ABSTRACT:

Objective: To investigate potential of high sensitivity C-reactive protein (hs-CRP) and serum uric acid as markers of metabolic syndrome (MetS). Study design: Case control study. Place and duration: Conducted at department of physiology, university of Karachi, from January 2015 to September 2015. Material and methods: Study was conducted on 88 subjects both men and women divided in two groups 44 normal controls and 44 subjects of MetS (IDF criteria), aged between 20-40 years. Anthropometric measurements, lipid profile, hs-CRP and serum uric acid were performed. Data were analyzed on SPSS version 22. $P < 0.05$ is regarded as significant. Results: hs-CRP was detected significantly higher in MetS cases as compared to controls ($5.43 \pm 1.3$ vs $1.47 \pm 1.2$ mg/L, $P=0.000$ in men and $4.64 \pm 1.2$ vs $1.78 \pm 1.4$ mg/L, $P=0.000$ in women). Partial correlation analysis indicated positive correlation of hs-CRP with body mass index (BMI), $r=0.745$, waist circumference (WC) $r=0.689$, Systolic blood pressure (SBP) $r=0.578$, Diastolic blood pressure (DBP) $r=0.508$, fasting blood glucose (FBG) $r=0.784$, serum insulin $r=0.804$ with $P < 0.001$ in men and BMI $r=0.701$, serum insulin $r=0.765$, $p < 0.001$; WC $r=0.599$, DBP $r=0.446$ in women. Significant negative correlation was seen with high density lipoprotein cholesterol (HDL-C) in both sexes. AUC (Area under curve) for ROC (Receiver operating characteristic) curve was significant with $P < 0.05$ in each, men and women. Serum uric acid level was significantly higher in subjects as compared to controls ($5.13 \pm 0.6$ vs $3.94 \pm 0.5$ mg/dl, $P=0.000$ in men and $5.21 \pm 0.3$ vs $4.3 \pm 0.6$ mg/dl, $P=0.000$ in women). Partial correlation analysis presented positive correlation with BMI $r=0.804$, WC $r=0.784$, FBG $r=0.565$, HOMA-IR $r=0.652$, serum insulin $r=0.672$ with $p < 0.001$ in men and BMI $r=0.853$, FBG $r=0.798$, serum insulin $r=0.862$, HOMA-IR $r=0.854$, WC $r=0.759$ with $p < 0.001$, DBP $r=0.384$ with $p < 0.05$ in women. AUC for ROC curve was significant with value $< 0.05$ in both men and women. Conclusion: Analysis of study data shows that hs-CRP and uric acid have the potential for prediction of MetS, in either sex.
**Key words:** High-sensitivity C-reactive protein, uric acid, marker, metabolic syndrome

**INTRODUCTION**
Metabolic syndrome (MetS) is a bunch of risk factors like abdominal or central obesity, raised blood pressure, abnormal lipids, insulin resistance or intolerance of glucose that leads to diabetes and cardiovascular disease. Gradual increase in all populations in the world has been observed and identified as major public health problem. High prevalence is a worldwide trend and available data indicates that 28% to 30% of adult population in most countries can be labeled as having MetS. With no consensus agreement upon definition the prevalence in Pakistan is 18% to 46% according to available definitions. Increase in rate of diabetes and cardiovascular cases has put burden on medical budget; so it warrants to detect easy and cost-effective way for diagnosis of MetS. It is obligatory to work upon the factors involved in increase of MetS and investigate the possible markers that can detect syndrome.

Increased plasma concentration of various inflammatory markers has been linked with MetS. High-sensitivity C-reactive protein (hs-CRP) has been observed as marker of chronic systemic inflammation of low grade nature because of its significant association with MetS. Hs-CRP has high predictive value for cardiovascular events and diabetes mellitus which come as a consequence of MetS in apparently healthy males and females. Significant increase in hs-CRP has been observed in children and adolescents when compared to normal weight group. It was further concluded that hs-CRP seems cardiovascular marker which could be used for early diagnosis of risk factors.

Purine metabolism in humans gives rise to production of uric acid; raised serum level is associated with MetS. Apart from MetS, hyperuricemia is associated with other cardiovascular problems. Increased level of uric acid in serum has been detected in MetS. Elevated uric acid predicts development of high blood pressure, obesity and type II diabetes. Hyperuricemia acts as substitute marker of MetS. Ethnic background has been suggested main contributor in determination of level of expression of various markers of metabolic disturbance and association with diabetes, MetS and cardiovascular diseases. In an attempt to detect MetS before appearance of its fully expressed components, uric acid was found most reliable biomarker for identification in obese subjects.

