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118

Original Research Article

A study of levels of oxidative protein modification markers AOPP and IMA with risk factors of metabolic syndrome

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Abstract

Objective: the study was designed to look in if there was any role for protein oxidation markers [like Advanced Oxidation Protein Products (AOPP) and Ischemia Modified Albumin (IMA)] in the pathogenesis of metabolic syndrome (MetS) and effect of number of risk factors of MetS on the level of these protein oxidation markers.

Method:
A total of 165 (n) patients were included in the study. Patients were divided into four groups based on presence or absence of 5 cardiovascular risk factors specified in revised NCEP (rNCEP) definition for MetS. Comparisons of the examined groups were statistically analysed by the application of Student's *t* test. Analysis of variance (ANOVA) followed by multiple comparison post hoc Fisher test were used for the analysis of differences between the groups with different numbers of risk factors of MetS.

Results: Both AOPP and IMA values significantly correlated with waist circumference, glucose, HDL-Ch and TG (p value < 0.05). AOPP and IMA values between control group and the rest of the groups showed significant difference (p value <0.05). Systolic BP however, did not show significant association with AOPP as well as with IMA (p values 0.139 and 0.083 respectively).

Conclusion: On the basis of the findings from the present study, it may be concluded that oxidative protein modification markers, both AOPP and IMA are increased in metabolic syndrome. In addition, increased AOPP and IMA even prior to the development of MetS, may be used to prevent the development of MetS. However, it requires confirmation by further studies.

Keywords: Oxidative protein modification, Advanced Oxidative Protein Products, Ischemia Modified Albumin, metabolic syndrome

1.Introduction

The metabolic syndrome (MetS) is a complex metabolic disease affecting a little more than one-third of adults in the united states and has become a leading health concern due to its link to cardiovascular disease (CVD)[1]. Asian Indians have been considered to be a "high-risk population" for both MetS and CVD as the prevalence of metabolic syndrome is varied by race and ethnicity[2]. In MetS, risk of all cause mortality is increased by 1.5 folds and risk of mortality due to CVD is increased by two folds[3].

MetS is a cluster of risk factors that consists of risk correlates of metabolic origin, pro-inflammatory and prothrombotic state. MetS

described for the first time by Reaven in 1988, has a number of definitions published by various organizations including the International Diabetes Federation, National Cholesterol Education Program (NCEP), and the World Health Organization, among others. Of these, definition of NCEP's Adult Treatment Panel is most widely used as it employs risk factors which are easily measurable[4]. According to revised NCEP (rNCEP) definition, presence of 3 or more of the 5 specified cardiovascular risk factors namely increased waist circumference, elevated Triglycerides (TG) or on treatment for dyslipidemia, diminished high density lipoprotein (HDL), systemic hypertension or on treatment for hypertension and elevated fasting glucose or on treatment for hyperglycemia constitutes MetS[5].

Mechanism of development of MetS is not fully understood, but hypertension, increased glucose level and dyslipidemia have been found to play role in the pathogenesis of MetS[6]. In addition, oxidative stress caused by increased free radicals constitutes the important component of pathogenesis of MetS[3].

Reactive Oxygen Species (ROS) formed normally as a natural by-product of the metabolism of oxygen play physiologically important role in signal transduction, but in excess either by dis-regulation of signal transduction or by oxidative modification of cellular macromolecules like lipids, proteins, DNA, RNA, and carbohydrates contribute to pathogenesis of diseases.[7]

Ischemia Modified Albumin (IMA) is formed by the modification of Albumin during ischemia as a result of increased generation of ROS and acidosis. Structural modification of N-terminal amino acid residues (Asp-Ala-His-Lys) of Albumin by ROS reduces its ability to bind to transition metal ions like Co^{2+}, Ni^{2+}, Cu^{2+} etc. Reduced ability of Albumin to bind to cobalt is used to measure IMA in biological fluids.[8]

Significantly increased protein modification by oxidative stress is observed in various pathological conditions including Diabetes Mellitus, obesity, Hypertension and Dyslipidemia.[9] Increased AOPP levels are also observed in various diseases including diabetes, obesity, atherosclerosis[10][12]. IMA is a marker of myocardial ischemia, which is increased in myocardial infarction and ischemic heart disease. In addition to ischemia, elevated levels of IMA are observed in exercise, diabetes, hypertension and atherosclerosis[9][13]. Considering the above said, the present study was designed to look into the levels of AOPP and IMA in MetS, followed by correlating

the risk factors of MetS with AOPP and IMA. We had also looked into the effect of increase in number of risk factors of MetS on AOPP and IMA levels as a part of this study.

