Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(3)

Measurement of Some Biochemical Parameters in Metabolic Syndrome

Suhair Abdul-Raheem Ahmed [1] and Israa Fadhil Ascar [2*]
[1,2]Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received: 23 September 2023 Accepted : 2 January 2024 Published: 20 July 2025
doi.org/10.30526/38.3.3750

Abstract

Patients with diabetes, high blood pressure (BP), and obesity have metabolic syndrome (MetS). The study aims to evaluate the levels of obestatin and osteopontin (OPN) as markers for detecting MetS and their relationship with other biomarker parameters at Al-Yarmuk Teaching Hospital in Baghdad. A total of 165 patients (125 with MetS and 40 healthy subjects as a control group) whose ages ranged from 20 to 80 years were examined and participated in this study. Body mass index (BMI), BP, fasting serum glucose (FSG), liver enzymes, total protein, and serum lipid profile were evaluated in each patient and control. In addition, obestatin and OPN were used with commercial ELISA kits according to the manufacturer's instructions. The study's results showed significant differences ($p < 0.05$) in BMI, BP, FSG, liver enzymes, and lipid profile between the MetS and control groups. The levels of obestatin were notably decreased in the MetS group compared to the control group (280.8 pg/mL vs. 1082.1 pg/mL). The level of OPN in patients was significantly higher than in controls (17.96 ng/mL vs. 5.47 ng/mL), respectively. The receiver operating characteristic (ROC) curve tests for obestatin and OPN are 0.981 and 0.999, respectively, which indicate that Obestatin and OPN are considered good diagnostic markers for subjects with MetS. It can be proposed that decreased levels of obestatin and increased levels of OPN in obese individuals may contribute to the onset of MetS and disrupted lipoprotein metabolism.

Keywords: Lipid profile, Liver enzymes, Metabolic syndrome, Obestatin, Osteopontin.

1. Introduction

Metabolic syndrome (MetS), also known as insulin resistance (IR) syndrome or syndrome X, is a group of characteristics that together increase the risk of type 2 diabetes mellitus (T2DM) and heart disease. These characteristics include obesity, high blood pressure (BP), elevated blood sugar levels, and high triglycerides (TG). Controlling weight, blood sugar levels, total cholesterol (TC), and TG levels can help extend life and reduce the risk of heart attacks and strokes (1). A significant waist circumference or an "apple-shaped" body, and elevated BP, they are diagnosed with hypertension. They are currently experiencing MetS (2). The underlying factors contributing to MetS include obesity, IR, physical inactivity, genetic predisposition, and advancing age (3,4). A peptide hormone called obestatin is a 23-amino acid peptide and is secreted from the stomach and acts in the spleen, intestine, breast

milk, mammary gland, as well as plasma (5). It was first discovered to interact with the orphan receptor GPR39, a receptor related to the ghrelin receptor subfamily, and is associated with weight gain, decreased food intake, gastric emptying, and reduced intestinal motility (6). It can be considered a significant indicator of an individual's nutritional status, providing insights into the presence of obesity and IR (7). Due to its considerable cardiovascular disease (CVD) as well as metabolic effects, obestatin is regarded as a promising therapeutic target for managing obesity in patients, particularly those with diabetes. Additionally, large amounts of these peptides are excreted in both fat- and lean-forms. Adipose tissue in overweight patients releases factors that promote angiogenesis (8). Furthermore, it has been demonstrated that obestatin not only upregulates genes related to β-cell regeneration, insulin production, and lipogenesis but also accelerates lipogenesis, increases β-cell mass, and improves lipid metabolism (9). Moreover, there is a frequent inverse correlation between circulating levels of obestatin in humans and the presence of obesity and DM. Additionally, studies have demonstrated that this peptide exhibits protective metabolic effects in diabetes models, indicating its potential therapeutic value in this context (10).

Detailed analysis using NMR and circular dichroism spectroscopy revealed that human and mouse obestatin, as well as human obestatin fragments: (6-23), (11-23), and (16-23), all adopt α-helical secondary structure. Although their order is different, their structures are distinct (11). The human obestatin gene is located on chromosome 3 (3p25-26) and was initially thought to consist of four exons (12); however, further studies identified additional upstream exons (13).

