake, and numerous risk factors associated with osteoporosis.

Calcium, the most abundant mineral in the body (approximately 1 kg), is predominantly stored in bones as calcium phosphate salts, accounting for 99% of its total. The extracellular fluid contains around 22.5 mmol/L of calcium, with roughly 9.0 mmol in serum. Serum calcium is more precisely classified as normal total calci-

um (2.2–2.6 mmol/L) and normal ionized calcium (1.1–1.4 mmol/L) (Campos et al., 2000). Parathyroid hormone (PTH), vitamin D, and calcitonin are key regulators of mineral metabolism. PTH, secreted by the parathyroid glands, functions to raise extracellular calcium levels by promoting bone resorption and enhancing renal calcium reabsorption (Suzuki et al., 2000). Vitamin D enhances intestinal calcium absorption and accelerates its active transport, thereby regulating calcium homeostasis. While calcidiol (25-OH D3) is biologically inactive, its active form, calcitriol (1α,25 OH_2 D_3), raises serum calcium through increased gastrointestinal absorption and renal reabsorption, reducing urinary calcium loss and stimulating bone calcium release via osteoclast activation.

Calcitonin, produced by the thyroid's parafollicular cells in response to elevated plasma calcium, decreases serum calcium by inhibiting osteoclast-mediated resorption and promoting urinary calcium excretion. Bone remodeling, an ongoing metabolic process, involves osteoclasts resorbing aged bone as osteoblasts form new bone. Imbalance in these processes can result in metabolic bone disorders (Mechevska Jovchevska, 2010; Adami, 2009). This balance can be assessed through circulating protein biomarkers in serum and urine, divided into formation and resorption markers (Blasiak, 1989). Osteocalcin (OS), a commonly analyzed formation marker, is a specific non-collagen protein produced by osteoblasts and is crucial to osteoblast function (Hofbauer et al., 2007; Bouillon et al., 1995). Low osteocalcin levels indicate reduced osteoblast activity (Campos et al., 2000; Brandao et al., 2007). Among resorption markers, β-CrossLaps (CTX) is widely analyzed due to its sensitivity and stability in serum and urine. Collagen cross-links, released from the bone matrix during resorption (at the onset of type 1 collagen degradation), are detectable through specific assays (Van Coeverden et al., 2002; Brandi, 2010).

Aspirin

Aspirin serves as the prototype for non-steroidal anti-inflammatory drugs (NSAIDs) due to its established antipyretic, analgesic, and anti-inflammatory properties. It achieves its effects by inhibiting all cyclooxygenase (COX) isoforms, forming an irreversible covalent bond with the serine 530 hydroxyl group (acetylation) that blocks arachidonic acid from accessing the enzyme (Fuster, 2011; Paez Espinosa, 2012). However, due to gastrointestinal bleeding risks, aspirin has largely been replaced by selective COX-2 inhibitors for managing fever, pain, and inflammation (Furst, 2012). Currently, it is often prescribed in low doses for its antiplatelet effects, commonly used to reduce the risk of cardiovascular events in high-risk

individuals (Bibbins-Domingo, 2016; Dehmer, 2016). Additionally, some studies suggest that low-dose aspirin might lower the risk of colorectal cancer. Data from the 2010 National Health Interview Survey in the United States indicated that, among 27,157 respondents aged 18 and older, 19% were regular aspirin users (defined as usage at least three times a week for over three months). This represented a 57% increase from 2005, possibly due to aspirin's reported cardiovascular benefits (Suda, 2004).

The widespread use of aspirin, particularly in older adults susceptible to bone mass loss, raises questions about its effects on bone health, especially given its influence on cyclooxygenase activity, which impacts prostaglandin E2 (PGE2), a regulator of bone metabolism. Osteoporosis, a common metabolic skeletal disorder in older adults, results from an imbalance in bone remodeling, where resorption surpasses formation, thereby elevating fracture risk and associated morbidity and mortality (Papaioannou, 2009; Feng, 2011; Ekegren, 2016; Lee, 2016). Since aspirin is frequently used for disease prevention in this age group, understanding its effects on bone health and on the biochemical and hematological parameters of the blood is increasingly essential.

