Middle East J Dig Dis. July 2023;15(3):175-179

doi 10.34172/mejdd.2023.340 http://mejdd.org

Middle East Journal of Digestive Diseases MEJDD

Original Article

Serum Alkaline Phosphate Level Associates with Metabolic Syndrome Components Regardless of Non-Alcoholic Fatty Liver; A Population-Based Study in Northern Iran

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Abstract

Background: Serum alkaline phosphatase (ALP) is an indicator of hepatobiliary disorders, such as metabolic syndrome (MetS). To assess the association between serum ALP levels and MetS, with or without non-alcoholic fatty liver disease (NAFLD), in a cohort study in northern Iran.

Methods: Data from approximately 5257 subjects aged more than 18 years participating in the Amol cohort were used. We extracted the required data and investigated the correlation between liver enzyme levels and MetS. Multiple logistic regression analyses based on the serum ALP quartiles were performed.

Results: Of them, 2860 were male with a mean age of 42.11 ± 16.1 years. A positive linear trend was observed between serum ALP levels and the number of MetS components in both sexes. In both sexes, systolic blood pressure, waist circumferences, and high-density lipoprotein (HDL) had a significant association with ALP. After adjusting for age, both sexes with NAFLD showed an increased risk of developing MetS. The risk of NAFLD increased in individuals with > 2nd quartile of ALP. Furthermore, higher ALP levels were associated with an increased risk of MetS in males (1.1014 [0.782–1.315]) and females (1.441 [1.085–1.913]).

Conclusion: There is a significant association between serum ALP levels and MetS, independent of fatty liver changes, suggesting that this marker can be considered as a feasible predictor of MetS.

Keywords: Fatty liver disease, Alkaline phosphatase, Metabolic syndrome

Cite this article as: Sohrabi M, Aghapour S, Khoonsari M, Ajdarkosh H, Nobakht H, Zamani F. Serum alkaline phosphate level associates with metabolic syndrome components regardless of non-alcoholic fatty liver; a population-based study in northern Iran. *Middle East J Dig Dis* 2023;15(3):175-179. doi: 10.34172/mejdd.2023.340.

Received: November 18, 2022, **Accepted:** May 27, 2023, **ePublished:** July 30, 2023

Introduction

The prevalence of *metabolic syndrome (MetS)* is increasing rapidly worldwide. MetS is associated with several morbidities, particularly non-alcoholic fatty liver disease (NAFLD).[1] Its increasing trend is a real threat to public health as it increases the *risk* of *diabetes* and *cardiovascular disease*.[2] Inflammation and insulin resistance may play an important role in this context. Hence, fatty liver disease and liver injury may develop in MetS that presents with elevated liver markers such as alkaline phosphatase (ALP).[3,4] Therefore, the low cost, *simplicity* of use, *non-invasive nature*, and wide acceptance by *patients* make liver markers a suitable method for diagnosing and predicting MetS.

ALP can be found in the serum as well as the external cell surface of various tissues, including the liver, bone, intestine, and placenta. Hence, the serum levels of ALP have been considered a hallmark of liver and bone pathologies.[4,5] Recent studies have shown that serum ALP may be associated with cardiometabolic diseases such as type 2 diabetes, dyslipidemia, hypertension, and peripheral arterial disease.[4,6] Its role in inflammation has been previously reported.[7] In this regard, the association between ALP and MetS has been considered, although this is a controversial issue.[4,8] Therefore, in this population-based study, we investigated the association between serum ALP levels and the risk of MetS and its components.

Materials and Methods

This study was a cross-sectional analysis of baseline data from the Amol Cohort Study (ACS). The ACS was a study conducted by the Gastrointestinal and Liver Disease Research Center (GILDRC) on the relationship between NAFLD, MetS, and their risk factors in the general population of urban and rural areas of Amol. The details of the study protocol have been previously published.[9] In brief, 6100 individuals participated in the baseline phase of the study, which was conducted between May 2008 and March 2011.