Osteopontin (OPN) is a glycosylated and hyperphosphorylated protein that was initially discovered in bone tissue and has also been identified in eggshells (14). The entity in question is a negatively charged structure, consisting of three hundred amino acids and featuring a cell-binding sequence composed of arginine, glycine, and aspartate. The specific location can be found on the long arm of chromosome 4, within the 4q13 region. The OPN protein is an acidic glycoprotein with a molecular weight of 34 kDa. Following its secretion from cells, it undergoes post-translational modifications, resulting in a molecular weight ranging from 44 to 75 kDa in various mammalian cells (15). The stromal cell protein serves as a mediator for various biological functions. It plays a role in regular physiological functions. It has been linked to the development of different disease conditions, such as atherosclerosis, cancer, glomerulonephritis, as well as several chronic inflammatory disorders (16). This study aims to examine the correlation between serum levels of obestatin and OPN in patients with MetS.

2. Materials and Methods

This study recruited 125 patients with a clinical diagnosis of MetS and 40 healthy volunteers aged 20-80 years from December 2022 to May 2023 at Al-Yarmuk Teaching Hospital in Baghdad. Blood samples were collected from 40 healthy individuals and 125 patients with MetS. Blood samples were obtained in sterile gel tubes and centrifuged at 1000 x g for 15 minutes. A small aliquot of the obtained serum was then isolated and stored at -20°C until the analysis time to measure biomarker parameters.

The height and weight of each subject were measured, and then used to calculate the body mass index (BMI) in kilograms per square meter (kg/m^2) according to the following equation: $BMI = Mass / (Height)^2$.

Systolic and diastolic BP (SBP and DBP) respectively, fasting serum glucose (FSG), liver enzyme [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) in U/L units], total protein in g/dL units, and lipid profile test including:

TC, TG, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein (VLDL) using (Linear, Spain) kits were measured for each patient and control. Determination of obestatin and OPN levels was performed using commercial ELISA kits according to the manufacturer's recommendations, USA.

2.1. Statistical analysis

Data were expressed as median (25^{th} and 75^{th} percentiles) for irregularly distributed numerical variables. The Mann-Whitney test was used to describe numerical variables that were not normally distributed. The significance level was set at a *p*-value of 0.05, using receiver operating characteristic (ROC) curve analysis; obestatin and OPN cut-off values were assessed.

3. Results

As shown in **Table (1)**, there were no notable changes in age. However, significant differences in BMI were observed among the study groups.

Table 1. The demographic characteristics of MetS and controls

Variables	MetS (n= 125)	Control (n= 40)	*p*-value
Age (Year)	53 (68-42.00)	49.5 (61-35.00)	N.S
BMI (kg/m^2)	39.5 (44.50- 35.42)	20.00 (22 -19)	0.001
SBP (mmHg)	16 (16-15)	12.00 (12 -12)	0.001
DBP (mmHg)	9 (9.50-9.00)	8 (8 -8)	0.001

*The collected data were analyzed by median ($25^{th}$and 75^{th} percentiles) via the man Whitney test at the 0.05 level.

Findings showed a significant difference in BMI between the MetS group (39.5 kg/m^2) and the control group (20 kg/m^2), but not between the ages of the two groups (53 and 49.5 years, respectively). Also, SBP and DBP were 16 mmHg and 9 mmHg, respectively, for the MetS group ($p = 0.001$).

The results in **Table (2)** show that the FSG for MetS was 117 mg/dL, which was significantly different ($p= 0.001$) from that of the control group, 99 mg/dL. There were significant differences ($p= 0.001$) in the levels of ALT, AST, and ALP in the MetS group, which were recorded as 38.6, 36.4, and 83.4 U/L, compared to 29.8, 28.9, and 70.6 U/L in the healthy control group. The exception was total protein, which was slightly higher in the healthy group (6.20 g/dL) than in the MetS group (5.89 g/dL).

