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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

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ABSTRACT

Levels of oxytocin (OXT) hormone and inflammatory biomarkers (leptin, resistin and adiponectin) were measured in 166 plasma samples of Metabolic Syndrome (MS) patients (1) that visited the outpatients' endocrinology clinics, Jordan University Hospital (JUH). Patients were subdivided into two arms according to their glucose profile status, subjects with glucose profile disturbances and normoglycemic subjects. MS biomarkers were significantly different ($P<0.01$) between study groups, mean OXT levels (ng/mL) were exceptionally higher ($p<0.01$) in MS-control group (2.25+0.85) than in MS-pre/T2DM group (1.20+0.50). Conversely, in comparison to MS-controls; MS-pre/T2DM patients had significantly higher adipocytokines plasma levels; namely resistin was 13 times higher (ng/mL) (82.05+ 32.44 vs. 6.45+4.39); leptin two times higher (ng/mL) (42.43+30.44 vs. 22.76+14.19) and adiponectin was 3-fold higher (µg/mL) (6.869+1.082 vs. 1.97+0.606). These findings were indicative for the potential pharmacologic benefit of this hormone in minimization of inflammatory markers chronic deleterious effect.

Keywords: Oxytocin (OXT), Metabolic Syndrome (MS), Adipocytokines, Leptin, Resistin, Adiponectin, Type 2 Diabetes Mellitus (T2DM).

1. INTRODUCTION

T2DM can be defined as a complex, multi-factorial, chronic metabolic and endocrine disorder that is associated with a combination of to insulin insensitivity, insufficiency and /or hyper insulin secretion [(2)]. Inflammatory processes are related to MS features, thus, patients that are older than 40 years old with metabolic disorders are considered to be more vulnerable to develop an impaired fasting glucose (IFG) [(3)]. Abdominal obesity is also linked to a low-grade chronic inflammation[(5)]. Abdominal white adipose tissue (WAT) accumulation can play an endocrine role by releasing high concentrations of bioactive compounds known as adipokines such as interlukin-6 (IL-6), tumor necrosis factor- alpha (TNF-α), leptin and adiponectin [(9)], all of which have a negative impact on insulin sensitivity[(10)], cardiovascular disorders (CVD) development, T2DM and non-alcoholic fatty liver diseases (NAFLD)[(7,9,10)] .

Recent epidemiological research has emphasized on novel biomarkers that are involved in the development of insulin resistance (IR) and the mostly considered adipokines biomarkers (adiponectin and leptin secreted from adipose tissue (AT), while the human studies showed that the adipocytes has a minimal role in resistin production instead it found to be produced from some of inflammatory cells such as macrophages.[(4)]While adiponectin has shown to exert an insulin sensitizing activity and anti-inflammatory role,

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leptin and resistin levels are increased in obese individuals and they are correlated to increased IR besides their pro-inflammatory effect [2,6].

Oxytocin (OXT) is a nine amino acid neuropeptide produced by the hypothalamus and it has a broad range of actions with no structural homologies between it as a metabolic hormone and any of adipocytokines biomarkers, the hormone has a well-known peripheral action in uterine contraction during labor and milk ejection [12] and a vital central role in energy regulation [18]. The chronic administration of OXT can reduce the food intake and body weight in diet induced mice and genetically induced obese rodents [3]. OXT and its analogues therefore can potentially have multiple therapeutic actions beyond the weight control and metabolism regulation such as lipid lowering, insulin sensitizing, insulin secretory effect[18], hyperphagia, food intake, lowering the visceral fat mass, ameliorate obesity, fatty liver, glucose intolerance and diabetes representing a new therapeutic avenue very similar to Glucagon like peptide (GLP-1) anti-diabetic agents [8,12].

2. EXPIREMENTAL

The association between OXT metabolic hormone , MS biomarkers (leptin, adiponectin and resistin) was evaluated in 166 MS patients[1] who visited the outpatient endocrinology clinics of JUH , all of them were completely naïve to anti-hyperglycemic medications and they were either overweight (≥25 kg/m^2) or obese (≥30 kg/m^2). Patients were subdivided to 77 apparently healthy MS participants were assigned as MS-control group (group 1) and 89 pre-diabetics or newly diagnosed with T2DM were assigned as MS-pre/T2DM (group 2) as illustrated in figure1.

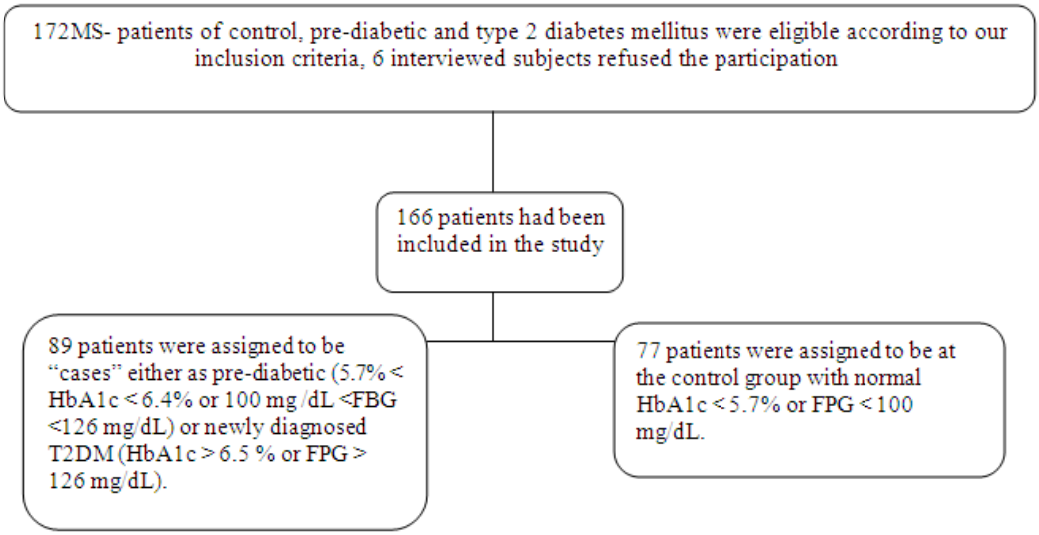


Figure 1: The study flow chart (consort diagram)