Inclusion and Exclusion Criteria

An adult population aged 18-80 years was enrolled in this study. The patients with the following problems were excluded from the study: those who had chronic liver

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diseases, seropositivity for hepatitis B virus surface antigen (HBS Ag), anti-HBC or hepatitis C virus antibodies (anti-HCV), known cases of autoimmune hepatitis or hereditary liver disease and alcohol consumption more than 30 g/day in men and more than 20 g/day in women.

Study Variables

Each participant received a self-administered general and lifestyle questionnaire that included detailed queries on sociodemographic variables, history of diseases, drug and substance abuse, smoking, alcohol consumption, nutrition, and physical activity.

Trained medical staff assessed characteristics such as standing height, waist circumference, weight, and *body mass index (BMI)*. Blood pressure was measured in a seated and relaxed position. Duplicate measurements were made using a standardized protocol, and the averages were used in the analysis.

Biochemical parameters were measured using a 12-hour fasting venous blood sample. Fasting plasma glucose (FPG), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), ALP, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured using an auto-analyzer (Bio-system kits). HBSAg, HBCAb, and HCVAb levels were assessed by ELIZA (Acon kit). Fasting serum insulin was measured by ELIZA (Monobind kit).

Abdominal ultrasonography (US) using Esaote May lab number 15 was performed for the liver and biliary ductus in all participants. An expert radiologist performed all sonographies.

Criteria for the NAFLD

NAFLD was established in the presence of fatty liver and absence of causes for secondary hepatic fat accumulation, such as alcohol consumption, seropositivity for HCV-Ab, HBS Ag or HBc antibody, hereditary liver disease, and drugs are known to be associated with fatty liver during the six months prior to enrollment.

Grading of Fatty Liver

According to transabdominal ultrasonography, fatty infiltration was classified into four grades; without evidence of fatty liver; mild: minimal diffuse increase in hepatic echogenicity with normal visualization of the diaphragm and border of intrahepatic vessels, moderate: increase of hepatic echogenicity with a slight impairment of intrahepatic vessels and diaphragm visualization, and severe: increase of hepatic echogenicity, poor penetration of the posterior segment of the right lobe and impaired visualization of the vessels and diaphragm.[10]

Criteria for the MetS

MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III, which was last updated in 2005 by the American Heart Association,[11] based on the presence of three or more of the following: (1) elevated waist circumference ≥ 102 cm in men and ≥ 88 cm in women; (2) elevated triglycerides or drug treatment for elevated triglycerides ≥ 150 mg/dL (≥ 1.7 mmol/L); (3) reduced HDL cholesterol or drug treatment for reduced HDL cholesterol < 40 mg/dL (< 1.0 mmol/L) for men and < 50 mg/dL (< 1.3 mmol/L) for women; (4) elevated blood pressure or antihypertensive treatment in a patient with a history of hypertension (systolic ≥ 130 mm Hg and/or diastolic ≥ 85 mm Hg), and (5) elevated fasting glucose or drug treatment of elevated glucose ≥ 100 mg/dL.

Statistical Analysis

Characteristic data are presented as mean ± standard deviation (SD). A t-test was used to compare means. Partial Spearman correlation was used to analyze the correlation between liver enzyme levels and the individual component of MetS adjusting for age. The results are presented as correlation coefficients. The trend was analyzed using Pearson correlation. ALP levels were categorized into four groups based on sex-specific 25th, 50th, and 75th percentiles. The cut-off points of quartiles for ALP levels were 139, 187, and 235 U/L in males and 144, 190, and 245 U/L in females.

Logistic regression (enter method) models were used to separately analyze the associations between ALP levels and the incidence of NAFLD and MetS in males and females. The results are presented as odds ratios (*ORs*) and 95% confidence intervals (*CIs*) in each quartile compared with the 1st quartile. In all analyses, $P < 0.05$ was taken to indicate statistically significant.

Results

Based on our criteria, 5257 individuals were enrolled in this study. The male and female sex distribution was 2860 and 2397 persons, respectively. The mean age of the patients was 42.11 ± 16.1 years. Of them, 1648 subjects had MetS.

Table 1 presents the basic characteristics of the participants. In this regard, individuals with MetS were older and heavier than healthy subjects.

We found significant but weak correlations between ALP levels and the individual components of MetS (Table 2); however, there was a positive linear trend between ALP levels and the number of MetS components in both sex groups (Table 3).

