Original Research Article

Correlation of C-Peptide and HbA1c in Type II Diabetes Mellitus

V. Aruna

Assistant Professor, Biochemistry, Guntur Medical College, Guntur, Andhra Pradesh, INDIA

ABSTRACT

Type 2 Diabetes Mellitus (Non Insulin Dependent Diabetes Mellitus-NIDDM) is a chronic metabolic disorder most prevalent in India with microvascular complications. Several studies were underway to identify biomarkers to detect complications associated with increasing duration of disease. The present study was done to understand the role of connecting peptide (C-peptide) in pathogenesis of microvascular changes and it's correlation to dyslipidemia of T2DM. The study was done at Government General Hospital, Guntur. In this study 61 known T2 DM patients and 51 age sex matched controls were selected. Their fasting blood samples were analysed for FBS, HbA1C, Urea, Creatinine, Total Cholesterol, High Density Lipoprotein Cholesterol and C-peptide. Dyslipidemia was not observed in Diabetic patients compared to controls (TC, HDLC & NonHDL p = 0.363, 0.294 & 0.336 respectively). HbA1C and C-peptide showed significant correlation between cases and controls (P = <0.00001). C-peptide showed significant correlation with lipid parameters & lipid ratios in individuals with poor glycemic control identified by HbA1c value of >9%. (ANOVA p = <0.0001) Pearson's correlation showed negative relation of HbA1c and C-peptide with lipid parameters TC, HDLC & Non HDLC (r = 0.378, 0.732, 0.313 and 0.81, 0.91 & 0.843 consecutively). C-peptide showed significant variation with Non HDL / HDL ratio & Non HDL / TC ratio (p = <0.002 & <0.00007) in diabetic patients with poor glycemic control identified by HbA1c of > 9%. We conclude that C-peptide would be a good marker to assess degree of function of pancreas and predictor of atherogenic complication in longstanding NIDDM.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterised by hyperglycemia, dyslipidemia due to deficiency or inappropriate functioning of Insulin, hypoglycemic hormone secreted by β-cells of pancreas. Its incidence is increasing in the last few years in India with growth rate of 12.5%.The prevalence of type 2 DM is 2.4% in rural population and 11.6% in urban population. [1]

Insulin resistance with relative Insulin deficiency is feature of T2DM. Proinsulin splits into Insulin and C-peptide (Connecting peptide) before it is released from endocytes of β - cells of pancreas. C-peptide can be estimated in the place of Fasting Insulin to measure endogenous secretion of insulin. C-peptide is com 31 amino acid polypeptide. 50% of insulin secreted into the portal vein is extracted in liver, hence fasting insulin assay reveals posthepatic concentrations rather than endogenous secretion. [2] C-peptide is not altered in liver & its assay determines endogenous insulin secretion accurately and discriminates injected insulin. In health normal serum C-peptide level is 0.78 - 1.89 ng/ml. C- peptide binds to receptor at the cell surface, activates G-protein coupled receptor and increases intracellular calcium. C-peptide improves glomerular filtration, increases production of nitric oxide (NO)3 and regulates proinflammatory markers like interleukin 6, tumor nuclear factor α (TNFα) .C-peptide .C-peptide stimulates migration

of T lymphocytes, macrophages and monocytes towards endothelium of capillaries. Increases smooth muscle cell proliferation in the small and medium sized arteries. Proatherogenic affects of C-peptide were studied by Marx et al; [4] Forst et al [5]

Hb undergoes nonenzymatic glycosylation in persons with persistant hyperglycemia and designated as HbA1c. HbA1c represents the integrated values of glucose over the preceeding 6-8 weeks and provides an additional criterion for assessing glucose control. In health it is 4% -7%.

In view of the different complications associated with T2DM several studies were underway to notify specific biomarkers in order to predict forthcoming issues like microangiopathy, metabolic syndrome and hypertension. The etiological factors leading to onset of T2DM include lifestyle, stress, obesity, environmental factors and heredity.

