The role of orexin hormone in sera of patients with metabolic syndrome of Kerbala province: Iraq

Hayder B. Abbas Al-Ghreaty,[a] Fadhil Jawad Al-Tu'ma,[a,*] Sattar J. Hatrosh[a]

[a]Department of Biology, Pure Sciences Education College, University of Karbala, Karbala, Iraq.
Correspondence to Professor Fadhil Jawad Al-Tu'ma (email: f_altoma_56@yahoo.com).
(*Submitted: 12 April 2017 – Revised version received: 05 May 2017 – Accepted: 23 May 2017 – Published online: 02 Oct. 2017*)

Aim The aim of this study is to investigate the association between orexin hormone in the sera of metabolic syndrome (MetS) patients with T2DM and without T2DM according to various parameters such as age, gender, BMI, blood pressure and duration of disease in the Kerbala province of Iraq.

Objectives MetS is a disorder consisting of various abnormalities such as dyslipidemia, obesity, hypertension and hyperglycemia. Over the past two decades, the prevalence of MetS has greatly increased, then increasing the risk of heart disease, stroke and diabetes, and it has become a global health problem. Orexin is a neuropeptide secreted by a group of hypothalamic neurons. Orexin neurons in the hypothalamus have widespread projections throughout the brain and peripheral structures.

Materials and Methods This study was conducted at Al-Hussein Teaching Hospital and Al-Zahraa Teaching Hospital of Al-Hussein Medical City, Kerbala Health Directorate, Kerbala, Iraq from June 2015 to March 2016. The 124 samples (52 males and 72 females) were randomly selected from MetS patients attending the abdominal consultation unit. The results obtained were compared between diabetic patients with non-diabetic group. The age range for all samples used in this study was between (35 and 65) years. Patients with MetS were divided into various groups depending upon the BMI and blood pressure. Body mass index, age, blood pressure, and fasting glucose were determined beside to the levels of the orexin hormone.

Results The results show that significant decrease ($P < 0.05$) in serum levels of orexin hormone in MetS with type 2 diabetic patients as compared with MetS without type 2 diabetes mellitus.

Conclusion The data observed indicate that the level of orexin hormone in MetS with and without T2DM was changed according to gender, BMI, age, duration of disease and blood pressure.

Keywords orexin, obesity, metabolic syndrome, type 2 diabetes mellitus

Introduction

Obesity is a major cause for the development of the metabolic syndrome (MetS), a state characterized by overweight, insulin resistance, hypertension and impaired lipid metabolism and body fat distribution. Individuals with MetS have marked risks for the development of type 2 diabetes. They possess high cardiovascular mortality.[1] The MetS is a cluster of risk factors predisposing to complications of obesity, including hypertension, hypercholesterolemia and impaired glucose tolerance. Patients with MetS are at high risk for diabetes and cardiovascular diseases. High blood pressure (BP), hyperlipidemia, smoking, family Hx and diabetes mellitus (DM) are predictive of less than half of all metabolic disorders and future cardiovascular events.[2] Type 2 DM arises due to β cell dysfunction or combined with concomitant insulin resistance.[3] The endocrine system confusions are the main factors in metabolic disturbance such as insulin resistance and type 2 diabetes, which are due to lifestyle changes, obesity, and aging.[3,4]

Orexin is a neuropeptide produced by a specific subset of neurons located in the lateral hypothalamic area. It regulates appetite and food intake. Orexin/hypocretin was first described in 1998 by De Lecea et al.[5]

Orexin-A and B (also known as hypocretin 1 and hypocretin 2) were discovered independently by two groups using different techniques. De Lecea et al.[5] identified the prohormone pre-prohypocretin and its peptide products hypocretin-1 (Hcrt-1) and hypocretin-2 (Hcrt-2) by nucleotide sequencing, whereas Sakurai et al.[6] used orphan receptor cloning technique for the discovery of orexins, i.e. orexin-A (Orx-A) and orexin-B (Orx-B).

Orexin A (OXA) was a hypothalamic peptide regulating food intake, wakefulness and sleep. Subsequent studies revealed that orexin A and its receptors are expressed in various tissues outside the central nervous system (CNS) such as the gastrointestinal tract and pancreas, where it modulates gastrointestinal motility and secretion of bicarbonate and insulin. It has been detected also in blood, yet source and the physiological role of circulating OXA is unknown. Orexin A increases food intake in rats.[6] These peptides were originated from the prepro-orexin (prepro-hypocretin) gene, which encodes a precursor (130 amino acids in rodents, 131 residues in humans) that is split into orexin A (synonymous with hypocretin-1; 33 amino acids) and orexin.[7]

The orexin has other various differ functions such as energy regulation, feeding, cardiovascular system control, neuroendocrine regulations, GI control, regulation of water balance, the modulation of pain, and the role of behaviour.[8,9] Orexin was known to excess food intake and regulating the sleep wake wakefulness, feeding behaviors and has display in their work in perilous pathological diseases such as inflammation, narcolepsy, depression and Alzheimer's.[10,11]

The aim of the presented work is to investigate the level of orexin hormone in sera of MetS patients with T2DM and without T2DM of Kerbala province of Iraq.

