The Levels of Oxytocin and Oxyntomodulin, Adiposity and Blood Indices in Pharmacotherapy Naive Diabetic and Non-Diabetic Patients with Metabolic Syndrome

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ABSTRACT

Aims: Oxyntomodulin (OXM) is a 37-amino acid peptide hormone released from the gut in postprandial state, while oxytocin(OXT) is a nine amino acid neuropeptide produced by the hypothalamus; both has a broad range of actions. Our study aimed to evaluate plasma peptide hormones; OXT and OXM; as well as conicity index (CI), waist circumference (WC), waist circumference to hip circumference ratio (WHR), red cell distribution width (RDW_CV%), mean platelet volume (MPV; fL) and triglyceride to high density lipoprotein-cholesterol (TG\HDL-C) ratio in metabolic syndrome (MetS) and Type-2 diabetes mellitus (T2DM) patients.

Methods: In a cross-sectional design, 30 normoglycemic lean subjects (control), 30 MetS and 29 MetS-Pre/T2DM subjects were enrolled. Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure plasma OXT and OXM. The correlations among these metabolic-biomarkers as well as patients' adiposity and hematological indices were examined.

Results: Median circulating levels of OXT (pg/ml) were lower in MetS and MetS-Pre/T2DM compared to control group ((median IQR) (MetS 1975.4and MetS-pre/T2DM 1403 *vs.* control 4176.6), p=0.009 and p=0.001, respectively). Conversely, median OXM (ng/mL) concentrations lacked any inter-group substantial variations (63023.6 (124670.05-13246.675); for the total study pool of recruits). Neither biomarker was described as substantially different in MetS vs. MetS-pre/T2DM (p>0.05). Principally, in both MetS and MetS-pre/T2DM groups; CI, TG\HDL-C ratio, WC/HC ratio and RDW-CV% were described as markedly higher (p< 0.001) vs. controls'. Median MPV in MetS-pre/T2DM (but not in the nondiabetic MetS group's) was significantly higher vs. controls' MPV. However, all above MetS-related indices were not ascribed any statistically pronounced variations in MetS vs. MetS-pre/T2DM groups.

Conclusions: In both MetS and MetS-pre/T2DM patients, OXT circulating concentrations were substantially decreased compared to lean controls, while there were not any significant variations in OXM levels between three groups.

Keywords: Oxyntomodulin (OXM); Oxytocin (OXT); Conicity index (CI); red cell distribution width (RDW) and mean platelet volume (MPV); triglyceride to high density lipoprotein-cholesterol (TG/HDL-C) ratio.

1. INTRODUCTION

Diabetes mellitus (DM) is a disease characterized by hyperglycemia; it is associated with relative or absolute impairment of insulin secretion or different degrees of peripheral resistance to the action of insulin[1]. There are three types of DM: type 1 diabetes mellitus, type 2 diabetes mellitus(T2DM) and gestational diabetes.T2DM is characterized by a combination of some degree of insulin

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resistance(IR) and a relative lack of insulin secretion[2]. Prediabetes occurs when blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes (Table 1) [3,4]. The metabolic syndrome (MetS) is a cluster of heart attack-risk factors, raised fasting plasma glucose (FPG), abdominal obesity, high cholesterol and high blood pressure (BP) (Table 2A) [5]. The underlying cause of the MetS continues to challenge the experts. Both IR occurs when cells in the body (liver, skeletal muscle and adipose/fat tissue) become less sensitive- and eventually resistant- to insulin) and central obesity are significant factors. Genetics, physical inactivity, ageing, a subchronic low grade proinflammatory state and hormonal changes may also have a causal effect [5].According to the new International Diabetes Federation (IDF) definition, for a person to be defined as having the MetS they must have central obesity (defined as waist circumference (WC) with ethnicity-specific values) plus any two of the following four factors enlisted in Table 2A.

Oxyntomodulin (OXM) is a 37-amino acid peptide hormone released from the gut in postprandial state that activates both the glucagon-like peptide-1 receptor (GLP1R) and the glucagon receptor (GCGR) resulting in superior body weight lowering to selective GLP1R agonists. OXM reduces food intake and increases energy expenditure in humans. While activation of the GCGR increases glucose production posing a hyperglycemic risk, the simultaneous activation of the GLP1R counteracts this effect. Acute OXM infusion improves glucose tolerance in T2DM patients making dual agonists of the GCGR and GLP1R new promising treatments for diabetes and obesity with the potential for weight loss and glucose lowering superior to that of GLP1R agonists [6].

Oxytocin (OXT) is a nine amino acid neuropeptide produced by the hypothalamus and it has a broad range of actions [7]. Hormone OXT has a well-known peripheral role in uterine contraction during labor and milk ejection during lactation; it has a different central role especially in energy balance regulation. The recent findings had confirmed that the chronic administration of OXT can reduce the food intake and body weight in diet induced obese (DIO).

