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ORIGINAL ARTICLE

Pulpal and periodontal diseases increase triglyceride levels in diabetic rats

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Abstract
Objectives The aim of this study was to evaluate triglyceride and cholesterol levels in diabetic rats and their relationship with pulpal and periodontal diseases.
Methods Eighty male rats (*Rattus norvegicus albinus*, Wistar) were divided into the following eight groups comprising ten animals each: normal rats (G1), rats with pulpal diseases (G2), rats with periodontal diseases (G3), rats with both pulpal and periodontal diseases (G4), diabetic rats (G5), diabetic rats with pulpal diseases (G6), diabetic rats with periodontal diseases (G7), and diabetic rats with both periodontal and pulpal diseases (G8). Diabetes was induced by injecting streptozotocin, periapical lesions were induced by exposing pulpal tissue to the oral environment, and periodontal diseases were induced by periodontal ligature. The animals were killed after 30 days, and lipid profile was enzymatically measured using Trinder's method. The total assessed values were statistically analyzed by analysis of variance and Tukey test ($p<0.05$).
Results The triglyceride levels of diabetic rats with periodontal disease and of diabetic rats with both periodontal and pulpal diseases were significantly higher than those of normal rats and nondiabetic group rats, respectively. The differences in the cholesterol levels among the groups were not significant.
Conclusions We found that the association of pulpal and periodontal diseases with diabetes increased triglyceride levels in rats.
Clinical significance Changes in lipid profile may be related to the presence of oral infections and diabetes.

Key words Lipid profile · Oral infections · Diabetes · Triglyceride levels · Cholesterol levels

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Introduction

Previous studies have shown that patients with periodontal diseases have higher total cholesterol and triglyceride levels than individuals with good oral hygiene [1–3]. Endodontic infections are very common oral infections worldwide, with an incidence similar to that of periodontal disease [4]. In addition, the occurrence of diabetes affects the severity of endodontic and periodontal infections [4–6] and may be associated with hyperlipidemia [7].

In recent years, various studies have shown that localized inflammation in the oral cavity is associated with increased levels of systemic disease markers [5, 8, 9]. These studies especially focused on the relationship between periodontal diseases, diabetes [8, 9], and cardiovascular diseases [10–12]. Several studies have shown that individuals with high blood pressure, obesity, and high plasma triglyceride and cholesterol

Table 1 Groups formed according to the experimental procedures

		Oral diseases			
		No oral diseases	Pulpal infections	Periodontal infections	Pulpal and periodontal infections
Systemic condition	Healthy rats	G1	G2	G3	G4
	Diabetic rats	G5	G6	G7	G8

levels have increased risk of developing diabetes [13] and coronary heart diseases [14, 15].

One study reported that patients with periodontitis had dyslipidemia and higher non-fasting serum glucose levels than the control population, suggesting a possible link between periodontitis, systemic inflammation, and a dysmetabolic state in otherwise healthy individuals [12]. Another study reported that a patient with pulpal infection showed sudden glucose intolerance and a rapidly increasing requirement for insulin [16]. However, no study about the interrelationship between pulpal infection and hyperlipidemia is available in the literature.

Studies have indicated that periodontal diseases, which cause an increase in the levels of proinflammatory cytokines in the serum and gingival crevicular fluid, are not only associated with the severity of lipid metabolism deterioration but also with worsening of the hyperlipidemic state of patients [17]. Furthermore, many studies have shown that pulpal infection is associated with increased proinflammatory cytokine levels [18, 19]. However, no studies are available on the interrelationship between the cytokine levels and lipid levels during pulpal infections.

Some studies have shown that the occurrence of periodontal diseases alone causes a change in the serum triglyceride or cholesterol levels [1–3]. In another study, patients who developed periodontal diseases also showed an increase in their cholesterol levels [20]. Furthermore, with the treatment of periodontal diseases, the levels of all the lipid parameters decreased; however, only the decrease in serum levels of total cholesterol was found to be statistically significant [17]. No studies have investigated the lipid profile patterns in animals or humans in relation with pulpal diseases and the association of these patterns with periodontal diseases and diabetes. The aim of this study was to evaluate the triglyceride and cholesterol levels in diabetic rats and their relationship with pulpal and periodontal diseases.

Methods

Eighty male *Rattus norvegicus albinus*, Wistar, each weighing 250–280 g, were used in the study. The animals were housed in temperature-controlled rooms and were given ad libitum access to water and food. The experimental protocol was approved by and conducted in accordance with the guidelines of the institutional ethical committee.

