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Original Article

Dentistry Section

Diabetes and Oral Changes: The Tryptophan Metabolism Link?

RISHABH KAPILA, KIKERI SEETARAMAIH NAGESH, ASHA IYENGAR, DIVYALAKSHMI

ABSTRACT

Objectives: The objective of this study is to determine whether the severity of periodontal disease is associated with an increase in the levels of tryptophan and its metabolites in the saliva of type 2 diabetic patients.

Materials and Methods: 40 subjects were selected for this study. Each subject of the study was subjected to a detailed case history and intraoral examination. Then, the glycosylated haemoglobin estimation, the CPI scoring and the assessment of the salivary tryptophan levels and the levels of its metabolite were done. The data which was obtained was subjected to statistical analysis.

Results: The mean salivary tryptophan metabolites were higher in diabetic individuals than non-diabetic individuals. An increase in the CPI score was associated with an increase in the levels of the salivary tryptophan metabolites.

Conclusion: A relationship exists between the severity of periodontal disease and the levels of salivary tryptophan and its metabolites in type 2 diabetic individuals. This study may add an insight into an alternate pathway for the development of periodontal disease in the type 2 Diabetes mellitus individuals and kynurenergic agents, which may act as new treatment modalities for such cases.

Key Words: Diabetes; Periodontal Disease; Tryptophan

INTRODUCTION

Diabetes mellitus is a metabolic disease which is characterized by the dysregulation of carbohydrate, protein and lipid metabolism. The primary feature of this disorder is the elevation in the blood glucose levels (hyperglycaemia), which results from either a defect in the insulin secretion from the pancreas or a change in insulin action, or both [1]. Diabetes could result, in part, in the activation of the tryptophan metabolism. This reduces the plasma tryptophan levels and elevates the kynurenine metabolite levels. Several products of the kynurenine pathway have anti insulin action [2].

Tryptophan metabolites, via the kynurenine pathway, play an important role in several fundamental biological processes, including neuronal excitability, antioxidant status, ultravioletprotection, cell growth and cell division [2]. The salivary kynurenine derivatives are also implicated in the onset and the development of periodontal disease in humans [3]. Diabetic patients are more susceptible to gingivitis and periodontitis than the healthy subjects. These diseases are commonly considered to be the oral complications of diabetes [4]. The oral conditions that are seen in individuals with diabetes may include a burning mouth, an altered wound healing and an increased incidence of infection. Enlargement of the parotid glands and xerostomia can also occur [5]. Thus, there is a possibility that the kynurenine pathway derivatives which are present in saliva may play a role in the onset and the development of periodontal disease in diabetic patients [2].

MATERIALS AND METHODS

An informed signed consent was obtained from all the subjects prior to their inclusion in the study. All the procedures were followed in accordance with the ethical standards and with the last update of the Helsinki Declaration.

A total number of 40 subjects were included in the study. The subjects who were selected included 22 males and 18 females of the age group 40 years and above. These subjects were then divided into four groups:

Group A: 10 patients with normal blood glucose levels. (Control group); Group B: 10 patients of confirmed type 2 diabetes with a glycosylated haemoglobin assay of < 7%; Group C: 10 patients of confirmed type 2 diabetes with a glycosylated haemoglobin assay between 7%–8%; and Group D: 10 patients of confirmed type 2 diabetes with a glycosylated haemoglobin assay of > 8%.

All the individuals were subjected to a thorough general and oral examination including their periodontal status by using the Community Periodontal Index (CPI). 2ml of saliva was then collected and sent for the estimation of the levels of the salivary tryptophan metabolites by the High Performance Liquid Chromatography (HPLC) method. The general examination of the subjects in both the non-diabetic and the diabetic groups included findings like retinopathy (vision changes, blindness), neuropathy, nephropathy, trophic ulcer and altered wound healing. In the intra-oral examination, the tongue and the buccal and the labial mucosa were examined for pallor and ulcerative lesions. The patients were questioned about any taste alterations or burning sensations. The salivary glands were assessed for any enlargement and for xerostomia or ptyalism. The history of dryness or stickiness in the mouth was noted. Then, the orifices of all the major salivary gland ducts were dried with sterile, dry gauze and the flow and the consistency of saliva was checked by milking the ducts.

