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ORIGINAL ARTICLE

# Association of Amylin Polypeptide and Inflammatory Biomarkers in Diabetic Coronary Artery Disease

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## Abstract

*Introduction:* Type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD) poses major threat worldwide contributing to excessive morbidity and mortality, these co-morbidities synergistically interact with inflammatory mechanisms. Amylin is fraternal twin of insulin, it has a great potential to fibrillation, aggregate formation, and deposition causes proteotoxicity to vital organs.

*Aim:* To find the association of amylin with inflammatory markers in T2DM with CAD subjects.

*Methodology:* Present cross sectional study conducted on 262 subjects (30–60 years) T2DM with angiographically proven CAD cases (n = 131) and healthy controls (n = 131). The diabetic and lipid profile were estimated and Serum Amylin, Insulin, hsCRP, TNF-α, and IL-6 levels were assessed by ELISA. Ethical approval and written informed consent were obtained. Subjects with other inflammatory diseases were excluded. Data were analyzed on SPSS-24 and descriptive statistics were performed for $p \leq 0.05$ (95% CI). Association between variables and area under the receiver operating characteristic curve (AUC of ROC) were performed to find the discrimination power.

*Results:* Blood Sugar Level-Fasting, Blood Sugar Level-Post Prandial, Glycosylated Haemoglobin (HbA1c), Insulin resistance (HOMA-IR), serum Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein-Cholesterol (LDL-C), very low density lipoprotein-Cholesterol (VLDL-C) and Non-HDL-C were significantly raised, whereas Insulin sensitivity check index (QUICKI) and high Density Lipoprotein-Cholesterol (HDL-C) decreased ($p \leq 0.001$) in T2DM-CAD compared with that of the control. The elevated level of amylin shows strong positive correlation with TG and also with raised level of hsCRP, IL-6, and TNF-α in T2DM-CAD ($p \leq 0.001$), which may due to cytokines released by monocytes/macrophages. Hyperamylinemia could promote amylin deposition in pancreas causing apoptosis of pancreatic β-cells and causes structural and functional changes of cardiac myocytes in heart.

*Conclusion:* Assessment of amylin with inflammatory cytokines may predict pancreatic as well as cardiac dysfunction and it is helpful in stratification of severity risk; may provide novel therapeutic target for DM-CAD patients.

**Keywords:** cardiac myocytes, hyperamylinemia, insulin resistance, inflammatory mechanism, proteotoxicity

## Introduction

Type 2 Diabetes Mellitus (T2DM) patients are more vulnerable to coronary artery disease (CAD), described as DM-CAD and it increases the rate of morbidity and mortality. Its pathogenesis is multifaceted, involving multiple factors like genetic predisposition, endogenous factor and different environmental factors, such as high fat diet, sedentary lifestyle, and chronic stress.[1] Type 2 diabetes with impaired glycemic regulation will result in alteration in cardiac pathophysiology independent of CAD and hypertension. It is assumed, increased body fat may affect the response of the body tissue to insulin, leading to insulin resistance and subsequent impairment of glucose and lipid homeostasis.

Cardiac disease is directly related to insulin resistance; the myocardial insulin response in diabetic patients is especially intact, indicating secondary to insulin resistance, may contribute to heart dysfunction in T2DM.[2] Insulin needs increase in the IR state, so that there is a rapid exhaustion of pancreatic islets of beta cells, due to overproduction of insulin to meet body requirement, contributing progressively to the insulin deficiency (ID). Accordingly, late T2DM recognized by both ID and IR, with hyperglycemia, resulting in endothelial dysfunction.[1] Individuals with BMI more than 30 kg/m$^2$ and ordinary insulin resistance have hyperinsulinemia and hyperamylinemia despite hyperglycemia and dyslipidemia; the amylin, a 37-amino acid peptide hormone obtained from amyloid aggregated in pancreatic islets of type 2 diabetic individuals.[3]

Amylin and insulin are released by pancreatic islets of ß-cells in response to dietary foodstuffs including glucose and fats. Amylin has vital function in controlling glucose homeostasis and restraining food intake via "meal ending satiation", probably by the activation of its receptor in brain's postrema region. In in-vitro experiments prove that amylin is seen to impede both basal and insulin stimulated glycogenesis in rodent muscle and repress glucose from being removed in liver cells. In further animal study, it appeared that, amylin could inhibit release of insulin by ß-cell apoptosis and glucose dysregulation in transgenic rodents.[4]

Amylin the amyloidogenic protein forms "oligomers, fibrils, and amyloid plaques" promptly at higher level in the circulation, and they were observed in the form of deposits in pancreas of 95% of subjects with non-insulin-dependent diabetes mellitus (NIDDM) in their histopathological findings. The toxic forms of amylin amyloid attaches to cellular membranes and induce $Ca^{2+}$ dys-homeostasis, cell dysfunction, and apoptosis.[2] In number of findings related to low grade systemic inflammation, it is an important mechanism for obesity pathogenesis and related metabolic conditions like T2DM and CVDs. Among these two comorbidities, amylin amyloid-mediated inflammation has synergetic interactions.