Dyslipidemia is feature of T2DM. Total cholesterol (TC) &other lipoprotein fractions were analysed routinely to predict coronary heart disease; commonly encountered complication of T2DM. In this study lipid ratios were considered as risk predictors rather than the true values of lipoproteins. Difference between TC and HDLC (High Density Lipoprotein Cholesterol) was considered as Non-HDL fraction of lipids. [6] Non HDL measures all apoB containing lipoproteins and correlates with circulating apoB levels. Considered a better marker for atherogenesis in individuals being treated with lipid lowering drugs like statins. [7] The ratios assessed in this study were cardiac risk ratio (CRR= TC/HDLC); Atherogenic coefficient (AC= Non HDL / HDL) and a novel marker ratio of Non HDL /TC.

Inclusion criteria: Known Type 2 Diabetic patients without complications in the age group of 20 - 60yrs both males and females

Exclusion criteria: Endocrine diseases, Pregnancy, Heart disease

MATERIAL METHODS

The study was done at GGH, Guntur from January 2020 to March 2020. T2DM patients 61 attending OPD for treatment and 51 age and sex matched healthy individuals as controls were selected for the study after oral informed consent. They were asked to withhold diabetic medication for 48hrs and attend central biochemistry lab in overnight fasting for sample collection.

Venous blood samples were collected under aseptic conditions. 2ml mixed with EDTA for estimation of HbA1c and 3ml collected in red vacutainers. Red vacutainers were centrifuged at 3000rpm for 5minutes & serum was seperated for anaysis of FBS, Total Cholesterol(TC)l, High Density Lipoprotein(HDLC), Urea and Creatinine on Beckman Coultier AU 480.

HbA1c was done by Ion Exchange resin method to assess the glycemic status of the subjects 6 weeks preceding the study.

C-Peptide was evaluated on Access 2 Beckman coultier by immuno radiometric assay (sandwich type). Normal range was 0.78 - 1.89ng/ml.

From TC and HDL the following lipid ratios were calculated
1. Cardiac Risk Ratio(CRR) = TC ÷ HDLC
2. Non HDL = TC - HDLC
3. Atherogenic coefficient (AC) = (TC-HDLC) ÷ HDLC
4. Non HDLC ÷ TC

Inclusion criteria:- Known cases of Type 2 Diabetes mellitus on oral hypoglycemic agents.

Exclusion criteria:- Endocrine diseases like thyroid disease, Coronary heart disease, Gestational diabetes and microvascular diseases associated with diabetes.

Statistical analysis

The biochemical values were analysed by NCSS 2021, v21.02 Continuous variables were expressed as mean ± standard deviation (SD). Analysis of Variance (ANOVA) was done to determine statistical difference between more than two variables. Pearson's correlation was done to investigate correlation between the

variables. Linear regression model analyses were done to determine trends of mean C-peptide, CRR, AC & NonHDL/TC ratio. P value of 0.05 was considered statistically significant.

RESULTS

Total numbers of 112 subjects were considered for the study - 61 known cases of Type 2DM and 51healthy age and sex matched controls. Among 61 diabetic individuals 31 were females and 30 were males in the age group of 20 - 60yrs.In the controls group 24 were men and 27 were women. Among 61 diabetics longevity of disease was - <5yrs 28 numbers (46%); > 5yrs -18 numbers (29%) and > 10yrs - 14 numbers (23%).

Table 1 shows Mean & SD of Biochemical parameters and Lipid ratios in Diabetic subjects and controls. FBS, HbA1c and C-peptide were elevated in Diabetic subjects compared to controls but TC, HDLC were lowered in controls and lipid ratios showed marginal difference between the cases and controls. Diabetic subjects were divided into 2 groups based on HbA1c values- as Poor glycemic control (HbA1c >9.0% N=31) and Good glycemic control (HbA1c 4% -7% N=30) Table 2 shows Mean & SD of Biochemical values and

