UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

Caroline Loureiro

TESE DE DOUTORADO

Perfil proteômico das infecções endodônticas em pacientes diabéticos

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CAROLINE LOUREIRO

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Tese apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Doutor em Ciência Odontológica, área de concentração em Endodontia.

Orientador: Prof. Ass. Dr. Rogério de Castilho Jacinto

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Resumo

Loureiro C. **Perfil proteômico das infecções endodônticas em pacientes diabéticos.** 2023**.** 73 f. Tese - Faculdade de Odontologia de Araçatuba, UNESP - Universidade Estadual Paulista, Araçatuba, Brasil, 2023.

RESUMO

Este estudo teve como objetivo determinar quantitativa e qualitativamente o perfil proteômico da periodontite apical (PA) em pacientes com Diabetes Mellitus tipo 2 (DMT2) em comparação com pacientes não comprometidos sistemicamente e correlacionar a expressão proteica de ambos os grupos com suas funções biológicas. A amostra foi composta por 18 pacientes com PA assintomática divididos em dois grupos de acordo com a presença de DMT2: grupo diabético - pacientes com DMT2 (n = 9) e grupo controle - pacientes sistemicamente saudáveis (n = 9). Após a coleta, as amostras do canal radicular foram preparadas para análise proteômica usando cromatografia líquida de fase reversa e espectrometria de massa. A análise proteômica quantitativa sem marcadores foi realizada pelo software Protein Lynx Global Service. A diferença na expressão proteica entre os grupos foi calculada através do teste *t* ($p < 0,05$). As funções biológicas foram analisadas usando o banco de dados *Homo sapiens* do UniProt. Um total de 727 proteínas humanas foram identificadas em todas as amostras. Entre elas, foram quantificadas 124 proteínas comuns aos dois grupos, das quais 65 proteínas do grupo diabético apresentaram diferenças significativas em relação ao grupo controle: 43 proteínas suprarreguladas ($p < 0,05$) e 22 subreguladas ($p < 0,05$). Nenhuma diferença significativa foi observada na

expressão proteica das 59 proteínas restantes (p > 0,05). A maioria das proteínas com diferenças na expressão estavam relacionadas à resposta imune/inflamatória. Lipocalina associada à gelatinase de neutrófilos, Plastin-2, Lactotransferrina e 13 isoformas de imunoglobulinas foram reguladas positivamente. Em contrapartida, a proteína S100-A8, a proteína S100-A9, a histona H2B, a defensina 1 de neutrófilos, a defensina 3 de neutrófilos e a proteína induzível por prolactina foram reguladas negativamente. Foram demonstradas diferenças quantitativas na expressão de proteínas comuns aos grupos diabético e controle, principalmente relacionadas à resposta imune, estresse oxidativo, apoptose e proteólise. Esses achados revelaram vias biológicas que fornecem a base para apoiar os achados clínicos sobre a relação entre PA e DMT2.

Palavras-chave: periodontite apical, diabetes mellitus, endodontia, interação hospedeiro-patógeno, proteômica.

Abstract

Loureiro C. **Proteomic profile of endodontic infections in diabetic patients.** 2023. 73 f. PhD Thesis – Araçatuba School of Dentistry, UNESP – São Paulo State University, Araçatuba, Brazil, 2023.

ABSTRACT

This study aimed to quantitatively and qualitatively determine the proteomic profile of apical periodontitis (AP) in Type 2 Diabetes Mellitus (T2DM) patients in comparison to systemically non-compromised patients, and to correlate the protein expression of both groups with their biological functions. The sample consisted of 18 patients with asymptomatic AP divided into two groups according to the presence of T2DM: diabetic group - patients with T2DM (n = 9) and control group - systemically healthy patients (n = 9). After sample collection, the root canal samples were prepared for proteomic analysis using reverse-phase liquid chromatography mass spectrometry. Label-free quantitative proteomic analysis was performed by Protein Lynx Global Service software. Differences in protein expression between groups were calculated using t-test ($p < 0.05$). Biological functions were analyzed using the *Homo sapiens* UniProt database. A total of 727 human proteins were identified in all samples. Among them, 124 proteins common to both groups were quantified, out of which 65 proteins from the diabetic group showed significant differences compared with the control: 43 up-regulated ($p < 0.05$) and 22 down-regulated ($p < 0.05$) proteins. No significant differences in protein expression were seen for the remaining 59 proteins ($p > 0.05$). Most proteins with differences in expression were related to

immune/inflammatory response. *Neutrophil gelatinase-associated lipocalin*, *Plastin-2*, *Lactotransferrin*, and 13 isoforms of immunoglobulins were up-regulated. In contrast, Protein S100-A8, Protein S100-A9, Histone H2B, *Neutrophil defensin 1*, *Neutrophil defensin 3*, and *Prolactin-inducible protein* were down-regulated. Quantitative differences were demonstrated in the expression of proteins common to diabetic and control groups, mainly related to immune response, oxidative stress, apoptosis, and proteolysis. These findings revealed biological pathways that provide the basis to support clinical findings on the relationship between AP and T2DM.

Keywords: apical periodontitis, diabetes mellitus, endodontics, host–pathogen interactions, proteomics.

Lista de Figuras

LISTA DE FIGURAS

		pág.
Figure 1	Recruitment flowchart	**3**
Figure 2	Venn diagram of exclusive and common human proteins identified in both groups, and the difference in expression of quantified common proteins	**11**
Figure 3	Interactions between differentially expressed common proteins comparing diabetic and control groups (string database). Protein-protein interaction (ppi) enrichment p value < 1.0 e-16. Biological processes with significative e-value were selected: immune system process (red), response to stress (blue), cellular oxidant detoxification (purple) and antimicrobial humoral response (green)	**17**

Lista de Tabelas

LISTA DE TABELAS

		pág.
Table 1	Demographic data of patients and teeth included in the study	**10**
Table 2	Description and biological function classification of differentially expressed human proteins, comparing diabetic with the control group	**12**
Table 3	Biological function classification of exclusive human proteins in the diabetic and control groups, and of common proteins (↑, ↓, SE) in the diabetic group in relation to the control group	**16**

Sumário

ARTIGO pág.

