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Serum Triglyceride and High Sensitive C - reactive protein Levels in Subclinical Atherosclerosis in Type 2 Diabetes Mellitus

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Abstract: Background: The elevated level of high-sensitivity C-reactive protein and Subclinical Atherosclerosis are associated with high mortality in type 2 diabetic patients. **Objective:** to detect serum levels of triglyceride and high sensitive C - reactive protein in type 2 diabetic patients and their relations to subclinical atherosclerosis in these patients. **Materials and Methods:** We studied 50 diabetic patients (age ranged 38 - 67years) who presented with subclinical atherosclerosis. Diabetic patients were divided according to HbA1C levels to good diabetic patients and poor diabetic patients besides 25 healthy individuals as a control. High sensitive C - reactive and triglyceride were measured and duplex of extra cranial carotid arteries performed for all patients. **Results:** We found a significant elevation of serum TG level in diabetic groups compared to control group and higher in poor controlled diabetic group than good controlled diabetic group. Serum TG level, serum hs-CRP level and IMT was significantly higher in uncontrolled diabetics compared to controlled diabetics. A significant positive correlation between TG level and the duration of DM, systolic BP, diastolic BP, BMI, FPG, PPG and IMT. **Conclusions:** Triglycerides and hs-CRP have a strong association with T2DM status. Subclinical atherosclerosis is a common and frequent complication of type 2 diabetes mellitus the possible implicated risk factors.
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1- Introduction:

Atherosclerosis and cardiovascular disease remains the principal cause of death and disability among patients with diabetes mellitus, especially in those with type 2 diabetes mellitus. [1]

Diabetes also has a close relation to subclinical atherosclerosis. Carotid atherosclerosis (CA) detected ultrasonically occurs in arteries with intimal hyperplasia. Carotid intima–media thickness (cIMT), which can be used to predict the risk of developing coronary artery stenosis in patients who are asymptomatic, is significantly greater in those with T2DM than in the general population. [2]

Measurement of cIMT is simple, noninvasive and a valuable means of identifying high-risk individuals both with and without diabetes. The presence of carotid plaque (CP) is an indicator of advanced atherosclerosis and can be used to predict cardiovascular risk, being associated with major adverse cardiovascular events in patients with T2DM. [3]

Diabetes mellitus and dyslipidemia commonly occur together, with lipid abnormalities affecting 60% to 70% of type 2 diabetes mellitus[4] and hyperglycemia accelerates atheroma formation in the setting of diabetic dyslipidemia. LDL cholesterol particles are more atherogenic in diabetes mellitus even in the absence of overt increased LDL concentration, with small, dense particles that are particularly prone to modification. [5]

Diabetic dyslipidemia is also characterized by elevated triglycerides, low high-density lipoprotein (HDL) cholesterol, and higher concentrations of apoB-containing particles,[6] suggesting either coassociations of independent disorders or a pathophysiologic role for insulin resistance, rather than hyperglycemia, in the development of diabetic dyslipidemia. [7]

Inflammation plays a major role in atherogenesis and cardiovascular outcomes has a significant impact on current management of coronary artery disease. Multiple risk markers have been evaluated in the past to improve risk stratification and prediction of adverse cardiac events. [8]

The carotid intima-media thickness (IMT) reflects the diffuse thickening of the intimal layer seen in atherosclerosis and has been validated as a measure of the risk for cardiovascular events and atherosclerotic disease burden. [9]

This study aim to detect the relationship between serum triglycerides and high sensitive c reactive protein levels in subclinical atherosclerosis in type 2 diabetes mellitus.

2- *Materials and Methods*

This Clinical based case control study has carried out in the Department of Internal Medicine, Faculty of Medicine, Zagazig University Hospital, included patients with type 2 diabetes mellitus diagnosed to be diabetic according to American Diabetes Association (ADA) criteria, 2017. Patients with clinically manifested atherosclerosis and Type 1 DM were excluded from the study also we excluded patient harbor any cause any elevate high sensitive CRP like infection, inflammation, surgery, renal failure or malignancy.

75 subjects were divided into three groups:

Group A: 25 healthy individual served as control group.

Group B: 25 well controlled diabetic patients without atherosclerosis.

Group C: 25 poor controlled diabetic patients with subclinical atherosclerosis.

Patients were subjected to Thorough history taking regarding age, sex, body mass index and diabetes index (diabetes duration, treatment modalities, and Macro/microvascular complications).

hs-CRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of CRP is directly proportional the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm. [10]

Triglycerides blood samples were obtained after an overnight fast 12h and triglycerides were assayed using enzymatic colorimetric test with lipid clearing factor. Triglycerides are determined after enzymatic hydrolysis with lipid clearing factor.

