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Original Research

Correlation of Plasma and Salivary Osteocalcin Levels with Nascent Metabolic Syndrome Components with and Without Pre/Diabetes Biochemical Parameters

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Abstract

Objectives: This study aimed to compare and correlate plasma and salivary levels of cardiometabolic risk biomarkers' of pharmacotherapy (appraised using colorimetric assays), adiposity, and atherogenicity indices. **Methods:** 61 Nascent MetS subjects vs. 30 lean normoglycemic and healthy controls were recruited in Family Medicine outpatient clinics/Jordan University Hospital (a referral medical center). Fasting blood and saliva specimens were collected. Clinical and anthropometric variables were determined along with atherogenecity and adiposity indices. **Results:** Among nascent MetS (metabolic syndrome) recruits, almost half were normoglycemic, 43% were prediabetic and 8% were diabetic. Pronouncedly Glycemic (FPG and Alc) and lipid parameters (TG, HDL-C and non-HDL-C), adiposity indices (BMI, WHR, WtHR, Conicity-index, BAI, LAP, VAI) and atherogenicity indices (AIP, TC/HDL-C, LDL-C/HDL-C, non-HDL-C/HDL-C and TG/HDL-C) were higher in the nascent MetS group (P<0.05 vs. controls). Markedly among the plasma cardiometabolic risk biomarkers (P<0.05 vs. controls) in the nascent MetS group, adipolin, cathepsin S, ghrelin, irisin, LBP, leptin, and osteocalcin were higher but plasma FGF1 levels were oddly lower. Significantly (P<0.05 vs. controls) nascent MetS –linked salivary levels of adipolin and LBP were higher as opposed to the lower cathepsin S. Only osteocalcin, amongst 9 metabolic risk biomarkers studied, had remarkably significant correlation between plasma and saliva levels, in both total sample and MetS patients (P<0.05). Markedly in the nascent MetS only group, both plasma and salivary osteocalcin correlated with FPG and A1c (P<0.05); salivary osteocalcin correlated with BMI and LAP (P<0.05). Likewise, in the total sample plasma osteocalcin correlated significantly with BMI, BAI, WHt R, SBP, DBP, TG, LAP, VAI, TG/HDL-C and AIP (P<0.05), while salivary osteocalcin had substantial correlations only with FPG and A1c (P<0.05). **Conclusion:** Association of nascent MetS-related plasma and salivary osteocalcin levels and clinical characteristics and indices propagate salivary osteocalcin as a non-invasive marker for clinical control of MetS-/preDM.

Keywords: adipolin, cathepsin; FGF1 (Fibroblast growth factor 1); ghrelin; irisin; klotho; LBP (lipopolysaccharide binding protein); Leptin, and Osteocalcin; adiposity and atherogenicity indices; cardiometabolic risk; nascent metabolic syndrome and prediabetes

INTRODUCTION

Obesity-linked complications and disorders, metabolic syndrome (MetS) and prediabetes (preDM) are presently major social and epidemiological health issues that can be, among others, strong risk factors for cardiovascular diseases and atherosclerosis.[1] Salivary clinical parameters (glucose[2]) cytokines, adipokines (adiponectin, IL(interleukin)-8 and -10, resistin, TNF (tumor necrosis factor)-α, MMP (Matrix metalloproteinase)-2 [3]) and proteins (pentraxin;[4] cystatin;[5]omentin and visfatin;[6] chemerin;[7] Myeloperoxidase;[8] HGF (hepatocyte growth factor)[9]) have been suggested to be discriminate non-invasive makers for clinical diagnosis, screen of development and progression of obesity related complications, MetS/preDM and, more importantly, prevention of gender related vascular complication.[10-11] Outstandingly; the 48h decreased saliva/serum irisin concentrations in the acute myocardial infarction was taken for a promising candidate biomarker for cardiomyopathy diagnosis.[12] Invariably the suggestive assessment of the variables influencing pathophysiologies of childhood can lead to improved control via early diagnosis in high-risk children.[13] Furthermore A practical, cost-effective, minimally invasive, salivary multimarker assessment platform has the potential to evade the limitation of conventional ''golden standard'' laboratory blood-based testing approaches, and thereby addressing a major unmet need in monitoring progress and management and of metabolic anomalies and derangement patients.[14-17] More so they can be pictured as future therapeutic targets associated with decreased morbidity and mortality through early diagnosis.

In this research we focused on adipose- and/or skeletal muscle-derived signaling as examples of metabotrophic factors (MTFs) involved in the pathogenesis of obesity and related cardiometabolic diseases.[18] Hence collectively a battery of adipolin, cathepsin, FGF1 (Fibroblast Growth Factor 1), ghrelin, irisin, klotho, LBP (Lipopolysaccharide Binding

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Protein), Leptin, and Osteocalcin proportionally associated with insulin resistance related adiposity and cardiometabolic risk factors. Hence it was the aim of this study to examine the early cardiometabolic risk associations of these peptides with adiposity – and atherogenecity –related insulin resistance in normoglycemic and dysglycemic MetS population. Scarcity of studies that investigated correlations between plasma and salivary cardiomatabolic biomarkers' levels in MetS patients is clearly noticeable. Moreover given that saliva biomarkers seem to be promising in the area of MetS detection and diagnosis due to less invasive nature, less expensive and faster sample collection in comparison to plasma biomarkers.16 Taken together it was this study aim to investigate the potential correlations between plasma and saliva levels of these cardiometabolic biomarkers for pharmacotherapy institution and follow up reasons of MetS patients with a defined cluster of adiposity and atherogenecity indices.

Adipolin (CTRP12), an insulin-sensitizing adipokine that inhibits gluconeogenesis and increases glucose uptake in hepatocytes and adipocytes; in addition to its anti-inflammatory action.[19] Among patients on hemodialysis, adipolin was significantly lower in the obese compared to the normal weight participants.[19]

Cathepsin S is a liver lysosomal cysteine protease that controls antigen presentation and is investigated extensively in autoimmune diseases.[20] Besides, cathepsin S controls adipocyte differentiation and improves glycemic control by reducing hepatocyte gluconeogenesis and glucose output.[20] Cathepsin S; as an adiposity biomarker expressed in white adipose tissue (WAT); its elevated circulating concentrations strongly and independently associated with MetS in overweight and obese Chinese adults.[20-21]

FGF1 (Fibroblast Growth Factor 1) is expressed liver, kidney, and brain, but most notably it is highly upregulated in WAT following a high fat diet (HFD) challenge.[22] FGF1 improves insulin resistance via repression of JNK (c-Jun N-terminal kinase)-mediated inflammation.[22]

Ghrelin is a neuropeptide hormone containing 28 amino acids released mainly by the parietal cells of the stomach and stimulating appetite.[23] Ghrelin possesses orexigenic and lipogenic effects and originally identified as a growth hormone secretagogue, with significant roles in glucose regulation; as the unacylated ghrelin counters hyperglycemia and enhances insulin sensitivity.[18] Distinctively plasma levels of low ghrelin in T2D patients but high levels in periodontitis were defined.[18]

Irisin is an insulin sensitizing adipomyokine released by both skeletal muscles and fat after exercise and shows a strong association with metabolic and cardiovascular diseases. Irisin stimulates the browning of WAT.[24] Irisin is regulated by Peroxisome Proliferator-Activated Receptor-γ Co-activator 1-α (PGC1-α) and hence it mediates thermogenesis by increasing uncoupling protein 1 levels.[24]

Klotho is a transmembrane protein enzyme, with essential components of endocrine Fibroblast Growth Factor (FGF) receptor complexes, and its blood circulating form affecting angiogenesis, energy metabolism, endothelial nitric oxide synthesis, antioxidant enzyme production, protection against endothelial dysfunction and aging.[25] Klotho is closely linked to many age-related biomarkers, cardiometabolic problems, kidney dysfunction, frailty, and functional disability.[26]

LBP (Lipopolysaccharide Binding Protein) is a 58-kDa glycoprotein synthesized in the liver that is released into circulation as a type I acute-phase reactant shortly after bacteremia or endotoxemia; in the circulation, LBP forms a complex with lipopolysaccharide (LPS; endotoxin) that triggers a cascade of inflammatory cytokines.[27] There is evidence of increased secretion of LBP from adipose tissue of patients with MetS.[27] Obesity, and henceforth LBP; are associated with enhanced microbial translocation.[28]

Leptin; as an neuroendocrine peptide secreted by adipocytes; influences control of food intake, body weight and energy homeostasis, lipid metabolism and insulin sensitivity.[29] Leptin upregulates proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6; these are associated with insulin resistance and T2D.[1] Leptin has renal and sympathetic actions and is involved in the pathogenesis of obesity- and MetS- linked hypertension. Leptin resistance may result in insulin resistance and beta cell dysfunction, leading to T2D. Furthermore, leptin has proliferative, pro-inflammatory, pro-thrombotic, and pro-oxidative with lipotoxic effects.[29] Leptin also appears to regulate reproduction and puberty, to prevent ectopic lipid deposition, and to link the immune and endocrine systems.[29]

Osteocalcin, a small osteoblast-specific secreted protein, acts as a hormone by stimulating insulin production and increasing energy expenditure and insulin sensitivity in target organs. Its exogenous application in Animal studies prevented obesity and glucose intolerance with marked impact on regulation of glucose and energy homeostasis in humans.[30] Osteocalcin is a circulating biomarker; synthesized by vitamins K- and D-dependent pathways. It is indispensible to bone mineralization and calcium homeostasis.[19,31] Osteocalcin was assigned an essential role in glucose and fat regulation demonstrated in vitro and in animal models. Most Osteocalcin is incorporated into the extracellular bone matrix; however, its undercarboxylated fraction (ucOC) is released into the bloodstream. UcOC can act directly on pancreatic beta cells and on adipocytes, regulating insulin secretion and insulin sensitivity. These findings have assigned a new role to the bone as an endocrine organ with extra-skeletal functions.[32]

AIM

This cross sectional study meant to compare and correlate clinical parameters, adiposity and atherogenecity indices as well as plasma and saliva levels of 9 cardiometabolic risk biomarkers of pharmacotherapy (arranged alphabetically): adipolin, cathepsin S, FGF1, ghrelin, irisin, klotho, LBP, leptin, and osteocalcin in overweight (BMI >25 kg/m^2) or obese (BMI >30 kg/m^2) drug-naïve ''nascent'' MetS subjects (with 3 or more of MetS criteria).[33,34]

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METHODS

Patients and study design

This is a cross sectional study aimed to examine the relation between plasma and salivary levels of 9 cardiometabolic risk biomarkers in two groups of adult (18-75 years) Jordanian patients (Figure1).