In the logistic regression analysis (Table 4), after adjusting for age, the presence of NAFLD in both sexes led to a five-fold increase in ALP level. As illustrated in Table 5, individuals in the 2nd quartile of ALP had an approximately 2-fold increased risk of NAFLD, and the strength of this association increased to more than 3 and 4-fold in the 4th quartile in women and men, respectively. Furthermore, after adjusting for age, higher ALP levels were associated with an increased risk of MetS (Table 5), which persisted in women after adjusting for NAFLD (Table 6).

Discussion

In the present study, we revealed a positive association between increased ALP levels and the presence of MetS and NAFLD. We observed that, compared with normal ALP subjects, other participants had a significantly higher risk of NAFLD and MetS, and after adjusting for fatty liver, we observed an association between elevated levels of ALP and MetS. We hypothesized that elevated ALP levels are associated with a greater prevalence of MetS, regardless of liver disease.

Steatohepatitis is the most common cause of persistently elevated serum liver enzymes.[12] Higher ALP levels may be associated with MetS and liver disease.[13] The prevalence of MetS has been increasing worldwide. Although the exact rate is not clear, a study in the United States reported that approximately 24% of the adult population has MetS.[14] In Iran, the prevalence of MetS was estimated to be approximately 34.7% based on the ATP III criteria.[15,16] Moreover, NAFLD is associated with elevated serum ALP.[14] In our study, many of the subjects suffered from MetS as well as NAFLD. While the association of MetS with NAFLD has a great impact in this regard, there are not many studies considering the MetS and NAFLD together, according to ALP. In fact, many authors consider NAFLD to be a liver presentation of MetS, but it is not involved in all patients with NAFLD.[17,18] Hence, the association between MetS and NAFLD was recently defined as metabolic-associated fatty liver disease.[19]

The mechanisms underlying the association between ALP level and MetS have not been clarified. ALP controls intracellular lipid accumulation in human preadipocytes. In addition, it has been revealed that inhibition of tissue-specific ALP in preadipocytes blocks both ALP activity and lipid accumulation.[20] Accordingly, obesity appears to be a probable link to this association. Insulin resistance and subclinical low-grade inflammation play key roles in MetS development. Thus, insulin resistance may be an underlying mechanism linking serum ALP activity and MetS. Serum ALP is known to be associated with insulin resistance, especially hepatic steatosis.[21]

According to a study in which serum ALP levels were measured in 32,329 subjects, females who were more than 15% overweight had 20% higher ALP activity than lean subjects.[22] In another study confirming the positive relationship between BMI and ALP, Ali et al suggested that serum ALP, particularly liver ALP, is derived from adipose and hepatic tissues.[22,23]

Some observational studies have suggested that serum ALP activity may be higher in individuals with MetS than in those without. Our results are consistent with these findings. Krishnamurthy et al demonstrated a strong association between higher serum ALP levels and increased prevalence of the MetS among the US general population.[14] Data from the Insulin Resistance

Table 1. Baseline characteristics of the subjects grouped by sex

		No MetS		MetS		*P* value
		Mean	SD	Mean	SD	
Male	Age	39.17	17.55	48.17	15.73	0.000
	Waist	85.95	11.63	100.12	9.61	0.000
	Glucose	93.41	21.68	111.84	42.19	0.000
	TG	118.89	73.39	206.10	106.60	0.000
	HDL	46.38	11.21	35.64	8.02	0.000
	Diastolic BP	73.12	11.82	83.94	12.49	0.000
	Systolic BP	113.03	13.46	126.66	16.83	0.000
	BMI	25.05	5.26	29.85	4.24	0.000
Female	Age	38.15	15.53	52.60	12.87	0.000
	Waist	86.16	12.03	101.52	11.29	0.000
	Glucose	94.06	26.51	125.02	56.28	0.000
	TG	111.21	66.59	200.46	119.99	0.000
	HDL	49.59	11.77	39.48	9.49	0.000
	Diastolic BP	72.17	11.63	82.79	13.44	0.000
	Systolic BP	110.03	14.33	126.51	19.08	0.000
	BMI	27.91	5.87	32.93	4.97	0.000

TG, triglyceride; HDL, high-density lipoprotein; BMI, body mass index; BP, blood pressure; SD, standard deviation.