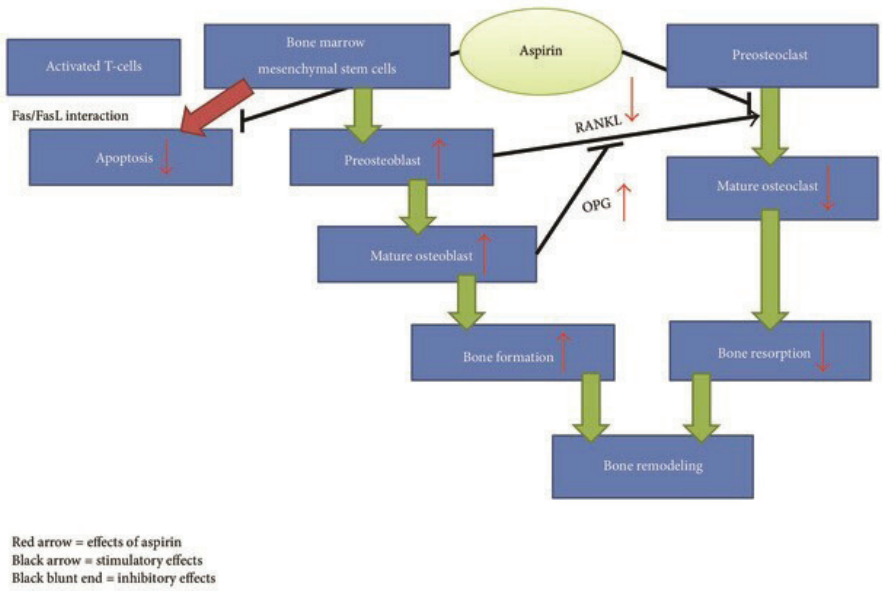
Effects of Aspirin on Bone Cells

Aspirin, depending on its dosage, significantly reduces the formation of tartrate-resistant acid phosphatase (TRAP)-positive cells derived from the RAW 264.7 macrophage cell line, as well as the expression of mRNA in key osteoclast markers: TRAP, cathepsin K (CTSK), matrix metalloproteinase 9 (MMP-9), and the calcitonin receptor (CTR). These inhibitory effects on osteoclast-like cells are mediated through the nuclear factor kappa-B (NFκB) signaling pathway (Zeng, 2016). NFκB, a transcription factor essential for synthesizing inflammatory cytokines, is activated via degradation of its natural inhibitor, NF-kappa-B inhibitor alpha (IKBα), followed by its nuclear translocation, where it initiates target gene transcription (Tak et al., 2001, Lawrence, 2009). Aspirin suppresses both the phosphorylation and degradation of IKBα, along with phosphorylation of the p50/p65 subunits and related signaling molecules such as ERK, p38, and JNK. Furthermore, the nuclear translocation of p65 is inhibited when RAW 264.7 cells are incubated with aspirin (Zeng, 2016).

In summary, aspirin may prevent osteoclastogenesis by inhibiting the NFκB pathway and may also enhance osteoblastogenesis by promoting the survival of progenitor stem cells and supporting preosteoblast differentiation (Fig. 2).

Figure 2

Effects of Aspirin on Bone Cells.



This doctoral project aims to examine blood parameters in patients from Kosovo with rheumatoid disease who are undergoing treatment with the non-steroidal anti-inflammatory drug (NSAID) aspirin. By analyzing these patients' blood samples, the study intends to gather data that can inform specific dietary and supplementation recommendations (Zhubi, 2024), potentially slowing disease progression and alleviating pain symptoms.

Literature Review

Link Between Aspirin Use and Bone Health in Epidemiological Studies

In a multicenter study involving 7,786 Caucasian women aged 65 and older, researchers found that bone mineral density (BMD) in the hip and spine was higher among those using aspirin 5-7 times per week compared to non-users. Additionally, women using aspirin for over a year exhibited higher BMD in both regions. However, this use did not correlate with an increased risk of hip fractures over

four years [relative risk ratio: 1.1 (95% CI: 0.7-1.6)] or any non-vertebral fractures [relative risk ratio: 1.0 (95% CI: 0.8-1.2)]. Noteworthy for its large sample size and adjustment for patient characteristics, the study recognized osteoarthritis as a factor that could enhance BMD. Nevertheless, the reliance on self-reported aspirin use may introduce bias, and the findings may not generalize to other populations as the cohort consisted solely of Caucasian women (Bauer et al., 1996).

Similarly, a Danish investigation focused on osteoporosis prevention included 2,016 women aged 45-58 years and discovered no significant differences in total body, lumbar spine, total hip, femoral neck, and distal forearm BMD between aspirin users and non-users. Although aspirin users initially showed a lower unadjusted decline in spinal BMD, this significance diminished after accounting for multiple factors. A ten-year follow-up indicated that aspirin use did not influence fracture risk [hazard ratio: 0.94 (95% CI: 0.66-1.33)]. While the study was extensive and featured a long follow-up, the homogeneity of the sample limited the applicability of the results (Vestergaard et al., 2012).

In the Health, Aging, and Body Composition study, which included 2,853 participants (50.5% men, 49.5% women; 43.1% African Americans, 56.9% Caucasians) with a mean age of 73.6 years, results showed that total body BMD was significantly higher among subjects using only aspirin and those combining aspirin with selective COX-2 NSAIDs. Additionally, participants using either aspirin or both types of NSAIDs exhibited increased cortical and trabecular BMD, as measured by quantitative computed tomography. For those on medication for at least one year, total body BMD was notably higher among aspirin users with selective COX-2 NSAIDs, while hip BMD was greater among those using aspirin with selective COX-1 NSAIDs (Carbone et al., 2003). Despite its strengths, this cross-sectional study could not establish causality, nor did it address fracture risk as an endpoint.