Control group that included 31 healthy individuals who were apparently healthy, normoglycemic (a fasting plasma glucose (FPG) <100 mg/dL or a hemoglobin A1c (A1C)<5.7% [35]) and lean (BMI<25 kg/m^2) and

Nascent MetS 61 recruits according to the new IDF (International Diabetes Federation) definition; for a person to be defined as having the MetS, they must have central obesity (defined as waist circumference with ethnicity specific values)* plus any two of four additional factors. These four factors are shown in Table 1. Nascent MetS participants were either:

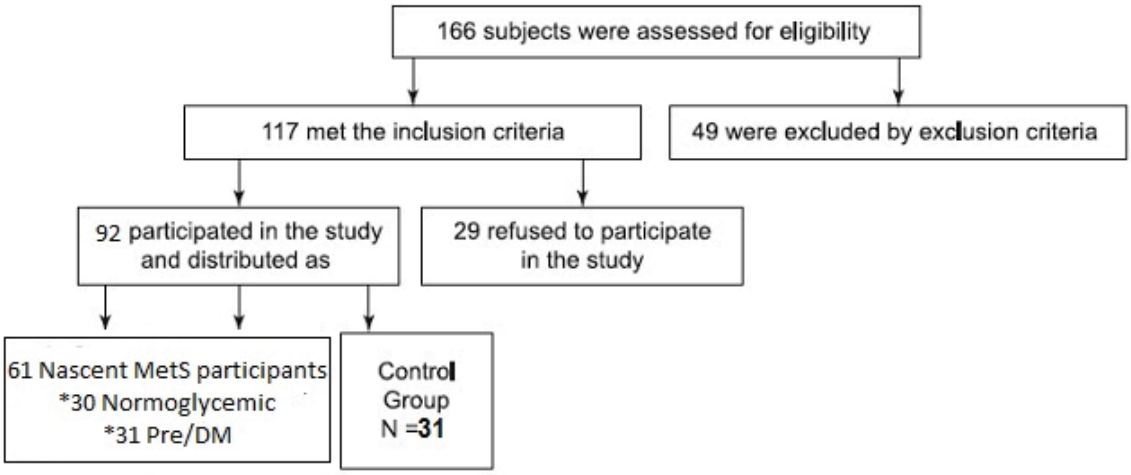

Figure 1. Recruitment Flowchart

Normoglycemic 30 cases - who were normoglycemic (FPG<100 mg/dL or a hemoglobin A1c (A1C)<5.7%[35])

Prediabetes 26 cases- a FPG of 100–125 mg/dL, or a 2-hour plasma glucose level of 140 mg/dL–199 mg/dL during a 75-g oral glucose tolerance test (OGTT), or A1C of 5.7%–6.4%; or

Type 2 diabetes mellitus 5 cases- a FPG level of ≥126 mg/dL, or OGTT, or a random plasma glucose of 200 mg/dL or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, or HbA1c level of 6.5% or higher.[35]

Table 1.IDF Metabolic Syndrome (MetS) World-Wide Definition	
For Eastern Mediterranean and Middle East population, the measure of central obesity include waist circumference of ≥ 94 cm for males and ≥ 80 cm in females. If body mass index is > 30 kg/m^2 then central obesity can be assumed, and waist circumference does not need to be measured.	
Raised triglycerides	≥ 1.7 mmol/l (150 mg/dL)or specific treatment for this lipid abnormality
Reduced HDL-cholesterol	< 1.03 mmol/l (40 mg/dL) in males < 1.29 mmol/l (50 mg/dL) in females or specific treatment for this lipid abnormality
Raised blood pressure	Systolic: ≥ 130 mmHg Or Diastolic: ≥ 85 mmHg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	Fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dL) or previously diagnosed Type 2 diabetes If > 5.6 mmol/l or 100 mg/dL, oral glucose tolerance test is strongly recommended but is not necessary to define presence of the syndrome

Exclusion criteria

Non-fasting individuals, Pregnant or breast feeding/lactating women

Any prior use of anti-diabetic or lipid lowering agents

Clinical evidence of autoimmune, inflammatory bowel disease, alcohol, drug abuse, and recently diagnosed and untreated endocrine disorder other than prediabetes or diabetes mellitus.

Study Protocol

The study was approved by the Jordan University Hospital (JUH) Institutional Review Board (IRB). All procedures performed in the study were in accordance with the ethical standards of the IRB, the Scientific Research Committee at the School of Pharmacy, JU and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The potential study participants were approached randomly during their visits to the Family Medicine Clinic at the JUH. Participants were interviewed and their medical files were reviewed in order to assess the inclusion and exclusion criteria and in order to distribute them into the study groups. The eligible participants were informed in detail about the study. All potentially eligible candidates were informed thoroughly about the study; and gave their written consent in Arabic. Participation in the study was voluntary. Patient recruitment took place between July 2017 and until May 2020; who were coded according to the study arm. Data collection of patients' medical and family history was conducted for all study participants alongside with reviewing their medical file to collect clinical information and laboratory data.

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Anthropometric measurements, Clinical settings, and colorimetric bioassays of cardiometabolic risk biomarkers of pharmacotherapy in both plasma and saliva specimens

The anthropometric data such as weight and height using a balance mounted stadiometer were measured at JUH Family Medicine outpatient Clinics. Waist circumference (WC) was measured using a non-stretchable tape at the midpoint between the last rib and the upper iliac crest, and hip circumference (HC) was measured around the widest section of the buttocks. Systolic blood pressure (SBP) and diastolic blood (DBP) pressures were measured using an electronic sphygmomanometer. Adiposity and atherogenecity indices were calculated using formulae.[36-38]A venous blood was drawn from each candidate after 12 hours fasting to assess the levels of FPG and lipid profile as well as the plasma and salivary levels of biomarkers. The biochemical analysis of fasting lipid profile (HDL-C, LDL-C, TG, and TC), FPG, and HbA1c were performed for each participant. Table 2 displays the indices that were used in this study.

Table 2. Comparison of demographic, clinical laboratory parameters, adiposity and atherogenicity indices between the study groups

Characteristic	**Total sample (N=92), N (%)**	**Controls (N=31), N (%)**	**MetS (N=61), N (%)**	**P**
Demographic and clinical characteristics				
Gender Female Male	 68 (73.9) 24(26.1)	 24 (77.4) 7 (22.6)	 44 (72.1) 17 (27.9)	 0.585#
Diabetes status among MetS patients Prediabetes Diabetes Normoglycemic			 26 (42.6) 5 (8.2) 30 (49.2)	
	Total sample (N=92), Mean (±SD) or Median [interquartile range]	**Control (N=31), Mean (±SD) or Median [interquartile range]**	**MetS (N=61), Mean (±SD) or Median [interquartile range]**	**P**
Age (years)	48.62 (11.21)	43.29 (11.72)	51.11 (10.16)	**0.001**
SBP (mm Hg)	131.98 (15.21)	116.62 (10.22)	139.16 (11.35)	**<0.001**
DBP (mm Hg)	81.88 (11.50)	72.38 (10.18)	86.31 (9.22)	**<0.001**
FPG (mg/dL)	91.90 [19.00]	86.46 (7.44)	96.70 [18.00]	**<0.001^**
A1c (%)	5.40 [0.8]	5.10 [0.50]	5.60 [0.70]	**<0.001^**
TG (mg/dL)	152.00 [141]	95.16 (30.54)	171.00 [125.00]	**<0.001^**
LDL-C (mg/dL)	138.41 (39.22)	128.05 (28.01)	143.25 (39.25)	0.094
HDL-C (mg/dL)	49.51 (15.93)	57.21 (112.37)	45.92 (16.24)	**<0.001**
Non-HDL-C (mg/mL)	159.03 (44.17)	147.00 [55.00]	169.49 (41.03)	**<0.001^**
TC (mg/dL)	208.54 (45.41)	193.23 (40.40)	215.41 (46.39)	0.054
Adiposity indices				
BMI (kg/m^2)	29.32 [9.99]	23.53 (1.67)	30.37 [10.84]	**<0.001^**
WHR	0.91 (0.06)	0.88 (0.07)	0.92 (0.05)	**0.002**
WHtR	0.62 (0.09)	0.54 (0.05)	0.66 (0.08)	**<0.001**
C-index	1.33 [0.13]	1.30 (0.11)	1.33 [0.09]	**0.01^**
BAI	35.37 [12.48]	30.10 (4.76)	39.30 (8.70)	**<0.001**
LAP	61.28 [75.28]	24.29 [23.84]	100.67 [81.47]	**<0.001^**
VAI	2.35 [2.38]	1.36 (0.56)	3.15 [3.05]	**<0.001^**
Atherogenicity indices				
AIP	0.49 [0.51]	0.22(0.22)	0.59 [0.39]	**<0.001^**
TC/HDL-C	4.31 [1.82]	3.08 [1.76]	4.71 [1.90]	**<0.001^**
LDL-C/HDL-C	3.01 [1.42]	2.48 (1.05)	3.14 [1.47]	**<0.001^**
Non-HDL-C/HDL-C	3.85 (4.44)	2.57 (1.18)	3.71 [1.91]	**<0.001^**
TG/HDL	3.11 [3.87]	1.86 (0.88)	3.91 [3.84]	**<0.001^**

Abbreviations: AIP – atherogenic index of plasma; A1c – glycated hemoglobin; BAI - body adiposity index;
BMI – body mass index; C-index - conicity index; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL- C – high-density lipoprotein-cholesterol; LAP – lipid accumulation product; LDL-C – low-density lipoprotein-cholesterol; MetS – metabolic syndrome; non-HDL-C – non—high-density lipoprotein-cholesterol; SBP –systolic blood pressure; TC – total cholesterol; TG – triglycerides; WHR - waist to hip ratio ; WHtR - waist-to-height ratio;*Normally distributed data are presented as means (SD), not normally distributed data are presented as median [interquartile range].#Comparison between MetS and control was made by Chi square. ^ Comparison between MetS and Control was made by Mann-Whitney test.

