DOI: 10.7860/JCDR/2020/42745.13488

Original Article

Internal Medicine Section

Assessment of Pentosidine as a Marker for Advanced Glycation End Products in Type 2 Diabetes Mellitus Patients

SARATH KUMAR JAGANATHAN JAISHANKAR[1], AMRUTHA MURALI[2], AMRIN MARZOOKA[3]

ABSTRACT

Introduction: With Diabetes Mellitus (DM) becoming a global burden, quantifying the number of people affected, both now and in the future, is vital so that the preventive component of diabetes and its complications can be emphasised. Many studies suggest that DM associated with persistent or uncontrolled hyperglycaemia leads to the production of Advanced Glycation End Products (AGEs) and Advanced Oxidation Protein Products (AOPPs) resulting in various micro and macrovascular complications. Pentosidine, an AGE, is a glycated protein product formed as a result of non-enzymatic glycation, mainly Maillard reaction between plasma glucose and plasma proteins.

Aim: To determine the efficacy of pentosidine as a marker for AGEs in Type 2 DM patients.

Materials and Methods: A case-control study was conducted in a tertiary care institution with approval of the institutional ethical committee August to September 2014. Forty four individuals who have been diagnosed with type 2 DM for more than five years were taken as cases and 44 healthy individuals who were age and gender matched were included as controls. The medications taken by cases were: Metformin (n=44); Glimepiride (n=26); Glibenclamide (n=7); Glipizide (n=4); and Atorvastatin (n=44). All the participants underwent routine and special (AGEs-pentosidine-ELISA) investigations. Data were analysed using the SPSS version 16 software.

Results: In this study, the mean level of pentosidine (AGE) among cases was 3.073 ng/dL, and among controls was 2.682 ng/dL. Although the levels of pentosidine (AGE) was found to be higher in cases than controls, this difference among the groups was not found to be statistically significant.

Conclusion: Levels of AGEs (pentosidine) in cases were higher than the controls, but not statistically significant. Although AGEs have been considered to play a role in the development of many micro and macro angiopathic complications both independently and synergistically in patients with DM, it must be conducted on a larger scale to extrapolate the results in order to assess the effectiveness of AGEs in supplementing routine investigations.

Keywords: Maillard reaction, Non-enzymatic glycation

INTRODUCTION

Diabetes is a grave, chronic condition [1]. In 2013, the global estimate of diabetes was found to be 382 million, and this number is expected to rise to 592 million by 2035 [2]. The number of people with DM, especially type 2 diabetes, is increasing at an alarming rate, and so are the complications associated with it [3,4], due to the population growth, ageing, urbanisation, increasing prevalence of obesity, and physical inactivity. Thus, quantifying the prevalence of diabetes, is essential so that the prevention aspect of it and its complications can be stressed upon [5,6].

Persistent hyperglycaemia in DM is associated with accelerated non-enzymatic protein-glucose condensation reactions, oxidative stress and carbonyl stress. These mechanisms result in the formation of AGEs such as Pentosidine, Carboxymethyl lysine, pyralline, GOLD-Glyoxal-lysine dimer, MOLD- Methyl glyoxal lysine dimer, Carboxy ethyl lysine, etc., and AOPP which inflict damages to biologically important compounds [5,7]. AGEs are formed when plasma glucose binds to serum albumin (plasma protein) and undergoes the Maillard reaction which is a non-enzymatic. AGEs and AOPPs have a role in the pathogenesis of retinopathy, atherosclerosis, neuropathy, nephropathy, diabetic embryopathy, impaired wound healing, etc. AGEs are related to the development and progression of heart failure in non-diabetic patients as well [8-10]. These complications, when looked at a cellular level occur as a result of interaction between AGEs, and certain specific and non-specific receptor systems. One of the main interactions which have been described by various studies is Receptor for Advanced Glycation End-Products (AGE-RAGE) interaction. This results in the activation of intracellular mechanisms responsible for the toxic effects [11].

As AGEs are irreversibly formed compounds [12], which are present even if the plasma glucose levels are normalised after persistent glycaemia, it is crucial to recognise methodologies to prevent the dreaded complications even before they show initial signs. Such methodologies, when found to prove the merits of this hypothesis, they should supplement routine investigations. Among the many AGEs being studied, pentosidine has been well characterised as a compound associated with diabetic complications. Hence, this study was conducted to the efficacy of pentosidine as a marker for AGE products in Type 2 DM patients.

