Clinical Epidemiology and Global Health 7 (2019) 313–316

Contents lists available at ScienceDirect

Clinical Epidemiology and Global Health

journal homepage: www.elsevier.com/locate/cegh

Association of A:O ratio with metabolic risk markers in North Indian women

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ARTICLE INFO

Keywords:
Metabolic syndrome
Obesity
Acylation stimulating protein
Orexin-A

ABSTRACT

Background: Plasma concentrations of Acylation stimulating protein (ASP) and Orexin-A are closely linked to body weight and energy homeostasis. The purpose of this study is to describe the associations of A:O with metabolic risk marker in women.

Methods: This is a case control study. Total 382 women were recruited for the study.192 women with metabolic syndrome (WmetS) &190 women without metabolic syndrome (WometS) according to NCEP-ATPIII guidelines. Serum ASP and Orexin-A level were determined by enzyme linked immunosorbent assay.

Results: Result indicated that Waist Circumference, Blood pressure, Lipid profile, Glucose (FPG), Insulin resistance (HOMA-IR), ASP and A:O ratio were significantly higher but HDL and Orexin-A level were significantly lower in WmetS than WometS. The correlation of ASP, A:O ratio were positively significant correlated with Waist Circumference (WC), Triglyceride (TG), Glucose (FPG) and negatively significant correlated with High density lipoprotein (HDL), however the orexin-A was negatively significant correlated with WC and TG in WmetS.

Conclusion: The study concluded that A:O ratio may be one of the potential biomarker for metabolic syndrome.

1. Background

Metabolic syndrome is a cluster of metabolic risk factors: abdominal obesity, dyslipidemia, raise blood pressure, and high level of glucose. Moreover, metabolic disturbance leading to development for cardiovascular disease and diabetes.[1] Women are often more likely to be overweight in general because of hormonal factor are responsible in weight gain and central obesity that increase fat mass of abdomen which is associated with dyslipidemia, insulin resistance, hypertension.[2] Adipose tissue is active metabolic regulator, secretes various adipocytokines and hormones alteration of these hormone, play a important role in the metabolic disorder.[3]

ASP and Orexin-A are two hormone are mainly secreted by the adipose tissue[4] and hypothalamus[5] respectively. Recognized as key regulators of various metabolic disorders, obesity and energy homeostasis.[6,7] Acylation stimulating protein (ASP) is an adipose tissue hormone. Which is similar to C3adesArg is a product of innate immunity, derived from the cleavage of complement C3 protein by carboxypeptidase. ASP is a linking protein immunity and metabolism. Recent research demonstrated that ASP play an important role in energy homeostatistis and lipid metabolism.[8]

ASP are responsible for stimulation of free fatty acid incorporation into adipose tissue by stimulating TG synthesis through activation of diacylglycerol acyltransferase and inhibition of lipoprotein lipase (LPL) activity.[7,8] ASP also increases glucose transport through enhanced translocation of glucose transporters. Orexin-A (hypocretin-1) is newly discovered neuropeptides synthesize in the brain, peripheral tissues and in the enteric nervous system.[5] Earlier Orexin-A were thought to be involved in appetite regulation, wakefulness and sleep mechanism.[9] Recent evidence shows that it may also play essential role in obesity, metabolic syndrome.[10,11]

Circulating ASP serum level are directly increased to the degree of adiposity while Orexin-A levels are inversely reduced in individuals with obesity, type 2 diabetes, metabolic syndrome, hypertention and cardiovascular disease.[10–13] Hence both the two biomarker ASP and Orexin-A interact with respect to energy homeostasis of adipositiy and metabolic related disease. Which is useful to identify the subject may reflect the functionality of adipose tissue. The rationale of this study was to consider may be the ratio of serum ASP: Orexin-A reported as a new surrogate marker in subjects with metabolic risk marker.

The study of A:O ratio is still opening much new insight into human physiology and driving the development of novel scientific approaches

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https://doi.org/10.1016/j.cegh.2018.09.008
Received 23 July 2018; Received in revised form 20 September 2018; Accepted 25 September 2018
Available online 26 September 2018

regarding metabolic disease. However at present relationship between A:O ratio and metabolic syndrome is poorly understood in human.

