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SIGNIFICANT DIFFERENT SERUM LEVELS OF INTERLEUKIN-6 AND ADIPOKINES IN PATIENTS WITH METABOLIC SYNDROME

Biochemistry

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ABSTRACT

Background & objectives: Several components of metabolic syndrome (MetS) facilitate its diagnosis, including abdominal obesity, hyperlipidemia, high blood pressure, and insulin resistance. The production of interleukin-6 (IL-6) and adipokines seem to be associated with MetS components. Elevated pro in flammatory cytokines show an emerging role in the patients with Metabolic syndrome. The production of interleukin-6 seems to be strong association in patients with Met-S. These components may form a target for novel treatment approaches. The main objectives of this study was to evaluate & estimate the serum levels IL-6 and adipokines with MetS and its components

Methods: We studied 45 subjects, with Metabolic syndrome, diagnosed by International Diabetes Federation (IDF) criteria and 45 healthy control subjects, age below 40 years, Male subjects. We compared the circulating levels of the serum IL-6 and adipokines levels were estimated using the ELISA in Metabolic Syndrome with onset of Type 2 Diabetes with clinical settings.

Results: Serum levels of IL-6 and adipokines were found to be significant difference between serum levels of IL-6 and adipokines in Metabolic Syndrome with type 2 diabetic cases than in control subjects (IL-6; 27.53 ± 2.61 vs 6.07 ± 1.76 pg/ml & $P < 0.001$). Further, serum levels of adiponect in were found significant decreased and serum level of leptin were found significantly increased in cases with Metabolic Syndrome and type 2 diabetes than in control group ($P < 0.001$)

Interpretation & conclusions: Patients with MetS had significantly greater serum IL-6 than the controls, supporting the evidence that inflammation plays an important role in the immunopathogenesis of the disease. Additionally, IL-6 and adipokines serum levels may predict MetS.

KEYWORDS

IL-6, Adipokines, Metabolic Syndrome components, Diabetes Mellitus.

INTRODUCTION

Metabolic syndrome (MetS), alternatively known as insulin resistance syndrome or syndrome x, is a set of metabolic disorders that increase patients risks for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). The main clinical symptoms of MetS include central obesity, hypertension, hyperglycaemia, low high-density lipoprotein (HDL) levels, and high triglycerides (1). Metabolic syndrome is defined by the International Diabetes Federation (IDF) as a waist circumference (WC) greater than 94 inches in Caucasian men and greater than 80 inches in Caucasian women plus at least two of the following risk factors: triglycerides greater than 150 mg/dl or taking lipid-lowering agents, HDL levels less than 40 mg/dl and 50 mg/dl in men and women, respectively, a systolic blood pressure above 130 mmHg or a diastolic blood pressure above 85 mmHg or taking medicine for high blood pressure, and a fasting blood sugar above 100 mg/dl or having T2DM (2). In the past it was believed that adipose tissue is inactive and only serves to store triglycerides; however, it has been well demonstrated that fatty tissues secrete bioactive proteins, generally termed adipokines, which appear to play key roles in energy homeostasis and inflammation. Recent evidence suggests that obesity, in particular chronic visceral adiposity, is associated with inflammatory markers including interleukin-6 (IL-6) (3, 4).Studies have shown that IL-6 and adipokines are secreted from infiltrated macrophages into adipose tissue and lipocytes and also play important roles in MetS, insulin resistance, non-alcoholic fatty liver disease, and atherogenesis (5-11). The current study aimed to evaluate IL-6 and adipokines serum levels in patients with MetS and healthy controls and determine their associations with MetS and MetS components.

MATERIALS AND METHODS

Selection of Met-S patients and controls

A total of 90 subjects, including 45 healthy controls from the This study was carried out at Department of Biochemistry in collaboration with the Department of General Medicine at S.P. Medical College and Associated group of P.B.M. Hospitals, Bikaner. All participants completed a medical history form and provided informed consent. The clinical history and disease status of each participant were taken. Metabolic syndrome was diagnosed based on the IDF definition (2). The body weights of MetS patients were measured on a digital scale with an accuracy of 100 g and recorded. Height was measured using a measuring tape while the patient's shoulders were in a natural state with an accuracy of 1 cm. Body mass index (BMI) was calculated according to the formula weight (kg)/(height (cm))^2. Waist circumference was measured at the slimmest point using a flexible tape with an accuracy of 1 cm while the participant was at the end of his/her natural expiration. Blood pressure was measured using a standard mercury column manometer. All measurements were taken from the left arm by the same Medicine Depatrment. Venous blood samples were collected after 12-14 hours of fasting to measure blood glucose and serum lipids levels. None of the healthy individuals had histories of autoimmune, allergic, cancerous, or infectious diseases and met no IDF criteria. The mean ages for male patients (36.92 ± 2.40 years), comprising 45 healthy control (male: aged 37.28 ± 2.05 years). The anthropometric parameters and clinical characteristics of the MetS patients are given in Table 1.

