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MESTRADO EM ODONTOLOGIA
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BALANÇO IMUNORREGULATÓRIO DE CÉLULAS TH1 NO SANGUE, FÍGADO E RIM DE RATOS NORMOGLICÊMICOS E DIABÉTICOS COM PERIODONTITE APICAL

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Projeto de pesquisa apresentado ao Programa de Pós-Graduação em Odontologia - Mestrado Acadêmico da Universidade de Uberaba, como requisito para obtenção do título de Mestre em Odontologia, na Área de Concentração Clínica Odontológica Integrada.

Orientadora: Prof. Dra. Renata Oliveira Samuel

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DEDICATÓRIA

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RESUMO

O objetivo deste estudo foi avaliar a influência da diabetes e/ou periodontite apical (PA) nos níveis de TNF-α e IL-6 nos tecidos hematológico, hepático e renal de ratos Wistar. Quarenta ratos machos foram divididos em quatro grupos: ratos normoglicêmicos (N), ratos normoglicêmicos com PA (N-AP), ratos com diabetes experimental (DE), ratos com diabetes experimental e periodontite apical (ED-AP). A diabetes foi induzida por injeção de estreptozotocina. O controle glicêmico foi realizado para confirmar o desenvolvimento da doença no sexto e último dia do período experimental. A infecção endodôntica foi induzida pela exposição do primeiro molar superior direito ao meio bucal. Após 30 dias, fragmentos de rim e fígado foram obtidos, bem como sangue venoso para quantificar os níveis de TNF-α e IL-6 pela técnica de captura de ELISA. Os valores obtidos foram tabulados e analisados estatisticamente por meio dos testes de análise de variância (ANOVA) e teste de Tukey ($p<0,05$). Os resultados mostraram níveis séricos de TNF-α aumentados no grupo N-AP quando comparado ao grupo N ($p<0,05$). Além disso, AP potencializou os níveis de IL-6 no fígado de ratos no grupo ED-AP quando comparado ao grupo N-AP ($p<0,05$). Entre os ratos diabéticos, o AP não alterou os níveis de TNF-α e IL-6 no plasma, nos tecidos renal e hepático ($p>0,05$). Além disso, o ED aumentou os níveis de TNF-α e IL-6 no plasma e no tecido renal quando comparado com ratos normoglicêmicos ($p<0,05$). Pode-se concluir que a AP pode promover alterações sistêmicas inflamatórias como o aumento dos níveis séricos de TNF-α e potencializar a produção de IL-6 em tecidos hepáticos de ratos diabéticos. Além disso, a diabetes pode aumentar os níveis de IL-6 e TNF-α no plasma e tecido renal.

Palavras-chave: Periodontite Periapical, Diabetes, IL-6, TNF- α

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ABSTRACT

The aim of this study was to evaluate the influence of diabetes and/or apical periodontitis (AP) in TNF-α and IL-6 levels in the hematologic, hepatic and renal tissues of Wistar rats. Forty male rats were divided into four groups: normoglycemic rats (N), normoglycemic rats with AP (N-AP), rats with experimental diabetes (ED), rats with experimental diabetes and apical periodontitis (ED-AP). Diabetes was induced by injection of streptozotocin. Glycemic control was performed to confirm the development of the disease at the sixth and last day of the experimental period. The endodontic infection was induced by exposure of the upper right first molar to the oral environment. After 30 days, fragments of kidney and liver were obtained, as well as venous blood to quantify TNF-α and IL-6 levels by ELISA capture technique. The values obtained were tabulated and analyzed statistically by means of two way analysis of variance tests (ANOVA) and Tukey test ($p<0.05$). The results showed increased TNF-α serum levels in the group N-AP when compared to the group N ($p<0.05$). Moreover, AP potencialized IL-6 levels in rat's liver in the group ED-AP when compared to the group N-AP ($p<0.05$). In diabetic rats, AP did not alter the levels of TNF- α and IL-6 in the plasm, renal and hepatic tissues ($p>0.05$). Furthermore, ED increased the TNF-α and IL-6 levels in plasm and renal tissue when compared with N rats ($p<0.05$). It may be concluded that AP may promote inflammatory systemic alterations as the increase of TNF-α serum levels and the pontencialize the IL-6 production in hepatic tissues of diabetic rats. In addition, the presence of diabetes may increase the levels of IL-6 and TNF-α in the plasm and renal tissue.

Key words: apical periodontitis, diabetes, IL-6, TNF-α

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INTRODUÇÃO

A inter-relação entre o impacto da infecção oral e a saúde sistêmica é um tema que já foi amplamente estudado na história da medicina (Hunter, 1900, Billings, 1914, Rosenow, 1928). A partir da comprovação da presença de bactérias na cavidade bucal por Miller, em 1890, vários pesquisadores começaram a relacionar infecções dentárias com prejuízos sistêmicos (Hunter, 1900, Billings, 1914, Rosenow, 1928). O tema era conhecido como 'teoria da infecção focal' (Mills, 1913).

Em meados da década de 40, era comum pesquisas e relatos de casos clínicos de cura de diversas doenças após extrações dentárias (Hunter, 1900, Billings, 1914, Price, 1925, Rosenow, 1928). O conceito de que a boca era um foco de doenças como esquizofrenia, artrite, alopecia, dentre tantas outras se popularizou. Era comum, nesta época, pessoas se submeterem a extrações múltiplas com o objetivo de prevenir ou tratar doenças (Hunter, 1900, Billings, 1914, Price, 1925, Rosenow, 1928).

A partir da década de 50, a teoria da infecção focal começou a ser desacreditada, uma vez que foi observado que pacientes que extraiam os dentes muitas vezes não tinham a cura de doenças (Easlick, 1951). Além disso, tanto pacientes com dentição saudável como pacientes com problemas dentários desenvolviam processos patológicos. Outro problema comum na época era o desenvolvimento de outros distúrbios gástricos decorrentes da ausência dos dentes, como a dispepsia (Vaizey, Clark-Kennedy, 1939).

