ORIGINAL RESEARCH PAPER
Volume-9 | Issue-4 | April-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

FASTING PLASMA INSULIN AND SERUM C-PEPTIDE IN OBESE/NON-OBESE SUBJECTS SUFFERING FROM DIABETES MELLITUS

Biochemistry

PriyankaTangri Associate Professor, Department of Biochemistry, Dr. Radhakrishnan Government Medical College, Hamirpur (Himachal Pradesh),

NitinTangri* Associate Professor, Department of Pulmonary Medicine, Dr. Radhakrishnan Government Medical College, Hamirpur (Himachal Pradesh), *Corresponding Author

Kuldip Singh Sodhi Professor, Department of Biochemistry, N.C Medical College, Israna, Panipat (Haryana),

Jagdish Ex-Professor, Department of Medicine, MM Institute of Medical Sciences & Research, Mullana, Ambala (Haryana),

ABSTRACT

Introduction: Globally, cardiovascular diseases (CVDs) are the leading cause of mortality owing to rapidly increasing incidence of obesity which in turn, is a well known risk factor for insulin resistance, diabetes mellitus (DM), dyslipidemia and hypertension. A common defect, insulin resistance, seen in both obesity and T2DM has been associated with higher levels of pancreatic hormones besides alterations in adipose tissue mass and metabolism. Elevated levels of pancreatic hormones namely insulin and C-peptide have been suggested to promote atherosclerosis thus posing greater risk of CVD for obese persons. **Aims and Objectives:** The present study was planned to estimate and compare the fasting blood levels of pancreatic hormones – plasma insulin and serum C-peptide in type 2 diabetes mellitus patients and non-diabetic subjects with and without obesity. **Materials and Methods:** A total of 400 subjects comprising 200 T2DM patients (with and without obesity) attending the OPDs and wards of Department of Medicine, M.M Institute of Medical Sciences and Research, Mullana, Ambala and 200 non-diabetic subjects (with obesity and without obesity) from amongst the attendants of the patients and volunteers in the age range of 30-70 years of either sex were selected for the study. In all the subjects included in the study, fasting plasma insulin and serum C-peptide levels were estimated using chemiluminescence immunoassay (CLIA) method. **Results:** It was observed that both plasma insulin ($p<0.001$) and serum C-peptide ($p>0.05$) levels werehigher in obese than non-obese diabetics. Similarly, obese non-diabetics showed significantly higher fasting plasma insulin and serum C-peptide levels than their non-obese counterparts ($p<0.001$). **Conclusion:**Estimation of plasma insulin and serum C-peptide levels may help in early screening of subjects with positive family history of DM as well as obese persons thereby creating awareness about various lifestyle modifications, which if implemented well in time may protect them against DM and its complications.

KEYWORDS

Cardiovascular disease, diabetes mellitus, obesity, insulin resistance, insulin, C-peptide.

INTRODUCTION

Insulin resistance, characterized by hyperinsulinemia, hyperglycemia and alterations in levels of adipokines, could lead to vascular endothelial dysfunction, an abnormal lipid profile, hypertension and vascular inflammation, all of which promote the development of cardiovascular disease. Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as a single-chain 86-amino acid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the amino-terminal signal peptide, giving rise to proinsulin. Cleavage of an internal 31-residue fragment from proinsulin generates the C-peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulphide bonds (Figure 1). The mature insulin molecule and C-peptide are stored together and co-secreted from secretory granules in the beta cells. Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters also influence insulin secretion [1].

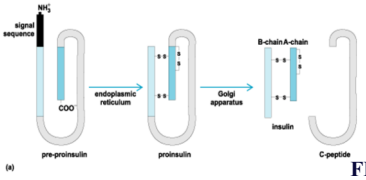
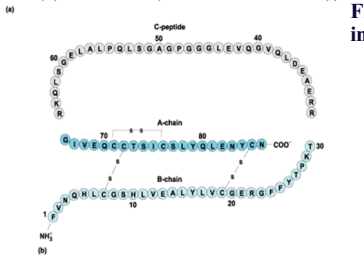


FIGURE 1: a) Synthesis of insulin from preproinsulin.



b) Structure of insulin.

