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Effect of Oral Infections on Serum Creatinine Levels in Diabetic Rats

Research Article

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Abstract

Aim: The aim of this study was to evaluate the relationship between pulpal and/or periodontal disease and serum creatinine levels in a rat model of diabetes mellitus.

Methods: Eighty male rats (Rattus norvegicus albinus, Wistar) were divided into the following 8 groups compris-ing 10 animals each: normal (G1), with pulpal disease (G2), with periodontal disease (G3), with both pulpal and periodontal disease (G4), diabetic (G5), diabetic with pulpal disease (G6), diabetic with periodontal disease (G7), and diabetic with both pulpal and periodontal disease (G8). Diabetes was induced by injecting streptozotocin, pul-pal disease were induced by exposing pulpal tissue to the oral environment, and periodontal disease was induced by periodontal ligature. After 30 days, blood was collected by cardiac puncture and the animals were killed. The maxillae were processed for histopathology. Serum creatinine levels were measured by the enzymatic method. The total assessed values were statistically analyzed by analysis of variance and Tukey's test ($p < 0.05$).

Results: Serum creatinine levels were significantly higher in diabetic rats than that in all normoglycemic rats ($p < 0.05$). The presence of pulpal and periodontal disease increased the serum creatinine levels in normoglycemic and diabetic rats, but there was no statistical difference between the groups ($p > 0.05$).

Conclusions: We found that the serum creatinine level was higher in diabetic rats and may be related to the pres-ence of oral infections.

Clinical significance: Changes in serum creatinine level may be related to the presence of oral infections and diabetes.

Keywords: Diabetes; Apical Periodontitis; Periodontal Disease; Creatinine Level

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Introduction

Oral infections such as pulpal and periodontal disease are frequently associated with diabetes mellitus [[1-3]. Periodontal disease is one of the most prevalent complications in diabetic patients [3-5]. It is also evi-dent in the increased severity of chronic periodontitis, periodontal abscess, and predisposition to infections in these individuals [6,7]. The reverse is also true, as periodontal infection is capable of exacerbating a dia-betic condition, which is another important aspect to be considered [8].

The current literature is replete with studies linking diabetes mellitus to periodontal disease alone [7,9-11]. However, information on pulpal disease, which is char-acterized by apical periodontitis due to infectious ne-crosis of the pulp, is still lacking [1,12,13].

The effect of diabetes mellitus in apical periodontitis has been investigated in animal models [14,15]. It was observed that diabetes accelerates the development and progression of periapical periodontitis in rats, as well as the relationship of periodontal disease [16]. However, information on the association between pul-pal and periodontal disease with diabetes is limited to a few studies [1,17], and the effects on systemic condi-tions have not been studied in detail [1].

One of the systemic complications for diabetic pa-tients is renal dysfunction [18], which can be diagnosed by measuring urea and creatinine levels in the blood [19]. Diabetic nephropathy, a major long-term compli-cation of diabetes mellitus, is the most common cause of end-stage renal disease worldwide requiring dialysis, and it is becoming a challenge to public healthcare sys-tems [20,21].

Creatinine is the preferred marker for renal dysfunc-tion and is readily available in clinical laboratory set-tings [22]. Being an indicator of decreased kidney function, serum creatinine levels are largely unaffected by a typical diet, and are thus used as markers of renal function [23,24]. Creatinine is a byproduct of muscle metabolism and typically remains in a steady state bal-anced out by renal elimination [22]. Despite their limitations, creatinine levels are currently used in clinical practice to estimate the glomerular filtration rate be-cause it is inexpensive, harmless, and easy to perform in patients [25].

High creatinine levels can relate to other infections such as acute infectious meningitis [25]. It has been suggested that creatinine levels increase due to glomer-ular hyperperfusion [26]. Experimental studies have shown that high creatinine levels are observed during the initial hyperkinetic phase of sepsis [27,28].

Lower serum creatinine represents a greater risk for developing diabetes [29]. The relationship between chronic kidney disease, diabetes, and oral health sta-tus has been reported [30]. Nephropathy is a chronic complication of diabetes mellitus and may be related to periodontal disease [31,32]. It is known that peri-odontal disease causes changes in serum creatinine lev-els [33]; however, to our knowledge, the association of this condition with diabetes or pulpal disease has not been explored thus far.

Thus, it seemed pertinent to evaluate the relationship between pulpal and/or periodontal disease and serum creatinine levels in a rat model of diabetes mellitus.