Metabolic inflammation promotes the aggregation of arterial lipids and relocation of smooth muscle cells, both aggravate the atherosclerosis. Therefore, the term atherosclerosis includes all immune aspects such as adhesion molecules, cytokines, chemokines, lymphocytes, endothelial cells, and macrophages. In both the early and the late phases of T2DM and the scaffolds among T2DM and CAD, metabolic inflammation subsequently assumed to be specific components behind the raised inflammatory processes and not well understood.[1]

The present study is preformed to find the relationship of circulatory amylin concentration with inflammatory cytokines in DM-CAD subjects. We hypothesized that the toxic amylin amyloid formed causes the destruction of pancreatic beta cells and alter the structure and function of cardiac myocytes, may lead to induce inflammation. Therefore, we studied circulatory levels of amylin, hsCRP, TNF-α, and IL-6 in T2DM with CAD population and compared with healthy controls.

## Material and Methods

The present cross sectional study was carried out from 2017 to 2019 with a total of 262 subjects of both gender aged between 30 to 60 years. Group 1 – included angiographically proven CAD with known T2DM patients (n = 131) and Group 2 – included healthy control (n=131), from MGM hospital, Navi Mumbai. Patients with history of chronic liver disease, kidney disease, malignancy, hypothyroidism and other inflammatory disorders were excluded from the study, since these conditions are known to influence the level of biomarkers. After selection of appropriate study subjects, written informed consent was obtained from all participants formerly explaining the details about the study protocol. The study was approved by Institutional Ethics

Committee (IEC registration number- MGMIHS/RS/2015-16).

### Sample size

According to the previous study the prevalence of T2DM in Mumbai was 9.3% and for CAD in Indian scenario taken as 9.3% (ranging from 9 to 10%).[5] Therefore, we have the calculated sample size by using the formula

$$N = Z^2 . P \frac{(1-P)}{d^2}$$

where, N = sample size, P = prevalence 5% = 0.093 for T2DM with CAD, Z = standard normal variable = 1.967, d = error 5% = 0.05. Hence, minimum samples = 130.54 (n = 131) for T2DM-CAD was considered and 1:1 ratio of healthy control to study group.

### Sample collection

After 10 to 12 h of fasting, 8–10 mL of blood samples were collected under aseptic condition by using BD vacutainers. Of which 2 mL fasting blood and 2 mL post prandial blood was collected in sodium fluoride bulb for estimation of glucose level by glucose oxidase method, 2 mL of blood was collected in Ethylene Diamine Tetra Acetic Acid (EDTA-anticoagulant) bulb for the measurement of glycated hemoglobin (HbA1c) by HPLC technique and remaining 4–6 mL of whole blood was transferred in plain vacutainers. After 30 min of blood collection in plain bulb, the serum was separated for estimation of lipid profile including total cholesterol, triglyceride, and HDL-C by cholesterol oxidase peroxidase-end point method, glycerol phosphate oxidase-enzymatic method and Immuno-inhibitions method respectively.

All these routine biochemical parameters were processed on the same day of sample collection on a fully automated system – auto-analyser Beckman Coulter AU480, (kits of Beckman Coulter, catalogue number: glucose – OSR6621, cholesterol – OSR6516, triglyceride – OSR 66118, and HDL–C – OSR6295). Whereas, LDL-C and VLDL-C were calculated by using Friedewald's formula as LDL = [TC-(TG/5)-HDL], VLDL-C = TG/5, and also Non-HDL-C = (Total cholesterol – HDL).

Residual serum was stored at –70°C for maximum 4–5 days and it was used to estimate fasting serum insulin, amylin, inflammatory markers including hsCRP, TNF-α, and IL-6 by sandwich Enzyme Linked Immunosorbent Assay (ELISA) (kits from Chemux Bioscience, RayBio, Calbiotech, & Krishgen BioSystems respectively) and read spectrophotometrically. Homeostatic Model of Assessment of Insulin Resistance was calculated by the following formula:-

HOMA-IR = fasting serum insulin (IU/mL) × fasting blood glucose (mmol/L)/22.5 and

Insulin sensitivity index was calculated as:

QUICKI = [1/log (fasting insulin μU/ml) + log (fasting blood glucose mg/dl)].

### Diagnostic Criterion

Diabetes was diagnosed with fasting plasma glucose ≥126 mg/dl or confirmed as per WHO criteria[6] or if there is a definite history of diabetes with records of treatment. Glucose tolerance was considered as normal with fasting plasma glucose was <100 mg/dl. For interpretation of serum lipid profile reference values were assessed based on the guidelines of National Cholesterol Education Programme (NCEP) and Adult Treatment Panel III (ATP-III). According to NCEP-ATP III guidelines, hypercholesterolemia is defined as total cholesterol level >200 mg/dl, high LDL-C when value >100 mg/dl; hypertriglyceridemia as TG >150 mg/dl and low HDL-C with <40 mg/dl.[7] Dyslipidemia was considered by presence of one or more than one abnormal serum lipid concentration.

