SERUM C-PEPTIDE LEVEL IN OBESE AND NON-OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

BACKGROUND

C-peptide is a reliable indicator of endogenous insulin secretion in type 2 diabetes mellitus. The worldwide explosion of obesity has resulted in an ever increasing prevalence of Type 2 Diabetes Mellitus (T2DM). Hyperinsulinaemia and insulin resistance are often emphasised as characteristics of T2DM. Obesity is associated with insulin resistance, thus highlighting the importance of life-style modifications in the management of T2DM.

MATERIALS AND METHODS

30 obese and 30 non-obese T2DM patients in the age group 18 - 65 years were selected for the study based on their body mass index. Their basal C-peptide level in serum was assayed by ELISA technique. Fasting blood glucose and HbA1c were assayed to assess the glycaemic status of the study subjects. Statistical analysis was done using SPSS version 22.0. Quantitative data were expressed as mean and SD and quantitative data for the two groups were compared with unpaired 't' test. Association between C-peptide and other parameters was assessed by Pearson correlation. P value less than 0.05 is considered significant.

RESULTS

The obese T2DM patients had higher basal C-peptide values compared to the non-obese patients. Mean C-peptide of obese group was 6.31 ± 2.2 ng/mL and that of the non-obese group was 3.53 ± 2.7 ng/mL. Mean C-peptide of the obese group was significantly higher than that of the non-obese group ($p < 0.05$). 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. The difference observed is statistically significant ($p < 0.05$). This is suggestive of poor control of glucose in the obese due to insulin resistance.

CONCLUSION

Higher C-peptide levels in obese T2DM patients denote insulin resistance, which highlights the importance of lifestyle modifications in the management of T2DM.

KEYWORDS

C-peptide, T2DM, Obesity, Insulin Resistance.

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BACKGROUND

Diabetes mellitus is described as one of the main threats to human health in the twenty first century. Type 2 Diabetes Mellitus (T2DM) comprises approximately 90% of all cases of diabetes mellitus.[1] According to WHO epidemiology reports, the number of adults with T2DM will rise from 135 million in 1995 to 300 million in 2025.[2] The prevalence of T2DM is rising rapidly because of increasing obesity and reduced activity levels as countries become more industrialised[3]; 60% - 80% of patients with T2DM are obese.[4] Obesity is associated with an increased risk of developing insulin resistance, which is a risk factor for T2DM.[5] Insulin resistance is characterised by decreased ability of insulin to act on peripheral tissues. Here, insulin concentration is rather higher than that in those with normal glucose tolerance indicating that insulin resistance rather than insulin deficiency is the fundamental defect in obesity and T2DM. This is followed by beta cell dysfunction in due course of time, as the beta cells fail to keep up with the body's need for insulin. Insulin resistance precedes the derangement in insulin secretion and clinical diabetes by as much as 20 years.[6] Obesity itself can accelerate the progressive decline in beta cell function in patients with T2DM.[7]

Insulin level in blood may be assayed to monitor the amount of insulin secreted by the beta cells and to check for insulin resistance.[8] With the rising incidence of T2DM in younger patients and development of new therapies aimed at preserving insulin secretion, the measurement of insulin secretion is becoming increasingly relevant. Assay of serum insulin as a measure of insulin secretion has several limitations. Recent evidence proves that C-peptide, a cleavage product released into the circulation during insulin synthesis can be used as an indicator of insulin secretion. C-peptide is a 31 amino-acid peptide that links the A chain and B chain in the proinsulin molecule. It facilitates the efficient assembly, folding and processing of insulin in the endoplasmic reticulum. It is cleaved from proinsulin and stored in secretory granules of beta cells of pancreas to be released into the blood stream in amounts equimolar with those of

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insulin. The physiology of C-peptide makes it appropriate for assessing insulin secretion. It is less susceptible to hepatic degradation with a half-life 2 - 5 times longer than that of insulin, is free from interference with insulin antibodies and allows discrimination of endogenous and exogenous sources of Insulin.[9] Hence, it is a more reliable indicator of insulin secretion than insulin itself.