ratios in the 2 groups respectively. C-peptide levels were higher with better glycemic control. The mean values of FBS, TC, HDL and lipid ratios showed little difference. HbA1c , C-peptide, Non HDL and Non HDL /TC ratio showed statistically significant (p values <0.00001, <0.00001, <0.03 and <0.008 respectively)Table 3 shows 11 individuals with good glycemic control(HbA1c 4% -7%) have C-peptide levels of <0.9 ng/ml but individuals (20%) with poor glycemic control(HbA1c 8.3%) have c-peptide levels >2.0ng/ml. Increasing fasting sugar values showed higher C-peptide levels in Diabetic individuals. Amongst diabetics 48% individuals have HbA1c levels of more than 9% indicating poor glycemic control. In the same group HbA1c and C-peptide were significantly correlated with lipid ratios - cardiac risk ratio   (CRR=TC/HDLC);   Atherogenic Coefficient (AC= TC-HDLC/HDLC) and ratio of Non HDLC /TC with p values <0.00001 Table 4. Table 2 shows correlation of biochemical values and lipid ratios in poor glycemic control to good glycemic control. HbA1c, C-peptide Non HDL, AC and nonHDL /TC ratio were statistically significant (p value <0.00001, <0.00001, <0.03, <0.05 and <0.008 respectively).

Table 1

Parameter	Normal values	Diabetics(N=61) Mean±SD	Controls(N=51) Mean±SD	P-value
FBS	70-110mg/dl	165.37± 50.4	86.86±9.78	<0.00001
HbA1c	4%- 7%	7.48± 2.74	5.26± 1.63	<0.00001
C-Peptide	0.78 - 1.89ng/ml	2.98± 1.66	4.96 ± 1.57	<0.00001
TC	150-200mg/dl	192.02±43.29	194.9±41.82	0.363
HDLC	35-60mg/dl	45.81±9.43	44.76±10.49	0.294
Non HDL		146.56±42.63	150.14±44.26	0.336
CRR	<4.5	4.31±1.49	5.14±4.41	0.090
AC	<3.0	3.32±1.199	3.57±1.599	0.176
Non HDL÷TC		0.75± 0.068	0.75±0.078	0.416

Table 2: BIOCHEMICAL VALUES IN DIABETIC SUBJECTS WITH POOR GLYCEMIC CONTROL & GOOD GLYCEMIC CONTROL

Parameter	Poor glycemic control(N=31) Mean±SD	Good glycemic controls(N=30) Mean±SD	P-value
FBS	167.74± 55.37	152.33 ± 48.94	0.127
HbA1c	9.55 ± 1.81	4.99 1±.29	<0.00001
C-Peptide	2.12 ± 1.40	3.77 ± 1.14	<0.00001
TC	197.81 ± 45.25	182.2 ± 38.71	0.07
HDLC	43.23 ± 6.64	47.0 ± 11.16	0.056
Non HDL	154.9 ± 43.3	135.53 ± 38.20	<0.03
CRR	4.45 ± 1.23	4.01 ±1.23	0.084
AC	3.58 ± 1.06	3.09 ± 1.24	<0.05
Non HDL/TC ratio	0.78 ± 0.06	0.74 ± 0.07	<0.008

Table 3: C-PEPTIDE levels in Diabetic subjects (N= Number of subjects =61)

Ng/ml	N	FBS	HbA1c
0-0.9	11	169.66 ± 27.32	6.88 ± 2.96
1 -2	41	156.48 ± 37.55	7.42 ± 2.57
>2	09	199.55 ± 92.81	8.3 ± 3.33

One way analysis of variance (ANOVA) of mean& SD of lipid ratios (AC & NonHDL/TCratio) with C-peptide were significant (p values <0.002 & <0.00007 respectively) in poor glycemic control compared to good glycemic control and HbA1c did not show any significant correlation with lipid ratios. Table 5. The same was not supported by other studies. [9] Diabetes Complications and Control Trial (DCCT) pointed out the influence glycemic status on dyslipidemia and HbA1c levels of <7% prevent microvascular complications. [10]. HbA1c correlated with FBS significantly (r=0.459, p = <0.009) in individuals with poor glyemic control.