Cytokine ELISA Assay

The serum levels of IL-6 and IL-17 were assessed using kits from the Bio Plex Human Cytikine Immuno-assay, Bio-Rad Lab. In. USA. The serum was separated and stored at -8ºC until further examination. ELISA method was followed by the manufacturer's protocol. The optical density was read at 460 nm with a microtiter plate by ELISA reader (Thermo Scientific-MULTISKAN FC).Serum cytokine levels were estimated with the help of standard curve and absorption as concentration was communicated as pg/ml.

Statistical Data Analysis

All Numerical data were presented as far as mean ± SD. Statistical data analysis of results was accomplished by common distribution 'Z' test. In this data analysis, variables presenting p- value under 0.05 and 0.001

were measured to be statistically significant and statistically highly significant respectively. Coefficient correlation (r value) was determined for final results correlation between two parameters by using with Pearson two-tailed data analysis. All statistical findings were done using SPSS software version 15.

RESULTS

The mean ages of Met-S patients and controls were 36.92 ± 2.40 and 37.28 ± 2.05 years, respectively. The serum levels of IL-6 in patients with Met-S were 27.53 ± 2.61, whereas in the healthy controls they were 6.07 ± 1.76 pg/ml. Serum IL-6 levels were significantly greater in the Met-S patients than in the controls ($P < 0.001$) (Table.1;Fig.1).Statistical analyses were performed to determine the relationships between IL-6 and adipokines serum levels in Met-S patients, and the variables are presented in Table 1 & Fig.1-4. Association of Weight index BMI and WC were significantly correlated with Age, BMI, WC, WHR, SBP, DBP, Fasting sugar, HbA1c, Total Cholesterol, LDL, HDL, VLDL, Triglycerides, Adiponectin, Leptin and Free fatty acid, IL-6 with respectively (BMI;WC) all correlation were seen to be significant statistically at the 0.01 level (Table-2).

DISCUSSION

The relationships between inflammatory biomarkers such as IL-6 and adipokines in Met-S have not been thoroughly investigated. This study showed that the serum IL-6 levels were significantly greater in Met-S patients than in controls. Interestingly, elevated serum TNF-α levels are associated with Met-S independent of Met-S components. In 2004, Moon et al. showed that serum IL-6 in obese adolescent Met-S patients correlated positively with BMI, WC, triglycerides, and diastolic blood pressures, and positive with HDL cholesterol (12).This study found significant correlations between serum IL-6 and each Met-S component, including triglycerides, HDL, low-density lipoprotein (LDL), fasting blood sugar (FBS), hypertension, and WC. Increased expression of serum IL-6 and its correlation with human obesity, insulin resistance in T2DM, and hypertension have been extensively studied previously (13-16). Previous studies revealed an association between IL-6 and systemic inflammation causing Met-S (17, 18). This study showed that serum IL-6 levels were significantly greater in Met-S patients than in controls. No significant correlations between serum IL-6 levels and Met-S components were observed. Previous studies showed that IL-6 is positively associated with BMI, fasting insulin, hypertension, and T2DM; however, such results disagree with our findings (19, 20). Sarbijani et al. reported that IL-6 serum levels were significantly greater in men with Met-S than in controls (21). They also observed a lack of correlation between IL-6 and Met-S components, which agrees with our findings. Additionally, Kitsios et al. showed that obese and overweight adolescents and children with Met-S had significantly greater serum IL-6 levels than their counterparts without Met-S (22). In contrast, some studies have shown that IL-6 serum levels are not associated with Met-S, which disagrees with our results (23, 24). Aldaham et al. showed that IL-6 serum levels were significantly affected not only by Met-S, but also by smoking and age (25). They showed that serum IL-6 levels were significantly greater in 71 male smokers than in former smokers. However, our study showed that serum IL-6 levels were significantly greater in non-smoking than in smoking patients. These contradictory results might be related to the low number of Met-S patients who were smokers in the current study. The present study showed that serum IL-6 levels were significantly less in patients who used narcotics than in those who did not. The effects of opioids on cytokine production have been investigated previously (26, 27). In addition, results of previous studies have been contradictory. For example, TNF-α production was increased by opioids in one study, but most studies showed its suppression; yet, another study showed opioids had no effect (28-31). Meijerink et al. recently showed that the production of IL-6, TNF-α, and several other cytokines was significantly suppressed in lipopolysaccharide- (LPS) induced whole blood from HIV-infected individuals who used heroin (32). One limitation of our study was the small number of patients who used narcotics. Further investigation with a larger sample size is needed to clarify the influence of narcotics on cytokine levels in Met-S patients.