Saliva Sampling

The patient was made to sit comfortably on the dental chair. He was then asked to wash his mouth with MilliQ water (prepared by

reverse osmosis) for 10 minutes. Then, 2 ml of non-stimulated, mixed saliva was collected from all the subjects (non-diabetic and diabetic) in Eppendorf tubes. The salivary samples were immediately treated with 2 M perchloric acid ($HClO_4$) and were transferred to centrifuge tubes. After 15 minutes of incubation with the acid at 4°C, the samples were centrifuged for 30 minutes at 12000 x g and the supernatant was collected for the measurement of the concentration of tryptophan and its metabolites by High Perfomance Liquid Chromatography (HPLC).

Evaluation of the Salivary Tryptophan Metabolites

The concentrations of tryptophan, anthranilic acid and kynurenic acid were determined by high performance liquid chromatography by using the supernatant which was collected after the saliva sampling.

The reversed–phase HPLC system consisted of a Waters Spherisorb S3 ODS2 150 × 2.1 mm column, an HP 1050 series pump and a Rheodyne injection valve which was fitted with a sample loop (5 µl). The column effluent was monitored by using a programmable fluorescence detector, HP 1046A.

The optimized conditions were determined by recording the fluorescence spectra by using a stop-flow technique. The excitation and emission wavelengths were set to 254/404 nm for tryptophan and kynurenic acid and 320/420 nm for anthranilic acid respectively. The output of the detector was connected to a ChemStation and to a computer which had the software for carrying out the measurements. The mobile phase consisted of 50 mM acetic acid and 0.25 M zinc acetate (pH –4.9) containing 1% of acetonitrile and it was pumped at a flow–rate of 0.18 ml/min. The chromatography was carried out at 21°C.

Chromatograms were obtained for tryptophan, kynurenic acid and anthranilic acid. These chromatograms were later used for estimating the peak area for the quantification of the data by using a data processor system. The width at half-height was obtained by multiplying the peak width at half-height by the peak height, to determine the area and the results were expressed in nanomoles per litre. These results indicated the salivary levels of tryptophan, kynurenic acid and anthranilic acid.

The Excel and SPSS (SPSS Inc, Chicago) software packages were used for data entry and analysis by using ANOVA and the Bonferroni test. p-values of < 0.05 were regarded as statistically significant.

RESULTS

A detailed history of the *general symptoms* of the individuals in the diabetic group revealed that 08 subjects (26.66 %) had vision changes and that 03 subjects (10 %) had peripheral neuropathy. A detailed history of the *oral symptoms* of the individuals in the diabetic group revealed that 7 subjects (23.33 %) had burning mouth symptoms, 4 subjects (13.33 %) had dysguesia, 3 subjects (10 %) had sialadenosis, 2 subjects (6.66%) had xerostomia and that 1 subject (3.33%) had oral ulcers. In the non-diabetic group, none of the above mentioned general and oral manifestations were present in the subjects.

The Community Periodontal Index (CPI) score was calculated for both the non-diabetic group and the diabetic group to determine the periodontal status. The mean CPI score was greater in the poorly controlled diabetic group (3.51 ± 0.53), followed by the moderately controlled group (2.67 ± 0.47), the well controlled group (2.67 ± 0.47) and finally, the non-diabetic group (1.89 ± 0.32). The results showed that the mean CPI values increased with the severity of diabetes and this difference was found to be statistically significant ($p<0.001$). However, no statistically significant difference was observed between the non-diabetic group and the well controlled diabetic group with respect to the mean CPI ($p>0.05$) [Table/Fig-1].

The mean salivary tryptophan and kynurenic acid levels within the diabetic study group and on comparison with the non-diabetic group were found to be statistically significant ($p< 0.001$) [Table/Fig-2 & 3]. A statistically significant difference was found between the mean salivary anthranilic acid levels of the poorly controlled diabetic group and the well controlled diabetic group as well as the non-diabetic group ($p<0.001$). However, the mean difference was not significant between the poorly controlled and the moderately controlled diabetic groups ($p>0.05$) and; between the well controlled diabetic and the control groups ($p>0.05$) [Table/Fig-4].

