ORIGINAL ARTICLE

ANALYSIS OF INSULIN RESISTANCE IN VARIOUS COMPONENTS OF METABOLIC SYNDROME

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ABSTRACT

Metabolic syndrome is a collection of health risks that increases chances of developing heart disease. The constellation of metabolic abnormalities includes glucose intolerance, central obesity, dyslipidemia, and hypertension. These conditions can occur in an individual more often than might be expected by chance. The aim of this study was to analyze the insulin resistance measured as Homeostasis model assessment of insulin resistance (HOMA-IR) in different variables of metabolic syndrome. In univariate analyses, insulin resistance was related to hypertension, low HDL-cholesterol, increased triglyceride concentration and diabetes, however, no relation of insulin resistance with LDL or total cholesterol could be established. Insulin resistance was found to be significantly higher in subjects with increase in number of risk factors of metabolic syndrome. According to HOMA-IR index all the diabetic subjects were insulin resistant. Even hypertensive, obese and first degree relative of diabetics was insulin resistant without having diabetes or impaired glucose tolerance. Maximum insulin resistance was observed in patients of T2DM with obesity, hypertension and microalbuminuria i.e. patients with multiple metabolic abnormalities. These results support the existence of metabolic syndrome and the relationship of that syndrome to multiple metabolic disorders by showing a strong association between insulin resistance and Syndrome X and suggest that hyperinsulinemia/insulin resistant may be the unifying pathophysiology underlying the syndrome.

Key words: Insulin resistance, Hyperinsulinemia, Metabolic syndrome

INTRODUCTION

Obesity, type 2 diabetes mellitus (T2DM), hypertension (HTN), and dyslipidemia are common metabolic disorders that afflict the majority of individuals[1]. Moreover, all of these common medical disorders occur with increasing incidence as the population ages[1]. Because obesity, HTN, T2DM and dyslipidemia occur frequently in the population at large, it is not surprising that any given individual, especially if he or she is > 50 years of age, might manifest two or more of these common medical problems. Reaven[2] used the term Syndrome X to refer to the association of dyslipidemia, hypertension, coronary artery disease, glucose intolerance and insulin resistance, more than two decades ago, but it has become the focus of considerable attention only in recent years. Most studies have assessed prediction of insulin resistance in individuals selected randomly with impaired glucose tolerance and diabetes. But very few studies across the globe have specifically assessed the prevalence of insulin resistance in different variables of metabolic syndrome i.e. subjects with one, two or multiple components of syndrome X and in first-degree relatives of type 2 diabetes mellitus patients who are at most risk of developing insulin resistance. Thus in this study, an attempt has been made to provide evidence that the common occurrence of the – obesity, glucose intolerance, hypertension and dyslipidemia in the same individual is more than a chance occurrence and is related to hyperinsulinemia/insulin resistance.

MATERIAL AND METHOD

240 subjects with either sex (122 males and 118 females) of varying age (range: 21 – 65 years) were enrolled from June 2010 to September 2011 and were categorized into six non-overlapping groups which include:

GROUP I: Healthy controls, i.e. subjects not suffering from diabetes, nor having any family history of diabetes, not suffering from hypertension or from any acute or chronic disease, nor taking any drugs believed to alter plasma glucose level or blood pressure status; n = 40 (20 males and 20 females).

GROUP II: Non-diabetic hypertensive patients defined on the basis of Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VII)[3]]; n = 40 (20 males and 20 females).

GROUP III: Non-diabetic, non-hypertensive overweight or obese patients [A BMI less than 25 kg/m^2 was considered as normal, BMI between 25 kg/m^2 and 30 kg/m^2 as overweight and greater than $30kg/m^2$ as obesity[4]]; n = 40 (18 males and 22 females).

GROUP IV: Non-diabetic, hypertensive microalbuminuric patients [Based on the presence of microalbuminuria in urine]; n = 40 (21 males and 19 females).

GROUP V: Type 2 diabetes mellitus patients defined as those having diagnosed diabetes after 30 years of age and did not require insulin during the first two years of diagnosis; n = 40 (23 males and 17 females).

GROUP VI: The first-degree relatives of type 2 diabetic patients, by definition, include individuals having 50% genome common to the group V patients i.e. they include parents, siblings and offspring. Though, it became impossible in our part to conduct genomic study to establish first-degree relatives of type 2 diabetes mellitus patients, they are recruited in the study according to the result obtained in pedigree chart analysis; n = 40 (20 males and 20 females).