CONCLUSION

Patients with Met-S in this study had significantly greater IL-6 and adipokines levels than healthy controls. These results support the evidence that inflammation plays an important role in the immunopathogenesis of the disease. Additionally, we suggest that IL-6 and adipokines serum levels be measured as valuable predicting factors for Met-S. The lack of association between serum IL-6 and adipokines levels and Met-S components remains to be investigated by further research.

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Table:1 Comparison of Mean±SD of Anthropometric Parameters of the Metabolic Syndrome and without Metabolic Syndrome Control Subjects

Clinical feathers	without Metabolic Syndrome (Control) (N= 45; M)	Metabolic Syndrome (Cases) (N= 45; M)
Anthropometric Parameters		
Age (Years)	37.28 ± 2.05	36.92 ± 2.40
BMI (kg/m^2)	23.10 ± 1.71	32.83 ± 3.62
WC (cms)	83.29 ± 2.69	90.38 ± 4.88
WHR	0.83 ± 0.03	0.94 ± 0.06
SBP (mm Hg)	124.88 ± 7.62	147.2 ± 10.31
DBP (mm Hg)	74.12 ± 3.54	84.8 ± 3.49
A. Biochemical Parameters		
FBS (mg/dl)	90.03 ± 12.55	182 ± 28.79
HbA1c (%)	4.42 ± 0.60	8.12 ± 0.96
TC (mg/dl)	180.8 ± 13.43	220.96 ± 23.72
TG (mg/dl)	104.8 ± 18.17	194.6 ± 32.43
HDL (mg/dl)	42.92 ± 2.96	38.52 ± 1.98
LDL (mg/dl)	116.0 ± 15.22	143.12 ± 19.45
VLDL (mg/dl)	22.0 ± 4.29	38.92 ± 6.49
B. Adipokines		
Adiponectin (µg/ml)	7.61 ± 0.76	5.96 ± 0.56
Leptin (ng/ml)	5.81 ± 0.50	12.49 ± 1.37
Free Fatty Acid (nmol/L)	0.62 ± 0.06	0.88 ± 0.18
C. Cytokines		
IL-6 (pg/ml)	6.07 ± 1.76	27.53 ± 2.61

MetS: Metabolic Syndrome; DM: Type 2 Diabetic Patients; M: Male Subjects; BMI: Body Mass Index; WC- Waist circumference, SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBG: Fasting Blood Glucose; HbA1c: Glycosylated Haemoglobin; TC: Total Cholesterol; HDL: High- Density Lipoproteins; LDL: Low-Density Lipoproteins; VLDL: Very Low-Density Lipoproteins; IL: Interleukin. Data are expressed as mean ± SD or median (interquintile range); Differences between CON and DM: *P<0.0001.

Table No.2 Correlation Coefficient between Variables with Anthropometric indices markers of insulin resistance and lipid profile with Metabolic Syndrome Cases

Correlation	BMI	WC
	r	R
Age	0.810**	0.721**
BMI	1.00	0.867**
WC	0.887**	1.00
Waist-Hip Ratio	0.743**	0.893**
SBP	0.827**	0.746**
DBP	0.834**	0.802**
FBS	0.874**	0.703**
HbA1c	0.745**	0.783**
Total Cholesterol	0.918**	0.925**
Triglyceride	0.817**	0.793**
HDL- Cholesterol	0.927**	0.921**
LDL- Cholesterol	0.875**	0.813**
VLDL- Cholesterol	0.971**	0.965**
Adiponectin	0.935**	0.761**
Leptin	0.877*	0.901**
Free Fatty Acid	0.958*	0.746*
IL-6	0.459	0.554

Pearson's correlation test r; correlation coefficient.
** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

HS* - Highly Significant

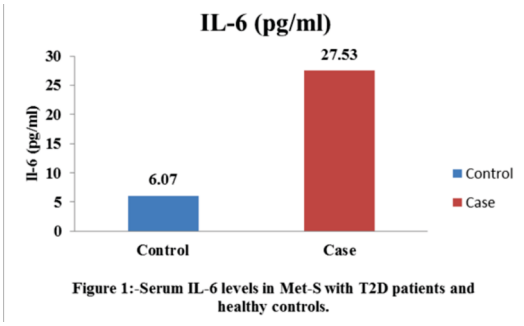

Figure 1:-Serum IL-6 levels in Met-S with T2D patients and healthy controls.

