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Serum Prolidase Activity in Metabolic Syndrome.

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ABSTRACT

Background: Metabolic syndrome (Mets) is defined as the existence of central obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia. Prolidase, a member of Matrix metalloproteinases (MMPs), plays an important role in collagen metabolism and extracellular matrix remodelling. Altered Matrix Metalloproteinase (MMPs) is the important factor in the pathogenesis of MetS. **Methods:** In this study 50 cases with metabolic syndrome diagnosed based on the Adult Treatment panel III (ATP III) criteria of the National Cholesterol & Education program (NCEP) and 50 age, sex matched healthy individuals were taken as control. Serum prolidase was estimated by ELISA, fasting plasma glucose, lipid profile, urea & creatinine were estimated by standard methods. **Results:** The prolidase levels was significantly higher in metabolic syndrome group (41.49±6.59) compared to control group (28.86±3.39) with a p value < 0.05. Statistical analysis were done by SPSS20 software. **Conclusion:** The serum prolidase values showed positive correlation with waist circumference, SBP, DBP, TG, LDL-C & negative correlation with HDL-C.

Keywords: Serum prolidase, Lipid profile and Metabolic syndrome.

INTRODUCTION

MetS is a cluster of interrelated metabolic disorders, including central obesity, insulin resistance, dyslipidemia, endothelial dysfunction, hypertension, hypercoagulation and chronic stress, that predisposes to type-2 diabetes and cardiovascular diseases (CVD) and is associated with considerable increase in all-cause mortality.[1] The worldwide prevalence of metabolic syndrome in adult population is estimated to be 20%-30% and is higher in the populations of developing country.[2-4] The worldwide prevalence of metabolic syndrome in adult population is estimated to be 20%-30% and is higher in the populations of developing country.[2-4] The adult Treatment Panel III (ATP III) criteria of the National Cholesterol and Education Program (NCEP) for Met S are the presence of any three of the following characteristics: abdominal obesity (AO), elevated blood pressure, dysglycemia, low plasma high-density lipoprotein cholesterol (HDL-C), increased triglycerides (TG).[5] The adult Treatment Panel III (ATP III) criteria of the National Cholesterol and Education Program (NCEP) for MetS are the presence of any three of the following characteristics: abdominal obesity (AO), elevated blood pressure, dysglycemia, low plasma high-density lipoprotein cholesterol (HDL-C), increased triglycerides (TG).[5] The diagnosis of MetS has been harmonized internationally using the NCEP ATP III criteria with the notable exeption of cutoffs for abdominal obesity for waist circumference, which differ by country, ethnicity,[6] and region.[7]Prolidase, a member of matrix metalloproteinases (MMP) family, is a cytosolic imidodipeptidase, which particularly cleaves imidodipeptides with C terminal proline or hydroxyproline. The enzyme plays an important role in the liberation and recycling of proline from imidodipeptides for resynthesis of collagen and other proline containing proteins.[8] In MetS, endothelial dysfunction may leads to cardiovascular risk with high morbidity and mortality.Altered MMPs is an important factor in the pathogenesis of metabolic syndrome.Prolidase has an important role in collgen metabolism and extracellular matrix remodelling.[8,9] Arteries of human body contain large quantities of collagen.[10] So any pathological processes affecting the vascular system will also affect the collagen cycle.[11] Fluctuations in prolidase activity can indicate a disruption in collagen metabolism which is characteristic of many diseases as well as disease progression.[12,13]Prolidase enzyme activity has been documented in plasma, erythrocytes, leukocytes, dermal fibroblasts and various organs such as kidney, brain, heart, thymus, uterus, lung, liver,

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spleen and pancreas, small intestine and stomach.[14] The aim of the study was to find the association between increase in serum prolidase activity with the metabolic syndrome.

MATERIALS AND METHODS

This is a cross sectional analytical study conducted in the Department of Biochemistry in collaboration with the Department of General Medicine, Burla. The study was approved by institutional ethical comittee and informed consent was obtained from individuals.

After complete history taking, anthropometric parameters were recorded.

Study population

The study included 50 numbers of cases between the age group of 25-50 years with MetS as per the guidelines of Adult Treatment Panel III, 2011 (ATP III) criteria of the National Cholesterol and Education Program (NCEP). According to this guidelines presence of MetS is evaluated having any three of the following :

1. Waist circumference: Male > 102 cm and Female >88 cm
2. Raised triglyceride: ≥150 mg/dl
3. Reduced HDL cholesterol: < 40 mg/dl in males and < 50 mg/dl in females
4. Raised blood pressure: systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg
5. Raised fasting plasma glucose: ≥ 100 mg/dl

Controls: 50 numbers of age, sex ,socio- economic status matched healthy individuals.

