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EVALUATION OF SERUM C-PEPTIDE LEVELS IN INDIAN TYPE 2 DIABETES INDIVIDUALS ATTENDING TERTIARY DIABETIC INSTITUTE

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It is a major cause of mortality and morbidity worldwide. While evaluating diabetes, C-peptide has been a widely used measure of pancreatic beta cell function. It is produced in equimolar amounts to endogenous insulin but is excreted at a more constant rate over a longer time. The measurement of C-peptide provides a better index of endogenous insulin production and pancreatic beta cell function than insulin measurements. C-peptide level is associated with diabetes type and duration of disease. It is useful for differentiating type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). C-peptide level may also correlate with microvascular and macrovascular complications. It has been proposed as one of the predictors of future use of insulin therapy, as well as likely response to other individual therapies. The current study was conducted to know the utility of c peptide levels in assessing the endogenous insulin secretory function in Indian patients with type 2 diabetes. **Methods:** The present study was conducted in 150 adult patients of type 2 diabetes mellitus presenting in outpatient department of Karnataka institute of endocrinology and research. Subjects with Type 1 diabetes, pregnant women with diabetes, subjects presenting with acute infections, septicemia, patients with acute or chronic pancreatitis, and subjects with pancreatic carcinoma were excluded from the study. In this study C-Peptide levels were estimated by electro chemiluminescence immunoassay method. **Results**: 150 type 2 diabetes subjects were selected randomly. Age group <30 to >70 years, 68.7% were males. Family history for diabetes was positive in 58.7% subjects. Duration of diabetes vary between new to > 10 years. 82% of subjects had BMI 18.5 to 30. 58% of subjects had waist circumference 90 to 110 centimeters. 49.3% had HBA1c 6 to 9% and in 49.3% HBA1c was >9%. Fasting C-peptide levels were normal in 20.7% and high in 70.3% of type 2 diabetes subjects. None of the patients fasting C-peptide was less than normal. BMI, waist circumference and triglyceride levels were high in type 2 diabetes subjects with high C-peptide levels. **Conclusions:** The current clinical role of C-peptide in diabetes is to differentiate type 1 diabetes from type 2 diabetes especially in subjects with overlapping clinical features. There is a limited role in the diagnosis of Type 2 diabetes with marked hyperglycemia where C-peptide testing may support a clinical decision on initial insulin therapy. Routine C-peptide testing in type 2 diabetes with poor glycaemic control can help to decide treatment modalities. It can also help in creating awareness about insulin resistance and need for aggressive lifestyle modification.

KEYWORDS: C-Peptide, HBA1c, triglyceride.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It is a major cause of mortality and morbidity worldwide.[1] Human insulin and c-peptide are synthesized as a single polypeptide chain known as proinsulin in the pancreatic islet by the beta cells. Proinsulin is cleaved proteolytically to form equimolar amounts of mature insulin and C-peptide and both are released in the portal vein. C peptide is a single peptide chain of 31 amino acids with molecular weight of 30200 g/mol. It is called as C-peptide because it connects the A and B chains of insulin in Proinsulin.[2] The majority of c-peptide is metabolized by the kidneys with 5–10% then excreted unchanged in the urine. This can make c-peptide measurement in individuals with chronic kidney disease inaccurate. Modern ultrasensitive c-peptide assays are able to detect c-peptide values as low as 0.0015–0.0025 nmol/l.[3] It is also important to be aware that cross-reactivity with proinsulin must be less than

10%, which is generally the case for modern assays. The presence of large numbers of anti-insulin antibodies that bind both proinsulin and c-peptide can give a falsely high c-peptide reading. In healthy individuals the plasma concentration of c-peptide in the fasting state is 0.3–0.6 nmol/l (0.9 -1.8 ng/ml), with a postprandial increase to 1–3 nmol/l (3-9 ng/ml). (1 nmol/l = 3 ng/ml).