1. INTRODUCTION **1**

2. MATERIAL AND METHODS **3**

   2.1. Study design **3**

   2.2.Patient selection **4**

   2.3.Sample collection **5**

   2.4.Root canal sample preparation **6**

   2.5.Shotgun label-free quantitative proteomic analysis **7**

   2.6.Bioinformatics analysis and protein classification **9**

3. RESULTS **9**

4. DISCUSSION **18**

5. CONCLUSIONS **23**

6. REFERENCES **23**

ANEXOS pág.

Anexo 1 – Guia de submissão revista International Endodontic Journal **33**

Anexo 2 – Comitê de Ética em Pesquisa em Humanos **48**

Artigo

1. Introduction

Diabetes mellitus (DM) is a complex metabolic disease caused by the absence or deficiency of pancreatic insulin production. The vast majority of DM cases can be categorised as resulting from either the destruction of β cells, which usually leads to absolute insulin deficiency (type 1), or from a combination of insulin resistance and inadequate response to compensatory insulin secretion (type 2) (Zimmet *et al*. 2016). Type 2 DM (T2DM) accounts for 90–95% of DM cases, and requires the use of insulin and/or other drugs to control blood glucose levels (American Diabetes 2013, DeFronzo *et al*. 2015).

Diabetic patients often have an impaired immune system and inflammatory response, which, combined with changes in blood microcirculation associated with DM, results in an increased susceptibility to bacterial infections (Bender & Bender 2003, Chakravarthy 2013). Oral health studies have shown that DM influences both the pathogenesis and prognosis of pulp and periapical diseases (Fouad 2003). Diabetes has been associated with an increased risk of endodontic infections, including apical periodontitis (AP) (Segura-Egea *et al*. 2005, Lopez-Lopez *et al*. 2011, Marotta *et al*. 2012), the presence of periapical radiolucencies in endodontically-treated teeth (Segura-Egea *et al*. 2016) and the extraction of teeth with root canal treatments (Cabanillas-Balsera *et al*. 2019). Furthermore, the presence of local inflammation caused by AP can increase blood glucose, thus intensifying DM and producing an uncontrolled diabetic state in the patient (Schulze *et al*. 2007).

DM and endodontic infections share fundamental molecular mechanisms in the development of a chronic inflammatory state (Sasaki *et al*. 2016). Immune/inflammatory pattern alterations characterised by neutrophil dysfunction, a proinflammatory macrophage profile and the elevated release of inflammatory mediators can produce a hyperresponsive immune/inflammatory system in diabetic patients (Bender & Bender 2003, Sasaki *et al*. 2016). Moreover, a hyperglycaemic state promotes the formation of advanced glycation end-products (AGEs), which increase cell apoptosis and the production of reactive oxygen species (ROS) (Graves *et al*. 2006).

The proteomic profile of endodontic infections has recently been investigated using mass spectrometry, as infected root canal contents allow for the study of the pathogen-host relationship by analysing human and bacterial protein expression. Bacterial proteomic profile of endodontic infections was addressed for the first time by Nandakumar *et al*. (2009), while human proteomic profile by Provenzano *et al*. (2013), using liquid chromatography-mass spectrometry. Human protein characterisation in endodontic infections of healthy patients has been determined by: the progression of endodontic pathogenesis (Loureiro *et al*. 2020, Silva *et al*. 2020); asymptomatic and symptomatic AP (Provenzano *et al*. 2013, Loureiro *et al*. 2021); post-treatment AP infections (Provenzano *et al*. 2016, Francisco *et al*. 2019); and acute apical abscesses (Provenzano *et al*. 2013, Alfenas *et al*. 2017). However, no study has yet used proteomic tools to investigate the molecular pathways of AP in patients with systemic disorders, such as diabetes. The objective of this study was

therefore to quantitatively and qualitatively determine the proteomic profile of AP in T2DM patients, compared with systemically non-compromised patients.

2. Material and Methods

This cross-sectional study was approved by the Research Ethics Committee of the School of Dentistry, Araçatuba - UNESP (N° 34694620.7.0000.5420). All patients were informed about the research procedures and agreed to participate by signing an informed consent form. This study followed the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (https://www.strobe-statement.org).

2.1. Study design

Figure 1 presents the recruitment flowchart showing the study design from patient screening to proteomic assessment.

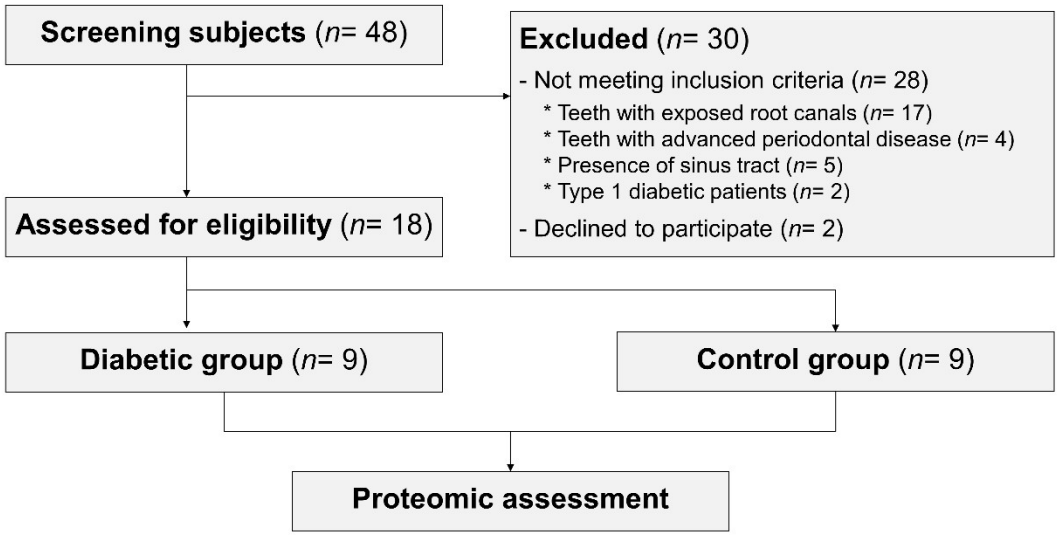

Figure 1. Recruitment flowchart.