Ultrasonographic analysis of carotid artery was performed with a high-resolution ultrasound scanner. The patients were examined in a supine position, extended neck and leaning the head to the opposite side of examination. Coronal and axis scan was done for B-mode examination. The color duplex study was done in some cases with significant morphological luminal narrowing. We examined each side for intimal medial thickness. [11]

Written Informed consent was taken from the patient to participate in the study. Approval for performing the study was obtained from internal medicine Department, Zagazig University Hospitals after taking Institutional Review Board (IRB) approval.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) and range. One way ANOVA test supplemented with Tukey HSD post hoc test was used to compare between more than two dependent groups of normally distributed variables while Friedman's test ranks test was used for non-normally distributed variables. All statistical comparisons were two tailed with significance Level of P-value $\leq$ 0.05 indicates significant, p <0.001 indicates highly significant difference while, P> 0.05 indicates Non-significant difference. Pearson's and Spearman's correlation coefficient were used for correlating normal and non-parametric variables. We consider (+) sign as indication for direct correlation i.e. increase frequency of independent lead to increase frequency of dependent & (-) sign as indication for inverse correlation i.e. increase frequency of independent lead to decrease frequency of dependent, also we consider values near to 1 as strong correlation & values near 0 as weak correlation. The significance Level for all above mentioned statistical tests done.

3- Results:

Demographic data of the studied subjects included 11 males and 14 females with mean age 47.28 years for Control group, 13 males and 12 females with mean age 50.08 years for Good Control diabetics group, and 12 males and 13 females with mean age 53.76 years for Poor Control diabetics group. There were highly significant difference regarding BMI between the three groups and a highly

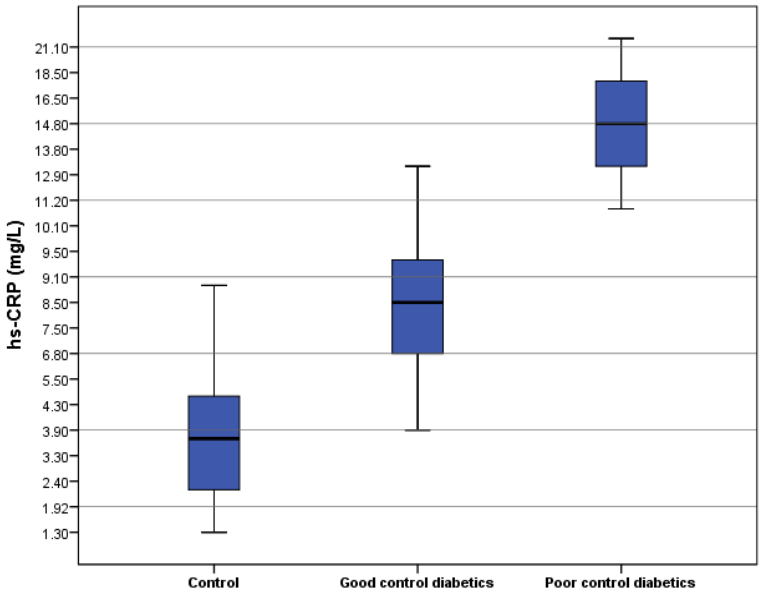

Figure 1. Box plot of high sensitive C - reactive protein level distributed between three studied groups

significant difference between well and poor diabetic patients.