Anthropometric measurements: Height in centimeters, weight in kilograms, and body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters (weight in kg/height in $m^2$). For normal weight, BMI is 18.5 to 24.9 $kg/m^2$; for overweight, BMI is 25 to 29.9 $kg/m^2$; and for obese 30 $kg/m^2$; or more.[8]

### Statistical Analysis

Statistical analysis was done by using software SPSS version 24.0. Testing the relationship between circulatory amylin concentrations with other

variables was done using Pearson's correlation (r). The descriptive statistics were expressed as Mean ± SD and the statistical significance level was considered as <0.05 at 95% CI. The association between the variables, the regression analysis and Area under curve the Receiver Operator Characteristic Curve (AUC-ROC curve) was performed to find specificity, sensitivity, and cut off value for assessment and stratification of risk or prediction of disease severity.

## Results

Figure 1 shows significant association of circulatory Amylin concentration with Triglyceride level in T2DM-CAD, r = 0.8838, **p≤0.01. We observed significant positive association between triglyceride to amylin level in T2DM with CAD, (r = 0.880, ** p≤0.01) . Figure 2 shows partial regression analysis of amylin with TG in DM-CAD, suggesting that T2DM with CAD patients may have genetic predisposition for hypertriglyceridemia, and hyperamylinemia; that can reduce chylomicron uptake leading to an increase in the concentration of TG rich lipoprotein remnants in the circulation.

Hyperamylinemia causes accumulation and deposition of amylin and form toxic insoluble fibril that leads to apoptosis and destruction of β cells. In support to our initial hypothesis, we observed strong positive association between inflammatory cytokine hsCRP and amylin (r = 0.790, p ≤ 0.01) in DM with CAD population (Figure 3).

**Table 1** Comparison of Age, BMI, Circulatory Amylin Level and Diabetic Profile in Healthy Control and T2DM with CAD Patients

| Parameters | Control | T2DM with CAD | P |
|---|---|---|---|
| **Age (years)** | 50.7 ± 5.9 | 55.06 ± 3.7 | >0.05 |
| **BMI (kg/m²)** | 21.2 ± 1.65 | 26.6 ± 4.3 | <0.05 |
| **Amylin (pmol/l)** | 10.68 ± 3.72 | 38 ± 12.5 | <0.01 |
| **BSL-F (mg/dl)** | 82.9 ± 8.21 | 249.5 ± 35.7 | <0.05 |
| **BSL-PP (mg/dl)** | 105.5 ± 7.43 | 277.5 ± 57.5 | <0.05 |
| **HbA1c (%)** | 4.6 ± 0.49 | 10.6 ± 2.9 | <0.05 |
| **Insulin (µU/ml)** | 6.8 ± 2.13 | 12.9 ± 3.4 | <0.05 |
| **HOMA-IR** | 1.71 ± 0.71 | 6.6 ± 2.6 | <0.05 |
| **QUICKI** | 0.4 ± 0.02 | 0.27 ± 0.01 | <0.05 |

Results are expressed as Mean±SD, p* ≤ 0.05 significant, p** ≤ 0.01 highly significant with 95 % CI, p > 0.05 is considered as Non-significant (NS).

**Table 2** Pearson's Correlation of Circulatory Amylin Level with BMI and Diabetic Profile in T2DM with CAD group

| Parameters | T2DM with CAD r -value |
|---|---|
| **BMI (kg/m²)** | 0.324* |
| **BSL-F (mg/dl)** | 0.868** |
| **BSL-PP (mg/dl)** | 0.874** |
| **HbA1c (%)** | 0.868** |
| **Insulin (µU/L)** | 0.851** |
| **HOMA-IR** | 0.868** |
| **QUICKI** | –0.868** |

Association of circulatory amylin level with study variables with statistical significance level at p* ≤ 0.05 and highly significant at p** ≤ 0.01 in T2DM with CAD groups.

**Table 3** Comparison of Circulatory Amylin Level and Lipid Profile in T2DM with CAD group

| Parameters | Control | T2DM with CAD |
|---|---|---|
| **Amylin (pmol/l)** | 10.68 ± 3.72 | 38 ± 12.5** |
| **T Cholesterol (mg/dl)** | 167.7 ± 25.6 | 228.2 ± 25.7* |
| **TG (mg/dl)** | 95.4 ± 20.2 | 199.6 ± 15.7** |
| **HDL-C (mg/dl)** | 56.8 ± 6.85 | 37.6 ± 3.6 ** |
| **LDL-C (mg/dl)** | 81.8 ± 18.9 | 154.5 ± 17.9** |
| **VLDL-C (mg/dl)** | 19.09 ± 4.0 | 39.92 ± 3.1** |
| **Non-HDL-C (mg/dl)** | 111 ± 32.2 | 179.8 ± 29.4** |
| **TG/HDL** | 1.7 ± 0.6 | 5.3 ± 0.8* |
| **LDL/HDL** | 1.6 ± 0.72 | 4.1 ± 0.8* |

Mean ± SD, p ≤ 0.05 significant, p** ≤ 0.01 highly significant with 95 % CI, comparison of lipid profile between control and T2DM with CAD group.