2.2. Patient selection

The sample consisted of 18 patients of both genders, aged between 18–70 years, who sought endodontic treatment at the School of Dentistry, Araçatuba - UNESP from July 2018 to December 2019. The study was divided into two groups according to the presence of T2DM: a diabetic group with patients diagnosed with T2DM (n = 9), and a control group with systemically healthy patients (n = 9). The sample size was based on our previous clinical study (Loureiro *et al.* 2021), in which proteomic analysis of root canal contents from patients with symptomatic or asymptomatic AP was performed.

Glycated haemoglobin (HbA1c) was recorded on the day of treatment using data from recent laboratory tests (up to 3 months); patients with a metabolic control status of HbA1c ≥ 7.5% were included in the diabetic group (American Diabetes Association 2019). Patients without T2DM who were reportedly healthy (i.e., without any systemic disease) were included in the control group. HbA1c was also recorded in control group patients through recent exams (up to 3 months) or exam requests.

Medical records, anamnesis and clinical/radiographic examinations were analysed for patient selection. Patients reporting smoking habits, uncontrolled arterial hypertension, autoimmune diseases, pregnancy, chronic liver disease, chronic kidney disease, transplant, use of antibiotics in the past 3 months or presenting with severe periodontal disease were excluded. T2DM patients presenting with controlled arterial hypertension, or who were overweight or obese could be included in the diabetic group.

Only asymptomatic teeth with primary endodontic infections that were associated with the presence of a periapical lesion visible on the radiograph and were diagnosed as chronic AP were included. Periapical radiography was performed using a digital radiographic sensor with measurement software (DentalMaster DICOM, V 1.0.11, Micro Imagem, Rio de Janeiro, RJ, Brazil). Only teeth with periapical lesions ≥ 2.5 mm in diameter as measured by the software were included. The exclusion criteria consisted of teeth with spontaneous pain and/or pain when biting/eating, and tenderness to percussion, teeth that could not be isolated with a rubber dam, teeth with root canals exposed to the oral cavity or sinus tract, a history of dental trauma or the presence of oedema in periapical tissues, advanced periodontal disease or incomplete root formation.

2.3. Sample collection

Samples were taken from root canal contents according to a protocol described in previous studies (Jacinto *et al*. 2008, Loureiro *et al*. 2021). After administration of local anaesthesia with 2% lidocaine and epinephrine at 1 : 100 000 (Alphacaine; Nova DFL Industria e Comercio S/A, Curicica, RJ, Brazil), the tooth was isolated using a rubber dam, followed by sealing of the crown/rubber dam interface with a light-cured gingival barrier (TopDam; FGM, Joinville, SC, Brazil). Disinfection of the tooth and the operative field was performed using 30% hydrogen peroxide (Merck KGaA, Darmstadt, Germany) and 2.5% sodium hypochlorite (Rioquímica) for 30 s each. Neutralisation was then performed using 5% sodium thiosulfate (Merck KGaA). Access cavity preparation was conducted using sterile high-speed diamond burs, without water spray. Cooling during this

stage was performed manually with sterile saline solution. Before accessing the pulp chamber, contaminants (restoration/carious tissue) were removed without exposing the root canals.

Samples were collected only from broad and rectilinear canals (minimum initial diameter of a size 15 K-File). Before root canal sample collection, a sterile K-file was introduced with minimal instrumentation to confirm root canal access. Three sterile paper points were then placed 1 mm from the apex and held in place for 60 s each. If the canal was completely dry, a drop of sterile saline was placed before removing the paper point. After collection, the paper points were stored in sterile, DNA- and RNA-free cryotubes, which were frozen at –80 °C until they were used for proteomic analysis.

2.4. Root canal sample preparation

Sample preparation for proteomic analysis was performed based on the protocol used by previous studies (Ventura *et al*. 2018, Loureiro *et al*. 2020, Loureiro *et al*. 2021).

The paper points of each sample were cut and pooled in biological triplicate (samples from 3 patients in the same group were combined in a single tube, comprising three pools per group). For protein extraction, an extraction solution containing 6 mol L^{-1} urea and 2 mol L^{-1} thiourea in 50 mmol L^{-1} NH_4HCO_3 pH 7.8 was added until the paper points were covered. The samples were vortexed for 10 min at 4 °C, then sonicated at 4 °C for 5 min and finally centrifuged at 20 817 ***g*** for 10 min at 4 °C. The supernatant was then collected, and this step was repeated twice. The pellets were placed in filter tubes

(Corning® Costar® Spin-X® Plastic Centrifuge Tube Filters; Sigma-Aldrich, New York, USA) and centrifuged at 20 817 ***g*** for 10 min at 4 °C. The total solution recovered was approximately 160 µL in each biological triplicate. After, 50 mmol L^{-1} NH_4HCO_3 (volume corresponding to 1.5 × the sample volume) were added to the samples, which were placed in Falcon Amicon Ultra-4 10k tubes (Merck Millipore, Tullagreen, CO, Ireland) and centrifuged at 4500 ***g*** at 4 °C to approximately 150 µL. For protein reduction, samples were incubated with 5 mmol L^{-1} dithiothreitol at 37 °C for 40 min, and subsequently with 10 mmol L^{-1} iodoacetamide at 37 °C for 30 min in the dark. The tryptic digestion was performed for 14 h at 37 °C by adding 2% (w/w) trypsin (Promega, Madison, WI, USA). 5% formic acid was then added to inhibit the action of trypsin, and the samples were desalted and purified with a C18 spin column (Thermo Scientific, Rockford, IL, USA). 1 µL of each sample was used for protein quantification according to the Bradford method (Bio-Rad Bradford Assays). The remnants were dried to approximately 1 µL in SpeedVac (Thermo Scientific), and were resuspended in 3% acetonitrile and 0.1% formic acid for submission for Nano Liquid Chromatography Electron Spray Ionization Tandem Mass Spectrometry – LC-ESI-MS/MS (Waters, Manchester, UK).

