Research Communication

Association between serum uric acid, high sensitive C-reactive protein and pro-oxidant-antioxidant balance in patients with metabolic syndrome

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Abstract

There is persuasive evidence that oxidative stress and inflammation are features of the metabolic syndrome (MetS). We have investigated the relationship between serum pro-oxidant-antioxidant balance (PAB), serum uric acid, and high sensitive C-reactive protein (hs-CRP) in 7,208 participants from the MASHAD study cohort, who were categorized as having MetS,

Abbreviations: *MetS, Metabolic syndrome; PAB, Pro-oxidant-antioxidant balance; hs-CRP, High sensitive C-reactive protein; IR, Insulin resistance; NAFLD, Non-alcoholic fatty liver disease; ROS, Reactive oxygen species; JNK, Jun NH_2-terminal kinase; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α, Tumor necrosis factor-α; IL-6, Interleukin-6; PDH, Pyruvate dehydrogenase; ACC, Acetyl co-A carboxylase; SOD, Superoxide dismutase; GSH, Glutathione; HO, heme oxygenase; BMI, Body mass index; PAL, Physical activity level; HDL-C, High-density lipoprotein cholesterol; AGEs, Advanced glycation end products assay; AOPP, Advanced oxidative protein products*

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or not, using International Diabetes Foundation (IDF) criteria. Serum hs-CRP was measured by Polyethylene glycol (PEG)-enhanced immunoturbidimetry method using an Alycon analyzer (ABBOTT, Chicago, IL, USA). A colorimetric method was used to determine serum PAB. Serum PAB values were significantly higher in the individuals with MetS compared to those without ($P<0.001$). Furthermore, there was a step-wise increase in mean serum PAB concentrations as the number of components of the MetS increased. The combination of features of MetS had different association with serum PAB and hs-CRP. Multiple linear regression analysis showed that body mass index (BMI, B = 2.04, $P<0.001$), physical activity level (PAL, B = 18.728, $P=0.001$), serum uric acid (B = −1.545, $P=0.003$), and serum C-reactive protein (B = 0.663, $P<0.001$) were associated with serum PAB in individuals with MetS. Multiple logistic regression analysis showed that serum PAB (B = 0.002, $P<0.001$, CI = 1.001–1.003), serum C-reactive protein (B = 0.007, $P<0.015$, CI = 1.001–1.013), and serum uric acid (B = 0.207, $P<0.001$, CI = 1.186–1.277) were all significantly associated with MetS. Serum PAB was strongly associated with serum uric acid and serum hs-CRP. Moreover, serum PAB as well as serum uric acid and serum hs-CRP were independently associated with MetS. Individual features of MetS were also associated with serum hs-CRP and PAB.

Keywords: *pro-oxidant; antioxidant; PAB; metabolic syndrome; hs-CRP; uric acid*

1. Introduction

Metabolic syndrome (MetS), also called syndrome X, is a clustering of cardiovascular risk factors that include abdominal obesity, insulin resistance (IR) [1], non-alcoholic fatty liver disease (NAFLD), dyslipidemia, hypertension, and a pro-inflammatory state [2]. Insulin resistance appears to have an important role in the pathogenesis of MetS, and oxidative stress may be responsible for initiating an inflammatory process and the development of insulin resistance (IR) [3]. Oxidative stress is a state that arises from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses, which detoxify the reactive intermediates. The four major cellular ROS include Mito-O_2^-, .OH, $^1O_2$, and H_2O_2 [4,5].

Reactive oxygen species (ROS) are vital for the normal immune response, and most ROS are produced at a low level during normal aerobic metabolism. An imbalance leads to excess ROS generation and may result in chronic disease such as cardiovascular disease, cancer, Parkinson's disease, and other neurological disorders [6]. Stress-signaling pathways, such as the Jun NH_2-terminal kinase (JNK), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathways may inhibit insulin signaling and insulin-mediated glucose uptake by tissues [7]. The activation of JNK and NF-kB, following the up regulation of various pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) are associated with IR [7–9]. In response to IR, the pancreas may release more insulin, and consequently hyperinsulinemia may result, which is also associated with hypertension, obesity, and dyslipidemia [9]. Insulin stimulates lipogenesis by activating pyruvate dehydrogenase (PDH) and acetyl co-A carboxylase (ACC), moreover increases sodium retention by renal tubules.

