Original Article

Serum levels of Activin A: Marker of insulin resistance and cardiovascular risk in prediabetics

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Abstract

Background: A substantial proportion of health burden in diabetic individuals can be attributed to cardiovascular complications. The increasing risk of cardiovascular complications along the spectrum of dysglycemia warrants the need to devise novel markers for early assessment and management. Activin A is a multifunctional cytokine with an important role in glucose homeostasis and vascular diseases. It can thus serve as a guide for early identification of cardiovascular disease (CVD) risk in prediabetes. **Objective:** The aim of the study was to measure serum levels of activin A in prediabetics, compare them with normoglycemic controls and find the correlation of activin A with markers of insulin resistance such as the homeostatic model assessment of insulin resistance (HOMA-IR). **Methods:** Sixty prediabetic patients and similar age-, sex-, blood pressure–, and BMI-matched controls were recruited in the study. In both groups, serum levels of fasting blood glucose and post prandial glucose, glycated hemoglobin (HbA1c) and fasting insulin were measured. HOMA-IR values were calculated. Serum activin A levels were measured in both groups using ELISA. The obtained values were compared between the two groups. **Results:** The median (IQR) of s. fasting insulin (mIU/L) in the case group was 15.3 (12.2–18.62) which was significantly higher than that in the control group, which was 6 (4.2–7.3). The median (IQR) of s. activin A (ng/mL) in the case group was 263.55 (227.18–279.56) which was significantly higher than that in the control group, which was 159.9 (150.73–178.75) ($P < 0.001$). There was a very strong positive correlation of s. activin A (ng/mL) with s. fasting insulin (mIU/L) and HOMA-IR (rho = 0.67 and 0.75, respectively, $P < 0.001$). **Conclusion:** Activin A, if combined with other atherosclerotic markers, might improve the assessment of insulin resistance and cardiovascular risk in prediabetics and lead to focus on lifestyle modifications and preventive medical therapy, thereby contributing to the prevention of CVD-related mortality and morbidity in these patients.

Keywords: Activin A, HOMA-IR, insulin resistance

Introduction

Diabetes mellitus is a chronic, metabolic condition characterized by elevated levels of blood glucose, due to defects in insulin secretion or insulin action, resulting from a genetic predisposition coupled with environmental factors. Prediabetes, a state of intermediate hyperglycemia and a harbinger of diabetes mellitus, includes individuals with fasting plasma glucose (FPG) of 100–125 mg/dl (impaired fasting glucose - IFG), two-hour post prandial blood glucose of 140–199 mg/dl (impaired glucose tolerance - IGT), or glycosylated hemoglobin (HbA1c) of 5.5%–6.4%.[1]

A substantial proportion of health burden in diabetic individuals can be attributed to cardiovascular complications. Currently, available evidence from a number of studies suggest that approaching dysglycemia as a spectrum and a continuous risk factor, much as BP and cholesterol levels, can be more useful for cardiovascular disease (CVD) risk assessment and prevention, instead of chasing specific cut-offs.[2] Christian Weyer *et al.*[3] conducted a longitudinal observational study and showed that insulin resistance and β cell failure coevolve simultaneously rather than sequentially, as was previously believed.

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The EpiDREAM cohort study found that the risk of cardiovascular complications and death increases progressively along the spectrum of normoglycemia, IFG or IGT and diabetes mellitus. An increase of 1 mmol/L in FPG corresponded to a 17% increase in risk of future cardiovascular events or death in the study participants.[4]

Activin A, a member of the TGF β superfamily, has been recognised as a multifunctional cytokine, expressed in a variety of tissues and having a wide range of roles in embryonic stem cell differentiation, wound repair, inflammation and glucose homeostasis. The levels of activin A have been found to be raised in individuals with abnormal glucose regulation (IFG/IGT), thus suggesting a role in glucose regulation.[5] Emerging evidence also indicates the role of activin A as a cardiovascular marker due to its association with coronary atherosclerotic burden and heart failure.[6]

This study aimed to measure the serum levels of activin A in prediabetics and compare with normoglycemic controls and also find the correlation with insulin resistance parameters.