2.5. Shotgun label-free quantitative proteomic analysis

Identification of peptides was performed using a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, UK). The nanoACQUITY UPLC was equipped with nanoACQUITY HSS T3, an analytical reverse-phase column (75 µm × 150 mm, 1.8 µm particle size (Waters, Manchester, UK). The column was

equilibrated with mobile phase A (0.1% formic acid in water). The peptides were then separated with a linear gradient of 7–85% mobile phase B (0.1% formic acid in acetonitrile) for 70 min at a flow rate of 0.35 µL min^{-1}. The column temperature remained at 55 °C. The Xevo G2 Q-TOF mass spectrometer was operated in positive nanoelectrospray ion mode, and data were collected using the MSE method at elevated energy (19–45 V), which allows data acquisition of both precursor and fragment ions in one injection. Source conditions used included capillary voltage of 2.5 kV, sample cone at 30 V, extraction cone at 5.0 V and source temperature at 80 °C. Data acquisition occurred over 70 min and the scan range was 50–2000 Da. The lock spray, used to ensure accuracy and reproducibility, was run with a [Glu1] fibrinopeptide solution (1 pmol/ µL min^{-1}) at a flow rate of 1 µL min^{-1}, as a reference ion in positive mode at m/z 785.8427. Protein Lynx Global Server (PLGS) version 3.0 software (Waters, Manchester, UK) was used to process and search for LC-MSE continuum data. Proteins were identified using the embedded ion accounting algorithm in the software and a search of the *Homo sapiens* database (UniProtKB/Swiss-Prot) downloaded on January 2020 from UniProtKB (http://www.uniprot.org/).

For label-free quantitative proteomic analysis, three raw MS files from the diabetic and control groups were analysed using the PLGS software. All proteins identified that had a score with confidence greater than 95% were included in the quantitative statistical analysis. Identical peptides from each technical triplicate sample were grouped based on mass accuracy (< 10 ppm) and time of retention tolerance (< 0.25 min), using the clustering software embedded in the PLGS. Differences in expression between the diabetic and control groups were

analysed using the t-test statistic, and the downregulation or up-regulation of protein expression was determined ($p < 0.05$).

2.6. Bioinformatics analysis and protein classification

For data analysis, reviewed and unreviewed proteins were analysed according to their accession number using the UniProt database (http://www.uniprot.org). Repeated proteins and fragments were excluded from the analysis, as were reverse proteins. The biological functions analyses were performed using the Homo sapiens proteome database (UniProtKB/Swiss-Prot). A Venn diagram was created using the Bioinformatics & Evolutionary Genomics platform (http://bioinformatics.psb.ugent.be/webtools/Venn/). For the interaction networks, the STRING® database (https://string-db.org/cgi/network.pl) was used to illustrate the interactions between common proteins showing different expression levels in the diabetic and control groups.

Protein classification was based on the methods of previous studies (Eckhardt *et al*. 2014; Loureiro *et al*. 2021). The identified proteins were divided into the following categories: metabolism and energy pathways, immune/inflammatory response, transport, structure, DNA/RNA regulation and repair, cell communication and signal transduction, cell growth and/or maintenance, differentiation of neural cells, apoptosis, stress response, and proteins of unknown function.

3. Results

A total of 18 patients participated in this study (11 of whom were male), and the demographics of these individuals are provided in Table 1. The mean age

of patients in the diabetic group was 60.3 ± 8.8 years, and 44.3 ± 11.4 years in the control group. In diabetic patients, the mean HbA1c was 8.75 ± 0.9. With the exception of one patient, all individuals in the diabetic group had controlled arterial hypertension associated with DM.

Table 1. Demographic data of patients and teeth included in the study.

Patient	Group	Medications	Body mass index (Kg/m^2)	Arterial hypertension	Age	Gender	HbA1c (%)	Teeth
P1	Diabetic	Captopril, metformin	24.4	Yes	48	Male	8.9	Canine
P2	Diabetic	Simvastatin, metformin	30.6	No	49	Female	7.5	Molar
P3	Diabetic	Losartan, metformin	23.0	Yes	68	Male	9.4	Canine
P4	Diabetic	Losartan, metformin	23.0	Yes	68	Male	9.4	Canine
P5	Diabetic	Captopril, metformin	22.1	Yes	68	Male	10.1	Incisor
P6	Diabetic	Losartan, metformin	32.4	Yes	52	Female	7.8	Incisor
P7	Diabetic	Losartan, metformin	23.6	Yes	70	Male	9.8	Incisor
P8	Diabetic	Olmesartan, metformin	23.2	Yes	60	Male	7.8	Premolar
P9	Diabetic	Losartan, metformin	25.2	Yes	60	Male	8.1	Premolar
P10	Control	-	33.9	No	46	Female	5.5	Premolar
P11	Control	-	24.5	No	32	Male	4.8	Incisor
P12	Control	-	20.3	No	40	Female	5.1	Premolar
P13	Control	-	23.1	No	35	Male	4.8	Incisor
P14	Control	-	22.4	No	43	Male	5.0	Incisor
P15	Control	-	24.5	No	42	Female	4.7	Premolar
P16	Control	-	23.7	No	36	Female	4.9	Canine
P17	Control	-	26.1	No	58	Male	5.4	Canine
P18	Control	-	27.0	No	67	Female	5.5	Premolar

Proteomic analysis of the samples revealed a total of 1901 accession numbers. After the exclusion of duplicates, human proteins were selected from the raw data obtained by nLC-ESI-MS/MS, as previously described by Loureiro *et al.* (2021). Following selection, all analysed samples accounted for 727 proteins of human origin in both groups. Among them, 350 proteins were present in the diabetic group, and 501 in the control group. The complete list of human

proteins identified is presented in the supplementary information (Table S1). The distribution of proteins in each group is shown in Figure 2.