Sample processing:

- 5 ml of venous blood was collected under aseptic precaution from the subjects at early morning after overnight fasting of 8-12 hours.
- For blood sugar estimation 0.5 ml of blood was transferred to fluoride tube .For serum, the rest of the blood was transferred to plane tube. Fluoride and plane tube were centrifuged to get plasma and serum.
- For Prolidase estimation 1ml of serum sample was stored at -200C.
- Sample were thawed to room temperature before every assay and repeated thaw was avoided.
- The biochemical parameters like FBS, lipid profile, urea, creatinine were estimated in Clinical auto analyser COBAS INTEGRA (C-311).
- Serum prolidase was estimated by ELISA KIT as per manufacturer's instruction in Lisa Scan EM (Erba Mannheim).

Statistics:

- All the results were expressed in mean± SD.
- The significance of difference between the two groups was done by unpaired student t-test.
- p value of <0.05 was considered statistically significant.
- Pearson correlation coefficient was used to evaluate any relationship between different variables.

Statistical analysis were done by SPSS-20 software.

RESULTS

Table 1: Age and Sex Distribution ofthe Study Group

Variables	Controls	Cases
Age	41.10 ± 6.39	43.57 ± 4.56
Sex (MALE: FEMALE)	34:16	32:18

The study was done between the age group of 25-50 yrs, with mean age of 38.85±7.37 and 43.57 ± 4.56 in control and case respectively. The ratio between male and female was 34:16 in control and 32:18 cases.

Table 2: Comparison ofBmi,Wc,Sbp,Dbp Among The Study Gruop

Variables	Control	Cases	Pvalue
BMI (kg/m2)	22.22 ± 1.78	26.95 ± 3.01	<0.0001
WC (cm)	92.00 ± 5.55	103.00 ± 8.25	<0.0001
SBP(mmHg)	119.52±9.44	132.92 ± 14.02	<0.0001
DBP(mmHg)	79.84±6.26	89.04 ±10.69	<0.0001

- The mean Body Mass Index (BMI) for control was 22.22 ± 1.78 kg/m2 and for cases it was significantly higher 26.95 ± 3.01 kg/ m2 with p value < 0.0001.The difference was statistically significant.
- The mean waist circumference in control group was 92.00 ± 5.55 cm, for cases it was 103.00 ± 8.25 cm (p <0.0001) which was statistically significant.
- The SBP was statistically significant (p<0.0001) higher in cases 132.92 ± 14.02 mmHg than control 119.52±9.44 mmHg.
- The DBP was statistically significant (p<0.0001) higher in cases 89.04 ±10.69 mmHg compared to control 79.84±6.26 mmHg.

Table 3: Comparision of Biochemical Parameters of the Study Group

Variables	Control	Cases	p value
UREA(mg/dl)	20.32±6.38	22.53±8.84	NS
CREATININE(mg/dl)	0.83±0.21	0.91±0.16	NS
FBS (mg/dl)	89.12 ± 9.99	120.48±28.48	<0.0001
TC (mg/dl)	157.88± 26.14	195.82±33.65	<0.0001
TG (mg/dl)	109.50±23.08	183.38±36.00	<0.0001
HDL-c (mg/dl)	41.4±8.88	37.7±6.26	<0.0179
LDL-c (mg/dl)	93.36±25.73	125.167±19.07	<0.0001

- The mean values of urea, creatinine showed no statistical difference between control and cases.
- The mean FBS in cases 120.48±28.48 mg/dl was higher as compared to control 89.12 ± 9.99 mg/dl which was statistically significant (p<0.0001).

- The mean serum total cholesterol in cases 195.82±33.65 mg/dl was higher as compared to control 157.88± 26.14mg/dl which was statistically significant (p<0.0001).
- The mean serum Triglyceride in cases 183.38±36.00 mg/dl was higher as compared to control 109.50±23.08 mg/dl which was statistically significant (p<0.0001).
- The mean serum high density lipoprotein cholesterol(HDL-C) in cases 37.7±6.26 mg/dl was lower as compared to control 41.4±8.88mg/dl which was statistically significant (p<0.0001).
- The mean serum low density lipoprotein cholesterol(LDL-C) in cases 125.167±19.07 mg/dl was higher as compared to control 93.36±25.73 mg/dl which was statistically significant (p<0.0001).