Oxidative stress is balanced by the action of enzymatic and non-enzymatic anti-oxidants that include superoxide dismutase (SOD), glutathione (GSH), and heme oxygenase (HO), and vitamin C and α-tocopherol [10]. Individuals with MetS often have elevated levels of pro-oxidants and reduced levels of antioxidants [11]. Most methods that are currently available, measure pro-oxidants and antioxidants individually. The pro-oxidant-antioxidant balance (PAB) assay attempts to provide an integrated measure of antioxidant and prooxidant activities, using a simple, rapid, and inexpensive technique [11]. Previous studies have reported elevated levels of pro-oxidant and reduced protective activity of antioxidants in individuals with MetS [11,12]. This current study was conducted to evaluate serum PAB, as a potentially marker of different categories of MetS assessed in individuals with different combinations of the features used to characterize MetS.

2. Material and Methods

2.1. Subjects and anthropometric measurements

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences and Tabriz University of Medical Science, and written consent was obtained from all participants. Demographic and clinical parameters are shown in Table 1. The criteria used for defining MetS were derived from the International Diabetes Foundation (IDF), and were used to categorize subjects into MetS+ and MetS–. A summary of the criteria were waist circumference >94 cm in men or >80 cm in women along with at least two of the metabolic abnormalities including blood pressure >130/85 mm Hg, fasting blood glucose > 100 mg/dL, fasting triglycerides >150 mg/dL, and high-density lipoprotein cholesterol (HDL-C) <40 mg/dL in men and 50 mg/dL in women. In the final cohort, there were 2,481 individuals with MetS, and 4,727 in the non-MetS group that were further divided into six sub-groups including: healthy controls (n = 1,896) without any feature of MetS, individuals with abnormal waist circumference (n = 687), abnormal waist

TABLE 1 ***Comparison of the demographic, anthropometric, and biochemical parameters for the participants with and without MetS***

	MetS−	*MetS+*
Age (y)	47.19 ± 8.1	49.56 ± 8.1
Sex (n) men	1,985 (43.1%)	766 (29.4%)
Female	2,619 (56.9%)	1,838 (70.6%)
Weight (kg)	69.53 ± 12.45***	75.8 ± 12.66
BMI (kg/m^2)	26.86***	29.76 ± 4.32
Waist circumference (cm)	93.36 ± 11.99***	100.38 ± 10.19
Hip circumference (cm)	102.14 ± 9.2***	106.61 ± 8.91
Uric acid (mg/dL)	4.52 ± 1.38***	4.9 ± 1.41
hs-CRP (mg/dL)#	1.46 (2.24)***	2.04 (3.01)
PAB [35] HK units	64.91 ± 53.56***	71.46 ± 56.87
FBG (mg/dL)	89.08 ± 35.04***	99.5 ± 45.58
Cholesterol (mg/dL)	186.88 ± 37.59***	199.61 ± 40.76
Triglyceride (mg/dL)#	101 (56)***	177 (97)
HDL (mg/dL)	44.95 ± 10.42***	39.12 ± 7.67
LDL (mg/dL)	115.78 ± 33.84***	118.03 ± 38.06
SBP (mm Hg)	117.38 ± 15.73***	129.69 ± 20.75
DBP (mm Hg)	76.67 ± 10.58***	83.65 ± 12.21
PAL	1.62 ± 0.29***	1.55 ± 0.26

Data presented as mean (SD) and median (IQR).
Independent sample t*-test and Mann-Whitney U Test were used in normal and non-normal# distributed data respectively.*
FBG: fasting blood glucose; LDL: Low density lipoprotein; HDL: high density lipoprotein; BMI: body mass index; SBP: systolic blood pressure; DBP: Diastolic blood pressure; hs-CRP: high sensitive C-reactive protein; PAB: pro-oxidant anti-oxidant balance; PAL: physical activity level; MetS: Metabolic Syndrome.
**P < 0.05, **P < 0.01, ***P < 0.001.*

circumference together with a low serum high density lipoprotein (HDL, n = 1,610) or raised fasting serum triglyceride (TG, n = 216) or high blood pressure (n = 170) or fasting hyperglycemia (n = 148) derived from the MASHAD study cohort (Fig. 1).