In order to find out whether a correlation existed between the Community Periodontal Index scores and the salivary tryptophan metabolites i.e. the salivary tryptophan, kynurenic acid and anthranilic acid levels, the Pearson's correlation test was applied. The results of the Pearson's correlation test suggested that with an increase in the severity of periodontal disease, the salivary tryptophan, kynurenic acid and anthranilic acid levels also increased and vice versa [Table/Fig-5].

DISCUSSION

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders which are manifested by abnormally high levels of glucose in the blood [6].The six main complications which are associated with diabetes mellitus are retinopathy, nephropathy, neuropathy, macrovascular disease (cardiovascular disease, cerebrovascular disease, peripheral vascular disease), altered wound healing and periodontal disease. Diabetes mellitus and periodontal disease are two common chronic diseases that have long been considered to be biologically linked [7]. Tryptophan, which is an essential amino acid, is required for the biosynthesis of proteins and it is the precursor for several biological compounds [8]. *The role of tryptophan and its metabolites in the oral physiology and pathology have not been studied as yet.* Tryptophan was found in significant amounts in the saliva of diabetic individuals in some studies, particularly tryptophan metabolites like kynurenine and anthranilic acid [2]. In a study which was conducted in a Chinese population, results which were similar to those of our study were noted, with an increase in the prevalence of the vision changes [9]. Studies have shown an association between diabetes and oral changes. The significant oral changes which were observed in diabetics were hyposalivation which led to oral dryness, altered taste, burning mouth sensation, angular cheilitis, glossitis, and stomatitis [10,11]. An association between diabetes and periodontal disease has long been looked into. Diabetes increases the likelihood of developing periodontal diseases to two- to five-fold [12]. To find out any such association, the Community Periodontal Index (CPI) score was calculated for both the non-diabetic and the diabetic groups. The results which were obtained were in accordance to those of studies which were conducted to investigate the associations between glycaemic control and the severity of periodontal disease. It has been shown that the gingival and periodontal score was statistically higher in the diabetic group than in the non diabetic group [13]. There was a strong association

Group	n	Mean	Std dev	Min	Max	F	P-value
Non-diabetic	10	1.71	0.32	1.10	2.16	37.835	<0.001
Well Controlled diabetic	10	1.89	0.32	1.40	2.55		
Moderately Controlled diabetic	10	2.67	0.47	1.66	3.12		
Poorly Controlled diabetic	10	3.51	0.53	2.50	4.00		

[Table/Fig-1]: Community Periodontal Index (CPI) Scores in the Non-diabetic and Diabetic group

Group	n	Mean	Std dev	Min	Max	F	P-value
Non-diabetic	10	220.22	6.34	210.20	230.20	270.535	<0.001
Well Controlled diabetic	10	261.51	21.80	232.20	292.60		
Moderately Controlled diabetic	10	363.38	19.74	329.90	388.20		
Poorly Controlled diabetic	10	457.71	27.84	422.60	492.60		

[Table/Fig-2]: Salivary Tryptophan Levels in the Non-diabetic and Diabetic group

Group	n	Mean	Std dev	Min	Max	F	P-value
Non-diabetic	10	4.16	0.89	3.10	5.40	120.963	<0.001
Well Controlled diabetic	10	6.43	0.37	5.80	6.90		
Moderately Controlled diabetic	10	7.41	0.27	6.90	7.70		
Poorly Controlled diabetic	10	8.33	0.25	7.90	8.70		

[Table/Fig-3]: Salivary Kynurenic Acid Levels in the Non-diabetic and Diabetic group

Group	n	Mean	Std dev	Min	Max	F	P-value
Non-diabetic	10	3.31	0.25	3.10	3.80	57.870	<0.001
Well Controlled diabetic	10	3.43	0.22	3.20	3.80		
Moderately Controlled diabetic	10	4.43	0.28	4.10	4.80		
Poorly Controlled diabetic	10	4.64	0.37	4.20	5.20		

