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Biochemistry

EVALUATION OF RISK MARKERS OF CVD IN METABOLIC SYNDROME

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ABSTRACT

Background: Metabolic syndrome is one of the major public health issues with 30–40% probability of developing type 2 Diabetes Mellitusand/or cardiovascular disease.On extensive search of literature we could hardly find any study which could find a strong correlation between basal C-peptide and insulin levels as a risk marker for CVD and whatever was available the workers have tried to establish that high C-peptide levels coexists with hyperinsulinemia in metabolic syndrome.So we tried to explore the levels of C-peptide and insulin as a risk marker of CVD in the patients of metabolic syndrome.

Methods: After obtaining ethical clearance, a total of 267 patients of metabolic syndrome were selected and enrolled for the present study. All the patients were subjected to complete general and systemic examination and findings noted. Waist circumference was also recorded in all of them. The special investigations like lipid profile, LFT, KFT, fasting blood glucose, C-peptide and Insulin levels were estimated in all the cases and findings recorded and statistically analysed.

Results: Out of the total of 267 subjects,15 (5.6%), 12 (4.5%) and 240 (89.8%) had C- peptide level <0.78 (mean± SD 0.49 ±0.24), 0.78-1.89 (mean± SD 1.35 ±0.45) and >1.89(mean± SD 6.14±3.47) respectively while in 3 (1.1%), 198 (74.1%) and 66 (24.7%) subjects, the insulin levels were found to be in <2 (mean± SD 1.8±0.0), 2-25 (mean± SD 13.05±26.58) and >25 (mean± SD 43.1±16.85) respectively.

Conclusion: In our study 240 (89.8 %) subjects were having elevated C-peptide which is statistically significant (p<0.001), whereas only 66 (24.75) subjects had evaluated insulin level with no statistical significance. Therefore we came to a conclusion that fasting serum C-peptide levels is a better risk marker of CVD rather than fasting insulin levels in patients with Metabolic Syndrome.

KEYWORDS : Metabolic Syndrome, fasting blood glucose, fasting C-peptide, fasting Insulin and biomarker

Introduction

Metabolic Syndrome (Met S) is clinical syndrome consisting of physical and metabolic abnormalities. It is commonly associated with increased risk for development of type-2 diabetes mellitus (T2DM), cardiovascular disease (CVD and other medical conditions.[1] Definition of MetS, as has been proposed by the International Diabetes Federation (IDF) in 2005[2] is widely accepted;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 parameters:

S. No.	Parameters	Description
1	Raised Triglycerides	≥150mg/dL (1.7 mmol/L) Or specific treatment for this lipid abnormality
2	Reduced HDL cholesterol	< 40 mg/dL (1.03 m mol/L) in males < 50mg/dL (1.29 m mol/L) in females or specific treatment for this lipid abnormality
3	Raised Blood pressure	systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension.
4	Raised fasting plasma glucose	FPG ≥100 mg/dL (5.6 m mol/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 Body Mass Index (BMI)is > 30kg/m^2, central obesity can be assumed and waist circumference is not required to be measured.

Table 1. Definition proposed by IDF 2005[2]

The IDF has proposed a new set of criteria with ethnic/racial specific cut-offs.[3] Worldwide prevalence of MetS ranges from <10% to 84 %. Higher socioeconomic status sedentary lifestyle and high Body Mass Index (BMI) were significantly associated with MetS. Furthermore, the prevalence is 1.5-2 times higher in women compared to men.

C-peptide is a 31 amino acids compound and is released from the pancreatic β-cells during cleavage of insulin from pro insulin, which is a single polypeptide chain of 86 amino acids and has three "C-C" cystine bonds, and eventually released into the bloodstream in amounts equimolar with those of insulin. Half life of C-peptide is 3-4 times longer than that of insulin and it is mainly excreted by the kidney. It links the A and B chains in manner that allows correct folding "C-C" disulfide bond formation insulin.[4]

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 risk marker of the cardiovascular risks associated with the MetS. [5,6]. The reference range of C-peptide is 0.78-1.89ng/mL (Conventional units).

Insulin being an anabolic hormone, promotes glucose uptake, glycogenesis, lipogenesis, and protein synthesis, in addition, insulin is important factor in the regulation of plasma glucose homeostasis, as it counteracts actions of glucagon and other catabolic hormones like epinerphrine, glucocorticoid, and growth hormone. The reference range of insulin is 2-25 ,IU/L (conventional Units).

The proposed central abnormality associated with MetS is insulin response (IR).[7] The term IR indicates the presence of an impaired biological response to either exogenously administered or endogenously secreted insulin7 and is associated with the progression to impaired glucose tolerance (IGT) and T2DM. 8.9.

