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Dental Science

Comparison of Salivary Cortisol Level in Type 2 Diabetic Patients and Pre-Diabetics with Healthy People

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Abstract

BACKGROUND: Cortisol is one of the important enzymes of saliva. Control of this hormone is an effective way to adjust the glucose level in diabetic patients.

AIM: The aim of this research is to compare the salivary cortisol level in type 2 diabetic patients and pre-diabetics with healthy people.

METHODS: In this case-control study (2018), the unstimulated salivary samples were collected from 44 patients with type 2 diabetes, 44 pre-diabetic people (case group), and 44 healthy subjects (control group), matched for age and gender. The samples were transferred to the laboratory, and salivary cortisol level was measured using ELISA. Data were analysed using SPSS 22 and Chi 2 tests.

RESULTS: The mean salivary cortisol level in type 2 diabetic patients was 3.14 ± 1.17, in pre-diabetic cases was 1.83 ± 0.68, and in healthy controls was 0.86 ± 0.43 ($P < 0.001$). The mean DMFT in type 2 diabetic patients was 19.6 ± 6.5, in the pre-diabetic group was 13.43 ± 4.5, and in healthy controls was 9.38 ± 3.72 ($P < 0.001$).

CONCLUSION: With regards to the results, salivary cortisol level in type 2 diabetic patients is more than pre-diabetic people, and in pre-diabetic people is more than healthy people. Also, there was a significant relation between salivary cortisol level and DMFT index.

Introduction

Diabetes mellitus is a multi-agent metabolic disease characterised by increased blood glucose and metabolic disorders of carbohydrates, fat and protein [1]. Increased blood glucose results from impaired secretion of insulin and liver gluconeogenesis [2], [3]. Pre-diabetes is a condition in which blood glucose is somewhat high, but the patient does not have all the criteria for diabetes. Fasting blood glucose in pre-diabetes is 100 to 125 mg/dL, while in diabetes, fasting blood glucose is 126 or above 126 mg/dL [4]. The two main types of diabetes include type 1 diabetes (insulin-dependent) and type 2 diabetes (non-insulin dependent). The global prevalence of diabetes is increasing, and it's predicted to reach from 180 million in 2000 to 320 million in 2025 [5], [6]. The prevalence of diabetes in Iran is close to its global value as about 5.5% of the population [7].

Diabetes Mellitus has a very complex clinical presentation and is associated with complications such as nerve, kidney and retina damage and cardiovascular disease [8], [9]. The oral manifestations of diabetes are variable and are commonly manifested in patients with poorly controlled blood glucose. Some oral complications of diabetes include dry mouth, gingivitis, periodontitis, dental abscesses and soft tissue lesions of the tongue and oral mucosa [10], [11].

Diabetic patients need to control blood

glucose levels to diagnose, treat and track their illness. Typically, a blood sample for analysis is obtained through the vein or by finger stick or other aggressive methods which often result in physical and mental stress and pain in the patient. Because of this, the use of other biological fluids, such as saliva, is desirable to determine the level of glucose in the blood and avoid these invasive methods [12]. Salivary glands dysfunction is one of the problems that is commonly referred to by diabetic patients. This can be a direct result of the patient's medical condition and inadequate control of their blood glucose [13]. Therefore, many salivary compounds, such as enzymes, can be reliably considered for diagnosis and prognosis because saliva is readily available [14], [15], [16], [17].

Studies have shown that the determination of salivary combinations in diabetic patients can be useful in the detection and control of oral complications of diabetes [18], [19], [20]. One of the compounds in the saliva is cortisol. Cortisol is a glucocorticoid hormone secreted from the adrenal cortex and plays a role in regulating mineralocorticoids, immune system function, blood pressure and metabolism. Conditions such as hypertension, hypercholesterolemia, central obesity and glucose intolerance are associated with increased levels of cortisol. Following the changes in blood cortisol levels, the amount of this hormone also changes in the saliva [21], [22]. Salivary cortisol level has recently been suggested as a valuable recommendation for blood cortisol analyses. Given that this method is non-invasive and requires not much laboratory procedures, it can be investigated in unlimited cases [23]. Shirzaii et al., a study in 2016 showed that salivary cortisol level is higher in people with type 2 diabetes compared to healthy subjects [24].

Dental caries is the most common disease which itself is the most common dental disease. In many cases, dental caries leads to tooth pulp infection, along with pain in the patient [25]. In 1938, a person by the name of Palmpour presented the DMFT index which was suggested by World Health Organization (WHO) and the International Dental Federation (FDI) to evaluate the variability of teeth health [26].