Table 4: STUDENT'S t TEST IN DIABETICS WITH POOR GLYCEMIC CONTROL

PARAMETER	T VALUE	P VALUE	SIGNIFICANCE
HbA1C - CRR	12.968	< 0.00001	SIGNIFICANT
HbA1C - AC	15.858	< 0.00001	SIGNIFICANT
HbA1C - NH/TC RATIO	26.973	< 0.00001	SIGNIFICANT
HbA1C -CP	18.064	<0.00001	SIGNIFICANT
CP- CRR	6.957	< 0.00001	SIGNIFICANT
CP - AC	4.621	< 0.0001	SIGNIFICANT
CP - NH/TC RATIO	5.326	< 0.00001	SIGNIFICANT

Table 5: One-Way Analysis of Variance IN DIABETICS WITH POOR GLYCEMIC CONTROL

PARAMETER	F RATIO	P VALUE	SIGNIFICANCE
CRR WITH C-PEPTIDE	1.061	0.448	NS
AC WITH C-PEPTIDE	4.138	0.002	Significant
NONHDL / TC RATIO WITH C-PEPTIDE	6.603	0.00007	Significant
CRR WITH HbA1C	0.213	0.995	NS
AC WITH HbA1C	0.064	0.999	NS
NON HDL /TC RATIO WITH HbA1C	0.060	0.999	NS
C-peptide with HbA1C	134.76	<0.00001	Significant

Pearson's correlation showed marked negative relation between TC, HDLC and Non HDLC fractions of lipids with HbA1c (p values 0.378, 0.732 & 0.313 respectively) and with C-peptide (p values 0..81, 0.91 & 0.843 respectively) Table 6. Evaluation of Non-HDL as an independent, novel coronary risk factor was under study. Multiple risk assessment systems like Prospective Cardiovascular Munster study (PROCAM) [11], Systemic Coronary Risk Estimation (SCORE) include lipid related markers to predict Coronary Artery Disease. But in this study the same could not be established.

Table 6: PEARSON'S CORRELATION COEFFIENT POOR GLYCEMIC CONTROL(N=31)

Correlation with HbA1c	R	P	SIGNIFICANCE
FBS	0.4597	0.009	SIGNIFICANT
TC	0.164	0.378	NS
HDL	-0.064	0.732	NS
NON HDL	0.187	0.313	NS
Correlation with C-Peptide			
HbA1C	-0.539	<0.001	SIGNIFICANT
TC	-0.047	0.81	NS
HDL	-0.0206	0.91	NS
NON HDL	-0.037	0.843	NS

LINEAR REGRESSION SCATTER PLOT
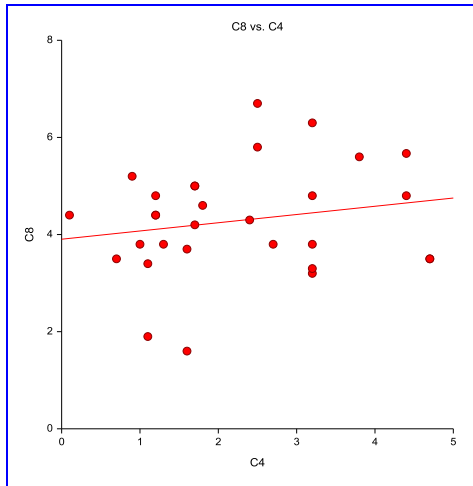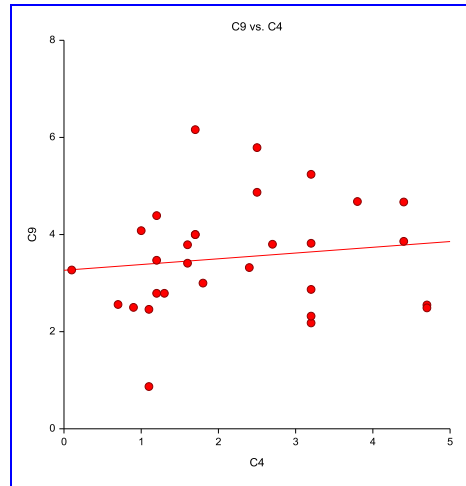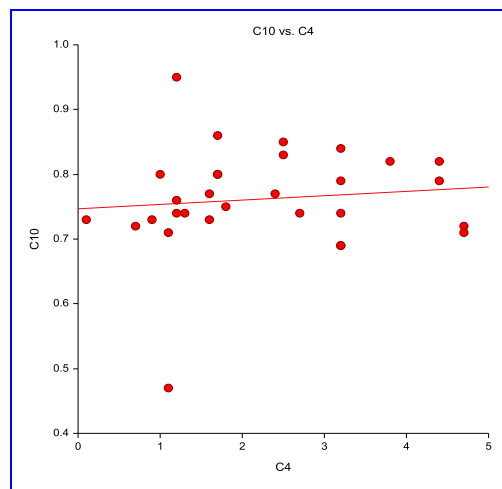**X-Axis - C-Peptide; Y-Axis = CRR (1) ; AC (2) & NonHDL / TC RATIO (3)**