Table 2. Serum glucose, liver enzymes, and total proteins levels of MetS and control group

Variables	MetS (n= 125)	Control (n= 40)	*p*-value
FSG (mg/dL)	117 (136-101)	99 (104-96)	0.001
ALT(U/L)	38.60 (42.38-32.55)	29.80 (39.55-22.65)	0.001
AST(U/L)	36.40(42.00-31.00)	28.90 (37.35-21.95)	0.001
ALP (U/L)	83.40(90.65-74.63)	70.60 (90.85-59.75)	0.001
Total protein (g/dL)	5.89(6.04-5.49)	6.20 (6.64-6.04)	0.001

FSG: Fasting serum glucose, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP*:* alkaline phosphatase

The serum lipid profile results for patients and healthy groups are shown in **Table (3**). There were significant differences ($p= 0.001$) between the MetS group and the control group regarding serum cholesterol, TG, LDL-C, and VLDL. While HDL-C was significantly

decreased (p=0.001) in the MetS group compared to the healthy group.

Table 3. Serum lipid profile of MetS and control group

Variables	MetS (n= 125)	Control (n= 40)	*p*-value
Cholesterol (mg/dL)	237.00 (257.00-229.00)	151.00 (158-135)	0.001
TG (mg/dL)	282.00 (304.00-265.50)	111.00 (145.00-97.00)	0.001
HDL-C (mg/dL)	29 (30.50-27.50)	47.5 (48.50-4600)	0.001
LDL-C (mg/dL)	150.00(168.00-142.00)	72.00 (86.00-64.00)	0.001
VLDL (mg/dL)	56.00(60.00-53.00)	23.50(31.50-20.00)	0.001

*The collected data were analyzed by median at the $p < 0.05$ level. TG: Triglycerides: LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, VLDL: Very low-density lipoprotein.

The results of **Table (4)** and **Figure (1**) showed a significant (p= 0.001) decrease in obestatin level in the MetS group (280.82 pg/mL) compared to the control group (1082.1 pg/mL). While the OPN level was significantly (*p*= 0.001) higher in the MetS group (17.96 ng/mL) as compared to the healthy control group (5.47 ng/mL), as shown in **Figure (2)**.

The ROC test results for the obestatin levels demonstrated ideal cut-off values, with a sensitivity of 97.58% and a specificity of 100.00%. The area under the curve (AUC) was calculated to be 0.981, indicating a high level of accuracy in diagnosing the condition (**Figure 3**). Similarly, the OPN levels exhibited a sensitivity of 98.39% and a specificity of 100.00%, with an AUC of 0.999 (**Figure 4**). These findings suggest that both obestatin and OPN are reliable diagnostic factors. The cut-off value for the obestatin level was determined to be ≤ 577.871, while for the OPN level it was > 6.974. Values below these thresholds are indicative of patients with the condition being tested.

Table 4. Serum obestatin and OPN of MetS and control group

Variables	MetS (n= 125)	Control (n= 40)	*p*-value
Obestatin (pg/mL)	280.82 (441.89-174.90)	1082.10 (1305.66-720.79)	0.001
OPN (ng/mL)	17.96 (20.74- 10.00)	5.47(5.68-5.15)	0.001

The collected data were analyzed by the median at the $p < 0.05$ level. OPN: Osteopontin.

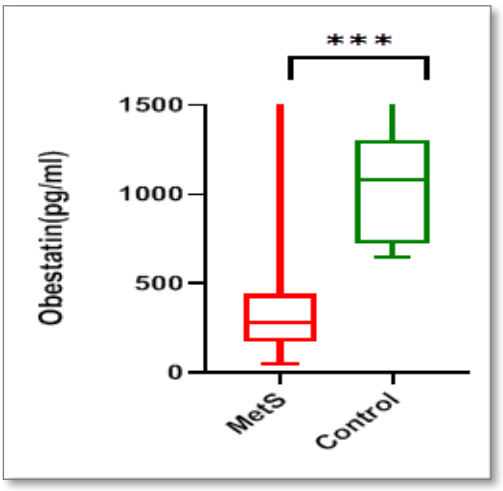

Figure 1. Median serum obestatin of MetS and control group

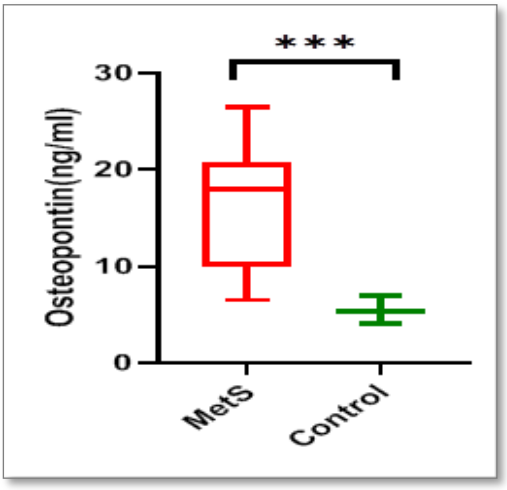

Figure 2. Median serum OPN of MetS and control group.