Joint effect of both hs-CRP and uric acid was studied; rise in level was found to be associated with increase in MetS components. In Chinese study hs-CRP correlated with uric acid and increase in prevalence was observed with rise in its level. In Japanese community-dwelling women rise of hs-CRP and uric acid was investigated and it was seen that both have co-ordinated effect as independent determinants of MetS and insulin resistance.

Keeping in view the outcomes of MetS the current study was designed to find out potential role of hs-CRP and serum uric acid as markers of MetS.

**MATERIAL AND METHODS**
This case-control study was designed and accomplished at the department of physiology, university of Karachi. A total of 88 subjects were finally selected. Two groups were formed, 44 subjects with MetS as per International Diabetes Federation (IDF) criteria as MetS group and 44 age and sex matched subjects as control from same population.

**Duration of study:** From January 2015 to September 2015. Approval of study was given by the board of advanced study and research (BASR) vide Resol.No 10(5)04 dated 15.8.2014 & 02.9.2014. Investigations were conducted as per declaration of Helsinki. Participants were included after their agreement. Verbal and written consent was sought.

**Inclusion criteria:** Central obesity (waist circumference for men ≥ 90cm and for women ≥ 80cm) plus any two from following four components: 1) Triglyceride (TG) ≥ 150 mg/dl 2) HDL-C less than 40 mg/dl in men and less than 50 mg/dl in women. 3) SBP ≥ 130 or DBP ≥ 85 mm Hg. 4) Fasting blood glucose ≥ 100 mg/dl.

**Exclusion criteria:** Subjects with elevated uric acid or taking medicine that increase serum uric acid, CVS disorders, chronic inflammatory disease, acute infections, cancer, smokers, alcoholics, pregnancy, chronic kidney disease and diabetes.

**Measurement of anthropometric parameters and blood pressure:**
Weight (Kg), height in meters, waist circumference (cm),
hip circumference (HC) in cm and BMI were measured with standard methods. Waist hip ratio (WHR) = waist circumference divided by hip circumference.

Body fat percentage = body fat mass [kg] divided by body weight [kg] multiplied by 100 with bio-impedance meter, model NO: BF 510 supplied by Omron corporation, Kyoto, Japan.

Blood pressure of subjects measured in seated position twice in the right arm second reading taken after rest of 10 min by mercury sphygmomanometer and average reading was used for analysis.

**Blood sample collection and analysis:**

After fasting of 12-14 hours, 6ml blood was drawn from vein in the morning under aseptic conditions in plain test tubes without anticoagulant. The blood was allowed to coagulate. The samples were centrifuged and serum was separated. Three aliquots were prepared, one for immediate assay of fasting glucose, uric acid and lipid profile. Other two were stored at - 80°C for subsequent analysis of insulin and hs-CRP.

Total cholesterol, HDL-C, TG and (FBG) were measured by assay kits from Human GmbH Germany using Metrolab 1600, SA. Low density lipoprotein cholesterol (LDL-C) level was detected by using Friedewald method by equation. Uric acid measured by Uricase-PAP method (Greiner Diagnostic GmbH, Germany) using Metrolab 1600. Human insulin (Cat no.10801) and high sensitivity C-reactive protein (cat no.10603) with enzyme immunoassay test kits supplied by Perfemed group, USA; Using EMP M 201 microplate reader. Insulin resistance (HOMA-IR) by HOMA calculator.

**DATA ANALYSIS**

All the collected data were gathered, arranged and then analysed by standardized statistical programs, including test of significance (independent T-test). Data were stored and analysed by using SPSS (version 22.0). Mean and standard deviation was computed for all
quantitative parameters, like age, BMI, WC, HC, WHR, SBP, DBP, body fat %, fasting glucose, serum insulin, total cholesterol, HDL-C, LDL-C, T.G, serum uric acid, hs-CRP and HOMA-IR. Study subjects were divided into two groups, control and MetS group. These groups were further divided by gender as well. They were compared by applying independent T-test on quantitative parameters of both groups. Independent T-test was used because this test is designed to compare two groups at a time.

Partial correlation was used to see the correlation of hs-CRP and uric acid with the components of MetS. All statistical tests were performed after testing the normality of the data, at 5% level of significance. Values were taken statistically significant at $p < 0.05$.