Table 2. Correlation between alkaline phosphatase quartiles and individual components of metabolic syndrome according to sex

		Diastolic BP	Systolic BP	TG	HDL	FPG	Waist
Male	Correlation	0.022	0.010	0.049	-0.005	0.024	0.096
	Significance (2-tailed)	0.220	0.577	0.007	0.006	0.179	0.000
Female	Correlation	0.011	0.044	0.035	-0.045	0.039	0.090
	Significance (2-tailed)	0.588	0.036	0.093	0.031	0.062	0.000

TG, triglyceride; HDL, high-density lipoprotein; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose.

Table 3. Trends of alkaline phosphatase level in relation to the number of MetS components

	Number of MetS' components												Trend test Pearson coefficient
	0		1		2		3		4		5		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Male	192.62	135.73	205.16	131.61	221.68	152.58	219.26	141.20	231.11	133.83	197.05	97.28	0.108
Female	185.19	113.78	217.29	150.43	228.99	174.51	229.10	163.66	207.22	90.89	226.39	138.13	0.089

SD, standard deviation; MetS, metabolic syndrome.

Atherosclerosis Study in the United States on 633 participants with an average 5.2 years follow-up disclosed a positive association between serum ALP activity and MetS.[24] Grethen et al also showed a significant difference between bone ALP in severely obese women and controls. In addition to ALP release from adipose tissue, bone turnover markers increased in these subjects.[25]

In the present study, the association between MetS and the female sex was more prominent, but the exact mechanism is unclear. However, the results may be affected by hormonal changes during a woman's lifetime, as well as by the total adipose tissue. Compared to men with the same BMI, women have more total adipose tissue, which can be a source of free fatty acids and inflammatory substances that may consequently be involved in the insulin resistance process and its consequent side effects. Moreover, the role of estrogen in metabolic inflammation and hemostasis must be considered.[26,27] However, after menopause, increased bone loss results in higher total ALP levels than in premenopausal individuals.[28] Kim et al revealed as association of ALP level with MetS in both sexes.[4] In contrast, a Korean study among men and postmenopausal women revealed that the association between ALP activity and MetS after adjusting for age, BMI, and osteocalcin was not statistically significant.[29] Moreover, among the MetS components, serum ALP levels were significantly associated with waist circumference and HDL levels in both sexes. Previous studies confirmed this association.[4,16]

Table 4. The association between NAFLD and metabolic syndrome adjusting for age

			MetS		OR (95% CI)
			No (%)	Yes (%)	
NAFLD	Male	No	1453 (72.7)	273 (31.7)	4.993 (4.182- 5.961)
		Yes	546 (27.3)	588 (68.3)	
		Total	1999	861	
	Female	No	1210	201 (25.5)	5.082 (4.106- 6.290)
		Yes	400	586 (74.5)	
		Total	1610	787	

OR, odds ratio; CI, confidence interval; MetS, metabolic syndrome; NAFLS, non-alcoholic fatty liver disease.

Strengths and Limitations

The study population was socially representative of the general urban and rural areas of Amol, and a follow-up plan was conducted in this population. We were able to take into account a wide range of disorders associated with predictable ALP levels. However, bone measurements and vitamin D levels were not performed in this study, and we cannot preclude this confounding factor, especially to explain the lack of association in men. Further studies are needed to better understand the link between ALP and MetS.

Conclusion

We found significant associations between serum ALP levels and NAFLD, as well as MetS, compared with the normal group. In addition, high ALP levels significantly increased the risk of MetS and NAFLD. The association between MetS and ALP levels is independent of fatty liver changes. Therefore, we suggest that these markers are feasible predictors of MetS.