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Cathepsin S, leptin, LBP (Lipopolysaccharide Binding Protein), FGF1 (fibroblast growth factor 1), and osteocalcin were procured from Abcam (Cambridge, MA, USA). Adipolin, ghrelin, irisin, and klotho were obtained from MyBiosourse, Inc. (San Diego, CA, USA). Markers' plasma and salivary levels were assayed according to manufacturers' instructions with intra- and interassay precisions of <10-<12% (UV-VIS spectrophotometer used was Spectro Scan 80D UV-VIS spectrophotometer (Sedico Ltd., Nicosia, Cyprus)). Harvested plasma (from lithium heparin collection tubes centrifuged at 4000 rpm for 10 minutes) were immediately stocked at -80°C until analysis. All saliva samples were collected via passive drool method into SalivaBio Saliva Collection Device (Salimetrics, Carlsbad, CA, USA).Immediately after collection, saliva samples were centrifuged for 15 min at 4000 rpm to remove any particles or sediments and supernatants using 2ml cryovials were stored at−70 ◦C until analysis.

Statistical analysis

Data were entered and analyzed via IBM SPSS© statistics 22 (SPSS, Inc., USA). Shapiro-Wilk test for was used for the assessment of normality of data distribution. Categorical data were expressed as numbers (%), normally distributed continuous data were expressed as mean (±SD), and not normally distributed continuous data were expressed as median [interquartile range]. Gender differences between the study groups were tested using Chi-square test. While comparing continuous independent variables between the study groups we used the independent sample t-test for normally distributed data and Mann-Whitney test for data that were not normally distributed. Spearman correlation test was used for the assessment of correlations between plasma and salivary metabolic risk biomarkers as well as of selected biomarkers and clinical and laboratory parameters in both the total study sample and the MetS patients alone. Correlations were considered very strong, if correlation coefficient was at least 0.8; moderately strong, if the coefficient was 0.6 up to 0.8; fair, if the coefficient was 0.3 to 0.5 and poor if the coefficient was less than 0.3.[39] For all statistical tests, p value < 0.05 was determined as statistically significant.

RESULTS

Demographic and Clinical Characteristics (Table 2)

About three quarters of the participants were females, with gender distribution similar between the two study groups (P=0.585). Among MetS patients, almost half were normoglycemic, about 43% were prediabetic and about 8% were diabetic. The average age of study participants was 48.6 years, with MetS group being significantly older than the control group (P value <0.001). In accordance to the study selection criteria, glycemic (FPG and A1c) and lipid parameters (TG, HDL-C and non-HDL-C), adiposity indices (BMI, WHR, WtHR, C-index, BAI, LAP, VAI) and atherogenicity indices (AIP, TC/HDL-C, LDL-C/HDL-C, non-HDL-C/HDL-C and TG/HDL-C) were all significantly higher in the MetS group compared to the control group (P value <0.05)

Plasma and salivary metabolic risk biomarkers levels in MetS patients (Table 3)

Among the plasma metabolic risk biomarkers, adipolin, cathepsin S, ghrelin, irisin, LBP, leptin, and osteocalcin were significantly higher (P value <0.05) in the MetS group compared to the control group. Notably, ghrelin concentration in the MetS group exceeded that of control more than 50,000-fold, while lipocalin concentration in the MetS group exceeded that

Table 3. Comparison of plasma and salivary cardiometabolic risk biomarkers between the study groups

Biomarker	Total sample (N=92), Mean (±SD) or Median [interquartile range]	Control (N=31), Mean (±SD) or Median [interquartile range]	MetS (N=61), Mean (±SD) or Median [interquartile range]	P value
p. Adipolin (ng/mL)	0.80 [0.35-1.12]	0.27 [0.15]	0.95 [0.55]	<0.001^
s. Adipolin (ng/mL)	2.30 [2.10]	1.31 [3.43]	2.44 (1.35)	0.013^
p.Cathepsin S (pg/mL)	45300.00 [24525.00]	40885.1 (10130.810	48250.00 [24750.00]	<0.001^
s.Cathepsin S (pg/mL)	18944.7.00 (10722.32)	23028.57 (11300.00)	17038.89 (10006.34)	0.033
p.FGF 1 (pg/mL)	301.25 [292]	393.62 (192.04)	250.00 [250.00]	0.027^
s.FGF 1 (pg/mL)	41.00 [60.00]	31.33 [30.00]	77.84 (71.66)	0.193^
p.Ghrelin (pg/mL)	17.00 [164.13]	0.00118 [0.620]	62.18 [210.12]	<0.001^
s.Ghrelin (pg/mL)	2.05 [22.5]	3.22 [13.20]	1.49 [37.9]	0.273^
p.Irisin (ng/mL)	167.97 [112.1]	118.50 (61.99)	183.38 [106.8]	0.001^
s.Irisin (ng/mL)	15.73 [5.80]	15.33 [5.8]	15.73 [6.10]	0.699^
p.Klotho (ng/mL)	0.70 [0.31]	0.74 [0.33]	0.66 [0.29]	0.176^
s.Klotho (ng/mL)	0.97 [0.28]	1.02 (0.27)	0.97 [0.23]	0.216^
p.LBP (ng/mL)	6446.42 (1784.43)	5100.58 (1312.25)	7074.47 (1628.90)	<0.001
s.LBP (ng/mL)	293.83 [182.72]	226.62 (80.17)	328.12 (116.23)	<0.001
p.Leptin (pg/mL)	2783.25 [3567.0]	1821.07 (1291.14)	3409.50 [4228.50]	<0.001^
s.Leptin (pg/mL)	102.50 [75.0]	124.38 (60.52)	95.00 [68.00]	0.086^
p.Osteocalcin (ng/mL)	26.62 [4.82]	23.90 (3.29)	27.91 [3.67]	0.001^
s.Osteocalcin (ng/mL)	0.25 [0.11]	0.26 (0.07)	0.25 [0.12]	0.894^

Abbreviations: FGF 1 – fibroblast growth factor 1; LBP – lipopolysaccharide binding protein; *Normally distributed data are presented as means (SD), not normally distributed data are presented as median [interquartile range]. #Comparison between MetS and control was made by Chi square. ^ Comparison between MetS and Control was made by Mann-Whitney test.

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of control more than 17-fold. On the other hand, the plasma FGF1 levels were significantly lower (P value <0.05) in the MetS group compared to the control group. Among the salivary biomarkers; adipolin and LBP were significantly higher (P value <0.05) in the MetS group compared to the control group, as opposed to cathepsin S that was significantly lower (P value <0.05) in the MetS group compared to the control group

Correlations between salivary and plasma levels of metabolic risk biomarkers (Table 4)

Only osteocalcin, amongst 9 metabolic risk biomarkers studied, had significantly fair correlation between plasma and saliva, in both total sample and MetS patients (P value <0.05)

Correlations of plasma and salivary osteocalcin with clinical and biochemical parameters, adiposity and atherogenecity indices (Tables 5-6)

Likewise, in the total sample plasma osteocalcin correlated significantly and appreciably with BMI, BAI, WHt R, SBP, DBP, TG, LAP, VAI, TG/HDL-C and AIP (P value <0.05), while salivary osteocalcin showed correlations only with FPG and A1c (P<0.05). In the MetS only group, both plasma and salivary osteocalcin correlated markedly and fairly with FPG and A1c (P value <0.05); additionally, salivary osteocalcin correlated pronouncedly and moderately with BMI and LAP (P value <0.05)

DISCUSSION

Saliva has been progressively studied as a non-invasive and relatively stress-free diagnostic alternative to blood and, hence, it may mirror alterations in systemic biomarkers' concentrations.[40] Very recently human saliva was found to contain irisin and its level was significantly higher than the serum levels in both obese and normal weight subjects.[12] Where assaying oxytocin levels in saliva was feasible;[40] it was reported that salivary oxytocin levels remain elevated for more than two hours after intranasal oxytocin administration.[40] Besides salivary oxytocin levels have been found to be correlated to plasma oxytocin levels.[41] Noninvasive salivary assessments of

Table 4. Correlations between plasma and salivary concentrations of cardiometabolic risk biomarkers of pharmacotherapy