2. Method

The present study includes total n = 382 women. Subjects were selected on the basis of NCEP-ATP III criteria. In this study the sample size obtained 192 for women with metabolic syndrome (WMetS) as cases and 190 for women without metabolic syndrome (WoMetS) as controls. The level of significance was assumed 0.05% (α), and power (1-β) of the test was taken as 95% calculated by N-Master software. Who had no cardiac, respiratory, inflammatory, endocrine diseases, Pregnant, lactating and women with any kind of gynaecological or obstetrical problems and on medication including hormone replacement therapy, on addictions like smoking, alcohol intake etc were excluded. The study was approved by the Ethics Committee of KGMU, Lucknow, U.P and the Indian Council of Medical Research, New Delhi, India **Ref.Code: 58 ECM II B/P31.** Written informed consent was obtained from all the participants completed to collect information regarding subjects' medical, personal, family, dietary, and menstrual history.

2.1. Criteria for metabolic syndrome

The diagnostic criteria for metabolic syndrome by NCEP-ATP III, are based on clinical and biochemical parameters. The women are classified having Metabolic Syndrome if they met three or more risk factors of the NCEP-ATP III criteria out of the following:

1. Waist circumference: > 88 cm (35 inch) in women.
2. Hypertriglyceridemia: Serum triglycerides level ≥150 mg/dl.
3. Low HDL cholesterol: < 40 mg/dl in men and < 50 mg/dl in women.
4. High blood pressure: Systolic blood pressure ≥130 mm Hg and/or Diastolic ≥85 mm Hg.
5. High fasting glucose: Serum glucose level ≥110 mg/dl.

2.2. Anthropometric measurements

All subjects were evaluated for Waist Circumferences, a good marker for measuring central/visceral obesity, WC was measured midway between the margin of the lowest ribs and the iliac crest, at the point of minimal inspiration. and blood pressure (BP) was measured with the Bp TRU device, 3 blood pressure readings were obtained, which uses the traditional oscillometric technique (Beckett & Goodwin, 2005).

2.3. Sample collection and biochemical estimation

Blood samples for measuring the biochemical parameters were obtained in the morning after 12 h of fast 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, Plasma insulin concentrations were determined using immune radiometric assay (IRMA) (Immunotech Radiova, Prague).Plasma glucose concentrations were determined by glucose oxidase-peroxidase method (Merck) usingsemi automated glucose analyzer (Microlab 300, Merck). ASP (MYBIO.com Catalog-MBS012694) & Orexin-A (Phoenix Pharmaceuticals inc. Catalog- EK-003-30) level was measured by enzyme-linked immunosorbent assay.

Insulin resistance Homeostasis model assessment (HOMA), an index of insulin resistance (IR) based on plasma levels of fasting glucose and insulin has been widely applied for quantifying insulin resistance calculated by:

HOMA -IR = [fasting Insulin (μU/I) x fasting glucose (mmol/l)/ 22.5]

2.4. Statistical analysis

Statistical analysis was conducted by using the software GraphPad Prism 5.03. Variables are presented as mean ± SD. All the data follows normal distribution so we used parametric tests. Comparisons between two groups were made by using Unpaired *t*-test. All statistical tests were two-tailed, and $p < 0.05$ was considered as statistically significant. Pearson's correlation was performed to observe the correlation of ASP, Orexin-A and A:O ratio with the metabolic risk markers.

3. Results

This is a case-control study, age between 20 and 40 years. Observation of present study shows, total n = 382 female subjects, divided in to two group n = 192 women with metabolic syndrome (WMetS) and n = 190 women without metabolic syndrome (WoMetS) as per National Cholesterol Education Program Treatment Panel (NCEPATP) guidelines.

3.1. Anthropometric and biochemical parameters in women with and without metabolic syndrome

The baseline characteristics of the two groups are summarized between women with metabolic syndrome and women without metabolic syndrome **(**Table 1**)** in terms of anthropometric and biochemical parameters. Statistically significant ($p < 0.001$) high values for TC (178.3 ± 33.6 *vs.* 148.24 ± 82.9, $p < 0.001$), FPI (14.41 ± 9.25 *vs.* 7.01 ± 3.91, $p < 0.001$), HOMA-IR (3.98 ± 3.44 *vs.* 1.59 ± 0.93, $p < 0.001$), ASP (23.41 ± 2.90 *vs.* 16.39 ± 3.91, $p < 0.001$), and significant low values for Orexin-A (26.07 ± 5.84 *vs.* 36.50 ± 10.42, $p < 0.001$), ratio were observed in women with metabolic syndrome compared to women without metabolic syndrome.

