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Original Research Article

Serum Pentosidine – A surrogate marker of vascular complications in Type 2 Diabetes Mellitus

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Abstract

Background: As a result of prolonged hyperglycemia in diabetic patients, glucose reacts with proteins non-enzymatically to produce Advanced Glycation End products (AGES) such as Pentosidine (PEN). These AGEs play a significant role in causing life threatening microvascular (diabetic retinopathy and diabetic nephropathy) and macrovascular complications in Type 2 Diabetes Mellitus (T2DM) patients as stated by previous studies earlier.The present study is designed to evaluate the association of PEN with the development of vascular complications and how it can be useful as a surrogate marker for diagnosing these complications in T2DM patients.

Materials and Methods: This case-control study was conducted between July 2022 to March 2025. A total number of 246 T2DM cases (123 without & 123 with vascular complications) who attended the out- patient department (OPD) of Medicine were recruited and matched with 246 healthy, age and gender-matched controls. Baseline parameters (age, gender, duration of disease and BMI) were recorded and serum levels of PEN, Glycated Hemoglobin, FBG and PPBG were analyzed. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version V30 and level of significance i.e p value <0.05 was considered as significant.

Results: Highly significant ($p<0.001$) differences were found in mean levels of PEN (299.97± 25.86 ng/ml), HbA1C (10.75± 0.56 %), FBG (132.31± 13.54 mg/dl) and PPBG (159.52± 14.60 mg/dl) in cases with complications when compared to cases without complications and controls. Pearson's correlation depicted significant ($p<0.001$) positive correlation of PEN with T2DM duration and HbA1C levels in both the T2DM cases. Linear regression analysis showed the significant ($p<0.05$) positive association of PEN levels with T2DM duration and Logistic regression analysis showed that PEN levels positively significantly ($p<0.05$) related to the vascular complications in T2DM cases.

Conclusion: In the present study, Pentosidine levels were found significantly positively associated with the development of vascular complications and T2DM duration. This strongly suggested the utility of Pentosidine as a surrogate marker of vascular complications in type 2 diabetes patients.

Keywords: Pentosidine, Vascular complications, Surrogate marker.

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1. Introduction

Diabetes mellitus (DM) is a widespread health concern impacting individuals across all the age groups. The World Health Organization (WHO) reported that roughly 180 million people globally are living with Type 2 DM (T2DM), which was previously known as adult-onset diabetes. The prevalence of T2DM is projected to be double by 2030.[1] In individuals with T2DM, the primary cause of morbidity and mortality is microvascular and macrovascular complications.[2,3] Despite the complex pathophysiology of T2DM, Advanced Glycation End Products (AGEs) are main contributors to the development of the disease and its consequences.[2,4]

The biogenesis of AGEs is a complex process that happens through several steps of reactions. The entire process that leads to the formation of AGEs is known as the Maillard reaction.[5] During non –enzymatic browning of proteins due to Maillard reaction, a fluorescent substance called Pentosidine (PEN) has been identified. Pentosidine is a fluorescent cross-link substance derived from pentose that is formed between arginine and lysine residues in collagen.[6]

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PEN levels have been found elevated in both macrovascular as well as microvascular complications in T2DM as suggested by previous studies.[7,8] The present study focussed on establishing the association of PEN with vascular complications and duration of T2DM and also evaluating the correlation of PEN with other biochemical parameters in T2DM cases with & without vascular complications in order to enhance the significance of PEN as a surrogate marker for diabetic vascular complications in a routine clinical setting.

2. Materials and Methods

This case-control study was carried out at tertiary care hospital of Central India with approval (155/IEC/2021)of Institutional Ethics Committee (IEC). Total 246 T2DM cases between age 35-74 years that were diagnosed for T2DM (without /with vascular complications) were recruited from out- patient department (OPD) of Medicine. Out of them, 123 T2DM cases were found without any complications & 123 were having either of the micro/macro vascular complications (Diabetic retinopathy, Diabetic nephropathy, Diabetic neuropathy, Coronary artery disease and Stroke). These were matched with 246 healthy, age and gender-matched controls. Participants with other forms of diabetes, any history of chronic/acute illness and age less than 35 years and more than 74 years were excluded. After briefly informing the study participants, appropriate written consent was obtained. 5 ml of blood sample was collected under all aseptic precautions, centrifuged at 3000 RPM for 15 min and separated as per the need of serum/plasma and stored at -20 ˚C in the deep freezer at Research Laboratory of Biochemistry Department and Multidisciplinary Research Unit (MRU) for further analysis.

