ASSOCIATION OF INSULIN RESISTANCE WITH REDOX IMBALANCE IN PATIENTS WITH NON DIABETIC HYPERTENSION

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ABSTRACT: Oxidative stress involved in the pathogenesis of several diseases. Hypertension itself acts as source of oxidative stress. Insulin resistance is involved in the pathogenesis of essential hypertension. In the present study an attempt was made to study the association between insulin resistance and oxidative stress in non diabetic hypertensive patients. Two hundred and three, non diabetic hypertensive patients and two hundred and ten, healthy normotensive subjects were enrolled in this study. Malondialdehyde, reduced glutathione, glutathione peroxidase, Fasting insulin and HOMA-IR levels were estimated in both groups. Fasting insulin, reduced glutathione, glutathione peroxidase and MDA shows significant difference between cases and controls. Among the patients HOMA-IR were significantly correlated with lipid peroxides and shows negative correlation between HOMA-IR and glutathione peroxidase. Increased HOMA-IR was found in non diabetic hypertensive patients. This study reveals the link between oxidative stress and insulin resistance.

Key words: Oxidative stress (redox imbalance), HOMA-IR, Essential hypertenstion, Malondialdehyde.

INTRODUCTION

Hypertension is reported to be the fourth contributor to premature death in developed countries and the seventh in developing countries (Deepa R, et al, 2003).The epidemiology of hypertension, in terms of its importance as a risk factor for cardiovascular diseases, continues to be a major area of research. A meta-analysis of hypertension prevalence rates in India (Gupta R.1997) was significantly higher in urban than in rural populations and the prevalence of hypertension was higher in urban compared to rural areas.

Hypertension is risk factor for atherosclerotic diseases, based on insulin resistance and/or the resultant hyperinsulinemia (Reaven GM, 1998, Kaplan NM,1989). Numerous cross-sectional studies have shown a close association between insulin resistance and hypertension (Staessen J et al., 1998, Haffner SM et al., 1994). In untreated essential hypertensive patients, fasting and postprandial insulin (INS) levels are higher than in normotensive controls, with a direct correlation between plasma INS concentrations and BP (Sowers JR, 2004). Several studies have shown correlation between hyperinsulinemia and free radical production in human fat cells and rats (Krieger-Brauer HI and Kather H, 1992, DeFronzo RA, Ferrannini E, 1991and Habib MP, 1994).

Recent findings suggest that linking hyperinsulinemia or insulin resistance to hypertension have been provocative, many inconsistencies remain and causal relationships have not been established. A recent hypothesis pointed out the possible role of oxidative stress as a key player in the pathogenesis of insulin resistance, β-cell dysfunction, and hypertension (Ceriello A and Motz E, 2004).

Oxidative stress has been recognized as a general mechanism relevant in the pathogenesis of several human diseases (Freeman BA and Crapo JD, 1982). It is triggered by exposure to exogenous factors or by chemicals producing reactive oxygen species and is associated with an overproduction of reactive oxygen species, as well as an impairment of antioxidant defence capacity (Halliwell B , 1996). Hypertension has been shown to be one of the conditions associated with decreased antioxidant capacity (Berry C, et al., 2001, Redon J, et al., 2003 and Touyz RM, 2004). Oxidative damage to unsaturated lipids is a well-established general mechanism for oxidant-mediated cellular injury (Slater AD et al., 2000). In addition to extensive experimental studies, increased lipid peroxidation has been reported in a wide variety of clinical and toxicological conditions (Yagi K , 1994).

Recent hypothesis pointed out the possible role of oxidative stress as a key player in the pathogenesis of insulin resistance, beta-cell dysfunction, and hypertension (Ceriello A and Motz E, 2004). Several studies have shown correlation between hyperinsulinemia and free radical production in human fat cells and rats (Krieger-Brauer HI and Kather H, 1992, DeFronzo RA, Ferrannini E, 1991and Habib MP, 1994).

The aim of the present study is to demonstrate the association between oxidative stress and insulin resistance in non diabetic hypertension patients.

MATERIALS AND METHODS

The subjects were selected from those who visited for a checkup and treatment at Narayana Medical College and Superspeciality Hospital, Nellore, Andhra Pradesh, India. Two hundred and three freshly diagnosed essential hypertension subjects of age between 35-60y (SBP ≥140mmHg and/ or DBP ≥ 90mmHg) were included in this study. Two hundred and ten normotensive(SBP≤ 120mmHg and/ or DBP≤ 80mmHg) healthy, age(35-60 y), sex matched subjects were selected as controls (n=210) only patients having mild to moderate hypertension were included in this study. Patient suffering from severe essential hypertension, secondary hypertension, diabetes mellitus, known cardiac abnormalities, renal, liver, nutritional disorders and pregnant women were excluded from this study. Institutional ethical committee of this medical college have approved the study and informed consent obtained from the patients.

Demographic data

Study subjects age, BMI, waist /hip ratio were recorded. Blood pressure was measured using mercury sphygmomanometer with the patient in the sitting position, legs and crossed. After 5 min of rest in the sitting position, BP was measured on both arms and the higher of the two was taken in to consideration with the systolic and diastolic pressure were in different categories, the higher of the two was used in the classification. They were classified as normotensive and hypertensive as per the recommendation of the JNC7 report.