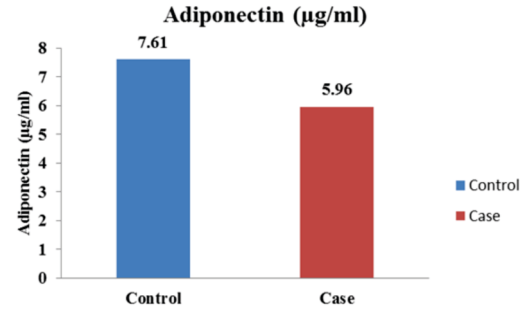

Figure 2:-Serum Adiponectin levels in Met-S with T2D patients and healthy controls

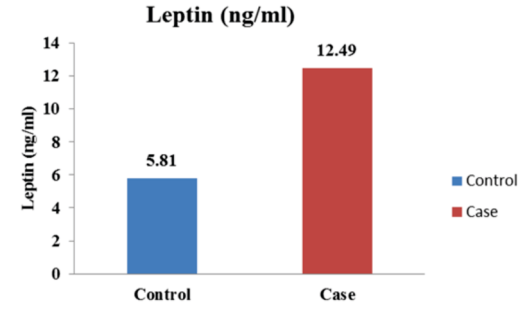

Figure 3:-Serum Leptin levels in Met-S with T2D patients and healthy controls

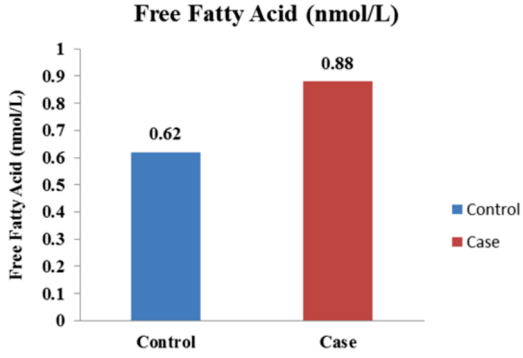

Figure 4:-Serum Free Fatty Acid levels in Met-S with T2D patients and healthy controls