Patients with overt albuminuria, congestive cardiac failure, pre-existing macrovascular condition, urinary tract infection, pregnant females or who had given birth within the preceding six weeks, lack of approval by physician and subjects showing disinterest were excluded from the study. All subjects were studied as outpatient. Participant's examination included interviews for medical and nutritional history. Present and past history of each case was recorded in detail regarding their general information i.e. name, age, sex, address, religion, occupation, economic status, nutritional and personal habits, education, medication and history suggestive of any systemic illness. Each subject was then examined for various anthropometric parameters: Weight (Kg) and height (meters) were measured (using Omron digital body weight scale HN-286 and SECA 206 wall mounted metal tapes respectively). Body Mass Index (BMI) was calculated by Weight (Kg) / height squared (m^2). All anthropomorphic measures reflect the average of 2 measurements (measured by same person on same instrument to avoid inter-instrument and inter personal variation). Blood pressure (BP) was measured two times in the seated position after 10 minutes of rest with a standard manual mercury sphygmomanometer (Diamond Deluxe Industrial Electronics and Products). The recorded pressure of the two measurements was averaged. Subjects were assigned to a category of hypertensive status according to the Seventh Report of the Joint National Committee (JNC 7)[3]. Hypertension(HTN) stage 2 was defined with a systolic blood pressure equal to or exceeding 160 mmHg or diastolic BP equal to or exceeding 100 mmHg, and those who had used BP lowering medications. Normal blood pressure was considered to be a systolic reading < 140 mmHg and a diastolic reading <90 mmHg. Readings between these levels were classified as stage 1 hypertension. Age was defined as the age at the time of interview (though no documentary proof had been entertained) and the date of diagnosis of disease was obtained from the patient. All subjects were asked to collect a random urine sample for analysis of albumin excretion. Urine collection was carried out during unrestricted daily life activity. The urinary albumin concentration was determined by Micral test using commercially available assay kits from Roche Diagnostics (Mannheim, Germany). The micral test is a test-strip method in which the color reaction is mediated by an antibody-bound enzyme[5]. The mean inter- and intra-assay coefficients of variation (CV) were 3.6 and 4.4%, respectively. Normoalbuminuria was defined as Albumin Excretion Rate (AER) < 30 mg/24 hr, and Microalbuminuria as AER 30- 300 mg/24 hr[6]. Results were confirmed after 2 measurements done in a space of 6 months. If the results of a 2nd measurement placed the patient in a different category from that based on the first measurement, a 3rd urine sample was obtained to confirm either the first or second measurement.

After an overnight fast of 12 hours, a standardized oral glucose tolerance test (OGTT) using 75 grams of glucose was performed following WHO guidelines[7], venous sampling was done after 0 and 120 minutes of glucose taking. Patients with a previous diagnosis of diabetes mellitus were not submitted to the OGTT. Glucose tolerance was assessed according to American Diabetes Association (ADA)[8] i.e. subjects with a fasting plasma glucose $\geq$ 126 mg/dl and/or a 2 hour plasma glucose level $\geq$ 200 mg/dl were considered to have diabetes; subjects with a fasting plasma glucose 110-125 mg/dl and with 2 hour plasma glucose level 140-199 mg/dl were considered to have impaired fasting glucose(IFG) and impaired glucose tolerance(IGT) respectively; and subjects with fasting plasma glucose < 110 mg/dl and 2 hour plasma glucose < 140 mg/dl were regarded as having normal glucose tolerance (NGT).

Serum and plasma was separated from blood sample and were subjected for analytical procedures: Plasma glucose was measured using the glucose oxidase method (GOD-POD, CV%: 3.4). HbA1c was measured with a DSS machine using the ion exchange chromatography method. Serum cholesterol by Cholesterol Oxidase p-aminophenazone (CHOD-PAP, CV%: 3.9)), serum triglycerides by Glycerol phosphate oxidase p-aminophenazone (GPO-PAP, CV%: 3.6) methods and high-density lipoprotein (HDL) cholesterol by precipitation method (CV%: 4.7). Low-density lipoprotein (LDL) cholesterol was calculated with Friedenwald's formula[9]. Adult Treatment Panel III (ATP III) criteria[10]were used to classify plasma lipid levels. Total cholesterol, triglyceride and LDL levels

exceeding 200, 150, and 100 mg/dl respectively, and HDL levels below 45 mg/dl were considered as abnormal. Plasma insulin was measured by a highly specific immunoradiometric assay (CV%: 4.1) with a two-site monoclonal antibody[11]. Biochemical tests were analyzed on a Bayer express plus auto analyzer. Quality was controlled using standard solutions. These experiments were approved by Ethical Committee. Homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate for the direct measurement of insulin resistance and was calculated as follows[12]:

HOMA-IR= [fasting insulin (μU/mL) ×fasting glucose (mmol/L)]/22.5.

