Association of Circulating Orexin-A Level With Metabolic Risk Factors in North Indian Pre Menopausal Women

Vani Gupta*, Sameeksha Mishra, Sandeep Kumar and Supriya Mishra

Department of Physiology,
King George’s Medical University,
Lucknow

Abstract

The present study was designed to investigate the association between circulating Orexin-A level with metabolic risk factors in North Indian adult women. 342 women were enrolled for the case-control study, 172 women were with metabolic syndrome (mets) and 170 healthy control women were without metabolic syndrome, (womets) according to (NCEP ATP III criteria). Circulating Orexin-A level was determined by enzyme-linked immunosorbent assay. Observations indicated low levels of orexin-A (26.06±6.09 ng/ml) in women with mets and other metabolic risk factors compared to women without metabolic syndrome (36.50±10.42 ng/ml). Further, in women with metabolic syndrome, circulating Orexin A was significantly associated with waist circumference, triglyceride (negative correlation) and hyperdensity lipoprotein (positive correlation). Our study shows that circulating Orexin A was found to be significantly associated with hyperlipidemia, obesity and obesity-related disorders in North Indian premenopausal women.

Introduction

Orexins A (hypocretin-1) and B (hypocretin-2) are newly discovered neuropeptides synthesized in brain, peripheral tissues and enteric nervous system (1). Earlier Orexins were thought to be involved in appetite regulation, wakefulness, and sleep mechanism but recent evidences showed that they play an essential role in obesity, metabolic syndrome and blood pressure (BP) regulation (2). In the hypothalamus, both Orexin A and Orexin-B are derived from the same precursor prepro-Orexin by proteolytic cleavage (3). Orexin neurons develop throughout the central nervous system and expressed mainly in endocrine cell, intestinal mucosa, pancreas and the male reproductive system (4, 5).

Orexin-A is a 33 amino acid peptide, detectable in human plasma/serum. Animal studies show that Orexin-A rapidly crosses the blood-brain barrier (6) and reach brain tissue by simple diffusion. Orexins bind to two types of receptors, Orexin-1 receptor (OX1R) and Orexin-2 receptor (OX2R). OXA selectively binds OX1, while OXB binds both OX1 and OX2 with slightly lower affinities. Orexin neurons are widely distributed in the brain and peripheral tissues due to which they play a significant role in appetite, blood regulation and metabolism (7-9). Orexin may play an important role in metabolic syndrome as it was earlier suggested that its concentration has
substantial effects on metabolic states, which is closely related to upper body obesity (10).

Previous studies show that low plasma Orexin-A was predominantly known for narcolepsy and sleep-related disorders, but subsequently it has been reported to have a variety of pharmacological actions such as energy expenditure and lipid metabolism (11, 12). The level of Orexin-A differs in individual with higher body mass index (BMI) (13), which implies Orexins involvement in body weight regulation. Moreover, genetic ablation of Orexin neurons in mice causes narcolepsy and late onset obesity despite a decrease in food intake (2). The above information suggest that the role of Orexin neurons is more significant in regulation of energy expenditure than food intake, and disturbance of energy homeostasis ultimately leads to metabolic syndrome. The study of Orexin biology has been and is still opening much new insights into the human physiology and driving the development of novel scientific approaches regarding metabolic diseases. However, at present relationship between Orexin and metabolic syndrome is poorly understood in human as well as in rodents.

Furthermore, women are often more likely to be obese because hormones play a role in weight gain and appetite regulation(14) which is one of the risk factors for metabolic syndrome and its related disorders like type 2 diabetes and atherosclerosis.

Considering the above facts the present study was designed to explore the association of circulating plasma Orexin-A levels with the metabolic risk markers in women with metabolic syndrome.

Methods

In this study we enrolled 342 women, who satisfied our exclusion and inclusion criteria. Out of which 172 women were with metabolic syndrome (wMets) according to NCEP-ATP III criteria and another 170 were age-matched apparently healthy women controls. A structured form was completed to collect the information regarding subjects’ medical, personal, family, dietary, and menstrual history. This study was approved by the Ethics Committee of this Institute and the Indian Council of Medical Research, New Delhi, India and “all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research”. Written informed consent was obtained from all participants.

Criteria for Metabolic Syndrome

The NCEP-ATP III criteria for metabolic syndrome (15) are based on simple clinical and biochemical parameters. The current subjects were classified as having metabolic syndrome if they had three or more risk factors, which included any 3 of the following: 1) waist circumference (WC) > 88 cm (35 in); 2) triglycerides (TG) ≥ 150 mg/dL (1.69 mM); 3) high density lipoprotein cholesterol (HDL-C) < 50 mg/dL (1.29 mM); 4) systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg; and 5) fasting plasma glucose (FPG) ≥ 110 mg/dL (6.1 mM).