PEN levels were analyzed using ELK Biotechnology Pentosidine kit (Catalog no. ELK7927) based on the principle of competitive ELISA technique.[9] While other routine biomarkers like HbA1C,FBG & PPBG were analyzed at Central Clinical Laboratory (CCL)of the institution by Beckman Coulter AU5800 Series Clinical Chemistry Analyzer.[10,11]

2.1. Statistical analysis

It was performed using SPSS (Statistical Package for the Social Sciences) software versionV30.[12] Statistical tests used were Independent‘t’ test, Pearson’s correlation, multiple linear regression and multiple logistic regression. Baseline and Biochemical parameters were expressed in terms of MEAN±SD and p value was considered as <0.001 significant level.

3. Results

Table 1: Baseline parameters in study subjects

Parameters	Controls (a)	Cases without Complications (b)	Cases with Complications (c)	P Value (a-b)	P Value (a-c)	P Value (b-c)
Age (Years)	48.48 ±8.99	50.20 ±9.58	57.60 ±9.64	0.091 (NS)	<0.001	<0.001
Male (n) (%)	130 (52.8%)	63 (51.2%)	70(56.9%)	-	-	-
Female (n) (%)	116 (47.2%)	60 (48.8%)	53(43.1%)	-	-	-
Duration of Diabetes (Years)	-	2.21±1.59	9.64±4.75	-	-	<0.001
BMI (Kg/ $m^{2)}$	24.54 ±3.15	26.68 ±2.57	26.95 ±2.80	<0.001	<0.001	0.431(NS)

Note:-BMI=Body mass index.Data is expressed in terms of Mean, Standard Deviation, number (n) & percentage (%)and level of significance i.e p value is considered as <0.001 as highly significant, <0.05 as significant &>0.05 as non-significant (NS).

Table 2: Biochemical parameters in study subjects

Parameters	Controls (a)	Cases without Complications (b)	Cases with Complications (c)	P Value (a-b)	P Value (a-c)	P Value (b-c)
PEN (ng/ml)	121.92±24.94	291.88±32.24	299.97±25.86	<0.001	<0.001	<0.05
HbA1C (%)	4.78±0.57	8.79±0.56	10.75±0.56	<0.001	<0.001	<0.001
FBG (mg/dl)	84.26±7.81	121.12±18.31	132.31±13.54	<0.001	<0.001	<0.001
PPBG (mg/dl)	124.26±7.81	147.82±16.75	159.52±14.60	<0.001	<0.001	<0.001

Note:-PEN=Pentosidine,HbA1C=Glycated hemoglobin, FBG=Fasting blood glucose, PPBG=Post prandial blood glucose.

Data is expressed in terms of Mean±SD and level of significance i.e p value is considered as <0.001 as highly significant, <0.05 as significant and >0.05 as non-significant (NS).

Table 3: Pearson's Correlation coefficients forcomparison of PEN with other parameters in study subjects

PEN v/s parameters	Cases w/o Complications (r value)	Cases with Complications (r value)
T2DM duration	0.765**	0.803**
BMI	0.053	0.008
HbA1C	0.744**	0.757**
FBG	0.682**	0.223*
PPBG	0.102	0.125

Note:-Data is expressed in terms of Correlation coefficient i.e. 'r' and level of significance i.e p value is considered-**as <0.001 as highly significant and *<0.05 as significant.