C-peptide was initially thought to be an inactive substance. Its role in diabetes management has been expanding for the past few decades. It has recently been shown to be a biologically active peptide which corrects vascular, neural and renal dysfunction in patients with Type 1 Diabetes Mellitus (T1DM). Replacement therapy with C-peptide has been shown to ameliorate the microvascular complications, especially nephropathy, neuropathy and retinopathy in T1DM.[10] Similar studies in T2DM patients are limited. A comparative study of basal serum C-peptide levels between obese and non-obese patients with T2DM will help us to compare the insulin secretion in the obese and non-obese patients as one C-peptide molecule is released into the circulation with the secretion of each insulin molecule. The role of obesity in the pathogenesis of insulin resistance and thereby T2DM can be assessed. A high C-peptide level denotes insulin resistance and a low level denotes beta cell dysfunction. This will help us to alter the modality of treatment by incorporating lifestyle changes that can reverse insulin resistance by increasing insulin sensitivity.

MATERIALS AND METHODS

This was a cross-sectional study carried out in the Diabetic Clinic of Government Medical College, Thiruvananthapuram. The study was conducted after getting approval from Institutional Ethics Committee according to the provisions of Helsinki declaration. A written informed consent was obtained from the participants. The study subjects included 60 diagnosed T2DM patients between 18 - 65 years of age. The study sample was selected on a random basis. They were grouped into obese and non-obese based on the WHO criteria of obesity in adult Asians.[11]

Body mass index was calculated from the formula: BMI = Body Weight in Kg/Height in m^2. Those with a BMI > 25 were grouped as obese and those with a BMI < 25 were grouped as non-obese.

Patients with acute infections, renal failure, hypertension, polycystic ovarian syndrome and pregnancy were excluded from the study.

Collection of Blood Samples

6 mL venous blood was drawn in a fasting condition, under strict aseptic precautions. Blood for C-peptide assay was collected in plain sample tubes, blood for FBS estimation in bottles containing sodium fluoride and blood for HbA1c estimation in EDTA bottles. Serum for C-peptide assay was separated by centrifugation and kept at -20^0C until analysis.

C-peptide assay was done using ELISA kit from DRG Industries. C-Peptide ELISA is a solid phase enzyme-linked immunosorbent assay, based on the principle of competitive binding. Microtitre wells are coated with anti-mouse antibodies, which bind a monoclonal antibody directed towards a unique antigenic site on the C-peptide molecule. Endogenous C-peptide of a patient sample competes with a C-peptide - horseradish peroxidase conjugate for binding to the coated antibody. The amount of bound peroxidase conjugate is inversely proportional to the concentration of C-peptide in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of C-peptide in the patient sample. Normal serum C-peptide value was found to be 0.5 - 3.2 ng/mL.[12]

Blood glucose was assayed by glucose oxidase method[13] in fully automated clinical chemistry analyser manufactured by Transasia Biomed.

HbA1c was estimated by Ion exchange resin method[14] to assess the glycaemic status of the patients over the previous 6 - 8 weeks.

Statistical analysis was done using SPSS version 22.0. Quantitative data were expressed as Mean and Standard deviation. Qualitative data were expressed as proportion and compared by chi-square test. Quantitative data for the two groups were compared with unpaired 't' test. Association between C-peptide and other parameters was assessed by Pearson correlation. P value less than 0.05 is considered significant. Multiple linear regression model was done to assess the independent predictors of C-peptide.

RESULTS

The study group consisted of 60 T2DM patients, of which 30 were obese and 30 were non-obese. The subjects in the present study were aged between 34 and 65 years. Out of these 23 were in the age group 50 - 59 years and 21 were over 60 years of age. Majority of the obese patients were in the age group 50 - 59 years (43.3%) and majority of the non-obese were above 60 years of age (46.7%). Mean age of the obese group is 52.3 ± 8.1 years and that of the non-obese group is 56.0 ± 8.0 years; the observed difference was not statistically significant ($p > 0.05$). There was a female preponderance in the study population. There were 46 females (76.7%) and 14 males (23.3%); 90% of the obese and 63.3% of the non-obese were females and 10% of the obese and 36.7% of the non-obese were males.

