

SERUM OSTEOPROTEGERIN FOR EARLY IDENTIFICATION OF CAD IN TYPE 2 DM PATIENTS

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ABSTRACT

Background:

Osteoprotegerin (OPG) is a well-known protein, it can reduce the osteoclast production and it also be used to identify people with CAD. The aim of this study was to investigate OPG levels, CIMT, and CAC measures in diabetic participants, as well as examine the link between blood OPG levels and early atherosclerosis such as CIMT, CAC, and GLS in patients with type 2 DM.

Method:

The study included 160 type 2 DM patients. All study were investigated GLS and venous blood specimens were collected to determine OPG. Serum OPG levels was determined using an ELISA. FPG, PPBS, and HbA1c values were assessed. CIMT were measured with B-mode ultrasonography, CAC were measured with CT.

Results:

The OPG levels were substantially higher in FBS >126 mg/dl group (1.64 ng/ml) compared to the FBS ≤126 mg/dl group (1.00 ng/ml). Mean OPG levels were higher in the PPBS ≥200 mg/dl group (1.47 ng /ml) compared to the PPBS <200 mg/dl group (0.93 ng /ml). The mean OPG level were substantially higher in abnormal CIMT (1.74 ng/ml) when compared to the normal CIMT (0.92 ng /ml). Mean OPG level was statistically significant in abnormal GLS (1.91 ng/ml) compared to the normal GLS group (1.15 ng/ml). Serum OPG levels also demonstrated a significant increase with disease severity. Mean levels ranged from 0.67 ng/ml in the "normal" group to 3.93ng/ml in the "severe" group, with a highly significant difference (F = 41.203, p= 0.000).

Conclusion:

Our study demonstrates the serum OPG levels are substantially associated with CAD severity, highlighting their potential as early biomarkers for CAD. This biomarker could complement existing diagnostic tools, allowing for earlier detection and better risk stratification in CAD patients.

Keywords: Osteoprotegerin (OPG), Carotid intima-media thickness (CIMT), Coronary Artery Calcium (CAC), Computer Tomography (CT), Global Longitudinal Strain (GLS), Diabetes mellitus (DM), enzyme-linked immunosorbent assay (ELISA), Fasting blood sugar (FBS), Postprandial Blood sugar (PPBS), coronary artery disease (CAD), Glycated haemoglobin (HbA1c).

INTRODUCTION:

T2DM causes vascular ageing and linked to increased complication, as well as mortality

and morbidity from coronary artery disease (CAD). (1,2) Diabetic individuals have significantly higher levels of serum

osteoprotegerin (OPG), motivating further inquiry in CIMT, CAC, and GLS. Previous investigations indicated that OPG may rise in reaction to arterial damage induced by endothelial dysfunction. (3) OPG is a newly discovered glycoprotein that is a soluble member of the TNF receptor family. (4) OPG promotes bone mineralisation by blocking RANKL receptors and osteoclast. (7-9) OS plays a significant role in the development of CAC by stimulating a trans-differentiation in endothelial cells into osteoblast-like cells, resulting in elevated levels of OPG. (5,6) Various sets of researchers have observed the serum OPG levels are dramatically raised in both type 1 and 2 DM individuals, (10-15) both diabetic and nondiabetic persons suffering from CAD. (14-17) Serum OPG levels were shown to be higher in patients with type 2 diabetes, with or without complications. (18-20) Our study has to evaluate and associate the serum osteoprotegerin with biochemical and less-invasive imaging methods that may pave the way for a novel developmental sensitive biomarker for early diagnosis and prediction of CAD in type 2 DM.

METHODS:

Our investigation is prospective cross-sectional study was carried out Kelambakam, at Chettinad Super Speciality Hospital. 160 DM patients were totally included in study. The study had been approved by IHEC. The period of this study is extended between January 2024 to February 2025. The study population presented with DM were carefully selected. Based on the inclusion and exclusion criteria every patient had been selected. Our study was association between OPG and coronary artery CAC, CIMT, and GLS in T2DM patients. **INCLUSION CRITERIA:** included age >18 years, both sex, patient with HbA1c

level >6.5%. **EXCLUSION CRITERIA:** included H/O CAD, percutaneous coronary intervention, CABG, AF and CKD. 5 individual that was involved in the study were excluded due to poor echo window and 3 individual was not willing to continue the study after excluding these individuals results for 152 people were used.