Marker	Spearman's correlation	Total sample	MetS patients
Adipolin (ng/mL)	Correlation Coefficient	0.180	-0.006
	Sig. (2-tailed)	0.098	0.962
	N	86	57
Cathepsin S (pg/mL)	Correlation Coefficient	0.020	0.049
	Sig. (2-tailed)	0.856	0.716
	N	87	58
FGF1 (pg/mL)	Correlation Coefficient	0.084	0.068
	Sig. (2-tailed)	0.449	0.621
	N	84	55
Ghrelin (pg/mL)	Correlation Coefficient	-0.177	-0.141
	Sig. (2-tailed)	0.098	0.292
	N	88	58
Irisin (ng/mL)	Correlation Coefficient	0.073	-0.005
	Sig. (2-tailed)	0.506	0.973
	N	85	57
Klotho (ng/mL)	Correlation Coefficient	-0.086	-0.13
	Sig. (2-tailed)	0.436	0.336
	N	84	57
LBP (ng/mL)	Correlation Coefficient	0.174	-0.037
	Sig. (2-tailed)	0.108	0.781
	N	86	59
Leptin (pg/mL)	Correlation Coefficient	-0.110	-0.031
	Sig. (2-tailed)	0.315	0.814
	N	86	59
Osteocalcin (ng/mL)	**Correlation Coefficient**	**0.231***	**0.341****
	Sig. (2-tailed)	**0.035**	**0.009**
	N	**84**	**57**

Abbreviations: FGF 1 – fibroblast growth factor 1; LBP – lipopolysaccharide binding protein

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Table 5a. Correlations of plasma and salivary osteocalcin with clinical and demographic parameters and adiposity indices in the total sample

		Age	C-index	BMI	BAI	WHR	WHtR	SBP	DBP	FBG	A1C	TG
p.Osteocalcin (ng/mL)	Correlation Coefficient	0.104	0.194	**0.431****	**0.374****	0.109	**0.377****	**0.321****	**0.278****	-0.029	0.048	**0.297****
	Sig. (2-tailed)	0.333	0.070	**0.000**	**0.000**	0.313	**0.000**	**0.002**	**0.009**	0.786	0.656	**0.005**
	N	88	88	**88**	**88**	88	**88**	**88**	**88**	88	88	**88**
s.Osteocalcin (ng/mL)	Correlation Coefficient	0.063	-0.141	0.114	0.126	-0.056	0.080	0.047	-0.016	**-0.324****	**-0.331****	0.170
	Sig. (2-tailed)	0.560	0.189	0.289	0.241	0.606	0.461	0.662	0.886	**0.002**	**0.002**	0.113
	N	88	88	88	88	88	88	88	88	**88**	**88**	88

Table 5b. Correlations of plasma and salivary osteocalcin with atherogenecity indices in the total sample (continued)

		LAP	LDL-Cl	HDL-C	TC	Non-HDL-C	NonHDL-C/ HDL-C	VAI	TC/HDL	LDL/HDL	TG/ HDL-C	AIP
p.Osteocalcin (ng/mL)	Correlation Coefficient	**0.406****	0.020	-0.112	0.107	0.147	0.174	**0.298****	0.171	0.087	**0.280****	**0.280****
	Sig. (2-tailed)	**0.000**	0.850	0.299	0.323	0.173	0.106	**0.005**	0.111	0.422	**0.008**	**0.008**
	N	**88**	88	88	88	88	88	**88**	88	88	**88**	**88**
s.Osteocalcin (pg/mL)	Correlation Coefficient	0.161	0.144	0.009	0.104	0.152	0.058	0.129	0.059	0.089	0.125	0.125
	Sig. (2-tailed)	0.134	0.182	0.936	0.334	0.156	0.590	0.230	0.582	0.410	0.245	0.245
	N	88	88	88	88	88	88	88	88	88	88	88

Abbreviations: AIP – atherogenic index of plasma; A1c – glycated hemoglobin; BAI - body adiposity index; BMI – body mass index; C-index - conicity index; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL- C – high-density lipoprotein-cholesterol; LAP – lipid accumulation product; LDL-C – low-density lipoprotein-cholesterol; MetS – metabolic syndrome; non-HDL-C – non—high-density lipoprotein-cholesterol; p - plasma; s - salivary; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; VAI – visceral adiposity index; WHR - waist to hip ratio -; WHtR - waist-to-height ratio

Table 6a. Correlations of plasma and salivary osteocalcin with clinical and demographic parameters and adiposity indices in 61 MetS patients

		Age	C-index	BMI	BAI	WHR	WHtR	SBP	DBP	FBG	A1C	TG
p.Osteocalcin (ng/mL)	Correlation Coefficient	-0.045	0.147	0.211	0.228	-0.010	0.202	0.227	0.148	**-0.376****	**-0.317***	0.118
	Sig. (2-tailed)	0.735	0.267	0.108	0.082	0.937	0.125	0.084	0.263	**0.003**	**0.014**	0.372
	N	59	59	59	59	59	59	59	59	**59**	**59**	59
s.Osteocalcin (ng/mL)	Correlation Coefficient	-0.023	-0.188	**0.264***	0.224	-0.146	0.131	0.069	-0.024	**-0.377****	**-0.465****	0.208
	Sig. (2-tailed)	0.865	0.154	**0.043**	0.088	0.268	0.322	0.605	0.855	**0.003**	**0.000**	0.114
	N	59	59	**59**	59	59	59	59	59	**59**	**59**	59

Table 6b. Correlations of plasma and salivary osteocalcin with atherogenecity indices in 61 MetS patients (continued)

		LAP	LDL-C	HDL-C	TC	Non-HDL-C	Non HDL-C/ HDL-C	VAI	TC/HDL	LDL/HDL	TG/HDL-C	AIP
p.Osteocalcin (ng/mL)	Correlation Coefficient	0.250	0.078	0.138	0.128	0.113	0.002	0.068	0.003	-0.060	0.056	0.056
	Sig. (2-tailed)	0.057	0.558	0.298	0.336	0.395	0.985	0.608	0.981	0.649	0.674	0.674
	N	59	59	59	59	59	59	59	59	59	59	59
s.Osteocalcin (ng/mL)	Correlation Coefficient	**0.277***	0.093	0.077	0.054	0.126	0.038	0.127	0.039	0.027	0.128	0.128
	Sig. (2-tailed)	**0.034**	0.485	0.561	0.685	0.342	0.777	0.337	0.771	0.838	0.334	0.334
	N	**59**	59	59	59	59	59	59	59	59	59	59

Abbreviations: AIP – atherogenic index of plasma; A1c – glycated hemoglobin; BAI - body adiposity index; BMI – body mass index; C-index - conicity index; DBP – diastolic blood pressure; FPG – fasting plasma glucose; HDL- C – high-density lipoprotein-cholesterol; LAP – lipid accumulation product; LDL-C – low-density lipoprotein-cholesterol; MetS – metabolic syndrome; non-HDL-C – non—high-density lipoprotein-cholesterol; p - plasma; s - salivary; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; VAI – visceral adiposity index; WHR - waist to hip ratio

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progression and clinical control of metabolic anomalies and derangements' make it feasible to conduct frequent monitoring in clinical settings, as well as in nonclinical-care settings such as at home-based testing thereby circumventing the limitation of conventional laboratory blood-based testing.[17-18]

Adipolin, Cathepsin S, and FGF1

To the best of our knowledge, this is the first work to investigate salivary levels of adipolin, cathepsin S, FGF1 and ghrelin in nascent MetS patients. In a remarkable consistency with our study outcomes[42] both plasma and salivary adipolin and plasma cathepsin S were significantly higher in nascent MetS (both non- and pre-DM) group compared to normoglycemic and lean controls. It was previously reported that coenzyme Q10 supplementation in overweight and obese patients with diabetes improved glucose homeostasis, via a decrease in both adipolin level simultaneously with HbA1c.[42] Noticeably in a community-based cohort study in elderly men; positive correlation was retained between higher cathepsin S with a higher risk for developing diabetes.[43] Conversely saliva cathepsin S levels (in an odd dissimilarity to its plasma levels) were decreased in MetS patients vs. controls. There was no correlation between the plasma and salivary levels of either adipolin or cathepsin S of in the whole study sample and the MetS only patients. Comparable to cystatin C findings in post-myocardial infarction patients;[43] we found lack of correlation between plasma and saliva levels of molecular predictive biomarkers (adipolin or cathepsin S), FGF1 or ghrelin, thereby lacking on differentiation between MetS and controls in the total sample. Interestingly markedly lower levels of plasma (but not saliva) FGF1 in nascent MetS patients were reported; with specifically further reductions in FGF1 circulation concentrations in the prediabetic (but not normoglycemic) MetS group.[44] Though highly unlikely; FGF-1 was increased in newly diagnosed type 2 diabetes patients and obese children and adolescents.[44]

Ghrelin

Substantially in comparison to controls; a significant thousands-fold ghrelin increase in plasma (but not saliva) of MetS patients was delineated. More specifically increased ghrelin plasma level was shown in preDM/MetS (but not normoglycemic MetS) participants vs. controls.[45] As opposed to our results, salivary concentrations of active and inactive ghrelin were more markedly decreased in obese T2D subjects than in the non-obese T2D or healthy controls.[45] However, our data agree with those of Benedix et al.[46] who found significantly lower serum ghrelin levels in lean, as compared to obese subjects, while there was no difference in the ghrelin concentrations in saliva between the groups. Likewise, it was found that, although the concentration of acylated or unacylated ghrelin was lower in diabetic saliva, the decrease was not significant.[18] Furthermore, a meta-analysis showed no difference in the concentrations of ghrelin, in saliva between individuals with and without obesity.[47] Our data are also matching those in a recent study in healthy non-obese individuals where there was no correlation between the fasted salivary and plasma ghrelin.[47] Similarly, Wang et al.[47] found lack of correlation between the sample means for fasted salivary and plasma ghrelin (r =0.099, p value = 0.637). Furthermore, there was no within-participant association between fasted salivary and plasma ghrelin.