Study sample size

Clinical settings and duration

This research has been conducted after having the approval from the scientific research committee at the faculty of pharmacy and approval from the JUH institiutional review board (IRB), researcher was avaliable at the outpatient endocrinology clinics JUH from Sunday-Wedenesday in the peroid between August-December 2014. For blood sample harvesting , 5 mL blood sample was collected from each participant, the process of sampling was completely ethical and every patient accepted the participation had signed the informed consent form which was written in arabic and all participants understood that their voluntary participation and the confidentiality is well preserved . Participants with any of the following was excluded from the study.

- Pregnant or lactating women.
- Any prior treatment with anti-diabetic agents.
- Clinical evidence of autoimmune or life threating disease (alcohol/drug abuse/dyslipdimia/recently diagnosed with untreated endocrine disorder).
- Individuals with inflammatory disease such as the inflammatory bowel disease.
- Obesity secondary to an endocrine dearrangement.

Researcher collected the demographic data consisting of (weight, height, waist circumeference and blood pressure) in co-operation with nursing team, biochemcal data composed of (HbA1c, Fasting Plasma Glucose (FPG), Fasting Lipid Panel (FLP)) were performed in biochemical laboratories of JUH. In addition, patients' medical data were evaluated for history of delivery of overweight neonates (>4 kg), polycystic ovarian syndrome (PCOS) in women ,as well as history of coronary artery diseases (CAD) and DM in first degree relatives, Lifestyle data such as smoking and physical activity were collected from patients themselves.

BMI was calculated by using the following equation: BMI= weight(kg)/height $(m)^2$,

Plasma samples were harvested by using the centifugation technique on fresh withdawn blood samples at 2000 round per minute (RPM) for 10 minutes at zero degree centigrade (C^o). Then, the plasma aliguotes were kept at -80 (C^o) till the time of biochemical analysis

Laboratory assay work principles

ELISA technique was applied to the previously kept human plasma samples for both involved groups in order to measure the levels of OXT metabolic hormone and each of the adipokines biomarkers (leptin, resistin and adiponectin), the dilution factor had been determined based on a previous pilot studies, OXT levels measurment did not require any dilution for the involved samples while both of resistin and adiponectin samples required 200 folds and leptin required 15 folds to be diluted to give the significant readings.

The ELISA kit procedure had been applied precisely. OXT in vitro competitive binding technique is designed for the accurate quantitive measurment of OXT in human plasma that means the intensity of yellow coloration is inversely propotional to the amount of OXT (ng/mL) ***captured in the plate*** Abcam®'s (Maryland,USA) ***while ELISA sandwich technique was applied for the other adipokines and the resulted yellow color was directly proptional with each of resistin (***ng/mL) (RayBiotech®, Georgia, USA), leptin(ng/mL) (RayBiotech®, Georgia, USA) and adiponetin (µg/ml) (Abcam® Human adiponectin ELISA. Maryland USA) captured amounts.

No technical replicates were performed and all the results of color intesity indicating concetrations were read by a plate reader (Bio-Tek instruments, USA).

Statistics

All biomarkers tests were carried out with 2 independent experiments , all data were tested for normality of distribution. All results are expressed as mean ± SD. Pre-coded data were entered into statistical package for the social science software release 20 (SPSS® Inc., Chicago, IL), Categorical data were expressed as frequency and percentage; while continuous variables were presented as mean + SD. Independent sample t-test was utilized to compare continuous data among two categories. Chisquare test was utilized as appropriate to compare two sets of categorical data. Correlations between biomarkers and clinical parameters were assessed using Spearman correlation due to non-normal data distribution. All probabilities were two tailed and p-value < 0.05 was regarded as statistically significant.

3. RESULTS

Patients' demographic data

The majority of participants were females (66.9%) and the mean age was 51±10.73 years old . Participants were obese (47.5%), morbidly obese (30.2%) or overweight (20.4%) with mean BMI 33.23±5.48 kg/m^2. BMI categories distribution between males was homogenous (p=0.104). This is also the case for females' BMI category distribution (p=0.368) and there were no difference in the demographic characteristics (age,

gender, BMI) (p>0.05) which confirm the homogeneity of the study arms (Table 1).