Table 4: Comparison of Serum Prolidase in the Study Group

Variables	Control	Cases	P-Value
SERUM PROLIDASE (ng/ml)	28.86±3.39	41.49±6.59	<0.0001

[Table 4] showing serum Prolidase in cases 41.49±6.59(ng/ml) was higher compared to control 28.86±3.39 (ng/ml) with p value<0.0001.The difference was statistically significant.

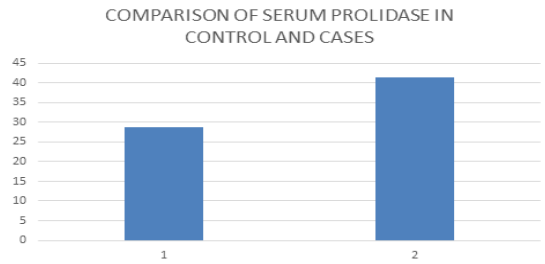
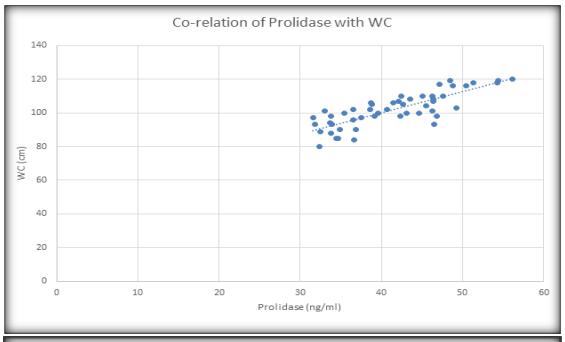
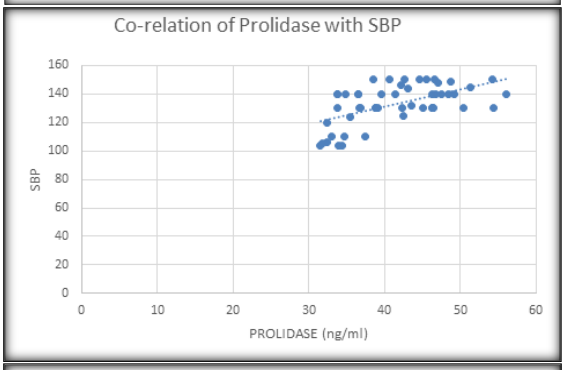


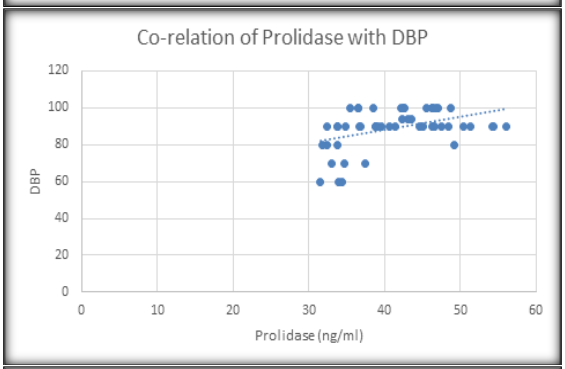
Table 5: Correlation Coefficient Of Serum Prolidase With The Components Of The Metabolic Syndrome

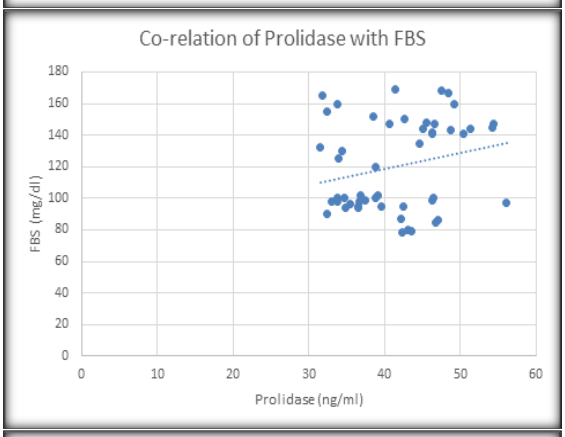
Components of the Metabolic Syndrome	Correlation Coefficient (r)
WAIST CIRCUMFERENCE	+0.872
SBP mmHg	+0.572
DBP mmHg	+0.430
FASTING BLOOD SUGAR mg/dl	+0.232
TRIGLYCERIDE mg/dl	+0.277
HDL mg/dl	- 0.229

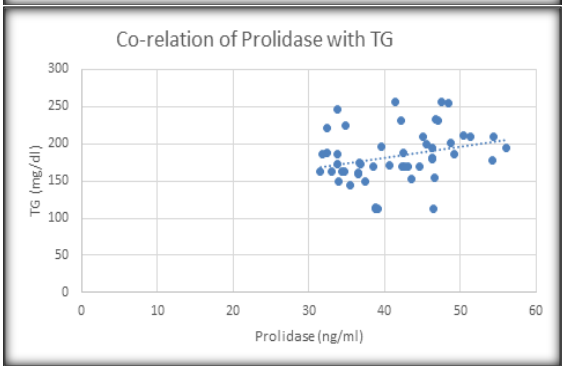
[Table 5] showing correlation between serumProlidase with different components of MetS. Serum Prolidase had positive correlation with waist circumference(WC),SBP, DBP,FBS,TG with r value of 0.872, 0.572, 0.430, 0.232, 0.277 respectively.
It had negative correlation with HDL-C with r value – 0.229.

