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Study of serum nitric oxide in insulin resistant subjects with varying degrees of metabolic syndrome

KEYWORDS: Metabolic syndrome, Insulin resistance, Nitric oxide, Endothelial damage

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ABSTRACT *Metabolic syndrome is the primary metabolic disorder associated with insulin resistance which can affect health of endothelium. The aim of this study was to assess the association between insulin resistance and serum nitric oxide in patients with varying degrees of metabolic syndrome. Total 150 subjects were enrolled for the study and were divided into four groups based on the presence of metabolic syndrome components as per NCEP ATP III criteria. Anthropometric and biochemical parameters were studied in all the subjects. Insulin resistance was calculated using HOMA IR model. Serum nitric oxide levels were estimated using modified Griess method. Comparison of all biochemical parameters between control and study group II, III and IV showed significant difference (p<0.05) except for serum nitric oxide which statistically insignificant on comparing group III levels with group IV. These finding indicate presence of three MS component is enough to cause damage endothelium.*

Study of serum nitric oxide in insulin resistant subjects with varying degrees of metabolic syndrome

The term MS refers to a cluster of correlated disorders that include glucose intolerance, insulin resistance, obesity, dyslipidemia, and hypertension.[1] Numerous metabolic abnormalities found in the metabolic syndrome, including hyperglycemia, excessive fatty acids and insulin resistance, cause an endothelial cell dysfunction by affecting nitric oxide synthesis or degradation.[2] Although the exact mechanism by which metabolic syndrome induces endothelial dysfunction remains to be clarified, there are many possibilities of vascular endothelial damage and increase in cardiovascular risk in these patients. It is stated that insulin resistance play an important role in endothelial dysfunction. Although in the physiological state insulin stimulates nitric oxide synthesis and increases nitric oxide- mediated vasodilation, this action is diminished or reversed in the case of insulin resistance, as is found in MS. Metabolic syndrome is defined by several definitions like WHO, the National Cholesterol Education Program's Adult Treatment Panel III report (NCEP) and the International Diabetes Federation (IDF). While the WHO definition emphasizes on insulin resistance and glucose intolerance, the IDF definition is based on central obesity, whereas all the factors considered equally in NCEP definition.4 Hence we have considered NCEP ATP III criteria identifying MS.

The present study was undertaken to study the association of various component of metabolic syndrome with insulin resistance and serum nitric oxide in order to find out which component has maximum effect on levels of serum nitric oxide in metabolic syndrome patients with IR.[3]

Research Design and setup

This was a cross sectional, experimental study design carried out in MGM Medical College & Hospital from January 2012 to January 2013. A total of 150 subjects were enrolled for the study.

According to ATP III Asian definition, the components of MS are (1) large waist circumference (LWC) ≥80 cm in female and ≥90 cm in male, (2) high triglyceride (HTG) ≥150mg/dl, (3) low HDL-cholesterol (HDL) <40mg in male and <50mg in female, and (4) high blood pressure (HBP) ≥130/85 mg or on medication, (5) Fasting glucose ≥ 110 mg/dl [6,7].

All the subjects were divided in to three groups.

Group I (n=50) - Healthy controls,

Group II (n=33) - Subjects with presence of two MS components (BMI, TG)

Group III (n=32) - Subjects with three MS components.
(BMI, Cholesterol, TG)

Group IV (n= 35)-Subjects with more than three MS components. (large waist circumference, high TG, low HDL, high BP, Fasting glucose.

A written informed consent was obtained from the subjects before commencing the study. The protocol was approved by the Institutional Ethics Research review committee.

Assessment: Homeostasis model of assessment for insulin resistance (HOMA index) was employed for evaluating insulin resistance using formula, fasting glucose (mmol/L) × fasting insulin (UI/L)/22.5.[4] Anthropometric parameters were noted for all the controls and subjects in the study which included measurement of body weight, height, BMI, waist- hip circumference and blood pressure. BMI was calculated (BMI = body weight/height (kg/m^2).

Venous blood sample was obtained after a 12-hour fast for biochemical analysis which included estimation of insulin by ELISA method, [5] fasting glucose by hexokinase method and lipid profile by an enzymatic method using commercial kits. Low density lipoprotein cholesterol (LDL-C) levels were determined using the Friedewald formula, as modified by De Long.[6] Serum nitric oxide was estimated indirectly by measurement of stable decomposition product (NO_2^-), employing Griess reaction according to the modified method of Mirinda et al.[7]

Table 1 Anthropometric parameters of various groups

Parameters	Group I	Group II	Group III	Group IV
No. of subjects	50	33	32	35
Age	50 ± 3.43	52 ± 4.02	54 ± 3.98	57 ± 5.43
BMI (kg/m^2)	23.31 ± 1.22	28.949 ± 1.39	29.56 ± 4.87	30.56 ± 3.39
Waist/ Hip ratio	0.86 ± 0.003	1.00± 0.003	1.00 ± 0.006	1.00 ± 0.005

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Table 2 Comparison of descriptive parameters between Group I and Group II