Table 1. Demographic characteristics of study pool participants*p*[c]

Parameter	**Total number of subjects N=166**	**group 1 N= 77 46.4%**	**group 2N= 89 53.6%**	
Age in years (mean+SD) Range (years)	51+10.73 (20-75)	49.95+10.03	51.91+11.28	0.241
Age in years (mean+SD) MalesN= 55	49.00 +11.1	48.08+11.32	49.93+10.51	0.532
Age in years (mean+SD) Females N= 111	51.88 +10.22	50.85+9.33	52.92+11.60	0.307
Gender, N[a] (%) Male Female	 55 (33.1) 111 (66.9)	 25 (32.5) 52 (67.5)	 30 (33.7) 59 (66.3)	 0.866
BMI(mean+SD)(Kg/m^2)	33.23+5.48	33.16+5.13	33.29+5.80	0.887
BMI(mean+SD)(Kg/m^2) Males N= 55	31.91 +5.43	32.41+3.25	31.41+4.71	0.379
BMI(mean+SD)(Kg/m^2) Females N=111	34.11 +5.91	33.52+5.82	34.69+4.82	0.258
BMI category, N(%)[a] Normal weight Overweight Obese Morbidly obese	 3 (1.9) 33 (20.4) 77 (47.5) 49 (30.2)	 2 (2.6) 14 (18.2) 41(53.2) 20 (26)	 1 (1.2) 19 (22.4) 36 (42.4) 29(34.1)	 0.104
BMI category, N (%)[a] Male Overweight Obese Morbidly obese	 16 (30.2) 28 (52.8) 9 (17)	 4 (16) 16 (64) 5 (20)	 12 (42.9) 28 (52.8) 9 (17)	 0.104
BMI category, N (%)[a] females Overweight Obese Morbidly obese	 10 (19.2) 49 (45) 40 (36.7)	 7 (12.3) 25 (48.1) 15 (28.8)	 17 (15.6) 24 (42.1) 25 (43.9)	 0.368

[a] percent within total. [c]*p* -value by independent-sample t-test for age and BMI; and by Chi square test for gender and BMI categories; SD: Standard deviation.

Differences in clinical characteristics and biomarkers levels in both study arms

The clinical characteristics and parameters levels of the study population are summarized in table 2. Kidney functions represented by creatnine levels were retarded among MS-pre/T2DM of participants (0.76+0.22 mg/dL) (*p*=0.008), glucose profile illustrated by HbA1c and FPG was significantly complicated among MS-pre/T2DM participants as well, HbA1c (7.24+8.20) (*p*=0.036), FPG (120.52+29.10) (*p*<0.001). In contrast high density

lipoprotein (HDL) registered higher level among MS-controlsparticipants (48.42+14.57 mg/dL) (p=0.034). OXT metabolic hormone mean levels (ng/mL) was 2-fold lower in MS-pre/T2DMpatients (2.25+0.85) than MS-controls (2.25+0.85) (p<0.001) (figure 2). Resistin inflammatory biomarker levels were 13-fold higher among MS-pre/T2DMpatients (ng/mL) (82.05+32.44) than MS-controls of participants (6.45+43.92) (p<0.01) (figure 3). Likewise, adiponectin mean level (µg/mL) was 3.5 folds higher in MS-pre/T2DM of study (6.86+1.08) than MS-controls of patients (1.97+14.19) (p<0.01) (figure 4), similarly leptin mean level (ng/mL) was 2 folds higher among MS-pre/T2DM patients (42.43+30.44) than MS-controls participants (2.76+14.19) (p<0.01) (figure 5).

Table 2. Clinical parameters and inflammatory biomarkers of the study pool participants

Parameters	**Total sample N=162 (mean+SD)**	**MS-controls N=76 46.9% (mean+SD)**	MS-pre/T2DM **N=86 53.1% (mean+SD)**	***p***[b]
Systolic blood pressure SBP (mm Hg)	136.32+18.85	132.75+18.28	139.40+18.90	0.23
Diastolic blood pressure DBP (mm Hg)	80.83+11.60	78.87+10.97	82.53+11.92	0.42
Waist circumference (cm)	104.68+12.08	103.71+11.79	105.52+12.34	0.339
Serum creatnine (mg/dL)	**0.72+0.21**	**0.67+0.19**	**0.76+ 0.22**	**0.008**
HbA1c	**6.31+6.02**	**5.27+0.34**	**7.24+8.20**	**0.036**
FPG (mg/dL)	**111.83+26.47**	**101.69+18.60**	**120.52+29.10**	**<0.001**
Total cholesterol (mg/dL)	198.69+43.43	196.24+44.17	200.76+42.99	0.542
LDL-C (mg/dL)	137.92+75.00	139.08+103.30	136.90+36.25	0.857
HDL-C (mg/dL)	**46.01+13.57**	**48.42+14.57**	**43.80+12.27**	**0.034**
TG (mg/dL)	173.79+140.07	179.04+184.36	169.15+84.57	0.657
OXT (ng/ml)	**1.69+0.86**	**2.25+0.85**	**1.20+0.50**	**<0.001**
Resistin (ng/ml)	**48.13+44.81**	**6.45+4.39**	**82.05+32.44**	**<0.001**
Leptin (ng/ml)	**34.46+26.89**	**22.76+14.19**	**42.43+30.44**	**<0.001**
Adiponectin (µg/ml)	**4.66+2.60**	**1.97+0.60**	**6.86+1.08**	**<0.001**
Leptin Adiponectin Ratio LAR	0.105+0.222	0.0126+0.088	0.009+0.0276	0.344

[b]*p*-value by independent-sample t-test , SD: standard deviation, FPG: fasting plasma glucose, HbA1c: hemoglobin glycosylated A_{1C}, HDL: high density lipoprotein, LDL: low density lipoprotein, OXT: oxytocin, TG: triglyceride