Height, weight, and waist circumference were measured twice using a standard method. Subjects wore light clothes without shoes, and were measured to the nearest 0.1 Kilogram on a calibrated digital scale in Kilogram (scale SECA 813, Hamburg, Germany) and height was measured in centimeters and the nearest 0.1centimeter using a stadiometer (SECA 217, Hamburg, Germany). The body mass index (BMI) was calculated as weight divided by height squared (m^2). A standard mercury sphygmomanometer was used to assess blood pressure, twice with a 30-minute interval for any participant while seated and rested. The average of two measurements was recorded as the blood pressure. Health related behavior factor, such as levels of physical activity and drugs taken by each participant was obtained by questionnaire.

2.2. Physical activity level

Physical activity level (PAL) of participants was assessed using a standard questionnaire modified from the SHHS/MONICA questionnaire. Extremely inactive subjects were considered as those with a PAL < 1.4, and other categories as follows: sedentary (1.4–1.69), moderately active (1.7–1.99), vigorously active (2.00–2.4) or extremely active > 2.4 based on their PAL score.

2.3. Routine biochemical analysis and high sensitive C-reactive protein

A blood sample was collected after a 14 hours over-night fast, and was used to rule out impaired glucose tolerance, and was also used to assess lipid profile, serum uric acid, serum high sensitive C-reactive protein (hs-CRP), and serum PAB. Aliquots of sera were frozen at −80°C until analysis. Serum lipid, fasting blood glucose, and serum uric acid concentrations were measured enzymatically using commercial kits on a BT-3000 autoanalyzer (Biotechnical, Rome, Italy). Serum hs-CRP was measured by a PEG-enhanced immunoturbidimetry method using an Alycon analyzer (ABBOTT, Chicago, IL, USA).

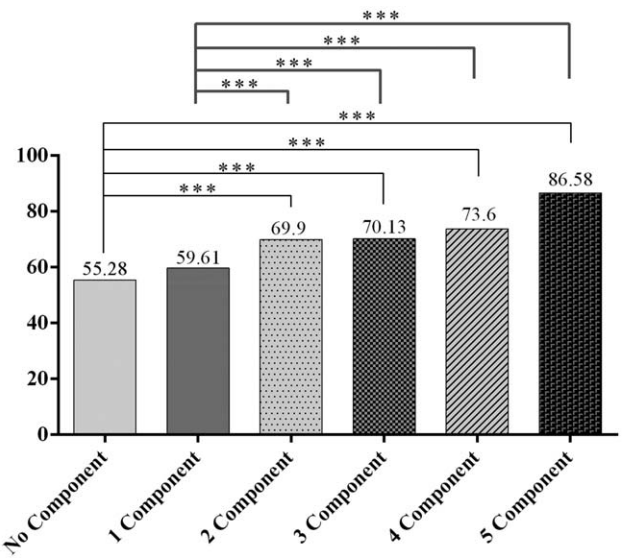

FIG 1 *ANOVA test was used to compare the means of PAB values in 16 different groups with GraphPad Prism version 3. WC = waist circumference; HDL = high density lipoprotein; TG = triglyceride; HTN = hypertension (blood pressure >130/85 mm Hg); G = blood glucose; **P < 0.01; ***P < 0.001.*

2.4. Chemicals for the PAB assay

TMB powder (3,3′,5,5′-tetramethylbenzidine, Sigma), peroxidase enzyme (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). All the other regents used were reagent grade and were prepared in double distilled water [9].