A case-control study conducted by Vestergaard et al. involving 124,655 cases and 373,962 controls found that the risk of any fractures decreased with low-dose aspirin usage [odds ratio: 0.93 (95% CI: 0.91-0.96)]. In the adjusted model, taking more than one defined daily dose (DDD) per day was linked to an increased risk of fractures [odds ratio: 1.17 (95% CI: 1.02-1.34)] [38]. Low doses of aspirin, at or below 0.5 DDD/day [odds ratio: 1.10 (95% CI: 1.01-1.20)] and between 0.51 and 1 DDD/day [odds ratio: 1.17 (95% CI: 1.08-1.27)], were also associated with hip fractures. The limitations of this case-control study included the inability to account for confounding factors like medication adherence.

Overall, human epidemiological studies indicate a slight positive impact of aspirin on BMD, while its effects on fracture risk vary from null to increased risk. Since all studies mentioned are observational, their findings primarily generate hypotheses. Given the potential minor effects and adverse outcomes of aspirin, conducting randomized controlled trials to assess the influence of aspirin on BMD and fracture risk poses challenges. More extensive prospective studies are necessary to substantiate aspirin's effects on bone health.

Methods and Materials

This doctoral project employed a previously developed Dietary Supplement Questionnaire to assess a sample of 100 patients (57 females and 43 males, aged 42-89 years) from Kosovo diagnosed with rheumatoid disease—osteoporosis. The questionnaire primarily addressed educational level, employment status, family history of osteoporosis, and place of residence. Patients were classified based on their body mass index (BMI). Additionally, data on smoking habits and the consumption of oral anticoagulants, particularly aspirin, were included as important variables for the study.

The questionnaire also explored the use of dietary supplements (DS) and the specific types consumed by participants, as well as their intake of various vitamins and calcium from diverse dietary sources. Notably, some respondents were also COVID-19 positive (Zhubi, 2024).

Alongside the survey, a blood analysis was conducted on the osteoporosis patients included in the study, measuring nine parameters, as presented in Table 1, along with their reference values. All samples were collected following the approval of the institutional ethics committee and with the informed consent of the patients. Blood samples were drawn into heparinized tubes, and the analysis of blood parameters occurred between January and June 2024, with participation being voluntary.

The study evaluated vitamin D levels, serum calcium, serum iron, sedimentation rate, glucose, triglycerides, cholesterol, AST, and ALT. The primary goal was to analyze the blood test results to determine whether the values fell within the reference range or exhibited deviations. Furthermore, the study aimed to assess significant differences in the quality of blood tests among specific patient groups. If such differences were identified, the research sought to characterize their common traits and investigate the sources contributing to these discrepancies based on the responses gathered from the survey.

Table 1

Tested Blood Parameters.

Parameter	Reference Values
Vitamin D	20-60 ng/mL
Serum Calcium	8,5-10,5 mg/dl
Serum Iron	10,5-26 mmol/L
Sedimentation rate	0-20 mm/hr
Blood Glucose	3,9-5,6 mmol/L
Triglycerides	<1,7 mmol/L
Cholesterol	<5 mmol/L
AST	≤40 IU/L
ALT	≤40 IU/L

The statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS). The Kruskal-Wallis test was applied to assess equality, serving as the non-parametric equivalent of one-way ANOVA.

Results and Discussion

Biochemical Analysis of Blood Samples in Patients With Rheumatoid Disease

In addition to the survey conducted with patients from the Republic of Kosovo diagnosed with rheumatoid disease, the study also included an analysis of specific blood parameters. Following a complete blood count for all 100 patients, the descriptive statistics of the obtained results are summarized in Table 2.

Table 2

Descriptive Statistics of Blood Parameters.

Indicator	Vitamin D	Serum Iron	Serum Calcium	Sedimen-tation	Glucose	Triglycer-ides	Choles-terol	AST	ALT
Reference	20-60 ng/ml	10.5-26 mmol/L	8.5-10.5 mg/dl	0-20 mm/hr	3.9-5.6 mmol/L	<1.7 mmol/L	<5 mmol/L	≤40 IU/L	≤40 IU/L
Mean	21.21	12.54	8.63	8.17	4.79	1.78	5.1	42.45	43.34
Median	21	12	8.6	7	4.7	1.6	4.9	40	40
Maximum	33	15.5	9.3	24	6.3	2.7	6.0	60	87
Minimum	8	10.2	7.4	2	3.9	1.6	4.9	11	40
Std. Dev.	6.64	1.73	0.36	4.15	0.67	0.28	0.29	7.22	7.67
Skewness	0.05	0.45	-0.76	0.99	0.34	1.72	1.27	-0.67	3.95
Kurtosis	2.13	1.65	4.97	4.58	1.85	5.51	3.42	9.55	21.82
Jarque-Bera	3.18	11.04	25.8	26.9	7.46	75.9	27.6	186	1,737

The analysis of blood parameters revealed that the mean values of nearly all measured parameters fell within the reference ranges, with notable exceptions in the AST and ALT levels, where average and median values approached or slightly exceeded the upper limit of 40 IU/L.