Correlation between MS biomarkers and Clinical Parameters in MS-Participants

The correlation of clinical parameters with biomarkers of all MS-sample subjects are summarized in Table 3. Further correlations among MS-control group and MS-pre T2DM group are summarized in Table 4. Resistin had a positive correlation with age (*p=0.025)* however it had a negative correlation with DBP of MS subjects (p=0.043). adiponectin was positively correlated with SBP (p=0.033), DBP (p<0.01), serum creatnine (p=0.019) and Fasting blood glucose (p=0.016)). DBP had a positive correlation with waist circumference (p=0.002)**,** BMI (p=0.022) and SBP (p <0.001). A positive correlation was also found between leptin and BMI (p <0.001) and waist circumference (p < 0.001). OXT was negatively correlated with serum creatnine (p=0.037) and positively correlated with both adiponectin (p=0.011) and resistin (p=0.30). HbA1c has a negative correlation with HDL (P=0.027). FPG has positive correlations with both resistin (p=0.011) and

leptin/adiponectin ratio (LAR) (p=0.022) but negatively correlated with HDL (p=0.029).

A positive correlation was found between resistin and LDL (p=0.001), LDL and TG ($p < 0.001$). In contrast OXT was negatively correlated with HDL (p=0.034). Resistin was found to be correlated positively with TG (p=0.004) and adiponectin was positively correlated to LAR ($p < 0.001$).

Table 3. Correlation between the clinical parameters and MS biomarkers (OXT, resistin, leptin and adiponectin) in all MS-participants

Correlation / **Clinical parameters**		**OXT (ng/mL)**	**Resistin (ng/mL)**	**Leptin (ng/mL)**	**Adiponectin (µg/mL)**	**LAR**
Age	Correlation	-0.041	0.116	0.039	0.118	-0.028
	Sig (2-tailed)	0.600	0.148	0.636	0.139	0.736
	N	162	156	148	160	146
SBP	Correlation	0.004	0.141	0.079	0.137	-0.024
	Sig (2-tailed)	0.957	0.078	0.330	0.084	0.778
	N	162	156	148	160	146
DBP	Correlation	-0.044	0.139	**0.198**	0.130	0.048
	Sig (2-tailed)	0.583	0.083	**0.016**	0.101	0.563
	N	162	156	**148**	160	146
BMI	Correlation	-0.005	0.078	**0.514**	0.041	**0.350**
	Sig (2-tailed)	0.949	0.340	**<0.001**	0.611	**<0.001**
	N	158	152	**144**	156	**142**
Waist circumference	Correlation	-0.096	0.115	**0.285**	0.071	0.153
	Sig (2-tailed)	0.222	0.152	**<0.001**	0.371	0.066
	N	162	156	**148**	160	146
Creatnine	Correlation	**-0.050**	0.065	0.021	**0.194**	**-0.174**
	Sig (2-tailed)	**0.011**	0.693	0.809	**0.018**	**0.043**
	N	**149**	145	136	**147**	**135**
HbA1c	Correlation	**-0.307**	**0.383**	**0.191**	**0.708**	**-0.383**
	Sig (2-tailed)	**<0.001**	**<0.001**	**0.021**	**<0.001**	**<0.001**
	N	**162**	**156**	**145**	**157**	**143**
FPG	Correlation	**-0.307**	**0.383**	0.058	**0.305**	**-0.175**
	Sig (2-tailed)	**<0.001**	**<0.001**	0.498	**<0.001**	**0.041**
	N	**152**	**147**	139	**150**	**137**
Total cholesterol	Correlation	-0.007	0.061	0.097	0.149	0.011
	Sig (2-tailed)	0.934	0.486	0.283	0.086	0.905
	N	137	132	125	135	124
LDL	Correlation	0.045	0.157	0.055	**0.216**	-0.045
	Sig (2-tailed)	0.581	0.057	0.520	**0.008**	0.601
	N	153	148	140	**151**	138
HDL	Correlation	-0.021	-0.098	**-0.185**	-0.161	**0.302**
	Sig (2-tailed)	0.800	0.238	**0.029**	0.050	**<0.001**
	N	151		**139**	149	**137**
TG	Correlation	-0.026	0.111	-0.005	0.156	-0.134
	Sig (2-tailed)	0.746	0.176	0.957	0.054	0.112
	N	156	151	143	154	141

OXT	Correlation	1.000	**-0.568**	**-0.209**	**-0.480**	**0.222**
	Sig (2-tailed)	.	**<0.001**	**0.012**	**<0.001**	**0.008**
	N	162	**155**	**144**	**156**	**142**
Resistin	Correlation	**-0.568**	1.000	**0.335**	**0.732**	**-0.249**
	Sig (2-tailed)	**<0.001**		**<0.001**	**<0.001**	**0.003**
	N	**155**	156	**145**	**150**	**143**
Adiponectin	Correlation	**-0.480**	**0.732**	**0.304**	1.000	**-0.400**
	Sig (2-tailed)	**<0.001**	**<0.001**	**<0.001**	.	**<0.001**
	N	**156**	**150**	**146**	160	**146**
Leptin	Correlation	**-0.209**	**0.335**	1.000	**0.304**	**0.678**
	Sig (2-tailed)	**0.012**	**<0.001**	.	**<0.001**	**<0.001**
	N	**144**	**145**	148	**146**	**146**
LAR	Correlation	**0.222**	**-0.249**	**0.678**	**-0.400**	1.000
	Sig (2-tailed)	**0.008**	**0.003**	**<0.001**	**<0.001**	
	N	**142**	**143**	**146**	**146**	146

Spearman correlations. BMI: body mass index, FBG: fasting Blood glucose, DBP: diastolic blood pressure, HbA1c: hemoglobin glycosylated A1C, HDL: high density lipoprotein, LAR: leptin/adiponectin ratio, LDL: low density lipoprotein, SBP: systolic blood pressure, TG: triglycerides.