Materials and Methods

Location and Duration of the Study

This study was conducted at Al-Hussein Medical City - Al-Hussein Teaching Hospital - Karbala l Iraq from June 2015 to March 2016.

Samples, Age and Experimental Design and Grouping

All the 124 samples (52 males and 72 females) were randomly selected from MetS patients who were attending the abdominal consultation unit. The results obtained were compared between diabetic patients with non-diabetic patients. The age range for all samples used in this study was ranged between 35 and 65 years. Patients with MetS were divided into the following groups depending upon the BMI and BP.

The working criteria developed by the NCEP: ATPIII has been criticized a new set of criteria that included waist circumference, blood lipids, blood pressure, BP, and fasting glucose. MetS was diagnosed based on clinical and/or histopathologic criteria. The data collected included age, duration of diabetes, gender, blood pressure, measurement of body mass index (BMI) using the formula (weight in kg/height in m^2), duration of diabetes.

Bio-markers determined include serum orexin hormone level and fasting blood sugar (FBS), which were measured within 24 h after blood withdrawal, serum orexin hormone levels were measured using the new orexin ELISA assay kit, which was designed for the determination of Orexin in human serum or plasma samples (BioSite, Sweden). Blood pressure was obtained after the subject had been seated for at least 5 min. Systolic and diastolic pressures were measured twice, with values averaged, by the use of an automated blood pressure measurement device. Patients who suffered from corticosteroid or thyroxin treatment, liver disease, thyroid trouble, and patients with kidney failure were excluded from the current study.

The mean ± Standard Errors of all parameters measured from groups G1 and G2 were determined with serum Orexin, BP, FBS, BMI and other biochemical parameters measured.

Table 1. **Mean ± Standard Error (SE) values of serum orexin and fasting serum glucose in DM in MetS group compared with non-diabetic MetS patients' group in both gender at ($P < 0.05$)**

Parameter	Gender	Metabolic syndrome	Mean ± SE	*P* value
Orexin, ng/mol	Male ($N = 72$)	With T2DM ($N = 27$)	212.17 ± 25.6	$P < 0.05$
		Without T2DM ($N = 52$)	455.3 ± 68.2	
	Female ($N = 52$)	With T2DM ($N = 27$)	216.87 ± 16.5	$P < 0.05$
		Without T2DM ($N = 52$)	347.2 ± 51.6	
FBG, mg/100 ml	Male ($N = 72$)	With T2DM ($N = 27$)	268.9 ± 15.5	$P < 0.05$
		Without T2DM ($N = 52$)	101.68 ± 4.12	
	Female ($N = 52$)	With Diabetic ($N = 27$)	250.47 ± 12.06	$P < 0.05$
		Without T2DM ($N = 52$)	102.6 ± 3.2	

FBG, Fasting blood glucose; SE, Standard Error.

Results

Table 1 shows the results obtained for serum orexin and fasting blood glucose in type 2 DM in MetS group compared with

Table 2. **Mean ± Standard Error (SE) values of serum (Orexin) in DM in MetS group compared with individuals of non-diabetic MetS patients' group according to age at ($P < 0.05$)**

Age range	Metabolic syndrome	Orexin, ng/mol Mean ± SE	*P* value
35–44 year ($N = 31$)	With T2DM ($N = 18$)	184.19 ± 26.5	$P < 0.05$
	Without T2DM ($N = 13$)	358.58 ± 54.3	
45–54 year ($N = 56$)	With T2DM ($N = 26$)	245 ± 25.7	$P < 0.05$
	Without T2DM ($N = 30$)	412.89 ± 67.08	
55–65 year ($N = 37$)	With T2DM ($N = 29$)	207.58 ± 20.56	$P < 0.07$
	Without T2DM ($N = 8$)	420.5 ± 69.23	
Total $N = (124)$	With T2DM ($N = 73$)	215.1 ± 14	$P < 0.05$
	Without ($N = 51$)	400 ± 42.8	

Table 3. **Mean ± Standard Error (SE) values of serum orexin in DM in MetS group compared with individuals of non-diabetic MetS patients group in duration of disease at ($P < 0.05$)**