O tema gerou tantos problemas na comunidade médico-odontológica, que ficou esquecido por muitos anos. No entanto, na década de 90, outras pesquisas começaram a relacionar novamente a possível relação entre infecções periodontais e a saúde sistêmica (Genco, Löe, 1993; Clarke, Hirsch, 1995). Só que desta vez as pesquisas eram mais minuciosas e a proposta não era tão radical como antes. As pesquisas começaram a evidenciar que pacientes com doença periodontal descontrolada tinham mais propensão a ter piora de quadro de algumas doenças autoimunes, tais como diabetes, lúpus, psoríase, doenças cardiovasculares, entre outras (Genco, Löe, 1993; Clarke, Hirsch, 1995). No entanto, desta vez, concebeu-se que a possível relação entre as infecções orais e a saúde sistêmica não se

dá somente pela liberação de bactérias via corrente sanguínea. Na verdade, foi observado que esta relação é bidirecional, ou seja, a doença periodontal pode interferir na patogênese de doenças autoimunes assim como doenças autoimunes podem interferir na patogênese da doença periodontal. E foi consolidado que esta relação ocorre, na maioria das vezes, não pela simples presença de bactérias, mas sim pela ativação de células e mediadores inflamatórios comuns tanto nas infecções locais como em doenças sistêmicas (Prabhu *et al.* 1996). As vias biológicas para potencializar estas doenças são as mesmas. Assim, em um paciente com diabetes descompensada, por exemplo, haverá grande liberação de mediadores inflamatórios no sangue e, consequentemente, onde existir outro foco de infecção, como a doença periodontal, a resposta do organismo será mais exacerbada (Prabhu *et al.* 1996).

Neste contexto, milhares de estudos já deixaram bem clara a relação entre a infecção periodontal e a saúde sistêmica. No entanto, a abordagem agora diverge da abordagem do passado. Antes, para se evitar os possíveis problemas sistêmicos de alterações bucais, era indicada a extração dentária (Hunter, 1900, Billings, 1914, Price, 1925, Rosenow, 1928). Hoje, está comprovado que o tratamento e acompanhamento do paciente já traz benefícios no controle das doenças potencializadas pela periodontite (Sanz *et al.* 2018). Isso contribui para uma visão mais holística do paciente na Odontologia: a boca faz parte da manutenção de saúde do indivíduo.

No entanto, estudos relacionando a infecção endodôntica com a saúde sistêmica só surgiram recentemente. Antes, era mais comum estudos relacionando infecções endodônticas com casos pontuais de endocardites e abscessos esporádicos. Ainda há lacunas para preencher quanto aos mediadores inflamatórios liberados e possível semelhança com o impacto que a doença periodontal traz para saúde sistêmica. Sabe-se que tanto a doença periodontal como a infecção endodôntica possuem semelhanças, tais como a presença de bactérias gram positivas e negativas, a liberação de células e mediadores inflamatórios em comum que geram, como consequência, a reabsorção óssea (Gazivoda *et al.* 2009, Ogle, 2017).

É muito comum pacientes com infecções endodônticas permanecerem com periodontites apicais por muitos anos sem tratamento devido a ausência de dor. No entanto, manter este processo infeccioso pode ser prejudicial. Na patogênese da lesão apical são

liberadas células inflamatórias, tais como linfócitos, que possuem diferentes linhagens, que tem como consequência, a ativação de citocinas pró e antiinfamatórias. Os linfócitos T helper 1 (Th1) atuam na liberação de citocinas pró-inflamatórias. Quanto maior a quantidade de linfócitos Th1, maior será a liberação de interleucinas (IL), fator de necrose tumoral alfa (TNF-α), dentre outras citocinas que, de forma geral, tem a função de "ativar a inflamação" tanto na patogênese local da infecção, como em regiões distantes do local de origem. A ativação da inflamação localmente na periodontite apical, pode levar, por exemplo, a maior atividade osteoclástica, aumentando a reabsorção óssea da região (Marçal *et al*. 2010; Azuma *et al.* 2014). Por outro lado, estes mediadores podem atuar também de forma sistêmica, ativando processos inflamatórios em locais que já exista uma pré-disposição para tal (Azuma *et al.* 2017).

A diabetes é uma das doenças que pode ser afetada em um organismo em que haja maior liberação destes mediadores pró-inflamatórios sistemicamente (Costa et al. 2023). Por se tratar de uma doença auto-imune, citocinas como a IL-6 e TNF- α podem aumentar ainda mais a resistência insulínica, piorando o controle hiperglicêmico e potencializando a doença (Costa *et al*. 2023). Estudos em animais, demonstraram que em ratos com PA, há maior concentração de hemoglobina glicosilada que em animais diabéticos sem PA (Cintra *et al.* 2014). Além disso, estudos relacionando a doença periodontal com a diabetes, constataram que após o tratamento da doença periodontal, há melhora significativa da condição hiperglicêmica. Inclusive, há atualmente estratégias de promoção de saúde que visam uma atenção integrada a pacientes diabéticos visando a manutenção da saúde periodontal para que haja maior controle de doenças autoimunes (Herrera *et al.* 2023).

Diferente da doença periodontal, no entanto, ainda faltam estudos que ilustre a magnitude que a infecção endodôntica pode afetar a saúde sistêmica. Uma das formas de fazer esta avaliação é realizando estudos em animais. A vantagem destes estudos é que, diferente de um estudo em humanos, é possível isolar e deixar como única variável entre os espécimes a presença ou ausência de periodontite apical. Diante do exposto, o objetivo deste estudo é quantificar IL-6 e TNF-α no sangue, fígado e rins de ratos portadores de PA.

PROPOSIÇÃO

O objetivo deste estudo foi avaliar o impacto da PA na saúde sistêmica de ratos Wistar por meio da quantificação das citocinas pró-inflamatórias TNF-α e IL-6 no sangue, fígado e rim.

