Jordan Journal of Pharmaceutical Sciences, Volume 12, No. 2, 2019

Correlations of Oxytocin, Fibroblast Growth Factor-21 and Hepatocyte Growth Factor in Diabetic and non-Diabetic Metabolic Syndrome Patients: Acase- Control Study from Jordan

Khaled Al-Musa[1], Violet Kasabri[1]*, Nailya Bulatova[1], Amani Al-Rawashdeh[1], Nahla Khawaja[2], Haider Bustanji[2], Dana Hyasat[2], Ayman Zayed[3], Munther Momani[3], Sundus Alalawi[1]

[1] Department of Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, PO Box 11942, Amman, Jordan.
[2] National Center for Diabetes, Endocrinology and Genetics, Queen Rania Street, PO Box 13165 Amman, 11942 Jordan.
[3] School of Medicine, The University of Jordan, Queen Rania Street , PO Box 11942, Amman, Jordan.

ABSTRACT

Background and aims: Oxytocin (OXT), hepatocyte growth factor (HGF) and fibroblast growth factor-21 (FGF-21) were shown to play key roles in different aspects of metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM). Nevertheless, how OXT correlates – if at all - with the plasma levels of both FGF-21, and HGF is still to be investigated in MetS-pre/T2DM patients.

Methods: In a cross-sectional study, 85 MetS-normoglycemic (control) and 90 MetS-preT2DM patients (BMI-, gender- and age-matched) were enrolled. Plasma OXT levels were measured using competitive binding enzyme-linked immunosorbent assay (ELISA), while FGF-21 and HGF levels were measured using sandwich ELISA. The correlations between these biomarkers and between biomarkers and patients' clinical parameters such as HbA1c, FPG, blood pressure, lipid profile, and body mass index were evaluated.

Results: Mean circulating levels of both HGF and FGF-21 were substantially higher in the MetS-pre/T2DM group than in the MetS-control group; HGF (pg/mL) means±SD were 98.06±59.78 vs. 58.8±25.51, $p<0.05$, and for FGF-21 (ng/mL) 0.41±0.33 vs. 0.28±0.25, $p<0.05$. Conversely, mean OXT plasma levels (pg/mL) were lower in the MetS-pre/T2DM group than in the MetS-control group (1231.26±555.91 vs. 2201.54±867.50, $p<0.05$). In the total pool of MetS-participants, plasma OXT levels correlated inversely with both HGF and FGF-21 plasma levels; (Spearman correlation coefficient r = -0.403, $p<0.05$, N=162) for HGF, and (r = -0.222, $p<0.05$, N=159) for FGF-21, also HGF plasma levels correlated directly with those of FGF-21 (r = 0.203, $p<0.05$, N=152). In the total MetS-sample, HGF plasma levels correlated positively whereas OXT levels correlated inversely with HbA1c and FPG ($p<0.05$; N=175 the whole MetS swtudy population).

Conclusions: Unlike the decreased OXT levels HGF and FGF-21 were increased in MetS-pre/T2DM. A direct correlation was found between HGF and FGF-21, and an inverse correlation was observed between OXT and these two biomarkers separately. Furthermore, HGF and OXT levels correlated with the degree of glycemic control. Our findings may have potential to be utilized in the therapeutics of MetS-pre/T2DM.

Keywords: Oxytocin (OXT); Hepatocyte Growth Factor (HGF); Fibroblast Growth factor-21 (FGF-21); Metabolic Syndrome (MetS); Type 2 Diabetes Mellitus (T2DM); Enzyme Linked Immunosorbent Assay (ELISA).

* hotice162@gmail.com
Received on 24 /11 /2017 and Accepted for Publication on 5 / 9/2018.

1. INTRODUCTION

Diabetes mellitus is a cluster of metabolic diseases marked by hyperglycemia produced by impairments in insulin production, insulin action, or both. In diabetes chronic hyperglycemia is correlated with long-term damage, dysfunction, and failure of different organs.[1] Patients whose glucose levels do not match the criteria for diabetes, but are too elevated to be regarded normal, are considered to have prediabetes and have an increased risk for developing diabetes and cardiovascular disease.[1] Impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) are termed as prediabetes, and are frequently associated with obesity, hypertension and dyslipidemia with high trigylcerides and/or low HDL-C (Table 1).[1] According to the guidelines from the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA),[2] MetS is diagnosed when a patient meets at least 3 of the following 5 criteria: waist circumference ≥102 cm (40 in) in men or ≥88 cm (35 in) in women; fasting glucose ≥100 mg/dL (or receiving drug therapy for hyperglycemia), blood pressure ≥130/85 mm Hg (or receiving drug therapy for hypertension), triglycerides ≥150 mg/dL (or receiving drug therapy for hypertriglyceridemia), and HDL-C < 40 mg/dL in men or < 50 mg/dL in women (or receiving drug therapy for reduced HDL-C).

Table (1)
Categories of increased risk for prediabetes* [(1)]

FPG 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (IFG)
2-h PG in the 75-g OGTT 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) (IGT)
A1c 5.7-6.4%
*For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at higher ends of the range.

FPG: fasting plasma glucose, IFG: impaired fasting glucose, PG: plasma glucose, OGTT: oral glucose tolerance test, IGT: impaired glucose tolerance, A1c: glycosylated hemoglobin

Oxytocin (OXT) is a hypothalamic neuropeptide. Dietary obesity is associated with enhanced negative regulation of OXT release. Inhibiting the negative regulation of OXT protects against dietary obesity by normalizing the release of OXT and energy balance in cases of chronic nutritional excess. Moreover, inhibiting OXT release from hypothalamic neurons and antagonizing it centrally in mice are similarly obesogenic.[3] The significance of OXT neurons in feeding regulation was supported by a study done on Sim1+/- mice which proposes reduced OXT neuropeptide as one of the mechanisms that mediate the hyperphagic obesity in Sim1+/- mice.[4] Through what are likely to be impairments in food intake and energy expenditure, disruptions in OXT signaling contribute to obese phenotypes in diet-induced obese (DIO) mice.[5]

Fibroblast growth factor 21 (FGF-21) is a polypeptide of 181 amino acids; it is secreted by many tissues involved in glucose and lipid metabolism such as the liver (mainly), the pancreas, adipose tissue, and skeletal muscle.[6] FGF-21 was suggested to have an important role in the pathogenesis of T2DM, hepatic and whole-body insulin resistance, as plasma FGF-21 levels were found to be elevated in insulin resistance states and were associated with insulin resistance in the liver and in the whole body (muscles).[7] On the other hand, the association between FGF-21 serum levels and metabolic parameters was examined in a study on Japanese subjects; serum FGF-21 levels correlated significantly with pulse pressure, systolic blood pressure (BP), diastolic BP, triglycerides (TG) levels, body mass index (BMI), fasting plasma glucose (FPG) levels, age, and total cholesterol levels. Multiple regression analysis after adjusting for gender, age, and BMI, showed an independent and significant association between FGF-21 levels on one side and TG levels and high systolic BP on the other.[8] Hiratsuka *et al*.[9] indicated that, independent of liver function, there is a strong association between serum HGF levels and MetS and its components. Also, an increase in circulating Hepatocyte Growth Factor (HGF) levels is observed in obesity and it correlates linearly with body mass index (BMI)[10] and insulin resistance. [11]

2. EXPERIMENTAL

2.1. Study design

A case-control study was conducted in the National Center for Diabetes, Endocrinology and Genetics (NCDEG) with the aim to establish the relationship between OXT, FGF-21, and HGF blood levels in MetS patients. In the MetS-pre/T2DM group there were 90 overweight or obese patients (BMI > 25 kg/m^2 or BMI > 30 kg/m^2, respectively) newly diagnosed with either prediabetes or T2DM (ADA 2014; Table 1) and were drug-naïve to antidiabetic agents. On the other hand, the MetS-control group consisted of 85 normoglycemic overweight or obese individuals (BMI > 25 kg/m^2, or BMI > 30 kg/m^2, respectively).

2.2. Sample size

Based on the results of Qian *et al.* [12] the sample size was calculated by the formula:

$N = 2 * SD^2 (Z_\alpha + Z_\beta)^2 / \Delta^2$ [13]

where:

N: Sample size.

Z_α: Type one error= 1.96 when $\alpha = 5\%$.

Z_β: Type two error= 1.28 when $\beta = 10\%$.

SD = Standard deviation of controls (normoglycemic obese subjects with mean OXT = 9.23 ng/L) and equals 1.14 [12]

Δ = the ambitious difference yielded between the means of case and control pools [12] study equals 9.23-7.16= 2.07 ng/L

By using the aforementioned equation, the minimal required number of subjects per each study arm = 6 participants.

2.3. Clinical setting and duration

The study began after obtaining approval from the Scientific Research Committee at the School of Pharmacy and from the Institutional Review Board (IRB) and patients' recruitment took place between August 2014 and January 2015. Anthropometric measurements (weight, height and waist circumference), blood pressure and biochemical analyses of HbA1c, fasting blood glucose and fasting lipid profile were performed for each participating patient. Blood samples (3 mL) were collected in lithium heparin test tubes and were centrifuged to obtain their plasma portions, which were stored at a -80^o C freezer until the time of ELISA measurement of OXT, HGF and FGF-21 plasma levels. Clinical waste management took place via the Jordan University Hospital incinerator.