Figure 4 shows significant positive association between amylin and IL-6 in T2DM with CAD subjects (r = 0.903, p ≤ 0.01).

## Discussion

Metabolic syndrome (MtS) includes "obesity, dyslipidemia, hypertension and hyperglycemia". These are typical causal factors for the progression of type 2 diabetes and cardiovascular diseases.[4] In this cross sectional study, we enrolled total of 262 participants of which 131 were healthy control, and the other 131 angiographically diagnosed cases of T2DM-CAD, aged 30 to 60 years were of both gender. Table 1 shows T2DM-CAD subjects with BMI more than 25 kg/m², and they have significantly increased circulatory levels of amylin

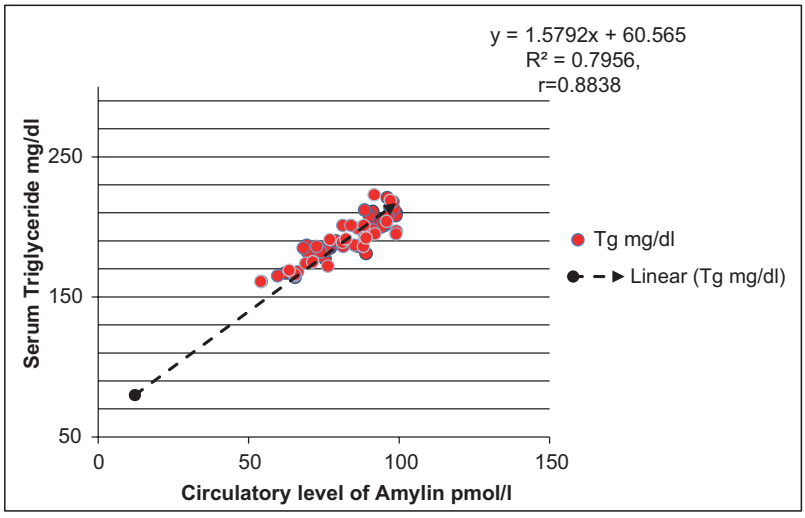


**Figure 1** Correlation of Amylin with Triglyceride in T2DM with CAD

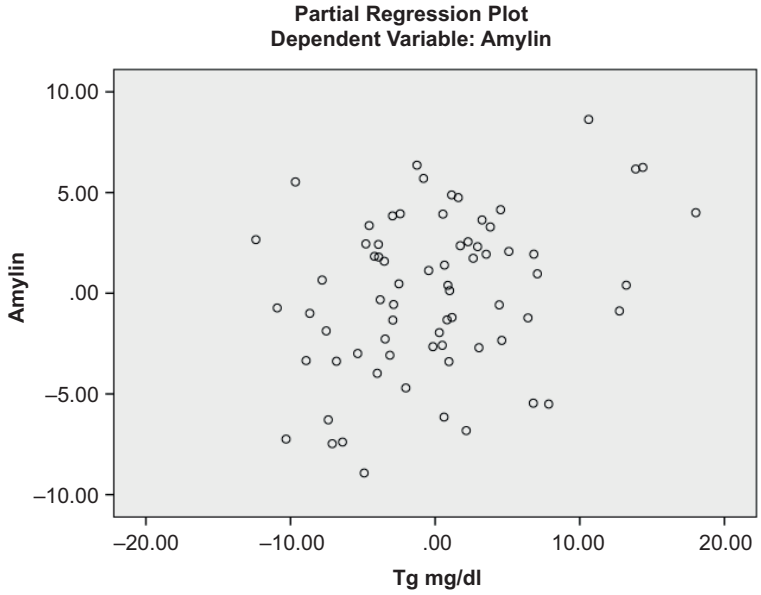


**Figure 2** Partial Regression Analysis between Amylin and Triglyceride in T2DM with CAD

**Table 4** Pearson Correlation of Amylin with Lipid Profile in T2DM and T2DM with CAD group

| Parameters | T2DM with CAD r –value |
|---|---|
| **Cholesterol (mg/dl)** | 0.273NS |
| **Triglyceride (mg/dl)** | 0.880** |
| **HDL-C (mg/dl)** | –0.790** |
| **LDL-C (mg/dl)** | 0.377 * |
| **VLDL-C (mg/dl)** | 0.880** |
| **Non-HDL-C (mg/dl)** | 0.396** |

[Statistical significance level at p* ≤ 0.05 and p**≤0.01 (95 % CI) and NS-p > 0.05] The present observation shown in Table 4 indicates significant positive association of amylin with lipids including Cholesterol, TG, LDL, and VLDL, whereas negative association with HDL-C in T2DM with CAD subjects.