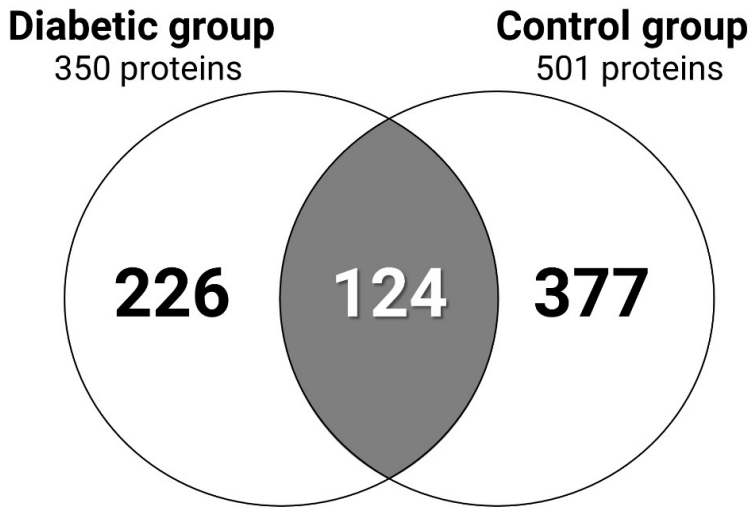

Figure 2. Venn diagram of exclusive and common human proteins identified in both groups, and the difference in expression of quantified common proteins.

All 124 common proteins were quantified for both groups. 65 proteins showed significant differences in expression in the comparison between the diabetic group and the control group: 43 proteins were up-regulated and 22 were down-regulated in the diabetic group, compared to the control group (Table 2). No significant difference in expression was observed in 59 quantified proteins (Figure 2). Most proteins that were differentially expressed belonged to the immune/inflammatory response category. Neutrophil gelatinase-associated lipocalin (NGAL), Plastin-2, Lactotransferrin and 13 isoforms of immunoglobulins were among the proteins up-regulated in the diabetic group. In contrast, Protein S100-A8, Protein S100-A9, Histone H2B, Neutrophil defensin 1, Neutrophil defensin 3 and Prolactin-inducible protein were down-regulated. The greatest increases in expression were observed for Haemoglobin subunit beta (26-fold

higher), Haemoglobin subunit zeta (15-fold higher), Haemoglobin subunit delta (9-fold higher) and Serine/threonine-protein kinase 31 (6-fold higher).

Table 2. Description and biological function classification of differentially expressed human proteins, comparing diabetic with the control group.

Expression differences	Ratio D/C	Accession number	Description	Biological function
↑	**26.05**	**P68871**	**Hemoglobin subunit beta**	**Transport [C]**
↑	**15.80**	**P02008**	**Hemoglobin subunit zeta**	**Transport [C]**
↑	**9.12**	**P02042**	**Hemoglobin subunit delta**	**Transport [C]**
↑	**6.23**	**Q9BXU1**	**Serine/threonine-protein kinase 31**	**Catabolic process [A]**
↑	**5.81**	**P01877**	**Immunoglobulin heavy constant alpha 2**	**Adaptive immunity [B]**
↑	**5.05**	**P01876**	**Immunoglobulin heavy constant alpha 1**	**Adaptive immunity [B]**
↑	**4.44**	**A0M8Q6**	**Immunoglobulin lambda constant 7**	**Adaptive immunity [B]**
↑	**4.35**	**P0CG04**	**Immunoglobulin lambda constant 1**	**Adaptive immunity [B]**
↑	**4.26**	**B9A064**	**Immunoglobulin lambda-like polypeptide 5**	**Defense response to bacterium [B]**
↑	**4.22**	**P0DOY3**	**Immunoglobulin lambda constant 3**	**Defense response to bacterium [B]**
↑	**4.18**	**P0DOY2**	**Immunoglobulin lambda constant 2**	**Defense response to bacterium [B]**
↑	**4.10**	**A0A075B6Z2**	**T cell receptor alpha joining 56**	**Unknown [K]**
↑	**3.67**	**P0CF74**	**Immunoglobulin lambda constant 6**	**Adaptive immunity [B]**
↑	**3.13**	**Q14980**	**Nuclear mitotic apparatus protein 1**	**Cell division [G]**
↑	**2.66**	**Q06830**	**Peroxiredoxin-1**	**Response to oxidative stress [J]**
↑	**2.56**	**S4R460**	**Immunoglobulin heavy variable 3/OR16-9**	**Adaptive immunity [B]**
↑	**2.53**	**A0A4W8ZXM2**	**Immunoglobulin heavy variable 3-72**	**Adaptive immunity [B]**
↑	**2.53**	**G3V1N2**	**HCG1745306_ isoform CRA_a**	**Transport [C]**
↑	**2.51**	**P32119**	**Peroxiredoxin-2**	**Response to oxidative stress [J]**
↑	**2.23**	**Q8WXA9**	**Splicing regulatory glutamine/lysine-rich 1**	**mRNA processing [E]**
↑	1.86	P13796	Plastin-2	Interleukin-12-mediator [B]
↑	1.86	A6NIW5	Peroxiredoxin 2_ isoform CRA_a	Response to oxidative stress [J]
↑	1.84	P13797	Plastin-3	Cytoskeleton component [D]
↑	1.77	P11678	Eosinophil peroxidase	Response to oxidative stress [J]
↑	1.70	P04264	Keratin_ type II cytoskeletal 1	Structural activity [D]
↑	1.60	P01023	Alpha-2-macroglobulin	Differentiation/Protease inhibitor [G]
↑	1.55	P05164	Myeloperoxidase	Response to oxidative stress [J]
↑	1.55	X6R8F3	Neutrophil gelatinase-associated lipocalin	Neutrophil degranulation [B]
↑	1.55	Q5TEC6	Histone H3	DNA binding [E]
↑	1.54	P60709	Actin_ cytoplasmic 1	Constituent of cytoskeleton [D]
↑	1.54	P63261	Actin_ cytoplasmic 2	Constituent of cytoskeleton [D]