2.4. Study participants

The following inclusion and exclusion criteria were used to select the study participants.

Inclusion criteria (patients should meet all):

- Adult patient (male or female older than 18 years of age) with MetS[(2)] either overweight (BMI > 25 kg/m^2) or obese (BMI > 30 kg/m^2) and fasting blood glucose levels > 100 mg/dL (applicable for MetS-cases but not MetS-controls).

1. For the MetS-pre/T2DM group: antidiabetic-drug naïve patients, newly diagnosed with either prediabetes or T2DM (Table 1).[(1)]
2. For the MetS-control group: normoglycemic individuals, matched by age (in decades), gender, & BMI category e.g. [(overweight: BMI > 25 kg/m^2) or (obese: BMI>30 kg/m^2)] with MetS-pre/T2DM patients.

Exclusion criteria

1 Pregnant or breast feeding females.

2 Presence or history of any inflammatory or autoimmune conditions or other serious diseases (apart from cardiovascular diseases or diabetes complications).

3 Obesity secondary to endocrine derangement other than DM.

2.5. Determination of OXT levels using colorimetric ELISA technique

Abcam's (USA) OXT *in vitro* competitive binding ELISA kit was used for the quantitative measurement of OXT in human serum. The generated yellow color was read at 405 nm (Bio-Tek Instruments, USA). The intensity of the yellow coloration is inversely proportional to the amount of OXT captured in the plate.

2.6. Determination of FGF-21 levels using colorimetric sandwich ELISA technique

Abcam's (USA) FGF-21 human *in vitro* sandwich ELISA kit was used for the quantitative measurement of human FGF-21 in heparinized plasma. The density of yellow coloration at 450 nm in a plate reader (Bio-Tek Instruments, USA) is directly proportional to the amount of FGF-21 captured in plate. The results were inferred from the calibrated reference curve as a standard.

2.7. Determination of HGF levels using colorimetric sandwich ELISA technique

Abcam's (USA) HGF human *in vitro* sandwich ELISA kit is was used for the quantitative measurement of human HGF in heparinized plasma. The intensity of the color is measured at 450 nm in a plate reader (Bio-Tek Instruments, USA). The results were inferred from the calibrated reference curve as a standard.

2.8. Statistics

Biomarkers (OXT, HGF, and FGF-21) tests were carried out with 2 independent experiments and each of the experiments consisted of 2 technical replicates. Pre-coded data were entered into the Statistical Package for the Social Sciences software release 20 (SPSS Inc., Chicago, IL). Categorical results were expressed as count and percentages; while continuous variables were presented as mean ± SD (standard deviation). All data were assessed for normality of distribution. Independent Student's t-test was utilized to compare continuous data between the two groups while Chi-square test was utilized to compare two sets of categorical data. Correlations between biomarkers and clinical parameters were assessed using Spearman correlation. All probabilities were two-tailed, and p values < 0.05 were regarded as statistically significant.

3. RESULTS

The total number of enrolled patients was 175 as shown in the study flowchart (Figure 1).

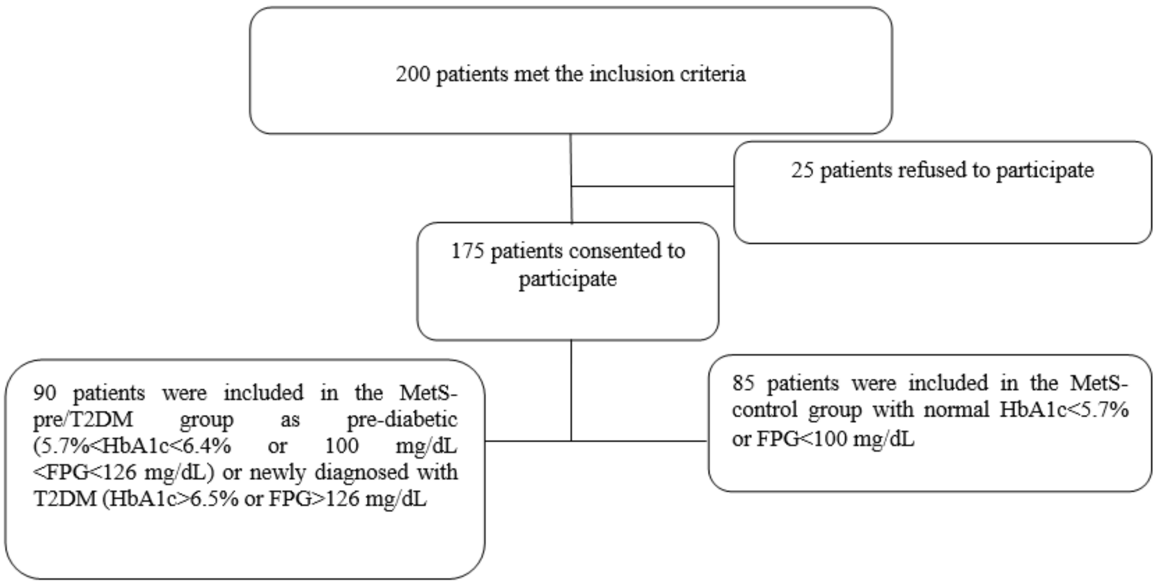


Figure (1): The study recruitment flowchart

3.1. Demographic data of patients

Table 2 summarizes the demographic characteristics of the study sample. All patients were Caucasian, the majority of them were females (68%), and the mean age was 51.07±10.61 years. Most patients were obese (48.80%), while the rest were either morbidly obese (29.40%) or overweight (19.40%); mean BMI was 33.33±5.26 kg/m^2. There were no statistically significant differences in the demographic characteristics such as age, gender, and BMI between the MetS-control and MetS-pre/T2DM groups (*p*>0.05 for all; Table 2).

Table (2)
Demographic characteristics of the study MetS-participants

Parameter	Total number of subjects N= 175	MetS-Control group n= 85	MetS-pre/T2DM group n= 90	*p* [b]
Age in years (mean ± SD) Range (years)	51.07±10.61 (20 – 75)	50.18±9.91 (24 – 71)	51.92±11.21 (20 – 75)	0.278
Gender, N (%)[a*]				
Male	56 (32)	27 (31.80)	29 (32.20)	0.948
Female	119 (68)	58 (68.20)	61 (67.80)	
BMI (mean ± SD) (kg/m^2)	33.33±5.26	32.98±5.33	33.68±5.20	0.388
BMI category, N (%)[a*]				
Normal weight	4 (2.40)	3 (3.50)	1 (1.20)	
Overweight	33 (19.40)	14 (16.50)	19 (22.40)	0.213
Obese	83 (48.80)	47 (55.30)	36 (42.40)	
Morbidly obese	50 (29.40)	21 (24.70)	29 (34.10)	

[a] Percent within total. [b] *p*-value by independent-sample t-test for age, BMI, and gender. *By Chi-square test. BMI: body mass index.

3.2. Clinical characteristics of study groups

Table 3 summarizes the clinical characteristics of the study MetS-participants. The mean systolic blood pressure was 135.99±18.71 mm Hg, and the mean diastolic blood pressure was 80.65±11.52 mm Hg. The mean waist circumference was 104.37±11.84 cm; 106.43±10.59 cm for males and 103.39±12.32 cm for females, *p*=0.114. The following parameters were significantly higher in the MetS-pre/T2DM group compared to the MetS-control group: systolic BP (139.24±18.86 vs. 132.55±18.03 mmHg: *p*=0.018); diastolic BP (82.50±11.86 vs. 78.68±10.87 mmHg; *p*=0.028); creatinine (0.76±0.22 vs. 0.67±0.20 mg/dL; *p*=0.008); HbA1c 6.39±0.81 vs. 5.29±0.33; *p<0.05*; and FPG (119.84±30.26 vs. 101.65±18.04 mg/dL; *p<0.05*).

3.3. Plasma levels of OXT, HGF, and FGF-21 in study groups

Table 3, Figures 2 and 3 demonstrate that the mean circulating levels of both HGF and FGF-21 were substantially higher in the MetS-pre/T2DM group than in the MetS-control group, where mean HGF plasma levels were 98.06±59.78 pg/mL and 58.8±25.51 pg/mL, respectively, *p<0.05* and mean FGF-21 plasma levels were 0.41±0.33 ng/mL and 0.28±0.25 ng/mL, respectively, *p*=0.007; Conversely, mean OXT plasma levels were lower in the MetS-pre/T2DM group than in the MetS-control group, 1231.26±555.91 and 2201.54±867.50 pg/mL, respectively; *p<0.05*; (Table 3 and Figure 4).