The association of obesity with T2DM has been long recognized. 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), 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 as a risk marker of CVD at our place and find out their correlation.

Material and Methods:

The present study was conducted in the department of Biochemistry, Subharti Medical College, Meerut from June 2016 to May 2017. Patients attending the Metabolic OPD of ChatrapatiShivajiSubharti hospital associated with Medical College were screened for MetS and enrolled for the present study. Informed consent was taken from each patient. Study group included 267 subjects of MetS who fulfilled the criteria of MetS proposed by IDF 2005 within the age group of 21 to 70 years.

Patients having uncontrolled Hypertension, Diabetic Ketoacidosis, Fructose or Galactose intolerance, Congestive Cardiac Failure, Pregnancy or having blood urea and serum creatinine in abnormal range or taking drugs like Insulin, Corticosteroids, Levodopa and Oral contraceptives, were not included in the study.

Waist circumference was recorded in all subjects. General information and detailed medical history was recorded from each subject and they were subjected to complete physical and systemic examinations. 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 estimated in all the cases and findings recorded.

After 10 to 12 hours of fasting, venous blood sample was collected under all aseptic conditions, 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 20-30 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.

Results and Observations:

Out of the total study group of 267 subjects, 144(53.9%) were males and 123(46.1%) were females.

According to the levels of C- peptide, all 267 patients were divided into three groups; patients with values as <0.78ng/ml, 0.78- 1.89ng/ml and >1.89ng/ml, while according to the levels of Insulin, they were divided in to three groups; patients with values as <2mIU/L, 2- 25 mIU/L and >25 mIU/L.

15 (5.6%), 12 (4.5%) and 240 (89.8%) subjects had C- peptide level <0.78 (mean± SD 0.49 ±0.24), 0.78-1.89 (mean± SD 1.35 ±0.45) and >1.89(mean± SD 6.14±3.47) respectively while in 3 (1.1%), 198 (74.1%) and 66 (24.7%) subjects, the insulin levels were found to be in <2 (mean± SD 1.8±0.0), 2-25 (mean± SD 13.05±26.58) and >25 (mean± SD 43.1±16.85) respectively.The results and observation are shown in tables 1 to 5 below:

TABLE 1: DISTRIBUTION OF SUBJECTS ACCORDING TO GENDER

SEX	FREQ	%
MALE	144	53.9
FEMALE	123	46.1
TOTAL	267	100.0

TABLE 2: DISTRIBUTION OF SUBJECTS ACCORDING TO PARAMETERS

PARAMETERS	LEVELS OF PARAMETERS	FREQ	%
WAIST CIRCUMFERENCE (cm)	*Male ≥ 90*	144	53.9
	Female ≥ 80	123	46.9
SYSTOLIC BP (mm of Hg)	*<130*	36	13.5
	≥130	231	86.5
DIASTOLIC BP (mm of Hg)	*<85*	180	67.4
	≥85	87	32.6
BLOOD SUGAR (mg/dl)	*<100*	99	37.1
	≥100	168	62.9
HDL_C (mg/dl)	*Male <40 & Female <50*	159	59.6
	Male ≥40 & Female ≥50	108	40.4
TRIGLYCERIDE (mg/dl)	*<150*	108	40.4
	≥150	159	59.6

TABLE3: DISTRIBUTION OF SUBJECTS ACCORDING TO LEVELS OF C-PEPTIDE AND INSULIN (n=267)

PARAMETER	RANGE	FREQ (%)	MEAN ± SD
C- PEPTIDE	*<0.78*	15 (5.6)	0.49 ±0.24
	0.78-1.89	12 (4.5)	1.35 ±0.45
	>1.89	240 (89.8)	6.14±3.47
INSULIN	*<2*	3 (1.1)	1.8±0.0
	2-25	198 (74.1)	13.05±26.58
	>25	66 (24.7)	43.1±16.85

TABLE 4: CORRELATION BETWEEN C-PEPTIDE AND INSULIN (n=267) WITH REFERENCE TO C-PEPTIDE LEVELS

RANGE (C_PEPTIDE)	r-value	p-value
<0.78	-0.475	0.419(NS)
0.78-1.89	0.046	0.971(NS)
>1.89	0.465	<0.001(SIG.)
Total	0.471	<0.001(SIG.)

NS- not significant, SIG- significant

TABLE 5: CORRELATION BETWEEN C-PEPTIDE AND INSULIN (n=267) WITH REFERENCE TO INSULIN LEVELS

RANGE (INSULIN)	r-value	p-value
<2	--	--
2-25	0.194	.118(NS)
>25	0.405	.061(NS)
Total	0.471	<0.001(SIG.)