Acknowledgments

This study was funded by the Gastrointestinal and liver diseases research center, Iran University of medical sciences

Authors' Contribution

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Table 5. The risk of NAFLD and MetS in each sex group according to different quartiles of ALP levels adjusted to age

		Q1 OR (95% CI)	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)
NAFLD	Male	1 (Ref)	2.642 (2.081- 3.353)	2.307 (1.812- 2.937)	4.135 (3.267- 5.234)
	Female	1 (Ref)	1.965 (1.492- 2.588)	2.035 (1.540- 2.691)	3.287 (2.485- 4.322)
MetS	Male	1 (Ref)	1.370 (1.072- 1.749)	1.279 (0.997- 1.641)	1.573 (1.238- 2.000)
	Female	1 (Ref)	1.563 (1.194- 1.046)	1.477 (1.126- 1.937)	1.637 (1.250- 2.144)

OR, odds ratio; CI, confidence interval; MetS, metabolic syndrome; NAFLS, non-alcoholic fatty liver disease.

Table 6. The risk of MetS in each sex group according to different quartiles of ALP levels adjusted to age and grade of fatty liver

		Q1 OR (95% CI)	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)
MetS	Male	1 (Ref)	0.997 (0.766- 1.297)	0.946 (0.724- 1.238)	1.1014 (0.782- 1.315)
	Female	1 (Ref)	1.442 (1.084- 1.918)	1.343 (1.007- 1.792)	1.441 (1.085- 1.913)

OR, odds ratio; CI, confidence interval; MetS, metabolic syndrome; NAFLS, non-alcoholic fatty liver disease.

Competing Interests

The authors declare no conflict of interest related to this work.

Ethical Approval

The study protocol was approved by the Board of Ethics of the GILDRC, which included gastroenterologists, pathologists, forensic specialists, oncologists, clinical nutritionists, epidemiologists, and surgeons. Before enrollment, the protocol was explained to the participants, and written informed consent was obtained.