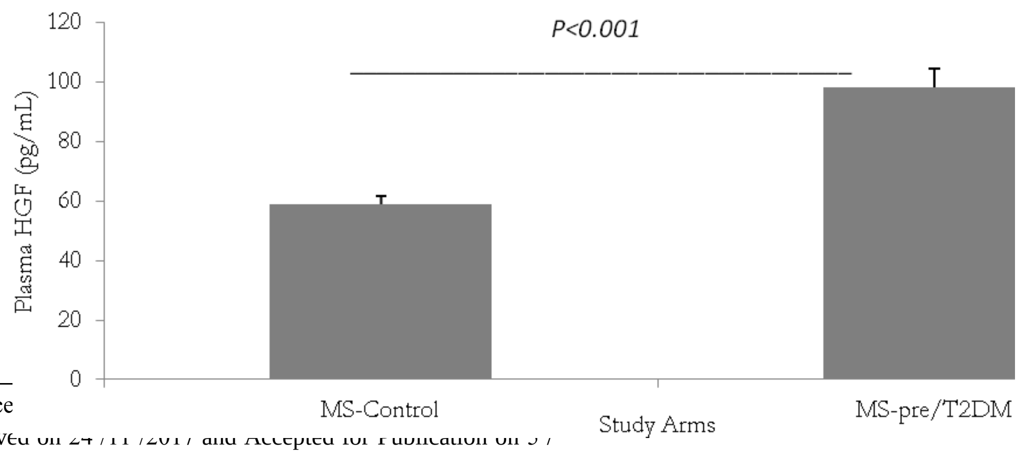
* hotice162@gmail.com
Received on 24 /11 /2017 and Accepted for Publication on 5 / 9/2018.

Table (3)

Clinical characteristics of MetS-participants and comparison between both study groups.

Parameter (Unit)	**Total sample, N=175 (Mean ± SD)**	**MetS-Control group; n=85 (Mean ± SD)**	**MetS-pre/T2DM group; n=90 (Mean ± SD)**	***p*** [a]
Systolic BP (mmHg)	**135.99±18.71**	**132.55±18.03**	**139.24±18.86**	**0.018**
Diastolic BP (mmHg)	**80.65±11.52**	**78.68±10.87**	**82.50±11.86**	**0.028**
Waist circumference (cm)	104.37±11.84	103.49±11.43	105.19±12.23	0.345
Males	106.43±10.59	107.56±8.58	105.38±12.23	
Females	103.39±12.32	101.56±12.15	105.10±12.33	
Serum creatinine (mg/dL)	**0.72±0.22**	**0.67±0.20**	**0.76±0.22**	**0.008**
HbA$_1$c (%)	**5.84±0.83**	**5.23±0.33445**	**6.39±.81**	**<0.001**
FPG (mg/dL)	**111.02±26.63**	**101.65±18.04**	**119.84±30.26**	**<0.001**
Total cholesterol (mg/dL)	197.26±45.86	197.04±44.79	197.48±47.21	0.953
LDL-C (mg/dL)	132.29±37.28	128.07±37.85	136.26±36.52	0.159
HDL-C (mg/dL)	45.79±13.30	47.79±14.13	43.81±12.2	0.056
TG (mg/dL)	160.36±87.30	151.72±90.12	168.60±84.23	0.211
FGF-21(ng/mL)	**0.35±0.30**	**0.28±0.25**	**0.41±0.33**	**0.007**
HGF (pg/mL)	**78.43±49.87**	**58.79±25.51**	**98.06±59.78**	**<0.001**
OXT (pg/mL)	**1707.89±872.03**	**2201.54±867.5**	**1231.26±555.91**	**<0.001**

[a]*p*-value by independent-sample t-test and comparison is significant at the 0.05 level (2-tailed). FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin (A$_1$c), HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides. BP: blood pressure.



* hotice
Received on 24 /11 /2017 and Accepted for Publication on 5 / 9/2018.

Figure (2): The circulating HGF plasma levels in the study arms. Results are mean ± SD. MetS: Metabolic Syndrome. MetS-pre/T2DM: Metabolic syndrome patients with prediabetes or type 2 diabetes mellitus. *Comparison is significant at the 0.05 level (2-tailed).

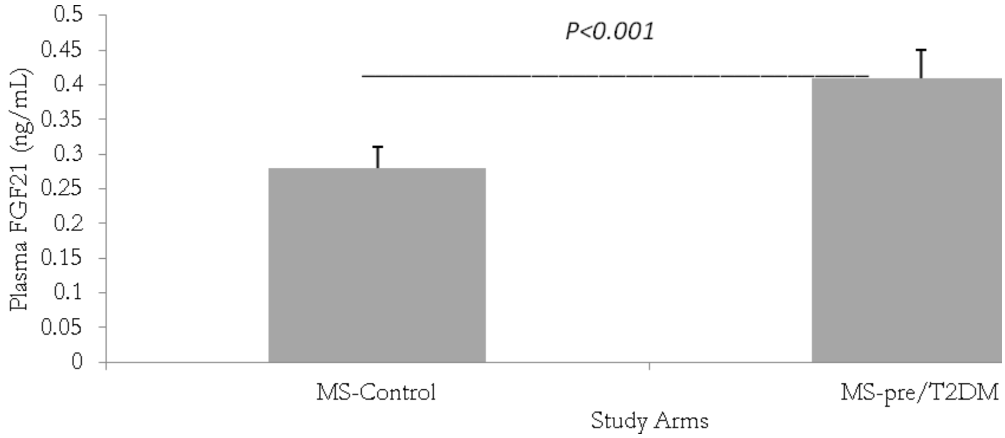


Figure (3): The circulating FGF-21 plasma levels in the study arms. Results are mean ± SD. MetS: Metabolic Syndrome. MetS-pre/T2DM: Metabolic syndrome patients with prediabetes or type 2 diabetes mellitus. *Comparison is significant at the 0.05 level (2-tailed).

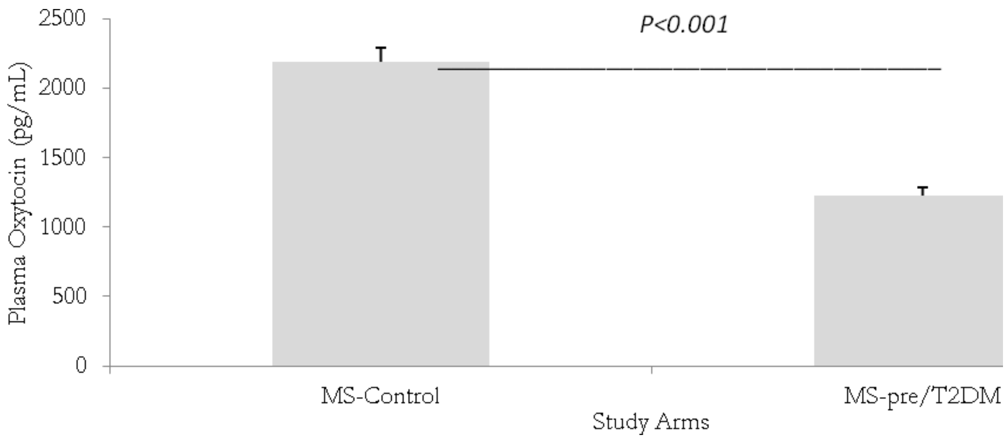


Figure (4): The circulating OXT plasma levels in the study arms. Results are mean ± SD. MetS: Metabolic Syndrome. MetS-pre/T2DM: Metabolic syndrome patients with prediabetes or type 2 diabetes mellitus. *Comparison is significant at the 0.05 level (2-tailed).

3.4. Correlations between OXT, HGF, and FGF-21 levels and between these biomarkers and clinical parameters

Table 4 shows that plasma OXT levels correlated inversely with both HGF and FGF-21 levels in the total pool of participants, $p<0.05$ and $p=0.005$, respectively.

OXT also correlated inversely with HbA1c, *p<0.05*, fasting plasma glucose (FPG), *p<0.05*, and creatinine, *p*=0.037 in the total pool of MetS-participants, but correlated directly with TG (*p*=0.010) in the MetS-control group, and also directly with LDL-C (*p*=0.009) in the MetS-pre/T2DM group.

Interestingly, HGF in the total population of the MetS-study correlated directly with FGF-21, *p*=0.012, and with all of the following parameters: waist circumference, *p*=0.022, creatinine, *p<0.05*, HbA1c, *p<0.05*, and FPG, *p<0.05*, while in the MetS-control group it correlated directly only with waist circumference, *p*=0.034. FGF-21 correlated directly with TG in the total MetS-population and in the MetS-pre/T2DM group, *p*=0.005 and *p*=0.003, respectively, and also correlated directly with total cholesterol, *p*=0.035, in the pre/T2DM group.