Capítulo 1 – **Artigo que será submetido a Archives of Oral Biology**

Apical periodontitis and diabetes increase inflammatory mediators in plasm, hepatic and renal tissues

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ABSTRACT

The aim of this study was to evaluate the influence of diabetes and/or apical periodontitis (AP) in TNF-α and IL-6 levels in the hematologic, hepatic and renal tissues of Wistar rats. Forty male rats were divided into four groups: normoglycemic rats (N), normoglycemic rats with AP (N-AP), rats with experimental diabetes (ED), rats with experimental diabetes and apical periodontitis (ED-AP). Diabetes was induced by injection of streptozotocin. Glycemic control was performed to confirm the development of the disease at the sixth and last day of the experimental period. The endodontic infection was induced by exposure of the upper right first molar to the oral environment. After 30 days, fragments of kidney and liver were obtained, as well as venous blood to quantify TNF-α and IL-6 levels by ELISA capture technique. The values obtained were tabulated and analyzed statistically by means of two way analysis of variance tests (ANOVA) and Tukey test ($p<0.05$). The results showed increased TNF-α serum levels in the group N-AP when compared to the group N ($p<0.05$). Moreover, AP potencialized IL-6 levels in rat's liver in the group ED-AP when compared to the group N-AP ($p<0.05$). In diabetic rats, AP did not alter the levels of TNF- α and IL-6 in the plasm, renal and hepatic tissues ($p>0.05$). Furthermore, ED increased the TNF-α and IL-6 levels in plasm and renal tissue when compared with N rats ($p<0.05$). It may be concluded that AP may promote inflammatory systemic alterations as the increase of TNF-α serum levels and the pontencialize the IL-6 production in hepatic tissues of diabetic rats. In addition, the presence of diabetes may increase the levels of IL-6 and TNF-α in the plasm and renal tissue. Clinical significance AP is capable of promote systemic inflammatory alterations alone or when is associated with diabetes.

Key words: apical periodontitis, diabetes, IL-6, TNF-α

INTRODUCTION

Apical periodontitis (AP) is an infectious lesion characterized by inflammatory bone destruction that is mediated by various cytokines secreted from immunocompetent cells that have infiltrated the periapical tissues in response to intracanal bacterial infection (Kawashima *et al.* 1996). TNF-α and IL-6 are proinflammatory cytokines that play an important role in initiating and coordinating the cellular events that make up the immune system's response to infection (Kabashima *et al.* 2002). Previous studies have related the role of these cytokines in the AP pathogenesis (Gazivoda *et al.* 2009; Martinho *et al.* 2012; Azuma *et al.* 2014), specially related with their capacity to activate osteoclastic bone resorption (Kawashima *et al.* 2007; Silva *et al.* 2007, Torres-Monjarás *et al.* 2023).

In addition, TNF-α and IL-6 plays an active role in the diabetes pathogenesis (Sun *et al.* 2010; Ahad *et al.* 2014; Volpe *et al.* 2014; Turner *et al.* 2014, Hosseine *et al.* 2023), specially exacerbating the consequences of this disease, as diabetic nepropathy (Navarro-Gonzalez *et al.* 2008; Ahad *et al.* 2014, Mansoor *et al.* 2022) and the development of oral complications (Sun *et al.* 2010; Marigo *et al.* 2011; Zhao *et al.* 2011; Cintra *et al.* 2014, Martinho *et al.* 2021). Other studies have shown that AP associated with periodontal disease altered systemic conditions in diabetic rats, represented by an increase of triglyceride levels (Cintra *et al.* 2013), glycaemia (Cintra *et al.* 2014a), inflammatory cells (Cintra *et al.* 2014b) and IL-17 serum levels (Cintra *et al.* 2014c). Nevertheless, the literature needs more information about the relationship between AP and systemic health. Thus, the aim of this study was to evaluate the influence of diabetes and/or AP in TNF-α and IL-6 levels in the hematologic, hepatic and renal tissues of Wistar rats.

METHODS

Experimental design

Forty three-month-old male Wistar rats, each weighting 250-280g, were used in the study. The rats were divided into four groups: N- normoglycemic rats; N-AP- normoglycemic rats with apical periodontitis; ED- experimental diabetes; ED-AP- experimental diabetes with apical periodontitis, which were housed in mini-isolator for rats (Alesco, São Paulo, Brazil) in temperature-controlled rooms (25 ±1ºC) and were given ad libitum access to water and food. The experimental protocol was approved by and conducted in accordance with guidelines of the institutional ethical committee (CEEA 023/ 2017/ Uniube).

The rats were fasted overnight (14-16h), and tail-tip blood was used to measure the fasting blood glucose monitoring system (Accu-Check® Performa; Roche Diagnostics Corporation, IN, USA). Subsequently, the rats were intramuscularly anesthetized with ketamine (87 mg/kg; Francotar; Virbac do Brasil Ind. Com. Lta., SP, Brazil), and xylazine (13 mg/kg; Rompum; 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 (0.01 M, pH 4.5) (groups N and N-AP; n=20) or with streptozotocin (Sigma- Aldrich Corp., MO, USA) (groups ED and ED-AP; n=20). Streptozotocin was dissolved in citrate buffer solution at 35 mg/kg body weight for experimental induction of diabetes (Cintra *et al.*, 2013; Cintra *et al.*, 2014).

Six days after diabetics induced, blood samples were collected from each rat to determine their blood glucose levels. The rats with blood glucose levels of more than 200 mg/dL were used in this study (Garber *et al.* 2009).

Induction of endodontic infection

After confirmation of hyperglycemia, animals were anesthetized for the induction of AP with ketamine (87 mg/kg; Francotar; Virbac do Brasil Ind. Com. Lta., Brazil), and xylazine (13 mg/kg; Rompum; Bayer S.A., São Paulo, Brazil) intramuscularly.

For the development of AP, the pulps of the right upper first molars were exposed on the mesial surface using surgical round burs (Broca Ln Long Neck- Maillefer; Dentisply Ind. Com. Ltda, RJ, Brazil) (groups N-AP and ED-AP; n=20) (Garber *et al.* 2009).

