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Original Research Article

Study of age, sex and body mass index wise variation in C-peptide level among urban population of Northern part of Bihar

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ABSTRACT

Background: Different factors regulate the insulin level in blood. Earlier C-peptide was considered as by product of insulin biosynthesis and its role in body seems to be negligible. C-peptide at low physiological concentrations mimics the effects of insulin. However, in the presence of elevated level of insulin concentrations concomitant raised level of C-peptide may blunt peripheral effects of insulin age. This study was carried out to verify the age, sex, and BMI wise variation in level of C-peptide among apparently healthy individual and its contribution in fine-tuning of the tissue's metabolism under different physiological conditions.
Methods: This is a prospective cross-sectional observational study. Estimation of serum c-peptide level was done by solid phase direct sandwich enzyme linked immunosorbent assay (ELISA) kit method. Glucose is estimated by Glucose oxidase peroxidase (GOD-POD) end point colorimetric method and HbA1C was estimated by ion exchange resin method (kit method).
Result: Serum C-peptide level is significantly high in advance age and males in compared to younger age and females respectively. The mean level of serum C-peptide is higher in higher body mass index (BMI) group compared to lower, but this difference is statistically insignificant.
Conclusions: In normal subjects, the level of C-peptide shows positive correlation with age and BMI. Males have higher level of C-peptide in comparison to females. Apart from these variation C-peptide contributes to different biological effects.

Keywords: Age, Sex, BMI, C-peptide

INTRODUCTION

Different factors regulate the insulin level in the blood, such as aging of skeletal muscle, mitochondrial dysfunction, intramyocellular lipid accumulation, increased inflammation, oxidative stress, changes in the activities of enzymes that regulate insulin sensitivity, endoplasmic reticulum stress, decreased autophagy, sarcopenia, and over-activated RAS. These all induce insulin resistance. These processes can impair skeletal muscle insulin sensitivity and increase the risk of insulin resistance and diabetes.[1]

In 1968 Chance et al isolated and characterized proinsulin from crystalline porcine insulin and identified the sequence of 33 amino acid peptides which links the insulin A and insulin B chains. These 33 amino acid peptide chains were designated as 'connecting peptide' or 'C-peptide'.[2] Earlier C-peptide was considered a by-product of insulin biosynthesis and its role in the body seems to be negligible. It was regarded as an inert by-product that only plays a vital role in insulin synthesis by interlinking A and B chains of insulin for correct folding and interchain disulfide bond formation.[3] Later, in 1996 after positive experimental evidence, it was concluded that C-peptide is a biologically active hormone. In humans, C-peptide is

composed of 31 amino acids, having a molecular weight of about 3021 Daltons.[4] The human C-peptide chain of 31 amino acids contains 5 acidic amino acids while it is devoid of basic as well as aromatic amino acids. About half of the C-peptide (approximately 50%) produced by beta cells is removed by the kidney. The majority of which is degraded via peritubular uptake while approximately 5-10% of total C-peptide produced is excreted unchanged in the urine (>85% excreted after metabolizing in the kidney).[5,6]

C-peptide measurement can be used strongly for the assessment of insulin secretion in a person without any renal impairment.[7] C-peptide binds to the cell surface receptor for its biological action. Following the binding of C-peptide to the cell surface receptor its intracellular signal transduction is initiated by activation of the Ca^{2+} ion-dependent pathway with subsequent stimulation of $Na^{+}K^{+}$ATPase and eNOS activities.[8-10] By improving these enzyme systems, C-peptide improves renal function, increases blood flow, augments glucose utilization, and improves autonomic and somatic nerve functions.[11,12] C-peptide at low physiological concentrations mimics the effects of insulin however in the presence of elevated level of insulin concentrations concomitant raised level of C-peptide may blunt peripheral effects of insulin.[13,14] Some studies on diabetic patients and animals have proven that treatment with C-peptide alone or in combination with insulin has improve physiological functions and this is beneficial in preventing complications associated with diabetes mellitus.[15] This study was carried out to verify the age, sex, and BMI-wise variation in the level of C-peptide among apparently healthy individuals and its contribution to fine-tuning of the tissue's metabolism under different physiological conditions.

METHODS

This was a prospective cross-sectional observational study carried out on 60 healthy individuals, in the department of biochemistry, Darbhanga medical college, Laheriasarai from March 2017 to November 2017 after approved by institutional ethical committee with submission no DMC/209/26122016. All biochemical investigations were performed in the postgraduate research laboratory, department of biochemistry, Darbhanga medical college, Laheriasarai a tertiary care teaching center.

Inclusion criteria

Healthy adults of the 18-60 years age group of both sexes (Doctors, college students, and college staff) were included in the study subject.

Exclusion criteria

Pregnant or lactating females, known cases of diabetes mellitus, hypertension, chronic kidney disease, pre-existing chronic liver disease as well as patients on corticosteroids and lipid-lowering drugs were excluded.