MATERIALS AND METHODS

A case-control study was conducted in a tertiary care institution with the approval (2014/1977/9) of the institutional ethical committee during the period of August to September 2014 among 88 individuals after obtaining informed consent. The number of participants selected was calculated with an error margin of 3% and a confidence interval of 95% of the total sample size of 96 based on the ELISA test requirements (total 96 strip wells). A total of 44 individuals of both genders who have been diagnosed with type 2 DM for more than 5 years, regardless of age with no apparent complications due to diabetes, were taken as cases, and 44 healthy individuals who were non-smokers, non-alcoholic, with no DM and hypertension, age and gender matched were included as controls in the study. Patients with type 1 DM, hypertension, coronary artery disease, and subjects taking anti-oxidants were excluded from the study. The medications taken by cases were: Metformin (n=44); Glimepiride (n=26); Glibenclamide (n=7); Glipizide (n=4); and Atorvastatin (n=44).

The samples were collected from the Department of Diabetology and analysed in the Department of Biochemistry. From all the participants, 5 mL fasting venous blood sample was collected, and serum was separated by centrifugation at 3000 rpm for 10 minutes, aliquoted into Eppendorfs, and stored at -20°C for analysis. All the participants underwent routine investigations like fasting blood sugar using GOD/POD method, fasting lipid profile which included total cholesterol using CHOD-PAP method, triglyceride using GPO-PAP method, HDL cholesterol using direct Immuno-turbidimetry method, LDL cholesterol using direct Immunoturbidimetry method [13-16] and special investigation- AGEs-pentosidine-ELISA (bioassay technology laboratory- Cat. No E0004 Hu).

STATISTICAL ANALYSIS

Data was analysed using the SPSS version 16 software. Descriptive statistics were reported as mean (SD) for continuous variables and frequency (percentage) for categorical variables. Spearman's rank correlation was used for univariate analysis. The groups were compared using analysis of Variance and t-test. A p-value <0.05 was considered as statistically significant.

RESULTS

The study population consisted of 88 individuals- 44 cases with type 2 DM with no associated medical complications, for more than five years and 44 normal healthy controls. The study groups were matched for age (53±7 years), sex and risk factors. Comparison of biochemical parameters between the study groups are listed in [Table/Fig-1,2].

Variables	Group	No	Mean	Std. Dev	T-score	p-value
Blood glucose (mg/dL)	**Case**	**44**	**183.258**	**70.468**	**7.618**	**<0.001 S**
	Control	**44**	**95.635**	**29.237**		
Total cholesterol (mg/dL)	Case	44	205.890	51.787	0.636	0.526 NS
	Control	44	213.628	61.854		
TGL (mg/dL)	Case	44	180.519	56.264	0.368	0.714 NS
	Control	44	174.462	93.953		
HDL (mg/dL)	Case	44	63.143	12.391	1.803	0.075 NS
	Control	44	68.665	16.103		
LDL (mg/dL)	Case	44	108.760	33.799	1.794	0.076 NS
	Control	44	95.484	35.618		
VLDL (mg/dL)	Case	44	36.106	11.253	0.368	0.714 NS
	Control	44	34.106	18.791		

[Table/Fig-1]: Comparison of fasting blood glucose and lipid profile between the study groups.
p-value <0.05: significant; S: Significant; NS: Not significant

Variables	Group	No	Mean	Std. Dev	T-score	p-value
AGEs (ng/dL) (Pentosidine)	Case	44	3.073	4.214	0.573	0.568 NS
	Control	44	2.682	1.660		

[Table/Fig-2]: Independent samples t-test of 'Case' and 'Control' on AGE (pentosidine).
p-value <0.05: significant; S: Significant; NS: Not significant

In the cases, blood sugar levels were found to be significantly higher than the controls (p< 0.001). Lipid levels of cases were higher than controls except the total cholesterol as all the cases were under statin therapy.

[Table/Fig-2] indicates the mean pentosidine level (AGE) in cases of 3.073 ng/dL and controls with a mean value of 2.682 ng/dL. Although the levels are higher in the cases relative to the controls, they were not statistically significant.

[Table/Fig-3] indicates that there is no significant correlation between glucose levels and pentosidine.

Overall correlation			
		Glucose	AGE
Glucose	Pearson correlation	1	-0.017
	Sig. (2-tailed)		0.876
	N	88	88
AGE-Pentosidine	Pearson correlation	-0.017	1
	Sig. (2-tailed)	0.876	
	N	88	88

[Table/Fig-3]: Correlation between glucose levels and AGE (pentosidine).
Overall: r= -0.17, p=0.87

DISCUSSION

This study was conducted to identify a marker for AGEs in type 2 DM patients, as many studies have suggested the role of AGEs in the development and progression of chronic diabetes complications. Among the many AGEs identified, pentosidine has been well-described to be a good predictor of various vascular complications associated with diabetes.