Once insulin is secreted into the portal venous system, about 50% is degraded by the liver. Unextracted insulin enters the systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the recruitment of intracellular signalling molecules, such as insulin receptor substrates (IRSs). IRSs and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin [1].Fasting insulin levels have been reported to be higher in overweight/obese subjects as compared to normal weight subjects [2].It is also known that significantly high insulin levels (hyperinsulinemia) with normal fasting blood glucose are features of insulin resistance which may further be implicated in the development of CVD [3].It is well documented that high levels of insulin are associated with elevated "connecting peptide" (C-peptide) levels as both are produced in equimolar amounts. C-peptide was initially considered an inert substance, but recent research findings have proven that it is a biologically active peptide that affects various cell membranes, including endothelial, renal and nerve cells [2].

In general, C-peptide is produced by a series of enzymatic cleavages of the precursor molecules-preproinsulin and proinsulin (Figure 1).C-peptide is composed of 31 amino acids and facilitates the correct folding of proinsulin to allow the cysteine disulphide bridges between the alpha chain and beta chain of insulin to form. Enzymatic cleavage of proinsulin by proconvertases and carboxypeptidases produces insulin and C-peptide which are released from beta cells into portal circulation. C-peptide has several conserved sequences despite some variability in different species, e.g. N-terminal acidic region, glycine rich central segment, and C-terminal pentapeptide [4].C-terminal pentapeptide gives full replacement of the entire molecule which is similar to other peptides with hormone function like gastrin and cholecystokinin. C-peptide is cleared by the kidney and has a half life of about 20-30 minutes compared to insulin which is cleared through liver and has a half life of about 3-5 minutes [5].The longer half life, renal clearance, and equimolar release of C-peptide make it an attractive proxy for estimating insulin secretion and beta cell function

[6].Until the development of C-peptide assays, evaluation of beta cell function in insulin treated patients was impossible as insulin assay is unable to discriminate between secreted and injected insulin. Further, C-peptide determination is disturbed, to lesser extent, than insulin measurement by the presence of insulin binding antibodies and insulin analogues that the patient undergoing insulin therapy uses [7].Thus, plasma C-peptide concentrations provide an indirect measure of the endogenous insulin secretory reserve.

Abdullah et al considered fasting C-peptide level of <0.6 ng/ml as an indicator of poor insulin reserve; thus suggesting the role of C-peptide as a useful guide in initiating therapy to prevent diabetic complications. The study also reported higher fasting C-peptide levels in the obese type 2 diabetics than in non obese diabetics. This increase in C-peptide levels was associated with increased plasma glucose due to insulin resistance inferring that majority of patients with elevated fasting blood sugar and fasting C-peptide levels were obese and also that obese patients were more insulin resistant than non obese [8].Number of other studies also observed that basal plasma glucose, insulin and C-peptide concentrations are higher in obese than non obese patients [2, 9]. So, the significance of routine C-peptide testing in obese and individuals with poor glycemic control resides in assessment of endogenous insulin reserve and also in altering the treatment modality based on it. It may help in early screening of subjects with positive family history and in creating awareness about lifestyle modifications and education to prevent obesity. Furthermore, the possible role of C-peptide as an additional biomarker in the prediction of the early development of CVD in obese subjects besides insulin has also been highlighted [2].

Keeping in view the above facts, the present study was planned to estimate and compare the fasting blood levels of pancreatic hormones – plasma insulin and serum C-peptide in T2DM patients and non-diabetic subjects with and without obesity.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala (Haryana). A total of 400 subjects in the age range of 30-70 years of either sex were selected for the study, out of which 200 patients were suffering from type 2 diabetes mellitus and 200 were normal subjects (included as controls) from amongst the attendants of the patients and volunteers. Patients were diagnosed as diabetic according to the diagnostic criteria for diabetes mellitus issued by American Diabetes Association (ADA) [10]. All the subjects included in the study were divided into 4 groups in the following manner:

Group I: 100 subjects having T2DM and obesity.
Group II: 100 subjects having T2DM and no obesity.
Group III: 100 subjects having only obesity and no diabetes.
Group IV: 100 subjects having no obesity and no diabetes.