After verification of the study subject for fulfilling the inclusion and exclusion criteria, their informed consent was taken and a detailed history of all the study subjects was recorded. Blood samples were collected in the morning after overnight fasting (minimum 8 hr) for estimation of serum fasting C-peptide, plasma fasting blood sugar (FBS), and HbA1C. After taking all aseptic and antiseptic precautions about 6 ml of venous blood sample was collected by using 22 gauze needles and a syringe. Among which 2 ml sample was transferred to the plain vial for estimation of serum C-peptide level while 2 ml blood was transferred to sodium fluoride vial for estimation of FBS and 2 ml blood was transferred to the EDTA vial for estimation of HbA1C. After collection of the sample, the plain vial sample was allowed to clot at room temperature for 30 min. then serum was separated from a plain vial by centrifugation at 2500 rpm for 20 min and plasma was separated from a sodium fluoride vial by centrifugation at 2500 rpm for 20 min by using a fixed rotor centrifugation machine of Scientex. hemolyzed samples were not used for estimation. If estimation was not done on the same day, samples were stored in aliquots at 2-8 ^{0}C in refrigerator. All tests were done within two days of collection. Estimation of serum c-peptide level was done by solid phase direct sandwich ELISA kit method. Glucose is estimated by GOD-POD endpoint colorimetric method on Erba semiautomatic machine after verification of single levels of internal quality control and HbA1C was estimated by ion exchange resin method (kit method). The normal reference range of our lab for fasting serum C-peptide, serum HbA1C and plasma FBS was respectively 0.5-3.0 ng/ml, 4.60 -6.20% and 70-110 mg/dl.

Statistical analysis

After verification of all the biochemical test results, out of 60, 10 subjects were excluded from the study due to their abnormally raised FBS level or HbA1C level, or both. Finally, Statistical analysis was done on the data collected from 50 study subjects**.** The obtained data were analyzed using SPSS statistical software (v 24; IBM Corporation, Armonk, NY, USA). Data were represented as the mean and standard deviation for the serum levels of C-peptide, insulin, FBS, and HbA1C of the study groups. $P<0.05$ was considered statistically significant.

RESULTS

Among the study group of 50 subjects, 12 (24%) subjects were in the 20-29 years of age group, 13 (26%) subjects were in the 30-39 age group, 12 (24%) subjects were in the 40-49 years of age group and 13 (26%) subjects were of 50-59 years of age group. Based on sex, out of 50, 29 (58%) subjects were male, and 21 (42%) subjects were females. Based on BMI, there are 20 (40%) subjects had a BMI <25 g/m^2 while 30 (60%) subjects had BMI ≥25 kg/m^2.

Table 1 show that among 50 subjects of our study group had serum C-peptide (ng/ml) range from 0.52-2.98 (ng/ml)

mean 1.83 (ng/ml) and SD±0.75, HbA1C range from 4.60-6.20% mean 5.40% SD±0.81 and plasma FBS range from 68.8-110 mg/dl mean 96.12 mg/dl and SD±10.56.

Table 2 shows that as age advances, the level of serum C-peptide rises in the study group. The mean level of serum C-peptide in the 50 -59-year age group is 2.15±0.6s43 ng/ml and in the younger age group is 1.56±0.845 ng/ml and the difference in the mean level of serum C-peptide is statistically significant (p<0.05).

The mean level of serum C-peptide is more in males than females in the study group as shown in Table 3. The mean level of C-peptide in males is 1.86±0.77 ng/ml and in females is 1.79±0.73 ng/ml. and this sex-wise difference in the level of serum C-peptide is statistically significant (p<0.05).

The mean level of serum C-peptide is more in higher BMI subjects (≥25 kg/m^2) than in lower BMI subjects (<25 kg/m^2) in the study group, as shown in Table 4. The mean level of C-peptide in higher BMI subjects is 2.01±0.69 ng/ml while in lower BMI subjects is 1.54±0.76 ng/ml. Although the mean level of serum C-peptide is higher in the higher BMI group, but this difference is statistically insignificant (p>0.05).

Table 1: Level of serum C- peptide (ng/ml), HbA1C (%) and plasma FBS (mg/dl) in study group.

No. of subjects	C-peptide (ng/ml)		HbA1C (%)		FBS (mg/dl)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
50	0.52-2.98	1.83±0.75	5.20-7.80	6.40±0.812	68-110	96.12±10.56

Table 2: Level of serum C-peptide (ng/ml) of different ages in the study group.

Age (In years)	No. of subjects	C-peptide (ng/ml)		T value	P value*
		Range	Mean ± SD		
20-29	12	0.52-2.82	1.56±0.84	14.50	0.00
30-39	13	0.54-2.86	1.72±0.78		
40-49	12	0.61-2.82	1.89±0.67		
50-59	13	0.84-2.98	2.15±0.64		

*P<0.05 considered statistically significant.