Parameters	Group I Control Group (n=50)	Group II With < 3 MS components(n=33)	P value
Systolic B.P. (mmHg)	115 ± 6.98	118.8 ± 5.89	0.000
Diastolic B.P. (mmHg)	76.7± 4.98	81.5 ± 4.041	0.000
Fasting Blood Glucose (mg/dl)	83.43 ± 8.54	88.67 ± 5.31	0.000
2 hr Post prandial Blood Glucose (mg/dl)	119.8 ± 6.01	123.43 ± 8.58	0.000
Fasting Insulin	12.2 ± 5.67	12.56± 4.76	0.76
HOMA IR	2.23 ± 0.69	3.67± 1.04	0.000
Total Cholesterol mg/dl)	145.3 ± 2.45	155± 7.43	0.000
Triglycerides (mg/dl)	110± 17.2	125.8 ± 15.12	0.000
HDL Cholesterol(mg/dl)	42.7 ± 1.77	40.76 ± 2.99	0.000
VLDL (mg/dl)	25.4 ± 4.12	30.26 ± 5.84	0.000
LDL Cholesterol (mg/dl)	88.78 ± 12.23	96.63 ± 16.97	0.000
Nitric oxide µmol/l	0.33 ± 0.04	0.22 ± 0.061	0.000

Table 3. Comparison of descriptive parameters between Group II and Group III

Parameters	Group II With < 3 MS components (n=33)	Group III With 3 MS components (n=32)	P value
Systolic B.P. (mmHg)	118.8 ± 5.89	128 ± 2.68	0.000
Diastolic B.P. (mmHg)	81.5 ± 4.041	86.5 ± 3.98	0.002
Fasting Blood Glucose (mg/dl)	88.67 ± 5.31	109.9± 3.76	0.000
2 hr Post prandial Blood Glucose	123.43 ± 8.58	135.81 ± 9.44	0.000
Fasting Insulin µIU/ml	12.56± 4.76	17.8 ± 5.2	0.001
HOMA IR	3.67± 1.04	4.65± 1.12	0.004
Total Cholesterol mg/dl)	155± 7.43	169.6 ± 3.23	0.000
Triglycerides (mg/dl)	125.8 ± 15.12	182.23± 51.07	0.000
HDL Cholesterol(mg/dl)	40.76 ± 2.99	37.89 ± 19.09	0.004
VLDL (mg/dl)	30.26 ± 5.84	45.95 ± 22.07	0.000
LDL Cholesterol mg/dl)	96.63 ± 16.97	128.73 ± 34.89	0.000
Nitric oxide µmol/l	0.22 ± 0.061	0.18 ± 0.059	0.03

Table 4. Comparison of descriptive parameters between Group III and Group IV

Parameters	Group III With 3 MS components (n=32)	Group IV With > 3 MS components (n=35)	P value
Systolic B.P. (mmHg)	128 ± 2.68	136 ± 2.86	0.000
Diastolic B.P. (mmHg)	86.5 ± 3.98	97 ± 2.60	0.000
Fasting Blood Glucose (mg/dl)	109.9± 3.76	117.4±2.97	0.000
2 hr Post prandial Blood Glucose	135.81 ± 9.44	143 ± 16.7	0.07
Fasting Insulin	17.8 ± 5.2	22.67 ± 4.5	0.000
HOMA IR	4.65± 1.12	6.84 ± 0.89	0.000
Total Cholesterol mg/dl)	169.6 ± 3.23	205.08 ± 33.14	0.000
Triglycerides (mg/dl)	182.23± 51.07	211.71 ± 77.01	0.11
HDL Cholesterol(mg/dl)	37.89 ± 19.09	36.87± 20.1	0.65
VLDL (mg/dl)	45.95 ± 22.07	47± 19.3	0.82
LDL Cholesterol mg/dl)	128.73 ± 34.89	137± 31.78	0.36
Nitric oxide µmol/l	0.18 ± 0.059	0.17± 0.04	0.40

Anthropometric parameters are described in table 1. Male and female subjects were matched for age and number in both the control and the study groups. Descriptive statistics of control and subjects with metabolic syndrome and their comparative analyses along with p values are presented in Tables 2,3,4 resp.All values are expressed as means ±SD. There is statistically significant difference in all biochemical parameter of group II and group III. HOMA IR in group II was 3.67 ±1.04 and group III was 4.65 ±1.12 which was significantly significant (p<0.005). Serum nitric oxide in group II was 0.22 ± 0.061 and in group III was 0.18 ± 0.059 which was statistically significant (p<0.01). HOMA IR in that of group III was 4.65 ±1.12 and that of in group IV was 6.48 ±0.89 which was statistically significant (p<0.0001) but serum nitric oxide levels of group III were 0.18 ± 0.059 and in group IV 0.17±0.04 were which was also statistically insignificant.