Vitamin D levels among the study participants ranged from 8 to 33 ng/ml, with a mean of 21 ng/ml, aligning closely with the lower limit of the reference range. The standard deviation for this parameter was 6.64 ng/ml. According to the Jarque-Bera statistic and corresponding p-value, vitamin D was the only parameter exhibiting a normal distribution, as illustrated in Fig. 3.

Serum iron levels for the osteoporosis patients ranged from 10.2 to 15.5 mmol/L, averaging 12.5 mmol/L with a standard deviation of 1.7 mmol/L, which is within acceptable reference values. Serum calcium levels were found to range from 7.4 to 9.3 mg/dl, with an average of 8.6 mg/dl and a standard deviation of 0.4 mg/dl, indicating that this measurement typically hovers around or slightly below the lower limit of the reference range.

Sedimentation rates ranged from 2 to 24 mm/hr, averaging 8.2 mm/hr, with a standard deviation of 4.2 mm/hr. While most values remained within the acceptable limit of 20 mm/hr, some patients did exhibit elevated levels.

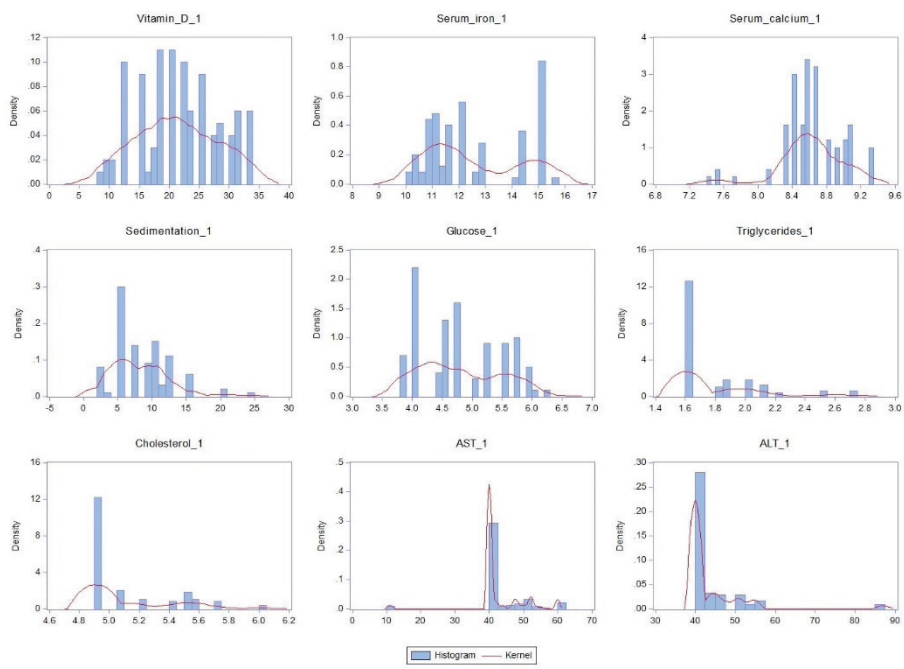
The average blood glucose level was 4.8 mmol/L, falling within the reference range of 3.9 to 5.6 mmol/L, although some patients displayed elevated glucose levels, ranging from 3.9 to 6.3 mmol/L.

Triglyceride levels were reported between 1.6 and 2.7 mmol/L, with a mean of 1.8 mmol/L and a standard deviation of 0.3 mmol/L. Notably, 37% of patients had elevated triglyceride values, exceeding the reference level of <1.7 mmol/L. Similarly, cholesterol levels ranged from 4.9 to 6 mmol/L, with an average of 5.1 mmol/L and a standard deviation of 0.3 mmol/L. Approximately 40% of patients exceeded the acceptable maximum of 5 mmol/L.

Analysis of AST and ALT indicated that 30% of the osteoporosis patients exhibited elevated values, specifically above the reference level of 40 IU/L. The maximum recorded AST level reached 60 IU/L, while ALT levels peaked at 87 IU/L.

Figure 3

A Graph Showing the Distribution of Blood Parameter Data.