OXT and its analogs have multiple therapeutic actions beyond the role of weight control and metabolism regulation. Impressively, it has lipid lowering, insulin sensitizing, and insulin secretory effects. Thus, it is closely tied with a marked pharmacologic value as a new class of anti-diabetic polypeptide agents, very similar to the (GLP-1), to treat diabetes cases whether related to obesity or not [7]. In other words, peripheral OXT treatment can control the hyperphagia, food intake, lowering the visceral fat mass, ameliorate obesity, fatty liver, glucose intolerance and diabetes. Taken together, it represents a new therapeutic avenue[9].

Interestingly, a significant correlation between some biomarkers and hematologic indices with diabetes and MetS was reported. In a randomized controlled trial, aimed to investigate the relationship between the MetS and new inflammatory markers as MPV and RDW, patients with MetS had significantly higher mean platelet volume (MPV) and red cell distribution width (RDW) when correspondingly compared to those without MetS. Moreover, patients meeting 5 MetS criteria had higher MPV and RDW than those meeting 3 criteria[10]. Recently, in a cross-sectional study, FPG, MPV, RDW, absolute lymphocyte count, absolute neutrophil count, total white cell count, RDW, Body Mass Index (BMI), and waist to hip ratio (WHR) were significantly higher among diabetic subjects compared to apparently healthy controls. This is a reflection of poor glycemic control and lifestyle changes. FPG is significantly correlated with total White blood cell (WBC), absolute lymphocyte and neutrophil counts, and MPV. The routine hematological profile checking of patients with T2DM may help to prevent complications associated with aberrations in hematological values[11]. A study suggested that high platelet activity enhances vascular complications in DM patients[12]. MPV is a marker showing platelet function and activation. Altered platelet morphology and function can be reflected as a factor for risk of microvascular and macrovascular diseases[13]. In a cross sectional study conducted in Pakistan, MPV was significantly increased in the impaired fasting glucose (IFG) group, when compared to the nondiabetic group, and

it increased further when compared to the DM and IFG groups [14]. Another study was conducted to measure MPV in 145 consecutive T2DM patients and 100 nondiabetic control subjects without known coronary artery disease, who had complete blood count on venous blood sample. The results show significantly higher MPV in diabetic patients than in the nondiabetic controls. This suggests that platelets may play a role in the micro- and macrovascular complications of diabetic patients[13]. Recently, in a cross-sectional study, adiposity markers (WC, WHR, waist-height ratio (WHtR), the conicity index (C-index) and the body adiposity index (BAI) were used in diabetic patients to identify the risk for possible cardiovascular disease (CVD) [15]. Another study showed that WC and BMI are not only the simplest to obtain, but also the most accurate surrogate markers of visceral adiposity in young adults, and are good indicators of insulin resistance[16]. Thus, in this cross-sectional study design, we aimed to compare OXT and OXM plasma levels, adiposity, blood and atherogenecity indices in MetS patients with or without diabetes.

2. METHODS

2.1. Overview of the study population

The type of research we had established was a cross-sectional one in which the relationship between the disease and exposure status of a population was determined once at specific time point. This method is cost-effective and can be achieved in a short duration of time even if the population is large, but causality between the exposure and disease cannot be reached. Our study sample size was calculated according to the findings of Qian, *et al.*[17] and the formula used was $N= 2*SD^2 (Z_\alpha+Z_\beta)^2 / \Delta^2$ [18] 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 OXT baseline from Qian, *et al.*[17] study and equals 1.38 ng/L.

Δ = the difference yielded between OXT levels of diabetic /pre-diabetic group vs. the control group after a study of 3 months treatment and equals to 2.07 ng/L[17]. Using this equation, the number required was 9 patients per each study arm but triple folds were recruited for maximal validity and impact of outcomes. Patients were recruited for one time visit without follow up.

2.2. Clinical setting and duration

Approval for the study was obtained from the Clinical IRB (Institutional Review Board) Committees affiliated with the National Center for Diabetes, Endocrinology, and Genetics (NCDEG) (101675/9/SM). Informed written consent was obtained from each participant. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants who attended at NCDEG for the first time were screened for potential recruitment and for collecting blood samples following signing the informed consent form (ICF) written in Arabic.

2.3. Protocol of sampling

A-inclusion criteria

The age of participants ranged between 18-75 years old. Our study population is divided into three groups based on values of BMI (Table 2A), glycemic parameters of participants [1] and the presence of at least 2 components of MetS in addition to central obesity (Table 2B):

Group 1 (Healthy lean control): Normoglycemic (with HbA1c <5.7% or FPG of <100 mg/dL) and Lean with 19.5 < body mass index (BMI) < 25 kg\m².

