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EFFECT OF METABOLIC PARAMETERS AND HS-CRP LEVELS ON CARDIAC AUTONOMIC FUNCTION IN PATIENTS WITH METABOLIC SYNDROME

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Abstract

AIM: The aim of the study was to evaluate cardiovascular autonomic function (CAF) in metabolic syndrome (MetS) patients and its association with hs-CRP and other metabolic parameters

.**Methods**: A total of 125 subjects of both sexes (control: 50; MetS: 75) were included for study. From anthropometric data body mass index (BMI) and waist circumference (WC) were measured. Blood pressure (BP) and biochemical analysis of blood glucose levels, lipid profiles and hs-CRP were done. Insulin resistance (IR) was calculated by HOMA-IR. To evaluate cardiac autonomic function we performed cold pressure test, heart rate response to standing and hand grip test were under sympathetic function, and deep breathing test, heart rate response to valsalva were performed under parasympathetic functions.

Results: The levels of hs-CRP were found to be elevated in obese with MetS subjects where it correlated significantly with its components including measures of Sympathetic and parasympathetic activity when compared to control groups. Positive correlation of hs-CRP was noted with obese MetS, SB/DP, TCHOL, TGL, VLDL, LDL, FBS, PPBS, INSULIN, HOMA IR, and Heart rate response to standing.

Conclusion: Obesity with MetS seem to be associated with elevated hs-CRP and CAF determination and probably increase cardiovascular risk in obese subjects.

Introduction

The incidence of metabolic syndrome, the most threatening epidemic in industrialized countries, is rapidly rising. More particularly, increased incidence of overweight and obesity have been recognized as predisposing factor for metabolic syndrome. Nonetheless, the biochemical and molecular mechanisms underlying obesity and its associated co morbidities like type 2 diabetes mellitus (T2DM), cardiovascular disease, and dyslipidemia are incompletely understood. In these days, life has changed considerably in both developed and developing countries. Food has become abundant, snacking frequency increased and the necessity for physical effort became appreciably reduced [1-6]. Furthermore, physical activity does not need to coincide with the light period anymore. As a result, the environment sensed by the brain has become metabolically compressed and arrhythmic. From the perspective of a longstanding evolutionary progress, this has been a hasty "environmental mutation." It is proposed that in such circumstances the susceptible brain loses its sensation for internal and external rhythm. Since the brain uses the autonomic nervous system to implement the internal rhythmcity, we propose an unbalanced and arrhythmic autonomic nervous system as one of the major causes of the metabolic syndrome [7].

The metabolic syndrome consists of visceral obesity, hyperglycemia, hyperinsulinemia, hypertension and dyslipidemia. A common pathophysiological denominator underlying these epidemiological correlations has not been well known. Inflammation plays a significant role in the development and progression of cardiovascular

diseases (CVDs). C-reactive protein (CRP) is a reflective predictor of cardiovascular events in healthy populations and in patients with coronary heart disease [8]. Recently, many epidemiological and interventional studies have indicated that high cardio respiratory fitness (CRF) is associated with lower levels of CRP and the inverse association between CRF and cardiovascular events is mediated in part by inflammatory factors.

A number of studies have shown that both T2DM and hypertension are strongly associated with increased insulin resistance (IR) and obesity, besides being powerful risk factors for CVDs. However, the role of IR and the involvement of low-grade inflammation and cardiac autonomic function (CAF) in the development of hypertension and CVDs is not yet been exposed. Thus, the present study was aimed to evaluate the effect of components of metabolic parameters and an hs-CRP level on cardiac autonomic function in patients with metabolic syndrome has been included.

Materials and methods

Study Design

The study design was cross-sectional. A total of 125 patients attending department of Endocrinology at Narayana Medical College (NMC), Nellore, Andhra Pradesh were recruited. The subjects were of both sexes, in the age group of above 18 years. After careful interview by using a structured questionnaire, the details were documented. This study on human research was approved by the Institutional ethical committee, NMC, Nellore.

Anthropometric measurements and Blood Pressure

Heights, weight, waist circumference of the subjects were recorded using standard scale and body mass index (BMI) was determined. Systolic and diastolic blood pressure was recorded using sphygmomanometer.