Table (4)

Correlations for plasma levels of OXT, HGF, FGF-21 and clinical parameters in the total study MetS-population, and the two study groups

Correlation		**Total MetS-population**			**MetS-Control group**			**MetS-pre/T2DM group**		
Clinical parameter		**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**
Systolic blood pressure	Correlation	-0.013	0.125	0.040	0.054	0.067	-0.041	0.168	0.106	0.025
	Sig.(2-tailed)	0.870	0.113	0.613	0.625	0.551	0.729	0.119	0.346	0.815
	N	171	162	162	84	81	74	87	81	88
Diastolic blood pressure	Correlation	-0.058	0.148	0.150	0.005	0.138	0.003	0.101	0.121	0.154
	Sig.(2-tailed)	0.451	0.060	0.057	0.962	0.220	0.983	0.352	0.284	0.151
	N	171	162	162	84	81	74	87	81	88
BMI	Correlation	-0.005	0.049	0.096	0.052	0.045	0.179	0.019	0.105	0.017
	Sig(2-tailed)	0.951	0.546	0.232	0.641	0.687	0.128	0.866	0.367	0.875
	N	166	157	157	84	81	74	82	76	83
Waist circumference	Correlation	-0.061	**0.181***	-0.016	-0.044	**0.238***	0.026	0.002	0.197	-0.082
	Sig.(2-tailed)	0.430	**0.022**	0.844	0.694	**0.034**	0.828	0.986	0.078	0.445
	N	170	**161**	162	83	**80**	74	87	81	88
Creatinine	Correlation	**-0.166***	**0.287****	0.086	0.021	0.123	-0.074	-0.120	0.166	0.133
	Sig.(2-tailed)	**0.037**	**0.000**	0.298	0.853	0.295	0.551	0.287	0.151	0.237
	N	**158**	**150**	149	77	74	68	81	76	81
HbA$_1$c	Correlation	**-0.469****	**0.553****	0.154	-0.059	0.083	0.010	0.102	-0.007	-0.153
	Sig.(2-tailed)	**0.000**	**0.000**	0.053	0.595	0.459	0.930	0.348	0.949	0.162

Correlation		**Total MetS-population**			**MetS-Control group**			**MetS-pre/T2DM group**		
Clinical parameter		**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**
	N	**171**	**162**	159	84	81	74	87	81	85
FPG	Correlation	**-0.310****	**0.361****	0.147	-0.179	0.220	-0.018	0.002	0.159	0.102
	Sig.(2-tailed)	**0.000**	**0.000**	0.071	0.118	0.058	0.880	0.986	0.174	0.364
	N	**159**	**150**	152	78	75	70	81	75	82
Total cholesterol	Correlation	0.010	0.066	0.113	-0.071	0.182	-0.028	0.158	-0.055	**0.248***
	Sig.(2-tailed)	0.908	0.438	0.183	0.544	0.127	0.822	0.183	0.652	**0.035**
	N	148	141	141	75	72	68	73	69	**73**
LDL-C	Correlation	0.026	0.099	0.111	0.050	0.133	0.036	**0.287****	-0.089	0.129
	Sig.(2-tailed)	0.742	0.225	0.171	0.664	0.253	0.769	**0.009**	0.442	0.246
	N	161	153	154	79	76	71	**82**	77	83
HDL-C	Correlation	-0.042	-0.095	-0.106	-0.210	-0.059	-0.165	0.004	0.097	0.014
	Sig.(2-tailed)	0.603	0.242	0.195	0.062	0.611	0.166	0.972	0.406	0.899
	N	159	153	152	80	77	72	79	76	80
TG	Correlation	-0.015	0.025	**0.221****	**0.286****	-0.088	0.084	-0.138	-0.178	**0.316****
	Sig.(2-tailed)	0.845	0.755	**0.005**	**0.010**	0.443	0.481	0.214	0.119	**0.003**
	N	164	156	**157**	**81**	78	73	83	78	**84**
OXT	Correlation	1.000	**-0.403****	**-0.222****	1.000	-0.204	-0.030	1.000	-0.032	-0.203
	Sig.(2-tailed)	0.	**0.000**	**0.005**	0.	0.068	0.802	0.	0.779	0.062
	N	171	**162**	**159**	84	81	74	87	81	85
HGF	Correlation	**-0.403****	1.000	**0.203***	-0.204	1.000	-0.005	-0.032	1.000	0.174
	Sig.(2-tailed)	**0.000**	0.	**0.012**	0.068	0.	0.970	0.779	0.	0.123
	N	**162**	162	**152**	81	81	72	81	81	80
FGF-21	Correlation	**-0.222****	**0.203***	1.000	-0.030	-0.005	1.000	-0.203	0.174	1.000
	Sig.(2-tailed)	**0.005**	**0.012**	0.	0.802	0.970	0.	0.062	0.123	0.
	N	**159**	**152**	162	74	72	74	85	80	88

By Spearman correlations, FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin (A1C), HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides. * Correlation is significant at the 0.05 level (2-

tailed).

3.5. Gender-based differences in clinical parameters and MetS-biomarkers in total MetS-population, and in both study groups

Table 5 demonstrates that in the MetS-pre/T2DM group, the mean BMI among MetS-females was significantly higher (34.7±5.10 kg/m^2) compared to MetS-males (31.49±4.79 kg/m^2), p=0.007. Furthermore, among the same group, gender-based BMI categories were statistically significantly different, p=0.013, with the majority of males in the group being obese 44.4%, while the majority of females being morbidly obese 43.1%. Nevertheless, in the MetS-controls; the MetS-males' mean BMI was 31.72±4.65 kg/m^2 while among MetS-females it was 33.56±5.57 kg/m^2, p=0.140, with no statistically significant differences in gender-based BMI categories, p=0.776. The majority of both males and females in the group were obese 63.0% and 51.7%, respectively (Table 5).

Table (5)
Gender-based differences in clinical parameters and MetS-biomarkers (OXT, HGF, and FGF-21) in the total study MetS-population, and in both study groups

Clinical parameter	MetS-total population			MetS-control			MetS-pre/T2DM		
	MetS-Males, n=56 (mean±SD)	**MetS-Females, n=119 (mean±SD)**	***p*** **[b] value**	**Males, n=27 (mean±SD)**	**Females, n=58 (mean±SD)**	***p*** **[b] value**	**Males, n=29 (mean±SD)**	**Females, n=61 (mean±SD)**	***p*** **[b] value**
Age (years)	49.05±10.73	52.03±10.46	0.084	47.89±10.91	51.24±9.32	0.148	50.14±10.64	52.77±11.47	0.301
SBP (mm Hg)	138.71±17.63	134.71±19.14	0.188	137.48±15.08	130.26±8.94	0.086	139.86±19.92	138.95±18.50	0.832
DBP (mm Hg)	81.98±10.58	80.02±11.93	0.294	80.41±7.51	77.88±12.10	0.321	83.45±12.76	82.05±11.49	0.604
BMI (kg/m^2)	**31.61±4.67**	**34.13±5.35**	**0.003**	31.72±4.65	33.56±5.57	0.140	**31.49±4.79**	**34.70±5.10**	**0.007**
BMI category, N(%)[a]									
Normal weight	1 (1.9)	3 (2.6)	0.053^	1 (3.7)	2 (3.4)	0.776^	**0 (0)**	**1 (1.7)**	**0.013^**
Overweight	15 (27.8)	18 (15.5)		4 (14.8)	10 (17.2)		**11 (40.7)**	**8 (13.8)**	
Obese	29 (53.7)	54 (46.6)		17 (63.0)	30 (51.7)		**12 (44.4)**	**24 (41.4)**	
Morbidly obese	9 (16.7)	41 (35.3)		5 (18.5)	16 (27.6)		**4 (14.8)**	**25 (43.1)**	
Waist circumference (cm)	106.43±10.60	103.39±12.32	0.114	**107.56±8.58**	**101.56±12.15**	**0.024**	105.38±12.23	105.10±12.33	0.920

Clinical parameter	MetS-total population			MetS-control			MetS-pre/T2DM		
	MetS-Males, n=56 (mean±SD)	**MetS-Females, n=119 (mean±SD)**	***p* [b] value**	**Males, n=27 (mean±SD)**	**Females, n=58 (mean±SD)**	***p* [b] value**	**Males, n=29 (mean±SD)**	**Females, n=61 (mean±SD)**	***p* [b] value**
Serum creatinine (mg/dL)	**0.86±0.23**	**0.65±0.17**	**<0.001**	**0.82±0.23**	**0.60±0.14**	**<0.001**	**0.91±0.22**	**0.70±0.19**	**<0.001**
HbA_1c (%)	5.91±0.88	5.81±0.81	0.472	5.29±0.31	5.29±0.35	0.972	6.49±0.84	6.33±0.80	0.404
FPG (mg/dL)	113.32±30.47	109.91±24.64	0.445	105.03±22.38	99.99±15.46	0.246	121.31±35.21	119.14±27.93	0.761
Total cholesterol (mg/dL)	195.94±44.66	197.83±46.57	0.816	201.26±41.95	194.96±46.39	0.568	189.60±47.94	200.55±47.01	0.371
LDL-C (mg/dL)	136.29±36.34	130.45±37.72	0.352	134.39±40.94	124.85±36.16	0.290	138.34±31.34	135.40±38.68	0.737
HDL-C (mg/dL)	**40.37±12.20**	**48.26±13.09**	**<0.001**	**42.49±13.98**	**50.43±13.56**	**0.016**	**37.98±9.56**	**46.23±12.42**	**0.005**
TG (mg/dL)	171.25±110.15	155.48±74.89	0.280	176.64±126.47	139.49±63.38	0.079	165.42±91.60	169.90±81.78	0.824
OXT (pg/mL)	1605.53±843.52	1757.73±884.90	0.285	2120.41±821.67	2239.97±892.89	0.558	1126.17±526.40	1283.81±567.20	0.214
HGF (pg/mL)	88.60±50.85	73.62±48.90	0.074	**72.16±35.64**	**52.83±16.57**	**0.001**	103.82±58.29	95.17±60.84	0.543
FGF-21 (ng/mL)	0.33±0.27	0.36±0.31	0.506	0.32±0.30	0.26±0.21	0.309	0.33±0.25	0.44±0.36	0.126

[a] Percent within total. [b] *p*-value by independent-sample t-test, and ^ by Chi-square test. BMI: body mass index, DBP: diastolic blood pressure, SBP: systolic blood pressure, FPG: fasting plasma glucose, HbA1c: hemoglobin glycosylated A_1C, HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides, SD: standard deviation.