Table 3: Level of serum C-peptide (ng/ml) of different sex in the study group.

Sex	No. of subjects	C-peptide (ng/ml)		T value	P value*
		Range	Mean ± SD		
Male	29	0.54-1.70	1.86±0.77	52.14	0.01
Female	21	0.52-2.86	1.79±0.73		

*P<0.05 considered statistically significant

Table 4: Level of serum C-peptide (ng/ml) of different BMI in the study group.

BMI (kg/m^2)	No. of subjects	C-peptide (ng/ml)		T value	P value*
		Range	Mean ± SD		
<25	20	0.54-2.98	1.54±0.76	7.55	0.08
≥30	30	0.52 - 2.98	2.01 ± 0.69		

*P<0.05 considered statistically significant.

DISCUSSION

For this study, whole subjects were subdivided based on their age, sex, and BMI. The biochemical parameter of serum C-peptide levels was studied in these subdivisions and their correlation was analyzed statistically.

In the present study, this was found that, as age advances, the level of serum C-peptide rises in study subjects (Table 2). This indicates that serum C-peptide in a normal person is high at the extremes of age due to increasing insulin resistance with age. Short et al also found that normally as age increases, insulin resistance increases, and it is associated with impaired glucose tolerance, one of the commonly observed phenomena among elderly adults which results in a compensatory rise in insulin level in the blood so that compensatory rise in serum C-peptide level in blood also occurred.[16] Petersen et al also reported that advancement in age is associated with detrimental changes in body composition, which persists even when elderly adults are matched to younger adults for BMI.

They also reported that adiposity, in particular abdominal adiposity, is well accepted as a determinant of insulin resistance and therefore may be a key mediator for the development of age-related insulin resistance despite an inverse relationship that exists between age and insulin sensitivity.[17]

In this study, the mean level of serum C-peptide was more in males than females in the study group (Table 3). This shows, a high level of insulin secretion along with C-peptide in males in comparison to females. Macotela et al found that there are significant differences in insulin sensitivity in adipose tissue of males and females, particularly concerning the intra-abdominal fat depot, which is regulated by physiological levels of sex steroids.[18] Mittendorfer et al found that the increased sensitivity to insulin and lipogenesis which was observed in adipocytes from females may account for their lower level of insulin resistance and diabetes risk in comparison to males despite their similar or higher fat content than in males. they also reported that insulin sensitivity differs between males and females.[19] Geer et al also found that in humans, men accumulate more visceral fat, whereas women accumulate more subcutaneous fat and have a higher percentage of body fat compared with men, they also concluded that in comparison to females, males have higher lean mass and more visceral and hepatic adipose tissue, whereas females have general adiposity and subcutaneous adipose tissue. These differences in adipose tissue distribution may provide a more insulin-sensitive environment in females, as visceral and hepatic adiposity is associated with increased insulin resistance.[20] Garaulet et al found that estrogen may also play an important role in these gender differences because it has a favorable effect on insulin and glucose homeostasis, adipose tissue distribution, and proinflammatory markers.[21]

Mean level of serum C-peptide in this study was more in higher BMI subjects (≥30 kg/m^2) than in lower BMI subjects (<25 kg/m^2) in study group (Table 4). Hotamisligil et al observed that obese patients generally suffered from low-grade inflammation, which is considered a potential risk factor for the emergence of type 2 diabetes and other associated complications.[22] Different studies show that obesity was generally associated with higher IL-6 and leptin concentrations. Moreover, adiponectin concentrations were significantly correlated to improved insulin sensitivity. Increased inflammatory cytokines and decreased total and HMW adiponectin concentrations have been associated with obesity, intra-abdominal fat accumulation, insulin resistance, and metabolic syndrome, and insulin levels typically increase to maintain normal glucose tolerance.[23-27] Polonsky et al found that insulin secretion rates are 3-4 times higher in obese insulin-resistant subjects than in lean.[28] Chiu et al found that body produces insulin under conditions of insulin resistance β cells in pancreas subsequently increase their production of insulin, further contributing to high blood insulin level along with high level of C-peptide.[29] Ramachandran et al found that Asian Indians have lesser insulin levels along with lower level of C-peptide in lower BMI subjects compared to higher BMI subjects.[30]

CONCLUSION

In normal subjects, the level of C-peptide increases with age and BMI. Males have a higher level of C-peptide in comparison to females. Apart from these variations, different biological effects suggest that it may act as a hormone to contribute to fine-tuning of the tissue's metabolism under different physiologic conditions.

Limitations

This study was performed on a very small sample size (Control part of the main study) and with limited no of parameters due to limited resource. Large sample size with more biochemical markers may give more fruitful results.

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