ORIGINAL RESEARCH PAPER INTERNATIONAL JOURNAL OF PURE MEDICAL RESEARCH

Physiology

KEYWORDS: Orexin, Reproductive age group Women, Body Mass Index, Fasting Blood glucose, Serum Insulin levels, Serum Orexin levels.

ASSOCIATION OF METABOLIC STATUS WITH SERUM OREXIN LEVELS IN YOUNG FEMALES

Volume - 8, Issue - 11, November - 2023 ISSN (O): 2618-0774 | ISSN (P): 2618-0766

Shikha Jain* MD Physiology, Associate Professor, Department of Physiology, NDMC Medical college & Hindu Rao Hospital, Malka Ganj, New Delhi-110007 *Corresponding Author

Amit Goel DNB Anesthesiology, PDCC, EDIC, Director & Head, Critical Care Medicine, Yatharth Super Speciality Hospitals, Greater Noida West, UP, India.

INTERNATIONAL JOURNAL OF PURE MEDICAL RESEARCH

ABSTRACT

Aim-

Metabolic fuel detectors regulate energy balance at peripheral and central levels. The aim of this study is to assess association of serum Orexin levels with Metabolic risk markers in women of reproductive age group.

Material And Methods-

The study was conducted in campus of King George Medical University (KGMU), Uttar Pradesh Lucknow, INDIA. 150 apparently healthy women of reproductive age group i.e. 20-40 years of age were randomly selected. Fasting serum orexin and Serum insulin levels were measured using ELISA and weight, Body mass index, Fasting blood glucose, Lipid profile and Blood pressure were estimated in women.

Result-

The results show that Serum orexin levels has significant positive correlation with fasting blood glucose, weight ,BMI and waist circumference along with a weak negative correlation with serum Insulin level.

Conclusion-

In present study Serum orexin levels significantly correlate with weight , BMI, Fasting blood glucose and weak negative correlation with serum Insulin levels. serum orexin levels did not correlate with lipid profile of women of reproductive age group.

INTRODUCTION

Metabolic Syndrome is a combination of medical disorders that, when occurring together, increases the risk of developing cardiac diseases and disturbed glucose homeostasis.[1,2] The term "metabolic syndrome" dates back to at least the late 1950s, but came into common usage in the late 1970s to describe various associations of risk factors with diabetes that had been noted as early as the 1920s. Metabolic syndrome has always been linked with obesity and a sedentary lifestyle caused by Excess energy intake and decreased energy consumption both of which are modifiable. The adverse impact on health of this syndrome is due to induction of metabolic derangements.

For biological reasons, women at all ages throughout the world are generally found to have a higher mean BMI and higher rates of obesity than men.[3] The metabolic status of an organism is transmitted to the brain through metabolic fuel detectors regulating food intake. There are many of these detectors at both the peripheral (eg. leptin, insulin and ghrelin hormone) and central (NPY, Orexin, melanocortin) levels.

Orexin A and B are recently discovered hypothalamic neuropeptides, involved in regulation of feeding behavior, sleep wakefulness and neuroendocrine homeostasis. Orexin promote both waking and feeding. Maintenance of body weight depends on the balance between energy intake and energy expenditure. Energy intake is food intake; and energy expenditure is derived from complex thermogenesis processes that include basal metabolism, adaptive thermogenesis, and physical activity of individual.

Hormones play a very important role in regulating energy balance by signals that are integrated in the brain centers, including the hypothalamus which in turn modulates feeding and energy expenditure. The hypothalamus, a key component of the system for regulation of energy homeostasis, continuously monitors signals that reflect energy status and initiates appropriate behavioral and metabolic responses.[4] Identification of novel factors involved in the control of appetite, such as, orexin A caused emergence of the concept of new perspectives on energy expenditure. Orexin A is 33 amino acid peptides with an N terminal pyroglutamyl residue and C terminal amidation residue. Orexin is a neuropeptide produced by a specific subset of neurons located in the lateral hypothalamic area. It regulates appetite, food intake and energy expenditure. Orexin/hypocretin was first described in 1998 by De Lecea et[5]

The lateral hypothalamus is the feeding center and ventromedial hypothalamus is the satiety Centre. The satiety center is the primary center that controls food intake by inhibiting the feeding center. The cells of the ventromedial nuclei act as satiety center due to their functioning as Glucoreceptors, which sense glucose in blood. The Glucoreceptors in VMH are different from rest of the brain cells in that they require insulin for glucose utilization.