[Table/Fig-4]: Salivary Anthranilic Acid Levels in the Non-diabetic and Diabetic group

		CPI (mm)	Tryptophan	Kynurenic Acid	Anthranilic Acid
CPI (mm)	Pearson Correlation	1	0.863	0.838	0.721
	Sig. (2-tailed)		<0.001	<0.001	<0.001
Tryptophan	Pearson Correlation	0.863	1	0.983	0.886
	Sig. (2-tailed)	<0.001		<0.001	<0.001
Kynurenic Acid	Pearson Correlation	0.838	0.983	1	0.898
	Sig. (2-tailed)	<0.001	<0.001		<0.001
Anthranilic Acid	Pearson Correlation	0.721	0.886	0.898	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	

[Table/Fig-5]: Correlation of Community Periodontal Index with Salivary Tryptophan and its Metabolites in the Diabetic group

between poorly controlled diabetes and severe periodontitis [14-19]. Moreover, a study had already indicated that all the type 2 diabetic patients should be informed about their increased risk for periodontal disease [20,21]. Tryptophan, an essential amino acid, is metabolized via the kynurenine pathway to form metabolites like kynurenine, kynurenic acid and anthranilic acid [22]. Recently, tryptophan and its metabolites such as kynurenic acid and anthranilic acid, have been implicated in a number of disorders including diabetes and periodontal disease. The results of our study were in accordance to those of a study which was done to estimate certain tryptophan derivatives in the saliva of patients with diabetes type 2. The increased concentrations of kynurenine and kynurenic acid in patients with diabetes and hypertension as compared to those in the healthy volunteers suggested an altered metabolism of tryptophan in these diseases [2].

A correlation between the community periodontal index and salivary tryptophan and its metabolites suggested that with an increase in the community periodontal index score (suggesting the severity of the periodontal disease), the levels of tryptophan and its metabolites also increased. Moreover, an increase in the salivary tryptophan metabolites was also associated with an increase in the severity of periodontal disease. This correlation between diabetes mellitus, periodontal disease and the salivary tryptophan metabolite levels could be related to the impaired kynurenine pathway metabolism of tryptophan. There are so far no documented studies to the best of our knowledge, that correlate the severity of periodontal disease with the salivary tryptophan metabolites between non-diabetic and diabetic individuals and within the 3 diabetic groups.

A considerable number of pharmacological tools have recently been made available to probe the kynurenine pathway experimentally. Some of these kynurenergic agents have been envisioned to be of therapeutic value, especially in the treatment of diseases that are associated with the impaired kynurenine pathway metabolism [23].

CONCLUSION

The role of the metabolites of tryptophan via the kynurenine pathway has been speculated in type 2 diabetes due to its anti-insulin action. The salivary levels of tryptophan and its metabolites may have some bearing in the progress of the periodontal disease in diabetic individuals.

Certain agents are available, that tap the kynurenine pathway i.e. kynurenergic agents and these can be envisioned to be of therapeutic value, especially in the treatment of diseases that are associated with the impaired kynurenine pathway metabolism. *This may open new avenues for the management of the periodontal disease in the Diabetes mellitus type 2 cases.*

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AUTHOR(S):

1. Dr. Rishabh Kapila
2. Dr. Kikeri Seetaramaih Nagesh
3. Dr. Asha Iyengar
4. Dr. Divyalakshmi

PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer,
   Department of Oral Medicine and Radiology,
   Guru Nanak Dev Dental College and Hospital,
   Sunam, Punjab, India
2. Professor, Head and Principal,
   Department of Oral Medicine and Radiology,
   D.A.P.M.R.V. Dental College and Hospital,
   Bangalore, Karnataka, India.
3. Professor, Department of Oral Medicine and Radiology,
   D.A.P.M.R.V.Dental College and Hospital, Bangalore,
   Karnataka, India.
4. Reader, Department of Oral Medicine and Radiology,
   D.A.P.M.R.V.Dental College and Hospital, Bangalore,
   Karnataka, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Rishabh Kapila
House Number 110, Sector 12-A
Panchkula, Haryana - 134109, India.
Phone: +91-925663774
E-mail: rishabh_kapila@rediffmail.com

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