As per the kit instruction the procedure of serum OPG ELISA had been performed.

CLINICAL MEASUREMENTS:

- All the T2DM patients were subjected to less-invasive methods to measure CIMT using B-mode USG , Coronary calcium score (CCA) imaging will be performed using multidetector computed tomography(CT) and GLS using Echocardiography the surrogate markers of atherosclerosis.

CIMT:

The distance between the leading edge of the first bright line (the blood-intima interface) of the far wall and the leading edge of the second bright line (media adventia interface) will indicate the intima medial thickness. Normal value of >0.7mm will be considered as aberrant and used as an early indicator of atherosclerosis.

CAC SCORE:

Coronary calcium on CT will be measured using the Agatston score. The total Agatston calcium score is calculated by adding the calcium from the right coronary, left anterior descending, and left circumflex arteries. CACs were categorised as zero, minimal ($0 \leq 10$), mild ($>10 \leq 100$), moderate ($>100 \leq 400$), and severe (>400).

GLS:

The peak systolic longitudinal strain will be measured using automated function imaging. GLS <17% is abnormal, GLS >17% is normal.

RESULTS:

Patient Demography:

This prospective cross-sectional study included a total of 152 patients, among them 74 are male and 78 are female patients.

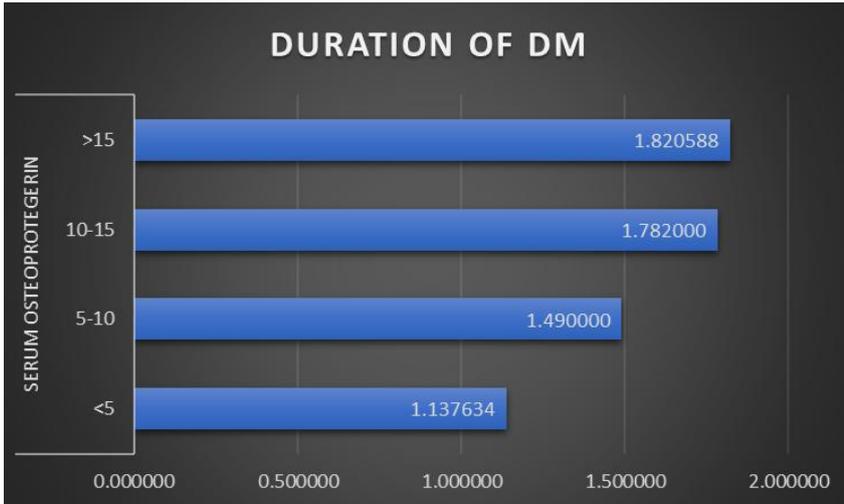
When compared to age distribution, The mean serum OPG level was greater in males (1.43 Pg /ml) when compared to females (1.23 Pg /ml). However, the variation was not statistically significant (F =0.44, p =0.50).

TABLE 1:

Variables	P value (<0.05)
Age	0.506
BMI	0.725
Duration of DM	0.003
HbA1c	0.001
Systolic BP	0.783
Diastolic BP	0.397
HDL	0.029
LDL	0.559
TGL	0.036
Total Count	0.025
FBS	0.006
PPBS	0.250
CIMT	0.011
CT-CAC	0.000
GLS	0.020

