

The Effect of Vitamin D Supplements on Antioxidant Parameters of Serum and Saliva in Diabetic Rats

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Article Type	ABSTRACT
Research Paper	Background and Objective: Vitamin D deficiency plays an important role in the process of diabetes and it may cause the appearance and exacerbation of symptoms by destroying and inducing different pathways. Vitamin D also has antioxidant properties and inhibits the production of free radicals. Therefore, this study was conducted to determine the role of vitamin D supplements on antioxidant parameters of serum and saliva and to compare them in diabetic rats. Methods: This experimental study was conducted on 28 male Wistar rats that were randomly divided into 4 groups (C group: healthy, CV group: healthy with vitamin D, DC group: diabetic without vitamin D, and DV group: diabetic with vitamin D). Streptozocin (65 mg/kg) was used intraperitoneally to make the rats diabetic. After 10 weeks, saliva (stimulated with pilocarpine) and blood from the eye vein were sampled. The antioxidant level was evaluated by measuring the serum and salivary levels of antioxidant factors (CAT, TAC, SOD, MDA). Findings: Vitamin D injection had a significant effect on salivary SOD level (6.44 ± 1.02 U/ml) and serum TAC level (25862.22 ± 4382.06 μmol/l) ($p < 0.05$). In addition, diabetes decreased the salivary level of CAT and TAC ($p < 0.001$). The findings did not show a significant difference in the serum and salivary levels of MDA. Moreover, vitamin D decreased fasting blood sugar in diabetic rats (211.14 ± 18.9) ($p < 0.05$) and the rats that received vitamin D gained less weight at the end of the study (71.67 ± 13.13 gr). Conclusion: The results of the study showed that oral intake of vitamin D supplement, along with its reducing effect on blood sugar, can reduce the oxidative stress caused by diabetes in serum and saliva levels. Keywords: Vitamin D, Serum, Saliva, Diabetes.
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Introduction

Vitamin D deficiency can be an important factor in type 2 diabetes (T2DM) because it affects various pathways involved in diabetes and its complications (1). These pathways include pancreatic insulin secretion disorder, insulin resistance, reduction in insulin receptor gene expression, sterile inflammation, and autoimmune disorders (2). Type 2 diabetes causes hyperinsulinemia, hyperglycemia and the release of free radicals and leads to a decrease in antioxidant capacity. Various studies have shown that vitamin D strengthens antioxidant systems and can be an option for the treatment of type 2 diabetes and helps to improve blood sugar control, prevent recurrence and complications of diabetes (3).

In periodontal diseases, increased levels of oxidative markers in saliva, serum, and gingival crevicular fluid are well known. This indicates the destruction of tooth and mucosa supporting tissues. In addition, oxidative damage in inflamed periodontal tissue leads to proliferation of antioxidant enzymes. As a result, metabolic diseases that cause periodontal tissue destruction can increase serum and salivary levels of oxidative stress agents (4). One study showed that three-month administration of vitamin D significantly improved HbA1C levels and reduced oxidative markers (5). Rahsepar et al. showed that women with polycystic ovary syndrome had lower vitamin D concentrations than the control group. Fasting insulin, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) and malondialdehyde (MDA) levels were also higher in this group (6). However, in the study of Cojic et al. on T2DM patients, the effect of vitamin D on HOMA-IR index, malondialdehyde levels and TG/TBARS index was not statistically significant (5). In another study, patients with periodontitis and diabetes showed lower total antioxidant capacity in their saliva compared to groups without both diseases or with either of them (7).

Gümüş et al., by studying patients with type 1 diabetes (T1DM), T2DM and systemically healthy patients, all of whom had periodontal inflammatory disease, showed that there was no significant difference in salivary antioxidant concentration between T1DM and T2DM patients and control group, and in each diabetic group, the decrease in saliva glutathione concentration showed a significant positive correlation with the depth of the probe, and the total antioxidant capacity was also correlated with the saliva flow rate (8). Moreover, in a double-blind trial, the use of vitamin D supplements for three months did not show beneficial effects on biomarkers of oxidative stress status (9). However, another study on 178 T2DM patients showed that vitamin D can reduce oxidative stress and inflammation by regulating glutathione production (10). Despite the available data from studies conducted on vitamin D injections, the results still need to be clarified (11). Some studies have shown a significant effect on the reduction of fasting blood sugar, while some studies have shown reduction of glycosylated hemoglobin and others have not reported a considerable effect (12).