Group 2 (MetS patients): Non-diabetic subjects as well as overweight of BMI ≥25 kg/m²or obese of BMI ≥ 30 kg/m² having three or more of the MetS components as delineated by IDF[5] (Table 2A).

Group 3 (MetS-Pre/T2DM patients): Drug naïve newly diagnosed patients with prediabetes or T2DM as well as overweight or obese having three or more of the MetS components as delineated by IDF[5] (Table 2B).

B-exclusion criteria

Interviewed subjects were excluded if they were 1)-pregnant or breast-feeding women; 2)-individuals on any

hypoglycemic agents; 3)- individuals with autoimmune or inflammatory or life-threatening diseases; 4)-individuals with obesity secondary to endocrine disorders other than diabetes; 5)- alcohol or drug abusers.

2.4. The demographic characteristics and laboratory data of population

For consenting eligible subjects, the demographic and clinical characteristics were collected, which included code name, age, weight, height and medical history. MetS components (inclusive WC, hip circumference (HC) and BP) and laboratory data were as follows:1)- biochemical tests (HbA1c, fasting glycemia and lipid panel (triglyceride (TG), Total Cholestrol (TC), Low Density Lipoprotein-cholesterol (LDL-C) and High Density Lipoprotein-cholesterol (HDL-C); 2)- automated complete blood count (CBC) (RDW-CV% and MPV); 3)- CI, WHR, BMI and TG\HDL-C ratio are calculated according to equations described previously.

Based on Tonding *et al*., [15] we calculated:

- WC/HC ratio= waist circumference (cm)/ hip circumference (cm)
- C-index = waist circumference/ (0.109 square root of weight/height)

According to Manco, *et al*., [19] we calculate:

TG/HDL-C ratio (Atherogenic index) =TG (mg/dl)/ HDL-C (mg/dl)

For interviewed individuals, who met the inclusion criteria of the study and signed (ICF), the blood samples in lithium heparin tubes were centrifuged at 0 °C at 2000 round per minutes for 10 minutes and the plasma harvested was in deep freeze at -80 ° C in labeled eppendrofs with participants' code names and date until analysis by Enzyme-linked-immunosorbent assay (ELISA) in accordance with manufacturers' protocols.

2.5. Measurement of metabolic biomarkers (OXT and OXM) plasma levels using ELISA technique

Abcam's (USA) OXT in vitro competitive binding ELISA kit is designed for the accurate quantitative measurement of OXT in human plasma, whereas OXT concentrations increase, the optical density produced is fainter. The kit protocol was performed according to manufacturer's instructions, and the color generated was read at 405nm on a plate reader (Bio-Tek Instruments, USA). The immunoplate of OXM kit procured from Phoenix Pharmaceuticals®, INC. (USA) and assay was performed according to manufacturer's instructions. The standard curve can be established by plotting the measured optical density at 450nm on plate reader (Bio-Tek® Instruments, USA).

2.6. Statistical analysis

All data were coded, entered and analyzed using SPSS© 22 (SPSS, Inc., USA). Normality of data had been checked by Shapiro-Wilk test and based on findings of this test, the findings of our study were expressed as median ± Interquartile range (IQR). Since our data were not normally distributed, Kruskal-Wallis test was used to check if there were significant variations in parameters of study between at least 2 continuous groups of data. Mann-Whitney test as post –hoc analysis was implemented to detect significant differences between every two groups. Chi-square test was employed to evaluate if there are inter-group significant variations in gender distribution. Multivariate analysis of covariance (MANCOVA) was used to eliminate the effect of age as a covariate on the relationship between the independent groups of variables and the continuous dependent variables. Moreover, Spearman's correlation was used as a non-parametric test to estimate the association between the two variables. For all statistical analyses, a *p*-value of less than 0.05 was considered statistically significant. All tests were two tailed.

3. RESULTS

3.1 Study participants

In this study, 177 subjects were approached and assessed for eligibility. The total number of participants who were eligible according to our inclusion criteria was 89. Fifteen of interviewed subjects refused to participate and another assessed 73 were not basically eligible (Figure 1).

Table 1. Diagnostic criteria of DM and prediabetes[4]

Result	**HbA1c**
Normal	less than 5.7%
Prediabetes	5.7% to 6.4%
Diabetes	≥6.5%
Result	**Fasting Plasma Glucose (FPG)**
Normal	less than 100 mg/dL
Prediabetes	100 mg/dL to 125 mg/dL
Diabetes	126 mg /dL or higher

