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OXIDATIVE STRESS IN METABOLIC SYNDROME

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ABSTRACT

As antioxidants play a protective role in the pathophysiology of diabetes and cardiovascular diseases, understanding the physiological status of antioxidant concentration among people at high risk for developing these conditions, such as Metabolic Syndrome, is of interest. In present study out of 187 first degree non-diabetic relatives and 192 non-diabetic spouses, 33.1 % and 19.7 % were found to have metabolic syndrome respectively. Subjects with metabolic syndrome ($\geq$ 3 risk factors) had poor antioxidants status as reflected by significantly low levels of vitamin A, C & E and significantly increased ($p < 0.01$) oxidative stress as compared to those without metabolic syndrome. At the same time serum insulin levels and insulin resistance were found to be significantly high ($p < 0.001$) in metabolic syndrome. A strong positive correlation ($r=0.946$; $p<0.001$) between oxidative stress and insulin resistance was observed in metabolic syndrome. Low levels of antioxidants and increased oxidative stress with insulin resistance in metabolic syndrome suggests that besides therapeutic life style changes (TLC) as suggested in ATP III guidelines inclusion of antioxidant vitamins, fruits and vegetable could be beneficial to ward off the consequences of metabolic syndrome.

KEY WORDS

Diabetes, NIDDM, CAD, Metabolic syndrome, Insulin Resistance, Antioxidants, Oxidative Stress

INTRODUCTION

Oxidative stress results due to disturbed equilibrium between pro oxidants and antioxidants and play a role in pathophysiology of Diabetes and Cardiovascular diseases (1, 2, 3). Consequently, the question, whether antioxidants could have a beneficial effect on reducing the risk of these conditions, has been intensively investigated, but the results remain inconclusive (4, 5). If antioxidants play a protective role in the pathophysiology of diabetes and cardiovascular disease, understanding the physiological status of antioxidants among people at high risk for developing these conditions is of interest, however, little is known about this. People with metabolic syndrome are at high risk for developing diabetes and cardiovascular disease (6, 7, 8). The metabolic syndrome is conceptualized as a constellation of metabolic and anthropometric abnormalities (9, 10), which include excess weight, hyperglycemia, hypertension, low concentration of HDL cholesterol and hypertriglyceridemia. In addition, various other abnormalities of uric acid, inflammation, hemostasis and fibrinolysis are often considered part of this syndrome. Diabetes particularly NIDDM as known results from the interaction of genetic and environmental factors. The disease has long been recognized as showing familial aggregation (11, 12). In view of this present study was conducted to examine the antioxidants status and oxidative stress in non-diabetic subjects with and without genetic predisposition. Further correlation between insulin resistance and oxidative stress was also examined to explore the association between the two.

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MATERIALS AND METHODS

Present study was based on 544 subjects from Urban Indian families with either diabetic father or mother. The families comprised of 94 NIDDM subjects ranging in age from 45 to 80 years and above (mean 62.8 ±12.26, median 62 year) and their 258 first-degree relatives, which included off springs and 192 spouses of NIDDM subjects and their first-degree relatives (without family history of diabetes) in the age group of 27 - 71 years (mean 47.6 ± 14.23, median 48 year). Out of 258 first-degree relatives, 71 were diabetic in the age range 42 – 57 years (mean 49.2 ± 6.16, median 48 year) and 187 were non-diabetic ranging in age from 18 – 52 years (mean 37.6 ± 10.24, median 40 year). Care was taken to exclude subjects suffering from any other pathological conditions that could affect oxidative stress and metabolic syndrome. Subjects

