 DOI: 10.18231/2394-6377.2016.0030

C-Peptide and insulin levels in patients of metabolic syndrome

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Introduction

Metabolic Syndrome (MetS) is cluster of physical conditions and metabolic abnormalities commonly found in association with increased risk for development of type-2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other medical conditions.[1] Definition proposed by the International Diabetes Federation (IDF) 2005[2] is the most recent; according to which, a person is identified as having the MetS if he/she has central obesity (defined with ethnicity specific values) plus any two of the following: raised triglycerides; reduced HDL cholesterol; raised blood pressure; or raised fasting plasma glucose (Table 1 & 2). The IDF, having recognized the difficulties in identifying unified criteria for MetS that were applicable across all the ethnicities, has proposed a new set of criteria with ethnic/racial specific cut-offs.[3]

Worldwide prevalence of MetS ranges from <10% to as much as 84%. Higher socioeconomic status, sedentary lifestyle and high Basal Metabolic Index (BMI) were significantly associated with MetS. Furthermore, the prevalence is 1.5–2 times higher in women compared to men.

C-peptide is composed of 31 amino acids, released from the pancreatic □-cells during cleavage of insulin from proinsulin, which is a single polypeptide chain of 86 amino acids and has three "C_C" cystine bonds, stored in secretory granules, and eventually released into the bloodstream in amounts equimolar with those of insulin. It is mainly excreted by the kidney, and its half-life is 3-4 times longer than that of insulin. It has an essential function in the synthesis of insulin in that it links the A and B chains in a manner that allows correct folding and "C_C" disulfide bond formation.[4]

Table 1: Definition proposed by the International Diabetes Federation (IDF) 2005[2]

Central obesity (defined as waist circumference* with ethnicity-specific values) Plus any two of following four factors:	
Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality
Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality
Raised blood pressure	systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	(FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed T2DM If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

**If Basal Metabolic Index (BMI) is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured.*

Several studies have found a strong correlation between basal C-peptide and components of MetS. These studies have concluded that an elevated fasting serum C-peptide levels can constitute a clinically important biomarker of the cardiovascular risks associated with the MetS[5,6]. The reference range of C-peptide is 0.78 – 1.89 ng/mL (conventional units).

Table 2: Ethnic specific values for waist circumference

Country/Ethnic group		Waist circumference
Europids* In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male	≥94 cm
	Female	≥80 cm
South Asians Based on a Chinese, Malay and Asian-Indian population	Male	≥90 cm
	Female	≥80 cm
Chinese	Male	≥90 cm
	Female	≥80 cm
Japanese**	Male	≥90 cm
	Female	≥80 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africans	Use European data until more specific data are available	
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available	
**In future epidemiological studies of populations of Europid origin, prevalence should be given using both European and North American cut-points to allow better comparisons.* ***Originally different values were proposed for Japanese people but new data support the use of the values shown above.*		

Insulin is an anabolic hormone that promotes glucose uptake, glycogenesis, lipogenesis, and protein synthesis of skeletal muscle and fat tissue. In addition, insulin is the most important factor in the regulation of plasma glucose homeostasis, as it counteracts glucagon and other catabolic hormones-epinephrine, glucocorticoid, and growth hormone. The reference range of insulin is 2 –25 m IU /L (conventional units).

The proposed central abnormality associated with MetS is insulin resistance (IR).[7] The term IR indicates the presence of an impaired biological response to either exogenously administered or endogenously secreted insulin[7] and is associated with the progression to impaired glucose tolerance (IGT) and T2DM.[8,9]

The association of obesity with T2DM has been recognized for decades. It is seen in all ethnic groups and is found across the full range of body weights, across all ages, and in both sexes[1,7,10,11]. The central (intra-abdominal) adiposity is more strongly linked to insulin resistance and to a number of important metabolic variables, including plasma glucose, insulin, total plasma cholesterol, triglyceride concentrations, and decreased plasma high density lipoprotein (HDL)-cholesterol concentration, than is total adiposity.[7]

Several workers have established that high C-peptide levels coexist with hyperinsulinemia in metabolic syndrome and so we decided to explore the levels of C-peptide and insulin in patients of metabolic syndrome at our place.

Method and Materials

The present study was conducted in the department of Biochemistry, Subharti Medical College, Meerut after obtaining ethical clearance by the Institutional Ethical Committee. Patients attending the Metabolic OPD of Chatrapati Shivaji Subharti hospital associated with Medical College were screened for MetS and enrolled for the present study. Informed consent was taken from each individual patient. Study group included 89 subjects of MetS who fulfilled the criteria of MetS proposed by IDF 2005 within the age group of 16 to 65 years.