Materials and Methods

The study was conducted in the Department of Medicine and Biochemistry, ABVIMS, Dr RML Hospital, New Delhi. The study was approved on 22/10/19 by IEC of RML hospital.

Study Design: Cross sectional, observational study.

Study Size: The study group consisted of 60 consecutive patients of prediabetes and 60 control subjects with matching age, sex, blood pressure and body mass index (BMI), from department OPD, wards and emergency at ABVIMS and RML Hospital.

Study Period: 1 November 2019 to 31 March 2021.

Calculation of sample size

Primary objective: To compare the serum activin A levels in prediabetes and control groups.

To achieve the primary objective, the input for statistical sample size calculation was taken from a 2018 study by Kuo *et al.*[12]

Mean value of activin A levels in controls was 491.2 ± 165.3 and in prediabetics was 559 ± 178.5. Taking these values as reference, 80% power of study and 5% level of significance, the minimum required sample size is 101 patients in each study group.

Formulae used:

For comparing the mean of two groups the following equation was used:

$$N \geq 2\frac{(standard\ deviation)^2}{(mean\ difference)^2}(Z_\alpha + Z_\beta)^2$$

Where Z_α is value of Z at two-sided alpha error of 5%;

Z_β is value of Z at power of 80%; and

mean difference is difference in mean values of two groups.

$$\text{Standard Deviation} = \sqrt{\frac{(S_1)^2 + (S_2)^2}{2}}$$

Where S_1 is standard deviation of group 1, that is, cases;

S_2 is standard deviation of group 2, that is, controls.

Calculations:

$$\text{Standard deviation} = \sqrt{\frac{(163.5)^2 + (178.5)^2}{2}} = 172.026$$

$$N \geq 2\frac{172.026^2}{(67.8)^2}(1.96 + 0.84)^2$$

$N \geq 100.94 = 101$ (approximately)

The sample size had to be reduced to 60 cases and 60 controls later on because of decreased footfall of patients in the medicine and diabetic OPD due to the ongoing COVID-19 pandemic.

Inclusion criteria

Sixty cases of prediabetes aged 18–65 years as defined by fasting plasma glucose between 100 to 125 mg/dL, or 2-hour postprandial glucose/2-hour OGTT (after 75 gm of glucose solution ingestion) between 140 to 199 mg/dL, or HbA1c = 5.7%–6.4% (ADA 2019).

Sixty control subjects, matched for age, gender, blood pressure and BMI, with fasting blood glucose of less than 100 mg/dl, and 2-hour postprandial glucose/2-hour OGTT of less than 140 mg/dl, and HbA1c less than 5.7 with no known co-morbidities as per exclusion criteria.

Exclusion criteria

- Known hypertensive
- History of drug or alcohol abuse
- History of overt cardiovascular events (stroke, ischemic heart disease, chronic obstructive peripheral arteriopathy, or heart failure)
- Known case of hypertrophic or dilated cardiomyopathy
- Known case of inflammatory bowel disease
- Known case of inflammatory arthropathy
- Patients on drugs like statins or other anti-hyperlipidemic drugs and anti-platelet or anti-thrombotic drugs.
- Pregnant females.

Methods

All the cases and controls underwent the following tests and examination:

Clinical examination

The study participants were called to the department of medicine, Dr. RML Hospital and asked to fill a pre-determined

questionnaire which included baseline data about age, sex and family history of diabetes or hypertension.

They underwent a detailed clinical examination including measurement of height, weight and waist circumference. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. Resting systolic and diastolic blood pressures were recorded twice using an automated sphygmomanometer after a 5-min rest and mean of the two readings was taken.