FBS of the study population ranged from 87 to 360 mg/dL; 35% had an FBS between 100 and 150 mg/dL and 25% had an FBS between 150 and 199 mg/dL. Mean FBS of the study population was 175.53 ± 67.89 mg/dL. Mean FBS in the obese group was 190.23 ± 72.81 mg/dL and that in the non-obese group was 160.83 ± 60.23 mg/dL. There was no significant difference in FBS between the two groups ($p > 0.05$).

18% of the study population had an HbA1c level above 8%, which included 26.7% of the obese and 10% of non-obese patients indicating poor control of diabetes mellitus. There was significant difference in mean HbA1c level between obese and non-obese T2DM patients (Table 1). Obese T2DM patients had comparatively higher HbA1c levels than the non-obese ($p < 0.05$).

65% of the study population had a C-peptide value above 3.2 ng/mL and 35% had a value between 0.5 and 3.2 ng/mL; 93.3% of the obese patients had a C-peptide value above 3.2 ng/mL, while only 36.7% of the non-obese had a C-peptide value above 3.2 ng/mL. The observed difference in C-peptide value between obese and non-obese was statistically significant ($p < 0.05$). Mean C-peptide of the obese group was significantly higher than that of the non-obese group ($p < 0.05$) (Table 2). Correlation of C-peptide with other variables showed significant positive correlation of C-peptide with BMI

(Table 3, Figure 1) and FBS (Table 3, Figure 2) with a $p < 0.05$. Serum C-peptide showed weak correlation with age and HbA1c. Multiple linear regression model for C-peptide was done with the factors identified during univariate analysis. C-peptide is shown to be associated with obesity even after adjusting for HbA1c, age and sex differences between the groups and the adjusted difference is statistically significant ($b = 2.681$) ($p < 0.001$).

Study Group	N	HbA_1C		t	p
		Mean	SD		
Obese	30	6.9	1.4	2.585	0.012
Non-Obese	30	6.0	1.2		
Total	**60**	**6.45**	**1.37**		

Table 1. Comparison of Mean HbA1c among Obese and Non-Obese T2DM Patients

Study Group	Mean	SD	t	p
Obese	6.31	2.2	4.32	0.001
Non-Obese	3.53	2.7		
Total	**4.92**	**2.85**		

Table 2. Comparison of Mean C-Peptide Level among Obese and Non-Obese T2DM Patients

Variable	Pearson Correlation	p
Age	0.074	0.572
BMI	0.441	0.001*
FBS	0.334	0.009*
HbA1c	0.246	0.058

Table 3. Correlation of C-Peptide with Other Study Variables *-Significant

Variable	Regression Coefficient (b)	Std. Error	t	P value
Obesity	2.681*	0.713	3.762	< .001*
HbA1c	0.173	0.254	.681	.499
Age	0.064	0.041	1.583	.119
Sex	0.718	0.823	.872	.387