Exceptionally, waist circumference was only significantly different between males and females in the MetS-control group; 107.56±8.58 cm for males, and 101.56±12.15 cm for females (p=0.024).

Mean HDL-C levels were significantly higher in females 48.26±13.09 mg/dL compared to males in the total study MetS-population, 40.37±12.20 mg/dL ($p<0.05$). Similarly, across MetS-control group; HDL-C levels were markedly higher in females 50.43±13.56 mg/dL than in males 42.49±13.98 mg/dL (p=0.016). Consistently, in the MetS-pre/T2DM group HDL-C levels were 37.98±9.56 mg/dL, and 46.23±12.42 mg/dL for males and females, respectively, p=0.005 (Table 5).

Creatinine was also significantly different between males and females across MetS-control group, MetS-pre/T2DM group, and across the total study MetS-population with respective values of 0.82±0.23 mg/dL and 0.60±0.14 mg/dL, $p<0.05$, 0.91±0.22 mg/dL and 0.70±0.19 mg/dL $p<0.05$ and 0.86±0.23 mg/dL and 0.65±0.18 mg/dL $p<0.05$, (Table 5).

Plasma HGF levels were significantly higher in males than in females in the MetS-control group (72.16±35.64 pg/mL and 52.83±16.57 pg/mL, respectively; p=0.001). Gender-based differences were lacking between males and females of the whole MetS-population, p=0.074 and MetS-pre/T2DM group, p=0.543 for HGF plasma levels.

Unlike HGF, neither mean plasma OXT nor FGF-21 levels were markedly different among genders of MetS-population or its study arms (Table 5).

3.6. Gender-based correlations between the clinical parameters and the biomarkers in both study groups

Tables 6 and 7 demonstrate the correlations between the tested biomarkers themselves and between them and the clinical parameters among each gender; in males of the total MetS-population OXT correlated inversely with all of the following: HGF, p=0.049, FGF-21, p= 0.039, HBA1c, $p<0.05$, and FPG, p=0.012, while in the same group HGF correlated directly with HBA1c and FPG, p=0.016 and p=0.011, respectively.

Table (6)

Correlations between the clinical parameters and MetS biomarkers (OXT, HGF, and FGF-21) in MetS-male and MetS-female participants of the whole study sample

Correlation Parameter		MetS-males in the total population			MetS-females in the total population		
		OXT	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**
Age	Spearman Correlation	0.051	0.092	0.033	-0.154	**0.213***	0.035
	Sig. (2-tailed)	0.707	0.515	0.812	0.101	**0.026**	0.715
	N	56	52	53	115	**110**	109
SBP	Spearman Correlation	0.079	0.087	0.246	-0.056	0.118	-0.054
	Sig. (2-tailed)	0.564	0.540	0.076	0.553	0.221	0.579
	N	56	52	53	115	110	109
DBP	Spearman Correlation	-0.011	-0.002	0.217	-0.083	**0.194***	0.124
	Sig. (2-tailed)	0.936	0.991	0.118	0.378	**0.042**	0.200
	N	56	52	53	115	**110**	109
BMI	Spearman Correlation	0.136	-0.041	-0.017	-0.077	0.164	0.133
	Sig. (2-tailed)	0.329	0.777	0.908	0.417	0.092	0.173
	N	54	50	51	112	107	106
Waist circumference	Spearman Correlation	0.171	-0.104	0.018	-0.164	**0.271****	-0.014
	Sig. (2-tailed)	0.209	0.463	0.896	0.081	**0.004**	0.887
	N	56	52	53	114	**109**	109
Creatinine	Spearman Correlation	-0.018	0.170	0.072	**-0.204***	**0.237***	0.125
	Sig. (2-tailed)	0.901	0.253	0.633	**0.034**	**0.016**	0.211
	N	50	47	47	**108**	**103**	102
HbA1c	Spearman Correlation	**-0.466****	**0.333***	0.007	**-0.464****	**0.638****	**0.228***
	Sig. (2-tailed)	**0.000**	**0.016**	0.958	**0.000**	**0.000**	**0.019**
	N	**56**	**52**	53	**115**	**110**	**106**
FPG	Spearman Correlation	**-0.344***	**0.359***	0.127	**-0.269****	**0.329****	0.169
	Sig. (2-tailed)	**0.012**	**0.011**	0.380	**0.005**	**0.001**	0.090
	N	**53**	**49**	50	**106**	**101**	102
Total cholesterol	Spearman Correlation	-0.031	-0.123	0.120	0.037	0.171	0.102
	Sig. (2-tailed)	0.837	0.425	0.439	0.712	0.094	0.319

Correlation Parameter		MetS-males in the total population			MetS-females in the total population		
		OXT	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**
	N	46	44	44	102	97	97
LDL-C	Spearman Correlation	-0.033	0.029	0.092	0.062	0.104	0.135
	Sig. (2-tailed)	0.818	0.843	0.531	0.523	0.295	0.171
	N	52	49	49	109	104	105
HDL-C	Spearman Correlation	-0.067	-0.228	-0.123	-0.047	0.039	-0.130
	Sig. (2-tailed)	0.639	0.114	0.403	0.629	0.697	0.190
	N	51	49	48	108	104	104
TG	Spearman Correlation	-0.049	0.123	0.075	0.018	-0.023	**0.289****
	Sig. (2-tailed)	0.729	0.400	0.609	0.847	0.816	**0.002**
	N	52	49	49	112	107	**108**
OXT	Spearman Correlation	1.000	**-0.274***	**-0.284***	1.000	**-0.464****	**-0.207***
	Sig. (2-tailed)	.	**0.049**	**0.039**	.	**0.000**	**0.034**
	N	56	**52**	**53**	115	**110**	**106**
HGF	Spearman Correlation	**-0.274***	1.000	0.031	**-0.464****	1.000	**0.293****
	Sig. (2-tailed)	**0.049**	.	0.832	**0.000**	.	**0.003**
	N	**52**	52	50	**110**	110	**102**
FGF-21	Spearman Correlation	**-0.284***	0.031	1.000	**-0.207***	**0.293****	1.000
	Sig. (2-tailed)	**0.039**	0.832	.	**0.034**	**0.003**	.
	N	**53**	50	53	**106**	**102**	109

* Correlation is significant at the 0.05 level (2-tailed). FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin (A1C), HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides.

Table (7)
Correlations between the clinical parameters and MetS biomarkers (OXT, HGF, and FGF-21) in MetS-male and MetS-female participants of both study groups

Correlation		MetS-control males			MetS-control females			MetS-pre/T2DM males			MetS-pre/T2DM females		
Parameter		**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**	**OXT**	**HGF**	**FGF-21**
Age	Spearman Correlation	-0.007	0.049	-0.062	0.055	0.183	0.089	**0.398***	-0.065	0.154	**-0.392****	0.237	-0.059
	Sig. (2-tailed)	0.971	0.815	0.774	0.684	0.178	0.538	**0.032**	0.748	0.425	**0.002**	0.084	0.659
	N	27	25	24	57	56	50	**29**	27	29	**58**	54	59
SBP	Spearman Correlation	0.142	0.110	0.334	0.018	-0.005	-0.235	0.068	0.136	0.162	0.211	0.065	-0.049
	Sig. (2-tailed)	0.480	0.601	0.111	0.893	0.969	0.101	0.728	0.499	0.400	0.111	0.641	0.712
	N	27	25	24	57	56	50	29	27	29	58	54	59
DBP	Spearman Correlation	0.262	-0.015	0.212	-0.071	0.148	-0.110	-0.045	0.005	0.194	0.168	0.177	0.176
	Sig. (2-tailed)	0.187	0.944	0.320	0.602	0.275	0.449	0.817	0.980	0.312	0.209	0.201	0.183
	N	27	25	24	57	56	50	29	27	29	58	54	59
BMI	Spearman Correlation	0.272	-0.035	0.146	-0.035	0.122	0.200	-0.286	0.254	-0.095	0.088	0.172	-0.020
	Sig. (2-tailed)	0.170	0.869	0.496	0.798	0.370	0.164	0.149	0.221	0.637	0.522	0.227	0.885
	N	27	25	24	57	56	50	27	25	27	55	51	56
Waist circumference	Spearman Correlation	**0.397***	-0.232	0.004	-0.223	0.258	0.034	-0.314	0.225	0.048	0.154	0.213	-0.155
	Sig. (2-tailed)	**0.040**	0.265	0.985	0.099	0.057	0.814	0.097	0.260	0.804	0.248	0.122	0.240
	N	**27**	25	24	56	55	50	29	27	29	58	54	59
Creatinine	Spearman Correlation	0.170	0.126	-0.182	0.031	-0.062	-0.049	-0.072	0.078	0.318	-0.136	0.163	0.166
	Sig. (2-tailed)	0.415	0.568	0.416	0.828	0.666	0.748	0.734	0.718	0.122	0.317	0.248	0.222
	N	25	23	22	52	51	46	25	24	25	56	52	56
HbA1c	Spearman Correlation	-0.032	-0.204	-0.182	-0.040	0.164	0.082	0.160	-0.331	-0.094	0.077	0.051	-0.195
	Sig. (2-tailed)	0.873	0.327	0.395	0.768	0.229	0.570	0.406	0.092	0.629	0.566	0.715	0.151
	N	27	25	24	57	56	50	29	27	29	58	54	56
FPG	Spearman Correlation	-0.207	0.252	-0.047	-0.129	0.181	-0.031	-0.125	0.160	0.113	0.069	0.099	0.110
	Sig. (2-tailed)	0.311	0.234	0.831	0.362	0.204	0.835	0.535	0.444	0.574	0.620	0.495	0.423
	N	26	24	23	52	51	47	27	25	27	54	50	55
Total cholesterol	Spearman Correlation	-0.009	0.123	0.255	-0.081	0.221	-0.150	-0.068	-0.364	-0.008	**0.288***	0.113	**0.292***