**Table 5** Comparison of Circulatory level of Amylin and Inflammatory Biomarkers in Control and T2DM-CAD

| Parameters | Control | T2DM with CAD |
|---|---|---|
| Amylin (pmol/l) | 10.68 ± 3.72 | 38 ± 12.5** |
| hsCRP (mg/l) | 0.67 ± 0.34 | 9.0 ± 3.8 ** |
| TNF-α (pg/ml) | 11.62 ± 5.0 | 46.3 ± 9.4 ** |
| IL-6 (pg/ml) | 8.01 ± 5.13 | 36.2 ± 10 ** |

(Mean ± SD, the statistical significant level at p**≤0.01 with 95% CI) Table 5 shows comparison of serum Amylin, hsCRP, TNF-α and IL-6 between controls and T2DM with CAD group. The observations indicate the circulatory level of amylin significantly raised in T2DM with CAD groups as compared with that of healthy control group.

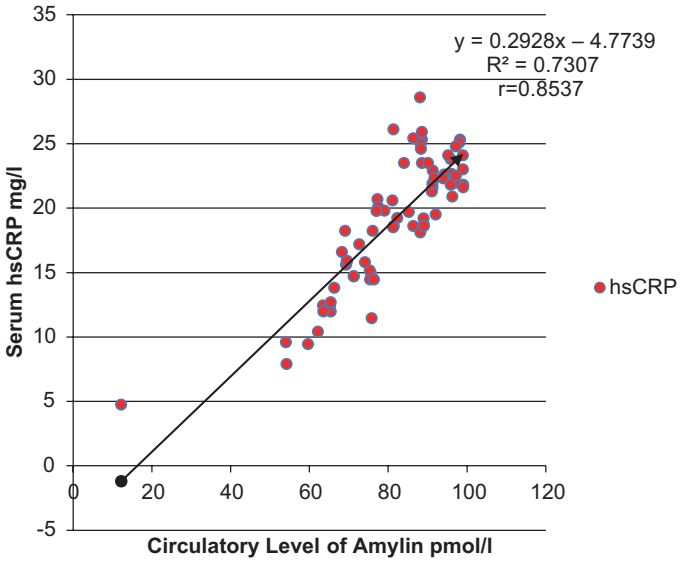


**Figure 3** Correlation of Amylin with hsCRP in T2DM-CAD

(38 ± 12.5 pmol/l) as compared to healthy control group (10.68 ± 3.72 pmol/l; p ≤ 0.01), and also shows positive association with BMI (r = 0.324, p-value 0.042) shown in Table 2. This may be due to less or decreased availability of functional amylin molecules to reduce the body weight. This is in line with Thomas R et al. They have studied such kind of relationship in obese children.[9] Among diabetic profile, all parameters significantly increased except insulin sensitivity index, which decreased (QUICKI-0.27 ± 0.01 vs 0.4 ± 0.02) in T2DM-CAD as compared with that of control group (p ≤ 0.01).

Despa S and colleagues reported that there was an increased circulatory level of amylin, and its accumulation in coronary arteries and cardiac parenchyma in human islet amyloid polypeptide diabetes due to a progressive defect in beta cell mass in rats transgenic for HIP (HIP rat).[2] They also conducted a study on human heart samples and observed the oligomerized amylin (i.e., trimers, tetramers, and octamers), significantly

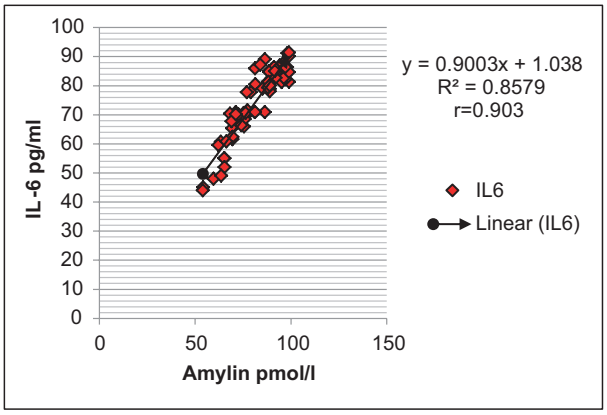


**Figure 4** Correlation of Circulatory Level of Amylin with Inflammatory Markers IL-6 in T2DM with CAD patients

**Table 6** Pearson's Correlation of Amylin with Inflammatory Markers in T2DM with CAD

| Parameters | T2DM with CAD r –value | P |
|---|---|---|
| **hsCRP (mg/l)** | 0.853** | ≤ 0.01 |
| **TNF-α (pg/ml)** | 0.306* | ≤ 0.05 |
| **IL-6 (pg/ml)** | 0.903** | ≤ 0.01 |

Statistical significance level at p* ≤ 0.05 and p** ≤ 0.01, we observed positive association of circulatory level of amylin with hsCRP, TNF-α, and IL-6 in T2DM with CAD patients in Table 6.