with strong smoking and alcoholic habits were also excluded. Each subject was assessed for Metabolic Syndrome according to Adult treatment Panel III (ATP III) criteria (10), a participant was considered to have the metabolic syndrome if he or she had three or more of the following criteria: 1) abdominal obesity: waist circumference (WC) > 102 cm in men and > 88 cm in women; 2) hypertriglyceridemia (TG): ≥150 mg/dl (1.695 mmol/l); 3) low levels of HDL cholesterol: < 40 mg/dl (1.036 mmol/l) in men and < 50 mg/dl (1.295 mmol/l) in women; 4) high blood pressure (HT): ≥130/ 85 mmHg; 5) high fasting glucose: ≥110mg/dl (≥6.1 mmol/l). The waist circumference was measured at the high point of the iliac crest at minimal respiration to the nearest 0.1 cm. Three readings of systolic and diastolic blood pressure were obtained from participants and the average of the last two measurements was used. The current use of antihypertensive medication was also considered as an indication of high blood pressure. Blood samples of the subjects were collected from anticubital vein after an overnight fast of 10-14 hours. The samples were analyzed for Fasting Blood Sugar, Triglycerides (TG) and HDL cholesterol (HDLC) on Merck Selectra auto analyzer using appropriate kits. Antioxidants status, oxidative stress and insulin resistance were evaluated in non-diabetic subjects with and without genetic predisposition. Vitamin A was measured after ultra violet irradiation subsequent to its extraction in n heptane (13), Vitamin C was estimated using dinitrophenyl hydrazine (14) and Vitamin E by spectrophotometry using bathophenanthroline (15). Lipid peroxide level in serum was measured by thiobarbituric acid assay and results were expressed as nmol of malondialdehyde (MDA) formed (16). Insulin was estimated by ELISA and Insulin resistance (IR) was calculated by Homeostasis model assessment method and expressed as HOMA-IR (17).

STATISTICAL ANALYSIS

Statistical analysis for the comparison of data was done using 'z' test and correlation coefficient (r) was calculated to establish correlation between insulin resistance and oxidative stress.

RESULTS AND DISCUSSION

The present study was conducted on 94 diabetic families consisting of 94 diabetic subjects, their 258 first-degree relatives and 194 spouses of either sex. The first-degree relatives were further divided depending upon those who had already developed diabetes and remaining without diabetes. All the participants were assessed for metabolic syndrome according to ATP III guidelines of NCEP expert panel 2001 (10). The purpose of conducting this study in diabetic families was with definite aim to examine the prevalence of metabolic syndrome, insulin resistance and oxidative stress in those with and without genetic predisposition. Diabetic subjects have greater probability of developing CVD and there are reports that in adult patients with diabetes, the risk of CAD is 3 to 5 folds greater than in non-diabetics despite controlling for other known risk factors (10, 18). Metabolic syndrome, which is considered as CHD equivalent in ATP III guidelines has almost 20% chances of developing CHD in 10 years and various components of metabolic syndrome-obesity, HT, hyperglycemia and dyslipidemia are found to be associated with insulin resistance. On one hand insulin resistance is a proven factor for CVD and on the other hand is oxidative stress which is involved in

Table 1. Prevalence of risk factors as per ATP III criteria

Subjects	WC > 102 cm in men & 88cm in women	HT ≥ 130/85 mmHg	TG ≥ 150 mg/dl	<HDL < 40 mg/dl in men & <50 mg/ dl in women	Hyperglycemia ≥ 110 mg/dl
I. NIDDM (n=94)	53 (56)	44 (47)	61 (64)	58 (62)	88 (94)
II. 1st Degree Relative					
i. Diabetic (n=71)	47 (66)	42 (59)	55 (77)	51 (72)	61 (85)
ii. Non Diabetic (n=187)	99 (53)	65 (35)	67 (36)	69 (37)	54 (29)
III. Non Diabetic Spouses (n=192)	69 (36)	61 (32)	56 (29)	38 (20)	40 (21)

WC- Waist circumference, HT- Hypertension, TG- Triglycerides, HDL- High Density Lipoprotein Cholesterol
Values in parenthesis are percentage