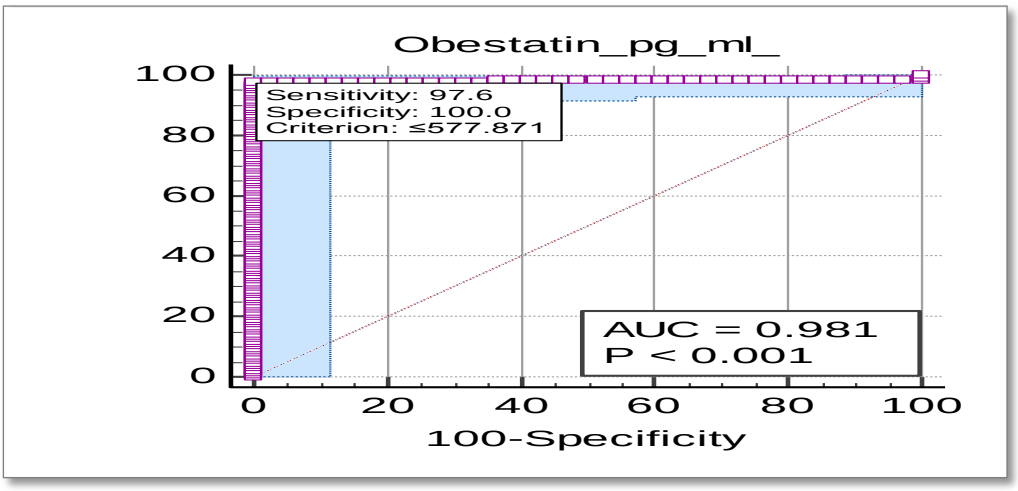

Figure 3. The ROC curve of obestatin.

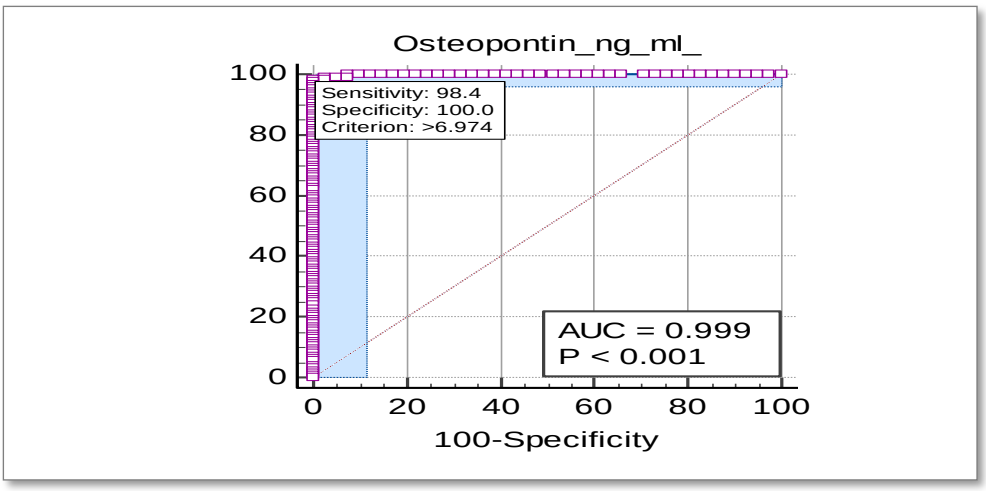

Figure 4. The ROC curve of OPN

4. Discussion

It has been proposed that MetS is a result of a complicated interplay between hereditary and environmental variables. Distress's pathophysiological causes important changes in metabolic processes. Numerous chronic disorders, including hypertension, chronic inflammation, oxidative stress, and hypertriglyceridemia, have been linked to these changes. Concern for T2DM risk factors ,i.e., obesity, IR, and fatty liver will arise from disruption of

cellular metabolic pathways, commonly known as MetS, which is particularly caused by abnormalities in carbohydrate metabolism (17,18).