**Table 7** Area under the Receiver Operator Characteristic Curve of Inflammatory Markers and Amylin in T2DM with CAD

| Variables | Sensitivity | Specificity | 95% CI | Associated criterion | AUC | p |
|---|---|---|---|---|---|---|
| **Amylin** | 97.5 | 86.8–99.1 | 100 | >24 | 0.998 | ≤0.01** |
| **hsCRP** | 65 | 48.3–79.4 | 95 | >8.1 | 0.787 | ≤0.01** |
| **TNF-α** | 42.5 | 27–59.1 | 100 | >46.1 | 0.697 | ≤0.01** |
| **IL-6** | 45 | 29.3–61.5 | 87.5 | >35.2 | 0.638 | 0.028* |

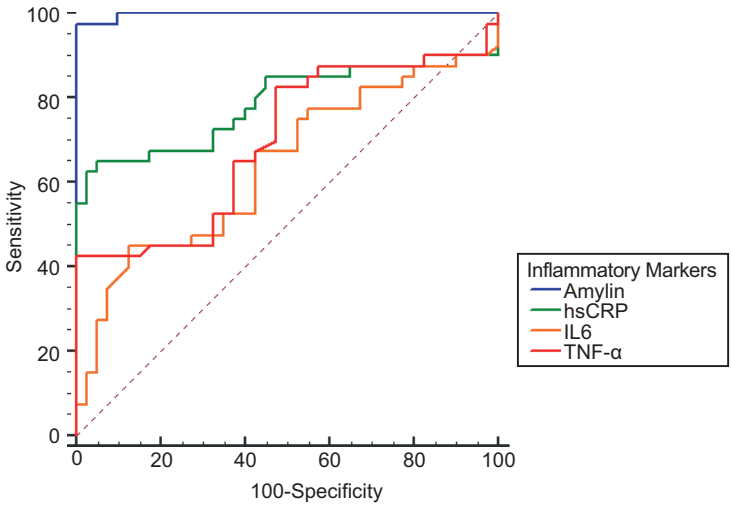


**Figure 5** Area under the Receiver Operator Characteristic Curve of Inflammatory Markers and Amylin in T2DM with CAD

higher in myocyte lysates from obese-heart failing group compared with those from lean-heart failing and lean-non-heart failing groups. Further, in the obese-non-failing group, they observed an elevated, aggregated form of amylin, including an early phase of protein aggregation.[10]

Another study of Despa S et al. also revealed that hyperamylinemia facilitates the accumulation and deposition of amylin amyloid in the heart, allowing the structural and functional changes in the cardiac myocyte. The possible mechanism of amylin amyloid cytotoxicity may involve, its oligomers interact with cardiac myocytes, results in greater transient sarcolemmal $Ca^{2+}$ leak and $Ca^{2+}$ transient leading to activation of pathological heart remodelling and diastolic dysfunction induced by $Ca^{2+}$ hypertrophic pathways.[2] As amylin is fraternal twin of insulin, hyperamylinemia advances hyperinsulinemia.[11]

Although, increased insulin secretion in obesity is consistent with the increased amylin concentration[9] and hyperamylinemic phase due to long-lasting exposure to hyperglycemia. The islets of pancreas facilitate selective amylin secretion and alters the secretory ratio of amylin to insulin.[11] These increased circulatory levels of amylin get accumulated and converts its soluble monomeric form into its non-soluble oligomeric fibrils, which has great potential and aggregates toxic nanoparticles. These toxic amylin amyloid gets accumulated into the islets of pancreas and causes destruction and apoptosis of beta cell, and decreases the total beta cell volume.

The accumulation of increased amylin concentration leads to toxic effect on the beta cell function in T2DM that alters cardiac function.[2] Diabetic is more vulnerable to coronary heart disease; thus rising obesity-related diabetic dyslipidemia (DD) comprises elevated concentrations of triglycerides with preponderance of high LDL-cholesterol and low HDL-cholesterol.[12]

In present study we found such kind of dyslipidemias in T2DM-CAD population (Table 3). These significantly raised the levels of total cholesterol, Triglyceride, LDL-C, VLDL-C, and decreased HDL-C ($p < 0.001$) in study subjects as compared with healthy control, and find their association with circulatory amylin concentration we performed Pearson's correlation and found positive association of circulatory amylin concentration with total cholesterol, TG, LDL-C, VLDL-C and negative association with HDL-C (Table 4). The dyslipidaemia in diabetic-CAD patients is on par with Nariman Mordi et al.[13]

To find better predictive power of variables the linear regression analysis was performed, and it was found that significant contribution of TG, in prediction of severity with dependent variable amylin in T2DM-CAD subjects with R square = 0.948, adjusted R square = 0.941 and standard error of estimate 3.14078, with significant F change is 0.001.