Table 2. Prevalence of metabolic syndrome as per ATP III criteria

	≥ 3 risk factors Metabolic syndrome	2 risk factors	0-1 risk factors
I. NIDDM (n=94)	57 (60.6%)	22 (23.4%)	15 (15.9%)
II. Ist Degree Relatives (n=258)	109 (42.2%)	58 (22.4%)	91 (35.2%)
i. Diabetic (n=71)	47 (66.1%)	13 (18.3%)	11 (15.4%)
ii. Non Diabetic (n=187)	62 (33.1%)	45 (24.0%)	80 (42.7%)
III. Non Diabetic Spouses (n=192)	38 (19.7%)	42 (21.8%)	112 (58.3%)

Table 3. Oxidative stress, antioxidants level & insulin resistance in relation to risk factors in first-degree non-diabetic relatives and spouses

	≥ 3 risk factors Metabolic syndrome	2 risk factors	0 or 1 risk factors
1st DEGREE NON-DIABETIC RELATIVES (n=187)			
VITAMIN-A (µg/dl)	17.08 ± 3.65 11.06 – 26.81	27.46± 5.43*** 17.83 – 36.16	28.93 ± 5.62 15.82 – 49.11
VITAMIN- C (mg/l)	5.09 ± 1.57 3.66 – 9.28	10.26 ± 3.71*** 4.30 – 16.81	10.79 ± 2.77 4.45 – 18.21
VITAMIN- E (mg/l)	7.17 ± 0.68 6.12 – 10.23	9.54 ± 1.17*** 7.06 – 10.91	9.79 ± 1.09 7.82 – 12.23
MDA (nmol/L)	260.1 ± 60.2 206.26 – 339.01	192.62 ± 42.6 ** 141.32 – 232.11	136.23 ± 30.6* 91.38 – 179.21
INSULIN (µU/ml)	26.34 ± 8.62 11.22 – 44.01	14.29 ± 8.47 *** 6.91 – 35.06	11.74 ± 7.26* 6.16 – 34.12
HOMA-IR	7.16 ± 2.9 3.42 – 13.56	4.48 ± 2.23 *** 2.56 – 8.67	2.59 ± 2.64* 1.34 – 8.56
NON DIABETIC SPOUSES (n=192)			
VITAMIN-A (µg/dl)	15.90 ± 3.97 10.01 – 27.26	18.68 ± 3.14** 14.92 – 28.61	19.30 ± 3.84 13.82 – 30.86
VITAMIN- C (mg/l)	4.81 ± 1.29 3.62 – 8.26	6.51 ± 1.99** 4.01 – 10.91	6.99 ± 2.77 3.23 – 17.11
VITAMIN- E (mg/l)	7.94 ± 0.58 7.01 – 9.11	8.48 ± 0.53* 8.06 – 9.73	8.65 ± 0.69 7.01 – 10.90
MDA (nmol/L)	186.24 ± 35.2 142.06 – 230.51	154.46 ± 32.4 ** 107.26 – 180.19	103.86 ± 26.2 ** 68.14 – 140.23
INSULIN (µU/ml)	23.52 ± 11.63 8.89 – 40.03	14.62 ± 7.00 *** 6.61 – 33.24	11.61 ± 8.05 * 6.63 – 37.06
HOMA-IR	6.66 ± 3.45 3.11 – 12.16	4.23 ± 2.88 ** 2.57 – 7.83	2.41 ± 2.09 * 1.13 – 6.91

Values are mean ± SD and range.
Statistical comparison was done between ≥ 3 and 2 risk factors; 2 and 0-1 risk factors and ≥ 3 risk factors in first-degree non-diabetics and spouses.
*** p< 0.001; ** p< 0.01; * p< 0.05 and rest not significant with P> 0.05

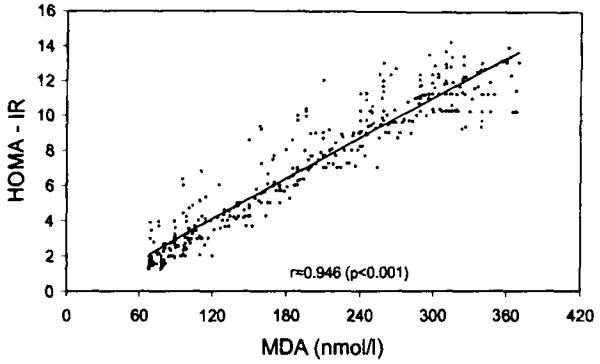

Figure 1. Correlation between Insulin resistance (HOMA-IR) and oxidative stress (MDA)

atherosclerotic cardiovascular diseases (3, 19), hence this study examined insulin resistance and oxidative stress in relation to metabolic syndrome, especially in first-degree non-diabetic subjects and spouses.