Laboratory investigations

Following investigations were performed:

- Fasting plasma glucose
- Two-hour postprandial plasma glucose
- HbA1c
- Fasting plasma insulin levels
- Serum lipid profile including triglycerides, total cholesterol, HDL-cholesterol, LDL- cholesterol, VLDL-cholesterol.
- Serum uric acid levels
- Samples for s. activin A levels were centrifuged at 3000 rpm for 10 minutes. Serum was separated and then stored in aliquots at −20°C in the department of biochemistry until batch analyzed by enzyme-linked immunosorbent assay (ELISA).
    - Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: HOMA-IR = (FPI × FPG)/22.5, where, FPI is fasting plasma insulin (mIU/L) and FPG is fasting plasma glucose (mmol/L)
      Or
      HOMA-IR= (FPI × FPG)/405, where FPI is fasting plasma insulin (mIU/L) and FPG is fasting plasma glucose (mg/dl).

Serum activin A

The kit used a double-antibody sandwich ELISA for determination of activin A concentration in serum, plasma, cell culture supernatant or any other biological fluid. The specified assay range was 7.8–300 ng/mL.

Test procedure

1. All the reagents and samples were brought to room temperature.
2. Standard was diluted with standard diluent using method of multiple proportion dilution.
3. Blank wells, standard wells, and test sample wells were set up as follows:
    - Standard wells: 50 μL standard was added to standard wells.
    - Test sample wells: 40 μL of special diluent and 10 μL of sample was added. (The sample dilution was five times and final result calculation was multiplied by five times.)
    - 50 μL of horseradish peroxidase (HRP) was added to each well, except blank well. Then plates were sealed, gently shaken and incubated for 60 minutes at 37°C.
4. Excess liquid was discarded, wells were dried and filled with diluted washing liquid, mixed and shaken for 30 seconds. Washing liquid was then discarded and plate was tapped into absorbent papers to dry. This procedure was repeated five times, and then patted dry.
5. 50 μL of chromogen solution A was added to each well, and then chromogen solution B was added to each well. The plates were then shaken and incubated for 10 minutes at 37°C, away from light.
6. Then stop solution of 50 μL was added into each well to stop the reaction (blue changed into yellow immediately).
7. Final measurement: The blank well was set zero, ELISA reader (at Biochemistry lab RML Hospital) was used to measure optical density (OD) at 450 nm wavelength within 15 minutes after adding the stop solution.
8. According to standards' concentration and the corresponding OD values, standard curve linear regression equation was calculated. Then OD values of the sample were applied on the regression equation to calculate the corresponding sample's concentration.
9. Values were obtained in ng/mL and multiplied by 5 to obtain the final s. activin A concentration of each sample.

Statistical analysis

Categorical variables are presented in number and percentage (%), and continuous variables are presented as mean ± SD and median (IQR). Normality of data was tested using Kolmogorov–Smirnov test. If the normality was rejected, then non-parametric tests were used.

Quantitative variables were compared using unpaired *t*-test or Mann–Whitney *U* test (when the data sets are not normally distributed) between the two groups. Qualitative variables were compared using Chi-squared test or Fischer's exact test. Pearson correlation coefficient or Spearman rank correlation coefficient were used to correlate quantitative parameters with each other. A P value of < 0.05 was considered as statistically significant. The data was entered in Microsoft Excel spreadsheet, and analysis was done using Statistical Package for the Social Sciences (SPSS) version 21.0.

Results

The aim of this study was to assess the serum levels of activin A in prediabetics and compare them with normoglycemic controls. It was a case–control study, and 60 cases and 60 controls were enrolled, matching in age, gender, BMI and blood pressure. The following observations were made [Tables 1 and 2].