A person's BMI, serves as a measure of overall health and is often associated with metabolic disease (19). This result is consistent with previous research (20), which found that the patient group had a BMI of 27.5 kg/m^2, while the control group had a BMI of 22.6 kg/m^2.

The hypertension is a serious health risk factor with disproportionate prevalence in emerging countries, contributing to increased mortality in both developed and developing countries globally (21). Due to changed lipid excretion, metabolic illnesses, and disorders, BP is intimately related to metabolic alterations and occurs in obesity (22), which is in a greement with previous study (23). So, it found an association between MetS and hypertension. A straightforward equation may be used to determine how metabolism is directly impacted by IR and how much more VLDL is produced (24). The liver excretes VLDL, which prevents the apolipoprotein B protein from being broken down and raises TG levels. When lipoprotein lipase secretion is reduced due to a high proportion of VLDL, the ratio of TG is seen to rise as the body fills with these particles. Low-density lipoprotein cholesterol counts sharply increase, offsetting a startling decline in HDL-C values (25, 26).

It has been confirmed that individuals with MetS exhibit significantly elevated liver enzyme levels compared to healthy individuals. This finding further strengthens the association between liver enzymes and the risk of developing MetS. This result is consistent with the results of the literature (27), which confirmed that the liver enzyme levels in patients with metabolic syndrome were much higher than those in healthy people, further confirming the link between liver enzymes and MetS risk.Patients with MetS are at high risk for CVD and have elevated TG levels in addition to elevated LDL-C. They also have low HDL-C levels and large numbers of small, dense LDL (sd-LDL) particles (28).

The obestatin is an appetite-suppressing hormone that reduces food intake and delays gastrointestinal motility, thereby preventing weight gain (29). This result is agreed with results of (30), who found that the serum level of obestatin was lower significantly in Mets patients as compared to the controls. While OPN is a versatile protein that plays a crucial role in the regulation of inflammatory processes, which have been implicated in the pathogenesis of atherosclerosis and MetS (31). This result is consistent with that of (32), who found elevated expression of OPN in obese individuals compared with healthy controls.The OPN levels are thought to be elevated in obesity-related diseases, suggesting that OPN may affect lipid absorption and metabolism (33). The ROC analysis, is a curve showing the discriminative power of a dual classifier as a function of the discriminative threshold (34). Results obtained from the ROC analysis suggest that these parameters are considered good diagnostic markers for MetS.

5. Conclusion

The results of this study indicate that alterations in metabolic status may impact the concentrations of serum obestatin and OPN. Hence, assessing this parameter can provide valuable insights into the diagnosis of MetS and the dysregulation of lipoprotein metabolism in individuals with obesity.

Acknowledgment

The authors thank the medical staff at Al-Yarmuk Teaching Hospital in Baghdad for their assistance in completing this work.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

None.

Ethical Clearance

This research was approved by the Scientific Committee in College of Science for Women, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment. A verbal agreement was obtained from each person included in the survey on December 13, 2022.