Potential uses of c-peptide are broad and include arriving at appropriate diagnosis, guiding therapy choices, and predicting morbidity in diabetes. Stimulated c-peptide sampling is a sensitive and specific test that can determine type and duration of diabetes. C-peptide is a useful indicator of beta cell function, allowing discrimination between insulin-sufficient and insulin-deficient individuals with diabetes. There is limited evidence with respect to predicting rarer forms of primary diabetes, such as MODY or LADA. It is also hypothesised that a lower c-peptide, can most likely predict requirement for insulin. Lower c-peptide values have also been shown to correspond with increased incidence of microvascular complications. This suggests that c-peptide levels may be used as an essential diagnostic and monitoring tool in type 2 diabetes.[4] Further, with the advent of newer drugs to overcome insulin resistance, it has become all the more important to know about the pathophysiology of diabetes, whether endogenous insulin secretion is normal and to know about insulin resistance. Keeping this perspective in mind, the current study was conducted to know the utility of c peptide levels in beta cell insulin reserve in patients with type 2 diabetes.

MATERIALS AND METHODS

The present study was conducted in 150 adult subjects with Type 2 Diabetes Mellitus presenting in outpatient department of Karnataka Institute of Endocrinology and Research Bangalore. Informed consent was taken from all the subjects included in the study and the approval from hospital ethical committee was taken. Subjects with Type 1 diabetes, pregnant women with diabetes, subjects presenting with acute infections, septicaemia, patients with acute or chronic pancreatitis, and subjects with pancreatic carcinoma were excluded from the study.

Diagnosis of diabetes mellitus was made according to ADA criteria if: HBA1C>6.5% or, fasting plasma glucose greater than 126 mg/dl and in a patient with classic symptoms of hyperglycemia with plasma glucose ≥200mg/dl on more than one occasion. After taking informed consent of the patient, detailed history was taken. Complete general physical examination was done with due emphasis on anthropometry. BMI was calculated by dividing the weight in Kg and the square of the height in meters.

A fasting and post prandial blood sample was taken for estimation of plasma glucose by Hexokinase method and serum lipids using a Hitachi C 311 autoanalyser (Roche Diagnostics, Mannheim, Germany). A1C was measured by the high-performance liquid chromatography method using the Bio-rad Variant 2 turbo analyser.

C-peptide was estimated by electro chemiluminescence immunoassay. In this study, c peptide levels of less than 0.5 ng/ml were considered less than normal, between 0.5 and 3.2 ng/ml as normal and more than 3.2 ng/ml as more than normal.

Study Design: An observational clinical study

STATISTICAL METHODS

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data are made: Dependent variables should be normally distributed, samples drawn from the population should be random, and cases of the samples should be independent.

Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Leven`s test for homogeneity of variance has been performed to assess the homogeneity of variance. A t-test is a statistical test that is used to compare the means of two groups. It is often used in hypothesis testing to determine whether a process or treatment actually has an effect on the population of interest, or whether two groups are different from one another with the null hypothesis (H_0) is that the true difference between these group means is zero and the alternate hypothesis (H_a) is that the true difference is different from zero.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for Qualitative data analysis. Fisher Exact test used when cell samples are very small.

Significant figures were considered on the basis of below values:
+ Suggestive significance (P value: 0.05<P<0.10)
* Moderately significant (P value: 0.01<P ≤ 0.05)
** Strongly significant (P value: P≤0.01)
The Statistical software namely SPSS 22.0, and R environment ver.3.2.2 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

150 type 2 diabetes subjects were selected randomly from patients attending outpatient department of Karnataka institute of endocrinology and research Bangalore. Of these subjects, mean age of the subjects was: 53.85±10.82 years. 39.3% of subjects were in the

age group of 51 -60 years, 22% were in age group of 41-50 years. 68.7% of study subjects were males. Family history for diabetes was positive in 58.7% subjects. (Table 1 to 3).

Table 1: Age distribution of patients studied.

Age in years	No. of patients	%
<30	3	2.0
30-40	17	11.3
41-50	33	22.0
51-60	59	39.3
61-70	28	18.7
>70	10	6.7
Total	150	100.0

Table 2: Gender distribution of patients studied.