Table 4: Regression analysis for association of PEN, HbA1C, FBG & PPBG with T2DM duration

Parameters	Coefficient	SE	95%CI	t value	P value
PEN	0.02	0.008	0.003 -0.037	2.35	0.0195*
HbA1C	2.9938	0.23	2.53 -3.4548	12.79	<0.0001**
FBG	0.02919	0.015	-0.001-0.059	1.86	0.0631
PPBG	-0.008689	0.016	-0.040 to 0.023	-0.53	0.5958

Note:-Multiple Linear Regression was performed taking 'T2DM duration' as *dependent* variable.Level of significance i.e p value is considered-**as <0.001 as highly significant, *<0.05 as significant and >0.05 as non-significant

Table 5: Regression analysis for association of PEN, HbA1C, FBG and PPBG with vascular complications

Parameters	Coefficient	SE	Wald test	P value	Odds Ratio	95% CI
PEN	12.5	0.04	4.55	0.0328*	1.09	1.00- 1.19
HbA1C	5.70	1.10	26.45	<0.0001**	300.0	34.13 - 2636.54
FBG	0.11	0.05	4.54	0.0330*	1.11	1.00 -1.24
PPBG	0.033	0.03	0.85	0.3555	1.03	0.96- 1.11

Note:-Logistic Regression was performed taking 'vascular complications' as dependent variable. Level of significance i.e p value is considered-**as <0.001 as highly significant, *<0.05 as significant and >0.05 as non-significant.

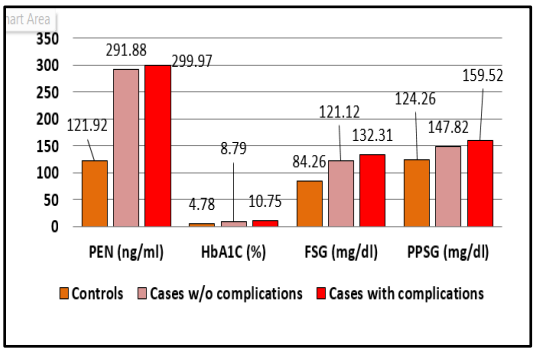

Figure 1: Mean Levels of PEN, HbA1C, FBG&PPBG levels in study subjects.

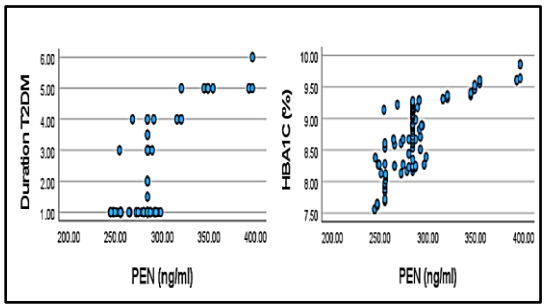

Figure 2: Correlation of PEN with T2DM duration and HbA1C levels in T2DM cases without complications

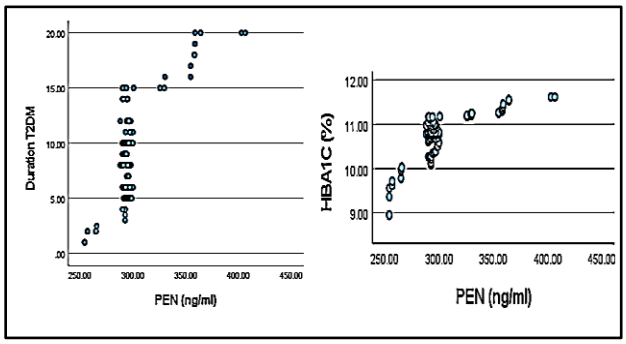

Figure 3: Correlation of PEN with BMI and HbA1C levels in T2DM cases with complications

The present study was conducted to establish an association of AGEs i.e (PEN) with vascular complications and T2DM duration, to find the correlation of PEN with other parameters and to compare the levels of PEN and other routine biochemical parameters in T2DM cases without complications and with complications compared to healthy, age and gender matched controls. **Table 1** showed the comparison of baseline parameters in study subjects. Independent't' test analysis showed significant (p<0.001) variations in mean levels of Age in controls, cases without complications and with complications. BMI was found insignificantly (p>0.05) higher in cases with complications as compared to without complications. **Table 2** and **Figure 1**