Exclusion criteria

The exclusion criteria included patients with concurrent acute illnesses as well as infectious diseases within the past 2 weeks. Pregnant, lactating women and those with malignancy, active immunological diseases, tuberculosis and with a history of using corticosteroids were also excluded from the study.

Biochemical analysis of blood glucose and lipid profiles

Blood samples were collected in sterilized tubes in the morning after 8 to 12 hour overnight fasting; serum was separated and stored at 4^{o}C until assayed. Most of the chemicals and kits used are of excellent quality obtained from Diasys diagnostics systems GmbH Alt Stasse 9 65558 Holzheim Germany. Fasting blood sugar (FBS), postprandial blood glucose level was measured by an automated enzymatic method using glucose hexokinase kit. Serum total cholesterol, HDL-C and serum triglycerides were measured using CHOD-PAP, Immuno-inhibition and Colorimetric method using glycerol-3 phosphate oxidase respectively. LDL-C and VLDL were estimated by simple calculation using Friedewald formula.

Estimation of HbA1c, insulin and hs-CRP,

HbA1c was estimated from blood using immunoturbidimetric test (chemistry analyzer bs400). Serum insulin was measured by a fully automated standard chemiluminescence platform test (Access 2 Immunoassay System by Beckman Coulter, USA). High-sensitivity C-reactive protein (hs-CRP) as an inflammatory marker was measured using particle-enhanced immunological agglutination procedure which has a sensitivity of 0.2 mg/L.

For insulin resistance, the homeostasis model assessment (HOMA-IR) was followed and the following formula was used: {fasting insulin (µU/mL)×fasting glucose (mmol/L)}/22.5

Autonomic function tests

Deep breathing test

Subject was made to lie down comfortably in supine position with head elevated to 30^{0}. Subject was instructed to maintain deep breathing at a rate of six breaths (allowing 5 seconds each for inspiration and expiration) per minute. ECG electrodes were connected for recording lead II ECG. While subject was breathing deeply, maximum and minimum heart rates were recorded with each respiratory cycle.

Valsalva Maneuver

Subject was informed to lie down in a semi recumbent or sitting position and nostrils were closed manually. Mercury manometer's mouth piece was introduced into the mouth of the subject. ECG machine was switched on for continuous recording. Subject was asked to exhale forcefully into the mercury manometer and asked to maintain the expiratory pressure at 40 mm of Hg for 10-15 seconds. ECG recordings were measure throughout the procedure, 30 seconds before and after the procedure.

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Cold pressure test

Subject was instructed about the test. Blood pressure was recorded under basal conditions. Cold water was taken in a container and the subject was asked to submerge one of his upper limbs in cold water for 1 minute. Blood pressure was recorded at the end of 60 seconds of submersion of the limb. Interestingly, submersion of the limb in ice cold water increased systolic blood pressure by about 10-20 mm of Hg and diastolic blood pressure by about 10 mm of Hg.

Heart rate response to standing

To measure heart rate, subject was made to lie down in supine posture. ECG electrodes were connected from the subject to the cardiowin system. Subject was asked to relax completely for a minimum period of 10 minutes and basal heart rate was recorded by using Cardiowin system. Then, subject was asked to stand up immediately and change in heart rate was noted from the monitoring screen of Cardiowin. Heart rate response to standing was determined by using the formula: heart rate in standing position-heart rate in supine position**.**

Hand Grip Test

Subjects were prior instructed about the test. They were made to lie down in semi recumbent position. ECG was recorded using electrodes for to know basal heart rate and blood pressure was recorded using sphygmomanometer. Subjects were informed to maintain 30% pressure of the maximum activity in the hand grip dynamometer for about 5 minutes. Heart rate, changes were recorded in SBP, DBP.

Statistical analysis

The data is represented as mean ± standard deviation (SD). Student's t test was performed to compare the intergroup means. Pearson's coefficient of correlation was used to determine correlations between continuous variables. *p* value of <0.05 was considered as significant for all the above tests. For statistical analysis SPSS (software) version 16 was used.