Table 4. Multiple Linear Regression Model for C-Peptide *-Significant

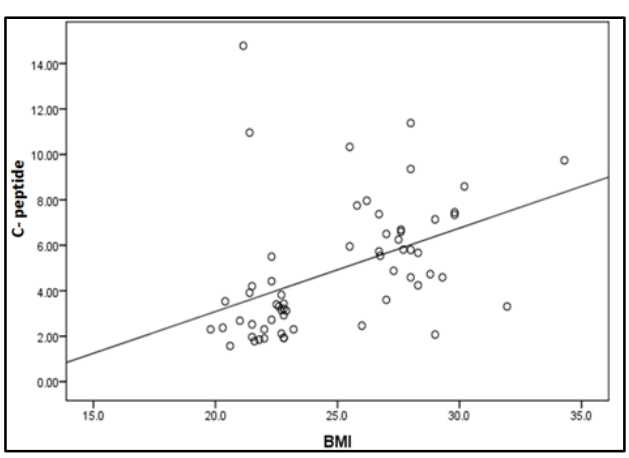

Figure 1. Correlation of C-Peptide with BMI

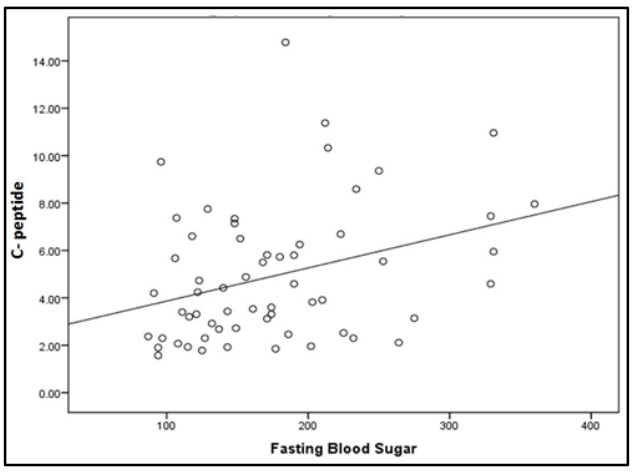

Figure 2. Correlation of C-Peptide with FBS

DISCUSSION

The present study has demonstrated that basal C-peptide level is significantly higher in obese T2DM patients compared to non-obese T2DM patients. This reflects a higher insulin secretion in them. This finding is in accordance with those reported by Sung Tae Kim et al, which showed that basal C-peptide concentration is mainly influenced by BMI.[15] Similar results reported by Michael H. Shanik et al concludes that obese patients are hyperinsulinaemic.[16]

Assay of fasting and post-glucose load of C-peptide levels in obese subjects by Enzo Bonora et al showed that the high C-peptide level seen in obesity is due to hyperinsulinaemia, which depends on pancreatic hypersecretion of insulin and C-peptide in the fasting state.[17] The findings are in accordance with the observation by Olefsky et al that insulin resistance and hyperinsulinaemia are associated with obesity and both abnormalities improve after weight loss, thus highlighting the importance of lifestyle modifications in the management of T2DM.[18] Correlation of C-peptide with other variables showed positive correlation of basal C-peptide with BMI and FBS. As BMI increases, serum C-peptide also increases. The increased insulin indicated by elevated C-peptide is unable to control the blood sugar, probably due to insulin resistance. This is indicated by a high HbA1c level in the obese. A cross-sectional study in the North Karnataka population yielded similar results.[19]

To prevent the development of T2DM, early detection of impaired glucose regulation may represent an appropriate strategy, as subjects with impaired glucose tolerance are at increased risk of developing T2DM.[20] Intervention studies have demonstrated that adoption of a healthy lifestyle characterised by healthy eating, regular physical activity and subsequent modest weight loss can prevent the progression of impaired glucose tolerance to clinical diabetes.[21]

The Diabetes Prevention Program Research Group suggests that future approaches to diabetes prevention should preferably include approaches that enhance insulin sensitivity.[22] Lifestyle modifications aimed at weight loss may result in providing a better glycaemic status in established T2DM. Prospective studies are needed to confirm the hypothesis that early therapeutic interventions aimed at reducing work load on beta cells and preserving even small residual beta cell secretion may modify the natural development of diabetes. Patients with adequate reserve may be managed with diet and exercise modalities to improve

insulin sensitivity. C-peptide in replacement doses for short duration has been shown to improve the early stage functional and structural abnormalities of kidneys and peripheral nerves.[23] Further studies are required to assess the long-term therapeutic effects of C-peptide as T2DM is associated with loss of beta cell function in due course of time. C-peptide has been shown to cause disaggregation of insulin leading to enhanced physiological effects of insulin.[24] This needs to be proven by further studies, such that incorporation of C-peptide in the insulin schedule can be attempted for better glycaemic control.

CONCLUSION

The present study has succeeded in demonstrating higher levels of C-peptide in obese T2DM patients, which proves the presence of higher levels of insulin secretion in them compared to the non-obese group. In spite of this hyperinsulinaemia, control of blood sugar is poor in the obese group as suggested by the significantly higher HbA1c values in them. This positively demonstrates the presence of insulin resistance in this group. Thus, we conclude that obesity leads to insulin resistance and is an important risk factor for poor glycaemic control in T2DM.

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