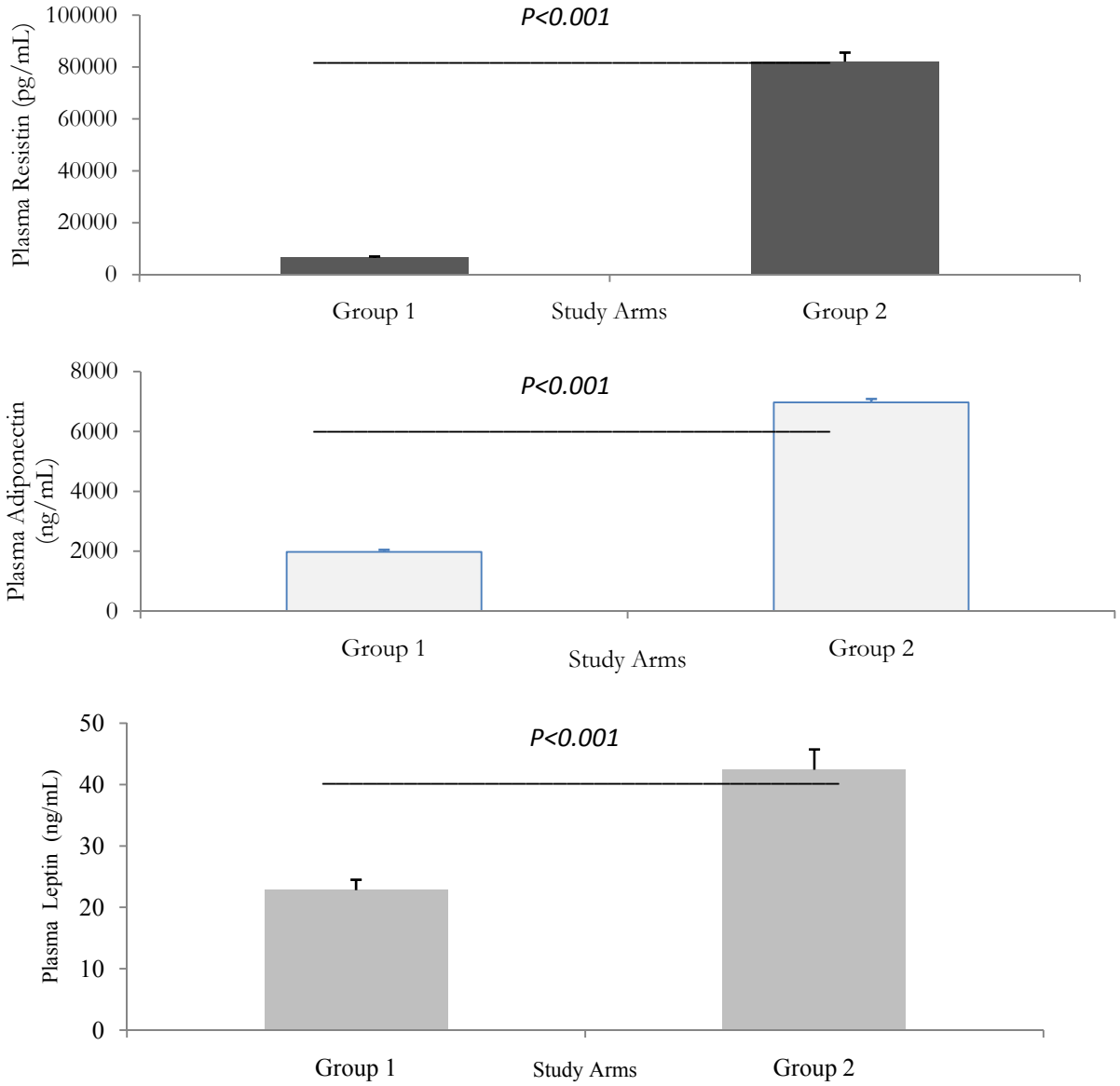
Table 4. Correlations between the clinical parameters and MS biomarkers (OXT, resistin, leptin and adiponectin) in MS-controls (Group 1) and MS-pre/T2DM (Group 2) participants