Results and discussion

Anthropometric measurements

In the present study, there were 20 males and 55 females in the obese metabolic syndrome (cases) group and 19 males and 31 females in the non metabolic syndrome (controls) group. Table 1 shows there was statistically significant difference in anthropometric parameters like body mass index [31.0(4.16) vs. 24.08 (3.25) cm/kg^2, p=0.001] and waist circumference [102.28(7.5) vs. 79.7 (5.0) cm, p=0.001] and age [36.36(10.30) vs. 35.88 (3.79) in years between cases and control groups respectively. The observed values for systolic blood pressure and diastolic blood pressure were [(SBP 134.73(9.16) vs. 122.62(7.64) mmHg, p=0.001] [(DBP 85.16(5.47) vs.71.34 (9.27) mmHg, p=0.001] between cases and control groups.

Table I. BMI, WC and blood pressure between controls and cases

Parameters	**Obese metabolic syndrome N=75**	**Non metabolic syndrome N=50**	***p* value**
Age (years)	36.59 ± 10.30	35.88 ± 3.79	0.001
Sex(Male/Female)	20/55	19/31	
BMI(kg/m^2)	31.0 ± 4.16	24.08 ± 3.25	0.001
WC(cm)	102.28 ± 7.5	79.7 ± 5.01	0.001
SBP(mmHg)	134.73 ± 9.16	122.62 ± 7.64	0.001
DBP(mmHg)	85.16 ± 5.47	71.34 ± 9.27	0.001

BMI: Body Mass Index; WC: Waist Circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

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Baseline biochemical parameters in different groups

We evaluated blood glucose, HbA1c, lipid profile and Hs-CRP in controls and cases. There was a significant ($p < 0.05$) elevation in levels of blood glucose, HbA1c, total cholesterol, TGs, LDL, insulin and Hs-CRP in obese metabolic cases when compared to normal controls as shown in table 2.

Table II. Baseline biochemical parameters in different groups

Parameters	Obese metabolic syndrome N=75	Non metabolic syndrome N=50	*p* value
FBS(mg/dl)	118.57 ± 23.94	84.76 ± 9.22	<0.05
PPBS(mg/dl)	158.49 ± 29.0	130.18 ± 11.86	<0.05
HbA1c (%)	7.02 ± 0.91	5.25 ± 0.39	<0.05
T.Cholesterol (mg/dl)	208.65 ± 39.42	171.06 ± 23.9	<0.05
HDL(mg/dl)	45.92 ± 6.66	44.40 ± 4.75	0.166
TGL(mg/dl)	169.02 ± 46.7	116.38 ± 36.6	<0.05
VLDL(mg/dl)	33.8 ± 9.3	23.2 ± 7.33	<0.05
LDL(mg/dl)	128.92 ± 31.5	103.38 ± 18.4	<0.05
Insulin(µIU/ml)	13.25 ± 5.15	7.56 ± 2.14	<0.05
HOMA-IR	4.01 ± 2.0	1.59 ± 0.55	<0.05
hs-CRP(mg/L)	7.25 ± 2.74	1.59 ± 0.68	<0.05

FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1c: Hemoglobin A1c; T. Cholesterol: Total Cholesterol; HDL: High Density Lipoprotein; TGL: Triglycerides, VLDL: Very Low Density Lipoprotein; LDL; Low Density Lipoprotein; hs-CRP: high sensitive C-reactive protein.

Evaluation of autonomic functions

We evaluated both sympathetic and parasympathetic parameters in controls and cases. As shown in table 3, under sympathetic parameters, considerable drop in blood pressure, hand grip and heart rate response (HR) was noticed when cold pressure test, hand grip test and HR response to standing was performed, in MetS cases when compared to control groups. Similarly, significantly reduced values in deep breathing test and HR response to valsalva was noticed in MetS cases when compared to controls under parasympathetic group.

Table III. Evaluation of autonomic functions in both groups

Parameter	Obese metabolic syndrome N=75	Non metabolic syndrome N=50	*p* value
Sympathetic			
Cold presser test Change in SBP(mmHg)	**11.04 ± 12.86**	**14.92 ± 3.00**	**0.38**
Change in DBP(mmHg)	**7.15 ± 12.65**	**11.16 ± 2.74**	**0.029**
Hand grip test Change in DBP(mmHg)	**4.43 ± 13.09**	**13.28 ± 2.79**	**0.001**
HR response to standing	**4.71 ± 7.0**	**16.2 ± 3.5**	**0.001**
Parasympathetic			
Deep breathing test	**1.2 ± 0.18**	**1.6 ± 0.12**	**0.164**
HR response to valsalva	**1.22 ± 0.24**	**1.6 ± 0.12**	**0.001**

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart Rate.