↑	1.52	P62736	Actin_ aortic smooth muscle	Constituent of cytoskeleton [D]
↑	1.51	Q6S8J3	POTE ankyrin domain family member E	Unknown [K]
↑	1.42	P07737	Profilin-1	Cytoskeleton organization [D]
↑	1.40	P02788	Lactotransferrin	Immunity, Osteogenesis [B]
↑	1.39	P62805	Histone H4	Metabolic process [A]
↑	1.39	P0CG39	POTE ankyrin domain family member J	Unknown [K]
↑	1.36	P01860	Immunoglobulin heavy constant gamma 3	Defense response to bacterium [B]
↑	1.30	P14618	Pyruvate kinase PKM	Catalytic activity [A]
↑	1.26	P68133	Actin_ alpha skeletal muscle	Constituent of cytoskeleton [D]
↑	1.21	P04406	Glyceraldehyde-3-phosphate dehydrogenase	Oxidoreductase activity [J]
↑	1.20	P01857	Immunoglobulin heavy constant gamma 1	Defense response to bacterium [B]
↑	1.13	P01834	Immunoglobulin kappa constant	Adaptive immunity [B]
↓	0.79	P02768	Serum albumin	Apoptotic process [I]
↓	0.78	C9JKR2	Albumin_ isoform CRA_k	Transport [C]
↓	0.77	P12273	Prolactin-inducible protein	Immune system process [B]
↓	0.76	P06733	Alpha-enolase	Glycolytic process [A]
↓	0.63	P68032	Actin_ alpha cardiac muscle 1	Constituent of cytoskeleton [D]
↓	0.61	P63267	Actin_ gamma-enteric smooth muscle	Constituent of cytoskeleton [D]
↓	0.52	P59665	Neutrophil defensin 1	Cellular response to LPS [B]
↓	0.52	P59666	Neutrophil defensin 3	Cellular response to LPS [B]
↓	0.50	P06899	Histone H2B type 1-J	Antibacterial response [B]
↓	**0.41**	**Q5T3N0**	**Annexin**	**Adaptive immune response [B]**
↓	**0.40**	**P0CG38**	**POTE ankyrin domain family member I**	**Unknown [K]**
↓	**0.38**	**P69905**	**Hemoglobin subunit alpha**	**Transport [C]**
↓	**0.34**	**Q96AB3**	**Isochorismatase domain-containing protein 2**	**Catalytic activity [A]**
↓	**0.29**	**Q562R1**	**Beta-actin-like protein 2**	**Constituent of cytoskeleton [D]**
↓	**0.24**	**P06702**	**Protein S100-A9**	**Inflammatory response [B]**
↓	**0.16**	**A5A3E0**	**POTE ankyrin domain family member F**	**Unknown [K]**
↓	**0.14**	**P69891**	**Hemoglobin subunit gamma-1**	**Transport [C]**
↓	**0.14**	**P05109**	**Protein S100-A8**	**Inflammatory response [B]**
↓	**0.13**	**P69892**	**Hemoglobin subunit gamma-2**	**Transport [C]**
↓	**0.09**	**P02100**	**Hemoglobin subunit epsilon**	**Transport [C]**
↓	**0.04**	**P11532**	**Dystrophin**	**Response to growth factor [G]**
↓	**0.02**	**A0A2R8Y7X9**	**GLOBIN domain-containing protein**	**Oxygen transport [C]**

Differences in expression amongst the groups were expressed as ↑ for up-regulated proteins and ↓ for down-regulated proteins ($p < 0.05$); Ratio D/C = ratio between diabetic and control group proteins. Superscript letters indicate the biological function of each protein. [A] Metabolism and energy pathways; [B] Immune/inflammatory response; [C] Transport; [D] Structural; [E] Regulation and repair of DNA/RNA; [F] Cellular communication and signal transduction; [G] Cell growth and

maintenance; [H] Differentiation of neural cells; [I] Apoptosis; [J] Stress response; [K] Unknown. Human proteins that were more than 2-fold higher or lower are in bold.

Two hundred and twenty-six proteins were found to be exclusive to the diabetic group, and had distinct biological functions. This group had several proteins related to immune/inflammatory response processes (e.g. Homeobox protein Hox-A2, Interleukin-7, Interferon gamma, Arachidonate 15-lipoxygenase, Centrosomal protein of 192 kDa and Centrosomal protein of 290 kDa); oxidative stress (e.g. Egl nine homolog 1, Fe2OG dioxygenase domain-containing protein, Lysine-specific demethylase 4C, Methylenetetrahydrofolate reductase, Putative oxidoreductase GLYR1 and Ribosomal protein S6 kinase alpha-4); apoptotic processes (e.g. Bcl-2-modifying factor, Caspase recruitment domain-containing protein 14, Nischarin, Programmed cell death 6-interacting protein, Protein Wnt-10b and Rho GTPase-activating protein 10); and proteolytic processes, such as proteases (e.g. Tripeptidyl-peptidase 2, Dipeptidyl peptidase 3, Ubiquitin carboxyl-terminal hydrolase 34, Ubiquitin carboxyl-terminal hydrolase 40, Probable ubiquitin carboxyl-terminal hydrolase FAF-X and Probable ubiquitin carboxyl-terminal hydrolase FAF-Y) and protease inhibitors (e.g. Serpin E3, Calpastatin, Serine protease inhibitor Kazal-type 2 and Submaxillary gland androgen-regulated protein 3B).

Most of the identified proteins were involved in biological processes related to cellular communication and signal transduction, the immune/inflammatory response and metabolism and energy pathways, while common proteins were primarily involved in the immune/inflammatory response, transport and structural function (Table 3). In terms of localisation, 26.7% were

discovered in the cytoplasm/cytoskeleton, 25.0% in the nucleus, 17.7% in the plasma membrane and 13.9% in the extracellular region. Figure 3 shows the interaction network between differentially expressed common proteins, and considers all active interaction sources with a medium confidence score (0.4) (STRING® database) to show the strength of protein-protein interactions (p value < 1.0e-16). Regarding the biological processes revealed by protein-protein interaction, up-regulated proteins showed functional enrichment of immune system process and response to stress (Figure 3a), while down-regulated proteins describe cellular oxidant detoxification and antimicrobial humoral response (Figure 3b).