According to the established methodology, statistical equality tests were conducted to determine significant differences among various respondent groups, utilizing the Kruskal-Wallis test for equality (Table 3). This non-parametric test assesses the medians of two or more groups concerning each of the nine analyzed blood parameters.

The results presented in Table 3 indicate that differences were evaluated based on gender (males and females), living environment (rural or urban), and smoking and alcohol consumption status. Additionally, the analysis included respondents who used oral anticoagulants, specifically aspirin, versus those who did not.

Statistically significant differences were observed in the median levels of blood parameters between patients who consumed aspirin and those who did not. This was particularly evident for sedimentation, glucose, triglycerides, cholesterol, AST, and ALT. The Kruskal-Wallis (KW) statistic exceeded the critical value, and the corresponding p-value was less than 0.05, confirming the significance of these differences.

As illustrated in Fig 4, patients using aspirin generally exhibited higher values across the mentioned blood parameters. Moreover, gender differences in glucose levels were noted, with men displaying higher blood sugar levels (4.7 mmol/L) compared to women (4.5 mmol/L).

Regarding triglyceride levels, former smokers had higher average levels (1.8 mmol/L) compared to non-smokers and current smokers (1.6 mmol/L). However, no significant differences were found in the median levels of vitamin D, serum calcium, or serum iron among the evaluated groups, nor for other blood parameters relative to the independent variables of gender, living environment, and smoking and alcohol consumption.

To assess the relationship between patients' age and body mass index (BMI) with the measured blood parameters, Spearman's rank correlation coefficients were calculated for the two variables (age and BMI) against the nine blood parameters, as summarized in Table 4. The correlation analysis indicated no statistically significant relationship between BMI and the blood parameters, as evidenced by low t-statistics and p-values greater than 0.05, suggesting a lack of linear correlation between BMI and each of the measured blood parameters.

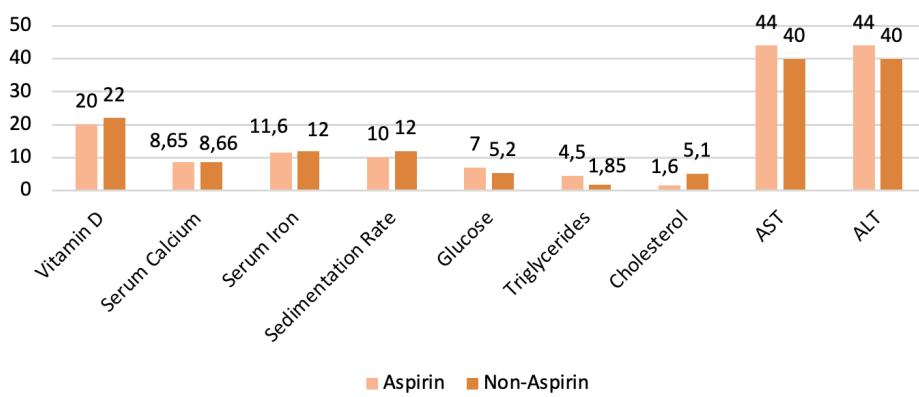
Table 3 *Results of the Conducted Kruskal-Wallis Tests for Equality of Medians.*

Variable	Category	Nr.	Vitamin D			Serum Calcium			Serum Iron		
Gender	Female		Med.	KB	p	Med.	KB	p	Med.	KB	p
Location	Male	57	22	0.005	0.945	8.6	2.654	0.103	12	0.519	0.471
Alcohol	City	43	20			8.6			12		
Cigarettes	Village	72	20	2.649	0.104	8.65	1.371	0.242	12	0.637	0.425
Anticoag-ulants	No	28	22.5			8.55			12		
	Yes	80	21	0.001	0.976	8.6	0.458	0.499	12	3.726	0.054
Variable	Smoker	20	21			8.65			11.5		
Gender	Non-smoker	23	20	1.645	0.439	8.7	2.086	0.352	12	0.087	0.957
Location	Former smoker	62	22			8.6			12		
Alcohol	Aspirin	15	18			8.7			11.7		
Cigarettes	No	30	20	0.225	0.636	8.65	0.828	0.363	11.6	2.010	0.156
Anticoag-ulants		70	22			8.6			12		
Variable	Category	Nr.	Sedimentation rate			Blood Glucose			Triglicerides		
	Female		Med.	KB	p	Med.	KB	p	Med.	KB	p
Gender	Male	57	7	0.490	0.484	4.5	5.183	0.023	1.6	0.299	0.585
Location	City	43	9			4.7			1.6		
Alcohol	Village	72	7	0.483	0.487	4.7	1.566	0.211	1.6	0.265	0.607
Cigarettes	No	28	9.5			4.7			1.6		
Anticoag-ulants	Yes	80	7	0.390	0.532	4.7	0.012	0.914	1.6	0.385	0.535
	Smoker	20	9.5			4.7			1.6		
Variable	Non-smoker	23	7	0.730	0.694	4.5	1.325	0.516	1.6	9.264	0.010
Gender	Former smoker	62	8			4.7			1.6		
Location	Aspirin	15	7			4.7			1.8		
Alcohol	No	30	10	5.775	0.016	5.2	6.330	0.012	1.85	5.775	0.016
Cigarettes		70	7			4.5			1.6		