Due to the limitations of the studies conducted on the role of vitamin D on salivary and serum levels of oxidative stress markers, this study was conducted with the aim of investigating the role of vitamin D injection on the serum and salivary levels of oxidative stress markers and comparing the increase or decrease in these two levels on rats.

Methods

After being approved by the ethics committee of Babol University of Medical Sciences with the code IR.MUBABOL.HRI.REC.1400.091 and following the ethical guidelines, this experimental study was conducted on 28 male Wistar rats aged 6 to 7 weeks and weighing 220 ± 20 grams (13). After random selection of samples, the rats were divided into four groups of seven, including a group of healthy rats without vitamin D treatment (control group C), a group of healthy rats treated with 1000 U/rat/w vitamin D

(Dana, IRAN) (CV group), a group of diabetic rats without vitamin D treatment (DC group) and a group of diabetic rats treated with 1000 U/rat/w vitamin D (Dana, Iran) (DV group). They were kept in an animal house at a temperature of $22\pm 2^{\circ}\text{C}$, 55% humidity and 12 h light/dark cycle with free access to food and water (13).

After fasting for 16 hours, two groups of rats were randomly injected with one dose of streptozocin (Sigma-Aldrich Co. St Louis, MO, USA) (STZ 65 mg/kg) intraperitoneally (IP). The other two groups were injected with one milliliter of placebo (0.9% normal saline) as a single dose and intraperitoneally. After 72 hours, the rats were examined in terms of becoming diabetic, and rats with blood sugar (BS) above 250 mg/dL were considered diabetic (14). During the study period (10 weeks), vitamin D was given as oral drops with a concentration of 1000 IU every week (500 IU per rat in two sessions) to each rat in CV and DV groups. Water was given to C and DC groups. On the final day, the weight and BS of fasting rats (without water and food during the night) were measured.

To prepare saliva and serum, 5 mg/kg pilocarpine was injected subcutaneously into the rats in each group and saliva was collected by micropipette. On the same day, blood samples (5 cc) were collected in glass tubes containing heparin through the eyes of rats. Biochemical measurement of oxidative parameters in saliva and serum samples, as well as total antioxidant capacity (TAC), activity of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) measurements were done using a commercially available kit (Teb Pajohan Razi Company, Iran) according to the manufacturer's instructions (15, 16).

In order to analyze the results obtained from the histological examinations, SPSS version 22, One Way ANOVA and Tukey's multiple comparison test were used, and $p\leq 0.05$ was considered significant.

Results

The results of measuring the weight of rats showed a significant difference between all groups except CV and DV groups (Figure 1). Group C had the highest weight gain and DV had the lowest weight gain. Comparing the BS of rats before and after the intervention with vitamin D, BS was significantly reduced only in the diabetic group receiving vitamin D supplements ($p<0.05$) (Table 1).

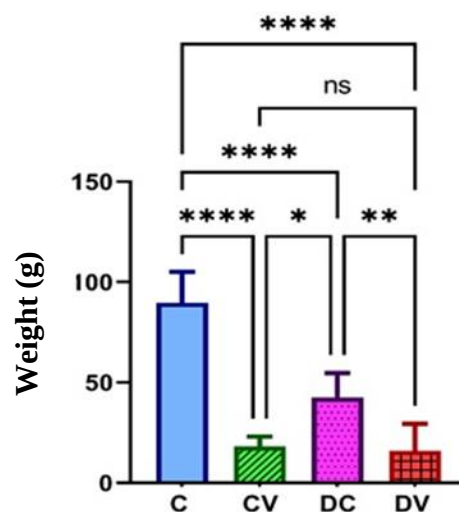


Figure 1. Intergroup comparison of weight gain in C (control), CV (control+vitamin D), DC (diabetic) and DV (diabetic+vitamin D). * $p<0.05$, ** $p<0.01$, **** $p<0.001$, ns: not significant