Duration of disease	Metabolic syndrome	Orexin, ng/mol Mean ± SE	*P* value
year 1< ($N = 30$)	With T2DM ($N = 20$)	169.47 ± 23	$P < 0.05$
	Without T2DM ($N = 12$)	522.11 ± 115	
5–1 year ($N = 58$)	With T2DM ($N = 35$)	210.56 ± 18.7	$P < 0.05$
	Without T2DM ($N = 22$)	318.51 ± 50.9	
>5 year ($N = 36$)	With T2DM ($N = 18$)	264.25 ± 30.9	$P < 0.05$
	Without T2DM ($N = 17$)	426.32 ± 72.4	
Total ($N = 124$)	With T2DM ($N = 73$)	215.1 ± 14	$P < 0.05$
	Without T2DM ($N = 51$)	400 ± 42.8	

individuals of non-diabetic MetS patients' group according to gender ($P < 0.05$).

Table 2 shows the results obtained for serum orexin in DM type 2 in MetS group compared with individuals of non-diabetic MetS patients' group according age at ($P < 0.05$).

Table 3 shows the results obtained for serum orexin in DM in MetS group compared with individuals of non-diabetic MetS patients' group according to duration of the disease at ($P < 0.05$).

Table 4 shows the results obtained for serum oerxin in DM in MetS group compared with individuals of non-diabetic MetS patients' group according to BMI at ($P < 0.05$).

Table 4. **Mean ± SE values of serum orexin in DM in MetS group compared with individuals of non-diabetic MetS patients' group in BMI at ($P < 0.05$)**

BMI	Metabolic syndrome	Orexin, ng/mol Mean ± SE	*P* value
Kg/m²25 ($N = 30$)	With T2DM ($N = 18$)	359.78 ± 24.1	$P < 0.05$
	Without T2DM ($N = 12$)	794.61 ± 87.2	
Kg/m²29.9–25 ($N = 32$)	With T2DM ($N = 20$)	177.18 ± 24.2	$P < 0.05$
	Without T2DM ($N = 12$)	299.55 ± 68.6	
Kg/m² >30 ($N = 62$)	With T2DM ($N = 35$)	162.44 ± 10.3	$P < 0.05$
	Without T2DM ($N = 27$)	269.71 ± 30	
Total ($N = 124$)	With T2DM ($N = 73$)	215.1 ± 14	$P < 0.05$
	Without T2DM ($N = 51$)	400 ± 42.8	

BMI, body mass index; SE, Standard Error.

Table 5 shows the results obtained for systolic blood pressure, diastolic blood pressure and BMI in DM in MetS group compared with individuals of non-diabetic MetS patients group in gender at ($P < 0.05$).

Discussion

In this study, we observed that serum orexin levels were reduced in type 2 DM with MetS patients as compared with non-diabetic type 2 MetS patients. In addition, results show a decrease in serum orexin levels in male patients with type 2 DM of MetS as compared with non-diabetic MetS male patients.

The observed data show that serum orexin levels were lower in MetS patients with type 2 DM in accordance with their age, duration of disease and BMI as compared with non-diabetic MetS patients.

Serum orexin levels were also lower in overweight/obese MetS patients associated with hypertension and type 2 DM when compared with individuals of non-diabetic MetS patients' serum. These data were in agreement with others which found that the action of OX neurons is controlled by energy status as specified by levels of glucose (7) and to inhibit the electrical excitability of OX neurons in the mouse LH hypoglycemia and blood glucose inhibits prepro-orexin mRNA expression in these cells.[12]

The activity of orexin neurons is tightly regulated by various hormones, neurotransmitters and nutrients.[13,14] Intriguingly, elevated glucose concentration can prevent or silence the activity of orexin neurons. This may show that purveyance orexin neurons with other energy-attached molecules, such as pyruvate and lactate, can stop glucose from blocking orexin neurons. This indicates that orexin neurons only control the changes in glucose levels while the levels of other energy molecules are low, whereas high-energy levels can stop glucose from regulating orexin.[15]

The lateral hypothalamic area LHA include glucose sensitive neurons that are stimulated by falls in circulating glucose and inhibited by prandial signals, such as the entity of food in the gut and/or a rise in portal glucose concentration preceding

Table 5. **Mean ± SE values for systolic blood pressure (SBS), diastolic blood pressure (DBS) and BMI in DM in MetS group compared with individuals of non-diabetic MetS patients' group in gender at ($P < 0.05$)**