References

1. Khoonsari M, Mohammad Hosseini Azar M, Ghavam R, Hatami K, Asobar M, Gholami A, et al. Clinical manifestations and diagnosis of nonalcoholic fatty liver disease. *Iran J Pathol* 2017;12(2):99-105.
2. Wang HH, Lee DK, Liu M, Portincasa P, Wang DQ. Novel insights into the pathogenesis and management of the metabolic syndrome. *Pediatr Gastroenterol Hepatol Nutr* 2020;23(3):189-230. doi: 10.5223/pghn.2020.23.3.189
3. Godoy-Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr* 2020;12:60. doi: 10.1186/s13098-020-00570-y
4. Kim JH, Lee HS, Park HM, Lee YJ. Serum alkaline phosphatase level is positively associated with metabolic syndrome: a nationwide population-based study. *Clin Chim Acta* 2020;500:189-94. doi: 10.1016/j.cca.2019.10.015
5. Price CP. Multiple forms of human serum alkaline phosphatase: detection and quantitation. *Ann Clin Biochem* 1993;30(Pt 4):355-72. doi: 10.1177/000456329303000403
6. Bilski J, Mazur-Bialy A, Wojcik D, Zahradnik-Bilska J, Brzozowski B, Magierowski M, et al. The role of intestinal alkaline phosphatase in inflammatory disorders of gastrointestinal tract. *Mediators Inflamm* 2017;2017:9074601. doi: 10.1155/2017/9074601
7. Estaki M, DeCoffe D, Gibson DL. Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity. *World J Gastroenterol* 2014;20(42):15650-6. doi: 10.3748/wjg.v20.i42.15650
8. Lallès JP. Recent advances in intestinal alkaline phosphatase, inflammation, and nutrition. *Nutr Rev* 2019;77(10):710-24. doi: 10.1093/nutrit/nuz015
9. Motamed N, Sohrabi M, Poustchi H, Maadi M, Malek M, Keyvani H, et al. The six obesity indices, which one is more compatible with metabolic syndrome? A population based study. *Diabetes Metab Syndr* 2017;11(3):173-7. doi: 10.1016/j.dsx.2016.08.024
10. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011;54(3):1082-90. doi: 10.1002/hep.24452
11. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation* 2005;112(17):2735-52. doi: 10.1161/circulationaha.105.169404
12. Oh RC, Hustead TR, Ali SM, Pantsari MW. Mildly elevated liver transaminase levels: causes and evaluation. *Am Fam Physician* 2017;96(11):709-15.
13. Zhang L, Ma X, Jiang Z, Zhang K, Zhang M, Li Y, et al. Liver enzymes and metabolic syndrome: a large-scale case-control study. *Oncotarget* 2015;6(29):26782-8. doi: 10.18632/oncotarget.5792
14. Krishnamurthy VR, Baird BC, Wei G, Greene T, Raphael K, Beddhu S. Associations of serum alkaline phosphatase with metabolic syndrome and mortality. *Am J Med* 2011;124(6):566.e1-566.e7. doi: 10.1016/j.amjmed.2010.11.030
15. Delavari A, Forouzanfar MH, Alikhani S, Sharifian A, Kelishadi R. First nationwide study of the prevalence of the metabolic syndrome and optimal cutoff points of waist circumference in the Middle East: the national survey of risk factors for noncommunicable diseases of Iran. *Diabetes Care* 2009;32(6):1092-7. doi: 10.2337/dc08-1800
16. Fatahi A, Doosti-Irani A, Cheraghi Z. Prevalence and incidence of metabolic syndrome in Iran: a systematic review and meta-analysis. *Int J Prev Med* 2020;11:64. doi: 10.4103/ijpvm.IJPVM_489_18
17. Reccia I, Kumar J, Akladios C, Virdis F, Pai M, Habib N, et al. Non-alcoholic fatty liver disease: a sign of systemic disease. *Metabolism* 2017;72:94-108. doi: 10.1016/j.metabol.2017.04.011
18. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015;313(22):2263-73. doi: 10.1001/jama.2015.5370
19. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020;73(1):202-9. doi: 10.1016/j.jhep.2020.03.039
20. Ali AT, Penny CB, Paiker JE, Psaras G, Ikram F, Crowther NJ. The effect of alkaline phosphatase inhibitors on intracellular lipid accumulation in preadipocytes isolated from human mammary tissue. *Ann Clin Biochem* 2006;43(Pt 3):207-13. doi: 10.1258/000456306776865179
21. Sesti G, Fiorentino TV, Hribal ML, Sciacqua A, Perticone F. Association of hepatic insulin resistance indexes to nonalcoholic fatty liver disease and related biomarkers. *Nutr Metab Cardiovasc Dis* 2013;23(12):1182-7. doi: 10.1016/j.numecd.2013.01.006
22. Schiele F, Henny J, Hitz J, Petitclerc C, Gueguen R, Siest G. Total bone and liver alkaline phosphatases in plasma: biological variations and reference limits. *Clin Chem* 1983;29(4):634-41.
23. Ali AT, Paiker JE, Crowther NJ. The relationship between anthropometry and serum concentrations of alkaline phosphatase isoenzymes, liver-enzymes, albumin, and bilirubin. *Am J Clin Pathol* 2006;126(3):437-42. doi: 10.1309/9n346gxx67b6px5w
24. Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB Jr, Haffner SM. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes* 2005;54(11):3140-7. doi: 10.2337/diabetes.54.11.3140
25. Grethen E, Hill KM, Jones R, Cacucci BM, Gupta CE, Acton A, et al. Serum leptin, parathyroid hormone, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, bone alkaline phosphatase, and sclerostin relationships in obesity. *J Clin Endocrinol Metab* 2012;97(5):1655-62. doi: 10.1210/jc.2011-2280
26. Monteiro R, Teixeira D, Calhau C. Estrogen signaling in metabolic inflammation. *Mediators Inflamm* 2014;2014:615917. doi: 10.1155/2014/615917
27. Hevener AL, Zhou Z, Drew BG, Ribas V. The role of skeletal muscle estrogen receptors in metabolic homeostasis and insulin sensitivity. *Adv Exp Med Biol* 2017;1043:257-84. doi: 10.1007/978-3-319-70178-3_13
28. Crilly RG, Jones MM, Horsman A, Nordin BE. Rise in plasma alkaline phosphatase at the menopause. *Clin Sci (Lond)* 1980;58(4):341-2. doi: 10.1042/cs0580341
29. Bae SJ, Choe JW, Chung YE, Kim BJ, Lee SH, Kim HY, et al. The association between serum osteocalcin levels and metabolic syndrome in Koreans. *Osteoporos Int* 2011;22(11):2837-46. doi: 10.1007/s00198-010-1504-y