Thomas R et al., have studied amylin and its relation with lipids in obese children and observed that there was a strong positive relation of amylin with TG and observed $r = 0.49$, and $p \leq 0.01$.[9] This may be due to hyperamylinemia which can lead to the development of hypertriglyceridemia. Through amylin, we can decrease the uptake

of chylomicrons, mostly, either directly or by modulating the insulin activity by controlling lipoprotein receptors. Increased amylin levels may lead to increased triglyceride rich lipoprotein remnants.[9]

It remains controversial, whether elevated amylin specifically or implicitly induces dyslipidemia or vice versa; Smith and colleagues reported bolus infusion with amylin and noted increased plasma TG levels significantly and TG-rich lipoprotein clearance decreased by 45 % approximately.[14] Ye et al showed that amylin infusion raises the concentration of non-esterified fatty acids and glycerol, with increased concentrations of liver triacylglycerols in animal model.[12]

Our findings show that the increased circulatory level of amylin may be due to increase in triglyceride rich lipoprotein contents in DM-CAD. However, structured long-term human studies are needed to demonstrate mechanism for linking hyperamylinemia with diabetic dyslipidemia. Hypertriglyceridemia may be key factor for a number of other lipid disruptions, since TG-rich lipoprotein clearance and sd LDL-C formation may be affected.

Obesity stays to catabolize of the TG-rich lipoproteins due to reduced mRNA in adipose tissues – Lipoprotein lipases (LPL), weakened LPL action in the skeletal muscle, and competes with VLDL and chylomicrons. Increased post prandial lipemia causes rise in free fatty acids in the circulation, which removes LPL from endothelial surface. LPL can eventually succumb to VLDL and IDL (intermittent density lipoprotein), which leads to further degradation of TG. Finally, the replacement by CETP [cholesteryl ester transfer protein] of TG from these remnants for cholesterol esters from HDL with definitive action of hepatic lipase led to small dense LDL-C (sdLDL) formulation. The cholesterol-ester content of LDL declines in the case of hypertriglyceridemia, while the TG content of LDL rises through the CETP action.[15]

Nevertheless, the rise of LDL-TG in plasma contributes to hydrolysis by hepatic lipase, which allows the sd-LDL formation. The metabolism of sd-LDL takes place steadily, and it gets more atherogenic. Remnants of chylomicron and LDL may relocate into the sub-endothelium and then become stuck and it may be taken up by monocytes/macrophages. sdLDL has improved sensitivity to arterial proteoglycans leading to increased persistence of sub-endothelial lipoproteins.[15] Furthermore, in comparison to native LDL, sub-endothelial remnants of chylomicrons and VLDL should not have to be altered to enable acquisition by macrophages scavenger receptors.

Boudewijn et al.,[15] have stated that small dense-LDLs are more prone to oxidation, partly due to less free cholesterol and reduced anti-oxidant content. It was noticed that the size of lipoprotein was a limiting factor for mobility through the endothelium and LDL particles migrate more efficiently than the remnants of chylomicron. The amount of particulates transferred does not inherently result into cholesterol deposition, as chylomicron remnants produce 40 times more cholesterol per particle as compared with that of LDL. Conversely, the LPL-enriched residues of chylomicrons and VLDL can be dispersed to tissues, which lead to fragment removal through interaction with proteoglycans and lipoprotein receptors. Several individuals with CAD have normal or marginally higher LDL-C levels, but they show low HDL and/or high TG concentrations. Thus, many high-risk patients may not be reliably diagnosed by focusing solely on LDL-C as a measure of risk in these individuals.[15]

In lipid lowering treatment by NCEP, Non-HDL-C is the secondary target after LDL-C with raised TG level.[16] Non-HDL-cholesterol can be determined effectively, since it does not need to prepare patient for fasting. In this investigation we noticed significant relationship of Non-HDL-C ($179.8 \pm 29.4$) with amylin $r = 0.396$, $p \leq 0.01$ (Table 4). Several efforts are made to investigate existing cardiovascular risk components for a better diagnosis of cardiovascular disease, and atherogenic indices are depicted in an undertaking to enhance the predictive capability of the lipid profile. These markers offers understanding of risk factors those are difficult to measure by routine assessments and can reflect both the metabolic and therapeutic relationship of lipid fraction more precisely.

The interaction of atherogenic dyslipidemia with inflammatory processes in diabetic individuals are more common. To test our initial hypothesis and scarcity of direct evidence regarding the relationship between circulatory level of amylin and inflammatory markers, we have selected T2DM with CAD study population. Low-grade inflammation is well-recognized in primary pathways linked with obesity, IR, T2DM and/or with CVDs; the conditions often co-exist with hyperamylinemia.[4]

Table 5 shows levels of inflammatory cytokines and circulatory amylin concentration in T2DM with CAD patients. We found significantly elevated

hsCRP (9 ± 3.8 mg/l), TNF-α 46.3 ± 9.4 pg/ml), IL-6 (36.2 ± 10pg/ml) and amylin (38 ± 12.5pmol/l) in T2DM with CAD population as compared to healthy control (0.67 ± 0.34, 11.62 ± 5, 8.01 ± 5.13 and 10.68 ± 3.72 respectively) with $p \leq 0.01$. We observed a significant association of circulatory amylin level with the inflammatory markers (Table 6), including hsCRP $r = 0.857$, $p \leq 0.01$ (Graph 3), TNF-α ($r = 0.306$, $p \leq 0.05$, and IL-6 ($r = 0.903$, $p \leq 0.01$, shown in Figure 4). As per these results, we have proved our initial hypothesis, that is, there is association between inflammation and amylin level.