Waist circumference was recorded in all. General information and detailed medical history was recorded from each individual subject and they were subjected to complete physical and systemic examinations. Routine investigation like Hemoglobin (Hb), Total Leucocytes Count (TLC), Differential Leucocytes Count (DLC), Erythrocyte Sedimentation Rate (ESR), Liver Function Test (LFT), Kidney Function Test (KFT), and the special investigations like, lipid profile (TG, TC, HDL-C, LDL-C, VLDL-C), fasting blood glucose (FBG), fasting C-peptide and fasting Insulin were done in all the cases and findings recorded.

Patients with Insulinoma and recent administration of drugs like insulin, corticosteroids, Levodopa and oral contraceptives, having severe uncontrolled hypertension, Diabetic ketoacidosis, fructose or galactose intolerance, congestive cardiac failure, pregnancy or having blood urea and serum creatinine in the abnormal range were excluded from the study by doing ECG, measuring blood pressure,

performing chemical and enzymatic analysis of urine and blood and by performing other specific concerned tests.

Sample Collection: After 12 to 14 hours of fasting, venous blood sample was collected under all aseptic conditions, 2ml in EDTA vacutainer for routine investigations, 4ml in plain vacutainer for special investigation and 2ml in Sodium fluoride vacutainer for fasting blood glucose. Plain and Sodium fluoride vacutainers were allowed to stand for 30-60 minutes. Serum was separated by centrifugation for 5 minute at 1500 rpm. The serum from plain and plasma from sodium fluoride vacutainer was transferred in different properly labeled aliquots and stored at -20ºC for estimation of lipid profile and Fasting blood glucose levels, Fasting Serum c-peptide and, Fasting Serum insulin. Serum C-peptide and Serum insulin were estimated by ELISA. DRG® C-peptide / insulin ELISA kits were used for estimation of C-peptide and Insulin.

Result and Observation

Out of the total study group of 89 subjects {48(53.9%) males and 41(46.1%) females}, 80 (89.8%), 5 (5.6%) and 4 (4.49%) subjects had c-peptide level >1.89ng/ml (mean± SD 6.14±3.47), <0.78ng/ml (0.49 ±0.24) and ≥0.78- ≤1.89ng/ml (1.35, ±0.45) respectively and insulin levels were found to be >25 mIU/L (43.1±16.85), <2 mIU/L(1.8±0.0) and ≥2-<25 mIU/L (13.05±26.58) in 67 (24.71%), 1(1.1%) and 66 (74.1%) subjects respectively which was statistically significant.

The results and observation are shown in Tables 3 & 4 below

Table 3: Distribution of Subjects according to sex

Sex	Subjects	
	(n)	(%)
Male	48	53.9
Female	41	46.1
Total	89	100

Table 4: Distribution of Subjects according to Parameters of Metabolic Syndrome

Parameters of metabolic syndrome	Levels of Parameters (as per cut off values)	Subjects	
		(n)	(%)
Waist Circumference (cm)	Male ≥ 90	48	53.9
	Female ≥ 80	41	46.9
Systolic BP (mm of Hg)	<130	12	13.5
	≥130	77	86.5
Dystolic BP (mm of Hg)	<85	60	67.4
	≥85	29	32.6
Blood Sugar (mg/dl)	<100	33	37.1
	≥100	56	62.9
(mg/dl)	Male <40 & Female <50		
	Male ≥40 & Female ≥50	36	40.4
Triglyceride (mg/dl)	<150	36	40.4
	≥150	53	59.6

(subject: 89) (%: 100). All samples are fasting

Table 5: Distribution of Subjects according to levels of C-Peptide and insulin

Parameter	Range Value	Subjects (n) (%)	Mean±SD
C- Peptide (ng/ml)	<0.78	5 (5.6)	0.49±0.24
	≥0.78-≤1.89	4 (4.49)	1.35±0.45
	>1.89	80 (89.8)	6.14±3.47
Insulin (mIU/L)	<2	1(1.1)	1.8±0.0
	≥2- <25	66(74.1)	13.05±26.58
	≥25	22(24.71)	43.1±16.85
Total		89 (100.0)	

Discussion

MetS is one of the major public health issues of this century.[12] MetS is cluster of physical conditions and metabolic abnormalities commonly found in association with increased risk for development of T2DM, CVD and other medical conditions.[1] If current trend continues, death and disabilities resulting from these conditions in both developed and developing countries will increase the financial burden on them. The frequency of MetS is variable depending on the definition used to determine it, as well as age, sex, ethnic origin and lifestyle.