2.5. Measurement of serum pro-oxidant-antioxidant balance (PAB) assay

The PAB assay was as previously described [13]. Standard solutions were prepared by mixing varying proportion (0–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 Mm NaOH). TMB powder (60 mg) was dissolved in 10 mL dimethyl sulphoxide (DMSO). The acetate buffer (20 mL, 0.05 M buffer, pH 4.5) was used to prepare the TMB/DMSO (400 μL) cation, then 70 μL of fresh cholramine T (100 mM) solution was added to the acetate buffer and mixed well. This was then incubated for 2 hours at room temperature in a dark place, 25 U of peroxidase enzyme solution was added to 20 mL of the TMB cation, and stored in 1 ml aliquots at −20°C. About 200 μL TMB/DMSO and acetate buffer (10 mL, 0.05 M buffer, pH 5.8) was well mixed to prepare the TMB solution; a working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution and incubating for 2 minutes in the dark at room temperature. About 10 μL of each sample, blank (distilled water) or standard, was mixed with 200 μL of the working solution and after 12 minutes incubation in dark place at room temperature 100 μL of N HCl was immediately added to each 96-well plate then enzyme linked immunosorbent assay (ELISA) plate reader at 450 nm with reference wavelength of 620 or 570 nm applied for measuring absorbance. A standard curve was obtained using standard sample for each plate to determine values for the serum samples.

2.6. Statistical analysis

SPSS version 18(SPSS Inc. Chicago, IL, USA) was used for all statistical analyses. After assessing normality using the Kolmogorov-Smirnov test, *t*-tests, and Mann-Whitney U tests were used for comparing parameters between MetS+ and MetS– groups. A two-sided *P*-value of <0.05 was consider statistically significant. Multiple linear regression analysis was used to determine the parameters that significantly affected the serum PAB value. Multiple logistic regression analysis was applied to identify independent parameters that were related to MetS. Multiple regression analysis was used to assess the impact of predictor variable on serum level of PAB. Analysis of variance (ANOVA) and Kruskal-Wallis tests with a Bonferroni post hoc correction were used to determine statistical differences between healthy control subjects and individuals with MetS, or with different combination of features of MetS.

3. Results

Demographic and anthropometrics parameters are shown in Table 1. As expected, participants in the MetS+ group had significantly higher value for serum PAB, body weight, BMI, waist circumference, hip circumference, uric acid, fasting blood glucose (FBG), hs-CRP, blood pressure, and for factors in the lipid profile, apart from HDL. Subjects who were in the MetS– subgroup had higher self-reported PAL compare to those in the MetS+ group. Logistic regression analysis showed that serum PAB values (B = 0.002, $P < 0.001$, CI = 1.001–1.003) as well as serum hs-CRP (B = 0.007, $P < 0.001$, CI = 1.007–1.013) and serum uric acid (B = 1.207, $P < 0.001$, CI = 1.186–1.277) were independently associated with the presence of MetS (Table 2). Bivariate correlation between serum PAB values and the aforementioned parameters were assessed in the overall study population as well as in the MetS+ and MetS– subgroups (Table 3). Serum PAB values were significantly related to BMI (r = 0.109, $P < 0.001$), waist circumference (r = 0.029, $P = 0.01$), hip circumference (r = 0.05, $P < 0.001$), serum low density lipoprotein (LDL, R = 0.034, $P = 0.01$), FBG (r = 0.044, $P < 0.001$), serum uric acid (r = −0.05, $P < 0.001$), serum total cholesterol (r = 0.049, $P < 0.001$), serum TG (r = 0.034, $P = 0.001$), serum serum LDL (0.034, $P = 0.008$), serum hs-CRP (r = 0.095, $P < 0.001$), and serum PAL (r = 0.047, $P < 0.001$) in the whole population (Table 3). Multiple linear regression analysis was applied to the determine differences in serum PAB values in the MetS+ and MetS– groups. Multiple regression analysis in the MetS– subgroup showed that serum PAB values were significantly related to several traditional cardiovascular disease (CVD) risk factors including BMI (B = 2.189, $P < 0.001$), waist circumference (−0.478, $P = 0.001$), hip circumference (0.056, $P = 0.02$), serum uric acid (B = −2.999, $P < 0.001$), and serum hs-CRP (B = 0.684, $P < 0.001$); whilst serum cholesterol (B = 0.047, $P = 0,09$) and LDL (B = −0.041, $P = 0.885$) were not significant correlated with serum PAB values. In addition, weight (B = −0.501, $P = 0.003$), BMI (B = 2.04, $P < 0.001$), waist circumference (B = −0.431, $P < 0.001$), serum hs-CRP (B = 0.663, $P < 0.001$), serum uric acid (B = −1.545, $P = 0.003$), and serum PAL (B = 18.728, $P = 0.007$) were found to be significant determinants of serum PAB in the MetS+