Figure -1

Figure - 2

Figure-3

Table 7: LINEAR REGRESSION REPORT IN DIABETIC SUBJECTS (N=61)

VARIABLE	REGRESSION COEFFICIENT	Std. ERROR	t	P
CRR - CP	4.64	0.128	36.125	<0.00001
AC - CP	3.713	0.101	36.778	<0.00001
TC-HDL/TC -CP	0.746	0.0304	24.555	<0.00001
LINEAR REGRESSION IN DIABETICS WITH GOOD GLYCEMIC CONTROL (N=30)				
CP -CRR	5.217	0.769	6.782	<0.00001
CP - AC	4.206	0.7799	5.394	<0.00001
CP - TC-HDL / TC	0.799	0.0424	18.845	<0.00001
LINEAR REGRESSION IN DIABETICS WITH POOR GLYCEMIC CONTROL (N=31)				
CP - CRR	3.9105	0.427	9.154	<0.00001
CP -AC	3,262	0.437	7.459	<0.00001
CP - TC-HDL/TC	0.746	0.0304	24.55	<0.00001

Table 7 shows Linear regression model for C-peptide with lipid ratios viz CRR, AC & NONHDL/TC ratio in Diabetic subjects (N=61), Diabetics with good Glycemic control (HbA1c 4.99% ±1.29 N=30) and Diabetics with poor glycemic control (HbA1c 9.55% ± 1.81; N=31).C-peptide was shown to be associated with lipid ratios irrespective of Glycemic status and the adjusted difference was statistically significant with p value of <0.00001.

DISCUSSION

The present study included Type 2DM patients in the age group of 20-60yrs with equal number of males and females. The concentration of C-peptide was shown to be 50% in Diabetic subjects (2.98 ±1.66) compared to controls (4.96 ± 1.57).

The present study proved C-peptide levels were within normal range (0.78 - 1.89ng/ml) in Type 2DM individuals irrespective of duration of disease.50% individuals were known diabetics for > 5yrs & 25% for >10yrs. Glycemic status denoted by HbA1C levels correlated well with C-peptide (P<0.001 Table 6). C-peptide was associated with lipid ratios as shown in linear regression model of cases with different HbA1c levels. Linear regression analysis demonstrated a strong correlation of c-peptide with lipid ratios (p<0.00001)

Lipid fractions and corresponding lipid ratios were on higher range in controls compared to Diabetic subjects (Table 1). Dyslipidemia of Type 2DM was not observed in this study. TC, HDLC & NonHDL showed negative correlation with HbA1c (Table 6) in spite of the value being >9.0% (r = 0.164, 0.064 & 0.187; p = 0.378, 0.732 & 0.313 consecutively).

Increasing HbA1c levels showed corresponding raise in C-peptide levels (Table 3). Negligible individuals have C-peptide below the low normal values ie. <0.9ng/ml. Significant correlation observed between c-peptide and HbA1c if the mean exceeded 9% p value <0.001. The same is true with atherogenic coefficient and nonHDL /Tc ratio by ANOVA p value <0.002 & <0.00007 consecutively. [12] We observed poor association of Non HDL levels among controls and diabetics in this study (p 0.336) where as strong association was demonstrated by Weiquan Lu, et al. [13]

In accordance with Linear regression analyses the lipid ratios, indicators of coronary atherosclerosis showed strong association with C-peptide in Type 2Diabetic patients irrespective of their HbA1c levels (p<0.00001) This finding strongly favours assessment of C-peptide in all Type 2 Diabetic patients irrespective of their glycemic status to predict cardiovascular risk. Finally we could not detect any gender difference with the gravity of the situation.

CONCLUSION

Peptide is a good tool at base level to predict risk of development of Type 2 Diabetes as well as coronary Heart Disease.