3.2 Demographic and clinical characteristics of study groups

Table 3 summarizes the demographic characteristics of the study sample. All participants were Caucasian, the majority of them (61.8%) were females. The median age was 41.5(29-52) years and the median BMI was 27.4 (23.77-32.755) kg/m^2. There were inter-group statistically significant variations in the demographic characteristics, such as age and BMI in both MetS and MetS-pre/T2DM groups (p<0.05 vs. lean controls). This demonstrates the heterogeneity between previous arms. There was not any statistically significant difference between the MetS BMI vs. MetS-pre/T2DM BMI (p>0.05). Intra- and inter-group gender distribution had not any statistically significant variations (p>0.05; Table 3). In perfect alignment with the inclusion criteria, FPG (mg/dL) and HbA1c (%) were significantly higher (p< 0.001) in MetS-pre/T2DM when compared to both normoglycemic control and nondiabetic MetS groups (Table 3). In abiding by MetS constellation of components, the following parameters were significantly higher (p< 0.001) in both nondiabetic MetS and MetS-pre/T2DM groups when compared to the control group: Systolic Blood Pressure (SBP) (mmHg); Distolic Blood Pressure (DBP) (mmHg), TG (mg/dL) as well as LDL-C levels (mg/dL). Conversely, HDL-C concentrations (mg/dL) were markedly lower (p< 0.001) in both MetS and MetS-pre/T2DM groups vs. healthy lean controls (38(34.5-40) and 38.4(34.6-42) vs. 50(44-70.5), Table 4). However, all the above MetS-related parameters (by definition) were not significantly different in MetS-pre/T2DM group when compared to those of MetS group. Additionally, Total Cholestrol (TC) (mg/dL) was significantly higher (p = 0.025) in MetS group when compared to control group (207(163.5-230.5), vs. 169 (131.25-209.75)). Interestingly, MetS-pre/T2DM group's total cholesterol was not significantly different from either control or MetS groups' (Table 3).

3.3. Adiposity and hematological indices, plasma levels of OXM and OXT in study groups

Table 4 displays the comparison of study groups with regard to MetS-related adiposity and hematological indices as well as molecular metabolic biomarkers.

Principally, MetS-related conicity index (CI) and TG/HDL-C ratio were significantly higher (p< 0.001) in both nondiabetic MetS and MetS-pre/T2DM groups when compared to the control group. Moreover, MetS-related WC/HC ratio and RDW-CV% were also proven substantially higher in both nondiabetic MetS and MetS-pre/T2DM groups vs. control group (Table 4).

Table 2A. Categories of BMI[5]

Underweight	15-19.9
Normal weight	20-24.9
Overweight	25-29.9
Class I obesity	30-34.9
Class 2 obesity	35-39.9
Class 3 obesity	≥40

Table 2B. IDF Definition of metabolic syndrome[5]

1-Central obesity assessed by waist circumference (WC)	**Females** **WC ≥ 80 cm**	**Males** **WC ≥94 cm**
plus any two components or more of following		
2-Raised Triglycerides (TG)	≥150 mg/dL(1.7 mmol/L) or specific treatment for this lipid abnormality.	
3-Reduced HDL-C	<40 mg/dL (1.03 mmol/L) in males <50 mg/dL (1.29 mmol/L) in females or specific c treatment for this lipid abnormality	
4-Raised blood pressure (BP)	Systolic BP (SBP) ≥ 130 or diastolic BP (DBP) ≥ 85 mmHg or treatment of previously diagnosed hypertension.	
5-Raised fasting plasma glucose (FPG)	≥100 mg/dL (5.6 mmol/L), or previously diagnosed T2DM	

Table 3. Demographic and clinical characteristics of all participants and comparison between three study groups

Parameter	Total sample N=89 Median(IQR)	Control group N=30 Median(IQR)	MetS group N=30 Median(IQR)	MetS-Pre/T2DM N=29 Median(IQR)	P1[a]	P2[a]	P3[a]
Age (years) (18-75)	41.5 (29-52)	28 (22.5-30.5)	46 (37.75-54.25)	52 (43.5-55)	**<0.001**	**<0.001**	.081
Gender N (%)* Male Female	34(38.2) 55(61.8)	8(26.7) 22(73.3)	11(36.7) 19(63.3)	15(51.7) 14(48.3)	0.163		
BMI (kg/m2)	27.4 (23.77-32.755)	22.21 (19.93-24.47)	31.17 (27.19-35.83)	31.8 (27.5-35.2)	**<0.001**	**<0.001**	0.705
SBP (mm Hg)	135 (120-140)	120 (115-126.25)	135 (127.5-141.25)	140 (135-147.5)	**<0.001**	**<0.001**	0.065
DBP (mm Hg)	80 (75-85)	75 (70-80)	84 (76.5-85.5)	85 (77.5-90)	**<0.001**	**<0.001**	0.634
FBG (mg/dL)	88 (81.5-100.5)	87 (79.5-90)	86 (79.75-93.25)	112 (103-118.3)	0.706	**<0.001**	**<0.001**
HbA1c (%)	5.5 (5.2-5.7)	5.25 (5.1-5.43)	5.4 (5.2-5.53)	5.9 (5.73-6.8)	0.072	**<0.001**	**<0.001**
TG (mg/dL)	148 (79.18-210.85)	72 (49.25-94.25)	199 (156.25-262)	179.5 (127.7-242.5)	**<0.001**	**<0.001**	0.308
TC (mg/dL)	185.5 (150.25-218.05)	169 (131.25-209.75)	207 (163.5-230.5)	187.9 (158.9-254)	**0.025**	0.058	0.755
LDL_C (mg/dL)	112.45 (83.25-147.25)	88 (74.25-119)	111.5 (84.5-154.98)	130.5 (101.7-174.3)	**0.026**	**<0.001**	0.228
HDL_C (mg/dL)	40 (36-48)	50 (44-70.5)	38 (34.5-40)	38.4 (34.6-42)	**<0.001**	**<0.001**	0.571