Gender	No. of patients	%
Female	47	31.3
Male	103	68.7
Total	150	100.0

Table 3: Family History of patients studied.

Family History Diabetics	No. of patients	%
No	62	41.3
Yes	88	58.7
Total	150	100.0

Duration of diabetes vary between new to > 10 years. 24.7% subjects had duration of >10 years, and 24% of the subjects had duration of diabetes between 2 to 5 years. 82% of subjects had BMI 18.5 to 30. 58% of subjects had waist circumference 90 to 110 centimetres. (Table 4 to 5)

Table 4: Duration - frequency distribution of patients studied.

Duration	No. of patients	%
New	20	13.3
Up to 2yrs	29	19.4
2-5yrs	36	24.0
5-10yrs	28	18.7
>10yrs	37	24.7
Total	150	100.0

Table 5: BMI/WC-frequency distribution of patients studied.

	No. of patients (n=150)	%
BMI (kg/m^2)		
• <18.5	1	0.7
• 18.5-25	57	38.0
• 25-30	66	44.0
• >30	26	17.3
Waist circumference		
• <90	56	37.3
• 90-110	87	58.0
• >110	7	4.7

49.3% had HBA1c 6 to 9% and in 49.3% HBA1c was >9%. Fasting C-peptide levels were normal in 20.7% and high in 70.3% of type 2 diabetes subjects. None of the patients fasting C-peptide was less than normal. BMI, waist circumference and triglyceride levels were high in type 2 diabetes subjects with high C-peptide levels. HDL levels were lower in type 2 diabetes subjects with higher c-peptide levels and it was statistically significant (Table 6 to 10)

Table 6: Blood Glucose parameters -frequency distribution of patients studied.

	No. of patients (n=150)	%
FPG		
• <120	31	20.7
• 120-150	35	23.3
• >150	84	56.0
PPG		
• <200	27	18.0
• 200-300	65	43.3
• >300	58	38.7
HbA1c%		
<6	2	1.3
6-9	74	49.3
>9	74	49.3

Table 7: FASTING C- PEPTIDE-frequency distribution of patients studied.

FASTING C-PEPTIDE	No. of patients	%
0.5 to 3.2 nanogram/ml	31	20.7
>3.2 nanogram/ml	119	79.3
Total	150	100.0

Table 8: Comparison of clinical variables according to fasting C-peptide of patients studied.

VARIABLES	FASTING C PEPTIDE		Total	P value
	0.5 to 3.2 nanogram/ml	>3.2 nanogram/ml		
Age in years	54.68±9.54	53.63±11.16	53.85±10.82	0.633
BMI (kg/m^2)	23.52±4.53	27.62±4.74	26.78±4.97	<0.001**
Waist circumference	89.26±10.35	93.50±9.24	92.63±9.60	0.028*
SBP (mm Hg)	127.16±19.06	137.20±20.98	135.13±20.94	0.017*
DBP (mm Hg)	79.10±10.18	81.68±10.85	81.15±10.73	0.234

FPG	211.29±80.99	167.26±58.22	176.36±65.76	0.001**
PPG	341.68±116.04	273.15±90.86	287.31±100.12	0.001**
HbA1c%	10.19±2.37	8.79±1.72	9.08±1.95	<0.001**

Table 9: Comparison of lipid profiles in relation to fasting peptide of patients studied.

LIPIDS	FASTING C PEPTIDE		Total	P value
	0.5 to 3.2 nanogram/ml	>3.2 nanogram/ml		
Total Cholesterol (mg/dl)	186.39±46.61	182.8±45.69	183.54±45.75	0.699
LDL (mg/dl)	117.87±41.22	117.96±38.82	117.94±39.19	0.991
HDL (mg/dl)	42.52±8.31	37.73±7.04	38.72±7.55	0.001**
TGL (mg/dl)	165.29±92.29	193.08±116.29	187.33±112.04	0.220

Table 10: Pearson Correlation.