Assessment of TNF-α and IL-6 in hematologic, hepatic and renal tissues

After 30 days, the rats were killed with an overdose of the anesthetic solution. The left kidneys of each rat and a fragment of the liver were collect and were immediately preserved in liquid nitrogen to avoid cytokine degradation. Fragments of kidney and liver were obtained to quantify TNF-α and IL-6 by ELISA capture technique. For this, 0,2g of tissues and 800 µl of sterile PBS, pH 7.0, were kept in ice and ground in a tissue homogenizer (Ultraturrax T8; IKA, Germany) for approximately 5 min. The resulting homogenate was centrifuged at 10,000 x g for 15 min at 4ºC and the supernatant was immediately stored at -80ºC (Revco, USA).

The blood were collected by cardiac puncture and were centrifuged immediately after collection at 1,800×g for 15 min at 4 °C to obtain plasma. The plasma (200 µL), which was cooled immediately to −80 °C, was used to determine the plasma TNF-α and IL-6 levels. The capture ELISA was performed using extract of kidney and liver and blood plasma with rat anti-mouse monoclonal antibody produced in cat and biotin rat anti-mouse monoclonal antibody produced in cat (BD Pharmingen™, CA, USA). Plates with 96 wells (Costar™**,** Washington DC, USA) were sensitized with 1µg/ml and 4µg/ml for TNF-α and IL-6, respectively. The detection antibody were with the 1µg/ml and 4µg/ml concentrations for TNF-α and IL-6, respectively. Rat recombinant of TNF–α and IL-6 (BD Pharmingen™, CA, USA) were used to generate a standard curves. The test was developed with 3,3',5'-tetramethylbenzedine-TMB (BD Pharmingen™, CA, USA) in accordance with manufacture's instructions, and plates were read by the microplate EZ read 400 (Biochrom, MA, USA) with 450nm filter.

STATISTICAL ANALYSIS

The total assessed values were tabulated for each experimental group. Two-way analysis of variance (ANOVA) 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

The results may be seen in table 1 and figures 1 and 2. It may be seen that the normoglycemic rats with AP (N-AP) showed higher TNF-α levels in the hematologic tissue when compared to normoglycemic rats without AP (N) ($p<0.05$). There is no statistical difference in the TNF-α levels between diabetic rats without apical periodontitis (ED) and diabetic rats with apical periodontitis (ED-AP) ($p>0.05$). The AP did not alter the TNF-α levels in renal tissues ($p>0.05$) of diabetic rats as much as normoglycemic rats ($p>0.05$).

On the other hand, the presence of diabetes and/or AP did not alter the TNF-α levels in hepatic tissue ($p>0.05$). However, it may be observe a significance increase in the TNF-α levels in hematologic and renal tissues of diabetic rats (ED and ED-AP) when compared to normoglycemic rats (N and N-AP) ($p<0.05$).

Regarding the IL-6 levels, it may be observe a significance increase in the IL-6 levels in hematologic, hepatic and renal tissues of diabetic rats (ED and ED-AP) when compared to normoglycemic rats (N and N-AP) ($p<0.05$). The AP alone did not alter the IL-6 levels in normoglycemic (N and N-AP) nor in diabetic rats (ED and ED+AP) ($p>0.05$).

DISCUSSION

The rats used in this study had uniform body weights and were normoglycemic. Diabetes mellitus was induced by injecting the rats with streptozotocin. The glucose levels were found to be approximately six fold higher than those observed in normoglycemic rats. 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 (Kohsaka *et al,* 1996). 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.

A model of oral infection were used as described previously (Garber *et al.* 2009; Cintra *et al.* 2013; Cintra *et al.* 2014 abc; Samuel *et al.* 2019). 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 (Kohsaka *et al,* 1996).

The data obtained and the statistical analysis showed that the presence of diabetes and/or endodontic infection did not alter TNF-α and IL-6 levels in hepatic tissues ($p>0.05$) when compared to other bodies and more studies should be performed for explain these results. In the renal tissues, we may observe an increase of TNF-α and IL-6 levels in diabetic rats (ED and ED-AP) when compared with nomorglycemic rats (N and N-AP). It may occurs because the kidney is the major organ to have damage in diabetic conditions, represented by diabetic nephropathy. Diabetic nephropathy is a serious renal lesion, characterized by renal dysfunction, fibrosis and glomeruloesclerosis (Heerspink & Zeeuw. 2011; Franceschini *et al.* 2012; Meguro *et al.* 2012). Although the diabetic nephropathy has many factors, dyslipidemia and lipotoxicity further play an important role in the development of pathological process (Athyros *et al.* 2010; Rutledge *et al.* 2010; Kim *et al.* 2013). The dyslipidemia associated with diabetes leads to excessive accumulation of lipids in the kidneys, which causes serious kidney damage as well as increased insulin resistance, oxidative stress and inflammation (Wahba & Mak *et al.* 2007; Murea *et al.* 2010; Das *et al.* 2019). The inflammatory response has also been considered a major mechanism by which lipotoxicity causes diabetic oxidative stress disorder and structural abnormalities in the kidney, through the release of various inflammatory factors (Lin *et al.* 2008; Navarro-

Gonzalez & Mora-Fernandez. 2008; Rivero *et al.* 2009). On the other hand, AP did not alter the TNF-α and IL-6 levels in renal tissue ($p > 0.05$), both in diabetic rats and normoglycemic rats, neither the IL-6 levels in plasm. However, there are studies in the literature that showed that the periodontal disease may increase the TNF-α and IL-6 serum levels in normoglycemic patients (Khanna & Mali 2010; George *et al.* 2013; Danielsen *et al.* 2023) and in diabetic patients (Sun *et al.* 2010; Sun *et al.* 2011; Marigo *et al.* 2011; Zhao. 2011). It is known that the pathogenesis of periodontal disease is similar to the AP pathogenesis, represented by the organism response against aggressor agent, resulting in the release of inflammatory mediators and consequent bone resorption (Silva *et al.* 2007). It shows us that more researchers needs to be conducted regarding the influence of apical periodontitis in tissues, during the presence or absence of systemic diseases.