2. Materials and methods

This hospital based study was done at Father Muller Medical College Hospital Mangalore, which is located in Karnataka state of India. The study duration was from June 2011 to January 2013. Patients visiting the outpatient department for routine health check up were the subjects of this study. The age group of the patients was 21 to 68 years, and both males and females were included in the study.

2.1 Patient data collection:

This study was conducted by including the patients visiting Father Muller Medical College Hospital; Mangalore, Karnataka, India. Based on the number of patients visiting the hospital and fitting in for the study, the study was designed and the protocol stated. The patients were informed about the study and their consent was taken before they were then included in the study. The study design and protocol has been approved by the institutional ethical committee.

2.2 Sample size and categorization:

A total of 165 (n) patients were included in the study. Patients were divided into four groups based on presence or absence of 5 cardiovascular risk factors specified in revised NCEP (rNCEP) definition for MetS. The first group or control group contained patients without any of the 5 cardiovascular risk factors namely increased waist circumference, elevated Triglycerides (TG) or on treatment for dyslipidemia, diminished high density lipoprotein (HDL), systemic hypertension or on treatment for hypertension and elevated fasting glucose or on treatment for hyperglycemia. The second group had one, the third had two and the fourth or MetS group contained three or more risk factors.

2.2.1 Inclusion criteria

After an informed consent blood samples were collected from apparently healthy normal subjects who visited the hospital for routine health check-up. Triglycerides, HDL Cholesterol, glucose, AOPP, IMA were estimated in the fasting blood samples collected from these patients. BP, height and weight were recorded; BMI was calculated using height and weight.

2.3 Statistical analysis

Central tendency and variation of data are expressed as mean and standard deviation respectively. The statistical analysis was performed using SPSS (Version 16). Shapiro–Wilk test was

used to assess the normality of distribution of variables. Comparisons of the examined groups were performed by Student's *t* test. Analysis of variance (ANOVA) followed by multiple comparison post hoc Fisher test were used for the analysis of differences between the groups with different numbers of risk factors of MetS. Relationship of AOPP and IMA was tested by using Pearson's correlation. Multivariate analysis was performed to identify independent factors for the presence of MetS. A value of *p* less than 0.05 was considered as statistically significant.

3. Results

In this study an attempt is made to relate the risk factors of CVD with AOPP and IMA the marker of protein oxidation and marker of ischemia respectively. The participants in our study were grouped into normal (without risk factor), with one, with two and with three or more (metabolic syndrome) groups based on the presence of 5 specified cardiovascular risk factors namely increased waist circumference, elevated Triglycerides (TG) or on treatment for dyslipidemia, diminished high density lipoprotein (HDL), systemic hypertension or on treatment for hypertension and elevated fasting glucose or on treatment for hyperglycemia constitutes MetS.[5] All the risk factors of cardiovascular diseases used for defining MetS in the study participants (given in Table 1) varied between patients with MetS and the control group. The BMI and waist circumference, systolic and diastolic blood pressure, fasting glucose as well as triglycerides were significantly higher and HDL cholesterol was lower in MetS patients than the control group. Like MetS group, the second and third groups also had BP and all biochemical parameters increased except HDL which is decreased.

Table 1: Levels of risk factors in different groups

|	Control group without any risk factors (N = 40)	Group with one risk factor (N = 40)	Group with two risk factors (N = 41)	Metabolic syndrome (N =44)
	Mean±SD	Mean ±SD	Mean ±SD	Mean ±SD
BMI (Kg/sq. m.)	23.25±2.35	23.57±3.95	24.21±2.77	26.78±3.36
Waist circumference(cm)	83.02±6.09	84.51±8.62	85.62±6.92	89.44±8.12
Glucose (mg/dl)	91.77±7.42	98.28±11.73	103.27±35.28	105.2±18.11
HDL (mg/dl)	51.6±7.94	47.6±8.02	42.71±10.61	42.59±9.98
Triglycerides (mg/dl)	85.0±28.69	108.85±33.59	136.46±65.16	166.88±78.58
AOPP (Chl. T eq.)	23.16±5.12	30.92±6.95	36.03±11.84	40.99±16.77
IMA (ABSU)	0.281±0.038	0.291±0.047	0.300±0.058	0.308±0.061

[SD – Standard Deviation]

Protein modification product AOPP and marker of ischemia IMA levels also increased progressively and highest values were observed in metabolic syndrome (Table 1).