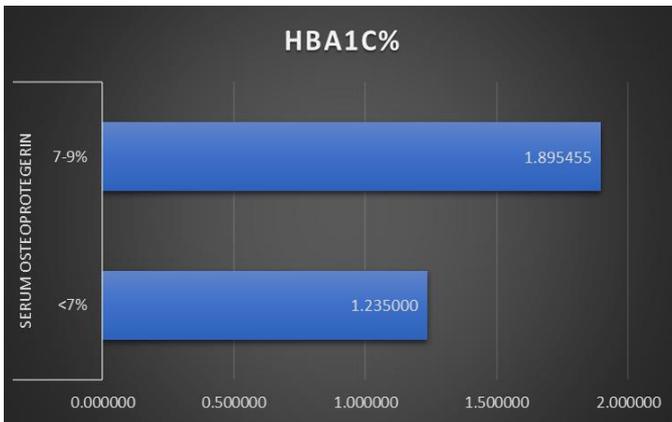
DURATION OF DM:

The duration of DM demonstrated a positive connection with serum OPG levels. Differences across groups were highly significant (F = 4 .99 2, p = 0 .00 3).



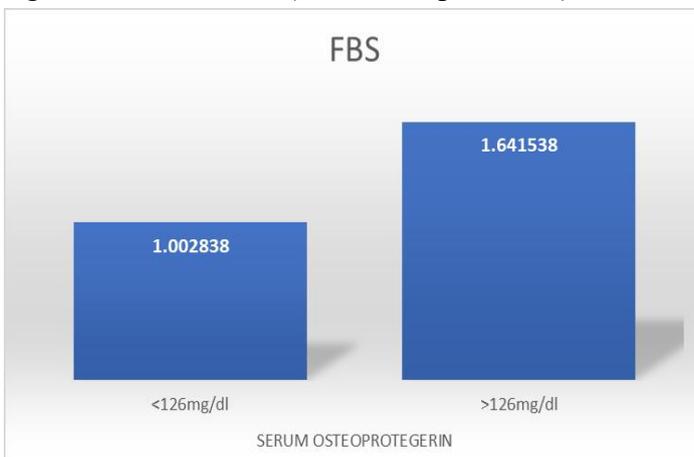
HbA1c:

HbA1c has a positive correlation with serum OPG levels. P value is statistically significant (p=0.001)



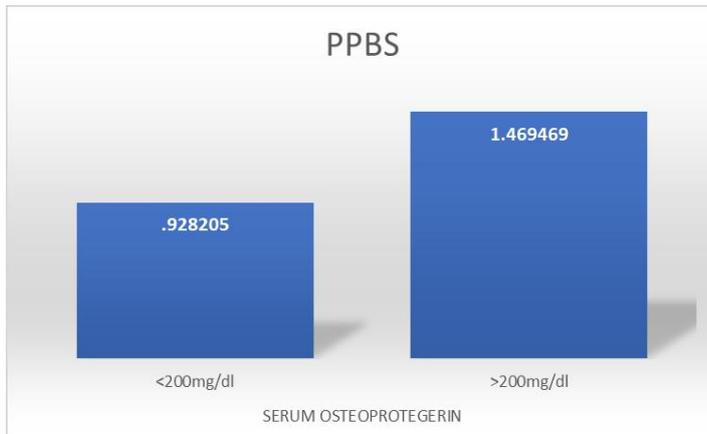
FBS:

Serum OPG levels was significantly greater in the FBS >126 mg/dl group (1.64 ng/ml) compared to the FBS ≤126 mg/dl group (1.00 ng/ml), with a statistically significant difference (t = -4.878, p = 0.006).



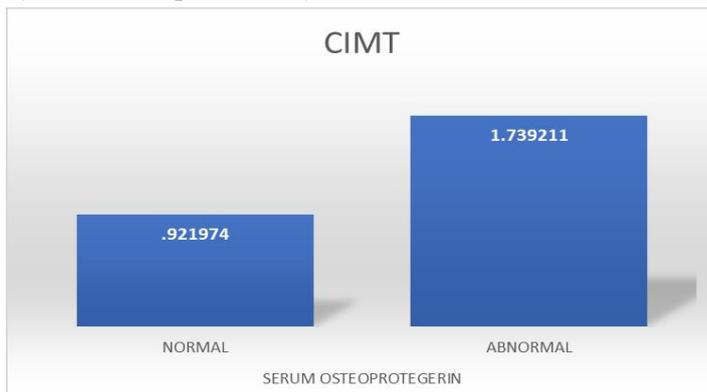
PPBS:

Mean OPG levels was higher in the PPBS ≥ 200 mg/dl group (1.47 ng /ml) compared to PPBS < 200 mg/dl group (0.93 ng /ml). However, The difference was likewise not statistically significant ($t = -3.490, p = 0.250$).