Discussion

The existence insulin resistance syndrome (IRS) is charac-

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terized by series of disorders which occurs together more often. The data presented herein support the occurrence of IRS in study groups. All the subjects were ranging in age from 45 to 58 years. The present study shows that all healthy controls were normotensive and had BMI less than 25kg/M^2.The participants in group II, III& IV had high BMI, waist to hip ratio, hypertension, increased fasting insulin concentration & higher HOMA-IR values compared to control. The comparison of biochemical parameters, fasting insulin and HOMA IR and serum NO of group II with III has shown statistically significant difference indicating that metabolic syndrome subjects with three feature of MS has more disturbed glucose and lipid homeostasis with lower levels of serum nitric oxide. These finding indicate that extent of endothelial damage is more in subjects with presence of three MS components. When comparison of group IV with was carried out with group III, it is observed that lipid parameters and serum NO did not differ significantly but fasting glucose, fasting insulin and HOMA IR showed significant difference. These finding reveals that subjects with greater number of MS components have high insulin resistance but has no effect on serum nitric oxide and lipid profile levels indicating that more than three metabolic syndrome component may not affect these parameters.

Our finding of Group II and III are in accordance with studies conducted by Zuvaroni, Orchard TJ. They showed that subjects who had three and more than three metabolic components had higher fasting insulin concentration and HOMA-IR index than those who had less than three MS components (Group II, Table 2).

Haffner et al have shown that pre diabetic subjects with insulin resistant had significantly higher body mass index, waist circumference, triglyceride concentration and blood pressure and lower HDL cholesterol than non converters. Our data has shown that maximum insulin resistance in patients with three and more than three MS components. Another important observation of our study is reduced levels of serum nitric oxide in both study groups (III& IV). [8]

Insulin is a vasoactive hormone, Insulin increases muscle blood flow in a time and concentration-dependent fashion through a mechanism that involves binding to the insulin receptor on the endothelial cell membrane. Insulin is known to have a direct vasodilatory effect mediated through stimulation of nitric oxide production in endothelial cells.[9]

At normal physiologic concentrations, insulin increases skeletal muscle blood flow in healthy, insulin-sensitive people and its effect to vasodilate skeletal muscle vasculature is directly proportional to its ability to stimulate glucose uptake.[10] In other words, insulin sensitivity and vasodilation are linked such that the most insulin sensitive individuals exhibit the greatest degree of vasodilation in response to insulin.

Insulin's effect on the endothelium is mediated through its own receptor and insulin signaling pathways, resulting in an increased production and/or release of NO. NO in muscle is produced by NOS,located in both vascular endothelium (eNOS)56andmyocytes (nNOS).[11] Insulin has been shown to activate directly a signaling cascade in cultured endothelial cells via insulin receptor substrate 1, phosphatidylinositol 3-kinase and protein kinase B, which can then phosphorylate and activate eNOS. [12-14]

But insulin-resistant people such as those who are obese and NIDDM patients exhibit blunted vasodilatory responses to insulin. Thus in insulin resistant state there are increased plasma levels of soluble adhesion molecules, augmented adhesiveness of circulating leukocytes, and endothelium-dependent, NO-mediated vasodilation is markedly impaired. In addition, activation of the sympathetic nervous system and increased renal sodium retention lead to hemodynamicchanges in those who are insulin resistant. [15-24]

Thus the mechanisms by which insulin resistance leads to endothelial dysfunction are certainly multiple and complex. All major abnormalities that are part of the insulin resistance syndrome, such as hyperglycemia, hypertension, dyslipidemia, and altered coagulation/fibrinolysis, are directly and independently linked to endothelial dysfunction.[25]

Conclusion

The components of metabolic syndrome increases risk of developing type 2 diabetes.

Assessment of Insulin resistance, HOMA IR, dyslipidemia and nitric oxide have been shown to be associated with endothelial dysfunction. The presence of endothelial dysfunction in insulin resistant subjects suggest that metabolic and vascular abnormalities are intimately linked at a fundamental level. Since microvascular endothelial dysfunction is closely associated with hypertension and CVD, improvement of microvascular function should be one of the first targets. This can be monitored by measuring serum NO level regularly.

In conclusion, this study has shown that increased fasting insulin concentrations is a risk factor for future cluster of metabolic disorders including dyslipidemia (especially low HDL-cholesterol and increased triglyceride concentration), hypertension and glucose intolerance. This indicates a strong correlation between insulin resistance and Syndrome X and suggests that insulin resistance may be the unifying pathophysiology underlying the syndrome. Persons with metabolic syndrome are at increased risk of incidence of diabetes and cardiovascular disease relative to people without the symptoms of Syndrome X. In a sense, insulin resistance can be viewed as a large iceberg submerged just below the surface of water. The physicianrecognizes only the tips of iceberg- diabetes, obesity, hypertriglyceridemia, hypertension, diminished HDLcholesterol and atherosclerosis—which extrude above the surface of and the complete insulin resistance syndrome may be missed. With the recognition that insulin resistance consists of a cluster of disorders and biochemical abnormalities, it is important for the scientific community to focus their attention on defining the mechanism(s) responsible for the defect in insulin-mediated glucose metabolism in type 2 DM.

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