There are controversial reports about the role of cortisol in the aetiology of diabetes, and few studies have been conducted on the evaluation of salivary cortisol levels, and also there have been no studies on the evaluation of salivary cortisol levels in pre-diabetic patients and the relationship between salivary cortisol level and DMFT index. Therefore, the present study aimed to compare salivary cortisol level in diabetic and pre-diabetic individuals with healthy subjects and its relationship with DMFT index.

Material and Methods

This is a case-control study which was conducted on patients referring to the health centre of Sari city in 2018. The sample size was estimated as 31 subjects for each group based on Shirzaii et al., study while considering the results of the study (mean and standard deviation of salivary cortisol levels in diabetic patients were 1.73 and 1.017 and 1.08 and 0.643 in the control group respectively), confidence level of 95% and power of 90% were estimated using the formula for comparing the two meanings in the G-power software. Regarding the fact that the number of studied groups was 3, the final sample size was adjusted according to the following formula, and it was determined as 132 subjects (44 in each group) [24].

$$n' = n * \sqrt{g-1}$$

The ethics code to this research was considered as IR. MAZUMS. REC. 1397.1188 after being approved by the Medical Ethics Committee. Samples were selected by simple sampling method, and written consent was obtained from patients for participation in the study. In this study, subjects were divided into three groups with 44 subjects in each. The first group consisted of all people with type 2 diabetes whose fasting blood glucose was 126 or greater than 126 mg/dL and had complete medical file and records and at least 5 years history of diabetes, had no other systemic diseases and weren't taking any other medications other than glucose controlling ones. The second group consisted of pre-diabetic subjects whose fasting blood glucose was 100 to 125 mg/dL and the third group consisted of healthy subjects without symptoms of diabetes (obesity and familial history of diabetes) or history of taking medications in the last three months that were matched with case group in terms of age and gender.

Exclusion criteria included: Body Mass Index more than 30, pregnancy, tobacco use, drugs and alcohol, Addison's systemic disease, Cushing's syndrome and thyroid disorders, history of injury and surgery in the past four weeks, history of malignancy, use of corticosteroid or hormone therapy and mental and sleep disorders.

All the participants had a blood test in the recent last month. All data, including gender, age, FBS, HbA1C and DMFT, were recorded. To evaluate the DMFT index, dental surfaces were dried using air syringe and carefully examined for the presence of tooth-coloured and non-tooth-coloured restorations. In this study, M represents the number of teeth lost due to decay. Whenever there is a lesion that appears on the smooth dental surfaces or inside pits and grooves that are embedded in the enamel or is softened on the floor and around it, the tooth is considered decayed (D). In this study, teeth that have cavities and are

dressed with temporary filling materials, as well as teeth with restorations that still contain caries are classified as Decayed teeth. Damaged teeth or treated decayed teeth whose appearance, function and size are restored to normal, are considered as Filled (F) which is referred to as the number of teeth that have undergone root canal therapies or are filled with restorative materials. After recording the mentioned items, the obtained numbers are summed, and the result determines the DMFT in each individual. The examination of teeth was conducted under the light on a dental chair [27].

To determine salivary cortisol level, subjects were asked not to eat, drink and brush 90 minutes before sampling. Subjects' total non-stimulatory salivary samples (by spitting) were obtained while sitting on a chair upright with their head slightly tilted forwards in a calm environment between 10 to 12 A. M. (near the peak of serum cortisol level). The collected samples were immediately put into the numbered test tubes and transferred to the immunology laboratory of the Baghban clinic of Sari. At the laboratory, saliva samples were centrifuged at centrifugal speeds of 2000 rpm for 10 minutes, and then the supernatant was stored at -20°C until the test. The level of salivary cortisol was measured by ELISA method using the Diameter kit (made in Italy).

Results

In this study, 44 diabetic patients, 44 pre-diabetics and 44 healthy people participated. There were 34 females (77.3%) and 10 males (22.7%) in all groups. The mean age in the diabetic group was 54 years and 48/07 in the pre-diabetic group, and the healthy group it was 42.86 years. The mean FBS in the diabetic group was 188.91 mg/dL, in the pre-diabetic group, it was 108.75 mg/dL, and in the healthy group it was 88.59 mg/dL. The mean salivary cortisol level in people with diabetes was reported as 3.13 mmol/dL, in pre-diabetics as 1.83 mmol/dL and healthy subjects as 0.85 mmol/dL. The mean DMFT index in people with diabetes was 19.56, in pre-diabetic individuals was 13.43, and in healthy subjects it was 9/38 (Table 1).