ACKNOWLEDGEMENTS

Abbreviations

Type 2 Diabetes Mellitus	-T2 DM
Connecting peptide	- C-Peptide
Total cholesterol	- TC
High Density Lipoproteins	- HDLC
Cardiogenic Risk Ratio	- CRR
Atherogenic Coefficient	- AC

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

REFERENCES

1. Anjana R.M., Deepa M., Pradeepa R., Mahanta J., Narain K., Das H.K., Adhikari P., Rao P.V., Saboo B., Kumar A., et al. Prevalence of diabetes and prediabetes in 15 states of India: Results from the ICMR-INDIAB population-based cross-sectional study. Lancet Diabetes Endocrinol. 2017;5:585–596. doi: 10.1016/S2213-8587(17)30174-2. [PubMed]
2. Ferranini E, Cobelli C. The kinetics of insulin in man. Role of the liver. Diabetes Metab Rev 1987; 3:365-97.https://doi.org/10.1002/dmr.5610030201
3. Yosten GLC, Maric-Bilkan C, Luppi P, Wahren J. Physiological effects and therapeutic potential of proinsulin C-peptide. Am J Physiol Endocrinol Metab. 2014;307:E955–E968. doi: 10.1152/ajpendo.00130.2014. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
4. Marx N, Walcher D, Raichle C, et al. C-peptide colocalizes with macrophages in

early arteriosclerotic lesions of diabetic subjects and induces monocyte chemotaxis in vitro. Arterioscler Thromb Vasc Biol 2004; 24: 540–545.Google Scholar CrossrefPubMed

5. Forst, T., Kunt, T., Pfützner, A., Beyer, J., Wahren, J., 1998. New aspects on biological activity of C-peptide in IDDM patients. Exp. Clin. Endocrinol. Diabetes 106, 270–276 DOI: 10.1055/s-0029-1212190

6. Anan Huang, Xin Qi, Liping Wei, Mingyin Zhang, Shiqi Zhou, "Non-HDL-c/TC: A Novel Lipid-Related Marker in the Assessment of Severity of Coronary Artery Lesions and Cardiovascular Outcomes", Cardiology Research and Practice, vol. 2019,

7. R. Verbeek, G. K. Hovingh, and S. M. Boekholdt, "Non-high-density lipoprotein cholesterol," Current Opinion in Lipidology, vol. 26, no. 6, pp. 502–510, 2015.View at: Publisher Site | Google Scholar

8. H. E. van Deventer, W. G. Miller, G. L. Myers et al., "Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population," Clinical Chemistry, vol. 57, no. 3, pp. 490–501, 2011.View at: Publisher Site | Google Scholar

9. Indumati.V, Patil VS, Krishnaswamy, Kumar S, Vijay V, Mahesh S, Rajeshwari V. NON-HDL choleaterol and LDL-C/HDL-C Ratio In Type II Diabetic patients. International Journal of Pharma and Bio Sciences. 2011;2:71-7.

10. Gotto AM Jr. Cardiologist's role in improving glucose control and global cardiovascular risk in patients with type 2 diabetes mellitus. Am J Cardiol. 2007:99:3-5.

11. Buyken AE, von Eckardstein A, Schulte H, Cullen P, Assmann G Type 2 diabetes mellitus and risk of coronary heart disease: results of the 10-year follow-up of the PROCAM study.Eur J Cardiovasc Prev Rehabil. 2007 Apr;14(2):230-6DOI: 10.1097/HJR.0b013e3280142037

12. Nimmanapalli HD, Kasi AD, Devapatla PK, Nuttakki V. Lipid ratios, atherogenic coefficient and atherogenic index of plasma as parameters in assessing cardiovascular risk in type 2 diabetes mellitus. Int J Res Med Sci 2016;4: 2863-9.

13. Weiquan Lu, MD, Helaine E. Resnick, PHD, Kathleen A. Jablonski, PHD, Kristina L. Jones, MPH, Arvind K. Jain, MS, Wm. James Howard, MD, David C. Robbins, MD and Barbara V. Howard, PHDNon-HDL Cholesterol as a Predictor of Cardiovascular care in Type 2 Diabetes, Diabetic Care 2003 Jan; 26(1): 16-23 .https://doi.org/10.2337/diacare.26.1.16

******