NS- not significant, SIG- significant

C-Peptide and Insulin levels are statistically significant and are correlated with each other in all 267 patients as a whole (r-value=0.471), while C-Peptide is statistically significant only in group with >1.89 levels while insulin levels are not statistically significant in any of the three groups. (Table 4 and 5)

There is rather a negative correlation between C-peptide and insulin when C-peptide value is <0.78 and is statistically not significant (table 4 and 5), when C-peptide value is >1.89: the correlation between C-peptide and insulin (r-value=0.465) and significant (table 4 and 5).

Discussion:

MetS is one of the major public health issues of our century.12 This syndrome is cluster of physical conditions and metabolic abnormalities commonly found in association with increased risk for development of T2DM and 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. The frequency of MetS is variable depending on the definition used to determine it, as well as age, sex, ethnic origin and lifestyle.

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 peptide hormone beneficially affecting renal, nervous and microvasular 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 risk marker of the cardiovascular risks associated with the Metabolic Sundrome.5,6 On extensive search of literature we could find hardly any studies correlating C-peptide with insulin as a risk marker of CVD in MetS, and whatever available, have established that high C-peptide levels coexists with hyperinsulinemia in this condition.

We have tried to explore the levels of C-peptide and insulin in 267 metabolic syndrome patients; out of these,144 were males and 123 were females; indicating that the incidence of MetS, is 53.9% in males and 46.1 % in females (Table 1); males being involved more as

compared to females. In a study done by PilarGayoso-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,15In many other studies worldwide and in Indian subcontinent, male had a higher prevalence of metabolic sundrome.[16,17,18,19] As we found higher prevalence of metabolic syndrome in men in our study too, it is widely recognized that male gender is significantly associated with cardiovascular risk. [20,21] Factors protecting women against cardiovasc ular risk are not clear, but to some extent,this 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 etat24and Ramchandran A et al25,found gender preponderance of females over males.

In our study we divided C-peptide level into three groups: < 0.78, 0,78-1.89 and > 1.89 ng/ml; and found that 15 (5.6%) subjects were having Mean±SD as 0.49±0.24, 12 (4.5%) were having 1.35±0.45 and 240 (89.8%) were having 6.14±3.47; While insulin levels were also divided into three groups: < 2, 2-25 and > 25 mIU/L; and found that 3 (1.1%) subjects were having Mean±SD as 1.8±0.0, 198 (71.4%) were having 13.05±26.58 and 66 (24.7%) were having 43.1±16.85 (Table 3).

In our study we observed that C-peptide levels were statistically significantly in 240 (89.8%) patients of MetS with levels >1,89 (r-value 0.465), these values were also statistically significantly in the group as a whole (r- value 0.471) (Table 4).

Insulin levels were not found statistically significantly in any of the group but rather this was found statistically significantly in the group as a whole (r- value 0.471) (Table 5).Chen CH et. alhad also shown that serum C-peptide level is significantly elevated in patients with diabetes and MetS. [26]

C-Peptide and Insulin levels are statistically significant and are correlated with each other in all 267 patients as a whole (r-value=0.471), while C-Peptide is statistically significant only in group with >1.89 levels while insulin levels are not statistically significant in any of the three groups. (Table 4 and 5)

There is rather a negative correlation between C-peptide and insulin when C-peptide value is <0.78 and is statistically not significant (table 4 and 5), when C-peptide value is >1.89: the correlation between C-peptide and insulin (r-value=0.465) and significant (table 4 and 5).

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

Fating insulin level is a crude index of insulin secretion and insulin resistance 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 variables of MetS than it did to insulin, possibly suggesting that C-peptide is a better surrogate than insulin estimating insulin resistance in epidemiological studies[31].

C-peptide is commonly used in preference to insulin measurement when assessing β-cell function in clinical practice. In patients on insulin assessment, C-peptide measurement must be used, because 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 β–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 marker of pancreatic insulin synthesis, and several epidemiologic studies have utilized C-peptide as an alternate risk marker to insulin because it has longer half-life than insulin and therefore is more stable[36]. Findings of RanaUsmani and Bharat Kumar Gupta are also in accordance with these findings.[37]

Conclusion:

In our study 240 (89.8 %) subjects were having elevated C-peptide which is statistically significant (p<0.001), whereas only 66 (24.75) subjects had evaluated insulin level with no statistical significance. Therefore we came to a conclusion that fasting serum C-peptide levels is a better risk marker of CVD rather than fasting insulin levels in patients with Metabolic Syndrome.

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