The median (IQR) of s. fasting insulin (mIU/L) in cases was 15.3 (12.2–18.62) which was significantly higher than the control group with 6 (4.2–7.3) [Tables 2 and 3]. The median (IQR) of HOMA-IR in the case group was 4 (3.25–4.93) which was significantly higher than that in the control group with 1.2 (0.88–1.5) ($P < 0.001$) [Tables 2 and 4, Figure 1]. Also, 96.7% of cases had HOMA-IR >2 in comparison to 6.7% of

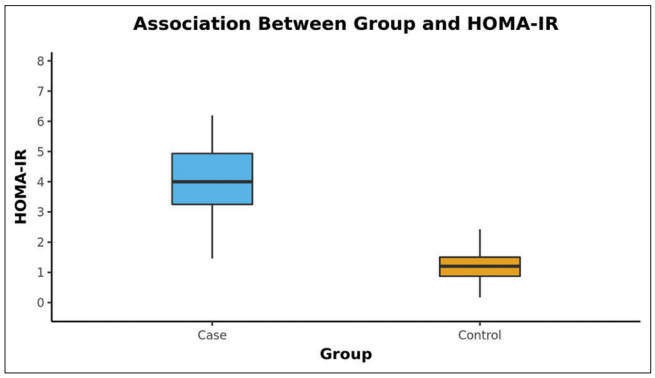

Figure 1: Comparison of HOMA-IR between cases and controls

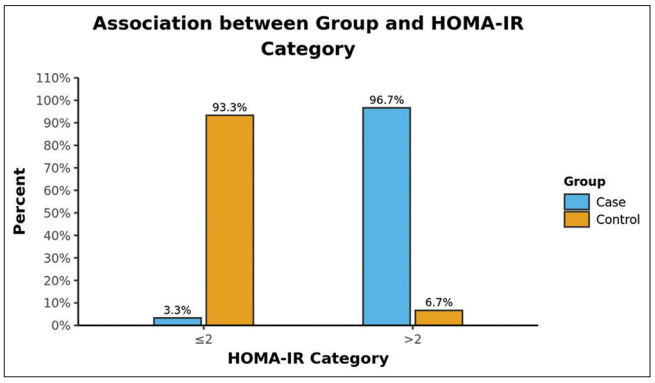

Figure 2: Comparison of HOMA-IR between cases and controls with cutoff of 2

Table 1: Demographic and anthropometric characteristics among cases and controls

Parameter	Cases (*n*=60)	Controls (*n*=60)	*P*
Age [Mean (SD)]	45.80 (10.06)	43.68 (9.66)	0.242
Gender (%)			
Male	24 (40.0%)	27 (45.0%)	0.580
Female	36 (60.0%)	33 (55.0%)	
BMI (kg/m^2) [Mean (SD)]	24.83 (3.02)	24.82 (2.58)	0.976
Waist Circumference (cm) [Mean (SD)]	87.92 (6.16)	87.17 (7.24)	0.542
Systolic BP (mmHg) [Mean (SD)]	117.20 (6.35)	118.13 (8.64)	0.265
Diastolic BP (mmHg) [Mean (SD)]	75.77 (4.70)	74.30 (4.77)	0.072

Table 2: Biochemical parameters among cases and controls

Parameter [Median (IQR)]	Cases (*n*=60)	Controls (*n*=60)	*P*
FBS (mg/dL)	107 (101.25-118)	84 (78-90)	<0.001
PPBS (mg/dL)	169 (155.5-182.25)	126 (114-132.25)	<0.001
HbA1c (%)	5.95 (5.8-6.21)	5.1 (4.8-5.32)	<0.001
Serum Fasting Insulin (mIU/L)	15.3 (12.2-18.62)	6 (4.2-7.3)	<0.001
HOMA-IR	4 (3.25-4.93)	1.2 (0.88-1.5)	<0.001
S. Activin A (ng/mL)	263.55 (227.18-279.56)	159.9 (150.73-178.75)	<0.001