REFERENCES

1. Cheung BM, Wat NM, Man YB, Tam S, Cheng CH, Leung GM, et al. Relationship Between the Metabolic Syndrome and the Development of Hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2). The American Journal of Hypertension. 2008; 21: 17-22.
2. International Diabetes Federation Press Conference: The IDF consensus worldwide definition of the metabolic syndrome [article online], 2005. Available from http://www.idf.org/webdata/docs/IDF_Met asyndrome_definition.pdf. Accessed 14 April 2005.
3. Nakayama Y, Komuro R, Yamamoto A, Miyata Y, Tanaka M, Matsuda M, et al. Rho A induces expression of inflammatory cytokine in adipocytes. Biochemical and Biophysical Research Communications. 2009; 379: 288-92.
4. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. European Cytokine Network. 2006; 17: 4-12.
5. Arner P. Insulin resistance in type 2 diabetes-role of the adipokines. Current Molecular Medicine. 2005; 5(3):333-9.
6. Langenberg C, Bergstrom J, Scheidt-Nave C, Pfeilschifter J, Barrett-Connor E. Cardiovascular Death and the Metabolic Syndrome Role of adiposity- signaling hormones and inflammatory markers. Diabetes Care. 2006;29(6):1363-9.
7. Devaraj S, Rosenson RS, Jialal I. Metabolic syndrome: an appraisal of the pro-inflammatory and procoagulant status. Endocrinology and Metabolism Clinics of North America. 2004;33(2):431-53.
8. Bastard J-P, Maachi M, van Nhieu JT, Jardel C, Bruckert E, Grimaldi A, et al. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both invivo and invitro. The Journal of Clinical Endocrinology and Metabolism. 2002;87(5):2084-9.
9. Anfossi G, Russo I, Doronzo G, Pomero A, Trovati M. Adipocytokines in atherothrombosis: focus on platelets and vascular smooth muscle cells. Mediators of Inflammation. 2010;2010.
10. Fisman EZ, Tenenbaum A. The ubiquitous interleukin-6: a time for reappraisal. Cardiovascular Diabetology. 2010;9(1):1.
11. Bautista L, Vera L, Arenas I, Gamarra G. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF- α) and essential hypertension. Journal of Human Hypertension. 2005;19(2):149-54.
12. Moon Y-S, Kim D-H, Song D-K. Serum tumor necrosis factor-α levels and components of the metabolic syndrome in obese adolescents. Metabolism. 2004;53(7):863-7.
13. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. The Journal of Clinical Investigation. 1995;95(5):2409.
14. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. The Journal of Clinical Investigation. 1996;97(4):1111.
15. Tzanavari T, Giannogonas P, Karalis KP. TNF- alpha and obesity. Current Directions in Autoimmunity. 2010;11:145-56.
16. Swaroop JJ, Rajarajeswari D, Naidu J. Association of TNF-α with insulin resistance in type 2 diabetes mellitus. The Indian Journal of Medical Research. 2012;135(1):127.
17. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circulation Research. 2005; 96(9):939-49.
18. Hung J, McQuillan BM, Chapman CM, Thompson PL, Beilby JP. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005; 25(6):1268-73.
19. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. The Journal of the American Medical Association. 2001; 286(3):327-34.
20. Lukic L, Lalic NM, Rajkovic N, Jotic A, Lalic K, Milicic T, et al. Hypertension in obese type 2 diabetes patients is associated with increases in insulin resistance and IL-6 cytokine levels: potential targets for an efficient preventive intervention. The International Journal of Environmental Research and Public Health. 2014; 11(4):3586-98.
21. Sarbijani HM, Khoshnia M, Marjani A. The association between Metabolic Syndrome and serum levels of lipid peroxidation and interleukin-6 in Gorgan. Diabetes and Metabolic Syndrome. 2015.
22. Kitsios K, Papadopoulou M, Kosta K, Kadoglou N, Chatzidimitriou D, Chatzopoulou F, et al. Interleukin-6, Tumor Necrosis Factor alpha and Metabolic Disorders in Youth. The Journal of Clinical Endocrinology and Metabolism. 2012;2(3):120-7.
23. Salmenniemi U, Ruotsalainen E, Pihlajamäki J, Vauhkonen I, Kainulainen S, Punnonen K, et al. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation. 2004;110(25):3842-8.
24. Choi K, Lee J, Lee K, Seo J, Oh J, Kim S, et al. Comparison of serum concentrations of C-reactive protein, TNF-α, and interleukin 6 between elderly Korean women with normal and impaired glucose tolerance. Diabetes Research and Clinical Practice. 2004;64(2):99-106.
25. Aldaham S, Foote JA, Chow H-HS, Hakim IA. Smoking Status Effect on Inflammatory Markers in a Randomized Trial of Current and Former Heavy Smokers. International Journal of Inflammation. 2015; 2015.
26. Mandler RN, Biddison W, Mandler R, Serrate S. beta-Endorphin augments the cytolytic activity and interferon production of natural killer cells. The Journal of Immunology. 1986;136(3):934-9.
27. Gein S, Gorshkova K, Tendryakova S. Regulation of interleukin-1β and interleukin-8 production by agonists of μ and δ opiate receptors in vitro.Neuroscience and Behavioral Physiology. 2009; 39(6):591-5.
28. Bastami S, Norling C, Trinks C, Holmlund B, Walz TM, Ahlner J, et al. Inhibitory effect of opiates on LPS mediated release of TNF and IL-8. Acta Oncologica. 2013;52(5):1022-33.
29. Bonnet M-P, Beloeil H, Benhamou D, Mazoit J-X, Asehnoune K. The μ opioid receptor mediates morphine-induced tumor necrosis factor and interleukin-6 inhibition in toll-like receptor 2- stimulated monocytes. Anesthesia and analgesia. 2008;106(4):1142-9.
30. Bhargava HN, Thomas PT, Thorat S, House RV. Effects of morphine tolerance and abstinence on cellular immune function. Brain Research. 1994;642(1):1-10.
31. Andjelkov N, Elvenes J, Martin J, Johansen O. Opiate regulation of IL-1beta and TNF-alpha in cultured human articular chondrocytes. Biochemical and Biophysical Research Communications. 2005;333(4):1295-9.
32. Meijerink H, Indrati A, Utami F, Soedarmo S, Alisjahbana B, Netea MG, et al. Heroin Use Is Associated with Suppressed Pro-Inflammatory Cytokine Response after LPS Exposure in HIV- Infected Individuals. PLOS ONE. 2015;10(4):e0122822.