The rats were fasted overnight (14–16 h), and tail-tip blood was used to measure the fasting blood glucose levels with an automatic blood glucose monitoring system (Accu-Check® Performa; Roche Diagnostics Corporation, Indianapolis, IN, USA). Subsequently, the rats were intramuscularly anesthetized with ketamine (87 mg/kg; Francotar; Virbac do Brasil Ind. e Com. Ltda., Roseira, Brazil) and xylazine (13 mg/kg; Rompun; Bayer S. A., São Paulo, Brazil). The rats were randomly assigned into groups and were endovenously injected in the penile vein with either citrate buffer solution

Table 2 Mean and standard deviation (mean ± SD) values of the lipid profile of the rats from the eight groups

	Blood glucose levels (mg/dL)		Lipid profile			
			Cholesterol levels (mg/dL)		Triglyceride levels (mg/dL)	
Groups	Mean	SD	Mean	SD	Mean	SD
G1	99.87 a	12.90	64.80 a	12.91	53.11 a	12.32
G2	102.19 a	17.34	67.40 a	12.50	57.66 a, b	11.16
G3	100.78 a	10.52	69.16 a	11.42	58.77 a, b	21.97
G4	97.65 a	18.67	70.80 a	10.56	60.44 a, b	16.10
G5	304.46 b	43.91	69.85 a	11.90	86.44 a, b, c	26.63
G6	299.13 b	39.77	70.87 a	8.45	90.42 a, b, c	25.82
G7	326.95 b	47.90	74.42 a	9.60	107.50 b, c	28.13
G8	361.64 b	53.23	75.83 a	13.41	129.00 c	26.77

Same letters indicate the absence of statistical difference among the groups ($p>0.05$)

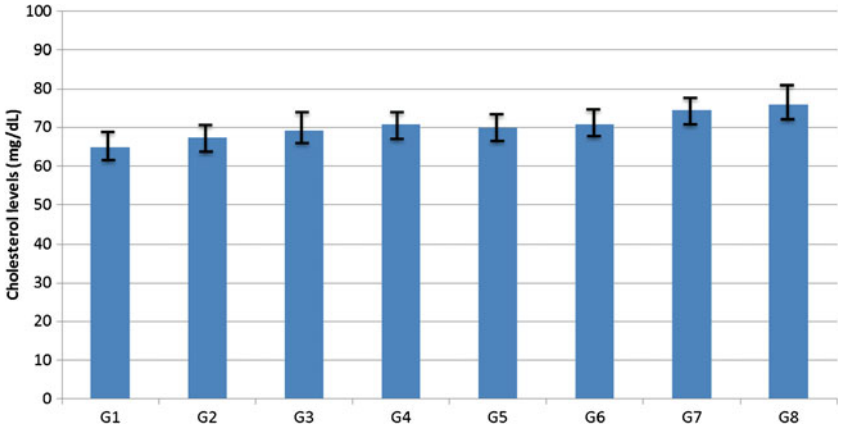

Fig. 1 Mean and standard deviation (mean ± SD) values of blood cholesterol levels (in milligrams per deciliter) of the rats from the eight groups

(0.01 M, pH 4.5; groups 1–4, n=40) or with streptozotocin (Sigma-Aldrich Corp., St. Louis, MO, USA). Streptozotocin was dissolved in citrate buffer solution at 35 mg/kg body weight for experimental induction of diabetes (groups 5–8, n=40) [21].

Six days after diabetes was induced, blood samples were collected from each animal to determine their blood glucose levels. The rats with blood glucose levels of more than 200 mg/dL were used in the study [22]. After confirmation of hyperglycemia, animals were sedated for the induction of oral infection.

For the development of pulpal infection, the pulps of the right upper first molars were exposed on the mesial surface using surgical round burs (Broca Ln Long Neck- Maillefer, Dentsply Ind. e Com. Ltda, Petrópolis, Brazil) (groups 2, 4, 6, and 8). To induce periodontal disease in rats from groups 3, 4, 7, and 8, sterile 4/0 silk ligatures (Ethicon; Johnson & Johnson, São Paulo, SP, Brazil) were placed around the second maxillary molar on the left [23]. After the infections were induced, the rats were divided into the groups as shown in Table 1.

After 30 days, the animals were killed with an overdose of the anesthetic solution. To determine the serum lipid profile, venous blood samples (50 μl) were collected via a cardiac puncture after the rats had fasted overnight for 8–12 h. The blood samples were centrifuged immediately after collection at 1,800×g for 15 min at 4 °C to obtain plasma [24].

Plasma total cholesterol and triglyceride levels were measured enzymatically by using a commercial kit (Cholesterol Liquiform Labtest® and Triglicérides Liquiform Labtest®, respectively—Labtest Diagnóstica Ind. e Com. Ltda, Lagoa Santa, MG, Brazil) as described by Trinder [25].

The total assessed values were tabulated for each experimental group, and a single calibrated operator analyzed the data in a blinded manner. One-way analysis of variance and the Tukey's test were used for statistical analysis, and a significance level of 5 % (p<0.05) was used to compare the mean values.