Table 3: Comparison of s. fasting insulin (mIU/L) between cases and controls

S. Fasting Insulin (mIU/L)	Group		*t*-test	
	Case	Control	*t*	*P*
Mean (SD)	15.34 (4.61)	5.93 (2.42)	14.004	<0.001
Median (IQR)	15.3 (12.2-18.62)	6 (4.2-7.3)		
Range	5.58-23.5	1-12.6		

controls [Table 5, Figure 2]. The median (IQR) of s. activin A (ng/mL) in the case group was 263.55 (227.18–279.56) which was significantly higher than that in the control group with 159.9 (150.73–178.75) ($P < 0.001$) [Tables 2 and 6, Figure 3]. There was a very strong positive correlation of s. activin A (ng/mL) with s. fasting insulin (mIU/L) and HOMA-IR, and this correlation was statistically significant (rho = 0.67 and 0.75, respectively, $P < 0.001$) [Table 7, Figures 4 and 5]. For every 1 unit increase in s. fasting insulin (mIU/L), the s. activin A (ng/mL) increased by 6.53 units. For every 1 unit increase in HOMA-IR, the s. activin A (ng/mL) increased by 28.89 units.

Discussion

In our study, activin A levels were significantly higher in the prediabetic group as compared to the control group. We also found a positive correlation of activin A with parameters related to insulin resistance, such as HOMA-IR and fasting plasma insulin levels.

Advances in research have led to the current understanding that a common pathophysiology drives the diabetic state throughout its natural history and across its varied clinical presentations, forming vicious cycles that aggravate end-organ damage. These include an interplay between the genetic predisposition, environmental influences, insulin resistance, inflammation and immune dysregulation.[7] The progression of insulin resistance to diabetes is paralleled by progression of endothelial dysfunction to atherosclerosis.[8]

Few previous studies have explored the role of activin A in glucose homeostasis and insulin resistance. The effect of activin A on glucose metabolism can be attributed to inhibition of peroxisome proliferator-activated receptor gamma (PPAR-γ), which increases insulin sensitivity via effects on hepatic glucose production and peripheral utilization.[9]

The studies available till date have not agreed upon a specific normal range of activin A that can be adopted into practice. The available data only suggests the comparison of activin A levels in certain study groups with normal controls.[10,11]

Kuo *et al.*[12] conducted a cross-sectional analysis and studied the levels of activin A. In line with our results, they also found significantly higher levels of activin A in prediabetics measuring 559 ± 178.5 pg/mL as compared to normoglycemic controls

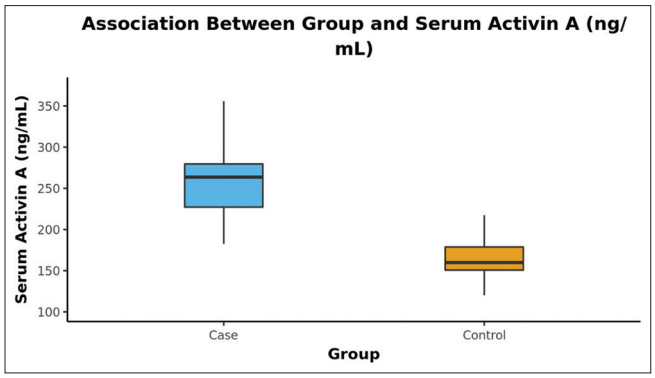

Figure 3: Comparison of serum activin A (ng/mL) levels between cases and controls

Table 4: Comparison of HOMA-IR between cases and controls

HOMA-IR	Group		*t*-test	
	Case (*n*=60)	Control (*n*=60)	*t*	*P*
Mean (SD)	4.08 (1.17)	1.23 (0.51)	17.252	<0.001
Median (IQR)	4 (3.25-4.93)	1.2 (0.88-1.5)		
Range	1.46-6.2	0.18-2.64		

Table 5: HOMA-IR distribution among cases and controls with cutoff of 2

HOMA-IR	Group			Chi-Squared Test	
	Case (*n*=60)	Control (*n*=60)	Total (*n*=120)	χ^2	*P*
≤2	2 (3.3%)	56 (93.3%)	58 (48.3%)	97.308	<0.001
2	58 (96.7%)	4 (6.7%)	62 (51.7%)		

measuring 491 ± 165.3 pg/mL ($P < 0.001$). Their findings also established a positive correlation between activin A values and HOMA-IR in prediabetics and diabetics (r = 0.137; $P = 0.0004$).