References

1. Stetic L, Belcic I, Sporis G, Stetic L, Starcevic N. Influence of physical activity on the regulation of disease of elderly persons with metabolic syndrome. Int J Environ Res Public Health. 2021; 18(1):275. https://doi.org/10.3390/ijerph18010275.
2. Shafeeq NK, Hussein TAA, Abass EAA. Metabolic syndrome. Ibn Al-Haitham J Pure Appl Sci. 2021; 34(3):26–38. https://doi.org/10.30526/34.3.2675.
3. Myers J, Kokkinos P, Nyelin E. Physical activity, cardiorespiratory fitness, and the metabolic syndrome. Nutrients. 2019; 11(7):1652. https://doi.org/10.3390/nu11071652.
4. Zamil AH, Amin SS. The prevalence of metabolic syndrome among university students in Wasit, Iraq. Saudi Med J. 2022; 43(11):1240–1247. https://doi.org/10.15537/smj.2022.43.11.20220558.
5. Karakoç Z, Topaloğlu U, Bayram B. Immunohistochemical distributions of leptin, ghrelin and obestatin hormones in bull and ram abomasum. Anat Histol Embryol. 2022; 51(3):411–418. https://doi.org/10.1111/ahe.12801.
6. Egido EM, Hernández R, Marco J, Silvestre RA. Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. Regul Pept. 2009; 152(1-3):61–66. https://doi.org/10.1016/j.regpep.2008.08.003.
7. Villarreal D, Pradhan G, Zhou Y, Xue B, Sun Y. Diverse and complementary effects of ghrelin and obestatin. Biomolecules. 2022; 12(4):517. https://doi.org/10.3390/biom12040517.
8. Tahir NT, Jedda WAA, ALfatlawi WR. Ghrelin and obestatin levels as a novel marker in Iraqi obese children. Baghdad Sci J. 2023;20(5):1654-1661. https://dx.doi.org/10.21123/bsj.2023.7103.
9. Mallikarjuna BG, Manjappara UV. Obestatin and rosiglitazone differentially modulate lipid metabolism through peroxisome proliferator-activated receptor-γ (PPARγ) in pre-adipose and mature 3T3-L1 cells. Cell Biochem Biophys. 2021; 79(1):73–85. https://doi.org/10.1007/s12013-020-00958-7.
10. Szentpeteri A, Lorincz H, Somodi S, Varga VE, Seres I, Paragh G, Harangi M. Human obestatin level and its correlations with metabolic syndrome components in non-diabetic obese patients. Atherosclerosis. 2017; 263:e250. https://doi.org/10.1016/j.atherosclerosis.2017.06.811.
11. Cowan E, Burch KJ, Green BD, Grieve DJ. Obestatin as a key regulator of metabolism and cardiovascular function with emerging therapeutic potential for diabetes. Br J Pharmacol. 2016; 173(14):2165–2181. https://doi.org/10.1111/bph.13502.
12. Green BD, Grieve DJ. Biochemical properties and biological actions of obestatin and its relevance in type 2 diabetes. Peptides. 2018; 100:249–259. https://doi.org/10.1016/j.peptides.2017.12.006.
13. Bora RR, Prasad R, Khatib MN. Cardio-protective role of a gut hormone obestatin: A narrative review. Cureus. 2023; 15(4):e37972. https://doi.org/10.7759/cureus.37972.
14. Lin EYH, Xi W, Aggarwal N, Shinohara ML. Osteopontin (OPN)/SPP1: From its biochemistry to biological functions in the innate immune system and the central nervous system (CNS). Int Immunol. 2023; 35(4):171–180. https://doi.org/10.1093/intimm/dxac060.