Results

Diabetic rats had significantly higher blood glucose levels than the control rats, indicating the development of hyperglycemia (Table 2). The changes in the plasma lipid concentration of the nondiabetic and diabetic rats are shown in Table 2. Total cholesterol levels were similar in the rats of all the groups (Fig. 1). No statistically significant differences were observed in the cholesterol levels of any groups (p=0.426). The

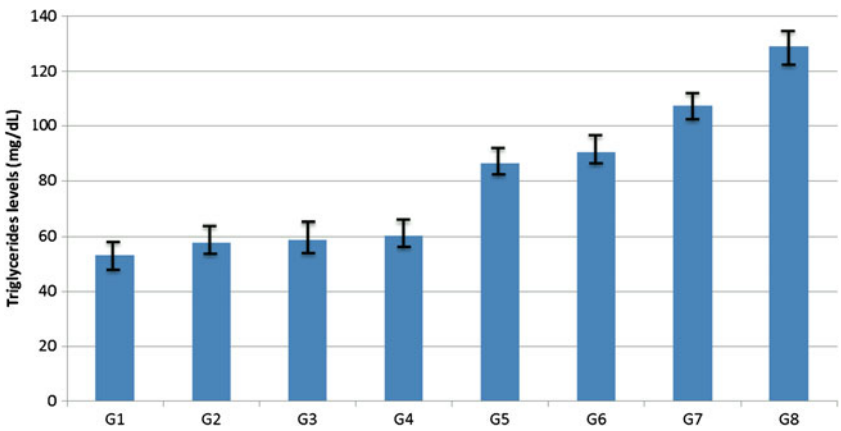

Fig. 2 Mean and standard deviation (mean ± SD) values of blood triglycerides levels (in milligrams per deciliter) of the rats from the eight groups

baseline cholesterol levels were higher in the rats in the diabetic groups than in the rats from the nondiabetic groups, but the differences in the levels were not significant (Table 2).

The total triglyceride levels were similar in the rats of all the nondiabetic groups ($p>0.05$). However, the rats in groups 1–4 had lower triglyceride levels than those in groups 5–8 (Fig. 2). The rats in groups 5–8 had the highest triglyceride levels. The triglyceride levels of diabetic rats with periodontal disease (G7) and of diabetic rats with periodontal and pulpal diseases (G8) were significantly higher than those of normal rats (G1) ($p=0.000$) and nondiabetic group rats (G1–G4) ($p=0.000$), respectively.

Discussion

The animals used in this study had uniform body weights, were normoglycemic, and did not have glycosuria. Diabetes mellitus was induced by injecting the rats with streptozotocin. The glucose levels were found to be approximately fourfold higher than those observed in the normal nondiabetic animals. Diabetic rats showed intense thirst, polyuria, and apathy. The overall metabolism of rats with streptozotocin-induced diabetes is very similar to the metabolism of human diabetic patients [21]. The blood glucose levels were higher in the rats of the diabetic model group than in those of the normal control group, indicating that hyperglycemia persisted in the diabetic rats.

Two models of oral infection were used [22, 23]. A previous study reported maximal active lesion expansion and bone destruction between days 7 and 15 after pulp exposure in a rat model system in which periapical lesions had been induced [21]. In the present study, periapical lesions were induced by pulpal exposure and were confirmed radiographically.

The process of inducing periodontal diseases by ligature placement is widely used in animal studies [23, 26]. Ligature favors the formation of bacterial plaques and induces an inflammatory response, thereby replicating many aspects of periodontal diseases in humans [23].

Oral infections, diabetes, and hypercholesterolemia have been closely associated with cardiovascular diseases [11, 27, 28]. Hypercholesterolemia induces pathologic manifestations such as decreased myocardial capillary density and increased endothelial damage [29]. Patients who develop periodontal disease show increased total cholesterol levels, low-density lipoproteins, and triglycerides levels [20, 30]. The results of some studies have shown that treatment of periodontal disease decreases the levels of all the lipid parameters; however, only the decrease in total cholesterol levels [7, 17] or oxidized low-density lipoprotein [31] was found to be statistically significant in the human model.

The results of some studies have indicated hypercholesterolemia in diabetic rats, which indicates the development of dyslipidemia in these rats [1–3]. In the present study, despite a small increase in the total cholesterol levels in diabetic rats with pulpal and periodontal diseases, the values were not found to be statistically significant.

Our results suggest that diabetes, pulpal infections, and periodontal lesions are correlated with higher levels of triglycerides, but a previous study has shown that high triglyceride levels are not correlated with the severity of attachment loss in patients with periodontitis [10]. Various studies have shown high triglyceride levels in diabetic rats, diabetic patients, and experimental periodontitis models [2, 3]. We suggest that the increased triglyceride levels observed in the diabetic rats in our study may be due to the lack of insulin, which normally activates the enzyme lipoprotein lipase [2, 3].

Although the impact of diabetes on periodontal diseases and periapical healing has been investigated previously, very few studies have reported the influence of pulpal infections alone or in association with periodontal diseases on blood cholesterol and triglyceride levels. The results of our study showed that the association of pulpal with periodontal diseases increases triglyceride levels in diabetic rats. Further studies are required for evaluating the interrelationship between diabetes, pulpal infections, and lipid levels and for confirming whether endodontic infections influence systemic health.

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