Table 3. Biological function classification of exclusive human proteins in the diabetic and control groups, and of common proteins (↑, ↓, SE) in the diabetic group in relation to the control group.

Biological function classification	Exclusive proteins		Common proteins		
	Diabetic	Control	↑	↓	NSE
Metabolism and energy pathways	24 (10.6)	47 (12.5)	3 (7.0)	2 (9.1)	4 (6.8)
Immune/inflammatory response	28 (12.4)	48 (12.7)	16 (37.2)	7 (31.8)	16 (27.1)
Transport	23 (10.2)	25 (6.6)	4 (9.3)	6 (27.3)	3 (5.1)
Structure	13 (5.8)	53 (14.1)	7 (16.3)	3 (13.6)	3 (5.1)
DNA/RNA regulation and repair	15 (6.6)	36 (9.5)	2 (4.7)	0 (0)	17 (28.8)
Cellular communication and signal transduction	53 (23.5)	62 (16.4)	0 (0)	0 (0)	6 (10.2)
Growth and/or cell maintenance	24 (10.6)	37 (9.8)	2 (4.7)	1 (4.5)	2 (3.4)
Differentiation of neural cells	8 (3.5)	10 (2.7)	0 (0)	0 (0)	1 (1.7)
Apoptosis	13 (5.8)	15 (4.0)	0 (0)	1 (4.5)	2 (3.4)
Stress response	9 (4.0)	19 (5.0)	6 (14.0)	0 (0)	2 (3.4)
Unknown	16 (7.1)	25 (6.6)	3 (7.0)	2 (9.1)	3 (5.1)
Total	226 (100)	377 (100)	43 (100)	22 (100)	59 (100)

↑ = up-regulated proteins ($p < 0.05$); ↓ = down-regulated proteins ($p < 0.05$); NSE = no significant expression in comparison to control group.

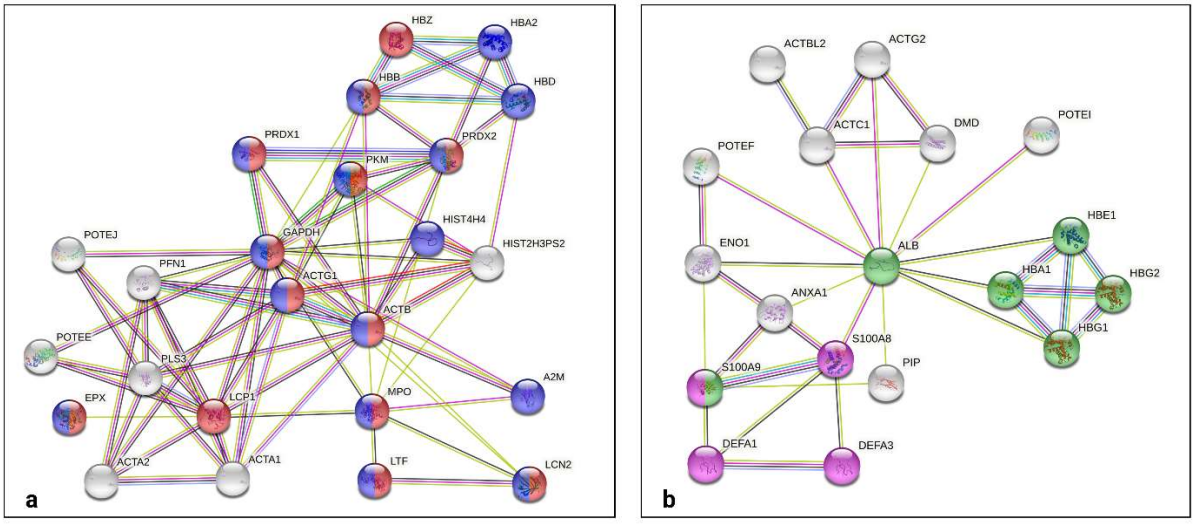

Figure 3. Interactions between differentially expressed common proteins comparing diabetic and control groups (STRING database). Protein-protein interaction (PPI) enrichment *p* value < 1.0 e-16. Biological processes with significative e-value were selected: immune system process (red), response to stress (blue), cellular oxidant detoxification (purple) and antimicrobial humoral response (green). (a) Up-regulated proteins – A2M: Alpha-2-macroglobulin; ACTA1: Actin, alpha skeletal muscle; ACTA2: Actin, aortic smooth muscle; ACTB: Actin, cytoplasmic 1; ACTG1: Actin, cytoplasmic 2; EPX: Eosinophil peroxidase; GAPDH: Glyceraldehyde-3-phosphate dehydro-genase; HBA2: Hemoglobin subunit alpha 2; HBB: Hemoglobin subunit beta; HBD: Hemo-globin subunit delta; HBZ: Hemoglobin subunit zeta; HIST4H4: Histone cluster 4 H4; HIST2H3PS2: Histone cluster 2 H3 pseudogene 2; LCN2: Neutrophil gelatinase-associated lipocalin; LCP1: Plastin-2; LTF: Lactotransferrin; MPO: Myeloperoxidase; PFN1: Profilin-1; PKM: Pyruvate kinase PKM; PLS3: Plastin-3; POTEE: POTE ankyrin domain family member E; POTEJ: POTE ankyrin domain family member J; PRDX1: Peroxiredoxin-1; PRDX2: Peroxiredoxin-2; (b) Down-regulated proteins - ACTBL2: Beta-actin-like protein 2; ACTC1: Actin, alpha cardiac muscle 1; ACTG2: Actin, gamma-enteric smooth muscle; ALB: Serum albumin; ANXA1: Annexin A1; DEFA1: Defensin, alpha 1; DEFA3: Neutrophil defensin 3; DMD: Dystrophin; ENO1: Alpha-enolase; HBA1: Hemoglobin subunit alpha 1; HBE1: He-moglobin subunit epsilon; HBG1: Hemoglobin subunit gamma 1; HBG2: Hemoglobin subunit gamma 2; PIP: Prolactin induced protein; POTEF: POTE ankyrin domain family member F; POTEI: POTE ankyrin domain family member I; S100A8: Protein S100-A8; S100A9: Protein S100-A9;

4. Discussion

The results of this study are the first to provide quantitative data on the differential expression of proteins in endodontic infections in patients with T2DM in comparison with systemically healthy patients. Qualitative data is also provided for the proteins found exclusively in each group, as well as information on their most relevant biological functions. By analysing protein expression and the corresponding biological pathways, our findings illuminate some of the main host mechanisms involved in AP in diabetic patients.