Anderson GO *et al.*[5] found higher baseline levels of activin A in their study subjects to be associated with a higher risk of development of abnormal glucose regulation (IFG/IGT/T2DM) at three-month follow up. Wu *et al.*[13] found significant correlation of activin A levels with HbA1c (R = 0.386; $P < 0.01$), fasting insulin (R = 0.399; $P < 0.001$ overall); ($r = 0.589$; $P < 0.05$ in IFG/IGT group) and HOMA-IR (R = 0.485; $P < 0.001$ overall); ($r = 0.547$; $P < 0.05$ in IFG/IGT group).

In addition to diabetes, the correlation of activin A with markers of insulin resistance has also previously been studied in nonalcoholic fatty liver disease (NAFLD) and obesity, with a speculated association with metabolic syndrome.[14,15] Perakakis *et al.*[16] reported a significant positive correlation of activin A with BMI and HOMA-IR ($r = 0.339$, $P < 0.001$; $r = 0.211$, $P < 0.05$, respectively) and concluded activin A to be implicated in glucose regulation as a part of a feedback loop including glucose or insulin.

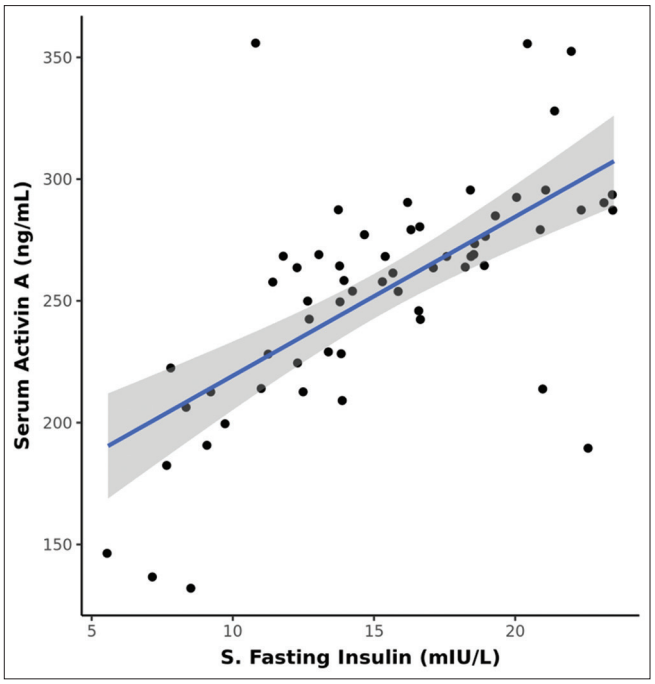

Figure 4: Correlation of s. activin A (ng/mL) with s. fasting insulin (mIU/L)

A number of studies have established the association of the levels of activin A with insulin resistance and coronary, as well as, non-coronary cardiac diseases, suggesting its role as a cardiovascular biomarker in dysglycemic states. Ueland T *et al.*[6] found elevated activin A levels to be associated with the severity of coronary atherosclerotic burden, thus concluding that increased activin A might reflect some chronic pathophysiological processes involved in the development of coronary atherosclerosis. Miyoshi *et al.*[17] found a significant association between serum concentration of activin A before PCI and peak s. creatinine kinase (CPK and CK-MB) concentrations as a marker of infarct size. Anastasilakis AD *et al.*[18] found that myocardial infarction (MI) patients had significantly higher activin A levels than controls ($P < 0.001$). Ofstad *et al.*[19] suggested that s. activin A concentrations had a predictive value for incident cardiovascular events and mortality, even after adjustment for conventional risk factors. Esposito P *et al.*[20] reviewed the role of activin A in atherosclerosis and vascular disease, suggesting rationale for promising therapeutic strategies targeting this pathway.