Statistical analysis: Data analyses were performed with the SPSS Version 15 statistical software. The results were expressed as mean ± SD if the variables were continuous, and as percentage, if categorical. The Chi square test was used for evaluating differences in proportions between groups. One-way analysis of variance was used for differences in continuous variables. Several variables have been log transformed to normalize distributions, and for these, geometric means are presented. The Kruskall-Wallis one-way analysis of variance was used to test other variables that were clearly non-normally distributed. To examine the joint effects of the variables associated with insulin resistance, logistic regression analysis was performed. For all analyses, the nominal level of statistical significance was<0.05.

RESULTS

All subjects were ranging in age from 21 to 65 years. The prevalence of diabetes and hypertension progressively increases from forth to sixth decade of life, while obesity was evenly distributed throughout the age spectrum. Age is an important factor and several workers[13,14] have examined the association between age and insulin resistance/serum insulin levels. In this study it was observed that as age advances person gradually develop insulin resistance, irrespective of the study group (Table II) which leads to hyperinsulinemia, a marker of Syndrome X.

All healthy controls and hypertensive subjects had BMI < 25 kg/m^2. However 67.5% diabetic, 92.5% group III participants and 10% first-degree relative of T2DM subjects have BMI > 30 kg/m^2. BMI was significantly higher in T2DM, and group III participants as compared to control subjects ($p<0.05$) (Table I) and in linear regression analysis, a positive correlation was found between BMI and insulin resistance in these groups (Table III), which indicates the high incidence of obesity among these conditions and it is one of the important risk factor for the development of coronary artery disease. However, in group VI and hypertensive subjects BMI remains elevated compared to normal subjects but the effect was no longer significant (Table I).

Table 1: Characteristics of different study groups

Characteristics	Group I	Group II	Group III	Group IV	Group V	Group VI	p value
No of Subjects	40 (20M;20F)	40 (20M;20F)	40 (18M;22F)	40 (21M;19F)	40 (23M;17F)	40 (20M;20F)	
BMI (kg/m^2)	22.7 ± 0.6	23.2 ± 0.7	32.2 ± 2.4*	23.8 ± 0.6	29.5 ± 1.2*	24.5 ± 1.1	* < 0.05
Glucose-F (mg/dl)	84.2 ± 7.9	88.7 ± 7.5	89.2 ± 10.2	86.7 ± 8.8	132 ± 13.5*	92.4 ± 8.3	* < 0.05
2 hour post Glucose(mg/dl)	124.2 ± 14.1	128.4 ± 11.9	125.6 ± 16.5	129.5 ± 11.3	--	131.8 ± 13.4	
Fasting Insulin(μU/ml)	17.4 ± 2.4	23.3 ± 4.5*	24.5 ± 5.1*	23.6 ± 4.9*	29.1 ± 7.2*	24.9 ± 6.8*	* < 0.05
HOMA IR	3.9 ± 1.3	7.8 ± 2.4*	8.3 ± 2.7*	7.9 ± 2.6*	10.1 ± 3.7*	8.7 ± 2.8*	* < 0.05
Total cholesterol (mg/dl)	172.4± 21.4	191.8± 38.5	196.8± 32.3	194.1± 32.3	202.7± 39.1	199.6 ± 26.7	
Triglyceride (mg/dl)	105.6 ± 14.6	140.9 ± 19.7*	164.8 ± 23.8*	141.7 ± 17.4*	181.5 ± 22.1*	132.7 ± 15.8*	* < 0.05
HDL-cholesterol (mg/dl)	46.3 ± 5.3	41.8 ± 6.1*	40.9 ± 6.9*	41.4 ± 5.4*	39.6 ± 5.1*	43.1 ± 4.1*	* < 0.05
LDL-cholesterol (mg/dl)	91 ± 18.1	119 ± 23.9	129 ± 21.3	121 ± 25.4	148 ± 31.7	101.9 ± 13.2	
Systolic B.P. (mmHg)	119 ± 11	138 ± 14*	132 ± 12*	140 ± 10*	142 ± 12*	128 ± 10	* < 0.05
Diastolic B.P. (mmHg)	78 ± 7	94 ± 13*	88 ± 9*	92 ± 8*	92 ± 10*	82 ± 8	* < 0.05

Hypertensive, obese, first degree relative of type 2 diabetic and group IV subjects were found to have relatively higher fasting and 2-hour post glucose levels as compared to normal healthy controls, but the difference was not significant.