The prevalence of risk factors in diabetics, their first-degree relative and spouses are given in Table 1. Besides, hyperglycemia, diabetic and their first-degree diabetic relatives showed very high prevalence of hypertension, elevated TG, low HDL cholesterol and raised waist circumference. The trend of these risk factors in order of decreasing prevalence was practically same in diabetic and their first-degree diabetic relatives: Hyperglycemia > TG> HDL> WC> HT. While in non-diabetics, the trend was – WC> HDL> TG> HT> Hyperglycemia and WC> HT> TG> Hyperglycemia> HDL in first-degree non-diabetic relatives and spouses respectively. The prevalence of individual risk factors was apparently less in non-diabetics as compared to diabetic subjects. However in first degree non-diabetic relatives and spouses central obesity remains an important factor for developing metabolic syndrome because of it's prevalence being 53% and 36% respectively (Table 1). Thirty three percent of non-diabetic first-degree relatives were found to have $\geq$ 3 risk factors hence were having metabolic syndrome, while 24% subjects had 2 risk factor and 43% subjects had 0-1 risk factors. However in spouses the prevalence of metabolic syndrome was 20% (Table 2). Interestingly only 25% of first-degree non-diabetic relatives and 22% of spouses were free from any risk factors while 57% of first-degree non-diabetic relatives and 42% spouses had risk of more than 10% in 10 years (2 risk factor - < 20% CHD risk in 10 years; > 3 risk factors - > 20% CHD risk in 10 years) irrespective of genetic background.

Individually each risk factor is also known to be associated with IR and when clustered together further aggravates resistance to insulin leading to CAD and NIDDM. As metabolic syndrome is associated with insulin resistance and compensatory hyperinsulinemia, subjects with $\geq$ 3 risk factors in both the groups i.e. first-degree non diabetic relatives and spouses had significantly higher ($p < 0.001$) levels of serum insulin as well as insulin resistance in comparison to those with < 3 risk factors. Simultaneously subjects with metabolic syndrome had significantly low concentration of nutrient antioxidant vitamins – A, C & E with significantly increased oxidative stress measured as MDA (Table 3). The elevated oxidative stress was further examined for its association with insulin resistance expressed as HOMA-IR. The value of correlation coefficient (r) being 0.946 ($p<0.001$) suggests a positive association between the two i.e. as insulin resistance increases there is simultaneous increase in oxidative stress and vice versa (Figure 1). Since oxidative stress is due to the imbalance between protective antioxidants and damaging free radicals (20), subjects with metabolic syndrome were found to have significantly low levels of antioxidant vitamins as compared to other groups (Table 3). The decreased concentration of antioxidant vitamins in metabolic syndrome may be attributed to lower intake of fruits & vegetables rich in antioxidants or increased use of antioxidants to counteract oxidative stress (21). However, no significant difference in antioxidant status, oxidative stress and insulin resistance could be made out in first-degree non-diabetic subjects and spouses with metabolic syndrome indicating that CHD risk due to prevailing contributory risk factor remains the same in those with or without genetic predisposition.

Low levels of antioxidants and increased oxidative stress with insulin resistance in metabolic syndrome suggests that besides therapeutic life style changes (TLC) which includes smoking cessation, weight control, taking reasonable exercise, proper diet etc. as suggested in ATP III guidelines (10) inclusion of antioxidant vitamins, fruits and vegetables could be beneficial to ward off the consequences of metabolic syndrome by reducing free radical formation and oxidative stress associated with this syndrome.

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