Table 1: Specifications of participants in the study

Title	Range	Mean ± Standard deviation	Groups	p-value
Age (year)	40-60	54 ± 5.70	Diabetics group	< 0/100
	31-60	48.07 ± 8.68	Pre-diabetics group	
	30-60	42.86 ± 11.90	Healthy group	
FBS (mg/Dl)	126-342	188.91 ± 48.91	Diabetics group	< 0/100
	100-124	108.75 ± 7.78	Pre-diabetics group	
	70-99	88.59 ± 7.49	Healthy group	
Cortisol (mmol/Dl)	1.4-6.8	3.14 ± 1.17	Diabetics group	< 0/100
	0.2-3.1	1.83 ± 0.68	Pre-diabetics group	
	0.3-1.8	0.86 ± 0.43	Healthy group	
DMFT	10-28	19.6 ± 6.5	Diabetics group	< 0/100
	6-28	13.43 ± 4.5	Pre-diabetics group	
	4-18	9.38 ± 3.72	Healthy group	

In a comparison between the two genders, the mean cortisol level in males was 2.34 mmol/dL, and in women it was 1.83 mmol/dL. According to the results, there is a significant difference in the cortisol levels between male and female diabetics (P = 0.005), but there are no significant differences in the cortisol levels between males and females in pre-diabetic and healthy groups (P = 0.051 and P = 0.624). In a comparison of cortisol levels between the three groups, the differences in salivary cortisol levels were significant among males (P < 0.001) and females (P < 0.001) (Table 2).

Table 2: Comparison of cortisol levels in both genders

Gender / Group	Female	Male	p-value
Diabetes	2.88 ± 0.97	4.02 ± 1.42	*0.005
Pre-diabetes	1.73 ± 0.69	2.2 ± 0.46	0/051
Healthy	0.88 ± 0.44	0.8 ± 0.39	0/624
Total	1.83 ± 1.09	2.34 ± 1.59	0/046*
p-value	* < 0.001	* < 0.001	------

*p < 0.05.

In a comparison of DMFT index between males and females, the mean DMFT index is 15.3 in males and 13.79 in females. According to the table below, there are no significant differences in the DMFT index between males and females in any of the participating groups. With the DMFT compared in the three groups, the DMFT index was significantly different among males (P < 0.001) and among females (P < 0.001) (Table 3).

Table 3: Comparison of DMFT index in both genders

Gender / Group	Female	Male	p-value
Diabetes	18.5 ± 6.7	22 ± 5.39	0/183
Pre-diabetes	13.1 ± 4.8	14.5 ± 2.72	0/396
healthy	9.41 ± 3.7	9.3 ± 3.97	0/935
total	13.79 ± 6.49	15.6 ± 6.65	0/280
p-value	* < 0.001	* < 0.001	---

*p < 0.05.

Regarding HbA1C, diabetic subjects were divided into two subgroups of controlled diabetes (7 > HbA1C) and uncontrolled diabetes (7 < HbA1C) and cortisol levels and DMFT index were evaluated. In the comparison between the two subgroups, 12 subjects were in the controlled group and 32 in the uncontrolled group. The mean cortisol level in the controlled group was 3.03, and in the uncontrolled group it was 3.49, and its P-value was reported as 0.94 by the Mann-Whitney test, which is statistically not significant. In a comparison of DMFT between the two groups, the mean DMFT was 16.83 in the controlled diabetes group and 20.6 in the uncontrolled diabetes group. Using the Mann-Whitney test, the P-value was reported to be 0.86, which is statistically not significant (Table 4).

Table 4: Comparison of cortisol levels and DMFT in diabetics group based on HbA1c levels in patients

	Number	DMFT	Cortisol level
Controlled diabetes	12	16.83 ± 6.10	3.03 ± 0.74
Uncontrolled diabetes	32	20.59 ± 6.47	3.49 ± 1.29
p-value	---	0.89	0.124

*p < 0.05.

Comparing the studied groups for age, FBS, salivary cortisol level and DMFT index, the results of the study showed a significant difference between the studied groups and the results are presented in Table 5.

Table 5: Significance level of inter-group relations

Treatment groups P-value	Age	FBS	Cortisol	DMFT
Healthy subjects & pre-diabetic patients	*0.022	*< 0.001	*< 0.001	*< 0.001
Healthy subjects & diabetic patients	*< 0.001	*< 0.001	*< 0.001	*< 0.001
Diabetic & pre-diabetic patients	*< 0.001	*< 0.001	*< 0.001	*< 0.001

*p < 0.05.