TABLE 2 *Multiple logistic regression analysis was applied to identify the independent parameters that are related to the presence of MetS*

	B	P	*Odd ratio*	*95%CI*
Serum uric acid (mg/dL)	0.207	<0.001	1.23	1.186–1.277
Serum hs-CRP (mg/dL)	0.007	0.015	1.007	1.001–1.013
Serum PAB [35]	0.002	<0.001	1.002	1.001–1.003
Constant	−1.715	<0.001	0.18	

Odds ratios with 95% confidence intervals (95% CI) obtained from multiple logistic regression tests.
hs-CRP: high sensitive C-reactive protein; PAB: pro-oxidant anti-oxidant balance.
Equation of logistic regression: $Log\frac{P}{1-P} = -1.715 + 0.207$ *uric acid-* $+ 0.007$ *hs-CRP* $+ 0.002$ *PAB.*

TABLE 3 ***Bivariate correlation, univariate, and multiple regressions between PAB values and different parameters***

	Bivariate correlation in total population	*Univariate regression B MetS–*	*Univariate regression B MetS+*	*Multiple regression B MetS– ($R^2 = 0.03$)*	*Multiple regression B MetS+ ($R^2 = 0.037$)*
Age	0.005	−0.048	−0.127		
Weight	0.011	0.118	−0.201*		−0.256
BMI (kg/m^2)	0.109***	1.435***	0.707**	2.189***	2.04***
Waist circumference	0.029**	0.187**	0.225*	−0.478**	−0.431**
Hip circumference	0.05***	0.395***	−0.063	0.0564*	
Uric acid (mg/dL)	−0.05***	−2.248***	−2.437**	−2.999***	−1.545**
hs-CRP (mg/dL)	0.102***	0.573***	0.769***	0.684***	0.663***
FBG (mg/dL)	0.044***	0.073**	0.032	0.01	
Cholesterol (mg/dL)	0.049***	0.085***	0.018	0.047	
Triglyceride (mg/dL)	0.048**	0.022	−0.001		
HDL (mg/dL)	−0.008	−0.003	0.22		
LDL (mg/dL)	0.034**	0.093***	−0.004	−0.041	
SBP (mm Hg)	0.011	−0.084	−0.008		
DBP (mm Hg)	0.005	−0.065	−0.078		
PAL	0.047***	3.608	24.56***		18.728**
Constant				120.36***	48.698**

FBG: fasting blood glucose; LDL: Low-density lipoprotein; HDL: High density lipoprotein; BMI: body mass index; SBP: systolic blood pressure; DBP: Diastolic blood pressure; hs-CRP: high sensitive C-reactive protein; PAB: pro-oxidant anti-oxidant balance; PAL: physical activity level; MetS: metabolic syndrome.
**P < 0.05, **P < 0.01, ***P < 0.001.*

subgroup, with weight (B = −0.201, $P = 0.04$) being a significant positive determinant of serum PAB in binary linear regression (Table 3).

There was an incremental rise in serum PAB with an increasing number of features of the MetS (Fig. 2). The presence of a combination of MetS traits and their number were investigated by ANOVA, and there was a statistically significant difference in serum PAB between the healthy group and subjects with abnormal serum HDL or TG or both abnormal waist circumferences. Moreover, subjects who had all the features of MetS had the highest levels of serum PAB in comparison to other groups; the difference in serum PAB for this group was also significantly different from the healthy group (Fig. 1). In individuals with 4 or 5 traits, that included abnormal waist circumference, HDL, TG, and glucose; or abnormal waist circumference, HDL, TG, and hypertension (HTN, blood pressure >130/85 mm Hg), there was a significant difference in serum PAB compared with healthy controls. Indeed, subjects who were categorized as having an abnormal waist circumference had significant differences in serum PAB compared to with individuals with three features: for example, waist circumference together with TG and HTN, or in those with two features, for example, abnormal waist circumference and high serum TG or low serum HDL (Fig. 1). A Kruskal-Wallis test demonstrated that there were significant differences in serum hs-CRP values between healthy individuals (without any criteria of MetS) and subjects who had abnormal waist circumference and low serum HDL or abnormal waist circumference and high serum TG (Fig. 3). Moreover, among all subjects with MetS, there was a significant difference in serum hs-CRP compared to healthy subjects and patients with high blood pressure, abnormal waist circumference, and raised serum TG. Although, the hs-CRP values in participants with four components of MetS including abnormal waist circumference, HDL, TG, and HTN were highest, there was no significant difference due to having within group difference.