Anthropometric indices including height, weight, hip and waist circumference were measured while subjects were in the standing position and wearing light clothing without shoes. Body Mass Index (BMI) was calculated by dividing body weight (in kilograms) by squared height (in square metres) according to WHO procedure [11].Waist to Hip Ratio (WHR) was calculated as waist circumference divided by hip circumference.Informed consent, both in English as well as vernacular language, was taken from all the participants included in the study. The study was approved by Institutional Ethics Committee of M.M Institute of Medical Sciences and Research, Mullana, Ambala.

EXCLUSION CRITERIA

1. The patients suffering from type 1 diabetes mellitus, rheumatoid arthritis and coronary artery disease.
2. The patients taking antioxidant drugs, corticosteroids, insulin.
3. Pregnancy.
4. Hyper or Hypothyroidism, Cushing syndrome or any other endocrinopathy besides diabetes mellitus.

Five milliliters (5 ml) of venous blood sample was collected in dry disposable syringe under aseptic conditions from ante-cubital vein of the subjects after an overnight fasting of 10-12 hours The plasma was separated for the estimation of fasting insulin levels while C-peptide levels were analyzed in the serum. Fasting plasma insulin and serum C-peptide levels were measured by Chemiluminescence immunoassay (CLIA)[12] using CLIA VAST enabled kit (Monobind Inc. USA) supplied by Lilac Medicare Private Limited.

Statistical analyses were carried out using SPSS version 20.0 software. Descriptive statistics were calculated for different characteristics of the subjects. Student t-test and one-way ANOVA (Analysis of Variance) were used to compare the statistical differences between continuous variables.

RESULTS

The physical parameters of both type 2 diabetic and non-diabetic subjects with and without obesity, i.e. age and body mass index (BMI) were reported previously. Briefly, the mean age (in years) of obese diabetics was lower than non-obese diabetics (50.68±9.41 vs. 51.69±9.41) while the BMI in diabetic, obese subjects (32.4±2.84 kg/m2) was higher than in non-obese subjects (23.55±1.77 kg/m2) with difference amongst two groups being statistically highly significant ($p<0.001$). [13, Int JRecent Trends in Sci and Tech].Similarly, the mean age (in years) of obese, non-diabetics (48.17±11.71) was comparable to that of non-obese, non-diabetics (48.49±11.26). In addition, there was statistically highly significant difference in BMI (kg/m2) amongst obese and non-obese, non-diabetics; BMI being higher in obese subjects (33.39±5.12 vs. 23.6±0.96). Table 1 shows levels of plasma insulin and serum C-peptide in obese and non-obese type 2 diabetic subjects.

TABLE 1: PLASMA INSULIN AND SERUM C-PEPTIDE IN DIABETIC SUBJECTS

PARAMETER	DIABETIC, OBESE SUBJECTS (N=100) Mean ± SD	DIABETIC, NON-OBESE SUBJECTS (N=100) Mean ± SD	p-value
INSULIN (μIU/ml)	11.94 ± 8.07	8.2 ± 6.68	p< 0.001
C-PEPTIDE (ng/ml)	2.51 ±1.60	2.26 ± 1.21	p>0.05

Plasma insulin levels were significantly higher ($p<0.001$) in obese diabetics while serum C-peptide levels were insignificantly higher ($p>0.05$) in obese group as compared to non-obesediabetic subjects.

Furthermore, the obese non-diabetics also showed significantly higher plasma levels of insulin than non-obese subjects without diabetes ($p<0.001$) [Table 2]. Similarly, serum C-peptideconcentration was found to be higher in obese as compared to non-obese diabetic subjects and the difference was found to be statistically highly significant ($p<0.001$).