a=P_{value} by Mann-Whitney Test; * =P_{value} by Chi-square test; P1=Control group compared to MetS group; P2=Control group compared to MetS-Pre/T2DM; P3=MetS group compared to MetS-Pre/T2DM

However, all the above MetS-related indices were not ascribed any statistically marked variations in nondiabetic MetS vs. MetS-pre/T2DM groups (Table 4). The significantly higher MPV median (fL, p = 0.015) was proven for MetS-pre/T2DM group (but not for the nondiabetic MetS group) when compared to healthy controls' MPV. Surprisingly, OXM plasma levels (ng/mL) lacked any statistically significant inter-group variation. The median-IQR plasma levels of OXM were 63023.6 (124670.05-13246.675). Controls' OXT plasma levels (pg/mL) were significantly higher vs. those of nondiabetic MetS and MetS-pre/T2DM groups. Remarkably, nondiabetic MetS' OXT was pronouncedly higher than in MetS-pre/T2DM patients (pg/mL; p< 0.015).

Table 4. Adiposity and hematological indices, plasma levels of biomarkers of all participants and comparison of three study groups

Parameter	Total sample N=88 Median(IQR)	Control group N=30 Median(IQR)	MetS group N=30 Median(IQR)	MetS-Pre/T2DM N=29 Median(IQR)	P1[a]	P2[a]	P3[a]
C- index	1.096 (0.98-1.17)	0.97 (0.87-1.05)	1.15 (1.08-1.22)	1.15 (1.06-1.18)	**<0.001**	**<0.001**	0.458
WC/HC ratio	0.89 (0.825-0.92)	0.82 (0.75-0.91)	0.91 (0.88-0.93)	0.9 (0.87-0.92)	**<0.001**	**0.006**	0.144
TG/HDL-C ratio	2.96 (1.49-5.62)	1.31 (0.94-1.97)	5.105 (3.79-6.75)	4.68 (2.16-6.55)	**<0.001**	**<0.001**	0.255
RDW_ CV%	13.3 (12.53-14.1)	13.1 (12.2-13.7)	13.3 (12.5-14)	13.6 (12.93-14.6)	**<0.001**	**0.011**	0.248
MPV (fL)	9.8 (8.97-10.53)	9.3 (8.55-9.9)	9.9 (9.2-10.6)	10.35 (9.08-11.03)	0.053	**0.015**	0.277
OXM (ng/mL)*	63032.6 (13246.675-124670.05)	46689 (12815.75-115258.55)	89024.8 (22532-143837.175)	48042.2 (10052.5-121639.25)	0.243	0.738	0.217
OXT (pg/mL)*	2314 (1272.2-4041.55)	4176.6 (2407.13-5243.3)	1975.4 (1522.25-3191.15)	1403 (1033.95-2567.3)	0.009	**0.001**	0.071

[a]=P_{value} by Mann-Whitney Test

* = P_{value} by Multivariate test

P1=Control group compared to MetS group

P2=Control group compared to MetS-Pre/T2DM

P3=MetS group compared to MetS-Pre/T2DM

Table 5. Comparison of our study OXT findings with most updated observational studies