Anticoag-ulants Variable	Category Female	Nr.	Cholesterol			AST			ALT		
			Med.	KB	p	Med.	KB	p	Med.	KB	p
Gender	Male	57	4.9	1.759	0.185	40	0.485	0.486	40	0.026	0.873
Location	City	43	4.9			40			40		
Alcohol	Village	72	4.9	0.074	0.785	40	0.144	0.704	40	0.130	0.718
Cigarettes	No	28	4.9			40			40		
Anticoag-ulants	Yes	80	4.9	0.028	0.867	40	0.058	0.809	40	0.475	0.491
	Smoker	20	4.9			40			40		
Variable Gender Location	Non-smoker	23	4.9	0.552	0.759	40	1.583	0.453	40	1.816	0.403
	Former smoker	62	4.9			40			40		
	Aspirin	15	4.9			40			40		
Alcohol	No	30	5.1	4.974	0.026	44	11.739	0.001	44	11.003	0.001
	Category	70	4.9			40			40		

Figure 4

Graph Showing the Median Values of Blood Parameters Based on the Use of Oral Anti-coagulants (Aspirin).



On the other hand, a statistically significant, moderate, negative linear relationship was identified between vitamin D levels and the age of patients with osteoporosis. The correlation coefficient for these variables was -0.27, indicating that as patients' age increases, there is often a corresponding decrease in blood levels of vitamin D.

Table 4 *Correlation Coefficients Between the Age and Body Mass Index of Patients With Osteoporosis and the Values of the Blood Parameters.*

Parameter	Age			BMI		
	Correlation Coefficient	t-statistic	p-value	Correlation Coefficient	t-statistic	p-value
Vitamin D	-0.27	-2.77	0.0066	-0.05	-0.50	0.6181
Serum Iron	0.06	0.61	0.5415	-0.09	-0.91	0.3636
Serum Calcium	-0.08	-0.82	0.4164	-0.01	-0.07	0.9472
Sedimentation rate	0.19	1.95	0.0536	-0.03	-0.29	0.7741
Blood Glucose	0.19	1.92	0.0575	-0.07	-0.74	0.4598
Triglicerides	0.38	4.02	0.0001	-0.09	-0.94	0.3491
Cholesterol	0.24	2.40	0.0185	0.07	0.67	0.5034
AST	0.06	0.62	0.5353	0.05	0.50	0.6178
ALT	0.04	0.43	0.6648	0.09	0.91	0.3669

Additionally, a statistically significant, moderate positive relationship was observed between age and the levels of triglycerides and cholesterol in the blood among patients with osteoporosis. The correlation coefficients for these parameters were 0.38 and 0.24, respectively, indicating that an increase in patient age is often associated with elevated triglyceride and cholesterol levels.

Furthermore, a weak positive relationship was noted between age and the levels of sedimentation and glucose, with correlation coefficients of 0.19 for both variables. This suggests that as patients age, there may be a slight increase in sedimentation and glucose levels.

Conversely, the analysis revealed no linear correlation between the age of patients with osteoporosis and serum iron, serum calcium, AST, and ALT levels. This indicates that age does not significantly influence these specific blood parameters in this population.

Future Directions

Bone health is influenced by several factors, with a well-balanced diet being crucial. Adequate intake of protein, calcium, vitamin D, and other nutrients supports bone metabolism. A recommended dietary approach includes consuming low-fat dairy

products, fruits, vegetables, and appropriate amounts of meat, fish, and poultry. It's essential for healthcare professionals and nutritionists to monitor mineral and vitamin supplementation, as excessive or insufficient amounts can negatively impact bone health.

Proper nutrition, particularly regarding protein, calcium, and vitamin D, is vital for maintaining bone mass and strength, especially in adults and the elderly. Attention to diet is also a critical aspect of rehabilitation programs for patients with osteoporotic fractures. For frail elderly individuals who have sustained hip fractures, good nutritional status is essential for recovery; poor nutrition can impede healing and increase the risk of further fractures (International Osteoporosis Foundation, 2006).

Numerous studies have explored the relationship between diet and osteoporosis, providing valuable insights for healthcare providers. Strengthening the understanding of how dietary supplements can protect against osteoporosis is crucial for developing new intervention strategies. Research in this area not only illuminates the connection between diet and osteoporosis pathogenesis but may also lead to innovative nutritional therapies. Therefore, studies summarizing the current evidence on the interaction between diet and osteoporosis are of significant importance.