In addition, this study showed that the TNF-α and IL-6 serum levels increased in diabetic rats (ED and ED-AP) when compared to normoglycemic rats (N and N-AP) ($p<0.05$), which agrees with other studies in the literature, where it was observed an increase in TNF-α and IL-6 serum levels in the presence of diabetes (Sun *et al.* 2010; Turner *et al.* 2014; Volpe *et al.* 2014; Danielsen et al., 2023). This increase interfered negatively in diabetics glycemic control (Sun *et al.* 2010; Sun *et al.* 2011; Marigo *et al.* 2011; Danielsen et al., 2023). Furthermore, normoglycemic rats with apical periodontitis (N-AP) showed higher TNF-α serum levels as compared with normoglycemic rats ($p<0.05$), which is in agreement with other study performed in normoglycemic with apical periodontitis (Astolphi *et al.* 2013, Giorgiou *et al.* 2023), as well as in patients with periodontal disease (Khanna & Mali. 2010). There was no statistical difference between TNF-α serum levels in diabetic rats (ED) compared to diabetic rats with apical periodontitis (ED+AP) ($p>0.05$). It may be explained because the increase of TNF-α serum levels was insignificant when compared to the large increase in rats with diabetes. It may be concluded that the presence of diabetes may increase the levels of IL-6 and TNF-α in hematologic and renal tissue.

The presence of apical periodontitis may promote systemic alterations as the increase of TNF-α serum levels. In addition, the presence of apical periodontitis may potencialize the IL-6 production in hepatic tissues of diabetic rats. These results show the importance of the dental health for the body hemostasis.

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REFERENCE

1.A K DAS, S KALRA, M TIWASKAR, S BAJAJ, K SESHADRI, S CHOWDHURY, et al. Expert Group Consensus Opinion: Role of Anti-inflammatory Agents in the Management of Type-2 Diabetes (T2D) **J Assoc Physicians India**. 2019. Dec;67(12):65-74.

2.AHAD A, GANAI AA, MUJEEB M, SIDDIQUI WA. Chrysin, an anti-inflammatory molecule, abrogates renal dysfunction in type 2 diabetic rats. **Toxicol Appl Pharmacol**. 2014 Aug 15;279(1):1-7.

3.ASTOLPHI RD, CURBETE MM, COLOMBO NH, SHIRAKASHI DJ, CHIBA FY, PRIETO AK, CINTRA LT, BOMFIM SR, ERVOLINO E, SUMIDA DH. Periapical lesions decrease insulin signal and cause insulin resistance. **J Endod**. 2013;39(5):648-52.

4.ATHYROS VG, MITSIOU EK, TZIOMALOS K, KARAGIANNIS A, MIKHAILIDIS DP. Impact of managing atherogenic dyslipidemia on cardiovascular outcome across different stages of diabetic nephropathy. **Expert Opin Pharmacother**. 2010;11(5): 723-30.

5.AZUMA MM, GOMES-FILHO JE, PRIETO AK, SAMUEL RO, LIMA VM, SUMIDA DH et al. Diabetes increases interleukin-17 levels in periapical, hepatic, and renal tissues in rats. **Arch Oral Biol**. 2017 Nov;83:230-5.

6.AZUMA MM, SAMUEL RO, GOMES-FILHO JE, DEZAN-JUNIOR E, CINTRA LT. The role of IL-6 on apical periodontitis: a systematic review. **Int Endod J**. 2014 Jul;47(7):615-21. doi: 10.1111/iej.12196. Epub 2013 Nov 14.

7.BILLINGS F. Mouth infection as a source of systemic disease. **J Am Med Assoc**. 1914;63:2024.

8.CINTRA LT, FACUNDO AC, PRIETO AK, SUMIDA DH, NARCISO LG, BOMFIM SR, OLIVEIRA E SILVA C, DEZAN-JÚNIOR E, GOMES-FILHO JE. Blood profile and histology in oral infections associated with diabetes. **J Endod**. 2014 Aug;40(8):1139-44. a

9.CINTRA LT, FACUNDO AC, AZUMA MM, SUMIDA DH, ASTOLPHI RD, BOMFIM SR, NARCISO LG, GOMES-FILHO. JE.Pulpal and periodontal diseases increase triglyceride levels in diabetic rats. **Clin Oral Investig.** 2013; 17(6):15959.

10.CINTRA LT, SAMUEL RO, AZUMA MM, RIBEIRO CP, NARCISO LG, LIMA VM, SUMIDA DH, COCLETE GA, DEZAN-JÚNIOR E, GOMES-FILHO JE. Apical periodontitis and periodontal disease increase serum IL-17 levels in normoglycemic and diabetic rats. **Clin Oral Investig.** 2014 Dec;18(9):2123-8. B

11.CINTRA LT, SAMUEL RO, FACUNDO AC, PRIETO AK, SUMIDA DH, BOMFIM SR, SOUZA JC, DEZAN-JÚNIOR E, GOMES-FILHO JE. Relationships between oral infections and blood glucose concentrations or HbA1c levels in normal and diabetic rats. **J Endod**. 2014; 47:228-37. C

12.CLARKE NG, HIRSCH RS. Personal risk factors for generalized periodontitis. **J Clin Periodontol**. 1995;22(2):136-45. Review.

13.CLAUSE BT. The Wistar Rat as a right choice: establishing mammalian standards and the ideal of a standardized mammal**. J Hist Biol**. 1993 Summer;26(2):329-49.

14.COSTA R, RÍOS-CARRASCO B, MONTEIRO L, LÓPEZ-JARANA P, CARNEIRO F, RELVAS M. Association between Type 1 Diabetes Mellitus and Periodontal Diseases. **J Clin Med**. 2023 Feb 1;12(3):1147. doi: 10.3390/jcm12031147.

15.DANIELSEN AK, DAMGAARD C, MASSARENTI L, ØSTRUP P, RIIS P, HOLMSTRUP P, NIELSEN CH. B-cell cytokine responses to Porphyromonas gingivalis in patients with periodontitis and healthy controls**. J Periodontol**. 2023 Jan 30. doi: 10.1002/JPER.22-0438.