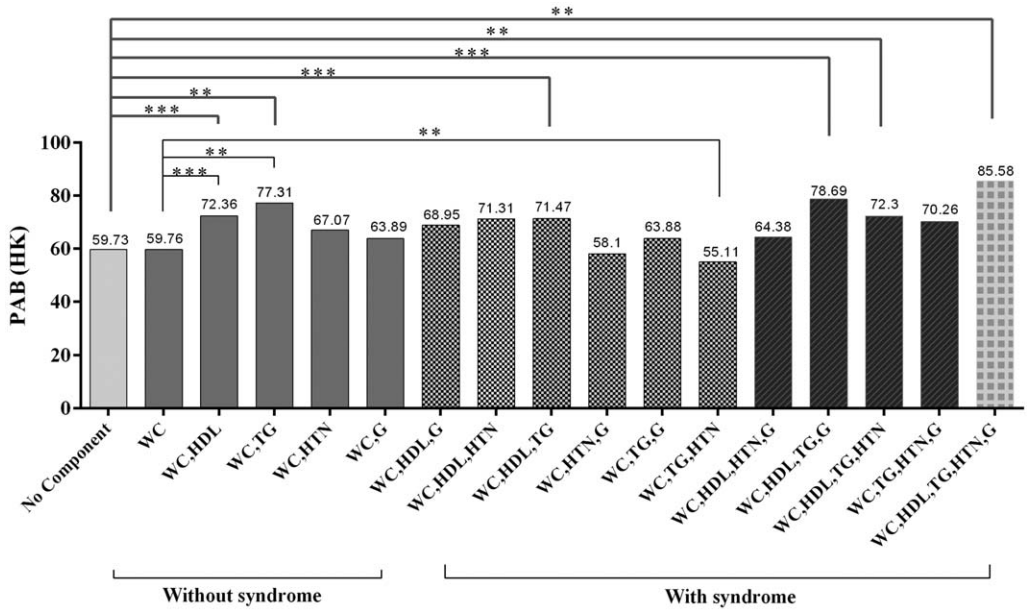

FIG 2 *ANOVA test was used to compare the means of PAB values in different groups with GraphPad Prism version3. ***P <0.001.*

4. Discussion

To the best of our knowledge, this is the first study evaluating the relationship between combination of MetS traits with serum hs-CRP and PAB in a large population cohort. As shown in Figs. 1 and 3, we did not find an additive, or synergistic effect of increasing features of the MetS on serum PAB and hs-CRP, as this appeared to vary dependent on the precise combination of MetS components. Previous studies have suggested that there is a continuum of risk and those individuals with one or two features of the MetS are at increased risk compared to individuals without any features of MetS. According to Fadini et al., one crucial point in defining the clinical entity of MetS is whether the risk associated with it is higher than sum of its single traits and not all combination of traits that comprise the MetS are associated with the same clinical outcomes [14]. Few studies have investigated the potential synergism and/or additive effects of the components of MetS, although the results have not been consistent, they suggest that the risk of MetS for cardiovascular disease is not greater than its individual traits [14,15]. However, we have found that MetS is associated with an alteration in serum hs-CRP, a biomarker of inflammation, and PAB, a measure of prooxidant-antioxidant status and like insulin resistance.