Anthropometric Measurements

WC and BP were evaluated in all the subjects (WC was measured at the narrowest point superior to the hip and was divided by the circumference of the hips measured at their greatest gluteal protuberance). Using an appropriate cuff size, physician measured the blood pressure on the right arm in sitting position after 5 min of rest. The first and fourth Korotkoff sounds were recorded as systolic and diastolic BP. Blood pressure was measured again after 5 min of rest, and the average was used in the analysis.

Laboratory measurements

Blood samples for measuring the biochemical parameters were obtained in the morning after 12 hours of fasting on the 10th day to rule out the hormonal variation because of menstruation, serum and plasma were separated from 6.0 ml of the blood, estimation of plasma glucose was done by a glucose oxidase-peroxidase method (Randox Laboratories Ltd., Antrim, UK) and serum lipid profile was done by enzymatic method (Randox Laboratories Ltd., Antrim, UK).
Determination of serum Orexin-A level

The serum concentration of Orexin A was measured with a Orexin-A/hypocretin (human, rat, mouse, bovine) Elisa kit (Phoenix Pharmaceuticals inc. Catalog- EK-003-30), according to the manufacturer’s protocol.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism version 5.03 (24). Quantitative variables are presented as mean±standard deviation (SD). Unpaired t-test was performed to assess the difference in various biochemical parameters. Pearson’s correlation was performed to observe the correlation of Orexin-A with the metabolic risk markers.

Results

The present study is based upon 172 women with metabolic syndrome (study group) and 170 women without metabolic syndrome (control group). All the metabolic markers differ significantly between study and control group (Table I).

Co-relation study

Pearson’s correlation (r) and multiple regression analysis were conducted to observe the association between Orexin A level and different metabolic risk markers. Orexin-A showed a significant inverse correlation with WC \( (r = -0.60, \ p = <0.001) \), TG \( (r = -0.74, \ p = <0.001) \), and direct correlation with HDL \( (r = 0.87, \ p = <0.001) \). Moreover, Orexin-A had a very weak negative correlation with FPG \( (r = -0.013, \ p = 0.86) \) which was not significantly associated in study group (Table II).

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Metabolic Risk markers, circulatory Orexin-A in North Indian premenopausal women with and without metabolic syndrome.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic markers</td>
<td>Study Group</td>
</tr>
<tr>
<td>Age</td>
<td>32.81±3.57</td>
</tr>
<tr>
<td>WC</td>
<td>90.37±13.83</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>109.96±22.39</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>165.28±15.30</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.68±6.06</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>91.69±10.11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>143.01±12.55</td>
</tr>
<tr>
<td>Orexin-A (pg/ml)</td>
<td>26.06±6.09</td>
</tr>
</tbody>
</table>

A value of p<0.05 was considered statistically significant.

The significant results were also observed in Correlation figure with linear regression (Fig. 1, Fig. 2, Fig. 3).

Discussion

This case-control study reveals an association between circulating Orexin-A and the premenopausal women with metabolic syndrome. The results of this
involved in energy expenditure than the stimulus of food intake (16). Earlier studies have explained that narcoleptic patients have higher BMI, which indicates that low circulating Orexin A level closely related to body weight regulation (17, 18). The current study also shows a negative association between WC and Orexin- A circulating level which is similar to the previous study that shows 50% patients have higher WC with low Orexin A level (19). The observations of this study are also in agreement with the studies that reveal a low level of circulating Orexin-A strongly associated with obesity, similar to what has been observed in obese rodent (20). Evidence shows that low level of circulating Orexin-A is also associated with obesity and type2 diabetes in men (21). A recent study reveals that Orexin reduces obesity through brown adipose tissue development and differentiation (22).

Orexin expression is down regulated in the lateral hypothalamus (LHA) in genetically obese mice as well (23). Poly F et al., (19) showed that the low levels of circulating Orexin-A was negatively correlated with serum triglyceride level and positively correlated with HDL level. In contrast, an observation was seen in another study that the Orexin expression rises with elevated lipids and obesity on a fat-rich diet in the animal model (24) suggesting a functional relationship between these measures.

The result of our study also shows negative association between serum Orexin and fasting glucose level in women with mets, similar to the findings in other studies which suggest that the wake-promoting activity of Orexin neurons are inhibited by a rise in glucose, and stimulated by a fall in glucose (hypoglycaemia) (25). The results obtained in the present study supports the previously conducted studies (26, 27) that Orexin control short-term regulation of energy homeostasis by initiating feeding in response to falls in glucose and terminating it after food ingestion. Thus, Orexin plays an important role in short term glucostatic feeding mechanism, however the exact phenomenon is still unknown.

Orexin-A level and SBP or DBP, similar results were
found in a study which shows that most narcoleptic patients are hypertensive as well (9, 19). In conclusion, the results show that the low level of Orexin-A negatively associated with metabolic risk markers in premenopausal women with mets. Further studies are still required to find out the pathological role of Orexin-A in premenopausal women with metabolic syndrome.

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