3.2. Metabolic risk markers in women with and without metabolic syndrome

Observation of metabolic risk factors in women with metabolic syndrome as per NCEPATP−III−2001 criteria (Study group) and women without metabolic syndrome (control group) **(**Table 2**)**. in terms of WC (93.24 ± 13.6 *vs.* 70.00 ± 7.70, $p < 0.001$), FPG (112.17 ± 18.76 *vs.* 91.58 ± 10.44, $p < 0.001$), TG (144.39 ± 39.72 *vs.* 95.64 ± 27.65) SBP (138.25 ± 13.95 *vs.* 115.08 ± 7.70, $p < 0.001$), DBP (89.44 ± 9.68 *vs.* 75.36 ± 7.37, $p < 0.001$), and low HDL (40.6 ± 5.40 *vs.* 43.71 ± 6.35, $p < 0.001$).

Table 1
Anthropometric, biochemical parameters in women with and without metabolic syndrome.

Anthropometric & biochemical parameters	Study Group (n = 192)	Control Group (n = 190)	p- value
Age	32.81 ± 3.57	33.49 ± 2.83	0.593
TC (mg/dl)	178.3 ± 33.6	148.24 ± 82.9	< 0.001*
FPI	14.41 ± 9.25	7.01 ± 3.91	< 0.001*
HOMA-IR	3.98 ± 3.44	1.59 ± 0.93	< 0.001*
ASP (nM/l)	23.41 ± 2.90	16.39 ± 3.91	< 0.001*
Orexin-A (ng/ml)	26.07 ± 5.84	36.50 ± 10.42	< 0.001*
A:O ratio	0.948 ± 0.307	0.502 ± 0.211	< 0.001*

Data are presented as mean ± SD, A value of ***p** < **0.05** was considered statistically significant; **TC:** Total Cholesterol; **FPI:** Fasting Plasma Insulin; **HOMA-IR**: Homeostatic Model Assessment - Insulin Resistance **ASP**: Acylation Stimulating Protein.

Table 2
Metabolic Risk markers in women with and without metabolic syndrome.

Metabolic markers	Study Group (n = 192)	Control Group (n = 190)	p- value
WC	93.23 ± 13.6	70.00 ± 7.70	< 0.001*
FPG (mg/dl)	112.17 ± 18.76	91.58 ± 10.44	< 0.001*
TG (mg/dl)	144.39 ± 43.93	95.64 ± 27.65	< 0.001*
HDL (mg/dl)	40.6 ± 5.40	43.71 ± 6.35	< 0.001*
DBP (mmHg)	89.44 ± 9.68	75.36 ± 7.37	< 0.001*
SBP (mmHg)	138.25 ± 13.95	115.08 ± 7.70	< 0.001*

Data are presented as mean ± SD, A value of *$\mathbf{p < 0.05}$ was considered statistically significant **WC:** Waist Circumference; **FPG:** Fasting Plasma Glucose; **TG:** Triglyceride; **HDL:** High Density Lipoprotein; **DBP:** Diastolic Blood Pressure; **SBP:** Systolic Blood Pressure.

3.3. Clinical and anthropometric parameters and their correlations with the A:O ratio in women with and without metabolic syndrome

Pearson's correlation (r) analysis were conducted to observe the correlation of ASP, Orexin-A and A:O ratio with different metabolic risk markers. ASP was positive significant correlated with WC (r = 0.498, p = < 0.001), FPG (r = 0.702, p = < 0.001), TG (r = 0.383, p = < 0.001), HOMA-IR (r = 0.315, p = < 0.001) and negative significant correlated with HDL (r = −0.614, p < 0.001) in women with metabolic syndrome. A:O ratio was positive significant correlated with WC (r = 0.632, p = < 0.001), FPG (r = 0.257, p = < 0.001), TG (r = 0.780, p = < 0.001) and negative significant correlated with HDL (r = −0.394, p < 0.001) in women with metabolic syndrome. However Orexin-A was negative significant correlated with WC (r = −0.487, p = < 0.001), TG (r = −0.602, p = < 0.001) in women with metabolic syndrome. **(**Table 3**).**

The significant results also show in correlations figure with linear regression (Figs. 1–4).