Parameters	Findings by AL-Nouaaimi *et al*., (2016)			Our findings					
	MetS N=76 (mean±SD)	MetS-Pre/T2DM N=86 (mean±SD)	P[b]-value	Control N=30 Median (IQR)	MetS N=30 Median (IQR)	MetS-Pre/T2DM N=29 Median (IQR)	P1[a]	P2[a]	P3[a]
SBP (mm Hg)	132.75+18.28	139.40+18.9	0.23	120 (115-126.25)	135 (127.5-141.25)	140 (135-147.5)	**<0.001**	**<0.001**	0.065
DBP (mm Hg)	78.87+10.97	82.53+11.92	0.42	75 (70-80)	84 (76.5-85.5)	85 (77.5-90)	**<0.001**	**<0.001**	0.634
HbA_1c (%)	5.27+0.34	7.24+8.20	0.36	5.25(5.1-5.43)	5.4 (5.2-5.53)	5.9 (5.73-6.8)	0.072	**<0.001**	**<0.001**
FPG (mg/dL)	101.69+18.60	120.52+29.10	**<0.001**	87 (79.5-90)	86 (79.75-93.25)	112 (103-118.3)	0.706	**<0.001**	**<0.001**
Total cholesterol (mg/dL)	196.24+44.17	200.76 +42.99	0.542	169 (131.25-209.75)	207 (163.5-230.5)	187.9 (158.9-254)	**0.025**	0.058	0.755
LDL-C (mg/dL)	139.08+103.3	136.90+36.25	0.857	88 (74.25-119)	111.5 (84.5-154.98)	130.5 (101.7-174.3)	**0.026**	**<0.001**	0.228
HDL-C (mg/dL)	48.42+14.57	43.80+12.27	**0.034**	50 (44-70.5)	38 (34.5-40)	38.4 (34.6-42)	**<0.001**	**<0.001**	0.571
TG (mg/dL)	179.04+184.3	169.15+84.57	0.657	72 (49.25-94.25)	199 (156.25-262)	179.5 (127.7-242.5)	**<0.001**	**<0.001**	0.308
OXT (pg/mL)	2253.71+851.24	1206.28+507.68	**<0.001**	4176.6 (2407.13-5243.3)	1975.4 (1522.25-3191.15)	1403 (1033.95-2567.3)	**0.009**	**0.001**	0.071

p[b]-value by independent-sample t-test; a=P_{value} by Mann-Whitney Test; P1=Control group compared to MetS group; P2=Control group compared to MetS-Pre/T2DM; P3=MetS group compared to MetS-Pre/T2DM