ROC curve analysis was carried out for the determination of efficacy in the identification of hs-CRP and uric acid levels of subjects with MetS. Curves depicted, area under curve, sensitivity, specificity and optimal cut-off points with $p$-value.

**RESULTS**

All the subjects, under study, were between the ages of 20-39 years, with the mean age 29.9 years. Study includes 88 subjects, 58 (65.9%) men and 30 (34.09%) women, equally divided into two groups, control and MetS group. Quantitative as well as qualitative parameters were compared between both groups (control and MetS group) by using independent T-test. There were a number of parameters, which were found to be
significantly different between control and MetS group, including anthropometric indices, fasting glucose, serum Insulin, lipid profile, serum uric acid, hs-CRP, HOMA-IR.

BMI, WHR, blood pressure, body fat%, fasting glucose, serum Insulin, total cholesterol, LDL cholesterol, T.G, serum uric acid, hs-CRP, HOMA-IR levels were significantly increased in MetS group while HDL cholesterol in MetS group was significantly deceased.

Baseline measurements
All quantitative parameters of men and women from both groups were compared by independent T-test with P value <0.05 taken as significant.

Men
It was found that men (from MetS group) had significantly high levels of BMI, WHR, blood pressure, body fat%, fasting glucose, serum insulin, serum uric acid, hs-CRP, HOMA-IR and the lipid profile, while HDL-C was found to be significantly low.

Women
On the other hand, women with MetS had significantly high levels of BMI, diastolic blood pressure, body fat%, fasting glucose, serum insulin, serum uric acid, hs-CRP, HOMA-IR and the lipid profile except T.G which was not significant, while HDL-C was found to be significantly low as compared to women in control group.

PARTIAL CORRELATIONAL ANALYSIS
Partial correlation coefficient between hs-CRP, uric acid and the components of MetS according to gender are shown in table 2.

The results presented significant positive correlation of hs-CRP and uric acid with most of the components of MetS. No correlation with TG and significant negative correlation with HDL-C in either sex.

ROC curves for identification of MetS Subjects
Determination of efficacy in the identification of hs-CRP and uric acid levels of men and women with MetS. ROC curves exhibited, area under the curve, sensitivity, specificity, optimal cut-off points and P-value(Fig 1,fig2).

MetS prediction in men: AUC, optimal cut-off points, sensitivity% and specificity% for hs-CRP were found to be 0.979, .4.45mg/L, 0.897 and 0.034 respectively. For uric acid 0.924, 4.55mg/dl, 0.828 and 0.172 respectively. Results were significant with P value <0.05.

For prediction of MetS in women: AUC, optimal cut-off points, sensitivity% and specificity for hs-CRP were found to be (0.76, 3.7mg/L, 0.733 and 0.133) respectively for uric acid (0.931, 4.05mg/dl, 0.667 and 0.333) respectively. Results were significant with P value <0.05.

DISCUSSION
Modernism and elevation of living standards has brought metabolic imbalance leading to obesity, impaired blood sugar, increased blood pressure, dyslipidemia and insulin resistance collectively called MetS. This syndrome has shown strong prediction of risk for diabetes and cardiovascular disease with increase of morbidity and mortality. High prevalence of MetS in Pakistani population has been detected regardless of definition applied which warrants immediate steps to stop increasing risk of diabetes and cardiovascular disorders that would bring possible rise in cost of handling human health.

The study was conducted on section of population in southern Pakistan. All cases had statistically significant higher levels of BMI, WC, HC, body fat%, DBP, FBG, TC, LDL-C, uric acid, hs-CRP and HOMA-IR when compared to control group in both sexes while WHR, SBP and TG were statistically significant in males but not in females.

In current study statistically significant higher levels of BMI, WC, HC, WHR, body fat%, FBG, blood Pressure and HOMA-IR found in cases are consistent with the findings of a great deal of numerous previous studies.

In this study significant difference in level of hs-CRP was seen in MetS group when contrasted with control group in either sex (5.43± 1.3 vs 1.47± 1.2 mg/L, p=000 in males and 4.64± 1.2 vs 1.78± 1.4 mg/L, p=000 in females). This finding of having significant difference between MetS subjects and controls is reported in number of studies.