Table 2: HOMA IR index according to various age groups in study groups

Age (years)	Group II	Group III	Group IV	Group V	Group VI
< 30	5.6 ± 1.4	6.2 ± 1.9	4.8 ± 0.9	7.6 ± 2.1	6.7 ± 1.2
30 – 40	6.2 ± 1.2*	6.5 ± 1.4*	5.9 ± 1.4*	8.8 ± 4.2*	7.3 ± 1.1*
> 40 – 50	7.1 ± 1.7*	7.3 ± 1.2*	6.9 ± 2.1*	10.3 ± 3.3*	8.1 ± 1.2*
> 50 – 60	9.4 ± 2.3*	9.1 ± 2.7*	9.8 ± 1.8*	12.1 ± 4.6*	9.9 ± 2.7*
> 60	10.1 ± 3.4*	11.1 ± 3.1*	10.8 ± 2.7*	12.8 ± 4.7*	11.0 ± 2.1*
p value	* < 0.05	* < 0.05	* < 0.05	* < 0.05	* < 0.05

Other important finding was observed that group VI subjects had higher glucose levels (fasting as well as 2 hour post glucose) relative to group II, III and IV, however the rise was not significant. In T2DM subjects significantly elevated fasting glucose level was observed compared to normal and other study subjects (Table I) and the mean HOMA IR was 7.8 ± 2.4, 8.3 ± 2.7, 7.9 ± 2.6, 10.1 ± 3.7 and 8.7 ± 2.8 μU/ml for group II, III, IV, V and VI respectively.

Table 3: HOMA IR index according to BMI in Group III and V subjects

	Group III		Group V	
BMI (kg/m²)	**n**	**HOMA IR**	**n**	**HOMA IR**
< 25	0	-	4	9.3 ± 1.8
25-30	3	7.4 ± 1.9	9	9.8 ± 2.1
> 30	37	10.9 ± 2.7	27	12.4 ± 2.2
p value		* < 0.05		* < 0.05

According to ATP (III) criteria, 55% hypertensive, 62.5% obese, 52.5% group IV, 82.5% T2DM and 42.5% first-degree relatives of T2DM subjects had abnormal lipid profile parameter of some degree, and the magnitude of impairment was highest in T2DM subjects and lowest in first-degree relative of T2 DM subjects. Observed trend was T2DM > Obesity > Hypertension with microalbuminuria > Hypertension > First-degree relative of T2DM. Significant positive correlation was observed in fasting insulin level and hypertriglyceridemia and low HDL-cholesterol ($p<0.05$). However, no association between HOMA-IR and the elevated LDL or total cholesterol could be established. The elevated coronary heart disease risk affecting and hypertensive patients may be attributed to a combined dyslipidemia. The dyslipidemia induced by insulin resistance in Syndrome X contribute to macrovascular complications.

DISCUSSION

The existence of a syndrome such as insulin resistance syndrome (IRS) requires both that a series of disorders occur together more often than would be expected by chance alone and furthermore that some common etiological factor underlines the phenomenon. The data presented herein support the occurrence of IRS. The present study shows that participants with increased fasting insulin concentrations and higher HOMA-IR values were hypertriglyceridemia, low HDL-cholesterol and were diabetic (Table 1I). However, the association between insulin and hypertension was independent of adiposity (Table I: As data shows that Group II subjects had BMI 23.2 ± 0.7 and B.P. 138 ± 14/94 ± 13, whereas Group III subjects had BMI 32.2 ± 2.4 and B.P. 132 ± 12/88 ± 9). This observation suggests that fasting insulin concentrations may be a stronger risk factor for hypertension in lean subjects. No association between fasting insulin concentrations and elevated LDL or total cholesterol was observed (Table 1)