The Role of Nutrition in the Development of Osteoarthritis

Osteoarthritis (OA) is understood to result from multiple factors, including age, obesity, mechanical loading, inflammation, joint injuries, and genetic predisposition (Cicuttini, 1996). However, the precise mechanisms behind the onset and progression of OA remain unclear, and no effective interventions exist to significantly delay disease progression or repair damaged cartilage (Chen et al., 2017). Current medical approaches focus primarily on pain management, while non-pharmacological strategies emphasize weight loss and physical activity. For those with advanced OA, surgical options may also be considered (Ronn, 2011).

Recently, there has been growing interest in the role of diet and nutrition in preventing and managing OA. Obesity is a significant modifiable risk factor for OA, not only due to its impact on joint mechanics but also because adipose tissue releases inflammatory mediators, contributing to chronic low-grade inflammation (Gambari et al., 2023). Research has shown a correlation between dietary fatty acids and inflammation, highlighting an increased ratio of omega-6 to omega-3 polyunsaturated fatty acids (PUFAs) as particularly detrimental (Liput, 2021). High consumption of omega-6 PUFAs is associated with synovitis and cartilage degradation in obese individuals due to chronic inflammation (Patterson, 2012).

Conversely, diets rich in omega-3 PUFAs have been found to reduce systemic inflammation (Gil, 2002), alleviate pain, and enhance joint function in OA patients (Deng, 2023). Therefore, incorporating omega-3 PUFAs into the diet presents a promising preventive and therapeutic strategy for managing obesity-related OA by facilitating better calcium and phosphorus absorption in the intestines. Vitamin D, which is converted into its active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], plays a critical role in stimulating mineral transport in the gut. This active form binds to the vitamin D receptor (VDR) to promote the expression of genes responsible for calcium and phosphorus transport (DeLuca, 1986). It suggests that 1,25$(OH)_2$D might directly influence OA cartilage and also operate through the endocrine system (Park, 2019). Supplementation with 25$(OH)_2$D may help relieve pain and potentially mitigate radiological signs of OA in individuals with low vitamin D levels (<50 nmol/L) (Sanghi, 2013).

Another avenue for addressing obesity-related OA is the gut microbiome. The systemic inflammation that exacerbates OA due to obesity is now linked to alterations in gut microbiota (Portune, 2017). Research by Schott et al. (2018) demonstrated that correcting gut dysbiosis associated with obesity can be achieved by fostering a healthy microbial community. By using the indigestible fiber oligofructose as a prebiotic dietary supplement, they restored a healthy gut microbiome in obese mice, which led to reduced systemic inflammation and protection against cartilage loss, highlighting a novel approach for treating obesity-related OA (Schott, 2018).

Additionally, antioxidant supplements like vitamin C, vitamin E, and curcumin have been suggested to alleviate OA symptoms. Antioxidants combat oxidative stress by neutralizing free radicals produced in the body (Chaudhary, 2023). Animal studies show that vitamin C can reduce OA symptoms, whether taken as a dietary supplement or via intra-articular injections, and several clinical studies support its chondroprotective effects. However, excessive vitamin C may produce adverse effects, indicating that its therapeutic role in OA management is still uncertain (Dunlap, 2021). Vitamin E has also been evaluated for its potential to prevent or treat OA, but results are mixed, with some studies showing positive effects on joint health while others report negligible or negative impacts (Chin, 2018). Recent clinical trials have indicated that curcumin, at doses of 160 to 2000 mg/day, effectively reduces knee OA symptoms, demonstrating comparable efficacy and better tolerability than traditional non-steroidal anti-inflammatory drugs (Gupte, 2019; Shep, 2019).

Future Directions: Nutrition as a Key Role in the Therapy of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease characterized by synovial hyperplasia, autoantibody production, cartilage and bone destruction, and malformations and destruction of multiple joints (Epstein, 1990). The pathogenesis of RA is complex and involves both genetic and environmental factors (Symmons, 2003).

Recent literature suggests that nutrition plays a crucial role in the therapy of RA by managing inflammation, immunity, and oxidative stress. Considering that specific dietary interventions can be beneficial support for patients suffering from RA, a nutritional pyramid has been created to help patients easily understand what is best for them to eat.

The proposed pyramid illustrated in Figure 5, Rondanelli et al. and Bach-Freis et al., closely resembles that recommended for the general population shown in Figure 6, with notable differences:

Base Composition: The foundation includes 5 servings of fruits and vegetables, highlighting the most beneficial vegetable types for consumption, rather than carbohydrates.

Carbohydrate Preference: It notes a preference for gluten-free carbohydrates for patients with RA.