Biochemical analysis

Fasting venous blood was collected immediately after enrollment in tubes containing EDTA. Blood samples were centrifuged at 2000×g for 10 min. serum samples were stored in a freezer at -20^{0}C for futher biochemical analysis. Samples were analyzed for fasting Glucose, Serum Creatinine, Total cholesterol, HDL- cholesterol, LDL- cholesterol, Triglycerol, analyzed by using Humaster 300(GmBh) Autoanalyser.

Analysis of serum Insulin

Serum Insulin levels was determined by using Chemiluminescence immunoassay (Beckmann coulter, USA). The homeostatic model assessment (HOMA) index was used to estimate insulin resistance, and calculated as fasting serum insulin (µU/mL)× fasting serum glucose (mM /22.5)(Mathews DR et al, 1985).

Determination of MDA

Malondialdehyde (MDA) was measured using the established thiobarbituric acid (TBARS) method (Satoh K, 1978**)**. This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, a colorless product of lipid peroxidation, measured at 532nm. MDA values are calculated using the extinction coefficient of MDA- thiobarbituric acid complex 89.74 × A, at 532nm and expressed as µmol/ml.

Determination of Glutathione Peroxidase (GPX)

Red blood glutathione peroxidase activity was assayed by the method of Rotruck et al (1973). The activity is expressed as U/gm Hb

Determination of Glutathione (GSH)

Red blood reduced glutathione activity was determined by the method of Beutler and Kerley (1963) . The method is based on the development of yellow color due to reaction of 5, 5- dithio(bis) nitrobenzoic acid(DTNB) with compound containing sulphydryl group. The absorbance was read at 412nm, the activity is expressed as mg/dL of RBC.

Statistical analysis

All results are shown as mean ± SD. The statistical significance of between-group differences was evaluated using Student's t-test. Simple correlations were determined by Pearson's correlation analysis. P-value of <0.05 was selected as the point of minimal statistical significance.

RESULTS

Clinical parameters of patients and controls were reported in Table-1 .Body mass Index, Waist/Hip ratio, Systolic Blood pressure (SBP), Diastolic Blood Pressure (DBP) shows statistically significant between patients and controls (p=0.0001). Whereas age and waist hip ratio were not statistically significant.

Table-1 : Demographic Data of the Patients and Control Subjects

Variables	Patients (n=208)	Controls (n=221)
Age	46.65±5.6	45.5±5
BMI (Kg/m2)	27.7±1.71*	22.84±2.2
W/H ratio	1.19±0.04*	0.97±0.09
SBP (mmHg)	147.95±9.8*	117.75±4.1
DBP (mmHg)	92.4±12.5*	78.5±3.5

Abbreviations

BMI= Body mass Index, WC= Waist circumference, HC=Hip circumference, WHR=Waist to Hip ratio, SBP=systolic blood pressure, DBP= diastolic blood pressure.

(p- value <0.05 was considered statistically significant*)

In Biochemical parameters except Blood glucose and serum creatinine all analytes were statistically significant in patients when compare to control subjects (p=0.0001.Fasting insulin, HOMA-IR, MDA, Glutathione peroxidise and reduced glutathione were significantly increased in hypertensive patients when compared to controls (p=0.001) as shown in the table-2.

TABLE-2: Biochemical Data of Patients and Control Subjects

Variables	Patients (n=208)	Controls (n=221)
Fasting blood glucose (mg/dl)	91.03±11.1	83.38±7.88
Serum Creatinine (mg/dl)	1.01±0.17	0.91±0.18
Total Cholesterol (mg/dl)	201.8±22.8*	167.68±37
HDL-Cholesterol (mg/dl)	40.87±7.4*	45.45±3.2
LDL-Cholesterol (mg/dl)	127.8±25.3*	102.63±12.9
Triglycerides (mg/dl)	160.6±25*	136.2±25.3
Fasting insulin (µ IU/ml)	24.41±15*	5.5±1.4
HOMA-IR (U)	4.5±2.5*	2.1±0.4
MDA(µmol/L)	7.6±2.5*	2.5±0.8
Glutathione peroxidise (U/g Hb)	44.5±9.2*	47.9±9.5
Reduced glutathione (µ mol/g Hb)	3.9±0.2*	5.6±0.8

Abbreviations:

HDL= High density lipoprotein, LDL= Low density lipoprotein, MDA= Malondialdehyde, HOMA-IR= Homeostatic Model Assessment – Insulin Resistance.

(p -value <0.05 was considered statistically significant*)

Univariate analysis showed that HOMA-IR were significantly correlated with MDA (r = 0.16, p = 0.001), further, HOMA-IR were found to have a significant negative correlation with glutathione peroxidase and (r=- 0.065, p = 0.001) table-3.