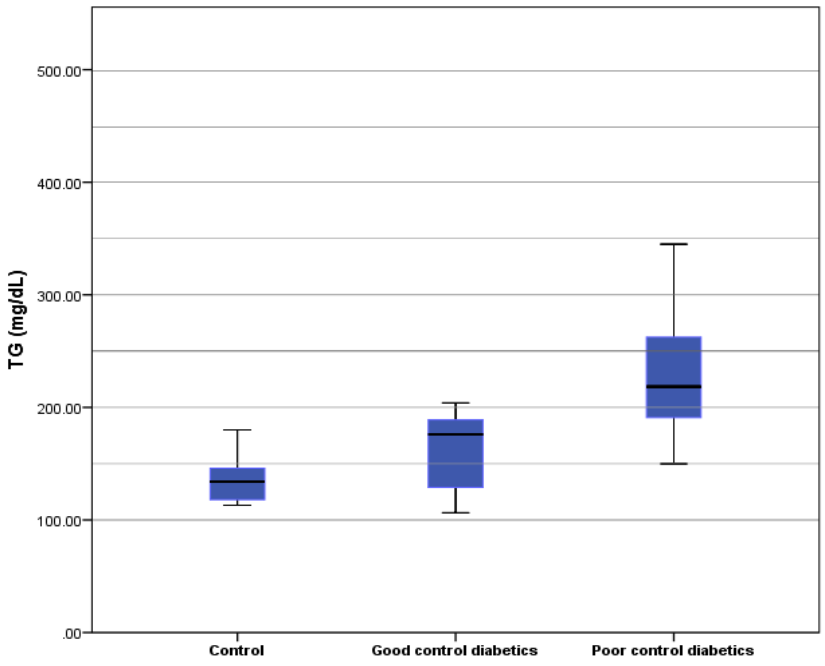

Figure 2. Box plot of TG level distributed between three studied groups

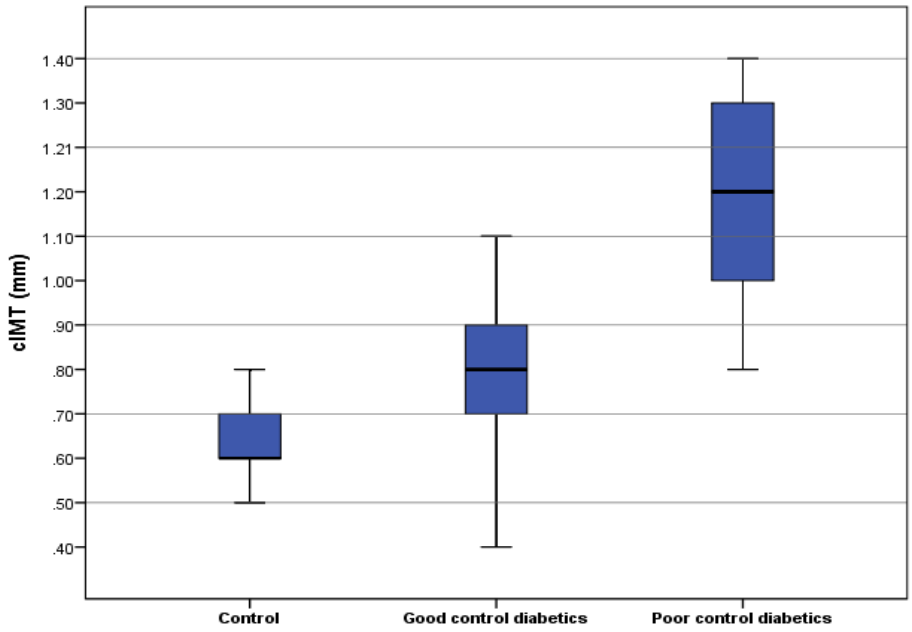

Figure 3. Box plot of cIMT distributed between three studied groups

There is highly positive significant correlations between triglyceride and hs-CRP levels in control group. Also, there is a positive significant correlations between high sensitive C - reactive protein and TG, FBS & PPS levels in Good control diabetics group.

There is a highly positive significant correlation between high sensitive C - reactive protein and TG & cIMT and a positive significant correlation between high sensitive C - reactive protein and Hb A1c levels in poor control diabetics group.

4- Discussion:

Diabetes is major risk factor for morbidity and mortality from cardiovascular diseases non-insulin dependent diabetes mellitus is frequently associated with premature atherosclerosis. The prevalence of atherosclerosis is markedly increased with diabetes mellitus and hypertension. An increased in thickness of carotid artery wall is a singe of early atherosclerosis.

This study showed that diabetes mellitus is atherogenic disease this proved from the finding that cIMT in diabetic groups is higher than control group , also we found that cIMT in poor control diabetic group is higher than good control diabetic group.

This result agree with the study done by Masson et al.[12] who measure CIMT in type2 diabetic patients and control group and show that cIMT increased in diabetic patients compared with control group

Also agree with the study done by Esposito et al.[13] that show diabetic patients had higher values of CIMT than did nondiabetic subjects; this was associated with higher serum concentrations of prion flammatory cytokines and CRP. CRP has been directly related to the rate of progression of carotid atherosclerosis.

Rubinat et al.[14] in their study showed that Subjects with type 2 diabetes mellitus are considered to be at high risk for cardiovascular disease. Carotid intima-media thickness (c-IMT) and carotid plaque prevalence were significantly greater in diabetic subjects than in the control group. Carotid plaques and c-IMT were more frequent in men than in women and increased with increasing age.

In our study we found that triglycerides increased in diabetic groups than control group, also we found that triglycerides increased in poor control diabetic group than good control diabetic group.

Zheng et al.[15] in their study showed that Patients of newly diagnosed T2DM with hypertriglyceridemia were younger, fatter and had worse lipid profiles, glucose profiles, and high insulin levels than those with normal TG. There is no difference in early phase insulin secretion among groups of newly diagnosed T2DM patients with different TG levels. The basal beta cell function (HOMA-β and MBCI) initially increased along rising TG levels and then decreased

as the TG levels rose further. The insulin sensitivity was relatively high in patients with a low level of TG and low with a high level of TG. Hypertriglyceridemia influences clinical characteristics and β-cell function patients with newly diagnosed T2DM. A better management of dyslipidemia may to some extent, reduce the effect of lipotoxicity, thereby improving glucose homeostasis in patients with newly diagnosed T2DM.