Correlation		**Group 1**					**Group 2**				
Clinical parameters		**OXT (ng/mL)**	**Resistin (ng/mL)**	**Adiponectin (µg/mL)**	**Leptin (ng/mL)**	**LAR**	**OXT (ng/mL)**	**Resistin (ng/mL)**	**Adiponectin (µg/mL)**	**Leptin (ng/mL)**	**LAR**
Age	Correlation	0.085	-0.042	**0.234**	-0.140	-0.192	-0.092	**0.230**	0.118	0.106	0.068
	Sig (2-tailed)	0.463	0.730	**0.048**	0.287	0.149	0.398	**0.033**	0.275	0.325	0.531
	N	76	70	**72**	60	58	86	**86**	88	88	88
SBP	Correlation	0.061	0.161	0.009	-0.031	-0.012	.190	0.031	0.006	0.089	0.086
	Sig (2-tailed)	0.600	0.182	0.937	0.817	0.929	0.080	0.779	0.952	0.412	0.426
	N	76	70	72	60	58	86	86	88	88	88
DBP	Correlation	0.028	0.074	-0.187	0.105	0.180	0.108	-0.038	0.008	0.134	0.130
	Sig (2-tailed)	0.810	0.545	0.115	0.425	0.176	0.320	0.729	0.940	0.215	0.229
	N	76	70	72	60	58	86	86	88	88	88
BMI	Correlation	0.056	0.133	-0.051	0.204	0.157	0.017	-0.015	0.069	**0.690**	**0.639**
	Sig (2-tailed)	0.629	0.274	0.668	0.118	0.239	0.880	0.890	0.531	**<0.001**	**<0.001**
	N	76	70	72	60	58	82	82	84	**84**	**84**
Waist	Correlation	-0.075	0.128	0.165	0.205	0.131	-0.011	0.078	-0.034	0.299	**0.252**
circumference	Sig (2-tailed)	0.518	0.292	0.166	0.116	0.326	0.920	0.476	0.752	0.005	**0.018**
	N	76	70	72	60	58	86	86	88	88	**88**
Creatnine	Correlation	-0.044	-0.235	0.105	0.133	0.101	-0.107	-0.006	-0.156	-0.171	-0.192
	Sig (2-tailed)	0.720	0.059	0.400	0.334	0.468	0.345	0.955	0.165	0.127	0.086
	N	69	65	66	55	54	80	80	81	81	81

HbA1c	Correlation	-0.050	-0.065	0.046	-0.065	-0.137	0.094	**-0.221**	-0.067	-0.152	-0.123
	Sig (2-tailed)	0.669	0.591	0.702	0.621	0.306	0.388	**0.041**	0.544	0.164	0.260
	N	76	70	72	60	58	86	**86**	85	85	85
FPG	Correlation	-0.187	0.173	0.074	-0.076	-0.118	0.039	-0.062	-0.061	-0.049	-0.014
	Sig (2-tailed)	0.118	0.164	0.550	0.577	0.396	0.726	0.583	0.584	0.661	0.897
	N	71	66	67	56	54	81	81	83	83	83
Total cholesterol	Correlation	-0.064	0.245	0.137	-0.102	-0.110	0.136	-0.090	0.129	0.187	0.131
	Sig (2-tailed)	0.618	0.064	0.298	0.482	0.452	0.249	0.448	0.271	0.108	0.263
	N	63	58	60	50	49	74	74	75	75	75
LDL	Correlation	0.055	**0.252**	0.087	-0.007	0.038	**0.328**	-0.030	0.112	0.038	-0.008
	Sig (2-tailed)	0.648	**0.040**	0.479	0.961	0.783	**0.003**	0.793	0.314	0.735	0.940
	N	72	**67**	68	57	55	**81**	81	83	83	83
HDL	Correlation	-0.214	-0.066	-0.033	0.052	0.065	0.001	0.086	-0.013	**0.366**	**0.381**
	Sig (2-tailed)	0.069	0.591	0.787	0.693	0.630	0.994	0.453	0.909	**<0.001**	**<0.001**
	N	73	68	69	59	58	78	78	80	**80**	**80**
TG	Correlation	**0.242**	0.104	0.078	-0.122	-0.114	-0.124	-0.076	0.052	-0.010	-0.066
	Sig (2-tailed)	**0.038**	0.395	0.522	0.357	0.397	0.266	0.498	0.635	0.931	0.550
	N	**74**	69	70	59	57	82	82	84	84	84
OXT	Correlation	1.000	-0.040	-0.126	0.079	0.101	1.000	0.037	0.172	-0.056	-0.084
	Sig (2-tailed)	.	0.742	0.294	0.554	0.455	.	0.736	0.115	0.611	0.444
	N	76	69	71	59	57	86**	86	85	85	85
Rsistin	Correlation	-0.040	1.000	-0.119	0.148	0.188	0.037	1.000	0.114	0.156	0.134
	Sig (2-tailed)	0.742	.	0.344	0.259	0.158	0.736	.	0.297	0.153	0.223
	N	69	70	65	60	58	86	86	85	85	85
Adiponectin	Correlation	-0.126	-0.119	1.000	-0.057	**-0.386**	0.172	0.114	1.000	0.135	-0.039
	Sig (2-tailed)	0.294	0.344	.	0.671	**0.003**	0.115	0.297	.	0.209	0.717
	N	71	65*	72	58	**58**	85	85	88	88	88
Leptin	Correlation	0.079	0.148	-0.057	1.000	**0.916**	-0.056	0.156	0.135	1.000	**0.949**
	Sig (2-tailed)	0.554	0.259	0.671	.	**<0.001**	0.611	0.153	0.209	.	**<0.001**
	N	59	60	58	60	**58**	85	85	88	88	**88**
LAR	Correlation	0.101	0.188	**-0.386**	**0.916**	1.000	-0.084	0.134	-0.039	**0.949**	1.000
	Sig (2-tailed)	0.455	0.158	**0.003**	**<0.001**	.	0.444	0.223	0.717	**<0.001**	.
	N	57	58	**58**	**58**	58	85	85	88	**88**	88

Spearman correlations. BMI: body mass index, FPG: fasting plasma glucose, DBP: diastolic blood pressure, HbA1c: glycosylated hemoglobin A1C, HDL: high density lipoprotein, LAR: leptin adiponectin ratio, LDL: low density lipoprotein, SBP: systolic blood pressure, TG: triglycerides

2



4. DISCUSSION

According to the previous studies[14], OXT finding were comparable to the results of our study, 166 patients were recruited and distributed into normoglycemic (NGT) versus newly diagnosed (T2DM), all patients were matched in their demographic information (age, gender and medical profile). All subjects were divided into 4 subgroups, namely NGT-normal weights versus NGT obese and normal weight T2DM versus obese T2DM, since our study is concerned with MS patients, thus the NGT obese patients versus T2DM obese patients were concerned for comparison with our results (Table 3).