Table 1. Comparison of weight gain of rat after vitamin D intervention and comparison of BS before and after vitamin D intervention

Group	Number	BS of rats (Mean±SD)		p-value*	Weight of rats	
		Before	After		Mean±SD	p-value*
C	7	107.58±7	108.71±8.4	0.8	84.20±58	-
CV	7	114.57±7.8	113.57±5	0.6	71.85±15.7	<0.001
DC	6	271.33±13.4	211.42±10	0.3	42.12±50.2	-
DV	7	311.42±84.7	211.14±18.9	0.01*	71.67±13.13	-

*Paired t-test

In the comparison between groups, no significant difference was observed in saliva and serum levels of MDA enzyme (Table 2). The measurement of SOD enzyme also showed that there was a significant difference in the salivary SOD level between DV and DC groups ($p<0.05$), but no significant difference was observed in the serum level of this factor in the groups (Figure 2).

There was a significant difference in saliva and serum levels of catalase enzyme between the groups. The level of salivary catalase in the control group was significantly higher than the diabetic group without vitamin treatment ($p<0.001$), while the serum level in the CV group was significantly higher than the DC group ($p<0.05$) (Figure 3). Moreover, vitamin D supplements had no effect on salivary TAC, and salivary TAC levels were lower in diabetic groups (DC and DV groups) compared to non-diabetic rats (C and CV groups). However, the serum level of TAC in both CV and DV groups was significantly higher than DC group (Figure 4).

Table 2. Intergroup comparison of MDA, SOD, CAT and TAC in four groups

Factor and group	Saliva		Serum	
	Mean±SD	p-value	Mean±SD	p-value
MDA (nmol/ml)				
C	0.25±0.0	0.393	0.94±0.04	0.061
CV	0.25±0.0		1.06±0.02	
DC	0.25±0.0		1.31±0.09	
DV	0.26±0.02		1.23±0.39	
SOD (U/ml)				
C	4.51±1.39	0.041	83.14±71.87	0.986
CV	4.06±1.57		85.21±71.59	
DC	3.63±1.84		82.17±52.93	
DV	6.44±1.02		83.80±23.77	
CAT (U/ml)				
C	71.89±18.53	0.013	289.48±48.01	0.027
CV	83.21±37.06		326.87±27.56	
DC	29.47±11.51		222.29±42.49	
DV	58.30±12.59		298.26±55.41	
TAC (μmol/L)				
C	9577±351.75	0.002	20073.34±7642.34	0.011
CV	9055±571.34		24584.44±6381.33	
DC	8012±694.21		178117.78±4209.5	
DV	8757±460.08		25862.22±4382.06	

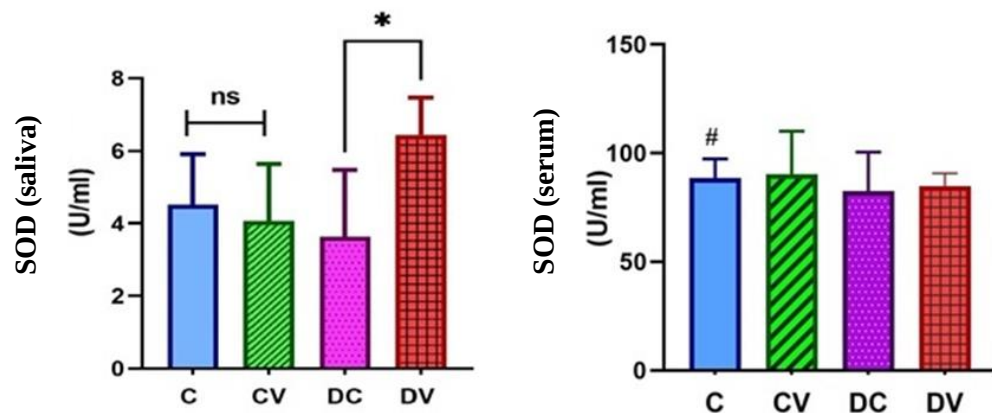


Figure 2. Intergroup comparison of saliva and serum SOD levels in four groups C (control), CV (control+vitamin D), DC (diabetic) and DV (diabetic+vitamin D). * $p < 0.05$, ns: non-significant, #: there was no significant difference between the groups and the control.