In a longitudinal study done by Marianne et al[6] on reproductive age group (RAG) women, the younger women are more likely than older women for transition from normal weight to overweight leading to metabolic complications. Hormonal factors in women play a role in appetite regulation.[7] Moreover role of Orexin neurons is more significant in regulation of energy expenditure than food intake, and imbalance of energy homeostasis may ultimately lead to metabolic complications.[8]

According to a study done by Kassie et al [3] on non-pregnant RAG women , unlike men, raised BMI, overweight and obesity in women are more prevalent. The activity of orexin neurons is regulated by energy balance.[9] Kastin AJ, et al [10] showed that because orexin-A rapidly crosses the blood-brain barrier and found in peripheral tissues, it might contribute to appetite control and energy expenditure in humans by acting peripherally in addition to its central role. Keeping in mind the above facts this study was planned with the aim to evaluate the association of serum orexin level with anthropometric variables in women of reproductive age group and to evaluate the association of serum orexin level with metabolic risk factors in women of reproductive age group.

MATERIAL AND METHODS

The study was conducted in campus of Chhatrapati Sahuji Maharaj Medical University (CSMMU), Uttar Pradesh Lucknow. Ethical clearance was taken from the ethics committee of this university 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.

The previous review of literature gave us an idea that the correlation coefficient between Body Mass Index and Orexin level is -0.43.Considering this correlation coefficient between Body Mass Index and Orexin level in the population with 80% power and 95% confidence. Sample size was 147 samples in our study. Sample size was calculated by using sample software n-Master.

Out of 153 subjects who were enrolled in our study 3 of them did not turn up for biochemical investigations contributing to 2% dropouts. Finally the Study group comprised of 150 apparently healthy women of reproductive age group recruited from women employees, nursing staff, and students of this university with no previous or current history of any kind of systemic disease. Complete information about medical history, complications associated with obesity were taken in the performa. All the participants of the study underwent a standardized interview using a questionnaire.

In all subjects various anthropometric measurements were taken by one investigator using standard techniques.

Waist Circumference was measured midway between the lowest rib and iliac crest at level of umbilicus with subject standing at the end of gentle expiration. Hip Circumference measured at level of greater trochanter.

Blood Pressure was measured Using an appropriate cuff size, the blood pressure measured in the right arm in sitting position after 5 min of rest using a standardized random zero sphygmomanometer. 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.

After overnight fasting, blood samples were collected from all the subjects at 8AM in the morning on day 10 of menstrual cycle to avoid diurnal and cyclic variation. Serum and plasma were separated as per requirement for biochemical investigations. Serum was separated by centrifugation at 3000 rpm.

Samples for fasting blood sugar were collected in fluoride vials. Serum total cholesterol, triglyceride and HDL cholesterol were analyzed using random access chemistry. Fasting sugar estimation[11] was done Based on enzymatic method using oxidase and peroxidase. Fasting insulin was measured by enzyme linked immunosorbent assay (ELISA). Fasting serum orexin levels was measured by OREXIN-A/HYPOCRETIN- (human, rat, mouse, bovine) EIA kit (PHOENIX PHARMACEUTICALS inc. catalog – EK- 003-30)

Statistical Analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD.

To compare the change in a parameter at two different time intervals paired "t" test was used. The ANOVA test was used to compare the within group and between group variances amongst the study groups. ANOVA provided "F" ratio, where a higher "F" value depicted a higher inter-group difference. To test the significance of two means the student 't' test was used . A p value <0.05 was considered significant. Pearson's correlation was performed to calculate the correlation of Orexin-A with continuous variables.

RESULTS:

A total of 150 women aged between 20 to 41 years were enrolled in the study. The demographic profile depicting the Age, BMI, Waist Hip Ratio, Systolic Blood Pressure and Diastolic Blood Pressure of 150 subjects finally selected for the study are given in table 1.