This case-control study consisted of 88 participants. Cases included 44 people who were diagnosed with Type 2 DM for more than five years, with no associated diabetic complications attending the diabetology out-patient department in a tertiary care teaching hospital in Chennai, India which caters to the patient population from various cities and towns nearby with varying socio-economic status, and controls included 44 healthy individuals, age and sex matched.

The main finding of this study was that the levels of pentosidine (AGE) were found to be higher in cases than controls, but this disparity between the groups was not found to be statistically significant (p=0.568). Other observations showed a significant difference in blood glucose levels between cases and controls (p≤0.001). Although the blood lipid levels except total cholesterol were higher in cases than controls, no statistically significant difference was found, as all patients (cases) were taking atorvastatin.

Although the levels of pentosidine were not significantly higher in cases than controls, the findings were in accordance with Sugiyama S et al., who found no difference in pentosidine levels between diabetic patients and healthy control subjects [17]. This study further suggests that plasma pentosidine levels are significantly influenced by renal function, and are independent of the duration or control of diabetes. Although there were no recorded renal complications in the patients included in the present study, renal function tests mainly creatinine could have been measured to confirm the findings.

Dolhofer-Bliesener R et al., also identified only a small increase in AGE levels in diabetic patients with normal renal function when compared to healthy subjects [18]. The theory of compromised renal function that plays a role in the reduction of pentosidine levels is further confirmed by a study conducted by Gohda T et al., [19].

While Yoshida N et al., found significantly higher pentosidine levels in diabetic patients than in controls, it was only in patients with diabetes having cardiovascular diseases than those without CVD [20]. This contributes to the fact that, while pentosidine is favourably linked to cardiac complications of diabetes, a cause and effect relationship can be established only when the complication is present and not before it evolves.

A study conducted by Kanazawa I et al., showed that six months of treatment with either metformin or pioglitazone lowered serum pentosidine levels in patients with type 2 DM [21]. Another similar study by Haddad M et al., also found that the plasma levels of pentosidine significantly decreased in patients receiving metformin compared to untreated patients with metabolic syndrome [22]. As the common drug taken by all the cases was metformin, it is likely that pentosidine levels were influenced by the medication, similar to the observations found in the above-mentioned studies.

In summary, although there was no statistically significant difference in pentosidine levels between cases and controls, it provides a clue that there were other factors causing an increase in pentosidine (AGE) levels that could be related to stress induced hyperglycaemia, exogenous dietary sources, life style factors etc. Thus, further larger long-term prospective studies giving priority to factors affecting the metabolism of pentosidine and other AGEs should be pursued to prove the cause and effect relationship between pentosidine and diabetic complications.

Limitation(s)

A study sample from different ethnic groups with a history of dietary patterns would have given us insight into the various factors that could affect the levels of AGE products. Renal function tests could have been performed among the study groups as pentosidine undergoes renal elimination. HbA1c measurement in the control group could have been done to confirm any pre-existing diabetic condition/glucose intolerance as continuous glucose monitoring rather than a single glucose test will give a dynamic value.

CONCLUSION(S)

In this study, the levels of AGE products (pentosidine) in cases were higher than controls but were not found to be statistically significant. While AGEs have been known to play a role in the development of many micro and macro angiopathic complications, both individually and synergistically, in patients with DM, it must be performed on a larger scale to extrapolate the results in order to determine the efficacy of AGEs in complementing routine investigations with emphasis given to factors affecting the metabolism of AGEs.

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PARTICULARS OF CONTRIBUTORS:

1. Medical Student, Department of Biochemistry, Kilpauk Medical College, Chennai, Tamil Nadu, India.
2. Medical Student, Department of Biochemistry, Kilpauk Medical College, Chennai, Tamil Nadu, India.
3. Medical Student, Department of Biochemistry, Kilpauk Medical College, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mr. Sarath Kumar Jaganathan Jaishankar,
Jains Westminster, Saligramam, Chennai, Tamil Nadu, India.
E-mail: drjjsarathkumar@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 02, 2019
- Manual Googling: Nov 05, 2019
- iThenticate Software: Jan 20, 2020 (16%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: No
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 31, 2019**
Date of Peer Review: **Sep 19, 2019**
Date of Acceptance: **Dec 21, 2019**
Date of Publishing: **Feb 01, 2020**