PAIR	r value	P value
FASTING C PEPTIDE **vs** Age in years	0.062	0.453
FASTING C PEPTIDE **vs** BMI (kg/m^2)	0.364	<0.001**
FASTING C PEPTIDE **vs** Waist circumference	0.252	0.002**
FASTING C PEPTIDE **vs** SBP (mm Hg)	0.084	0.304
FASTING C PEPTIDE **vs** DBP (mm Hg)	-0.010	0.906
FASTING C PEPTIDE **vs** FPG	-0.208*	0.010**
FASTING C PEPTIDE **vs** PPG	-0.287**	<0.001**
FASTING C PEPTIDE **vs** HbA1c%	-0.300**	<0.001**
FASTING C PEPTIDE **vs** Total Cholesterol (mg/dl)	-0.162*	0.048*
FASTING C PEPTIDE **vs** LDL (mg/dl)	-0.190*	0.020*
FASTING C PEPTIDE **vs** HDL (mg/dl)	-0.137	0.094+
FASTING C PEPTIDE **vs** TGL (mg/dl)	0.077	0.349
FASTING C PEPTIDE **vs** Creatinine (mg/dl)	0.142	0.082+

DISCUSSION

The estimation of C – peptide levels mainly allows differentiation between insulin-sufficient and insulin-deficient individuals with diabetes. Along with that, there is now evidence that suggests that c-peptide can also be useful in predicting future levels of glycemic control, response to hypoglycemic agents, and also the risk of future diabetes complications in type2 diabetes.[4]

Our study shows that 79.3% of the studied subjects with type 2 diabetes had fasting C-peptide concentrations above normal range. 20.7% subjects with normal c-peptide levels and no subjects had less than normal c-peptide levels. This indicates that the prevalence of insulin resistance is higher compared to beta cell dysfunction. In our study, it was noted that the C – Peptide levels didn't reduce as the duration of diabetes increased. This suggests that fasting C-peptide levels cannot be used as a marker for duration of type 2 diabetes. BMI, waist circumference and triglyceride levels were high in type 2 diabetes subjects with high C-peptide levels. This demonstrates that those overweight and obese type 2 diabetics have higher Insulin resistance. HDL levels were lower in type 2 diabetes subjects with higher c-peptide levels and it was statistically significant. This indicates that subjects with insulin resistance have low HDL levels.

C-peptide concentrations have been investigated in several studies to assess beta cell insulin reserves in patients with type 2 diabetes. A study by Diabetes Outpatient Clinics in Istanbul showed that 94% of patients with type 2 diabetes treated with insulin had borderline or sufficient beta cell reserve, and insulin resistance-related parameters were prominent in those with adequate beta cell reserve.[5]

In a study by Deep et al, patients with type 2 diabetes were categorized as having insufficient beta cell reserve (C-peptide <0.5 ng/mL) in 2%, sufficient beta cell reserve (C- peptide: 0.5-3.2 ng/mL) in 38%, and high beta cell reserve (C-peptide: >3.2 ng/mL) in 60% subjects. They recommended assessing C-peptide concentrations in patients with poor glycemic control to improve treatment choices.[6]

In a retrospective analysis of 179 patients with type 2 diabetes who were already diagnosed as having diabetes and were under treatment, the mean C-peptide level was found as 2.71 ng/mL, and they stated that 12 (6.7%) of patients had insufficient beta cell reserves (C-peptide <0.5 ng/mL), whereas 70 (39.1%) had borderline (C-peptide: 0.5-2 ng/mL) and 97(54.2%) had sufficient (C-peptide > 2 ng/mL) beta cell reserves.[7]

Another cross-sectional study was conducted in a tertiary hospital of Bangladesh to assess fasting serum c-peptide as a marker of the endogenous insulin secretory capacity in newly diagnosed subjects with type 2 diabetes mellitus (T2DM). 60 newly diagnosed type 2 diabetes subjects were investigated along with 30 age sex matched healthy controls Fasting c-peptide was significantly higher in