According to the results presented in Table 6, cortisol level was 0.97 units (P < 0.001) in pre-diabetic subjects and 2.28 (P < 0.001) in diabetic subjects higher than healthy subjects which are statistically significant. By adjusting the effects of variables such as age, gender and FBS, cortisol level in pre-diabetic subjects is 0.33 and in diabetic subjects is 0.62 units higher than healthy subjects, which is statistically significant. Based on the results of Kruskal-Wallis test, cortisol, as a dependent variable, as compared with independent variables in this study including gender, age, and FBS and significant results were reported. Based on the results of single-variable linear regression, for each unit of increase in age, cortisol level increases by 0.04 units, which was statistically significant (P < 0.001), but the effect of age on the cortisol level after adjusting other variables in this study was not statistically significant and considerable, and for each unit of increase in age, cortisol level decreases by 0.2 units (P = 0.713). In the assessment of the relationship between gender and salivary cortisol level, cortisol level in females was 51.1 mmol/dl less than males, which is statistically significant (P = 0.046). After adjusting other variables in the study, cortisol level in females was 0.15 units lower than males, which is still statistically significant (P = 0.01). In the assessment of the relationship between FBS and salivary cortisol level, for each unit of increase in FBS, the cortisol level increases by 0.02 units, which is reported as a significant relationship (P < 0.001). By adjusting the effects of other variables, the cortisol level increases by 0.28 units for each unit of increase in FBS, which is statistically significant (P = 0.006).

Ultimately, in the assessment of the relationship between cortisol level and DMFT, the correlation coefficient between cortisol level and DMFT was 0.5, and this correlation is statistically significant (P < 0.001).

Table 6: Investigation of factors related to cortisol level

Title	Multi-variable analysis		Single-variable analysis	
Variables	P-value	Beta factor	P-value	Beta factor
Pre-diabetics group (reference=healthy people)	*<0.001	0.33	*<0.001	0.97
Diabetics group (reference=healthy people)	*<0.001	0.62	*<0.001	2.28
Age	0.713	-0.02	*<0.001	0.04
Gender	*<0.010	-0.15	*0.046	-0.51
FBS	*0.006	0.28	*<0.001	0.02

*p < 0.05.

Discussion

This study was conducted to compare the salivary cortisol levels between the diabetic, pre-diabetic and healthy groups and also to investigate its relationship with DMFT. According to the results, the mean cortisol level in diabetic patients was higher than the pre-diabetic group and also in pre-diabetic group it was higher than in healthy subjects. These differences were statistically significant. Also, cortisol levels in patients with uncontrolled diabetes were more than those with diabetes, but this difference was not statistically significant. In a comparison between the two genders, the cortisol level in diabetic males was higher than diabetic females, but there were no significant differences in cortisol level between the two genders in the pre-diabetic and healthy group.

Investigating the DMFT index, the mean DMFT in diabetic patients was higher than pre-diabetic patients, and in pre-diabetic patients, it was higher than healthy subjects making these differences significant. In a comparison between the two genders, there were no significant differences in the DMFT index between males and females. There is a significant relationship between the mean DMFT and salivary cortisol levels in the studied subjects.

There have been some studies about the relationship between the blood glucose and salivary cortisol level increase, which are reviewed:

According to a study by Shirzaii et al., in Zahedan in 2016 with the aim of comparing salivary cortisol levels in type 2 controlled diabetic patients with healthy subjects, the mean salivary cortisol level in type 2 diabetic patients was 1.73 and in healthy subjects it was reported as 1/08 and it has been stated that there is a significant relationship between them and the mean cortisol level in diabetic patients was higher than healthy subjects. This result is similar to the present study. There have been no significant differences in the cortisol level in Shirzaii study between the two genders. Also, in this study cortisol levels in diabetic patients have been reported higher than Shirzaii study which is due to differences in sampling conditions, geographical location, and living conditions of the studied subjects [24].

In a study by Chiodini et al., In Italy in 2007, with the aim of evaluating salivary cortisol secretion in type 2 diabetic patients, the hypothalamus-pituitary-adrenal axis activity and cortisol secretion in diabetic patients was higher than healthy subjects (0001 > P) and it's been stated that it depends on the complications of diabetes. In this study, factors such as gender, duration of diabetes and HbA1c levels are deemed affect cortisol levels. These results are similar to the results of the current study with the difference that in Chiodini study no tests were conducted about pre-diabetic subjects [28].