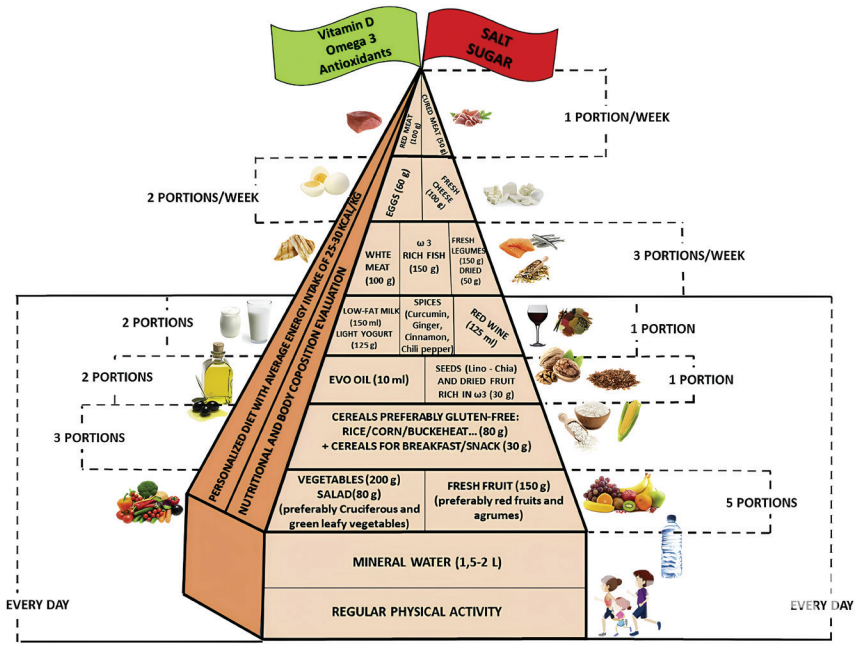
Inclusion of Seeds: Seeds are introduced as a daily consumption recommendation.

Health Alerts: Two flags are added, emphasizing that RA patients should avoid salt and simple sugars, and it is beneficial to take supplements of vitamin D, omega-3, and antioxidants.

Despite its potential utility, this nutritional pyramid is largely hypothetical due to the limited number of clinical trials, resulting in moderate to low levels of evidence supporting its recommendations. Nevertheless, it serves as a useful guide for researchers investigating the links between immunity, nutrition, and RA, as noted in Cochrane reviews (Hagen, 2009). Further research is crucial, particularly randomized clinical trials directly examining the relationships between diet, symptoms, and the progression of RA, to elucidate the mechanisms that connect immune regulation, inflammation, oxidative stress, and nutrition.

Figure 5

Ideal Nutrition Pyramid for Patients with Rheumatoid Arthritis (RA)



Source: Rondanelli, 2021

Figure 6

Mediterranean Diet Pyramid: A Lifestyle for Today.

Source: Bach-Faig, 2011

Conclusion

This study examined blood parameters in 100 patients diagnosed with osteoporosis who were also using aspirin. The results showed that vitamin D levels ranged from 8 to 33 ng/ml, with an average of 21 ng/ml, positioning patients at the lower limit of the reference range. Serum iron levels ranged from 10.2 to 15.5 mmol/L, with a mean of 12.5 mmol/L, while serum calcium levels ranged from 7.4 to 9.3 mg/dl, averaging 8.6 mg/dl. These findings highlight deficiencies in key nutrients associated with bone strength.

Inflammatory and metabolic indicators were also assessed. Sedimentation rates ranged from 2 to 24 mm/hr, averaging 8.2 mm/hr. Meanwhile, blood glucose levels averaged 4.8 mmol/L, which is within normal limits. About 37% of patients had elevated triglycerides (>1.7 mmol/L), with a range of 1.6 to 2.7 mmol/L and a mean of 1.8 mmol/L, while cholesterol levels averaged 5.1 mmol/L (range: 4.9–6 mmol/L), exceeding the reference limit in nearly 40% of patients. Increased AST and ALT levels were observed in 30% of participants, exceeding the reference level of 40 IU/L, suggesting potential liver stress or altered metabolism in this population.

Statistical analysis showed that patients using aspirin generally exhibited higher values in all tested blood parameters and reaching statistical significance. However, body mass index (BMI) did not show a significant correlation with any of the blood parameters. At once, increasing age was associated with lower vitamin D levels and higher levels of triglycerides, cholesterol, sedimentation, and blood glucose.

The findings indicate that patients using aspirin generally exhibit higher values for all examined blood parameters, with statistically significant differences observed. Correlation analysis revealed no significant correlation between body mass index (BMI) and blood parameter values. In addition, patient age was associated with decreased vitamin D levels and increased sedimentation, triglyceride, cholesterol, and glucose levels.

Given the numerous studies linking diet and osteoporosis, it is crucial to consolidate current evidence and pursue further research on their interaction. Strengthening scientific understanding of how dietary supplementation may protect against osteoporosis could pave the way for new nutritional therapies.

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