Parameter	Gender	Metabolic syndrome	Mean ± SE	*P* value
SBS, mmHg	Male ($N = 72$)	With T2DM ($N = 27$)	144.6 ± 2.9	$P > 0.05$
		Without T2DM ($N = 52$)	150.4 ± 3.52	
	Female ($N = 52$)	With T2DM ($N = 27$)	143.8 ± 2.4	$P > 0.05$
		Without T2DM ($N = 52$)	156.4 ± 4.9	
DBS, mmHg	Male ($N = 72$)	With T2DM ($N = 27$)	90.5 ± 1.34	$P > 0.05$
		Without T2DM ($N = 52$)	93 ± 1.65	
	Female ($N = 52$)	With T2DM ($N = 27$)	89.3 ± 1.16	$P > 0.05$
		Without T2DM (N = 52)	93.6 ± 3.13	
BMI, Kg/m²	Male ($N = 72$)	With T2DM ($N = 27$)	29.5 ± 0.98	$P > 0.05$
		Without T2DM ($N = 52$)	31.12 ± 1.24	
	Female ($N = 52$)	With T2DM ($N = 27$)	29.17 ± 0.8	$P > 0.05$
		Without T2DM ($N = 52$)	30.2 ± 1.05	

BMI, body mass index; SBS, systolic blood pressure; DBS, diastolic blood pressure mean ± Std. Error.

studies have suggested that orexin neurons might match to this neuronal population, since orexin neurons are stimulated by hypoglycemia.[16]

Orexin A was also detected as reduced level in obese patient and decreased plasma levels of orexin A were described in adult obese subjects compared to non-diabetic MetS patients of this study which in agreement others.[17] It was found that orexin levels were down-regulated in the hypothalamus of obese rodent subjects. In addition, clinical, reduction in plasma orexin-A (OX-A) levels in obese individuals were also apprised and lower OX-A plasma levels were raised during body weight loss.[8] This may indicate to understand how the orexin excretion in the brain is influenced by changes in glucose levels through different metabolic case, or in disease states such as diabetes and obesity.

Conclusion

The orexin levels in Iraqi patients of Kerbala province with MetS (with and without T2DM) was changed according to blood glucose, gender, BMI, age, duration of disease and blood pressure.

Conflict of Interest

None.

References

1. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002;288:2709–2716.
2. Alshoka MM, Al-Hamdani KJ. Conditioned risk factors in patients with coronary heart disease. J Contemp Med Sci. 2015;1:24–26.
3. Laaksonen DE, Lakka HM, Niskanen LK, Kaplan GA, Salonen JT, Lakka TA. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. Am J Epidemiol. 2002;156:1070–1077.
4. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415.
5. de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci. 1998;95:322–327.
6. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998;92:573–585.
7. Alizadeh A, Rahmani-Nia F, Mohebbi H, Zakerkish M. Acute aerobic exercise and plasma levels of Orexin A, Insulin, Glucose, and Insulin resistance in males with Type 2 Diabetes. Jundishapur J Health Sci. 2016;8:16–19.
8. Monda M, Viggiano A, Viggiano A, Mondola R, Viggiano E, Messina G, et al. Olanzapine blocks the sympathetic and hyperthermic reactions due to cerebral injection of orexin A. Peptides. 2008;29:120–126.
9. Jayashree V, Thenmozhi N. Orexin – a potential neurotransmitter: a review. Int J PharmTech Res. 2016;9:161–164.
10. Sellayah D, Bharaj P, Sikder D. Orexin is required for brown adipose tissue development, differentiation, and function. Cell Metab. 2011;14:478–490.
11. Cleeman JI. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). J Am Med Assoc. 2001;285:2486–2497.
12. Barson JR, Morganstern I, Leibowitz SF. Complementary roles of orexin and melanin-concentrating hormone in feeding behavior. Int J Endocrinol. 2013; 2013:983964.
13. Takahashi K, Lin JS, Sakai K. Neuronal activity of orexin and non-orexin waking-active neurons during wakesleep states in the mouse. Neuroscience. 2008;53:860–870.
14. Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron. 2005;46:787–798.
15. Venner A, Karnani M, Gonzalez J, Jensen T, Fugger L, Burdakov D. Orexin neurons as conditional glucosensors: paradoxical regulation of sugar sensing by intracellular fuels. J Physiol. 2011;589:5701–5708.
16. Ouedraogo R, Na¨slund E, Kirchgessner AL. Glucose regulates the release of Orexin-A from the endocrine pancreas. Diabetes. 2003;52:111–117.
17. Komaki G, Matsumoto Y, Nishikata H, Kawai K, Nozaki T, Takii M, et al. Orexin-A and leptin change inversely in fasting non-obese subjects. Eur J Endocrinol. 2001;144:645–651.