TABLE 3-Significant Correlation in the Patients Group

Variables	r= Value	P= value
HOMA-IR with-MDA	0.161	0.0001
HOMA-IR with-Gpx	-0.065	0.0001

Gpx:Glutathione peroxidise

DISCUSSION & CONCLUSION

Oxidative stress has been considered the major mechanism responsible for endothelial dysfunction in human hypertension , endothelial dysfunction being the early event in the pathogenesis of atherosclerosis (Williams IL et al., 2002 and Perticone F et al., 2000). Endothelial dysfunction may occur by reduced bioavailability of nitric oxide, and this mainly depends on the balance between nitric oxide production and its reaction with ROS. Several studies in human and rats shown correlation between hyperinsulinemia and free radical production (Krieger-Brauer HI and Kather H 1992, DeFronzo RA and Ferrannini E 1991, Habib MP et al., 1994). In the present study, there was a significant correlation between Insulin resistance and lipidperoxidation. This suggests that insulin resistance may play a role in the pathogenesis of oxidative stress. It was previously reported that insulin exerts a potent antiinflammatory effect and that it reduces ROS generation by mononuclear cells in hypertensive subjects (Dandona P et al, 2001). Shamir et al reported insulin-mediated reduction of oxidative stress in apolipoprotein E-deficient mice. These observations suggest that insulin may have a protective role against increased oxidative stress

On the other hand, oxidative stress can cause insulin resistance by increasing the serine phosphorylation of insulin receptor and IRS-1.Glutathione peroxidase being the first line of defence against oxidative stress is known to deplete the hydrogen peroxide needed for insulin signalling. Present study shows negative correlation between glutathione peroxidase and HOMA-IR. This study concludes the association between Insulin resistance and oxidative stress.

REFRENCES

1) Berry C, Brosnan MJ, Fennell J, Hamilton CA, Dominiczak AF. Oxidative stress and vascular damage in hypertension. Curr Opin Nephrol Hypertens 2001;10:247e255.
2) Beutler E, Duron O & Kelly BM. Improved method for the determination of blood glutathione, J.lab.clin.med.61:882- 8, 1963.
3) Ceriello A, Motz E: Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes and cardiovascular disease? The “Common Soil” hypothesis revisited. Arterioscler Thromb Vasc Biol 24: 816–823, 2004.
4) Deepa R, Shanthirani CS, Pradeepa R, Mohan V. Is the 'rule of halves' in hypertension still valid?--Evidence from the Chennai Urban Population Study. J Assoc Physicians India 2003; 51 : 153-7.
5) Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S 2001 Insulin inhibits intranuclear nuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 86:3257–3265.
6) DeFronzo RA, Ferrannini E 1991 Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194.

7) Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest 1982; 47: 412- 426.
8) Gupta R. Meta-analysis of prevalence of hypertension in India. Indian Heart J 1997; 49 : 43-8.
9) Haffner SM, Miettinen H, Gaskill SP, Stern MP: Metabolic precursors of hypertension: the San Antonio heart study. Arch Intern Med 1996; **156**: 1994–2000.
10) Habib MP, Dickerson FD, Mooradian AD 1994 Effect of diabetes, insulin and glucose load on lipid peroxidation in the rat. Metabolism 43:1442–1445.
11) Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr 1996;16:33e50.
12) Kaplan NM: The deadly quartet: upper-body obesity, glucose intolerance, hypertriglyceridemia and hypertension. Arch Intern Med 1989; **149**: 1514–1520.
13) Krieger-Brauer HI, Kather H 1992 Human fat cells possess a plasma membrane-bound H2O2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. J Clin Invest 89:1006–1013 .
14) Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 28: 412-419.
15) Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, Mattioli PL 2001 Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. Diabetes 50:159–165.
16) Redon J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Saez GT. Antioxidant activites and oxidative stress byproducts in human hypertension. Hypertension 2003; 41:1096e1101.
17) Roturck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG & Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase science 179: 588- 90, 1973.
18) Reaven GM: Role of insulin resistance in human disease.*Diabetes* 1988; **37**: 1595–1607.
19) Shamir R, Shehadeh N, Rosenblat M, Eshach-Adiv O, Coleman R, Kaplan M, Hamoud S, Lischinsky S, Hayek T 2003 Oral insulin supplementation attenuates atherosclerosis progression in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 23:104–110.
20) Satoh K. serum lipid peroxide in cardiovascular disorder determined by a new colorimetric method. Clin Chem Acta 1978; 90; 37- 43.
21) Slater AD, Bolton CS, Bailey AJ. The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 2000; 43: 550- 557.
22) Sowers JR: Insulin resistance and hypertension. *Am J Physiol Heart Circ Physiol* 286: H1597–H1602, 2004.
23) Staessen J, Fagard R, Amery A: The relationship between body weight and blood pressure. J Hum Hypertens 1998; 2: 207–217.
24) Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension. What is the clinical significance? Hypertension 2004;44:248e252.
25) Williams IL, Wheatcroft SB, Shah AM, Kearney MT 2002 Obesity, atherosclerosis and the vascular endothelium: mechanisms of reduced nitric oxide bioavailability in obese humans. Int J Obes Relat Metab Disord 26:754–764.
26) Yagi K. Lipid peroxides and related radicals in clinical medicine. Adv Exp Med Biol 1994; 366:1e15.

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