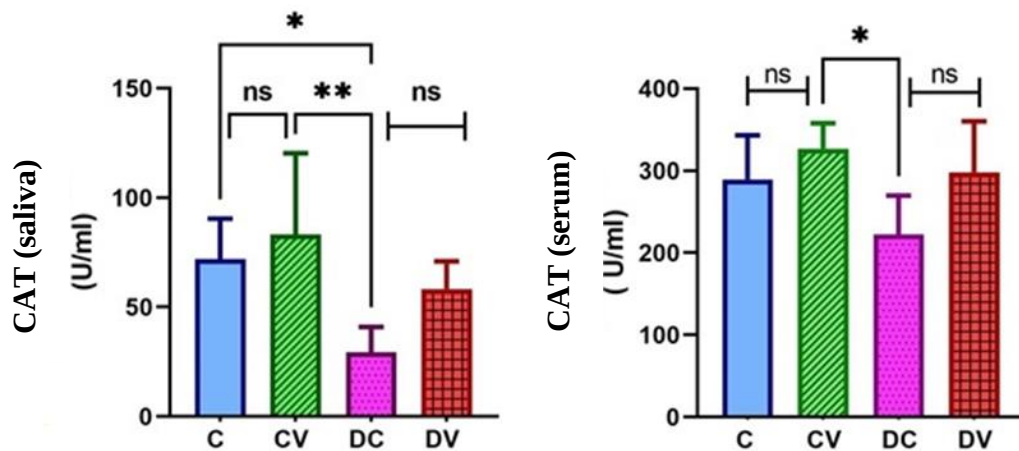


Figure 3. Intergroup comparison of CAT levels in saliva and serum in the groups C (control), CV (control+vitamin D), DC (diabetic) and DV (diabetic+vitamin D). * $p < 0.05$, ** $p < 0.01$, ns: not significant

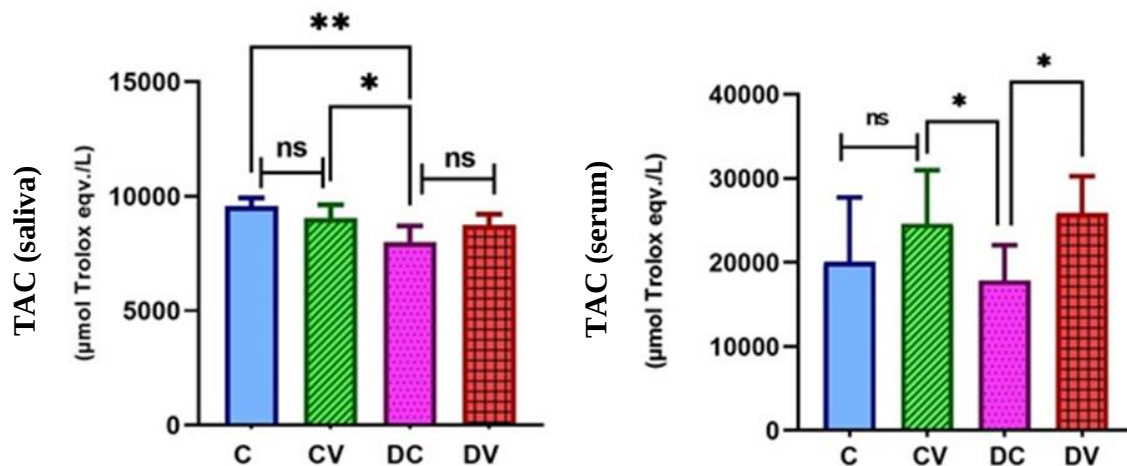


Figure 4. Intergroup comparison of saliva and serum TAC levels in four groups C (control), CV (control+vitamin D), DC (diabetic) and DV (diabetic+vitamin D). * $p < 0.05$, ** $p < 0.01$, ns: not significant