Methods

Eighty male Rattus norvegicus albinus Wistar rats, each weighing 250–280 g, were used in the study. The animals were housed in temperature-controlled rooms and given ad libitum access to water and food. The institutional ethical committee approved the experi-mental protocol, which was conducted in accordance with the institutional ethical committee guidelines. The animals were divided into 8 groups of 10 animals each, as shown in Table 1.

Induction of experimental diabetes

The animals 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 so-lution (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 65 mg/kg body weight for experimental in-duction of diabetes (groups 5–8, n = 40) [34].

Six days after diabetes mellitus was induced, blood samples were collected from each animal to determine their blood glucose levels. Rats with blood glucose lev-els exceeding 250 mg/dL were used in the study [35].

Induction of oral infections

For the development of apical periodontitis, 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)[36]. To in-duce periodontal disease in rats from groups 3, 4, 7, and 8, sterile 4/0 silk ligatures (Ethicon; Johnson & Johnson, São Paulo, Brazil) were placed around the left second maxillary molar [1].

Assessment of serum creatinine levels

To determine serum creatinine levels, venous blood samples (1 mL) were collected via cardiac puncture af-ter 30 days, and the animals were killed with an over-dose of the anesthetic solution.

The samples were placed in EDTA and immediately transferred to a technician who was blinded to case status. Serum creatinine was measured by the Jaffé method [19,24,25,29]. The Jaffé reaction is based on the yellow-orange color produced by creatinine reacting with alkaline picrate, which is measured at 492 nm. The amount of color formed is proportional to the concentration of creatinine in the sample. The sensi-tivity of the Jaffé reaction is 0.5 mg/dL creatinine in samples, and the entire detection time spans approxi-mately 30 min (Creatinina K Labtest®, Labtest Diag-nóstica S.A., Brazil).

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

Histopathological analysis

Maxillae were removed and immediately placed in 10% buffered formalin. Specimens were rinsed in sterile wa-ter and decalcified in 10% EDTA. They were rinsed again in sterile water, dehydrated in ethanol, cleared in xylene, and embedded in paraffin [1]. Six micron-thick serial sections of the molars and surrounding tissues were prepared and stained with hematoxylin and eosin

(HE). We made serial 6-μm thick paraffin sections of the mesial–distal aspects of all upper molars, and these were stained with HE. Morphological studies of the periapical and periodontal area were conducted.

Results

Thirty days after pulp exposure, pulps exhibited total necrosis and periapical lesions were established. Mod-erate to severe inflammatory reaction composed pri-marily of neutrophils (polymorphonuclear cells) and mononuclear cells was observed (Fig. 1a, b). Periodontal disease was observed based on the pres-ence of inflammatory cells and by measuring the distance from the cemento-enamel junction to the alveolar bone crest at the palatal surface

Table 1: Groups according to the experimental procedures

		Oral infections			
		None	Pulpal	Periodontal	Pulpal and periodontal
Systemic	Healthy	G1	G2	G3	G4
	Diabetic	G5	G6	G7	G8

of the mid-mesiopalatal root region of the second molar under ×100 magnification. We observed evidence of aggres-sive periodontitis with substantial periodontal bone loss (Fig. 1c, d).

Blood glucose levels were significantly increased in dia-betic rats as compared to that in control rats, indicating development of hyperglycemia (Table 2). The changes in the serum creatinine levels of non-diabetic and dia-betic rats are shown in Table 2.

Total serum creatinine levels were similar in the non-diabetic ($p > 0.05$) and in diabetic groups ($p > 0.05$). Conversely, diabetic rats had significantly higher serum creatinine levels than the control rats ($p = 0.000$) (Table 2).

Diabetic rats with pulpal and periodontal disease (G8) had high levels of serum creatinine, which was sta-tistically significant compared to all non-diabetic rat groups (G1–G4). Normoglycemic rats with pulpal and periodontal disease (G4) had serum creatinine levels similar to those of diabetic rats without oral infection (G5) (Table 2).

Discussion

There are a number of different rodent models for diabetes, particularly type 1 diabetes. Two models are commonly used: streptozotocin-induced diabetes in rats and non-obese diabetic (NOD) mice [37]. The ad-vantages of the streptozotocin model are that diabetes can be induced in a controlled manner in all animals, and animals in the diabetes and non-diabetes groups are of the same genetic background [38]. In this study,blood glucose levels were higher in rats in the diabet-ic model group than in those in the normal control group, indicating that hyperglycemia persisted in the diabetic rats.

Two models of oral infection were used [1]. Our re-sults are in agreement with that of other studies [14,39], which demonstrated histologically that induced periradicular lesions in diabetic rats were larger than that in non-diabetic rats. The induction of experimen-tal periodontitis stimulated inflammatory infiltrate formation in both diabetic and normoglycemic rats. In summary, diabetic rats had a higher degree of in-flammation and greater periodontal bone loss through enhanced resorption. These results also agree with that of previous studies [40,41].