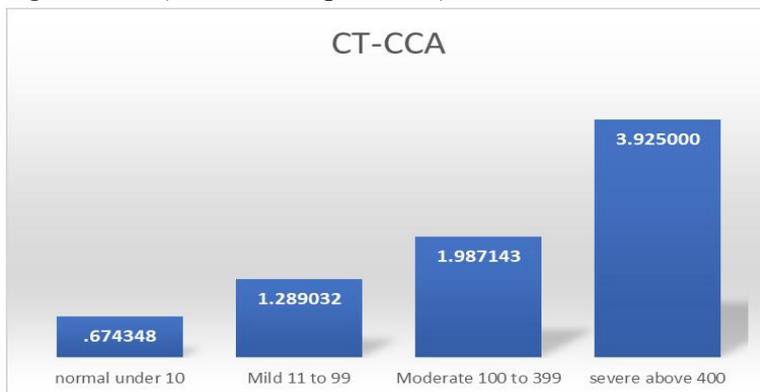
CIMT:

The mean OPG level was significantly greater in abnormal CIMT (1.74 ng/ml) compared to normal CIMT (0.92 ng /ml). There was a statistically significant difference ($t = -6.587, p = 0.011$).



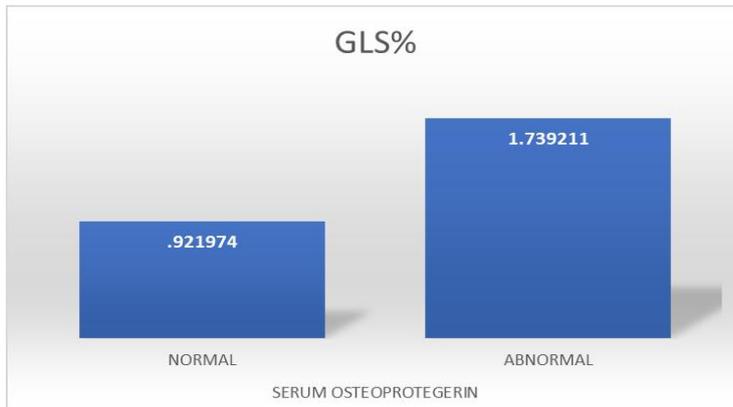
CT-CAC:

Serum OPG levels also demonstrated a significant increase with disease severity. Mean levels ranged from 0.67 ng/ml in the "normal" group to 3.93ng/ml in "severe" group, with highly significant ($F = 41.203, p= 0.000$)



GLS:

OPG levels was significantly high in abnormal GLS (1.91 ng/ml) compared to the normal GLS group (1.15 ng/ml) and there was a statistically significant difference ($t = -5.002, p = 0.020$).



DISCUSSION:

We performed a prospective cross-sectional study with 152 participants to assess the relationship between CIMT, CAC, GLS, and OPG levels in DM2. Patients with Type 2DM had greater levels of serum OPG in HbA1c, FBS, PPBS, CIMT, CAC, and GLS, which supported our theory. In our study there was a positive relationship between the serum OPG and HbA1c with significant p value. Previous research was a favourable association between serum OPG and HbA1c levels in type 2 diabetic patients.