15. Ascar IF. Biochemical study on a group of Iraqi patients with thyroid cancer in relation to osteopontin [dissertation]. Baghdad: University of Baghdad, College of Sciences for Women; 2019.
16. Kitamura K. Osteopontin. In: Takei Y, Ando H, Tsutsui K, editors. Handbook of hormones. Amsterdam: Elsevier; 2021; p. 597–599. https://doi.org/10.1016/B978-0-12-820649-2.00152-2.
17. Al-Husaini FK, Hywar FA, Elias NG, Falih IQ. Evaluation of chemerin in diabetic type 2 patients with metabolic syndrome in both genders. Iran J War Public Health. 2022; 14(4): 409–414. https://doi.org/10.29252/ijwph.14.4.409.
18. Parker J. Glucose metabolism, energy production and regulation of cellular and whole-body metabolism. J Australas Coll Nutr Environ Med. 2020; 39(1):29–33. https://doi.org/10.3316/informit.154784110868319.
19. Kunzova S, Maugeri A, Medina-Inojosa J, Lopez-Jimenez F, Vinciguerra M, Marques-Vidal P. Determinants of metabolic health across body mass index categories in Central Europe: A comparison between Swiss and Czech populations. Front Public Health. 2020; 8:108. https://doi.org/10.3389/fpubh.2020.00108.
20. Arnlov J, Ingelsson E, Sundström J, Lind L. Impact of body mass index and the metabolic syndrome on the risk of cardiovascular disease and death in middle-aged men. Circulation. 2010; 121(2):230–236. https://doi.org/10.1161/CIRCULATIONAHA.109.887521.
21. Amini M, Zayeri F, Salehi M. Trend analysis of cardiovascular disease mortality, incidence, and mortality-to-incidence ratio: Results from Global Burden of Disease Study 2017. BMC Public Health. 2021; 21(401):1–12. https://doi.org/10.1186/s12889-021-10429-0.
22. Alowfi A, Binladen S, Irqsous S, Khashoggi A, Khan MA, Calacattawi R. Metabolic syndrome: Prevalence and risk factors among adolescent female intermediate and secondary students in Saudi Arabia. Int J Environ Res Public Health. 2021; 18(4):2142. https://doi.org/10.3390/ijerph18042142.
23. Natesan V, Kim SJ. Lipid metabolism, disorders and therapeutic drugs–review. Biomol Ther. 2021; 29(6):596. https://doi.org/10.4062/biomolther.2021.122.
24. Vasse J, Lassartesse A, Marmontel O, Charrière S, Bouveyron C, Marrié N. Assessment of three equations to calculate plasma LDL cholesterol concentration in fasting and non-fasting hypertriglyceridemic patients. Clin Chem Lab Med. 2023; 62(2):270–279. https://doi.org/10.1515/cclm-2023-0360.
25. Tachebele B, Abebe M, Addis Z, Mesfin N. Metabolic syndrome among hypertensive patients at University of Gondar Hospital, North West Ethiopia: A cross sectional study. BMC Cardiovasc Disord. 2014; 14(177):1–9. http://www.biomedcentral.com/1471-2261/14/177.
26. Okafor CI. The metabolic syndrome in Africa: Current trends. Indian J Endocrinol Metab. 2012; 16(1):56–66. https://doi.org/10.4103/2230-8210.91191.
27. Zhang L, Ma X, Jiang Z, Zhang K, Zhang M, Li Y, Zhao X, Xiong H. Liver enzymes and metabolic syndrome: a large-scale case-control study. Oncotarget. 2015; 6(29):26782-26788. https://doi.org/10.18632/oncotarget.5792
28. Paredes S, Fonseca L, Ribeiro L, Ramos H, Oliveira JC, Palma I. Novel and traditional lipid profiles in metabolic syndrome reveal a high atherogenicity. Sci Rep. 2019; 9:11792. https://doi.org/10.1038/s41598-019-48120-5
29. Kosmas CE, Rodriguez Polanco S, Bousvarou MD, Papakonstantinou EJ, Peña Genao E, Guzman E, et al. The triglyceride/high-density lipoprotein cholesterol (TG/HDL-C) ratio as a risk marker for metabolic syndrome and cardiovascular disease. Diagnostics. 2023; 13(5):929. https://doi.org/10.3390/diagnostics13050929
30. Szentpéteri A, Lőrincz H, Somodi S, Varga VE, Paragh G Jr, Seres I, Paragh G, Harangi M. Serum obestatin level strongly correlates with lipoprotein subfractions in non-diabetic obese patients. Lipids Health Dis. 2018; 17(1):39. https://doi.org/10.1186/s12944-018-0691-y.
31. Sarac F, Basoglu O, Gunduz C, Bayrak H, Avci CB, Akcicek F. Association of osteopontin and tumor necrosis factor-α levels with insulin resistance in obese patients with obstructive sleep apnea syndrome. J Endocrinol Invest. 2011; 34(7):528–533. https://doi.org/10.3275/7287.

32. Bartosińska J, Przepiórka-Kosińska J, Sarecka-Hujar B, Raczkiewicz D, Kowal M, Chyl-Surdacka K, Bartosiński J, Kosiński J, Krasowska D, Chodorowska G. Osteopontin serum concentration and metabolic syndrome in male psoriatic patients. J Clin Med. 2021; 10(4):755. https://doi.org/10.3390/jcm10040755.
33. Ahmad R, Al-Mass A, Al-Ghawas D, Shareif N, Zghoul N, Melhem M, Hasan A, Al-Ghimlas F, Dermime S, Behbehani K. Interaction of osteopontin with IL-18 in obese individuals: implications for insulin resistance. PLOS One. 2013; 8(5):e63944. https://doi.org/10.1371/journal.pone.0063944.
34. Nahm FS. Receiver operating characteristic curve: Overview and practical use for clinicians. Korean J Anesthesiol. 2022; 75(1):25–36. https://doi.org/10.4097/kja.21209.