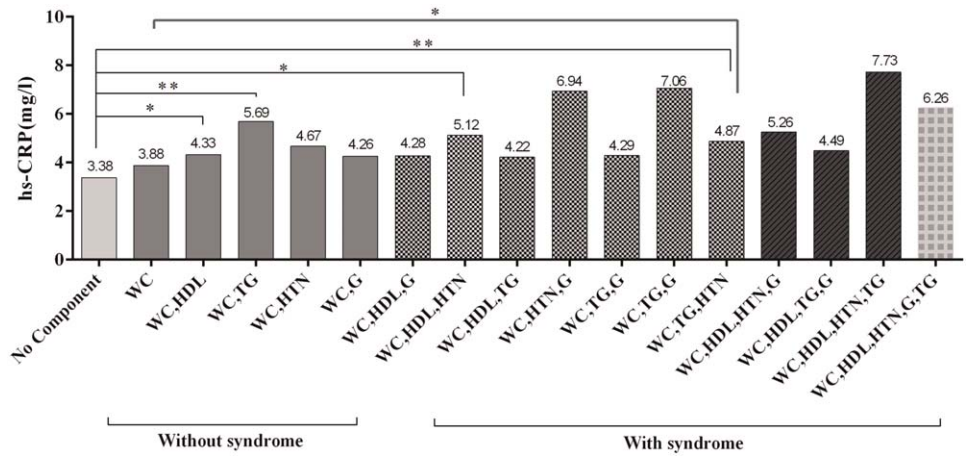

FIG 3 *Kruskal-Wallis test and Bonferroni Post-Hoc tests were used to compare serum CRP values in 16 different groups. WC = waist circumference; HDL = high density lipoprotein; TG = triglyceride; HTN = hypertension (blood pressure >130/85 mm Hg); G = blood glucose; *P < 0.05; **P < 0.01; ***P < 0.001.*

Hyperuricemia forms another consistent feature of MetS that has led to the suggestion that uric acid may be an additional feature of MetS [16]. Uric acid accounts for approximately 50% of the serum antioxidant activity and is a scavenger of ROS; it has been shown to be positively associated with serum PAB values (P-value < 0.001). We found serum uric acid was positively associated with serum PAB values ($P < 0.001$) [17]; however, serum urate levels are also strongly associated with the development of insulin resistance, obesity, hypertension, dyslipidemia, type II diabetes, and other manifestation of MetS [18]. While more evidence is needed to understand the association between uric acid, PAB, and hs-CRP as biomarker of systematic inflammation; one proposed mechanism is that uric acid upregulates CRP mRNA expression in human vascular smooth muscle cell and human umbilical vein endothelial cells by extracellular signal regulated kinase 44/2 that is involved in regulating the expression of CRP [19]. An increased serum PAB and hs-CRP has been previously reported in subjects with CVD [20], and higher levels of oxidative stress have been reported in individuals with MetS compared to healthy subjects [21]. Furukawa et al. found that systemic oxidative stress occurs in adipose tissue, the mRNA expression level of NADPH oxidase subunits are increased and mRNA expression level and activity of antioxidant enzymes decreased in obesity [21]. Increased production of ROS and inactivation or reduced synthesis of NO is an important pathogenic mechanism of uric acid in oxidative stress. Chronic hyper uremia is characterized by the accumulation of advanced glycation end products assay (AGEs) and advanced oxidative protein products (AOPP) and balance between oxidants and antioxidants and immune-inflammatory system well established. AGEs lead to activation of inflammatory cytokines, and subsequently lead to the generation of ROS, thereby enhancing the inflammatory processes get [22]. Inflammatory cascade signals may be initiated by the activation of monocytes by AGEs, furthermore AGEs and ROS directly induce CRP production in hepatocytes [22].

Furthermore, a serum urate >600 μM can induce free radical generation rather than providing a protection against them, by down regulating endothelial nitric oxide synthase (eNOS) production, NO normally plays a critical role in maintaining endothelial function, including the regulation of vascular tone, barrier function, inhibition of coagulation and thrombosis, suppression of inflammatory cell adhesion and migration, and angiogenesis [23]. Furthermore, uric acid promotes intracellular superoxide production [24,25]. Although a previous study did not support uric acid being a feature of MetS in an Asian population, the Transition and Health during Urbanisation in South Africa (THUSA) study found similar findings, as we have, with an association between hyperuricemia as independent factor for MetS [26]. The consensus of evidence with respect to the use of anti-oxidant supplements indicates that high doses of anti-oxidant supplements are either harmful or at best ineffective in chronic disease prevention [1,27–29]. However, a balance between anti-oxidants and pro-oxidants does appear to be crucial in maintaining health.