T2DM subjects than controls (8.97±5.96 vs. 1.69±0.66 ng/ml). None of the T2DM subjects had subnormal c-peptide, 19 (32%) had normal c-peptide, and 41 (68%) of them had elevated c-peptide levels. Higher fasting plasma glucose (FPG), plasma glucose 2-hours after 75gm oral glucose tolerance test (PG 2H-OGTT) and HbA1c levels were observed in T2DM subjects with elevated c-peptide in comparison to T2DM subjects having normal c-peptide. In T2DM subjects, c-peptide showed significant positive correlations with body mass index (BMI), FPG, PG 2H-OGTT, and HbA1c.[8]

An observational study of 30 obese and 30 non-obese subjects with T2DM in Kerala showed that the obese subjects with T2DM had higher basal C-peptide values compared to the non-obese subjects. Mean C-peptide of obese group was 6.31 ± 2.2ng/mL and that of the non-obese group was 3.53 ± 2.7 ng/mL ($p < 0.05$). There was no significant difference in FBS between the two groups ($p > 0.05$). Mean HbA1c of the obese group was 6.9 and that of the non-obese group was 6.0 ($p < 0.05$).[9]

A cross sectional study was done in 75 subjects with type 2 diabetes in the diabetic outpatient clinic of a tertiary care hospital, Bijapur. The fasting c-peptide levels and fasting plasma glucose levels in the obese patients were increased compared to the non obese individuals, indicating insulin resistance. HbA1c levels were increased more in obese patients indicating poor glycaemia due to insulin resistance. Unlike our study, these authors found that fasting c-peptide levels decreased as the duration of diabetes increased.[10]

In summary, our study adds to the pre existing evidence about C-peptide levels in type 2 diabetes. All the studied subjects with type 2 diabetes have adequate insulin reserve. The obese patients have higher c-peptide levels compared to the non obese patients. The C- peptide levels didn't consistently decrease with increasing duration of diabetes. HDL levels were lower in type 2 diabetes subjects with higher c-peptide levels and it was statistically significant.

The current clinical role of C-peptide in predicting treatment response is principally to exclude severe endogenous insulin deficiency in insulin-treated patients when considering insulin withdrawal or when considering the addition of therapies dependant on endogenous insulin for their action. There may be a limited role in the diagnosis of Type 2 diabetes with marked hyperglycaemia where C-peptide testing may support a clinical decision on initial insulin therapy. Evidence for a clinical role of C-peptide in predicting response to specific hypoglycaemic agents is weak. There is evidence that more insulin-resistant patients with higher C-peptide values have increased response to thiazolidinediones. This does not appear to be the case for metformin, sulphonylureas and dipeptidyl peptidase-4 (DPP-4) inhibitors.

Limitations of the study

One of the limitations of our study is the method we used to evaluate beta cell reserves. The preferred method for C-peptide measurement in the evaluation of endogenous insulin reserve is usually glucagon-stimulation C-peptide testing or mixed-meal tolerance test. The fact that C-peptide concentrations can be suppressed in extreme hyperglycemic conditions should also be taken into consideration when interpreting our results.

CONCLUSIONS

The current clinical role of C-peptide in diabetes is to differentiate type 1 diabetes from type 2 diabetes especially in subjects with overlapping clinical features. There is a limited role in the diagnosis of Type 2 diabetes with marked hyperglycemia where C-peptide testing may support a clinical decision on initial insulin therapy. Routine C-peptide testing in type 2 diabetes with poor glycaemic control can help to decide treatment modalities. It can also help in creating awareness about insulin resistance and need for aggressive lifestyle modification.

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ABBREVIATIONS

T2DM- Type 2 Diabetes mellitus.
BMI-Body mass index.
WCR-Waist circumference.
SBP-Systolic blood pressure.
DBP-Diastolic blood pressure.
FPG-Fasting plasma glucose.
PPPG-Post prandial plasma glucose.
HBA1C-Glycosylated haemoglobin.
C-Peptide-Connecting peptide.
OGTT-Oral glucose tolerance test.
ADA-American diabetes association.
LDL-Low density lipoprotein.
HDL-High density lipoprotein.
TGL-Triglycerides.

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