16.EASLICK K. An evaluation of the effect of dental foci of infection on health. **J Am Dental Assoc**. 1951;42: 615-97.

17.FOUAD A. Diabetes mellitus as a modulating factor of endodontic infections. **J Dent Educ**, v. 67, p. 459-466, 2003

18.FRANCESCHINI N, SHARA NM, WANG H, VORUGANTI VS, LASTON S. The association of genetic variants of type 2 diabetes with kidney function. **Kidney Int**. 2012; 82: 220–225

19.GARBER, S.E.; SHABAHANG, S.; ESCHER, A.P.; TORABINEJAD, M. The effect of hyperglycemia on pulpal healing in rats. Journal of Endodontics, v. 35, n. 1, p. 60-62, 2009.

20.GAZIVODA D, DZOPALIC T, BOZIE B, TATOMIROVIC Z, BRKIC Z, COLIC M. Production of proinflammatory and immunoregulatory cytokines by inflammatory cells from periapical lesions in culture. **J Oral Pathol Med**. 2009 Aug;38(7):605-11.

21.GENCO RJ, LOE H. The role of systemic conditions and disorders in periodontal disease. **Periodontol** 2000. 1993;2:98-116.

22.GEORGE AK, JANAM P. The short-term effects of non-surgical periodontal therapy on the circulating levels of interleukin-6 and C-reactive protein in patients with chronic periodontitis. **J Indian Soc Periodontol.** 2013 Jan;17(1):36-41.

23.GEORGIOU AC, WAAL SV, BUIJS MJ, CRIELAARD W, ZAURA E, BRANDT BW. Host microbiome interactions in apical periodontitis: the endodontic microbiome in relation to circulatory immunologic markers**. Int Endod J**. 2023 Mar 14. doi: 10.1111/iej.13912.

24.GROSS, J.L.; SILVEIRO, S.P.; CAMARGO, J.L.; REICHELT, A.L.; AZEVEDO, M.J. Diabetes Mellito: Diagnóstico, Classificação e Avaliação do Controle Glicêmico. **Arq Bras Endocrinol Metab**, v. 46, n. 1, 2002.

25.HEERSPINK HJ, ZEEUW D. The kidney in type 2 diabetes therapy. **Rev Diabet Stud**. 2011;8(3):392-02.

26.HERRERA D, SANZ M, SHAPIRA L, BROTONS C, CHAPPLE I, FRESE T, et al. Vinker S.Association between periodontal diseases and cardiovascular diseases, diabetes and respiratory diseases: Consensus report of the Joint Workshop by the European Federation of Periodontology (EFP) and the European arm of the World Organization of Family Doctors (WONCA Europe). **J Clin Periodontol.** 2023 Mar 19. doi: 10.1111/jcpe.13807.

27.HOSSEINI MOKHTARI Z, SALEHI ABARGOUEI A, MISHRA GD, AMANI R. Maternal circulating leptin, tumor necrosis factor-alpha, and interleukine-6 in association with gestational diabetes mellitus: a systematic review and meta-analysis. **Gynecol Endocrinol.** 2023 Dec;39(1):2183049. doi: 10.1080/09513590.2023.2183049.

28.HUNTER W. Oral sepsis as a cause of disease. **British Medical J**. 1900;2:215–6.

29.KABASHIMA H, NAGATA K, MAEDA K, IIJIMA T. Involvement of substance P, mast cells, TNF-alpha and ICAM-1 in the infiltration of inflammatory cells in human periapical granulomas. **J Oral Pathol Med**. 2002 Mar;31(3):175-80.

30.KAWASHIMA N, OKIJI T, KOSAKA T, SUDA H. Kinetics of macrophages and lymphoid cells during the development of experimentally induced periapical lesions in rat molars: a quantitative immunohistochemical study. **J Endod**. 1996; 22(6):311-6.

31.KAWASHIMA N, SUZUKI N, YANG G, OHI C, Okuhara S, NAKANO-KAWAWANISHI H, SUDA H. Kinetics of RANKL, RANK and OPG expressionWAs in experimentally induced rat periapical lesions. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod.** 2007 May;103(5):707-11.

32.KHANNA S, MALI AM. Evaluation of tumor necrosis factor-α (TNF-α) levels in plasma and their correlation with periodontal status in obese and non-obese subjects. **J Indian Soc Periodontol**. 2010 Oct;14(4):217-21

33.KIM MY, LIM JH, YOUN HH, HONG YA, YANG KS, et al. Resveratrol prevents renal lipotoxicity and inhibits mesangial cell glucotoxicity in a manner dependent on the AMPK-SIRT1-PGC1alpha axis in db/db mice. **Diabetologia**. 2013; 56(1):204-17.

34.KOHSAKA T, KUMAZAWA M, YAMASAKI M, NAKAMURA H. Periapical lesions in rats with streptozotocin-induced diabetes. **J Endod** 1996. 22:418–21.

35.LIN J, GLYNN RJ, RIFAI N, MANSON JE, RIDEKER PM, NATHAN DM, SCHAUMBERG DA. Inflammation and progressive nephropathy in type 1 diabetes in the diabetes control and complications trial. **Diabetes Care**. 2008;31(12):2338-43.

36.MARÇAL JR, SAMUEL RO, FERNANDES D, de ARAUJO MS, NAPIMOGA MH, et al. T-helper cell type 17/regulatory T-cell immunoregulatory balance in human radicular cysts and periapical granulomas. **J Endod.** 2010;36(6):995-9.

37.MARIGO L, CERRENTO R, GIULIANI M, SOMMA F, LAJOLO C, CORDARO M. Diabetes mellitus: biochemical, histological and microbiological aspects in periodontal disease. **Eur Rev Med Pharmacol Sci**. 2011 Jul;15(7):751-8.

38.MARQUINI, M. A.; MARQUINI, M. A.; CRAVEIRO, M. A.; TOGNETTI, V. M. CORRELAÇÃO ENTRE PERIODONTITE APICAL E DIABETES MELLITUS: UMA REVISÃO DA LITERATURA. **Ensaios USF**, *[S. l.]*, v. 4, n. 2, 2022. DOI: 10.24933/eusf.v4i2.175.Disponível:https://ensaios.usf.edu.br/ensaios/article/view/175. Acesso em: 24 mar. 2023.