Beshara et al.[16] in their study showed that every 10 mg/dL increase in triglyceride level significantly increased the risk of diabetes by 4% and of impaired fasting glucose by 2% ($p<0.001$). This association held true even when rising triglyceride levels remained within the accepted normal range (<150 mg/dL, $p<0.001$). Sustained increments in serum triglyceride level, even within the accepted normal range, are an independent risk factor for diabetes mellitus and impaired fasting glucose in normglycemic participants.

Also our study showed that there is highly positive significant correlation between triglyceride and CIMT in poor control diabetic group

This result agree with study done by Batluk et al.[17] that showed an Absolute non-fasting triglyceride levels were positively associated with cIMT. A final multiple regression model revealed that older age, more severe strokes, and higher levels of fasting triglycerides were significantly and independently associated with higher mean cIMT.

Reiner et al.[18] in their study proved that hypertriglyceridemia is an independent risk factor for CVD have occurred partly because elevated triglyceride levels are often a component of atherogenic dyslipidemia.

In our study we found that hs-CRP increased in diabetic groups than control group, also we found that hs-CRP increased in poor control diabetic group than good control diabetic group.

Also we found positive significant correlations between hs-CRP and HbA1c in poor control diabetic group

Pan et al.[19] in their study found that elevated plasma levels of hs-CRP were only positively associated with T2D among those already with high HbA1c levels, but not in those with low HbA1c levels. Therefore, elevated CRP levels may be a consequence of hyperglycemia, instead of being an etiological biomarker in T2D development.

Mahajan et al.[20] in their study showed that hs-CRP levels were significantly higher in both diabetic men and women as compared to their nondiabetic counterparts ($P < 0.001$). Elevated hs-CRP was positively associated with T2D (OR, 1.66; 95% confidence interval, 1.21–2.28; $P = 0.002$) even after adjusting for markers of obesity. After adjustments for age, sex, and BMI, HbA1c was the major correlate of hs-CRP in nondiabetic subjects ($\beta = 0.28$; $P = 0.03$). We observed that T2D patients were at higher risk for cardiovascular disease compared to nondiabetic subjects when classified into low-, intermediate- and high-risk groups based on hs-CRP levels (ptrend = 3.8×10^{-15}).

In our study we found highly positive significant correlations between hs-CRP and carotid intima medial thickness in poor control diabetic group

This result agree with Pokharel et al.[21] in their study who proved that Vascular inflammation is a key mechanism in the progression of atherosclerosis and acute coronary syndromes, Inflammatory markers, such as hs-CRP, have been shown to identify patients with increased risk for incident coronary heart disease, ischemic stroke and vascular mortality

Also Li et al.[22] in their study showed that elevated hs-CRP levels independently predicted all-cause and cardiovascular mortality risk in the general population. Individuals with the highest hs-CRP levels had a 75% and 2.03-fold increased risk of all-cause and cardiovascular mortality compared with the lowest category of hs-CRP levels. In addition, individuals with the highest hs-CRP levels appeared to have a 25% greater risk of total cancer mortality. Sensitivity analysis further confirmed the robustness of our pooling results.

A recent study by Pleskovič et al.[23] showed that T2DM patients with hs-CRP levels ≥ 2 mg/L had higher cIMT in comparison with T2DM patients with hs-CRP levels below 2 mg/L, and higher incidence of plaques/unstable plaques in comparison with T2DM patients with hs-CRP levels below 2 mg/L. so there is an association between the inflammatory marker hs-CRP and either cIMT or incidence of plaques/unstable plaques at the time of recruitment in patients with T2DM. Moreover, they found the association between hs-CRP levels and either cIMT progression rate or a change in the number of sites with plaques in a 3.8-year follow-up in subjects with T2DM.

Kurkowska-Jastrzebska et al.[24] in their study found a moderate positive correlation between CIMT and hs-CRP in patients with a history of MI. This correlation was independent of age, sex, hypertension, diabetes, and statin therapy. However, it was not found in control group .also showed that higher hs-CRP levels were higher coronary risk and more likely to indicate high-risk carotid atherosclerosis.

5- Conclusion:

Subclinical atherosclerosis is a common and frequent complication of type 2 diabetes mellitus the

possible implicated risk factors encountered in our work to explain atherosclerosis include hyperglycemia , duration of DM , obesity, hypertriglyceridemia and elevated hs-CRP level.

Routine measurement of serum TG level, serum hs-CRP, in all patients with type 2 DM in addition to duplex on extra cranial carotid arteries to detect early subclinical atherosclerosis is recommended. Early and proper treatment of DM and its complications, dyslipidemia and obesity help in CVD mortality reduction.

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Conflicts of interest:
There are no conflicts of interest.

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