Discussion

In the present study, while diabetes decreased the serum level of TAC (DC group), vitamin D supplements improved the TAC status in the serum of diabetic rats (DV group). In a similar study in diabetic rats, a significant decrease in serum TAC levels was observed compared to the control group, and treatment with ginger, cinnamon or their combination could significantly increase TAC in diabetic rats (17). In a study by Adelani et al. on healthy rats, lack of vitamin D in the diet increased the activity of antioxidant enzymes, while the group treated with vitamin D showed the effects of reducing oxidative stress due to the reduction of lipid peroxidation in the liver of rats (18). Reports show that increased ROS production or decreased ROS inhibition capacity contribute to the pathogenesis of diabetes complications (19, 20).

In this study, vitamin D supplements had no effect on salivary TAC, and salivary TAC levels were lower in diabetic groups (DC and DV groups) compared to non-diabetic rats (C and CV groups). Data on the total antioxidant capacity of saliva are conflicting. In one study, total antioxidant capacity in saliva samples was lower in women compared to men (21). However, some human studies reported no changes in serum and salivary TAC levels in diabetic subjects (22). These differences can be attributed to the lack of control of confounding factors affecting oxidative stress in human studies compared to animal studies and different TAC test methods (23).

In this study, similar to the results of a study by Adelani et al., no significant difference was observed in the serum level of SOD factor in the groups (18). However, these results were in contrast with the report of serum SOD levels in the study by Wei et al. (24). This discrepancy in SOD levels has also been observed in other studies focusing on diabetic patients. For example, Motawi et al. conducted a study on seventy patients with type 2 diabetes. Their findings showed that there was no significant difference in SOD activity between diabetic patients and healthy individuals (25). Other studies have reported an increase in SOD activity (26), a decrease in activity (25) or even an equal amount of SOD activity in diabetic patients compared to control groups (27). These variations may be due to differences in the early stages of diabetes or among patients who have had diabetes for a long time and are on long-term hypoglycemic therapy (25). Very few studies have investigated the changes of SOD activity in the saliva of diabetic rats (28). However, the findings of the present study showed that although vitamin D in healthy rats, which is not under oxidative stress, did not change salivary SOD, but the oxidative stress caused by diabetes can increase the level of salivary SOD in diabetic rats.

Another important antioxidant-related factor is catalase (CAT), which has one of the highest enzyme activities among all enzymes. In this study, similar to the study of Wenclewska et al. (29), salivary CAT levels were decreased in diabetic rats compared to healthy rats, but vitamin D intake had no effect on the serum and salivary levels of catalase enzyme. However, previous studies that examined this parameter at the tissue level reported positive effects of vitamin D intake (30, 31). MDA enzyme is one of the oxidative stress factors that is produced in response to tissue destruction and metabolic oxidative damage which is a chronic complication of diabetes (31, 32). Several studies have shown that vitamin D reduces MDA levels when examining tissue samples (33, 31). However, in our study, no difference was observed in serum and saliva MDA levels between diabetic and healthy rats, which shows that the concentration and duration of hyperglycemia in the studied rats did not cause extensive tissue destruction or necrosis to the extent that it affects MDA in serum and saliva. In the present study, administration of vitamin D caused less weight gain in diabetic and healthy groups. It is possible that vitamin D gavage caused loss of appetite or perhaps less food absorption in the studied rats (34). It is suggested that in future studies, the design of intervention groups be done with different concentrations of vitamin D, the diabetic FBS limit be higher than the current study and this amount be controlled during the intervention period. In addition, the duration of having diabetes before vitamin D intervention must increase.

According to the findings of the present study, the results of vitamin D consumption can be seen in both serum and saliva. In general, the consumption of vitamin D, along with its lowering effect on blood sugar, can have a positive effect on increasing anti-oxidative stress parameters such as SOD or improving the antioxidant status such as TAC enzyme in serum and saliva.

Conflict of interest: The authors declare that there is no conflict of interest.

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