Figure 1: Comparison of AOPP levels between the groups
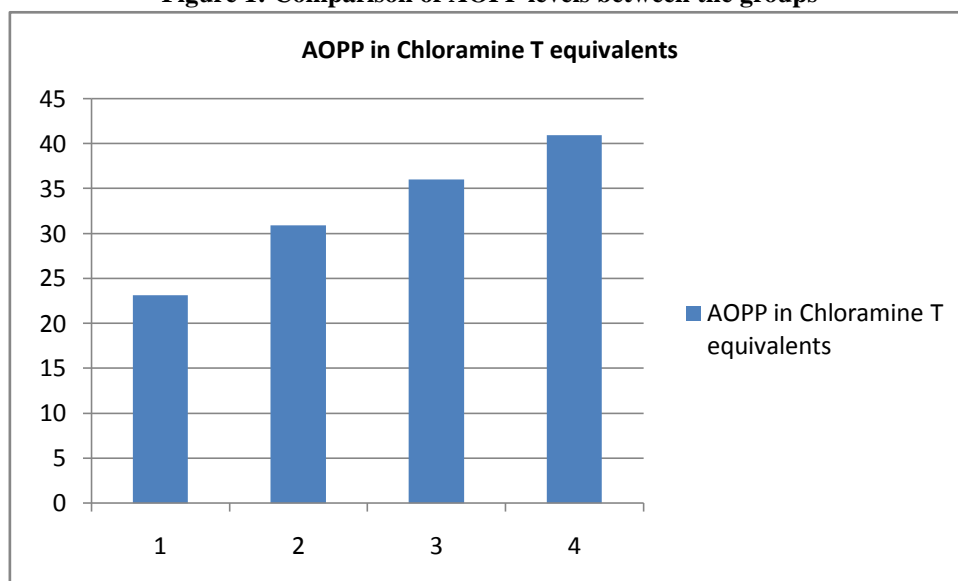
(Group 1- patients without any risk factors, group 2- patients with one risk factor, group 3- patients with 2 risk factors & group 4- patients with 3 or more risk factors).

Figure2: Comparison of IMA levels between the groups with differing number of risk factors
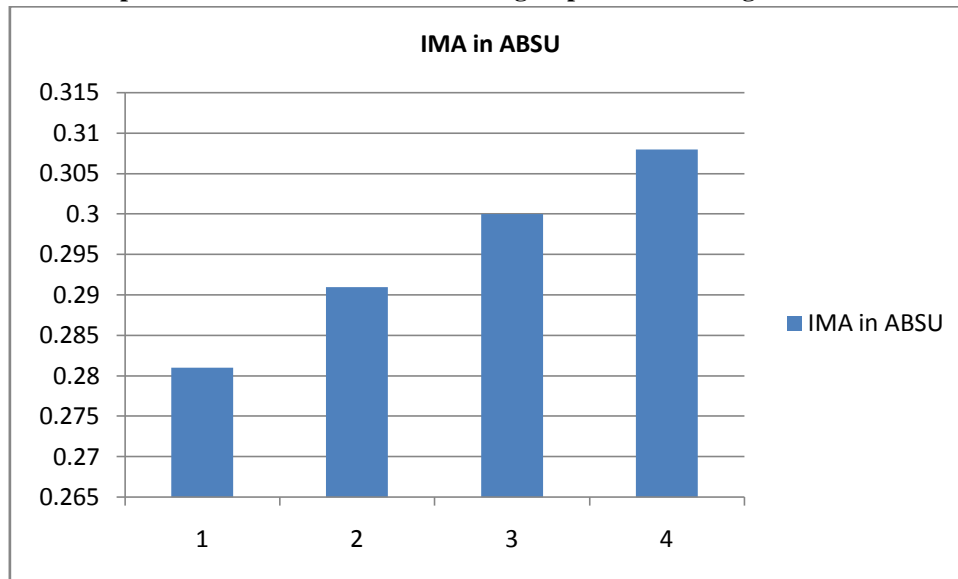
(Group 1- patients without any risk factors, group 2- patients with one risk factor, group 3- patients with 2 risk factors & group 4- patients with 3 or more risk factors).