Correlation		MetS-control males			MetS-control females			MetS-pre/T2DM males			MetS-pre/T2DM females		
Parameter		OXT	HGF	FGF-21	OXT	HGF	FGF-21	OXT	HGF	FGF-21	OXT	HGF	FGF-21
	Sig. (2-tailed)	0.967	0.576	0.241	0.575	0.128	0.324	0.769	0.105	0.973	**0.038**	0.443	**0.035**
	N	25	23	23	50	49	45	21	21	21	**52**	48	**52**
LDL-C	Spearman Correlation	-0.015	0.165	0.245	0.109	0.075	-0.080	0.135	-0.192	-0.106	**0.340****	-0.039	0.240
	Sig. (2-tailed)	0.942	0.430	0.248	0.440	0.599	0.591	0.521	0.368	0.614	**0.010**	0.781	0.069
	N	27	25	24	52	51	47	25	24	25	**57**	53	58
HDL-C	Spearman Correlation	**-0.412***	**-0.397***	0.124	-0.182	0.185	-0.246	0.130	0.162	-0.288	-0.075	0.141	0.060
	Sig. (2-tailed)	**0.033**	**0.049**	0.565	0.193	0.188	0.092	0.545	0.449	0.173	0.589	0.319	0.661
	N	**27**	**25**	24	53	52	48	24	24	24	55	52	56
TG	Spearman Correlation	0.193	0.281	-0.244	**0.391****	**-0.292***	0.256	**-0.506****	-0.140	**0.467***	0.005	-0.171	**0.267***
	Sig. (2-tailed)	0.336	0.173	0.250	**0.003**	**0.034**	0.076	**0.010**	0.515	**0.019**	0.968	0.218	**0.041**
	N	27	25	24	**54**	**53**	49	**25**	24	**25**	58	54	**59**
OXT	Spearman Correlation	1.000	0.005	-0.186	1.000	**-0.301***	0.084	1.000	-0.112	-0.350	1.000	0.011	-0.149
	Sig. (2-tailed)	.	0.983	0.384	.	**0.024**	0.560	.	0.579	0.063	.	0.938	0.274
	N	27	25	24	57	**56**	50	29	27	29	58	54	56
HGF	Spearman Correlation	0.005	1.000	-0.071	**-0.301***	1.000	-0.071	-0.112	1.000	0.227	0.011	1.000	0.231
	Sig. (2-tailed)	0.983	.	0.747	**0.024**	.	0.626	0.579	.	0.255	0.938	.	0.096
	N	25	25	23	**56**	56	49	27	27	27	54	54	53
FGF-21	Spearman Correlation	-0.186	-0.071	1.000	0.084	-0.071	1.000	-0.350	0.227	1.000	-0.149	0.231	1.000
	Sig. (2-tailed)	0.384	0.747	.	0.560	0.626	.	0.063	0.255	.	0.274	0.096	.
	N	24	23	24	50	49	50	29	27	29	56	53	59

*. Correlation is significant at the 0.05 level (2-tailed). FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin (A1C), HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides.

In males of the MetS-control group both OXT and HGF correlated inversely with HDL-C, p=0.033 and p=0.049, respectively. In males of the MetS-pre/T2DM group OXT correlated directly with age, p=0.032, and inversely with TG, p=0.010, while FGF-21 correlated directly with TG, p=0.019.

In females of the total MetS-population OXT correlated inversely with all of the following: HGF, $p<0.05$, FGF-21, p=0.034, HBA1c, $p<0.05$, FPG, p=0.005, and creatinine, p=0.034. In the same group HGF correlated directly with all of the following: FGF-21, p=0.003, age, p=0.026, DBP, p=0.042, waist circumference, p=0.004, creatinine, p=0.016, HBA1c, $p<0.05$ and FPG, p=0.001. Additionally, in the same group FGF-21 correlated directly with both HBA1c and TG, p=0.019 and p=0.002 respectively.

In females of the MetS-control group OXT correlated inversely with HGF, p=0.024, and directly with TG, p=0.003, while HGF correlated inversely with TG, p=0.034. On the other hand, in females of the MetS-pre/T2DM group OXT correlated directly with LDL-C and total cholesterol, p=0.010 and p=0.038, respectively, and inversely with age. p=0.002. In the same group (MetS-pre/T2DM), FGF-21 correlated directly with TG and total cholesterol, p=0.041 and p=0.035 respectively.

4. DISCUSSION

4.1. Importance of the study

Many studies investigated the effects of OXT in humans and animal models. OXT and its analogs have multiple therapeutic effects including weight control, lipid lowering and insulin sensitization, thus, OXT has potential role in treating obesity as well as diabetes.[14] In this study we have investigated for the first time the correlation between relatively decreased levels of OXT and dysregulation of some diabetes-MetS biomarkers i.e. (HGF, and FGF-21). Additional possible correlations of metabolic biomarkers with clinical parameters such as SBP, DBP, waist circumference, HbA1c, BMI, lipid profile in both MetS-control and MetS-pre/T2DM groups were investigated. It would be possible to provide a therapeutic suggestion about the neuropeptide OXT and/or, similarly, HGF and FGF-21 for problems like obesity and diabetes among the Middle Eastern populations.

4.2. Generalizability of the study findings

The number of people with diabetes is increasing throughout the world, and there are no signs of slowing of the rate at which it is increasing.[15] In Jordan the prevalence of T2DM and IFG is high and is alarmingly increasing.[(16)] Among the 175 patients enrolled in this study the majority (68%) of participants were females. The mean age of participants was 51.07±10.61 years which is relatively lower than that considered to be the mean age of Jordanian diabetics by Ajlouni *et al.*[16] (55.50±10.60 years). This could possibly be explained by our wider inclusion criteria: to recruit not only diabetics but also pre-diabetic and non-diabetic/obese patients) and narrower exclusion criteria: prior treatment with antidiabetic agents, history of autoimmune or inflammatory diseases, obesity secondary to endocrine derangements other than DM. Mean body mass index in our study population was 33.33±5.26 kg/m^2 which is slightly higher than that observed among Jordanian diabetics (32.20±5.81 kg/m^2) by Ajlouni *et al.*[16] which is in accordance with our study inclusion criteria of BMI> 25 kg/m^2.

4.3. Comparison of clinical characteristics and biomarkers between our study and other studies OXT findings

In the most recent study by Qian *et al.*[12] 176 patients were divided into four subgroups based on BMI: T2DM-obese, T2DM-normal weight, normal glucose tolerance (NGT)-obese, and NGT-normal weight. In our study; we are concerned with comparisons of our findings to those of T2DM-obese and NGT-obese subgroups in Qian *et al.*, [(12)] (Table 8). In the above study [12] both FPG and HbA1c were higher in the T2DM subgroup compared to the MetS-pre/T2DM group in our study due to inclusion of prediabetic patients in the latter. The levels of OXT in T2DM-obese patients were significantly decreased compared to patients with NGT-obese. Nevertheless, mean plasma OXT concentrations between men 8.69 (7.32~9.77) ng/L and women 8.90 (7.13~10.15) ng/L were not significantly different p=0.864[(12)] which is supported by our results. Gajdosechova *et al.*[17] reported that obesity was linked with reduced plasma OXT in obese Zucker rats, These outcomes emphasize the significance of the OXT system in the pathogenesis of obesity and propose oxytocinase inhibition as a potential approach in the therapy of obesity.