TABLE 2: PLASMA INSULIN AND SERUM C-PEPTIDE IN NON-DIABETIC SUBJECTS

PARAMETER	NON-DIABETIC, OBESE SUBJECTS (N=100) Mean ± SD	NON-DIABETIC, NON-OBESE SUBJECTS (N=100) Mean ± SD	p-value
INSULIN (μIU/ml)	12.81 ± 8.60	7.84 ± 4.04	p< 0.001
C-PEPTIDE (ng/ml)	2.71 ± 1.57	1.42± 0.39	p< 0.001

Analysis of Variance (ANOVA) revealed statistically highly significant difference amongst mean insulin and C-peptide levels of subjects included in four groups under study with $F = 9.517$ ($p<0.001$) and 19.14 ($p<0.001$) for insulin and C-peptide, respectively.

DISCUSSION

The age of diabetic subjects, both with and without obesity, was almost comparable with difference between two groups being statistically insignificant. A notable difference was found between obese and non-obese diabetics in terms of their BMI. The fasting plasma glucose levels were higher in non-obese than obese diabetics and the difference between the two groups was found to be statistically significant ($p<0.05$) as reported previously [13]. Paradoxically, obese, type 2 diabetics had significantly higher fasting plasma insulin levels than non-obese diabetics clearly indicating the presence of severe hyperinsulinemia and subsequent insulin resistance in obese than non-obese (Table 1). This finding pertaining to low insulin levels in non-

obese diabetics is in accordance with the findings of Barma et al who also reported lower insulin levels in lean type 2 diabetics than in obese diabetics [14]. Snehalatha et al conducted a study which analyzed the insulin secretion in Asian Indians and observed lesser insulin levels in non-obese compared to obese thereby suggesting that obese patients are hyperinsulinemic [15]. Obese diabetics had elevated C-peptide levels than non-obese but there was no significant difference in C-peptide levels between obese and non-obese diabetics ($p>0.05$) as revealed in Table 1. This again confirmed that obese patients are hyperinsulinemic. Similar results were reported by Abdullah et al and Barma et al [8, 14]. Even Jones et al also observed that plasma insulin and C-peptide concentrations are higher in obese than non-obese subjects [16]. Insignificant difference in C-peptide levels despite higher insulin levels observed in obese than non-obese diabetic patients may be due to excess extraction of insulin by the liver.

The mean age of obese, non diabetics was comparable to that of non obese, non diabetics. In addition, there was statistically highly significant difference in BMI amongst obese and non obese, non diabetics; BMI being higher in obese subjects. The fasting plasma glucose levels were well within the normal range for both obese as well as non obese subjects without diabetes [13].Furthermore, differences in fasting plasma insulin levels while comparing obese and non obese, non diabetics were found to be statistically highly significant in our study ($p<0.001$) (Table 2). These findings were similar to those observed by Carnethon et al in their study on Black and White adults and Urbanavicius et al while working on pre-diabetes and early type 2 diabetes [17, 18]. Interestingly, it is also known that significantly high insulin levels (hyperinsulinemia) with normal FPG, as seen in obese individuals are features of insulin resistance which may further be implicated in the development of CVD [3].

It is well documented that high insulin levels are associated with elevated C-peptide levels as both are produced in equimolar amounts but the significance of C-peptide estimation in obese individuals lies in its ability to assess endogenous insulin reserve which can help in early screening of subjects with positive family history and thus, creating awareness about lifestyle modifications to prevent obesity-related disorders in the future. Keeping in view the above fact, the serum C-peptide levels were estimated and compared between obese and non obese, non diabetic subjects. The serum C-peptide levels were found to be significantly higher in obese than non obese, indicating insulin resistance. These findings were similar to those obtained in study done by Abdullah et al and Abdullah BB et al [2, 8].

CONCLUSION

The higher fasting plasma insulin levels in obese diabetics as well as non-diabetics than non-obese diabetic and non-diabetic subjects clearly indicates the presence of severe hyperinsulinemia and subsequent insulin resistance in obese subjects. The notion was further confirmed by the elevated levels of serum C-peptide observed in obese subjects, both with and without T2DM in comparison to non-obese diabetics and non-diabetics. Therefore, determination of insulin and C-peptide in obese individuals may guide in assessing the risk of developing insulin resistance and thereafter, diabetes and CVD in such individuals. This implies that suitable preventive measures in terms of lifestyle modifications, if implemented, in such high-risk subjects, may protect them against DM and its complications.