Table 2: Correlation of AOPP and IMA with Risk factors

Risk factors	AOPP	IMA
	P value	P value
Waist Circumference	0.009**	0.021*
Systolic blood pressure	0.139	0.083
Glucose	0.009**	0.038*
HDL Cholesterol	0.002**	0.014*
Triglycerides	0.000***	0.037*

[* = p value < 0.05, ** = p value < 0.01, *** = p value<0.001]

We also correlated selected MetS risk factors with AOPP and IMA to see if there is association between protein oxidation by oxidative stress and the risk factors. Both AOPP and IMA were found to be significantly associated with waist circumference, glucose, HDL-Ch and TG. The p values of correlation of AOPP and IMA with the risk factors are given in the table no 2. Systolic BP however, did not show significant association with AOPP as well as IMA.

Table 3: Comparison of AOPP and IMA levels between different groups

Groups	Compared groups	AOPP	IMA
Control group (without any risk factor)(N = 40)	Group with one risk factor (N = 40)	0.015*	0.044*
	Group with two risk factors (N = 41)	0.000***	0.035*
	Metabolic syndrome (N=44)	0.000***	0.018*
Group with one risk factor(N = 40)	Group with two risk factors (N = 41)	0.262	0.366
	Metabolic syndrome (N=44)	0.000***	0.037*
Group with two risk factors(N = 41)	Metabolic syndrome (N=44)	0.273	0.093

[* = p value < 0.05, ** = p value < 0.01, *** = p value<0.001]

In addition, we investigated whether the presence of one or more risk factors had any influence on values of AOPP and IMA and the p values are given in table 3. We compared the values of AOPP and IMA of one group with that of the other groups to investigate the effect of risk factors. One-way analysis of variance showed that there were significant differences between control group (without any risk factor) and the rest of the groups including MetS group. Though both AOPP and IMA showed significant differences between group with one risk factor and metabolic syndrome group, no significant differences were observed between second and third groups. Comparison between third and

metabolic syndrome group however, did not show significant difference in both AOPP and IMA values.

4. Discussion

MetS is a collection disorders associated with obesity, which includes insulin resistance, hypertension and dyslipidemia, each one of which contributes to the risk for CVD. MetS is often characterized by oxidative stress due to imbalance between production of reactive oxygen species (ROS) by activated biochemical pathways and decreased activity of antioxidant defence enzymes. Though oxidative stress is observed in MetS, the role of ROS and the exact mechanism of development of MetS is still not clearly understood. ROS overproduction may be a cause for modification of proteins and their deposition in tissues, organs and vascular wall, as well as induction of insulin resistance promoting the pathogenesis of MetS.[14][15]

In this study, we have focused on AOPP and IMA, the biochemical parameters reflecting modifications of the albumin molecule induced by oxidative stress. Of the two protein modification products, AOPP is a marker of irreversible damage of proteins caused by oxidative stress and IMA is a marker of hypoxia-induced oxidative stress. We found significantly higher levels of both AOPP and IMA in patients with metabolic syndrome in comparison to the control group without any risk factor. Increased levels of oxidative stress induced protein oxidation products confirm that in patients with MetS, oxidative stress causes direct oxidative damage to proteins. Regarding protein modification by oxidative stress in patients with metabolic syndrome our findings are consistent with other investigations[16][17]

The mechanism involved in increased cardiovascular risk in metabolic syndrome is unclear. However, according to Word Health Organization and the European Group for the Study of Insulin Resistance, in metabolic syndrome, main reason for increased risk for CVD is due to insulin resistance. Insulin resistance triggers vasoconstriction, hypertriglyceridemia due to increased VLDL synthesis in liver, low HDL resulting in atherosclerosis[18]. Although main mechanism involved in metabolic alterations observed in MetS is insulin resistance, recent studies show that oxidative stress and chronic low level inflammation play important roles in MetS-related manifestations, including atherosclerosis, endothelial dysfunction and hypertension[19]. Moreover, data suggest that oxidative stress is not merely a consequence but it is an early event in the pathogenesis of chronic

diseases. In our study, both AOPP and IMA were increased progressively in all the groups containing increasing number of risk factors of CVD compared to control group. Subjects with more components of MetS may have a higher oxidative stress level[20]. Therefore, it is suggested that protein modification by oxidative stress is increased with increase in number of risk factors of CVD. However, no significant difference in AOPP and IMA values between patients with one risk factor and patients with two risk factors. This indicated that there is no much difference in magnitude of oxidative stress induced by one or by two risk factors. Of the two protein modification products, AOPP differed between the groups more significantly than IMA indicating that AOPP may be a better oxidative stress marker than IMA.