Table (8)
Comparison between the results of OXT in our study and the study by Qian *et al.*, [(12)]

Parameters Mean±SD	**Findings by Qian *et al.*, [(12)]**		**Our findings**	
	T2DM-obese	**NGT-obese**	**MetS-pre/T2DM**	**MetS-Controls**
Age (years)	46.19±11.06	45.21±9.24	51.92±11.21	50.18±9.91
Gender N (male/female)	46 (28/18)	42 (29/13)	90(29/61)	85(27/58)
BMI (kg/m^2)	27.49±2.03	27.78±2.66	33.68±5.20	32.98±5.33
SBP (mmHg)	128.39±14.48	128.93±16.03	139.24±18.86*	132.55±18.03
DBP (mmHg)	79.72±6.36	82.64±12.67	82.50±11.86*	78.68±10.87
Waist circumference (cm)	94.03±4.21	96.02±9.53	105.19±2.23	103.49±11.43
Serum creatinine (mg/dL)	-	-	0.76±0.22*	0.67±0.20
$HbA_{1}c$ (%)	9.25±1.91*	5.34±0.31	6.39±0.81*	5.29±0.33
FPG (mg/dL)	175.68±49.68*	90.18±8.28	119.84±30.26*	101.65±18.04
Total cholesterol (mg/dL)	197.21±45.24	176.33±25.52	197.48±47.21	197.04±44.793
LDL-C (mg/dL)	128.38±39.82	111.36±23.58	136.26±36.52	128.07±37.85
HDL-C (mg/dL)	41.37±7.34	46.79±12.37	43.81±12.20	47.79±14.13
TG (mg/dL)	231.17±25.77	157.66±82.37	168.60±84.23	151.72±90.12
OXT (pg/mL)	7.16 (6.45~8.82)*	9.23 (8.16~10.36)	1231.26±555.91*	2201.54±867.50

[a] Percent within total. FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin (A1C), HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TG: triglycerides. *Comparison is significant at the 0.05 level (2-tailed).

Interestingly, in the study by Qian *et al.*[12] serum OXT concentrations correlated inversely with HbA1c, FPG, BMI, waist circumference, TG, LDL-C, and total cholesterol in study population, $p<0.05$. Compared to the aforementioned study, OXT correlated inversely with FPG p=0.003 in the total MetS-population of our study which further confirms the effects of OXT in modulating insulin secretion, and insulin sensitization.

4.4. HGF findings

In a total of 1474 subjects of a general population free of liver, kidney, and lung diseases, HGF levels were indicated to be strongly associated with the MetS and all of its components, i.e. waist circumference, triglycerides, HDL-C. $p<0.05$, BP (p=0.002 for SBP and p=0.004 for DBP) and FPG, p=0.016 in a study by Hiratsuka *et al.*[9] Nevertheless, HGF correlated only with some of MetS components: waist circumference and TG, p<0.050 in 22 participants (10 women/12 men), BMI 20.6-34.5 kg/m^2 of the study by de Courten *et al.*[18] In our study HGF correlated directly with FPG, $p<0.05$ and waist circumference, p=0.022, and this comes in agreement with findings from Hiratsuka *et al.*[9] study, although we reported significant correlation of HGF with HbA1c levels, $p<0.05$ as well, not demonstrated in their study (p=0.208). . Nakamura *et al.*[19] investigated the relationship between HGF and HbA1c levels in KKAy mice (a rodent model of T2DM) and in humans. Interestingly, their results showed that serum HGF levels in KKAy mice were significantly lower than those in control mice, $p<0.05$. In human subjects of Nakamura *et al.*[19] and contrary to our findings, the serum HGF levels demonstrated a significant negative correlation with HbA1c $p<0.05$, while in our study we demonstrate a significant positive correlation between these two parameters, $p<0.05$.[19] Our study furthermore established a direct correlation between plasma levels of HGF and serum creatinine in the total MetS-population, $p<0.05$, which comes in harmony with findings from Hiratsuka *et al.*[9] study, where they also correlated levels of HGF directly with creatinine, $p<0.05$.

4.5. FGF-21 findings

FGF-21 has been well established as a protein associated with glucose and lipid metabolism, and represents a promising target for treatment of metabolic diseases. The level of endogenous FGF-21 is increased in patients with impaired glucose tolerance, probably as a compensatory response to the deterioration of metabolic imbalance.[20] Most recently, its serum levels independently and positively linked lower extremity atherosclerotic disease in a group of Chinese women with type 2 diabetes. The gender difference was linked to different estrogen levels in the same study.[21] Besides, FGF-21 serum levels correlated positively with waist circumference and systolic blood pressure in diabetic women suffering from lower extremity atherosclerotic disease and with triglyceride and C-reactive protein in diabetic men.[21] A recent clinical trial, demonstrated that exogenous FGF-21 mimetics that target FGF-21 signaling can achieve beneficial metabolic effects despite the already elevated FGF-21 levels. In addition, some clinically used medications such as fenofibrates and metformin may regulate energy and macronutrients metabolism by acting through FGF-21 signaling pathway.[20] The only significant correlation between FGF-21 and clinical parameters our study could find in the total MetS-population was its direct correlation with TG, p=0.005.

4.6. Correlations between OXT, HGF, and FGF-21

Our study has been unprecedented in establishing an inverse correlation between OXT and HGF levels in the total study MetS-population, $p<0.05$, as well as in both males, p=0.049 and females, $p<0.05$, of the MetS-population As for FGF-21 and OXT, our results demonstrated a negative correlation between the two biomarkers in the total study MetS-population, p=0.005, as well as in both males, p=0.039, and females, p=0.034, of the total MetS-population, contradicting the positive correlation between the two biomarkers in a group of amenorrheic athletes, p=0.024 demonstrated by Lawson *et al*.[22], and this could be explained by the typical physical differences that would be present between amenorrheic athletes and patients who met the inclusion criteria of our study. In our study FGF-21 also correlated positively with HGF in the total MetS-population, p=0.012, and in the females of the total MetS-population, p=0.003, which is another unprecedented finding.

5. CONCLUSIONS

The novelty in our study was that we investigated the correlation of OXT with HGF and FGF-21. In further, we demonstrated that OXT correlated inversely with both HGF and FGF-21 levels, and FGF-21 correlated directly with HGF. Our study showed a decrease in OXT levels, and an increase in both HGF and FGF-21 levels in MetS-pre/T2DM vs. apparently healthy MetS-controls. OXT concentrations correlated inversely with HbA1c, FPG, and creatinine. On the other hand, HGF concentrations correlated directly with FGF-21, HbA1c, FPG, waist circumference, and creatinine, and FGF-21 mean plasma levels correlated directly with TG.

Acknowledgement: The Deanship of Academic Research & Quality Assurance - The University of Jordan and Scientific Research Fund - The Ministry of Higher Education are graciously acknowledged for funding this work.

REFERENCES

(1) American Diabetes Association (ADA). (2014), Executive summary: Standards of medical care in diabetes—2014. Diabetes Care, 37(Supplement 1), S5-S13 and 1, S14-S80.

(2) Grundy, S.M. Cleeman, J.I. Daniels, S.R. Donato, K.A. Eckel, R.H. Franklin, B.A. Gordon, D.J. Krauss, R.M.

Savage, P.J. Smith, S.C. Jr, Spertus, J.A. Costa, F. American Heart Association, National Heart, Lung, and Blood Institute (2005), Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation, 112(17), 2735-2752.

(3) Zhang, G. Bai, H. Zhang, H. Dean, C. Wu, Q. Li, J. Guariglia, S. Meng, Q. and Cai, D. (2011), Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. Neuron, 69(3), 523-535.

(4) Kublaoui, B.M. Gemelli, T. Tolson, K.P. Wang, Y. and Zinn, A.R (2008), Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice. Molecular Endocrinology, 22(7), 1723-1734.

(5) Blevins, J. and Ho, J. (2013), Role of oxytocin signaling in the regulation of body weight. Reviews in Endocrine and Metabolic Disorders, 14(4), 311-329.

(6) Woo, YC. Xu, A. Wang, Y. and Lam, K.S. (2013), Fibroblast Growth Factor 21 as an emerging metabolic regulator: clinical perspectives. Clinical Endocrinology (Oxford), 78, 489-496.

(7) Chavez, A.O. Molina-Carrion, M. Abdul-Ghani, M.A. Folli, F. DeFronzo, R.A. and Tripathy, D. (2009). Circulating Fibroblast Growth Factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care, 32(8), 1542-1546.

(8) Jin, Q. Bando, Y. Miyawaki, K. Shikama, Y. Kosugi, C. Aki, N. Funaki, M. and Noji, S. (2014), Correlation of fibroblast growth factor 21 serum levels with metabolic parameters in Japanese subjects. The Journal of Medical Investigation, 61(1-2), 28-34.

(9) Hiratsuka, A. Adachi, H. Fujiura, Y. Yamagishi, S. Hirai, Y. Enomoto, M. Satoh, A. Hino, A. Furuki, K. and Imaizumi, T. (2005), Strong association between serum hepatocyte growth factor and metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism, 90(5), 2927--2931.

(10) Rehman, J. Considine, R. Bovenkerk, J. Li, J. Slavens, C. Jones, R.M. and March, K. L. (2003), Obesity is associated with increased levels of circulating hepatocyte growth factor. Journal of the American College of Cardiology, 41(8), 1408-1413.