In this study significant positive correlation has been observed between hs-CRP with BMI, W.C, DBP, FBG, serum insulin, HOMA-IR and body fat percent in both sexes and SBP only in men. Significant negative correlation with HDL-C in men and women and no significant correlation with TG in either sex. Numerous
studies have shown correlation of hs-CRP with components of MetS; significant positive correlation between hs-CRP and FBG and significant negative correlation with HDL-C. Excluding BP and FBG; hs-CRP was significantly correlated with components of MetS. Significant correlation of hs-CRP was observed with all components of MetS. In contrast to this correlation, a study from Karachi, urban Pakistan has observed no correlation between hs-CRP and fasting insulin level, insulin resistance and other parameters of MetS. This could be due to small scale study or ethnic background because our study subjects mostly belonged to rural areas.

In current study serum uric acid level (5.13± 0.6 vs 3.94± 0.5 mg/dl in men and 5.21± 0.3 vs 4.3± 0.6 mg/dl in women) was found statistically significant in MetS subjects compared with controls. These findings are consistent with results of studies conducted by other researchers; who have shown statistically significant increase in serum uric acid level in MetS subjects, both men and women when compared with controls. Similar findings of statistically significant increased uric acid in MetS subjects compared with non-MetS subjects have been observed in number of studies. Cross-sectional studies also have observed significantly increased serum uric acid level in MetS subjects. Taking normal reference range of serum uric acid level (2.95-7.74 mg/dl in males and 2.37-6.84 mg/dl in females) in Pakistani population aged between 15-40 years; the mean level of SUA in MetS subjects of this study lies in normal range. A concentration within normal range might be a risk factor and could predict development of MetS. The experimental study has provided strong evidence showing decrease in SUA level reverses the features of MetS in animal models.

The study has observed significant correlation of uric acid with BMI, WC, DBP, Body fat percent, FBG, S.insulin, HOMA-IR in both sexes and SBP only in men, a significant negative correlation with HDL-C in men and women and no significant correlation with T.G in either sex. This is in agreement with study of Hamedet al who have shown significant positive correlation of uric acid with BMI, HOMA-IR, insulin, WC, WHR and SBP in men and positive correlation between uric acid and T.G, BMI, HOMA-IR, insulin, WC and SBP in women.

In current study, for prediction of subjects with MetS; AUC for hs-CRP was 0.979 in men and 0.76 in women. The sensitivity % was 0.897 in men and 0.733 in women. The value of specificity % was 0.034 in men and 0.133 in women. The optimal cut-off point was 4.45 mg/L in men and 3.7 mg/L in women. Results obtained from ROC curves were significant with p-value <0.05. These results are in agreement with study by Grag et al who have shown best prediction value of hs-CRP at 2.6 mg/L with sensitivity of 71%, specificity of 78% and accuracy of 75%. They also observed increased sensitivity with increased cutoffs. In other study an hs-CRP level more than 3 mg/L predicted greater cardiovascular risk in MetS subjects in multivariate model. For diagnosing MetS in Japanese, the AUC of hs-CRP was found 0.71 in men and 0.74 in women; which is in agreement with current study.

In this study ROC curve for analysis of uric acid for prediction of metabolic syndrome shows AUC 0.924 in men and 0.931 in women. The value of sensitivity % in men was 0.828 and in women 0.667 with specificity of 0.172 and 0.333 in men and women respectively. The cut-off value was 4.55 mg/dl in men and 4.05 mg/dl in women. The results obtained from ROC curves were significant with p value of <0.05. A study on uric acid as a predictor of MetS has shown AUC from uric acid ROC curves as 0.56 in men and 0.706 in women with p< 0.001 in women. This study is showing agreement with present study only in case of women but not in men. This could be due to genetic differences and needs further investigations. The study by Zhang et al has observed that optimal cut-off values for uric acid to identify MetS were 6.3 mg/dl in men and 4.9 mg/dl in women respectively, with AUC 0.601 in men and 0.666 in women; compared to this results of current study are better.

CONCLUSION
The data analysis of this study has given encouraging results showing significant correlation of hs-CRP and the serum uric acid level with MetS components. The AUC of ROC curves have shown significance in identification of MetS.

Limitations:Like other studies there are some limitations. This was a small study and needs further work on large scale to explore the value of these emerging potential markers as a unique method for detecting subjects at high risk of MetS; with standardization of hs-CRP and uric acid measurements to get authentic results.
REFERENCES


Author’s Contributions

SS, SZ and TA have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. They are also involved in drafting the manuscript or revising it critically for important intellectual content. SS has been involved in the sample collection and bench work. All authors read and approved the final manuscript.

Conflict Of Interest

Authors declare that they have no competing interest.