Similar to studies elsewhere[15,16,17], this study shows that subjects who had multiple metabolic disorders (Group V) had higher fasting insulin concentration and HOMA-IR index than those who had only one or two disorder (Group II, III and IV; Table I). Swinburn et al[18] reported that insulin resistance was associated with lower weight gain in Pima Indians (r= - 0.38). Data reported by Valdez et al[19] from the San Antonia Heart Study also suggest that higher fasting insulin concentrations predict lesser degree of weight gain. These observations strengthen our conclusion that the relationship between hyperinsulinemia and the incidence of metabolic disorders is not due to concomitant changes in adiposity. Insulin resistance was found to be significantly higher in subjects with increase in the number of risk factors of metabolic syndrome. Maximum insulin resistance was observed in patients of T2DM with obesity, hypertension and microalbuminuria (Group V subjects). According to HOMA-IR index all the T2DM subjects were insulin resistant. Even hypertensive, obese and first degree relative of diabetics was insulin resistant without having diabetes or impaired glucose tolerance.

Result of this study, as regards to group VI subjects, is consistent with the study of Williams et al[20], showing occurrence of risk factor i.e. hyperinsulinemia within families, as the data (Table I) clearly reflect the prevalence of insulin resistance in normoglycemic first-degree relatives as compared to that in controls. Haffner et al[21] in their study had reported that subjects with increased insulin concentrations have an increased risk of type 2 DM compared to those with lower insulin concentrations. Our data had showed that maximum insulin resistance was observed in patients of T2DM with obesity, hypertension, microalbuminuria and dyslipidemia i.e. subjects with multiple metabolic disorders.

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 physician recognizes only the tips of iceberg- diabetes, obesity, hypertriglyceridemia, hypertension, diminished HDL-cholesterol 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.

BIBLIOGRAPHY

1. Park MJ, Boston BA, Oh M, Jee SH: Prevalence and trends of metabolic syndrome among Korean adolescents: from the Korean NHANES survey,1998-2005. J Pediatr 2009;155(4):529-534.
2. Reaven GM. Banting lecture: Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
3. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560-2572.
4. World health organization expert consultation: Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004:363;157-163.
5. Rodicio JL, Campo C, Ruilope LM. Microalbuminuria in essential hypertension. Kidney Int 1998;54:1523–755.
6. Rodicio JL, Campo C, Ruilope LM. Microalbuminuria in essential hypertension. Kidney Int 1998;54:1523–755.
7. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. Diabetes 1979;28:1039-57.
8. American Diabetes Association: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010;33:S62-S69.
9. Friedenwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
10. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486-2497.
11. Sobey WJ, Beer SF, Carrington CA. Sensitive and specific two site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. Biochem J 1989;20:1183-1197.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor GA, Treacher DF, Turner RL. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–19.
13. Kuk JL, Ardern CI. Age and Sex Differences in the Clustering of Metabolic Syndrome Factors. Diabetes Care 2010;33:2457–2461.
14. Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States,2003–2006. Natl Health Stat Report 2009;13:1–7
15. Zuvaroni I, Bonora E, Pagllera M, Dall AE, Luchotti L, Buonanno G, Bonati PA, Bergonzani M, Gundi L, Passeri M, Reaven G. Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Eng J Med 1989;320:702-06.
16. Orchard TJ, Becker DJ, Bates M, Kuller LH, Drash AL. Plasma insulin and lipoprotein concentrations : an atherogenic association? Am J Epidemol 1983;118:326-37.
17. Haffner SM, Fong D, Hazuda HP, Pugh JA, Patterson JK. Hyperinsulinemia, upper body adiposity and cardiovascular risk in non-diabetics. Metabolism 1988;37:338-45.
18. Swinburn BA, Nyomba BL, Saad MF, Zurlo FZ, Raz I, Knowler WC, Lilloja S, Bogardus C, Ravussin E. Insulin resistance associated with lower rates of weight gain in Pima Indians. J Clin Inves 1991;88:168-73.
19. Valdez R, Milchell BD, Hazuda HP, Morales PA. Correlates of weight change among type 2 diabetics and non-diabetics. Diabetes 1997;12:342-346
20. Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Barlow GK, Stephenson SH, Lalouel JM, Kulda H. Familial dtslipidemic hypertension : evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. JAMA 1988;259:3579-86.
21. Haffner SM, Stern MP, Mitchell BD, Hazuda HP, Patterson JK. Incidence of type II diabetes mellitus in Mexican Americans predicted by fasting insulin and glucose levels, obesity and body fat distribution. Diabetes 1990;39:283-89.