REFERENCES

1. Powers AC. Diabetes mellitus. In: Harrison's Endocrinology. Jameson JL, editor. 2nd edition. United States: McGraw-Hill, 2010:271-73.
2. Abdullah A, Hasan H, Raigangar V, Bani-Issa W. C-peptide versus insulin: Relationships with risk biomarkers of cardiovascular disease in metabolic syndrome in young Arab females. Int J Endocrinol 2012; 2012: 420792.
3. Grundy SM, Brewer HBJr, Cleeman JI, Smith SCJr, Lenfant C: National Heart, Lung, and Blood Institute; American Heart Association. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/ American Heart Association Conference on scientific issues related to definition. ArteriosclerThrombVascBiol 2004; 24(2):e13-8.
4. Munte CE, Vilela L, Kalbitzer HR, Garratt RC. Solution structure of human proinsulin C-peptide. FEBS J 2005;272(16):4284-93.
5. Marques RG, Fontaine MJ, Rogers J. C-peptide: much more than a byproduct of insulin biosynthesis. Pancreas 2004;29(3):231-8.
6. Sharma P, Sodhi KS, Jagdish. Interpreting the reference range of serum C-peptide. Indo Am J Pharm Res 2014; 4(09): 3630-33.
7. Sapin R. Insulin assays: previously known and new analytical features. Clin Lab 2003;49(3-4):113-21.
8. Abdullah BB, Patil BS, Thaseen A. Significance of C-peptide in type 2 diabetics- A study in North Karnataka population of India. Al Ameen J Med Sci 2010;3(1):65-70.
9. Jones CN, Abbasi F, Carantoni M, Polonsky KS, Reaven GM. Role of insulin resistance and obesity in regulation of plasma insulin concentrations. Am J PhysiolEndocrinolMetab 2000; 278(3):E501-8.
10. American Diabetes Association. Diagnosis and Classification of diabetes mellitus. Diabetes Care 2012; 35(Suppl 1): S64-S71.
11. Garrow JS, Webster J. Quenelle's index (W/H2) as a measure of fatness. Int J Obes 1985; 9: 147-53.
12. Kricka LJ. Optical techniques. In: Teitz Textbook of Clinical Chemistry and Molecular Diagnostics. Burtis CA, Ashwood ER, Bruns DE, editors. 4th edition. New Delhi: Saunders, 2008: p.85.
13. Tangri P, Tangri N, Sodhi KS, Jagdish. 'Diabesity' and Insulin Resistance: Results from a Study in a Rural Setting of Haryana, India. Int J Res Rev 2017;4(9):16-22.
14. Barma PD, Ranabir S, Prasad L, Singh TP. Clinical and biochemical profile of lean type 2 diabetes mellitus. Indian J EndocrinolMetab 2011; 15(Suppl 1): 40-3.
15. Snehalatha C, Satyavani K, Sivasankari S, Vijay V, Ramachandran A. Insulin secretion and action in different stages of glucose tolerance in Asian Indians. Diabet Med 1999; 16(5):408-14.
16. Jones CN, Abbasi F, Carantoni M, Polonsky KS, Reaven GM. Role of insulin resistance and obesity in regulation of plasma insulin concentration. Am J Physiol Endocrinol Metab 2000; 278(3):E501-8.
17. Carnethon MR, Palaniappan LP, Burchfiel CM, Brancati FL, Fortmann SP. Serum insulin, obesity, and the incidence of type 2 diabetes in black and white adults: the atherosclerosis risk in communities study: 1987-1998. Diabetes Care 2002; 25(8):1358-64.
18. Urbanavicius V, Abraitiene A, Vitkus D, Borovkiene R, Kucinskiene ZA. Adiponectin and insulin resistance in pre-diabetes and early type 2 diabetes mellitus. Gerontologija 2008; 9(1):7-14.