Two separate tests, or indeed several tests, are usually required to estimate the pro-oxidant/antioxidant balance. However, it can be estimated using the single PAB assay where we measure the balance between anti-oxidant and oxidant simultaneously by using 3,3',5,5'-tetramethylbenzidine (TMB) and two different types of reactions. These two reactions give us a redox when TMB cation is reduced to a colorless compound by antioxidants in a chemical reaction and chromogen TMB is oxidized to a color cation by peroxides in an enzymatic reaction. This assay was calibrated against different proportions of hydrogen peroxide and uric acid since they do not interact or neutralize the activity of each other and could be considered as golden standard for the existence of oxidative stress [30].

Previous studies have been compared other oxidative stress marker like AGEs and AOPP assay with PAB assay. The sensitivity and specificity of the PAB assay were 93% and 91% against carbonyl assay, respectively; 90% and 98% against the AGEs assay, respectively; 96% and 92% against the AOPP assay, respectively [30]. Moreover, the PAB assay was calibrated against the following standards: vitamin C (0–800 μM), trolox (0–800 μM), uric acid (0–6 Mm in 10 mM NaOH), glatathione (0–2,500 μM), bilirubin (0–32.5 mg/dL), hydrogen peroxide (0–1,000 μM), choleramine T (0–200 μM), tetrabuthylhydroperoxide (0–1,000 μM), and HClO (0–200 μM) in our previous study and the mean percentage recovery of adding vitamins C, E, glutathione, and hydrogen peroxide were; 91–111%, 91–125%, 96–111%, and 90–91%, respectively [13]. Trotti et al. proposed two different assay for estimating pro-oxidant burden and antioxidant capacity (oxyl-absorbant test) by using chromogen *N,N*-diethylparaphenylen-diamine in two different assays, although the same chromogen was used in both tests did not allow combined two tests in one assay because the second test necessitates the addition of pro-oxidants into the assay admixture while using uric acid and hydrogen peroxide did not show specific effect on PAB assay [13,31].

In spite of growing evidence for an association between higher serum PAB values in chronic disease, the PAB assay is not used routinely, because whilst providing an integrated value for the interacting pro- and antioxidant systems, it does not provide an assessment of specific antioxidants [30]. Previously we have shown that inflammatory markers (serum hs-CRP) are strongly associated with serum PAB and with several traditional risk factors of CVD [20], which supports our results on the inter-relationship between oxidative stress marker PAB and inflammation and traditional cardiovascular risk factors such as BMI, uric acid, and PAL. In a similar study, among Iranian adolescents (aged 10–18), an association was observed between serum hs-CRP and an oxidative stress marker previously [32].

Overall, our results indicate that in the general population, serum uric acid, PAB, and hs-CRP are associated with the features of MetS. Many studies have reported high levels of serum uric acid and hs-CRP in patient with MetS, or cardiovascular

disease. The serum PAB assay measures total antioxidant pro-oxidant capacity using a simple and rapid method [33]. In agreement with our finding //Glucan and colleagues investigated the relationship between the levels of total antioxidant capacity and PAB and advanced protein oxidation products (AOPPs) in 55 patients with MetS and 22 healthy subjects, and demonstrated increased serum PAB values reflected oxidative stress in patients with MetS [11]. Another study on 730 subjects, including normal weight (n = 207), overweight (n = 375), and obese subject (n = 151), showed that serum PAB values were positively associated with weight ($P < 0.005$), and obese subjects had significantly higher levels of PAB values compared with overweight and normal weight subjects; in addition, serum LDL was an independent predictor of PAB (B = 0.046, $P = 0.04$) [34].

5. Conclusion

In a large population sample derived from the MASHAD study cohort, serum PAB was found to be strongly associated with serum uric acid and hs-CRP. Furthermore, serum PAB, uric acid, and hs-CRP were independently associated with the presence of MetS. Multiple regression analysis showed that PAL in the MetS+ subgroup was a determinant of serum PAB values whilst in the MetS– subgroup this was not the case. In future, studies should consider a combination of MetS components and synergism and additive effect of these factors must be evaluated.

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Conflict of interest

The authors have no conflict of interest to disclose.

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Author Contributions

Design of the experiment (MGM, SRS, GAF), requirement of subjects (MGM, MRP, SD), measurement of the PAB (MA, ST), measurement of the C-reactive protein (MRP, MGM), statistical analysis (MA, MT, SD, HG), gathering data (MA,MM, ME, AHB, MRA), and manuscript preparation (all).

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