Evaluation of hs-CRP levels

Analysis of hs-CRP reveals that there exists a significant correlation between hs-CRP and blood pressure, blood glucose, insulin resistance and lipid profiles in MetS, as present in table 4.

Table IV. Correlation of hs-CRP with obese metabolic syndrome parameters

Parameters	***r* values**	***p* values**
SBP	0.399	<0.001
DBP	0.252	<0.029
FBS	0.448	<0.001
PPBS	0.437	<0.001
T.CHOL	0.317	<0.006
TGL	0.421	<0.001
VLDL	0.421	<0.001
LDL	0.269	<0.020
Insulin	0.327	<0.004
IR-HOMA	0.417	<0.001
Heart rate response to standing	0.302	<0.008

Table 4 shows a significant correlation of hs-CRP and other biochemical, insulin and cardiac autonomic function parameters. Hs-CRP had significantly positive correlation of SBP (r=0.399, p<0.001), DBP (r=0.252, p<0.029), T. Cholesterol (r=0.317, p<0.006), TGL (0.421, p<0.001), VLDL (0.421, r<0.001), LDL (r=0.269, p<0.020), FBS(r=0.448, p<0.001), PPBS(r=0.437, p<0.001), Insulin (r=0.327, p<0.004), IR-HOMA (O.417, p<0.001), Heart rate response to standing (0.302, p<0.008).

Discussion

Metabolic syndrome (MetS) is a swarm of metabolic risk factors that predispose to cardiovascular disease and type 2 diabetes. In the current study, we have measured Hs CRP levels, index of insulin resistance by HOMA-IR and cardiac autonomic function tests in 75 patients with metabolic syndrome and compared with 50 age and sex matched healthy controls. We found there is significant elevation in Hs CRP levels in patients with metabolic syndrome in comparison with controls. This is in accordance with previous studies [9-10]. In addition we have found HOMA-IR, a measure of insulin resistance index was significantly elevated in individuals with metabolic syndrome.

Previous studies have shown dysfunctional autonomous nervous system activity in patients with Met S and also shown that MetS is a state of peripheral sympathetic nerve hyperactivity [11-14]. In our study both sympathetic and parasympathetic parameters of cardiac autonomous nervous system were dysfunctional in MetS subjects which might support its role in the development of this syndrome.

In the current study, individuals with MetS had increased central adiposity as evidenced by increased waist circumference and increased overall BMI compared to healthy controls. BMI correlated to dyslipidemia, elevation in serum insulin and HOMA-IR increments. When obesity and HTN (hypertension) co-exists in an individual, the degree of sympathetic activation appears to be much greater than in an individual in whom the two conditions occur in isolation.

Hs-CRP was correlated with obesity and the index of insulin resistance HOMA-IR, which might explain the association of systemic inflammatory response in the pathogenesis of MetS and its future cardiovascular risk [15]. In addition high HsCRP levels also correlated positively with autonomus nervous system dysfunction which is seen in subjects with MetS. Therefore, the association of obesity, an increased CRP, insulin resistance and atherosclerosis can be explained. Previous studies have showed that central obesity is associated with high hs-CRP levels [16-17]. According to our data, hypertension and obesity have a significant correlation with increase in serum hs-CRP. Table 2 signifies, patients with MetS have a higher hs-CRP concentration than those without.

In this study, all individuals in the MetS groups demonstrated elevations in BP. The mean systolic and diastolic blood pressure recordings were significantly higher in the MetS group compared to control group. Altered BP responses in relation to autonomic dysfunction were also evident in our findings in individuals with MetS. Earlier studies have reported the prevalence of hypertension in MetS to 85% [18, 19].

Conclusion

Based on our study, we conclude that central obesity, blood pressure, lipid profiles and Hs CRP levels were significantly higher in patients with metabolic syndrome and associated with insulin resistance. In addition autonomic nervous system dysfunction was seen more in patients with metabolic syndrome compared with controls and also positively correlated with Hs CRP levels. From these findings, it can be concluded that systemic inflammatory response and autonomic nervous dysfunction plays an important role in metabolic syndrome. Hs-CRP was proved to be the strongest and most significant predictor of the risk of future cardiovascular ailments.

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