Activin A has also been linked to contractile dysfunction and insulin resistance in cardiomyocytes. Circulating activin A levels have been found to be associated with impaired myocardial glucose metabolism and high left ventricular mass/volume ratio (LVMV-ratio) in patients with uncomplicated type 2 diabetes (T2D), reflecting a potential detrimental role in early diabetic cardiomyopathy.[21]

There is plenty of research done worldwide implicating activin A in insulin resistance and cardiovascular diseases, but there is a paucity of Indian studies on the subject. Ours is probably the

Table 6: Comparison of serum activin A (ng/mL) between cases and controls

Serum Activin A (ng/mL)	Group		Wilcoxon, Mann-Whitney *U* Test	
	Case	Control	W	*P*
Mean (SD)	254.09 (46.24)	164.84 (25.84)	3379.000	<0.001
Median (IQR)	263.55 (227.18-279.56)	159.9 (150.73-178.75)		
Range	132.02-355.8	100.6-239.26		

Table 7: Correlation of serum activin A (ng/mL) with serum fasting insulin (mIU/L) and HOMA-IR

Correlation	Spearman Correlation Coefficient	*P*
S. Fasting Insulin (mIU/L) vs Serum Activin A (ng/mL)	0.7	<0.001
HOMA-IR vs Serum Activin A (ng/mL)	0.8	<0.001

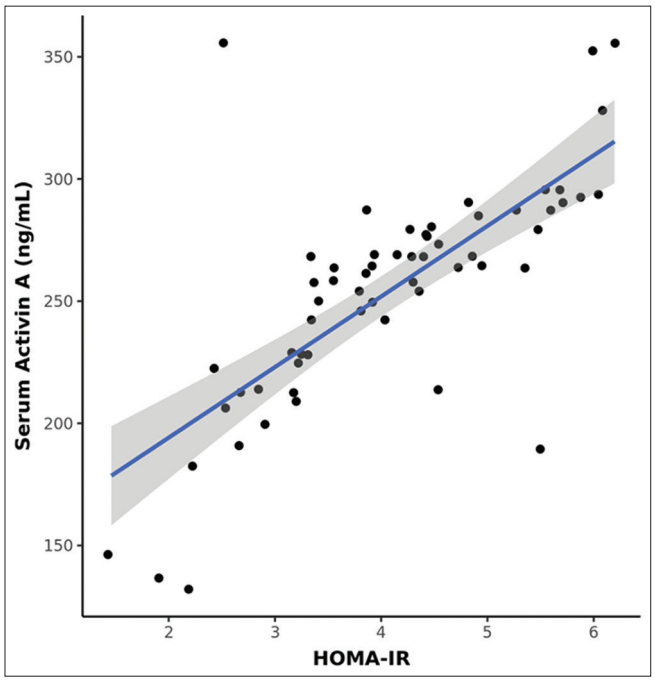

Figure 5: Correlation of serum activin A (ng/mL) with HOMA-IR

first case–control study of its kind in the Indian subcontinent to assess the role of a recently studied marker for insulin resistance activin A in prediabetes.

The spectrum of diabetes mellitus and its complications pose a vast and rapidly expanding health challenge in the current scenario. Keeping in mind the fact that activin A levels increase even before the development of overt diabetes and act as a marker of insulin resistance and cardiovascular risk, we suggest that assessment of this marker can identify patients with high CVD risk, thus paving a way for early management strategies including diet, lifestyle measures and pharmacological means.

An increasing understanding of the role of activin-signalling in insulin resistance and glucose homeostasis implicates that therapeutic interventions targeting this pathway may also provide novel strategies for management of related diseases.[22]

Conclusion

The spectrum of dysglycemic disorders, comprising of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus, is associated with cardiovascular complications, with an important role played by insulin resistance in the pathogenesis. Serum levels of activin A are increased in prediabetics and may be considered a predictor for early atherosclerosis. This molecule, if combined with other atherosclerotic markers can improve the cardiovascular risk assessment. It might lead to a focus on lifestyle modifications, dietary management and, if required, an aggressive preventive medical therapy, thereby contributing to primary prevention of diabetes, and CVD-related mortality and morbidity in these patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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