Table 1: Demographic Profile (n=150)

Variables	Mean±SD	Range	
		Min	Max
Age	26.47±5.81	20.00	41.00
BMI (kg/m2)	23.78±4.46	15.43	37.66
WHR	0.86±0.05	0.62	0.96
SBP (mm Hg)	116.21±9.62	100.00	160.00
DBP (mm Hg)	77.37±7.53	58.00	100.00

Table 2 shows the metabolic profile comprising Total Cholesterol, HDL Cholesterol ,Triglyceride levels, Fasting Blood Glucose ,Serum Insulin ,and Serum Orexin levels of the 150 subjects in study.

Table 2: Metabolic Profile (n=150)

Variables	Mean±SD	Range	
		Min	Max
Total cholesterol (mg/dl)	169.04±35.56	98.80	264.00
HDL-Cholesterol (mg/dl)	42.57±7.99	21.08	62.20
Triglyceride (mg/dl)	101.19±36.35	26.40	293.20
F. Blood glucose(mg/dl)	103.44±15.90	65.60	155.80
S. Insulin (µlU/ml)	10.94±5.38	1.25	39.56
S. Orexin(pg/ml)	49.90±13.84	9.00	80.00

Table 3 shows the correlation of different metabolic risk factors with Serum orexin levels. We find a significant correlation of Serum Orexin levels with weight , BMI, Waist circumference and Fasting Blood Glucose levels **($p < 0.05$).** Serum insulin levels show a weak negative correlation with serum orexin level though not statistically significant.

Table 3: Correlation between S. orexin levels and different anthropometric, biochemical parameters (n=150)

Parameters/Variables	"r"	"p"
Weight (Kg)	0.176	0.031
Height (cm)	0.064	0.436
BMI (kg/m2)	0.165	0.044
WC (Waist circumference) cms	0.265	0.001
WHR	0.141	0.085
S.Glucose (mg/dl)	0.727	<0.001
S. Cholesterol(mg/dl)	0.084	0.306
HDL(mg/dl)	-0.063	0.441
TG (mg/dl)	-0.010	0.899
SBP(mm Hg)	0.088	0.282
DBP(mm Hg)	0.032	0.693
Serum Insulin (µlU/ml)	-0.116	0.157

Figure1 below shows correlation of plasma glucose levels with serum orexin levels. The bivariate correlation shows a strong positive correlation between S. orexin and S. glucose levels ($r=0.727$; $p<0.001$) in all 150 women.

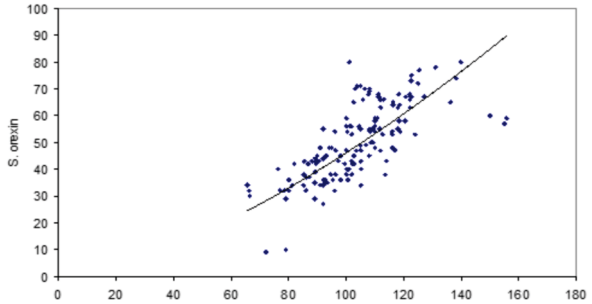

Figure 1: Correlation between plasma glucose and serum orexin level

The bivariate correlation shows a strong positive correlation between S. orexin and S. glucose levels (r=0.727; p<0.001) in all 150 women.

Figure 2 shows correlation of serum insulin levels with serum orexin levels.

Serum insulin levels shows a weak negative correlation with serum orexin levels in all 150 women though not statistically significant.

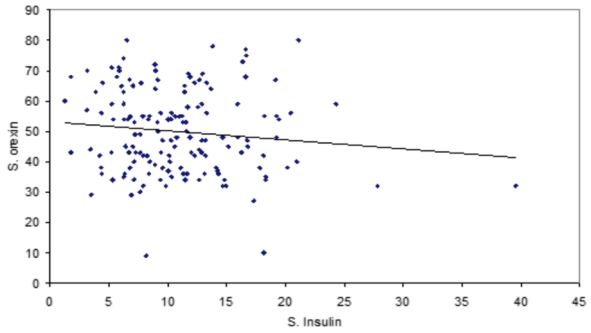

Figure 2: Correlation between S. Insulin and S. Orexin

Serum insulin levels shows a weak negative correlation with serum orexin levels in all 150 women though not statistically significant.