In a study by Roy et al., 1990 regarding

hypothalamic-pituitary-adrenal axis disorder in diabetic patients, glucocorticoid and cortisol secretion were higher in patients with type 2 diabetes mellitus and insulin resistance. Regarding this study, an increase in cortisol secretion leads to diabetes and makes metabolic control difficult. In the current study, similar to Roy study, cortisol secretion has been observed to be higher in people with diabetes than healthy subjects, but no results on the effects of cortisol secretion on diabetes occurrence have been achieved [29].

In a study by Liu et al., in the United States in 2005 regarding the level of salivary cortisol in soldiers with diabetes, similar to the current study, there is an increase in cortisol levels in diabetic patients [30].

In 1998, Roy et al., in a study on cortisol levels in diabetic subjects, found that diabetic patients with retinal damage and cardiovascular complications had higher levels of cortisol. They have pointed to rising cortisol levels in diabetic patients. However, the current study suggests that cortisol levels may still increase even if no diabetes complications occur, and blood glucose control is adequate [31].

In another study by Hackett et al., In 2014 about the association between the daily pattern of cortisol and type 2 diabetes, it was concluded that salivary cortisol levels in patients with type 2 diabetes increase only during sleep and is not much changed during the day, while the current study showed that salivary cortisol levels increase significantly in the morning compared to non-diabetic subjects. Measuring salivary cortisol levels frequently in a day can justify these differences [32].

Radahmadi et al., in a similar study in 2004 on the effects of psychical stress on exacerbation of diabetes mellitus, serum glucose, cortisol levels and body mass in rats found that cortisol secretion levels in people with diabetes mellitus increased significantly and the effects of diabetes on cortisol secretion is more than mental stress. Diabetes mellitus is a strong stimulant for the physiological system of the body [33].

All of the studies mentioned above have been based on the correlation between cortisol and blood glucose in diabetic patients and no studies have been conducted on pre-diabetic patients. According to our study, an increase in blood glucose levels in pre-diabetes may also increase salivary cortisol levels.

One of the oral complications of diabetes is dry mouth and increased glucose level of gingival crevicular fluid, which can increase dental caries occurrence. Another result of our study is the increase in DMFT in diabetic patients, which is reviewed in the following in some similar studies.

According to a study by Behbahani and Yasin in Ahvaz in 2017 with the aim of determining the relationship between DMFT index, FBS and HbA1C in type 2 diabetic patients, it is stated that there is a significant relationship between DMFT and blood glucose which is similar to the results of the current study. The prevalence of DMFT in the Behbahani study was 51.51, which is slightly lower than the current study. This difference can be due to differences in culture and the degree of oral hygiene in that area [34].

In 2010 in a study on the impact of diabetes on the prevalence of dental problems, Miko et al. stated that poor blood glucose control and preterm diabetes could increase the risk of dental caries. This finding is consistent with the results of the current study [35].

In a study by Miralles et al., in 2006 on the effects of systemic factors of diabetes on the development of dental caries, diabetes mellitus has been shown to increase dental caries, and dental caries has been more common in the diabetic population. These results are similar to the results of the current study [36].

All of the above studies have been conducted to investigate the relationship between diabetes and DMFT, but no studies have been done about the relationship between diabetes and DMFT on pre-diabetic subjects. According to the present study, it seems that increased blood sugar even in pre-diabetics may increase DMFT and pre-diabetes is a risk factor for dental caries.

Another finding from the present study is the relationship between salivary cortisol levels and DMFT. In a study by Golestannezhad et al., in Isfahan in 2014 on dental caries rampancy in patients with migraine, it has been stated that during migraine attacks, due to increased activity of the hypothalamus-pituitary-adrenal axis, salivary cortisol levels increase. It has also been stated that there is a significant relationship between migraine and increased dental caries [37].

The mentioned study above indirectly points out the relationship between cortisol and DMFT, but no studies have been done to assess the relationship between salivary cortisol levels and DMFT index so far. According to the findings of the current study, there is a significant relationship between salivary cortisol levels and DMFT index, and it seems that increased salivary cortisol levels increase the risk of dental caries. The exact mechanism of this association is unclear, but increased cortisol levels may increase the risk of dental caries by increasing the amount of glucose in the gingival crevicular fluid in diabetic and pre-diabetic subjects.

In a study by Farahat et al., in Yazd in 2013, on the status of DMFT index in patients with type 2 diabetes and its relationship with HbA1C, it has been observed that DMFT index in patients with uncontrolled diabetes is significantly higher than patients with controlled diabetes. In the present study, DMFT is higher in patients with uncontrolled diabetes than in patients with controlled diabetes, but this

difference was not statistically significant [38]. Also, no significant differences were found between salivary cortisol levels in these two groups, and no studies have been done on this issue so far.

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