Irisin

Our study showed that circulating irisin was significantly higher in nascent MetS patients compared to controls; emphasizing for the first time the lack of correlation between plasma and salivary irisin in our study. Specifically, in the MetS-pre/T2D patients; mean irisin plasma levels were also substantially higher vs. respective MetS-controls.[48-49] Apparently with progression of nascent MetS to T2D; irisin plasma levels are being consistently increased. Conversely plasma irisin levels (ng/mL) were significantly higher in the normoglycemic (but not prediabetic) MetS group vs. controls.[44] Intriguingly inconsistent data regarding irisin level were reported in different sets of MetS patients. Tang et al.[50] highlighted serum irisin levels to be significantly lower in nondiabetic overweight subjects compared with control; and as such irisin was not proven to be a predictor for MetS in logistic regression.[50] Yosaee et al.[51] demonstrated that obese patients with/out MetS had lower level of irisin than normal weight participants. Interestingly, irisin level showed a tendency to increase in prediabetes group compared to normal group (P value < 0.01) but showed a significant decrease when comparing diabetes from prediabetes group (P value < 0.001).[51] In a study by Tan et al.[52] that involved women with MetS components, circulating irisin levels were significantly higher than those in the healthy women. Additionally, FBG, WC, and TGs significantly correlated with the circulating levels of irisin.[52] Several studies have investigated salivary irisin levels in relation to cardiometabolic conditions[12, 52] including obesity;[12] our results showing lack of difference in salivary levels among the study groups are in disagreement with those of the latter study, where salivary irisin of the obese individuals was lower than that of the control; given salivary irisin association with oral pathology of chronic periodontal disease and recurrent aphthous stomatitis.[53]

Klotho

Strikingly circulating and salivary Klotho levels lacked any significant difference in between the nascent MetS vs. the controls. Using the Otsuka Long-Evans Tokushima Fatty (OLETF) rat which demonstrates multiple atherogenic risk factors (e.g., hypertension, obesity, severe hyperglycemia, and hypertriglyceridemia), it was shown that adenovirus-mediated klotho gene delivery can ameliorate vascular endothelial dysfunction, increase nitric oxide production, reduce elevated blood pressure, and prevent medial hypertrophy and perivascular fibrosis with lower risk of MetS.[54-55] Klotho concentration was significantly higher in the obese children than in the overweight and normal-body-weight subjects and was also significantly higher in insulin-resistant children than in insulin-sensitive children;[55] with inverse associations of serum klotho concentration and age, T2D, and HTN in Chinese renal transplantation patients.[55] No similar correlation could be retained between the blood and saliva klotho levels in our study arms; though determination of klotho salivary levels in nascent MetS patients was unprecedented.

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LBP (Lipopolysaccharide Binding Protein)

As of LBP (Lipopolysaccharide Binding Protein); there were significantly higher plasma and saliva LBP levels in patients with MetS compared to those of controls; This comes in accordance with substantially higher LBP level (ng/mL) in MetS (non- and pre-diabetic) patients.[56] Similarly, in a study of patients with nascent MetS and following adjustment for BMI and WC, LBP levels were pronouncedly increased in MetS, in addition, in correlation with numbers of MetS risk factors and increased risk of developing MetS, especially in normal-weight individuals.[27,57] Furthermore higher sera LBP levels[58] were reported in obese patients with MetS and pre-existing CVD, unlike those without MetS. Despite increase in both plasma and salivary LBP in MetS patients, we found lack of correlation between them. To the best of our knowledge, none of the previous works investigated LBP salivary levels in MetS patients.

Leptin

It is generally recognized that high leptin concentrations are associated with the obesity and resulting metabolic sequelae including insulin resistance, T2D and cardiovascular diseases.[29] MetS patients in our study had higher plasma leptin values than the control. Notably, in a previous study by our group, MetS-pre/T2D patients had two-fold leptin plasma levels in comparison to MetS-controls.[59] In the study in Saudi women leptin levels were also high in case of high BMI and waist circumference as compared to the counterparts with normal weight.[59] Similarly in a large Korean prospective study higher leptin levels were associated with an increased incidence of MetS.[59] In line with our results, there was no significant difference in salivary leptin levels between the underweight-normal and the overweight-obese Malaysian males.[60] Furthermore, a recent meta-analysis also showed no difference in the concentrations of leptin in saliva between individuals with and without obesity.[56] Our data on circulating and salivary leptin are also in line with those shown for Thai population where leptin levels were higher in plasma than in saliva and plasma leptin increased in patients with MetS compared to control, but saliva leptin levels were not different between healthy subjects and MetS patients, while salivary and plasma leptin had no correlation.[60]

Osteocalcin

Principally plasma (but not salivary) osteocalcin level was higher in MetS patients when compared to controls. Notably, osteocalcin level was markedly greater in normoglycemic MetS vs. both MetS-PreDM and controls.[61] In a cross-sectional study of subjects with MetS and central obesity, low osteocalcin was associated with diabetes but not adiposity; more specifically, patients with impaired fasting glucose had similar insulin resistance to diabetics but the same level of osteocalcin as non-diabetics. The median osteocalcin was noted to be highest in patients with impaired fasting glucose although not significantly different from non-diabetics.[62] In contrast, another study conducted in postmenopausal women showed significantly lower osteocalcin in the MetS, unlike controls.[31] In a cross-sectional study of MetS 235 patients aged 55–75 years, an inverse association was reported between undercarboxylated osteocalcin (ucOC) levels and cardiovascular risk in MetS patients without T2D.[32] Furthermore, serum ucOC levels were significantly lower in T2D patients than in MetS patients without T2D.[32] Most of the studies in MetS patients outlined the decrease in total OC levels in MetS patients in comparison to healthy subjects.[63] Nevertheless, other authors found no differences in total OC levels in postmenopausal women diagnosed with MetS vs. those without MetS.[64] Salivary concentrations of osteocalcin were higher in females than in males and were not related with periodontal status.[64] Cutando et al.[65] found that diabetic patients with chronic periodontitis showed higher salivary levels of osteocalcin than healthy subjects. For the first time; we showed a significant correlation between plasma and salivary osteocalcin levels in both the total sample and the MetS patients. Furthermore, in the total sample plasma osteocalcin correlated significantly with BMI, BAI, WHt R, SBP, DBP, TG, LAP, VAI, TG/HDL-C and AIP, while salivary osteocalcin showed correlations only with FPG and A1c. In contrast, a study by Guneyet al.[31] demonstrated that plasma osteocalcin levels negatively correlated with insulin resistance, and A1c in postmenopausal non-osteoporotic females with/without MetS. The fact that their sample differed in age and included only female gender may explain the difference in results. Intriguingly, in the MetS only group, both plasma and salivary osteocalcin correlated significantly with FPG and A1C. Furthermore, salivary osteocalcin correlated significantly with BMI and LAP. To the best of our knowledge, none of the previous studies investigated correlations between plasma and salivary osteocalcin or between the salivary osteocalcin and clinical characteristics before. Our data indicate that osteocalcin in plasma and saliva may be a promising biomarker used for the detection of MetS and the assessment of its clinical course and that measurement of salivary osteocalcin may serve as a non-invasive test for these purposes. Assessment of proteins from different functional classes is a plausible strategy to improve predictive ability of T2D.

Notably, low-molecular-weight proteins (<20 kDa) are more prevalent (14.5%) in the salivary proteome as compared to only 7% for the plasma proteome.6 In a study by Rao et al.[66] that involved individuals with T2D, salivary proteins demonstrated a greater than two-fold difference compared to controls; a majority of the differentially abundant proteins belong to pathways regulating metabolism and immune response. Importantly, the study found a trend of relative increase in the salivary proteins abundance with progression from the pre-diabetic to the diabetic state. On the other hand, in a study of 27 different cytokines involving 50 healthy adults there was little correlation between the plasma and salivary samples; therefore, it was concluded that substituting saliva for blood needs a great caution, and that relationships differ by biomarker.[66] We support the opinion that the data of salivary biomarkers' levels should be interpreted with caution as the type of sample (stimulated vs. unstimulated; whole vs. glandular), timing of sampling, sensitivity to preprocessing as well as presence of oral diseases are some of the confounding parameters may affect the biomarkers salivary levels;[5] Such cofounders including periodontitis, uneven salivary dilution

level, or other exogenous factors.[18]

CONCLUDING REMARKS and FUTURE PERSPECTIVES

Salivaomics of multiple biomarker candidates in swift and combined detection of nascent MetS related- expression changes and clinical relevance can improve accuracy, sensitivity, and reliability. Salivary markers can be also helpful in screening of high risk cases for practitioners to benefit of in clinical practice. Among 9 cardiometabolic biomarkers, we found correlations between the plasma and the saliva for osteocalcin. Salivary testing of osteocalcin may be the promising noninvasive parameter of early prediction/prognosis and prevention of nascent MetS.

LIMITATIONS

With a striking exception for osteocalcin; Fasting levels of certain markers in saliva (as of adipolin, cathepsin, FGF1, ghrelin, irisin, klotho, LBP, or leptin) are not always a reliable reflection of their blood levels in nascent MetS subjects.

Salivary composition can probably be influenced by the method of collection and degree of stimulation of salivary flow.

Changes in salivary pH may possibly interfere with concentration of salivary markers. Salivary proteolytic enzymes also can affect the stability of certain diagnostic markers

Inconsistency in salivary flow rate and variability in composition can be affected by frequent medications as well as systemic disorders.