Table 5. Comparison between the results of OXT in our study to the facts in the literatures

Age (Years)	46.19± 11.06	45.21 ± 9.24	0.287	51.91+11.28	49.95+10.03	0.241
Gender N (male/female)	46 (28/18)	42 (29/13)	-	89 (30/59)	77 (25/52)	0.866
BMI (kg/m^2)	27.49 ± 2.03	27.78 ± 2.66	-	33.29+5.80	33.16+5.13	0.887
SBP (mm Hg)	128.39 ±14.4	128.93 ±16.0	0.190	139.40+18.90	132.75+18.28	0.23
DBP (mm Hg)	**79.72 ± 6.36**	**82.64 ±12.67**	**0.036**	82.53+11.92	78.87+10.97	0.42
Waist circumference (cm)	94.03 ± 4.21	96.02 ± 9.53	0.392	105.52+12.34	103.71+11.79	0.339
HbA_1c (%)	**9.25 ± 1.91**	**5.34 ± 0.31**	**< 0.001**	**7.24+8.20**	**5.27+0.34**	**0.036**
FPG (mg/dL)	**175.68 ±49.6**	**90.18 ± 8.28**	**< 0.001**	**120.52+29.10**	**101.69+18.60**	**< 0.001**
Total cholesterol (mg/dL)	197.21 ± 45.24	176.334 ± 25.52	0.058	200.76+42.99	196.24+44.17	0.542
LDL (mg/dL)	128.38 ± 39.82	111.36 ± 23.58	0.051	136.90+36.25	139.08+103.3	0.857
HDL (mg/dL)	**41.37 ± 7.34**	**46.79 ±12.37**	**0.048**	**43.80+12.27**	**48.42+14.57**	**0.034**
TG (mg/dL)	**231.17± 125.77**	**157.66 82.37**	**< 0.001**	169.15+84.57	179.04+184.3	0.657
OXT (/mL)			**< 0.001**	**1206280 + 507680**	**2253710 + 851240**	**< 0.001**

[b]p-value by independent-sample t-test, SD: Standard deviation, FPG: fasting plasma glucose, HbA1c: hemoglobin glycosylated A_{1C}, HDL: high density lipoprotein, LDL: low density lipoprotein

The most updated cross sectional study had been conducted in 2015 to compare adipocytokines (leptin, adiponectin and LAR) levels between 450 chronic kidney disease patients (CKD) known as cases and 920 control participants, the homogeneity of demographic distribution between both study arms had been concerned[10]. Table 4 illustrates the comparison summary of our study to the reference study.

Table 6. Comparison between the results of adipocytokines (leptin and adiponectin) in our study to the literature review provided outcomes[8]

p-value	**Our findings**		**p-value**	**Findings by literature[10]**		**Parameters (mean±SD)**
	MS-controls	**MS-pre-/T2DM**		**Control**	**CKD cases**	
< .001	**22.76+14.19**	**42.43+30.44**	**< 0.001**	**9.7+11.5**	**16.9+20.2**	Leptin (ng/mL)
< 0.001	**1974.39+606.**	**6869.93+108**	**< 0.001**	**9200+4200**	**10400+7400**	Adiponectin (ng/mL)
0.344	0.0126+0.088	0.009+0.0276	**< 0.001**	**0.0010**	**0.0016**	LAR

A recent cross sectional study[17] had enrolled 73 Chinese subjects which were divided into two main groups, 38 patients with newly diagnosed T2DM and 35 subjects of non T2DM This study was designed to

measure salivary resistin and serum resistin in both groups of patients. (table.5).

Another observational study[5] had enrolled 67 morbidly obese patients with obstructive sleep apnea (OSA), divided in 3 main groups as: 23 diabetic patients, 27 patients with impaired glucose tolerance (IGT) and 17 subjects with normal glucose metabolism (NGM). Clinical outcomes of our study and the comparable studies are summarized in Table 5.

Table 7. Comparison between the results of adipocytokines (resistin) in our study and the studies[17] [5]

p-value	Our findings		p-value	Findings by literature[5]		p-value	Findings by literature[17]		Parameters
	MS-controls	MS-pre-/T2DM		NGM	Diabetic		Control	T2DM	
***P* <0.001**	**6454.73+ 4392.03**	**82053.49+ 32442.36**	***P*=0.043**	**3770+ 3230**	**6120+5930**	***P*<0.05**	**6100+600**	**12300+2700**	Serum resistin (pg/mL)
	-	-	-	-	-	***P*<0.05**	**1500+300**	**3400+400**	Saliva resistin (pg/ml)

The novelty of our study it is the one of its kind as a clinical cross sectional study that demonstrated the inverse correlation of OXT metabolic hormone with multiple adipocytokines biomarkers (leptin, resistin and adiponectin). We calculated LAR and its association with each involved clinical parameter and inflammatory biomarkers as well as OXT metabolic hormone. The correlation of clinical parameters among MS-participants and their attitudes in glucose profile disturbances had been studied. If we compared the results we achieved to the information from the previous population-based studies then we had demonstrated the elevation of adiponectin biomarker level among MS-pre T2DM paients unlike stated in literature were significant inverse association between this biomarker and IR were mainly over emphasized. We clarified the diminished levels of OXT in MS-pre T2DM patients which is in alignment with OXT hormone dual insulin secretory-sensitizer role. The hypothesis of obesity related elevation in leptin levels or leptin reisitance (hyperliptinemia) had been confirmed by our results (in specific with the female MS participants) indicating female gender sensitivity to leptin biomarker and we also matched the data showing resistin levels are associated with metabolic impairments and IR. Finally we had illustrated the association of OXT and several clinical parameters in light of its role in opposing obesity and insulin desensitization.