This may be due to the fact that over-secreted amylin oligomerizes, which are toxic to trigger inflammation in pancreatic islets, and contribute to T2DM. Oligomerized amylin aggregation is a potent source of inflammation in the pancreas; this is in line with Srodulski S and colleagues.[17] Xinwei Hou et al., showed a close relation of amylin with inflammatory markers and metabolic disorders, which is an identified risk factor for metabolic syndrome including obesity, inflammation, and insulin resistance.[4]

Also Despa S and colleagues conclude that amylin oligomers are bound to the sarcolemma, contribute to dysregulation of the myocyte $Ca^{2+}$, abnormal remodeling of the myocyte, and diastolic dysfunction, culminating from prediabetic state. Hyperamylinemia tend to accumulate amylin in heart, contributing to structural and functional modifications in cardiac myocyte.[2] This may likewise be recognized to higher glucose and FFA levels that show harmful effects on ß cells and activate numerous pro-inflammatory mediators. It has been recommended that cytokines be secreted by monocytes/macrophages, including TNF-α activated by stressed hyperglycemia.[18,19]

The TNF-α demonstrated a major inducer of amylin synthesis at mRNA, but had no impact on proinsulin expression. An elevated TNF-a and circulatory amylin levels were found in obese and insulin resistant state).[20] Nonetheless, TNF-α induces the expression of amylin under acute inflammatory conditions. Whereas, amylin may be elevated as a result of islet cell necrosis; it may also be caused by TNF-α, which plays a key role in inflammatory response in pancreatitis. In the up-regulation of amylin gene expression, some variables such as glucose and NEFA are recognizable.

Immunoreactivity was found in deposits of pancreatic islets amyloid, at proamylin flanking $NH_2$-terminal end. It was reported that impaired proamylin processing, contribute to hypersecretion of unprocessed or partially processed proamylin that can have a greater tendency to accumulate than mature amylin. Cai K, et al., found that glucose and TNF-α stimulated the secretion of pro-IAPP and IAPP in murine islets; while, glucose intensely up regulates pro-hormone convert [PC1/3 and PC2] translationally; the function of these enzymes may be not enhanced appropriately that resulting in increase in IAPP precursors. The disproportionate rise between PC1/3 and PC2 activity and proamylin expression may contribute to an increase in amylin precursors induced by TNF-α, however, further research is needed in depth. Upregulation of amylin by proinflammatory cytokine, TNF-α can lead to elevation of amylin and deposition of amylin amyloid. By direct involvement in inflammatory disruption and insulin resistance, TNF-α may play a major role in the over-expression of amylin during acute inflammatory stress.[20]

In the progression of CAD, a combination of metabolic, endothelial and pro-coagulant factors, and IL-6 are suspected to play major role. The impaired vessel-wall, smooth muscle cells increases the production and secretion of IL-6 as its gene transcript is such that in atherosclerotic legion there is an increased IL-6 expression, cardiovascular mortality forecast, and a potential MI incident[21]; also CRP increases circulatory IL-6.[20]

In Present study we found that serum IL-6 levels were significantly higher in T2DM with CAD group as compared with that of the healthy control, and it also found significant association with circulatory serum amylin level. Thus supporting the initial hypothesis, the inflammatory cycle may assume a causal part in the turn and anticipate diabetic and atherosclerotic disorder. Further study is required with more population, to affirm the causality and relationship between these inflammatory markers and circulatory amylin in diabetic-CAD population.

## Limitations and strengths of our study

This article is a primary cross sectional study that shows the elevated circulatory concentration of serum amylin and their association with inflammatory cytokines in T2DM with CAD patients; the incident cases are small therefore, further investigations in a large population size, age, and gender-wise distribution with follow-up monitoring

from zero day of disease diagnosis to the progression for at least 10 years are needed to confirm the study findings.

## Conclusion

Our study highlights the association of circulatory concentration of amylin and inflammatory mediators. The hyperamylinemia may be a strong indicator and predictor for future complications in DM-CAD, therefore, these variables may have the potential of predicting the severity of disease. Thus, identifying patients with diabetic dyslipidemia in association with increased serum level of amylin as well as increased inflammatory mediators provide an opportunity to reduce the incidence of future cardiac complications in diabetic subjects.

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## Conflict of interest

The authors have no conflicts of interest to declare.

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