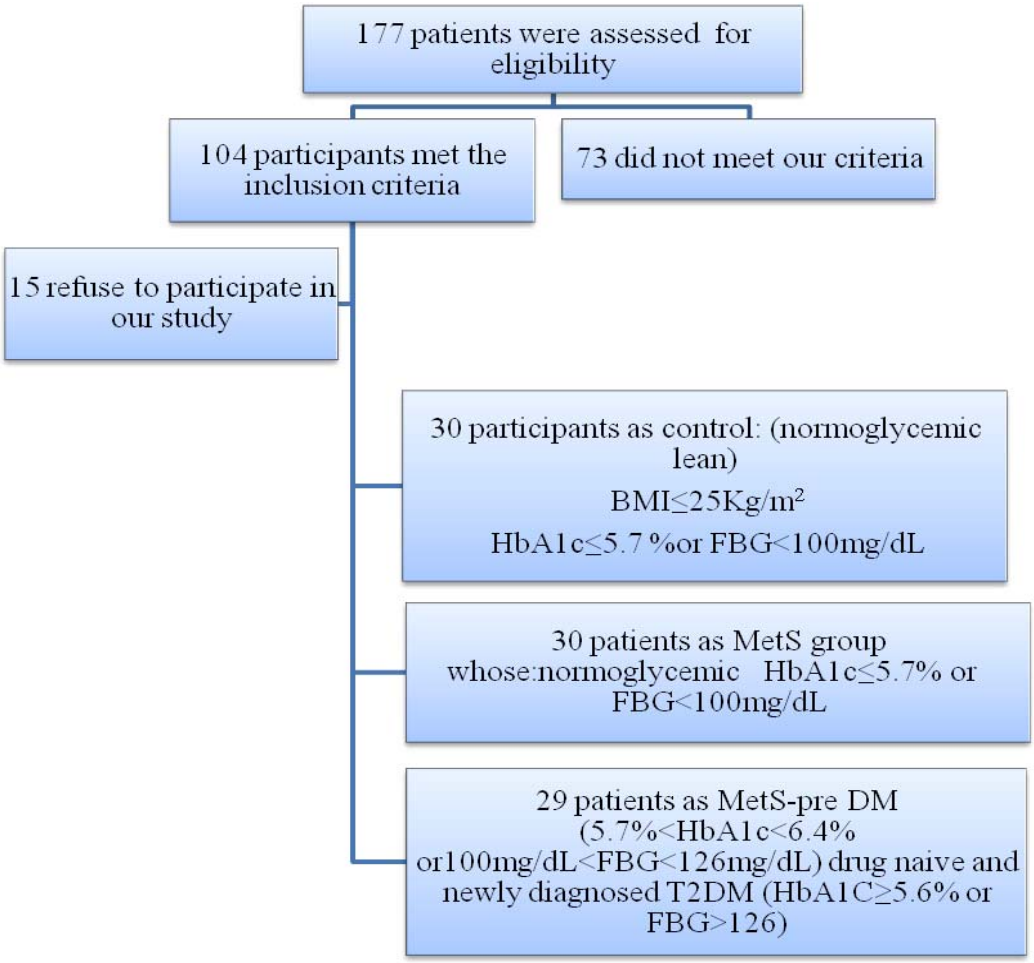

Figure 2: The study flow chart

4. DISCUSSION

Biomarkers are physical, functional or biochemical indicators of physiological or disease processes. They can be key indicators providing vital information in determining disease prognosis, in predicting response to therapies, adverse events and drug interactions, and in establishing baseline risk. In biomarkers translational research, the development and validation of surrogate, novel, and robust predictive, diagnostic and prognostic tests in modern medicine practices can be a thrilling drive and a challenging territory. Besides, biomarkers can play an increasingly important role in the discovery and development of new drugs. For the full utility of biomarkers to be realized, greater understanding of disease mechanisms and the interplay between disease mechanisms, therapeutic interventions and the proposed biomarkers is required[20].

Metabolic syndrome and T2DM represent an escalating global threat not due to hyperglycemia itself, but owing to its disabling complications (amputation, blindness, and dialysis). Disturbing quality of life, loss of productivity and being as family burden can be among the major destinations of diabetic patients. Thus, evaluating endogenous entities and indices of diabetes could suggest new pathophysiological lines of diabetes to be non/pharmacologically intervened. It is worth pointing out

that this is the first study in Jordan that investigates OXM levels in MetS-related T2DM population in Jordan. Furthermore, we also evaluated the adiposity and hematological indices (C index, WC/HC ratio, TG/HDL-C ratio, RDW-CV% and MPV) for the first time in the same population.

The effect of OXT has been investigated in human and animal models in many studies. It was found to be significantly decreased in obesity and T2DM patients. Moreover, serum OXT levels are closely correlated with glycolipid metabolism, IR [21]. These findings altogether indicate that OXT can effectively be used as a novel therapeutic agent against obesity and T2DM [21]. The mainstay of this study is that plasma levels of OXT and OXM are investigated to provide a therapeutic evidence for using OXT and/or OXM as drug targets in treating metabolic disturbances like obesity, IR, and DM.

In a recent cross sectional study conducted by Al-Nouaaimi *et al.* [22], 166 patients were enrolled; mainly 77 nondiabetic MetS patients and 89 MetS-Pre/T2DM patients. Table 5 displays comparisons of outcomes of AL-Nouaaimi *et al*.,[22] vs. the findings of our study. Notably, C-index, WC/HC ratio, TG/HDL-C ratio, RDW-CV% and MPV were left uninvestigated in Al-Nouaaimi *et al.* [22]. Comparable to our study design and outcomes, in Al-Nouaaimi *et al*. [22], there was a significant difference in FPG levels between nondiabetic MetS vs. MetS-Pre/T2DM. This can be inferred as generalizing both studies outcomes to Jordanian population. Importantly, our study MetS groups also demonstrated pronounced differences in fasting glycemia in diabetic/prediabetic MetS patients vs. normoglycemic lean controls. In addition, our reported findings of reduced OXT in both MetS groups (nondiabetic and pre/dianbetic subjects) aligned perfectly with reports on metabolic disorders and related complications [23-25].

Interestingly, Pocai [6] reports that acute OXM infusion improves glucose tolerance in T2DM patients acting as dual agonist of the GCGR and GLP1R. This offers new promising treatments for diabetes and obesity with the potential for weight loss and glucose lowering superior to that of GLP1R agonists. There were scarce studies to describe the effect of OXM in MetS or T2DM. Nevertheless, Wewer-Albrechtsen *et al.* [26] showed that the OXM in patients with T2DM was markedly reduced. Comparable significant OXM variation vs. controls' levels was not identifiable in either MetS-pre/diabetic or MetS-non diabetic patients in our study.

In conclusion, OXT circulating concentrations in both nondiabetic MetS and MetS-Pre/T2DM were evidently described as significantly lower vs. lean normoglycemic controls' OXT.

5. LIMITATIONS

- Small sample size.
- The study cross-sectional design can be a limitation as a causal relationship between changes in circulating biomarkers levels and the development of obesity-related T2DM cannot be conclusively determined.
- Circadian rhythmicity or pulsatile pattern of OXT at a different time points throughout the day may have not been granted sufficient focus.
- Stress may have equally affected plasma OXT levels.
- Study inclusion criteria to obtain MetS or MetS pre/T2DM were tight and demanding.