Future studies should emphasize on the possibility of synthesizing OXT hormone derivatives receptors agonists as a pharmacologic agent to suppress the deleterious CVD effect of inflammatory biomarker releasing by lipid tissue storage depot. Leptin analouges synthesis should be continued for maximum body weight control. More studies should investigate the adiponectin effect on MS-pre T2DM and finally gender distribution should be considered as male participants could be optimized to a half of the study sample population.

This study is limited by its cross-sectional design and does not infer a causal relationship between decreasing levels of circulating OXT and the development of obesity and T2DM. Also, Gender based differences were easily detectable because of unequal distribution of subjects; more female participants than males. Serial changes in OXT need to be measured at different time points during the day due to the daily and pulstaile pattern of OXT release and different mood status because serum OXT

levels may be affected dramatically by the time of the day and possible and variable acute stress experienced participants; respectively. We did not recruit normal subjects (normoglycemic and normal body weight) as a 3rd group of control participants beside the MS-population. Therefore, future endeavors should seek recruiting normoglycemic non-MS controls.

This is the *first clinical study* which examined the correlation of adipocytokines (leptin, adiponectin and resistin) and OXT.

Our study was powered enough and recruited a large study sample pool which helps in detecting all the possible correlations of clinical biomarkers and parameters. We had confirmed the fact of potential dual insulin secretory/sensitizer role of OXT according to the resulted diminished levels of OXT in MS-pre/T2DM patients. It also examined multiple adipocytokines in relation to OXT along their association with clinical parameters and inflammatory markers in the MS subjects, which is a metabolically unique group.

5. CONCLUSION

Our result demonstrated that there is inverse correlation of OXT metabolic hormone and adipocytokines (leptin, adiponectin and resistin).

The association of OXT and several clinical parameters confirms its role in opposing obesity and improving insulin desensitization. That's mean the possibility of this molecule formulation as a pharmacologic nasal spray agent should be considered in order to protect the MS-patients from the deleterious effects of the inflammatory cascade activation on the cardiovascular system.

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تأثير مستوى هرمون (الاوكسيتوسين) في بلازما الدم على ظهور مؤشرات الاضطراب الايضي (الليبتين، الاديبونيكتين والريسيستين) في مرضى السكري 2 في الأردن: دراسة مسحية

ميس النعيمي، فيوليت كسابري، امل العكور، نائلة بولاتوفا، أيمن عارف، منذر مومني، رولا سيلاوي، ياسر البستنجي[1]

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ملخص

قيست مستويات هرمون الاوكسيتوسين والمؤشرات الايضية الالتهابية (الليبتين، الريسستين والاديبونيكتين) في 166 عينة بلازما دم سحبت من مرضى مصابين بمتلازمة الاضطراب الايضي المزمنة والذين زاروا العيادات الخارجية للغدد الصماء، مستشفى الجامعة الأردنية. المرضى كانوا قد قسموا بحسب طبيعة البحث إلى مجموعتين أساسيتين على أساس مستويات سكر الدم لديهم، المرضى ذوي الاضطراب الايضي المصابين بالسكري والمرضى ذوي الاضطراب الايضي ذوي مستويات سكر طبيعية، كانت مستويات المؤشرات الالتهابية المرتبطة بالاضطراب الايضي مختلفة بين المجموعتين ($p<0.01$).

كان مستوى هرمون تنظيم الايض والطاقة مختلفا بشكل عالي ($p<0.01$) بين المجموعتين المشتركتين بالدراسة. معدل الاوكسيتوسين (ng/mL) كان يشكل الضعف في مجموعة الاضطراب الايضي– سكر طبيعي (2.25+0.85) ($p<0.01$) عن مجموعة الاضطراب الايضي– مرضى السكري/ ما قبل السكري (1.20+0.50) وعلى العكس فإن المؤشرات الالتهابية كانت أعلى في مجموعة مرضى السكري عن نظيرتها وبالشكل التالي: الريستتين كان أعلي بمعدل 13 مرة (ng/mL) (82.05+32.44 vs. 6.45+4.39)، الاديبونيكتين سجل فرقا يعادل الثلاثة اضعاف (µg/mL) (6.869+1.082 vs. 1.97+0.606)بينما الفرق في مستويات الليبتين كان يعادل الضعف بين المجموعتين (ng/mL) (42.43+30.44 vs. 22.76+14.19).

هذه النتائج تشير بوضوح لقيمة الفائدة الصيدلانية لهورمون تنظيم الطاقة الاوكسيتوسين لتقليل التأثير المزمن لمؤشرات الاضطراب الايضي على المدى البعيد.

الكلمات الدالة: الاوكسيتوسين. الاضطراب الايضي، اديبوسايتكينز. ليتبتين، ريسيستين، اديبونيكتين، مرضى السكري 2، فحص الانزيمات المناعية المرتبطة، المؤشرات الالتهابية.

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