C-Peptide, Insulin and Metabolic Syndrome

Increasing evidence has recently emerged from several laboratories that C-peptide has great potential relevance to the pathophysiology and treatment of diabetes, possibly acting as a peptide hormone beneficially affecting renal, nervous and microvascular functions in diabetic animals.[13,14] Several studies have found a strong correlation between basal C-peptide and components of metabolic syndrome. These studies have concluded that an elevated fasting serum C-peptide levels can constitute a clinically important biomarker of the cardiovascular risks associated with the Metabolic Syndrome.[5,6] On extensive search of literature we could find hardly such studies related to this and whatever was available several workers have established that high C-peptide levels coexists with hyperinsulinemia in metabolic syndrome.

We have tried to explore the levels of C-peptide and insulin in metabolic syndrome patients.

Demography

We studied 89 patients of MetS and noted the values of each parameter which comes under its diagnostic criteria (Table 4); out of which 48 were males and 41 were females; indicating that the

incidence of MetS in the present study is 53.9% in males & 46.1% in females (Table 3); males being involved more as compared to females. In a study done by Pilar Gayoso-Diz et.al[15] similar results were found. They found that in the overall data set, the MetS prevalence was 19.2% in men vs. 12.1% in women,[15] In many other studies worldwide and in Indian subcontinent, male had a higher prevalence of metabolic syndrome.[16, 17, 18, 19] As we found higher prevalence of metabolic syndrome in men in our study, it is widely recognized that male gender is significantly associated with cardiovascular risk.[20,21] Factors protecting women against cardiovascular risk are not clear, but to some extent may be explained by protective effect of endogenous estrogens against atherosclerosis in premenopausal females.[22] However studies done by Prasad *et al* (2012)[23], Peixoto C et al[24] and Ramchandran A et al[25] found gender preponderance of females over males in subjects with MetS.

Distribution of Subjects according to levels of C-peptide and insulin

In our study c-peptide level >1.89ng/ml, was significant in higher number of subjects. It was found that 80 (89.8%) subject had c- peptide level >1.89ng/ml. 67(24.71%) subjects were found to have insulin level <25 mIU/L and only22 (24.7%) were found to have Insulin level ≥25 mIU/L (Table 5). In our study Insulin level <25 mIU/L was seen in higher number of subjects. In many studies it was found that c-peptide level is higher in metabolic syndrome patients. Chen CH et. al in their study have also shown that the serum C-peptide level is significantly elevated in patients with diabetes and metabolic syndrome.[26]

Brambrink JK et al and Mikines KJ et al in their studies have also shown that serum C-peptide levels increase with increasing age, and previous studies of serum C-peptide levels have interpreted this as an age-related change in insulin secretion. Age-related elevated serum C-peptide levels are possibly a result of decreased total insulin clearance, and age-related decreases in β-cell mass and insulin resistance have been widely reported.[27,28]

Sung-Tae Kim et al in their study found that Basal C-peptide level has a strong association with insulin resistance. Thus, the direct correlations between C-peptide and three different MetS definitions (NCEP-ATP III, WHO, IDF) were verified. They found the basal C-peptide level was increased significantly in the MetS group with diabetes.[29]

Fasting insulin levels are a crude index of insulin secretion and insulin resistance and may underestimate the magnitudes of the associations between insulin resistance and components of MetS[30]. C-peptide appeared to correlate better to the well-known variables of MetS than it did to insulin, possibly suggesting that C-peptide is a better surrogate than insulin for estimating insulin resistance in epidemiological studies[31].

C-peptide is commonly used in preference to insulin measurement when assessing □-cell function in clinical practice. In patients on insulin, C-peptide measurement must be used as exogenous insulin will be detected by insulin assays.[32]

It has been proposed that C-peptide results are corrected for concurrent glucose measurement. While this appears to better correlate with □-cell mass and glucose intolerance after islet cell transplant, there are limited published data using this approach in clinical practice, making interpretation of this ratio difficult.[33,34,35]

C-peptide is a marker of pancreatic insulin synthesis, and several epidemiologic studies have utilized C-peptide as an alternate biomarker to insulin because it has a longer half-life than insulin and therefore is more stable.[36]

Conclusion

In our study we found that Metabolic syndrome effect more males (53.9%) than females (46.1%). C-peptide level was elevated in 90% of subjects which is statistically significant ($p<0.001$) whereas insulin level was within normal range in maximum number of patients; therefore C-peptide may be a better biomarker for high risk of CVD and T2DM in these patients.

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