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List of Abbreviations

ADA	American Diabetes Association
BAI	Body Adiposity Index
BMI	Body Mass Index
BP	Blood Pressure
C-Index	Conicity Index
CVD	Cardiovascular Disease
DIO	Diet Induced Obesity
DM	Diabetes Mellitus
ELISA	Enzyme- Linked Immunsorbent Assay
DM	Diabetes Mellitus
DBP	Diastolic Blood Pressure
FPG	Fasting Plasma Glucose
GCGR	Glucagon Receptor
GLP1R	Glucagon-Like Peptide-1 Receptor
HbA1c	Hemoglobin A1c
HDL-C	High Density Lipoprotein Cholesterol
ICF	Informed Consent Form
IDF	International Diabetes Federation
IQR	Interquartile Range
IR	Insulin Resistance
IRB	Institutional Review Board
LDL-C	Low Density Lipoprotein Cholesterol
MetS	Metabolic Syndrome
MPV	Mean Platlet Volume
NCDEG	National CenterFor Diabetes Endocrinology And
OXM	Oxyntomodulin
OXT	Oxytocin
RDW	Red cell Distribution Width
SBP	Systolic Blood Pressure
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholestrol
TG	Triglyceride
WHR	Waist- Hip Ratio
WHtR	Waist-Height Ratio

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قياس مستوى هرموني الاكستوسين والاكسنتوموديولين ومؤشرات السمنة ومؤشرات مكونات الدم في مرضى السكري النوع الثاني أو ما قبل السكري مع أو بدون متلازمة الأيض

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ملخص

هرمون الاكسنتوموديولين هو هرمون مكون من 37 حمضاً امينياً ببيتيداً يفرز من القناة الهضمية بعد الأكل، اما هرمون الاكسيتوسين فهو مكون 9 احماض امينية ببيتيدية يفرز من منطقة الغدة النخامية وله عدة وظائف في الجسم. في هذه الدراسة كان الهدف قياس مستوى هرموني الاكستوسين والاكسنتوموديولين، مؤشر السمنة، محيط الخصر ونسبة محيط الخصر الى محيط الحوض، توزع كريات الدم الحمراء، متوسط حجم الصفائح الدموية ونسبة الشحوم الثلاثية الى البروتين الدهني عالي الكثافة في مرضى السكري النوع الثاني او ما قبل السكري ومرضى متلازمة الأيض.

الطريقة: بطريقة الدراسة المقطعية تم اخذ عينة من30 شخصاً طبيعياً (نسبة سكر الدم طبيعية) و30 مريضاً يعاني من مرض متلازمة الأيض و29 مريضاً يعانون من مرض متلازمة الأيض مع السكري النوع الثاني أو ما قبل السكري. ثم تم استخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط لقياس نسبة الهرمونين بالإضافة الى مؤشرات السمنة والمؤشرات الدموية الأخرى لكل مريض.

النتائج: من هذه الدراسة تم استنتاج ما يلي: قيم الوسيط ولهرمون الاكسيتوسين (بيكوجرام/مليليتر) اقل عند مرضى متلازمة الأيض ومرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري مقارنة بالأشخاص الطبيعيين (مرضى متلازمة الأيض كان 1975.4 ومرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري كان 1403 بينما الأشخاص الطبيعيين كان 4176.6). ($p = 0.009$ و0.001) على الترتيب. أما تركيز هرمون اللأكسنتوموديولين (نانوغرام/مليليتر) لم يوجد اي اختلاف في تركيزه بين اي من المجموعات ((13246.675 – 124670.05) 63023.6). لم يوجد اختلاف في تركيز الهرمونين في مجموعة مرضى متلازمة الأيض مقارنة بمرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري ($p>0.05$).

بالنسبة لمؤشر السمنة ونسبة الشحوم الثلاثية الى البروتين الدهني عالي الكثافة ونسبة محيط الخصر الى محيط الحوض وتوزع كريات الدم الحمراء فقد لوحظ انهم اعلى بشكل واضح في مرضى متلازمة الأيض ومرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري مقارنة بالأشخاص الطبيعيين ($p< 0.001$).

اما متوسط حجم الصفائح الدموية فقد لوحظ انها اعلى بمرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري مقارنة بالأشخاص الطبيعيين، على الرغم من ذلك فقد لوحظ انه لا يوجد اي اختلاف احصائي في كل المؤشرات المتعلقة بمتلازمة الأيض بين مجموعة مرضى متلازمة الأيض ومرضى متلازمة الأيض والسكري النوع الثاني او ما قبل السكري.

الاستنتاج: تركيز هرمون الاكسيتوسين اقل في مجموعة مرضى متلازمة الأيض ومرضى متلازمة الأيض مع السكري النوع الثاني او ما قبل السكري مقارنة بتركيزه بالأشخاص الطبيعيين. بينما لم يوجد اي اختلاف يذكر في تركيز هرمون الاكسنتوموديولين في اي من المجموعات الثلاث.

الكلمات الدالة: الاكستوسين، الاكسنتوموديولين، مؤشر السمنة، نسبة محيط الخصر الى محيط الحوض، توزع كريات الدم الحمراء، متوسط حجم الصفائح الدموية، نسبة الشحوم الثلاثية إلى البروتين الدهني عالي الكثافة.

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