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ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

IMPACT OF FINDINGS ON PRACTICE

Establishment of correlation between the saliva and plasma biomarkers as well as of their correlation with clinical parameters in patients with metabolic syndrome and/or prediabetes would lead to a better understanding of pathogenesis of these conditions and potential development of new predictive/ therapeutic strategies in these related disorders.

COMPETING INTERESTS

The authors declare that there are no conflicts of interest

DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Materials supporting the findings are available from the corresponding author on reasonable request**.**

AUTHOR CONTRIBUTIONS

All authors contributed equally to this manuscript

ABBREVIATIONS

Adiposity indices (BMI, WHR (waist/Hip ratio); WtHR (waist/ Height ratio); Conicity-index; BAI (Body adiposity index); LAP (Lipid accumulation Product); VAI (Visceral adiposity Index) and atherogenicity indices (AIP (atherogenecity index of plasma); WAT (White adipose tissue)

References

1. Nabrdalik K, Chodkowski A, Bartman W, et al. Pentraxin 3 and atherosclerosis among type 2 diabetic patients. Open Life Sciences. 2017; 12(1): 92-98. https://doi.org/10.1515/biol-2017-0010
2. Shrestha S, Pokhrel S, Poudel A, et al. Implication of salivary biochemical parameters for diagnosis and prognosis of type 2 diabetes mellitus. International Journal of Analytical Chemistry. 2022;1781613. https://doi.org/10.1155/2022/1781613.
3. Zyśk B, Ostrowska L, Smarkusz-Zarzecka J. Salivary adipokine and cytokine levels as potential markers for the development of obesity and metabolic disorders. International Journal of Molecular Sciences. 2021; 22(21):11703. https://doi.org/10.3390/ijms222111703
4. Wetterö J, Jönsson F, von Löhneysen S, et al. Pentraxin-3 detected in human saliva shows limited correlation with biomarkers associated with systemic inflammation. ActaPathologica, Microbiologica, etImmunologicaScandinavica, (APMIS) 2021; 129(6):304-313. https://doi.org/10.1111/apm.13136.
5. Techatanawat S, Surarit R, Chairatvit K, et al. Salivary and serum cystatin SA levels in patients with type 2 diabetes mellitus or diabetic nephropathy. Archives of Oral Biology. 2019; 104:67-75.https://doi.org/10.1016/j.archoralbio.2019.05.020
6. KemerDoğan ES, Duran N. Is periodontal inflamed surface area associated with serum and salivary levels of IL-1β, visfatin, and omentin-1 in overweight/obese patients? Clinical Oral Investigations. 2022; 26:5351–5358.https://doi.org/10.1007/s00784-

022-04502-0
7. Gomathi GD, Gopalakrishnan S, Sudhakar U, et al. Effects of non-surgical periodontal therapy on saliva and gingival crevicular fluid levels of chemerin in periodontitis subjects with and without type 2 diabetes mellitus. Cureus. 2023; 15(1). https://doi.org/10.7759/cureus.33388.
8. Polizzi A, Torrisi S, Santonocito S, et al. Influence of myeloperoxidase levels on periodontal disease: an applied clinical study. Applied Sciences. 2020; 10(3):1037. https://doi.org/10.3390/app10031037
9. Anil S, Vellappally S, Preethanath RS, et al. Hepatocyte growth factor levels in the saliva and gingival crevicular fluid in smokers with periodontitis. Disease Markers. 2014;146974. https://doi.org/10.1155/2014/146974.
10. Waluś-Miarka M, Trojak A, Miarka P,et al. Correlates of pentraxin 3 serum concentration in men and women with type 2 diabetes. Innate Immunity. 2020; 26(5):351-357.https://doi.org/10.1177/1753425919891628.
11. Lee CH, Hsu KY, Lin CJ. IDF21-0219 Association of plasma and salivary cystatin-c levels and clinical characteristics in type 2 diabetes. Diabetes Research & Clinical Practice. 2022; 186(Suppl.1):109637. https://doi.org/10.1016/ j.diabres.2022.109637
12. (a). Aydin S, Aydin S, Kuloglu T, et al. Alterations of irisin concentrations in saliva and serum of obese and normal-weight subjects, before and after 45 min of a Turkish bath or running. Peptides. 2013; 50:13-18. https://doi.org/10.1016/ j.peptides.2013.09.011; (b). Aydin S, Aydin S, Kobat MA, et al. A Decreased saliva/serum irisin concentration in the acute myocardial infarction promising for being a new candidate biomarker for diagnosis of this pathology. Peptides.2014;56:141-147. https://doi.org/10.1016/j.peptides.2014.04.002.
13. Koopaie M, Salamati M, Montazeri R, et al. Salivary cystatin S levels in children with early childhood caries in comparison with caries-free children; statistical analysis and machine learning. BMC Oral Health. 2021; 21: 650. https://doi.org/10.1186/s12903-021-02016-x
14. Mamali I, Roupas ND, Armeni AK, et al. Measurement of salivary resistin, visfatin and adiponectin levels. Peptides. 2012; 33(1):120-124. https://doi.org/10.1016/j.peptides.2011.11.007
15. Brum RS, Duarte PM, Canto GL, et al. Biomarkers in biological fluids in adults with periodontitis and/or obesity: A meta-analysis. Journal of Indian Society of Periodontology. 2020; 24(3):191–215.https://doi.org/10.4103/jisp.jisp_512_19.
16. Tierney C, Bazou D, Lê G, et al. Saliva-omics in plasma cell disorders- Proof of concept and potential as a non-invasive tool for monitoring disease burden. Journal of Proteomics. 2021; 231:104015. https://doi.org/10.1016/ j.jprot.2020.104015.
17. (a).Beshay M, Rhee CM, Kalantar-Zadeh K. Novel monitoring of renal function and medication levels in saliva and capillary blood of patients with kidney disease. Current Opinion of Nephrology & Hypertension. 2022; 31(1):100-108.https://doi.org/10.1097/ MNH.0000000000000764; (b). Loo JA, Yan W, Ramachandran P, et al. Comparative human salivary and plasma proteomes. Journal of Dental Research. 2010; 89(10):1016–1023. https://doi.org/10.1177/ 0022034510380414.
18. (a).Srinivasan M, Meadows ML, Maxwell L. Assessment of salivary adipokinesresistin, visfatin, and ghrelin as type 2 diabetes mellitus biomarkers. Biochemistry Research International. 2018; 7463796; https://doi.org/10.1155/2018/ 7463796; (b). Srinivasan M, Blackburn C, Mohamed M, et al. Literature-based discovery of salivary biomarkers for type 2 diabetes mellitus. Biomarker Insights. 2015; 10:39–45. https://doi.org/10.4137/BMI.S22177.
19. (a).Fadaei R, Moradi N, Kazemi T, et al. Decreased serum levels of CTRP12/adipolin in patients with coronary artery disease in relation to inflammatory cytokines and insulin resistance. Cytokine. 2019; 113: 326–331. https://doi.org/10.1016/j.cyto.2018.09.019; (b). Alipoor E, Yaseri M, Mehrdadi P, et al. The relationship between serum adipokines and glucose homeostasis in normal-weight and obese patients on hemodialysis: a preliminary study. International Urology and Nephrology. 2020; 52(11): 2179–2187. https://doi.org/10.1007/s11255-020-02582-z.
20. (a).Rauner M, Föger-Samwald U, Kurz MF, et al. Cathepsin S controls adipocytic and osteoblastic differentiation, bone turnover, and bone microarchitecture. Bone. 2014; 64:281–287. https://doi.org/10.1016/j.bone.2014.04.022; (b). Karimkhanloo H, Keenan SN, Sun, et al. Circulating cathepsin S improves glycaemic control in mice. Journal of Endocrinology. 2021; 248(2):167–179. https://doi.org/10.1530/JOE-20-0408.
21. Chen L, Lu B, Yang Y, et al. Elevated circulating cathepsin S levels are associated with metabolic syndrome in overweight and obese individuals. Diabetes/Metabolism Research and Reviews. 2019; 35(3):e3117. https://doi.org/10.1002/dmrr.3117.
22. (a). Nies VJM, Sancar G, Liu W. Fibroblast Growth Factor Signaling in Metabolic Regulation. Front Endocrinol. (Lausanne) 2016; 6:193. https://doi.org/10.3389/fendo.2015.00193; (b). Fan L, Ding L, Lan J, et al. Fibroblast growth factor-1 improves insulin resistance via repression of JNK-mediated inflammation. Frontiers in Pharmacology.2019; 10:1478. https://doi.org/10.3389/fphar.2019.01478
23. Słotwińska SM. Ghrelin and oral diseases. Central-European Journal of Immunology. 2020; 45(4):433–438.https://doi.org/10.5114/ ceji.2020.103415
24. (a).Senesi P, Luzi L, Terruzzi I. Adipokines, myokines, and cardiokines: the role of nutritional interventions. International Journal of Molecular Sciences. 2020; 21(21): 8372. https://doi.org/10.3390/ijms21218372; (b) Binay Ç, Paketçi C, Güzel S, et al. Serum irisin and oxytocin levels as predictors of metabolic parameters in obese children. Journal of Clinical Research in Pediatric Endocrinology. 2017; 9(2): 124–131. https://doi.org/10.4274/jcrpe.3963
25. (a).Biyik I, Erten O, Isiklar O, et al. Comparison of serum human Klotho levels and thiol/disulfide homeostasis in women with polycystic ovary syndrome and in healthy women. Taiwanese Journal of Obstetrics &Gynecology. 2021; 60(3):487–491.https://doi.org/ 10.1016/j.tjog.2021.03.017; (b). Frohlich J, Chaldakov GN, Vinciguerra M. Cardio- and NeurometabolicAdipobiology:

Consequences and Implications for Therapy. International Journal of Molecular Sciences. 2021; 22(8):4137. https://doi.org/10.3390/ijms22084137
26. Wolf EJ, Morrison FG, Sullivan DR, et al. The goddess who spins the thread of life: Klotho, psychiatric stress, and accelerated aging. Brain, Behavior, and Immunity. 2019; 80:193-203. https://doi.org/10.1016/j.bbi.2019.03.007.
27. (a)Gonzalez-Quintela A, Alonso M, Campos J, et al. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. PLoS One. 2013; 8(1):e54600. https://doi.org/10.1371/ journal.pone.0054600; (b). Jialal I, Devaraj S, Bettaieb A, et al. Increased adipose tissue secretion of Fetuin-A, lipopolysaccharide-binding protein and high-mobility group box protein 1 in metabolic syndrome. Atherosclerosis. 2015; 241(1):130–137. https://doi.org/10.1016/j.atherosclerosis.2015.04.814.
28. Kheirandish-Gozal L, Peris E, Wang Y, et al. Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. Journal of Clinical Endocrinology & Metabolism. 2014; 99(2): 656–663.https://doi.org/10.1210/jc.2013-3327
29. (a).López-Jaramillo P, Gómez-Arbeláez D, López-López J, et al. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Hormone Molecular Biology and Clinical Investigation. 2014; 18(1):37–45. https://doi.org/10.1515/hmbci-2013-0053; (b).Jialal I, Devaraj S. Subcutaneous adipose tissue biology in metabolic syndrome. Hormone Molecular Biology and Clinical Investigation. 2018; 33(1). https://doi.org/10.1515/hmbci-2017-0074; (c).Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. Cytokine. 2019; 121: 154735. https://doi.org/10.1016/j.cyto.2019.154735; (d).Correia ML, Rahmouni K. Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. Diabetes & Obesity Metabolism. 2006; 8(6):603–610. https://doi.org/10.1111/j.1463-1326.2005.00562.x; (e). de Luis DA, Perez Castrillón JL, Dueñas A. Leptin and obesity. Minerva Medica. 2009; 100(3):229-236.
30. Mizokami A, Kawakubo-Yasukochi T, Hirata M. Osteocalcin and its endocrine functions. Biochemical Pharmacology. 2017; 132:1–8. https://doi.org/10.1016/j.bcp.2017.02.001.
31. Guney G, Sener-Simsek B, Tokmak A, et al. Assessment of the relationship between serum vitamin D and osteocalcin levels with metabolic syndrome in non-osteoporotic postmenopausal women. Geburtshilfe Frauenheilkunde 2019; 79(3): 293–299. https://doi.org/10.1055/a-0767-6572.
32. Riquelme-Gallego B, García-Molina L, Cano-Ibáñez N, et al. Circulating undercarboxylated osteocalcin as estimator of cardiovascular and type 2 diabetes risk in metabolic syndrome patients. Scientific Reports. 2020; 10:1840. https://doi.org/10.1038/ s41598-020-58760-7
33. International diabetes Federation (IDF). Worldwide definition of the metabolic syndrome. The IDF consensus worldwide definition of the Metabolic Syndrome 2006:1e19.
34. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021; 44(Suppl.1):S15-S33. https://doi.org/10.2337/dc21-S002. Erratum in: Diabetes Care. 2021; 44(9):2182.
35. Snouper A, Kasabri V, Bulatova N, et al. Plasma carnitine, choline, γ-butyrobetaine, and trimethylamine n-oxide, but not zonulin, are reduced in overweight/obese patients with pre/diabetes or impaired glycemia. International Journal of Diabetes in Developing Countries. 2022. https://doi.org/10.1007/s13410-022-01088-x
36. Bergman RN, Stefanovski D, Buchanan TA, et al. A better index of body adiposity. Obesity (Silver Spring). 2011; 19(5):1083-9. https://doi.org/10.1038/oby.2011.38.
37. Dobiášová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clinical Biochemistry.2001; 34:583–588. https://doi.org/10.1016/s0009-9120(01)00263-6
38. Alberti KG, Eckel RH, Grundy SM, et al.International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009; 120(16):1640-5. https://doi.org/10.1161/CIRCULATIONAHA. 109.192644.7S.
39. Chan YH. Biostatistics 104: Correlational analysis. Singapore Medical Journal. 2003; 44(12):614-619.
40. (a).Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin resistance. World Journal of Diabetes. 2014; 5(6): 730-738. https://doi.org/10.4239/wjd.v5.i6.730; (b).Kasabri V, Shawakri E, Akour A, et al. Cross-sectional correlates of increased IL-18 but reduced fetuin-A and oxytocin with adiposity and blood indices in metabolic syndrome patients with and without prediabetes. Therapeutic Advances in Endocrinology and Metabolism. 2018; 9(12):329-338. https://doi.org/10.1177/ 2042018818788802; (c).Grewen KM, Davenport RE, Light KC. An investigation of plasma and salivary oxytocin responses in breast- and formula-feeding mothers of infants. Psychophysiology. 2010; 47:625-632. https://doi.org/10.1111/j.1469-8986. 2009.00968.x; (d).Huffmeijer R, Alink LR, Tops M, et al. Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration. NeuroEndocrinology Letters. 2012; 33:21-25.
41. Bhandari R, Bakermans-Kranenburg MJ, van der Veen R, et al. Salivary oxytocin mediates the association between emotional maltreatment and responses to emotional infant faces. Physiology & Behavior. 2014;131:123-128.https://doi.org/10.1016/j.physbeh.2014.04.028

42. (a).Kasabri V, Al-Ghareeb MI, Saleh MI, et al. Proportional correlates of adipolin and cathepsin S in metabolic syndrome patients with and without prediabetes. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13(4):2403–2408. https://doi.org/10.1016/ j.dsx.2019.06.010; (b).Mehrdadi P, KolahdouzMohammadi R, Alipoor E, et al. The Effect of Coenzyme Q10 supplementation on circulating levels of novel adipokineadipolin/CTRP12 in overweight and obese patients with type 2 diabetes. Experimental & Clinical Endocrinology & Diabetes. 2017; 125(3):156–162.https://doi.org/10.1055/s-0042-110570.
43. (a).Jobs E, Risérus U, Ingelsson E, et al. Serum cathepsin S is associated with decreased insulin sensitivity and the development of type 2 diabetes in a community-based cohort of elderly men. Diabetes Care. 2013; 36(1): 163–165. https://doi.org/10.2337/dc12-0494; (b).Rathnayake N, Buhlin K, Kjellström B, et al. Saliva and plasma levels of cardiac-related biomarkers in post-myocardial infarction patients. Journal of Clinical Periodontology. 2017; 44(7):692–699. https://doi.org/10.1111/jcpe.12740.
44. (a).Saber GY, Kasabri V, Saleh MI, et al. Increased irisin versus reduced fibroblast growth factor1 (FGF1) in relation to adiposity, atherogenicity and hematological indices in metabolic syndrome patients with and without prediabetes. Hormone Molecular Biology and Clinical Investigation. 2019; 38(1): 20180063. https://doi.org/10.1515/hmbci-2018-0063; (b).Wang S, Yang Q, Yu S, eta l. Fibroblast growth factor 1 levels are elevated in newly diagnosed type 2 diabetes compared to normal glucose tolerance controls. Endocrine Journal. 2016; 63(4):359–365. https://doi.org/10.1507/endocrj.EJ15-0627; (c).Wang A, Yan X, Zhang C, et al. Characterization of fibroblast growth factor 1 in obese children and adolescents. Endocrine Connections. 2018; 7(8):932–940. https://doi.org/10.1530/EC-18-0141.
45. (a).AbuZayed R, Bulatova N, Kasabri V, et al. Correlates of zinc finger BED domain-containing protein 3 and ghrelin in metabolic syndrome patients with and without prediabetes. Hormone Molecular Biology and Clinical Investigation. 2019; 37(3):/j/hmbci.2019.37.issue-3/hmbci-2018-0052/hmbci-2018-0052.xml. https://doi.org/10.1515/hmbci-2018-0052; (b). Aydin S. A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. Journal of Biochemistry and Molecular Biology. 2007; 40(1):29–35. https://doi.org/10.5483/bmbrep.2007.40.1.029.
46. Benedix F, Westphal S, Patschke R, et al. Comparison of serum and salivary ghrelin in healthy adults, morbidly obese, and patients with metastatic carcinoma. Obesity Surgery. 2011; 21: 1265-1271.https://doi.org/10.1007/s11695-010-0161-8.
47. (a).Duffles LF, Hermont AP, Abreu LG, et al. Association between obesity and adipokines levels in saliva and gingival crevicular fluid: A systematic review and meta-analysis. Journal of Evidence-Based Medicine. 2019; 12(4):313–324. https://doi.org/10.1111/ jebm.12363; (b).Crabtree DR, Buosi W, Fyfe CL, et al. Salivary ghrelin response to drinks varying in protein content and quantity and association with energy intake and appetite. Physiology & Behavior. 2021; 242:113622. https://doi.org/10.1016/ j.physbeh.2021.113622.
48. Kahwaji R, Kasabri V, Bulatova N, et al. Evaluation of correlations of plasma levels of oxytocin, omentin-1 and irisin in diabetic and non-diabetic metabolic syndrome patients: a cross sectional study in Jordan. Jordan Medical Journal. 2017; 51(3): 97-810. https://doi.org/10.1016/j.dsx.2016.08.008
49. Tabak O, Simsek G, Erdenen F, Sozer V, Hasoglu T, Gelisgen R, Altunoglu E, Muderrisoglu C, Senyigit A, Uzun H. The relationship between circulating irisin, retinol binding protein-4, adiponectin and inflammatory mediators in patients with metabolic syndrome. Archives of Endocrinology and Metabolism. 2017; 61(6):515-523. https://doi.org/10.1590/2359-3997000000289.
50. (a).Tang L, Tong Y, Zhang F, et al. The association of circulating irisin with metabolic risk factors in Chinese adults: a cross-sectional community-based study. BMC Endocrine Disorders. 2019; 19(1):147.https://doi.org/10.1186/s12902-019-0479-8; (b).Hassan II, Hassan AB, Rajab HA, et al. Association of irisin and oxidative stress with biochemical parameters in patients with metabolic syndrome. Hormone Molecular Biology and Clinical Investigation. 2019; 39(1). https://doi.org/10.1515/hmbci-2019-0009.
51. (a).Yosaee S, Basirat R, Hamidi A, et al. Serum irisin levels in metabolically healthy versus metabolically unhealthy obesity: A case-control study. Medical Journal of the Islamic Republic of Iran. 2020; 34:46. https://doi.org/10.34171/mjiri.34.46; (b).Park K, Ahn CW, Park JS, et al. Circulating myokine levels in different stages of glucose intolerance. Medicine (Baltimore). 2020; 99(8):e19235. https://doi.org/10.1097/MD.0000000000019235
52. (a).Tan X, Hu W, Yang S, et al. Association of metabolic syndrome components with circulating levels of cytokine clusters in young women. Endocrine Connections. 2021; 10(1):66–75. https://doi.org/10.1530/EC-20-0569; (b).Hirsch HJ, Gross I, Pollak Y, et al. Irisin and the metabolic phenotype of adults with Prader-Willi Syndrome. PLoS One. 2015; 10(9):e0136864. https://doi.org/10.1371/journal.pone.0136864.
53. (a).Khan SU, Ghafoor S, Khaliq S, et al. Salivary Irisin and periodontal clinical parameters in patients of chronic periodontitis and healthy individuals: A novel salivary myokine for periodontal disease. Journal of Pakistan Medical Association. 2022; 72(1):27-33. https://doi.org/10.47391/JPMA.01367;(b).Altay DU, Korkmaz M, Ergun S, et al. Salivary irisin: potential inflammatory biomarker in recurrent apthous stomatitis patients. European Review for Medical and Pharmacological Sciences. 2021; 25(5):2252-2259. https://doi.org/10.26355/eurrev_202103_25257.
54. (a)Saito Y, Nakamura T, Ohyama Y, et al. In vivo klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. Biochemical and Biophysical Research Communications. 2000; 276(2):767–772. https://doi.org/10.1006/bbrc.2000.3470; (b).Luo L, Hao Q, Dong B, et al. The Klotho gene G-395A polymorphism and metabolic syndrome in very elderly people. BMC Geriatrics. 2016; 16:46. https://doi.org/10.1186/s12877-016-0221-6.
55. (a).Kim HJ, Lee J, Chae DW, et al. Serum klotho is inversely associated with metabolic syndrome in chronic kidney disease: results from the KNOW-CKD study. BMC Nephrology. 2019; 20:119. https://doi.org/10.1186/s12882-019-1297-y; (b). Socha-