4. Discussion

The observation of the present study shows that the association of the Acylation stimulating protein, Orexin-A and A:O ratio in women with metabolic risk factors, A:O ratio significantly correlated with metabolic variables like elevated triglycerides, hypertension etc. Indian women have excess body and abdominal fat these are ethnic factors affect the interaction between abdominal adiposity and metabolic risk marker.[14,15]

Studies have shown reported that ASP and Orexin-A are more susceptible to metabolic disturbance and the ratio of both might be responsible for the severity of the obesity[16,17] and regulation of energy homeostasis by fat storage in adipose tissue[17,18] ASP is increased and Orexin-A is decreased in obesity. Hence, imbalance in energy expenditure and energy intake, are important pathophysiological factors for the obesity and metabolic syndrome. Present study showed that elevated A:O ratio were found in WMetS compared to WoMetS and elevated A:O ratio may be contributing factor for the development of metabolic syndrome. Present finding is based on the studies of ASP and orexin.[19–21] In women with metabolic syndrome ASP levels are higher and Orexin-A levels are lower thus a higher A:O ratio.

Study shows that all the anthropometrical, biochemical and metabolic risk markers FPG, WC, SBP, DBP, TC, TG, Insulin, Insulin resistance (HOMA-IR), ASP and A:O ratio are significantly high mean value and low mean value of HDL and Orexin-A were found in women with metabolic syndrome than women without metabolic syndrome. Our study agreement with the other studies shows that elevated ASP

Table 3
Correlation of ASP, Orexin-A and A:O ratio with Anthropometric and biochemical Parameters in women with metabolic syndrome.

Variables	ASP (r-value)	p- value	Orexin-A (r-value)	p- value	A:O (r-value)	p- value
WC	0.498	< 0.001*	−0.487	< 0.001*	0.632	< 0.001*
FPG (mg/dl)	0.702	< 0.001*	0.067	0.412	0.257	< 0.001*
TG (mg/dl)	0.383	< 0.001*	−0.602	< 0.001*	0.780	< 0.001*
HDL (mg/dl)	−0.614	< 0.001*	0.088	0.213	−0.394	< 0.001*
Insulin	0.006	0.930	0.098	0.202	0.045	0.560
HOMA-IR	0.315	< 0.001*	0.111	0.154	0.073	0.314

A value of *$\mathbf{p < 0.05}$ was considered statistically significant.

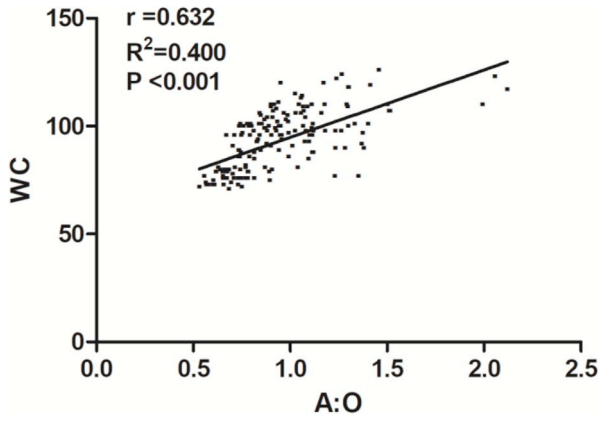

Fig. 1. Correlation between A:O ratio and WC in study group (n = 192).

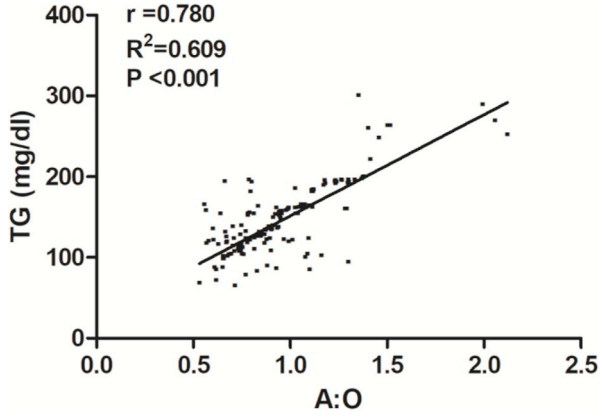

Fig. 2. Correlation between A:O ratio and TG (mg/dl) in study group (n = 192).