DISCUSSION

In our study 150 women of Reproductive age Group were selected in the study. This age group was selected as incidence of major weight gain was found to be greatest in women and men of 25-34 years of age [12] and women are prone to weight gain in a very short time period in younger age group leading to metabolic complications.[6]

Serum orexin levels showed significant positive correlation with different anthropometric and biochemical parameters such as weight, BMI, waist circumference, Hip circumference determining increased body fat that reflects unhealthy lifestyle and with serum glucose levels. The results are in liaison with the findings of Tomasik et al[13] Matsumura et al[14] and Heinonen et al[15] who also observed a significant positive association between BMI and serum orexin level which was measured using ELISA. Orexin- A has close association with raised BMI and obesity[16,17] and obesity is associated with overeating, Orexin A being an appetite inducing neuropeptide also influences the energy homeostasis.[8]. There are studies which show an inverse relation of waist circumference, BMI, Fasting blood glucose levels with orexin-A levels[18] and orexin-A was significantly lower in obese women and in women with metabolic syndrome as compared to non obese women[19] and those without metabolic syndrome.[8] Obesity is a worldwide health problem and is linked to a number of the leading causes of morbidity and mortality associated metabolic complications.

Serum insulin levels shows a weak negative correlation with serum orexin levels though not statistically significant.

No correlation of serum orexin levels were seen with lipid profile of the subjects.

The activity of orexin neurons is regulated by energy status and nutrient level of individual. Studies on mice show that orexin levels rise with elevated lipid levels and obesity on a fat rich diet in animal model.[20] Normally elevated glucose concentration increases the activity of orexin neurons to increase energy expenditure.[21]

The significant positive correlation between serum orexin level and fasting blood glucose levels along with a negative correlation with serum insulin levels is seen in this study.

The ventromedial nuclei of hypothalamus act as a satiety center and lateral hypothalamus acts as feeding Centre where orexin neurons are present. The satiety center being the primary center that controls food intake by inhibiting the feeding center. The feeding center is chronically active and its activity is inhibited by the activity in satiety center after ingestion of food .The cells of the ventromedial nuclei of hypothalamus act as a satiety center due to their functioning as glucoreceptors ,also called Glucostats i.e receptors which sense the glucose in the blood. The activity of satiety center is thus governed by the level of glucose utilization of cells within the center. If the glucoreceptors are inadequately supplied with glucose ,their activity is decreased.

The Glucoreceptors in the ventromedial nucleus are different from the rest of the brain cells in that they require insulin for glucose utilization. Low insulin leads to reduced cellular uptake of glucose and glucose utilization of the Glucoreceptors is low which simulates the condition of fasting, leading to unchecked activity of the feeding center releasing more and more of Orexin causing hyperphagia, obesity, altered lipid profile and disturbed glucose homeostasis resulting in vicious cycle of hyperorexinaemia, hyperglycemia, dyslipidemia, hyperphagia and polyphagia which may further trigger comorbid conditions like obesity, hypertension, and atherosclerosis ultimately leading to metabolic syndrome.

This leads to understand how the orexin secretion in the brain is influenced by changes in glucose levels through different metabolic states , or in disease states such as diabetes and obesity. Orexin system has emerged as a key target for therapeutic intervention in disorders associated with hypothalamic dysfunction, including Insomnia in which orexin antagonists are already acting as potential FDA approved drugs , and with the above contribution in study of orexin association with metabolic risk markers the orexin antagonists can prove to be Emerging targeted therapy for the treatment of Metabolic disorders due to abnormal body metabolism including Diabetes and also Metabolic syndrome.

CONCLUSION

In present study serum orexin levels showed significant positive correlation with different anthropometric and biochemical parameters such as weight, BMI, waist circumference, and serum glucose levels along with a weak negative correlation with serum insulin levels.

Conflict Of Interest-None

Funding-ICMR Sanction No- 52/4/2012-EMS ICMR New Delhi

Acknowledgements-

I am thankful to my subjects who gave me support and cooperated in sampling. Also thankful to pathology department of college for providing me opportunity to work there.

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