Banasiak A, Michalak A, Pacześ K, et al. Klotho and fibroblast growth factors 19 and 21 serum concentrations in children and adolescents with normal body weight and obesity and their associations with metabolic parameters. BMC Pediatrics. 2020; 20(1):294. https://doi.org/10.1186/s12887-020-02199-2; (c).Liu YC, Tsai JP, Wang LH, et al. Positive correlation of serum fibroblast growth factor 23 with peripheral arterial stiffness in kidney transplantation patients. Clinica Chimica Acta (International Journal of Clinical Chemistry.) 2020; 505:9–14. https://doi.org/10.1016/ j.cca.2020.02.014

56. Al-Qudah SA , Kasabri V , Saleh MI , et al. Cross-sectional correlates of nesfatin and lipopolysaccharide binding protein in metabolic syndrome patients with and without prediabetes. Hormone Molecular Biology and Clinical Investigation. 2018; 36(3). https://doi.org/10.1515/hmbci-2018-0035.
57. (a).Jialal I, Rajamani U, Adams-Huet B, et al. Circulating pathogen-associated molecular pattern - binding proteins and High Mobility Group Box protein 1 in nascent metabolic syndrome: implications for cellular Toll-like receptor activity. Atherosclerosis. 2014; 236(1): 182–187. https://doi.org/10.1016/j.atherosclerosis.2014.06.022; (b). Liu X, Lu L, Yao P, et al. Lipopolysaccharide binding protein, obesity status and incidence of metabolic syndrome: a prospective study among middle-aged and older Chinese. Diabetologia. 2014; 57(9):1834–1841. https://doi.org/ 10.1007/s00125-014-3288-7.
58. Lim PS, Chang YK, Wu TK. Serum lipopolysaccharide-binding protein is associated with chronic inflammation and metabolic syndrome in hemodialysis patients. Blood Purification. 2019; 47(1-3): 28–36.https://doi.org/10.1159/000492778.
59. (a)Al-Nouaaimi M, Kasabri V, Bulatova N, et al. Evaluation of the correlation of oxytocin plasma levels and metabolic syndrome biomarkers (leptin, adiponectin and resistin) in newly diagnosed type 2 diabetes patients in Jordan: a cross sectional study. Jordan Journal of Pharmaceutical Sciences. 2016; 9(2):115-128; (b).Al-Amodi HS, Abdelbasit NA, Fatani SH, et al. The effect of obesity and components of metabolic syndrome on leptin levels in Saudi women. Diabetes & Metabolic Syndrome. 2018; 12(3):357–364. https://doi.org/10.1016/j.dsx.2017.12.030; (c). Lee KW, Shin D. Prospective Associations of serum adiponectin, leptin, and leptin-adiponectin ratio with incidence of metabolic syndrome: the Korean genome and epidemiology study. International Journal of Environmental Research and Public Health. 2020; 17(9):3287. https://doi.org/10.3390/ ijerph17093287.
60. (a).Ibrahim Abdalla MM, Siew Choo S. Salivary leptin level in young adult males and its association with anthropometric measurements, fat distribution and muscle mass. European Journal of Endocrinology. 2018; 14(2):94-98. https://doi.org/10.17925/ EE.2018.14.2.94; (b).Thanakun S, Watanabe H, Thaweboon S, et al. Comparison of salivary and plasma adiponectin and leptin in patients with metabolic syndrome. Diabetology & Metabolic Syndrome. 2014; 6:19. https://doi.org/10.1186/1758-5996-6-19
61. Kasabri V, Albsoul-Younes A, Suyagh M, et al. Sirtuin 1, but not osteocalcin, correlates with lipid accumulation product, visceral adiposity and atherogenicity indices in newly diagnosed prediabetes-metabolic syndrome patients. Romanian Journal of Diabetes Nutrition and Metabolic Diseases. 2020; 27(3):220-236. https://doi.org/10.46389/rjd-2020-1034
62. Bador KM, Wee LD, Halim SA, et al. Serum osteocalcin in subjects with metabolic syndrome and central obesity. Diabetes & Metabolic Syndrome. 2016; 10 (1 Suppl. 1):S42–S45. https://doi.org/10.1016/j.dsx.2015.09.009
63. (a).Saleem U, Mosley TH, Kullo IJ. Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology. 2010; 30:1474–1478. https://doi.org/10.1161/ ATVBAHA.110.204859; (b).Bae SJ, Choe JW, Chung YE, et al. The association between serum osteocalcin levels and metabolic syndrome in Koreans. Osteoporosis International. 2011; 22: 2837–2846. https://doi.org/10.1007/s00198-010-1504-y; (c). García-Martín A, Cortés-Berdonces M, Luque-Fernández I, et al. Osteocalcin as a marker of metabolic risk in healthy postmenopausal women. Menopause. 2011; 18:537–541. https://doi.org/10.1097/gme.0b013e3181f8565e; (d). Lee SW, Jo HH, Kim MR, et al. Association between obesity, metabolic risks and serum osteocalcin level in postmenopausal women. Gynecological Endocrinology. 2012; 28:472–477. https://doi.org/10.3109/09513590.2011.633660
64. (a). Movahed A, Larijani B, Nabipour I, et al. Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: the crosstalk between bone and energy metabolism. The Journal of Bone and Mineral Metabolism. 2012; 30: 683–691. https://doi.org/10.1007/s00774-012-0367-z; (b).Gursoy UK, Liukkonen J, Jula A, et al. Associations between Salivary Bone Metabolism Markers and Periodontal Breakdown. Journal of Periodontology. 2016; 87(4):367-75. https://doi.org/10.1902/jop.2015.150399.
65. (a).Cutando A, López-Valverde A, Gómez-de-Diego R, et al. Effect of gingival application of melatonin on alkaline and acid phosphatase, osteopontin and osteocalcin in patients with diabetes and periodontal disease. Medicina Oral, Patología Oral y Cirugía Bucal. 2013; 18(4):e657–e663. https://doi.org/10.4317/medoral.18832; (b).Miricescu D, Totan A, Calenic B, et al. Salivary biomarkers: relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta Odontologica Scandinavica. 2014; 72(1):42–47. https://doi.org/10.3109/00016357.2013.795659.
66. (a).Rao PV, Reddy AP, Lu X, et al. Proteomic identification of salivary biomarkers of type-2 diabetes. Journal of Proteomic Research. 2009; 8(1):239–245. https://doi.org/10.1021/pr8003776; (b).Williamson S, Munro C, Pickler R, et al. Comparison of biomarkers in blood and saliva in healthy adults. Nursing Research & Practice. 2012; 2012:246178. https://doi.org/10.1155/2012/246178.