To investigate the association of risk factors with AOPP and IMA we correlated these risk factors with AOPP and IMA. Both AOPP and IMA showed significant positive correlations with waist circumference (WC), glucose, TG and negative correlation with HDL Cholesterol. These findings are consistent with the study conducted by Yubero-Serrano etal.[20] It has been postulated that increased triglycerides directly takes part in atherogenesis by increasing oxidative stress like in hyperglycemia.[21][21][22] In addition, mild inflammation and visceral cytokine production are also responsible for development of CVD in patients with increased WC. In hypertriglyceridemia as well as in increased WC, increased levels of AOPP and IMA partially account for the increase in the production of superoxide anions.[23] Oxidative stress induced by these anions play a key step in the pathogenesis of CVD by impairing endothelial dysfunction through decreased availability of nitric oxide (NO).[24] Regarding number of risk factors, each of these risk factors except systolic blood pressure increases oxidative stress in patients which in turn may increase protein modification. In our study, Systolic blood pressure did not correlate with either AOPP or IMA. Presence of at least one risk factor other than increased systolic blood pressure increased the levels of both AOPP and IMA significantly. ROS such as super oxide anion or hydrogen peroxide may induce vascular changes by activating redox sensitive signal pathways through oxidative modification of amino acid residues of proteins.[25]

Both AOPP and IMA are increased in metabolic syndrome and are believed to be formed by the action of oxidative stress on proteins. However, studies conducted on correlation between AOPP and

IMA showed mixed results. Study conducted by Demir *et al*. on AOPP and IMA in patients with cardiac syndrome X found positive correlation between AOPP and IMA in these patients. In contrast, Zurawska-Plaksej *et al*. did not find significant correlation between AOPP and IMA.[17] The results of our study did not show significant correlation like the study by Zurawska-Plaksej *et al*. This suggested that mechanism of formation of AOPP and IMA may be different from one another. Direct action of ROS on amino acid residues of proteins results in oxidation of these amino acid residues followed by formation of cross links to form AOPP. On the other hand, IMA is formed from Albumin under conditions of hypoxia. In MetS, it is suggested that observed increase in Albumin modification to IMA is due to decreased tissue perfusion as a result of pro-atherogenic environment. Increased IMA formation may possibly indicate progressive peripheral oxygenation insufficiency caused by vascular dysfunction in MetS patients. Compared to IMA level, in our study, AOPP level is more significantly increased indicating that AOPP is formed directly by the action of ROS on proteins.

Some limitations deserve to be mentioned in this study. First, the number of estimated patients was not too large and therefore we could not study the effect of risk factors in subgroups of MetS with particular numbers of risk factors. In addition, relatively small sample size does not ensure that the lack of significant difference for increased systolic blood pressure assessed really occurs in MetS. Therefore additional studies with more sample size and with different subgroups of MetS are needed to establish the relationship between the number of components of MetS and the degree protein modification.

5. Conclusion

On the basis of the findings from our study, oxidative protein modification markers, both AOPP and IMA are increased in metabolic syndrome. In addition, increased AOPP and IMA even prior to the development of MetS, may be used to prevent the development of MetS. Though pathophysiology of MetS is not fully understood, risk factors of CVD like hypertension, increased glucose level, dyslipidemia and oxidative stress have been found to play a role in the pathogenesis of MetS. In our study, regarding effect of number of risk factors of MetS on the level of these protein oxidation markers, presence of at least one risk factor other than increased systolic blood pressure increased the levels of both AOPP and IMA significantly. Therefore, oxidative stress and

protein oxidation are part of pathogenesis of MetS, markers of protein oxidation like IMA and AOPP can be used to identify people prior to the development of MetS. However, these findings require confirmation by further studies with increased sample size.

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