(21-23)

Our study showed the serum OPG level is positively correlated to FBS and PPBS but PPBS was not statistically significant when compared to FBS. However, research suggests a negative association between OPG and FBG in older male populations. (24) Alev et al., demonstrates that there is a significant correlation between serum OPG concentration and glycaemic control measures such as FBG, PPBS, and HbA1c, indicating long-term glucose management. (25)

In current study we found that, the serum OPG levels were significantly greater in abnormal GLS compared to normal GLS and it was statistically significant with P value. Diabetic

hypertensive individuals with poor LV systolic function (GLS ~ 18.5) showed higher DBP levels. In correlation analysis, we found no correlation. (27)

Our study demonstrates that OPG levels were significantly greater in the abnormal CIMT compared to the normal CIMT. Mutlu Gunes et al., shows that patients with DM2 had greater OPG and CIMT and lower FMD levels than healthy individuals. Diabetes and hypertension were linked to higher OPG levels and there was no correlation between CIMT, FMD, and OPG readings.

We observed the OPG level is closely and strongly connected with CAC patients. Serum OPG levels also demonstrated a significant increase with disease severity. As demonstrated in the prospective study, diabetic individuals were vulnerable to a greater risk of CAC incidence after the raised level of OPG, showing that high OPG levels were related to the length of T2M. (26) The aberrant CAC is considered an integrated factor showing the persons to various ages to CAD burden among all races and genders.

CONCLUSION:

Our current study demonstrates the serum osteoprotegerin are statistically proven with

CAD severity, highlighting their potential as early biomarkers for CAD. OPG concentration has been recommended as a helpful diagnostic for identifying aberrant CAC, CIMT, and GLS among T2DM patients. These biomarkers could complement existing diagnostic tools, allowing for earlier detection and better risk stratification in CAD patients. These indicators could be utilised in clinical settings to detect CIMT, CAC, and GLS early, allowing interventions can be developed by the lower CAD event rate in all patients. Future, research should focus on validating these findings in larger, diverse populations and exploring their utility in clinical practice.

REFERENCES:

1. Ndip A, Wilkinson FL, Jude EB, Boulton AJ, Alexander MY. RANKL–OPG and RAGE modulation in vascular calcification and diabetes: novel targets for therapy. *Diabetologia*. 2014 Nov;57:2251-60.
2. De Rosa S, Arcidiacono B, Chiefari E, Brunetti A, Indolfi C, Foti DP. Type 2 diabetes mellitus and cardiovascular disease: genetic and epigenetic links. *Frontiers in endocrinology*. 2018 Jan 17;9:2.
3. Akinci B, Demir T, Celtik A, Baris M, Yener S, Ozcan MA, Yuksel F, Secil M, Yesil S. Serum osteoprotegerin is associated with carotid intima media thickness in women with previous gestational diabetes. *Diabetes research and clinical practice*. 2008 Nov 1;82(2):172-8.
4. Østergård T, Nyholm B, Hansen TK, Rasmussen LM, Ingerslev J, Sørensen KE, Bøtker HE, Saltin B, Schmitz O. Endothelial function and biochemical vascular markers in first-degree relatives of type 2 diabetic patients: the effect of exercise training. *Metabolism*. 2006 Nov 1;55(11):1508-15.
5. Tóth A, Balogh E, Jeney V. Regulation of vascular calcification by reactive oxygen species. *Antioxidants*. 2020 Oct 8;9(10):963.
6. Kang JH, Ko HM, Moon JS, Yoo HI, Jung JY, Kim MS, Koh JT, Kim WJ, Kim SH. Osteoprotegerin expressed by osteoclasts: an autoregulator of osteoclastogenesis. *Journal of Dental Research*. 2014 Nov;93(11):1116-23.
7. Wu Y, Liu J, Guo H, Luo Q, Yu Z, Liao E, Zu X. Establishment of OPG transgenic mice and the effect of OPG on bone microarchitecture. *International Journal of Endocrinology*. 2013;2013(1):125932.
8. Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis*. 2009 Jun 1;204(2):321-9.
9. Liu W, Xu C, Zhao H, Xia P, Song R, Gu J, Liu X, Bian J, Yuan Y, Liu Z. Osteoprotegerin induces apoptosis of osteoclasts and osteoclast precursor cells via the Fas/Fas ligand pathway. *PLoS One*. 2015 Nov 16;10(11):e0142519.
10. Galluzzi F, Stagi S, Salti R, Toni S, Piscitelli E, Simonini G, Falcini F, Chiarelli F. Osteoprotegerin serum levels in children with type 1 diabetes: a potential modulating role in bone status. *European Journal of Endocrinology*. 2005 Dec;153(6):879-85.
11. Rasmussen LM, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A. Plasma osteoprotegerin levels are associated with glycaemic status, systolic blood pressure, kidney function and cardiovascular morbidity in type 1 diabetic patients. *European Journal of Endocrinology*. 2006 Jan;154(1):75-81.

12. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *The Journal of Clinical Endocrinology & Metabolism*. 2001 Feb 1;86(2):631-7.
13. Knudsen ST, Foss CH, Poulsen PL, Andersen NH, Mogensen CE, Rasmussen LM. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. *European Journal of Endocrinology*. 2003 Jul;149(1):39-42.
14. Avignon A, Sultan A, Piot C, Elaerts S, Cristol JP, Dupuy AM. Osteoprotegerin is associated with silent coronary artery disease in high-risk but asymptomatic type 2 diabetic patients. *Diabetes Care*. 2005 Sep 1;28(9):2176-80.
15. Anand DV, Lahiri A, Lim E, Hopkins D, Corder R. The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. *Journal of the American College of Cardiology*. 2006 May 2;47(9):1850-7.
16. Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, Nishizawa Y. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. *Circulation*. 2002 Sep 3;106(10):1192-4.
17. Schoppet M, Sattler AM, Schaefer JR, Herzum M, Maisch B, Hofbauer LC. Increased osteoprotegerin serum levels in men with coronary artery disease. *The Journal of Clinical Endocrinology & Metabolism*. 2003 Mar 1;88(3):1024-8.
18. Grant PJ. Diabetes mellitus as a prothrombotic condition. *Journal of internal medicine*. 2007 Aug;262(2):157-72.
19. Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, Van G, Kaufman S, Kostenuik PJ, Lacey DL, Boyle WJ. Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. *The Journal of experimental medicine*. 2000 Aug 21;192(4):463-74.
20. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999 Jan 28;397(6717):315-23.
21. Terekeci HM, Senol MG, Top C, Sahan B, Celik S, Sayan O, Kucukardali Y, Ipcioglu O, Cagiltay E, Oktenli C, Ozata M. Plasma osteoprotegerin concentrations in type 2 diabetic patients and its association with neuropathy. *Experimental and clinical endocrinology & diabetes*. 2009 Mar;117(03):119-23.
22. Xiang GD, Pu JH, Zhao LS, Sun HL, Hou J, Yue L. Association between plasma osteoprotegerin concentrations and urinary albumin excretion in type 2 diabetes. *Diabetic medicine*. 2009 Apr;26(4):397-403.
23. Knudsen ST, Foss CH, Poulsen PL, Andersen NH, Mogensen CE, Rasmussen LM. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. *European Journal of Endocrinology*. 2003 Jul;149(1):39-42.
24. Gannagé-Yared MH, Fares F, Semaan M, Khalife S, Jambart S. Circulating osteoprotegerin is correlated with lipid profile, insulin sensitivity, adiponectin and

- sex steroids in an ageing male population. *Clinical endocrinology*. 2006 Jun;64(6):652-8.
25. Altinova AE, Toruner F, Akturk M, Bukan N, Yetkin I, Cakir N, Arslan M. Relationship between serum osteoprotegerin, glycemic control, renal function and markers of atherosclerosis in type 2 diabetes. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2011 Jul 1;71(4):340-3.
26. Anand DV, Lahiri A, Lim E, Hopkins D, Corder R. The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. *Journal of the American College of Cardiology*. 2006 May 2;47(9):1850-7.
27. Zoroufian A, Razmi T, Taghavi-Shavazi M, Lotfi-Tokaldany M, Jalali A. Evaluation of subclinical left ventricular dysfunction in diabetic patients: longitudinal strain velocities and left ventricular dyssynchrony by two-dimensional speckle tracking echocardiography study. *Echocardiography*. 2014 Apr;31(4):456-63.