39.MARTINHO FC, CHIESA WM, LEITE FR, CIRELLI JA, GOMES BP. Correlation between clinical/radiographic features and inflammatory cytokine networks produced by macrophages stimulated with endodontic content**. J Endod.** 2012 Jun;38(6):740-5.

40.MARTINHO FC, LEITE FRM, ARRUDA-VASCONCELOS R, LOUZADA LM, DARVEU RP, GOMES BPFA. Influence of Bacterial Profiles in Cytokine and Clinical Features of Endodontic Disease. **J Endod.** 2021 Aug;47(8):1265-1271. doi: 10.1016/j.joen.2021.04.026. Epub 2021 May 18.

41.Miller WD. The human mouth as a focus of infection. **Dental Cosmos**. 1891;33:689–706.

42.MILLS A. Oral sepsis in general practice. **Br Med J**. 1913;1(2737):1264–1267.

43.MUREA M, FREEDMAN BI, PARKS JS, ANTINOZZI PA, ELBEIN SC et al. Lipotoxicity in diabetic nephropathy: the potential role of fatty acid oxidation. **Clin J Am Soc Nephrol**. 2010;5(12): 2373-9.

44.NAVARRO-GONZÁLEZ JF[1], MORA-FERNÁNDEZ C. The role of inflammatory cytokines in diabetic nephropathy**. J Am Soc Nephrol.** 2008 Mar;19(3):433-42.

45.OGLE OE. Odontogenic Infections. **Dent Clin North Am**. 2017;61(2):235-252. Review.

46.PRABHU A, MICHALOWICZ BS, MATHUR A. Detection of local and systemic cytokines in adult periodontitis**. J Periodontol**. 1996;67(5):515-22.

47.PRICE WA. Dental infections and related degenerative diseases. **J Am Med Assoc**. 1925;84:254.

48.RIVERO A, MORA C, MUROS M, GARCIA J, HERRERA H, NAVARRO-GONZÁLEZ JF. Pathogenic perspectives for the role of inflammation in diabetic nephropathy. **Clin Sci** (Lond). 2009; 116(6):479-92.

49.ROSENOW EC. Elective localization of bacteria in the animal body. In: **Jordan EO**, Falk IS, eds. The Newer Knowledge of Bacteriology and Immunology. 1928;576–89.

50.RUTLEDGE JC, NG KF, AUNG HH, WILSON DW. Role of triglyceride-rich lipoproteins in diabetic nephropathy**. Nat Rev Nephrol**. 2010; 6(6):361-70.

51.SAMUEL RO, ERVOLINO E, AZEVEDO ÍO, AZUMA MM, FERREIRA GT, CINTRA LTA.Th1/Th2/Th17/Treg Balance in Apical Periodontitis of Normoglycemic and Diabetic Rats. **J Endod.** 2019 Aug;45(8):1009-1015. doi: 10.1016/j.joen.2019.05.003. Epub 2019 Jun 19.

52.SANZ M, CERIELLO A, BUYSSCHAERT M, CHAPPLE I et al. Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International diabetes Federation and the European Federation of Periodontology. Diabetes Res **Clin Pract**. 2018;137:231-241.

53.SILVA TA, GARLET GP, FUKADA SY, SILVA JS, CUNHA FQ Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease**. J D Res**. 2007; (86): 306-19.

54.SUN WL, CHEN LL, ZHANG SZ, REN YZ, QIN GM. Changes of adiponectin and inflammatory cytokines after periodontal intervention in type 2 diabetes patients with periodontitis. **Arch Oral Biol**. 2010 Dec;55(12):970-4.

55.SUN WL, CHENA LL, ZHANG SZ, WU YM, REN YZ, QIN GM. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 diabetes and chronic periodontitis. **Intern Med**. 2011;50(15):1569-74. Epub 2011 Aug 1.

56.TORRES-MONJARÁS AP, SÁNCHEZ-GUTIÉRREZ R, HERNÁNDEZ-CASTRO B, GONZÁLEZ-BARANDA L, et al. Bacteria associated with apical periodontitis promotes in vitro the differentiation of macrophages to osteoclasts. **Clin Oral Investig.** 2023 Feb 17. doi: 10.1007/s00784-023-04920-8. Online ahead of print.

57.TURNER D, LUZIO S, KILDUFF LP, GRAY BJ, DUNSEATH G, BAIN SC, CAMPBELL MD, WEST DJ, BRACKEN RM. Reductions in resistance exercise-induced hyperglycaemic episodes are associated with circulating interleukin-6in Type 1 diabetes. **Diabet Med.** 2014 Aug;31(8):1009-13.

58.VAIZEY JM, CLARK-KENNEDY AE. **Dental Sepsis**: Anaemia, Dyspepsia, and Rheumatism. **Br Med J**. 1939;1(4094):1269-73.

59.VOLPE CM , ABREU LF, GOMES PS, GONZAGA RM, VELOSO CA, NOGUEIRA-MACHADO JA. The production of nitric oxide, IL-6, and TNF-alpha in palmitate-stimulated PBMNCs is enhanced through hyperglycemia in diabetes. **Oxid Med Cell Longev**. 2014; 2014

60.WAHBA IM, MAK RH. Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease. **Clin J Am Soc Nephrol**. 2007;2(3):550-62.

61.WAI T, ITOH H, ATSUMI H. Factors Associated with the Decline of Kidney Function Differ among eGFR Strata in Subjects with Type 2 Diabetes Mellitus. **Int J Endocrinol**. 2012; 2012.

62.ZHAO L, ZHOU Y, XU Y, SUN Y, Li L, CHEN W. Effect of non-surgical periodontal therapy on the levels of Th17/Th1/Th2 cytokines and their transcription factors in Chinese chronic periodontitis patients. **J Clin Periodontol**. 2011 Jun;38(6):509-16.