showed the significant (p<0.001) increased levels of PEN i.e 299.97± 25.86 pg/ml, HbA1C i.e 10.75± 0.56 %, FBGi. e 132.31± 13.54 mg/dl & PPBG i.e 159.52± 14.60 mg/dl in cases with complications in comparison to cases without complications and controls. **Table 3** & **Figure 2** showed the highly significant (r=0.765; p<0.001) positive correlation of PEN with T2DM duration and HbA1C (r=0.803; p<0.001) in cases without complications. Likewise in cases with complications, PEN showed highly significant (<0.001) positive correlation with T2DM duration and HbA1C. **Table 4** showed the significant positive association of PEN and HbA1C with T2DM duration while in **Table 5**, PEN, HbA1C and FBG showed significant positive association with vascular complications in T2DM cases. The ODDs ratio of PEN was found 1.09 which signified that for every one unit increase in PEN levels, the odds of developing vascular complications also gets increased by factor 1.09.

4. Discussion

In T2DM, chronic hyperglycemia leads to non-enzymatic glycation of proteins and lipids, known as the Millard reaction, resulting in the formation of AGEs like PEN. These AGEs inflict damage on small blood vessels, resulting into microvascular complications, such as retinopathy, neuropathy, and nephropathy, and macrovascular complications, including coronary artery disease, peripheral arterial disease, and cerebrovascular disease.[14]

As per the study done by Kerkeni M et al. (2012) on AGEs, s RAGE and PEN levels in microvascular complications in T2DM patients. They found that AGE,s RAGE and PEN levels were highly significantly increased in diabetic patients with retinopathy and nephropathy compared to control subjects ($p < 0.001$).[15]

The results showed similarities with the findings of the present study that showed highly significantly (p<0.001) increased levels of PEN in T2DM cases with complications as compared to cases without complications and controls. The findings of Hamid G.S et al. (2021) were also found related to the results of the present study. In their study, serum PEN levels were found to be significantly ($P < 0.001$) higher in Diabetic nephropathy patients as compared to the healthy control group.[16]

PEN levels were found highly significantly (p<0.001) positively correlated with T2DM duration and HbA1C incases without complications and in cases with complications, PEN showed insignificant (>0.05) positive correlation with BMI, but highly significant (p<0.001) with FBG levels in both the cases.Contrary to this, Jaishankar SKJ et al. (2020) reported no significant (p>0.05) correlation between fasting blood sugar levels and PEN levels.[17] Linear regression showed the significant (p<0.05) positive association of PEN levels with T2DM duration. This association was also stated by Sugiyama S et al. (1998),according to them, in patients with normal renal function, PEN levels were correlated with blood glucose control (HbAlC: $P = 0.0028$; fructoselysine: $P = 0.0133$), serum creatinine ($P = 0.029$), patient age ($P = 0.0416$), duration of diabetes ($P = 0.0431$), and total cholesterol ($P = 0.0056$) and LDL-cholesterol ($P = 0.0208$).[0]

Logistic regression analysis revealed that PEN levels positively significantly (p<0.05) influences the development of vascular complications in T2DM cases. For each one unit increase in PEN levels, odds of experiencing a complication increased by a factor 1.09 (OR=1.09).This means that T2DM cases with higher PEN levels have higher odds of developing vascular complications. Similar association between diabetic retinopathy (DR) and AGEs, specifically PEN and N-carboxy methyl lysine (CML) was found by Ghanem AA et al. (2010). They found significantly increased serum levels of PEN and CML in DR patients as compared to non-diabetic controls.

One of the important limitation of the present study was that other forms of AGEs such as Carboxy Methyl lysine (CML), Carboxy Ethyl lysine (CEL), fructosamine, etc. Could also be included to study the effect of these AGEs in different vascular complications in Type 2 Diabetes Mellitus.

5. Conclusion

A surrogate marker is an indicator that indirectly predicts the effect of any disease. In this study, Pentosidine levels were found significantly positively associated with the development of vascular complications and T2DM duration. This strongly suggested the utility of Pentosidine as a surrogate marker of vascular complications in type 2 diabetes patients. Therefore, it can be concluded that Pentosidine as an AGE, indirectly impacts the vascular changes in diabetics ultimately leading to the progression of life lasting vascular complications.

It can be also recommended that the Serum Pentosidine levels could be advised in diabetic patients to predict the chances of developing vascular complications in future.

6. Acknowledgement

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7. Conflict of Interest

There is no conflict of interest.

8. Source of Funding

None.

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