(11) Ara'ujo, T.G. Oliveira, A.G. Carvalho, B.M. Guadagnini, D. Protzek, A.O. Carvalheira, J.B. Boschero, A.C. and Saad, M.J. (2012), Hepatocyte growth factor plays a key role in insulin resistance-associated compensatory mechanisms. Endocrinology, 153(12), 5760-5769.

(12) Qian, W. Zhu, T. Tang, B. Yu, S. Hu, H. Sun, W. Pan, R. Wang, J. Wang, D. Yang, L. Mao, C. Zhou, L. and Yuan, G. (2014), Decreased circulating levels of oxytocin in obesity and newly diagnosed type 2 diabetic patients. The Journal of Clinical Endocrinology & Metabolism, 99(12), 4683-4689.

(13) Wang, H. (2007), Sample size calculation for comparing proportions. Wiley Encyclopedia of Clinical Trials, 1-11.

(14) Zhang, H. Wu, C. Chen, Q. Chen, X. Xu, Z. Wu, J. and Cai, D. (2013), Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. PLoS ONE, 8(5), e61477.

(15) Colagiuri, S. Borch-Johnsen, K. Glümer, C. and Vistisen, D. (2005), There really is an epidemic of type 2 diabetes. Diabetologia, 48(8), 1459-1463.

(16) Ajlouni, K. Khader, Y.S. Batieha, A. Ajlouni, H. and El-Khateeb, M. (2008), An increase in prevalence of diabetes mellitus in Jordan over 10 years. Journal of Diabetes Complications 22(5), 317-324.

(17) Gajdosechova, L. Krskova, K. Segarra, A.B. Spolcova, A. Suski, M. Olszanecki, R. and Zorad, S. (2014), Hypooxytocinaemia in obese Zucker rats relates to oxytocin degradation in liver and adipose tissue. Journal of Endocrinology, 220(3):333-343.

(18) De Courten, B. de Courten, M.P. Dougherty, S. Forbes, J.M. Potts, J.R. and Considine, R.V. (2013), Insulin infusion reduces hepatocyte growth factor in lean humans. Metabolism, 62(5), 647-650.

(19) Nakamura, S. Morishita, R. Moriguchi, A. Yo, Y. Nakamura, Y. Hayashi, S. Matsumoto, K. Matsumoto, K. Nakamura, T. Higaki, J. and Ogihara, T. (1998), Hepatocyte growth factor as a potential index of complication in diabetes mellitus. Journal of

Hypertension, 16(12 Pt 2), 2019-2026.

(20) Liu, J.J. Foo, J.P. Liu, S. and Lim, S.C. (2015), The role of fibroblast growth factor 21 in diabetes and its complications: A review from clinical perspective. Diabetes Research and Clinical Practice, pii: S0168-8227(15)00132-1.

(21) Zhang, X. Hu, Y. Zeng, H. Li, L. Zhao, J. Zhao, J. Liu, F. Bao, Y. and Jia, W. (2015), Serum fibroblast growth factor 21 levels is associated with lower extremity atherosclerotic disease in Chinese female diabetic patients. Cardiovascular Diabetology, 14, 32.

(22) Lawson, EA. Ackerman, KE. Slattery, M. Marengi, DA. Clarke, H. and Misra, M. (2014), Oxytocin secretion is related to measures of energy homeostasis in young amenorrheic athletes. The Journal of Clinical Endocrinology & Metabolism, 99(5), E881-E885.

ارتباط مستوى هرمونى الاكستوسين وعامل نمو خلايا الكبد (هيباتوسايت) وعامل نمو الخلايا الليفية-٢١ (فايبروبلاست-٢١) فى مرضى السكري النوع الثانى او ما قبل السكري مع او بدون متلازمة الأيض

خالد جمال الموسى[١]، فيوليت كسابري[٢]، نائلة بولاتوفا[١]، اماني الرواشدة[١]، نهلة خواجا[٢]، حيدر البستنجي[٢]، دانا حياصات[٢]، ايمن زايد[٣]، منذر المومني[٣]، سندس العلوي[١]

[١] قسم الصيدلة الحيوية والصيدلة السريرية، كلية الصيدلة، الجامعة الأردنية، عمان، الأردن.

[٢] المركز الوطني للسكري والغدد الصم والوراثة، شارع الملكة رانيا، عمان الأردن.

[٣] كلية الطب، الجامعة الأردنية، عمان، الأردن.

ملخص

أظهرت الأوكسيتوسن وعامل نمو خلايا الكبد (هيباتوسايت) وعامل نمو الخلايا الليفية-٢١ (فايبروبلاست-٢١) للعب ادوارا رئيسية في جوانب مختلفة من متلازمة الاضطراب الايضي وداء السكري النوع الثاني. ومع ذلك، كيف يرتبط الاوكسيتوسين مع مستويات البلازما من كل من الفايبروبلاست -٢١ والهيباتوسايت لا يزال يتعين تحت التحقيق في مرضى متلازمة الاضطراب الايضي و داء السكري النوع الثاني.

الطرق: في دراسة مسحية، التحق من مرضى الاضطراب الايضي (٨٥ شخص غير مصابين بالسكري و٩٠ مريضا بالسكري او ماقبل السكري). تم قياس مستويات بلازما الاوكسيتوسين باستخدام الفحص المناعي للربط الانزيمي التنافسي، في حين ان مستويات الفايبروبلاست-٢١ والهيباتوسايت تم قياسها باستخدام شطيرة الفحص المناعي للربط الانزيمي. تم تقييم الارتباطات بين هذه المؤشرات الحيوية والخصائص السريرية مثل نسبة السكر التراكمي، سكر صيام الدم، ضغط الدم، مستوى الدهون في الدم ومؤشر كتلة الدم. **النتائج:** إن متوسط مستويات الهيباتوسايت و الفايبروبلاست-٢١ كان أعلى بشكل واضح في مجموعة المرضى الذين يعانون من متلازمة الاضطراب الأيضي بالإضافة إلى مرحلة ما قبل السكري أو السكري من النوع الثاني بالمقارنة مع المرضى الذين يعانون من متلازمة الاضطراب الأيضي فقط؛ حيث كان: (المتوسط ± الانحراف المعياري)؛ (٩٨,٠٦±٥٩,٧٨) و(٥٨,٨٠±٢٥,٥٠) بالترتيب بالنسبة للعامل الأول (بيكوغرام/ مل) ($p<0.05$) ، وكان (٠,٤١±٠,٣٣) و (٠,٢٨±٠,٢٥) بالترتيب بالنسبة للعامل الثاني (نانوغرام/ مل) ($p<0.05$). وبالعكس فإن تركيز الأوكسيتوسين في البلازما (بيكوغرام/ مل) كان أقل بشكل واضح في المجموعة المذكورة أولاً (١٢٣١,٢٦±٥٥٥,٩١) مقارنة بالمجموعة المذكورة ثانياً (٢٢٠١,٥٤±٨٦٧,٥٠) ($p<0.05$). في الدراسة ارتبط مستوى الأوكسيتوسين عكسيا مع مستويات كل من الهيباتوسايت والفايبروبلاست-٢١ في العينة الكلية من المرضى؛ (معامل ارتباط سبيرمان =.403- ، $p<0.05$) و(معامل ارتباط سبيرمان = -.222 ، $p<0.05$) على الترتيب كما وارتبط مستوى عامل نمو خلايا الكبد طردياً مع مستوى الفايبروبلاست-٢١ (معامل ارتباط سبيرمان =.203 ، $p<0.05$). ومن الجدير بالذكر أنه في العينة كاملة ارتبط مستوى الهيباتوسايت في البلازما بشكل طردي مع كل من نسبة السكر التراكمي ومستوى سكر الدم للصائم، بينما ارتبط مستوى الأوكسيتوسين في البلازما عكسياً معهما ($p<0.05$).

الخلاصة: تزداد مستويات الهيباتوسايت والفايبروبلاست-٢١ لدى مرضى متلازمة الاضطراب الأيضي والنوع الثاني من السكري؛ بعكس مستويات الأوكسيتوسين والتي تنقص لدى نفس المجموعة. كما وجدنا أن هنالك علاقة عكسية بين مستويات هذين العاملين من جهة ومستويات الأوكسيتوسين من جهة أخرى ، وقد وجدنا أيضاً أن مستويات الأوكسيتوسين والهيباتوسايت لها علاقة بمدى السيطرة على مستويات السكر في الدم. قد تسهم نتائجنا في المساعدة على إيجاد حل جديد في مجال مداواة الاضطراب الأيضي ومرض السكري.

الكلمات الدالة: الأوكسيتوسين (OXT) ؛ عامل نمو خلايا الكبد (HGF) ؛ عامل نمو الخلايا الليفية ٢١ (FGF-21) ؛ متلازمة الأيض (MetS) ؛ داء السكري من النوع ٢ (T2DM) ؛ الفحص المناعي للربط الانزيمي (ELISA).

تاريخ استلام البحث 24 / 2017/11 وتاريخ قبوله للنشر 5/ 2018/9.