We measured creatinine levels in the rat serum with the Jaffé method, similar to other studies [19,24,25,29]. The traditional method for detecting creatinine is based on a modified Jaffé reaction, which is widely used in both laboratory and clinical detection. The en-tire detection time requires approximately 30 min. The reaction is carried out for a fixed period in order to minimize the interference of other substances reacting with the picric acid [42].

Creatinine is transported by the bloodstream to the kidney. There is little to no tubular reabsorption of creatinine by the kidney tubules. Therefore, creatinine levels in the blood may be used to calculate creatinine clearance, which reflects the glomerular filtration rate. Any condition that impairs the function of the kid-ney will likely increase the creatinine level in the blood. Changes in creatinine levels have been linked with dia-betes [29,43,44], periodontal disease [33], acute infec-tious meningitis [25], hyperkinetic phase of sepsis [23,24], kidney disease, kidney transplant, and oral health status [30,45,46].

In the present study, serum creatinine levels were sig-nificantly elevated in the diabetic group (0.73 ± 0.10 mg/dL) compared with that in the control group (0.56 ± 0.09 mg/dL). These results are also in agreement with that of previous studies [43,47]. These results confirm that streptozotocin-induced diabetes is a fac-tor for changes in creatinine levels. Conversely, lower serum creatinine levels increased the risk of type 2 dia-betes [29].

Some studies have shown that periodontal conditions may affect renal function [33,48-50]. However, severe periodontitis was not correlated with renal dysfunction in a studied population [51]. This difference between these studies may be related to methodology.

There is no related study regarding the relationship to pulpal in-

Figure 1. Histological overview (a, b) Intense acute inflammatory response (black arrows), tissue disorganization, and substantial bone resorption is observed adjacent to the tooth apex region (HE, original magnification ×25, inset magnification ×100). (c, d) Histological evidence of intense inflammatory cell concentra-tion, substantial bone loss, and root resorption (*) (HE, original magnification ×25, inset magnifica-tion ×100).

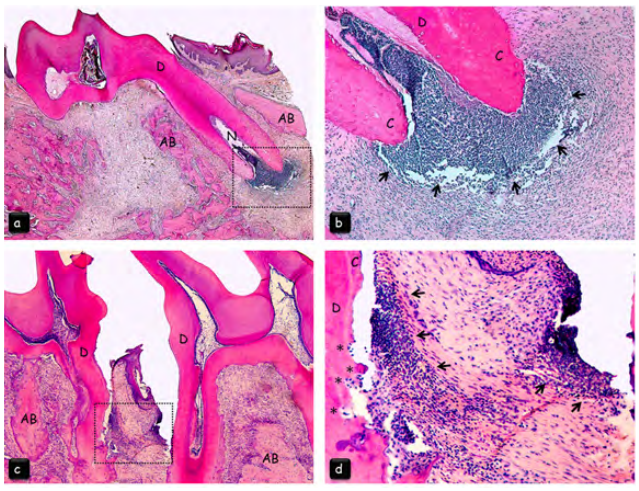

Table 2. Mean and standard deviation (mean ± SD) values of blood glucose and serum creatinine levels of each group

Group	Blood glucose (mg/dL)		Serum creatinine (mg/dL)		
	Mean*	SD	Mean*	SD	n
G1	101.34 a	11.86	0.56 a	0.09	9
G2	102.49 a	14.39	0.59 a	0.04	9
G3	100.89 a	12.33	0.59 a	0.05	9
G4	103,24 a	16.52	0.61 a, b	0.06	9
G5	301.63 b	36.82	0.73 b, c	0.1	8
G6	297.54 b	32.47	0.74 b, c	0.14	8
G7	312.60 b	37.99	0.73 b, c	0.08	9
G8	343.34 b	42.11	0.79 c	0.08	9

fection. In the present study, a higher mean serum creatinine level was associated with pulpal and periodontal disease in normal and diabetic rats (Table 2). In addition, a recent report demonstrated that pul-pal and periodontal disease increase triglyceride levels in diabetic rats, confirming the hypothesis that endo-dontic infections influence systemic health when as-sociated with periodontal disease [1].

We found that the presence of pulpal and periodon-tal disease increased serum creatinine levels in nor-moglycemic and diabetic rats; however, no statistical differences were found ($p > 0.05$). In conclusion, the present results suggest that serum creatinine levels are higher in diabetic rats and may be related to the pres-ence of oral infections.

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