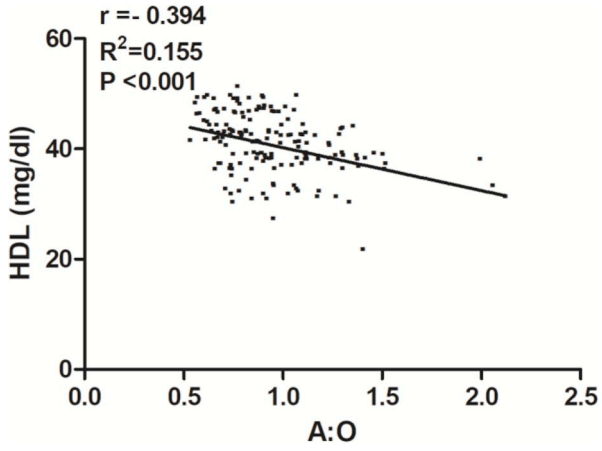

Fig. 3. Correlation between A:O ratio and HDL (mg/dl) in study group (n = 192).

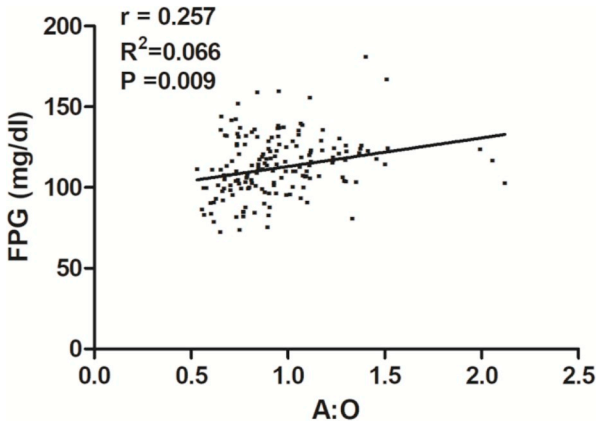

Fig. 4. Correlation between A:O ratio and FPG (mg/dl) in study group (n = 192).

and reduced orexin-A level involved in adiposity, hyperglycemia and lipid metabolism and it may serve as a atherogenic index in women with metabolic syndrome.[21–23]

Observation of this study also shows that increase A:O ratio have significant positive correlation with WC, TG, FPG, whereas HDL has negative correlation. Evidence shows that plasma ASP is positively correlated with Waist circumference[24] and Orexin-A level is negative correlated with WC[25] Which is strongly shows that high ASP and low circulating orexin-A level related with body weight regulation. Rationally A:O ratio could be relatively high, supporting the fact to explain the results obtained in the present study. Our findings strongly suggest that A:O ratio positively correlated with waist circumference, it may be a better index of central obesity related to metabolic syndrome in North Indian women. These results is best representative to evaluate central obesity is to measure waist circumference, as the excess of abdominal fat is more strongly associated with the metabolic risk marker.

Elevated ASP and decrease Orexin-A level lead to an increase triglyceride storage in adipose tissue are responsible for diabetes and cardiovascular disease that are associated with obesity and metabolic disorder.[16,26–28] Likewise, In the current study, obese women with metabolic syndrome have higher triglyceride was reported that high ASP level and low Orexin-A level are consequence of abnormal lipid metabolism. Since all of the subject in the current study were highly probable that these changes occur earlier in obesity. In the present study A:O ratio is directly associated with plasma lipid metabolism and the factors usually related to, diabetes, metabolic disorders and cardiovascular disease.

Furthermore, previous studies have not observed to find out the association between A:O ratio with metabolic syndrome and have not examined the A:O ratio in the population of India. In this study was observed A:O ratio shows positively associated with obesity and its related disorder lead to metabolic syndrome. Therefore A:O ratio might be effective parameter of the metabolic risk markers than ASP and Orexin-A alone. Conclusively observation of this study shows that increase A:O ratio could be a potential marker of metabolic syndrome, for this reason women with high ASP to Orexin-A ratio are more prone for development of metabolic syndrome.

Funding and acknowledgement

This study was funded by Indian Council of Medical Research No.3/1/2/20/12-RCH and No. 52/4/2012-BMS, New Delhi, India. We would like to thank faculty and staff of the Queen Mary Hospital, King George's Medical University, Lucknow for providing samples.

Conflicts of interest

There is no conflict of interest and no financial disclosure.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cegh.2018.09.008.

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