Table 1. TNF-α and IL-6 levels in hepatic and renal tissues and plasm. Different letters indicate significant statistical differences in the columns ($p<0.05$).

	TNF-α levels (pg/mL)			IL-6 levels (pg/mL)		
Groups	**Hepatic tissue**	**Renal tissue**	**Plasm**	**Hepatic tissue**	**Renal tissue**	**Plasm**
N	1357,832 ± 154,269[a]	1479,811 ± 209,636[a]	44,99 ± 26,085 [a]	62234,7± 7998[a]	34113,5 ± 7479,7[a]	4213,1±2703,7[a]
N-AP	1254,082 ± 293,200[a]	1407,812 ± 465,573[a]	233,944 ± 20,078 [b]	55880,1 ± 16870,7[a]	41600,6 ± 9535,4[a]	3153,5±1475,2[a]
ED	1450,227 ± 152,296[a]	1869,369 ± 439,230[b]	736,28 ± 249,389 [c]	72823,5 ± 7375,1[b]	57181,3 ± 8369,375[b]	22827,1±4306,8[b]
ED-AP	1330,693 ± 254,121[a]	2042,227 ± 246,540[b]	814,458 ± 171,325 [c]	73814,1 ± 6784,6[bc]	58203,3 ± 10212[b]	24237,8±3403,3[b]

Figure 1. TNF-α levels in hepatic and renal tissues and plasm.

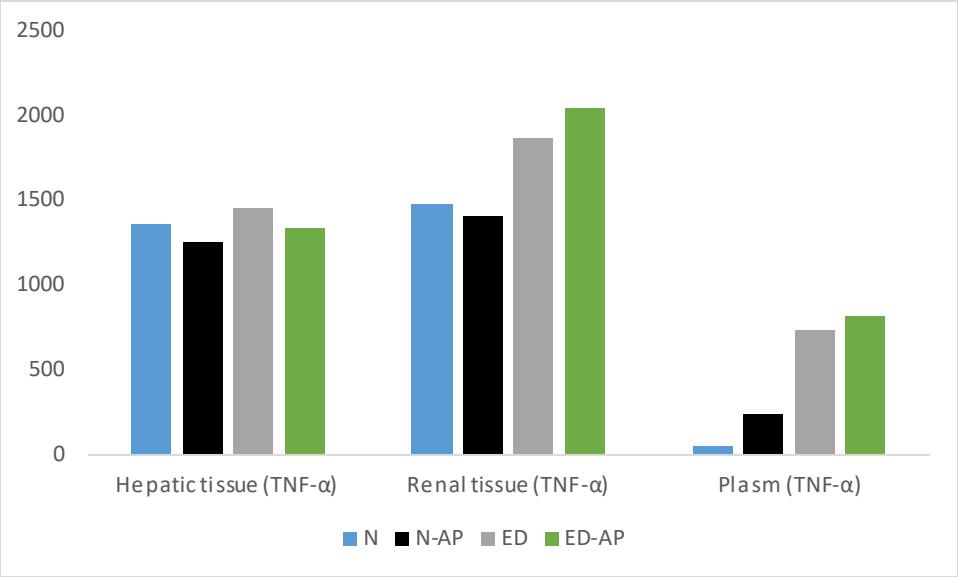
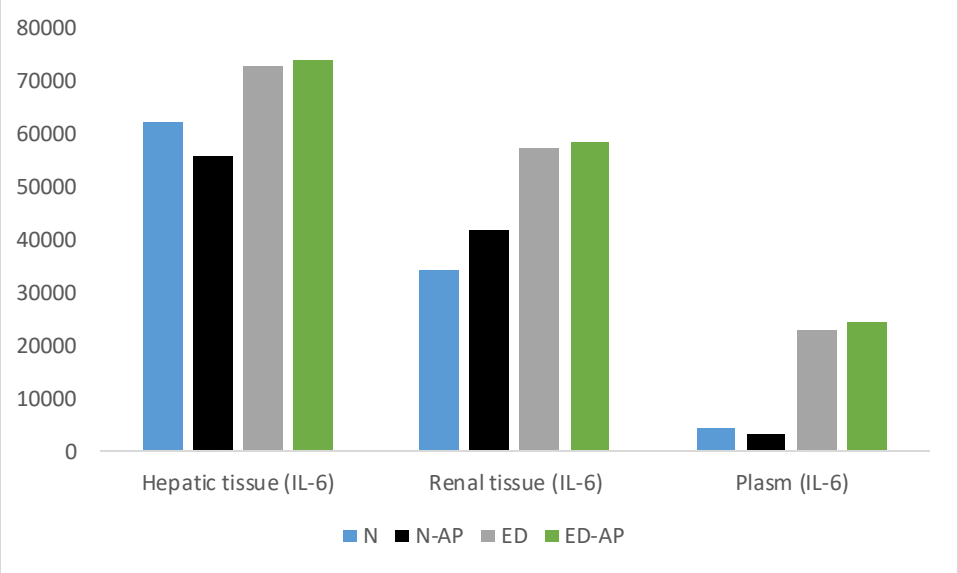


Figure 2. IL-6 levels in hepatic and renal tissues and plas



ANEXO

ANEXO 1- Normas da revista Archives of Oral Biology

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UNIUBE Educação e Responsabilidade Social — Comitê de Ética em Experimentação Animal

Ofício CEEA–064/2017 — Uberaba, 21 de novembro de 2017

Ilma Profa.

Renata Oliveira Samuel

Assunto: Encaminha processo nº 023/2017, sobre o protocolo de pesquisa *"Balanço imunorregulatório de células Th1/Th17/Treg em periodontites apicais de ratos normoglicêmicos e diabéticos".*

Prezado(a) Professor(a),

Em resposta a sua solicitação, informo que o protocolo acima referido foi submetido avaliação do CEEA-UNIUBE, na reunião do dia 23/10/2017, sendo considerado **aprovado.**

Atenciosamente,

Profa. Josely F. Figueiredo Bittar

Coordenadora do CEEA-UNIUBE