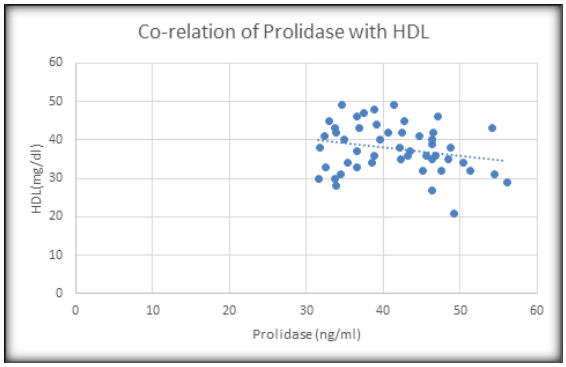












DISCUSSION

The current study was undertaken to evaluate the possible association between serum prolidase activity and metabolic syndrome. We found a significant increase in serum Prolidase activity in MetS group compared to control group. Serum Prolidase was positively correlated with WC, SBP, DBP, FBS, TG and negatively correlated with HDL-C. The International Diabetes Federation (IDF), National Cholesterol Education Program Adult Treatment Panel – III (NCEP ATP-III) guidelines and many others have defined metabolic syndrome (MetS) as a cluster of inter-connected metabolic abnormalities involving central obesity, elevated blood pressure,impaired glucose metabolism (diabetes mellitus) and lipid metabolism (hypercholesterolaemia and dyslipidaemia).[15]MetS increases the risk of type-2 diabetes, cardiovascular disease with increase mortality.[16] Components of metabolic syndrome like obesity,hypertension,impaired glucose metabolism and dyslipidemia all are associated with endothelial dysfunction. The mechanism behind these alteration of endothelial function by these risk factors are endothelial injury, inflammation,reactive oxygen species(ROS) production and disruption of Nitric Oxide(NO) function and bioavailability.[17] Endothelial dysfunction causes changes in the arterial vasculature which leads to micro and macrovascular complications, erosion and thrombosis.[18] In the human body all of the arteries consist of elastic lamellae, collagen fibrils and smooth muscle cells in the middle layer. So human arteries composed of large quantities of collagen.[10] Proline and hydroxyproline constitute 25% of the aminoacid residues in collagen thus collagen is chiefly a proline reservoir.[19]Prolidase is a unique enzyme capable of degrading dipeptides in which proline or hydroxyproline residue is located at the C-terminal position.[20]Prolidase has an important role in collagen metabolism, matrix remodelling and cell growth.[11] During protein breakdown, prolidase catalyse the end stage of the degradation of intracellular collagen, procollagen, and other proline-containing peptides in which proline containing dipeptides are hydrolysed releasing free proline into the cytoplasm.Free Proline again recycled for collagen biosynthesis.[21] Proline can participitate in metabolic signalling and acts as a stress substrate.Collagen breakdown generally occurs during conditions of metabolic stress such as compromise of the blood supply during tissue damage.[13] Previous evidence has shown that endothelial dysfunction can increase cardiovascular risk via pathologic variations, such as disturbing vascular tone, activating leucocyte migration, promoting blood clotting, and disrupting arterial homeostasis.[22-24] Increased serum Prolidase level in MetS group compared to control group indicates more collagen turnover which may be due to increase endothelial remodelling caused by hypertension, hypertriglyceridemia,impaired fasting glucose, obesity.Demirbag et al.[25] showed increased prolidase activity in hypertensive patients due to increased collagen turnover supporting our data. Yildiz et al.[26] has found positive correlation between serum Prolidase and SBP and negative correlation with HDL-C ,supporting our data.

CONCLUSION

From this study we had an insight to the role of serum prolidase in endothelial remodelling in metabolic syndrome and this could help in the development of drugs like matrix metalloproteinase inhibitors to improve endothelial function and prevention of cardiovascular diseases.However,further clinical studies are needed in larger groups to clarify the pathophysiological role of serum prolidase activity in metabolic syndrome.

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