Sixteen proteins related to immune/inflammatory function were found to have increased expression in the diabetic group. Thirteen immunoglobulins were found to be expressed at a high level (up to 5-fold higher), and these included 6 heavy chain and 7 light chain isotypes (lambda and kappa). Elevated levels of immunoglobulin light chains have been associated with inflammatory diseases (Brebner & Stockley 2013). Interestingly, increased immunoglobin light chains concentration has been shown to inhibit neutrophil apoptosis and stimulate mast cell degranulation, which may interfere with the normal resolution of inflammation, and thus contribute to a chronic inflammatory state (Cohen *et al*. 2003, Braber *et al*. 2012).

Another protein that was up regulated in the diabetic sample group was NGAL. This protein is involved in mediating the immune response to bacterial infections, the inflammatory process and apoptosis (Wang *et al*. 2007). Increased NGAL expression is associated with more severe periodontal disease (Tsuchida *et al*. 2013, Tan *et al*. 2020) and with specific modulation of the inflammatory

response to periodontitis in diabetic patients (Kido *et al*. 2020). The increased levels of NGAL in patients in the diabetic group may therefore be related to their hyperglycaemic state. It may be argued that DM influences periapical status by increasing the production of AGEs, due to protein glycation occurring as a result of hyperglycaemia (Ahmed 2005, Ilea *et al*. 2018). AGEs induce NGAL expression in human oral cells, which modulates IL-6 expression in neutrophils and promotes neutrophil migration, thus influencing the inflammatory condition in DM-associated periodontitis (Kido *et al*. 2020).

Proteins S100A8 and S100A9 showed lower levels of expression in the diabetic group. Although these proteins commonly appear overexpressed during infectious or inflammatory processes (Wang *et al*. 2018), the downregulation of Protein S100 may indicate a deficient immune response (Pouwels *et al*. 2015), which is consistent with the exacerbation induced by infection that is widely reported in diabetic patients (Bender & Bender, 2003; Chakravarthy 2013). Additionally, S100A8 and S100A9 have been suggested as biomarkers for the detection of periodontal diseases, and lower levels of these proteins may imply a higher susceptibility to the development of periodontal inflammation (Dommisch *et al*. 2019).

Regarding common proteins, it is important to note that those related to oxidative stress were up regulated in the diabetic group. These include Peroxiredoxin-1, Peroxiredoxin-2, Eosinophil peroxidase, Myeloperoxidase (MPO) and Glyceraldehyde-3-phosphate dehydrogenase. Increased levels of MPO have been reported in the dental pulp of diabetic rats (Catanzaro *et al*.

2006), in the gingival crevicular fluid of T2DM individuals with chronic periodontitis (Marinho *et al*. 2019) and in the progression of chronic inflammatory processes of T2DM patients with periodontitis (Peniche-Palma *et al*. 2019). MPO accumulation reveals the intensity of migration of polymorphonuclear leukocytes to the site of inflammation (Yamalik *et al*. 2000), and increased MPO may be related to the state of periodontal destruction and the enhancement of systemic conditions, including T2DM (Peniche-Palma *et al*. 2019).

Among the exclusive proteins of both groups, there were more proteins associated with oxidative stress in the diabetic group than in the control. ROS are unstable molecules formed from oxygen that react easily with other molecules. ROS are produced by host defence cells through the oxidation of crucial cell signalling proteins, acting as a mediator of inflammation (Mittal *et al*. 2014). ROS production increases the intensity of the immune/inflammatory response to pathogens by activating proinflammatory pathways (Schmidt *et al*. 1996). Prolonged release of ROS has been shown to exacerbate tissue destruction in rats, which may be related to the severity of AP associated with DM (Azuma *et al*. 2017; Prieto *et al*. 2017). Clinical data has also shown a positive association between diabetes and the incidence and severity of AP (Segura-Egea *et al*. 2012; Segura-Egea *et al*. 2005), as the imbalance of redox control in both diseases may act synergistically, thus amplifying their bidirectional courses (Allen *et al*. 2009).

Studies suggest that periapical lesions in diabetic patients are usually larger in size than those in healthy patients (Rudranaik *et al*. 2016), and take

longer to heal following endodontic treatment, particularly in subjects with high blood glucose levels (Arya *et al*. 2017; Ugur Aydin *et al*. 2021). This is likely due to the exacerbation of several processes by DM, including inflammation, oxidative stress and apoptosis. In this study, several proteins involved in the apoptotic process were identified exclusively in the diabetic group. Hyperglycaemia increases the generation and activity of AGEs (Mei *et al*. 2019), which in turn act directly to activate caspases, or indirectly on pathways that regulate apoptosis, such as oxidative stress or the expression of pro-apoptotic genes (Graves *et al*. 2006).

Proteolytic proteins were also identified more frequently in the diabetic group, which could be related to the increase in proteolytic processes caused by a hyperglycaemic state (Flakoll *et al*. 1993). Among these proteins were Tripeptidyl-peptidase 2 and Dipeptidyl peptidase 3. While Tripeptidyl-peptidase 2 may be involved in part of the pathway leading to the degradation of bone matrix proteins (Page *et al*. 1993), Dipeptidyl peptidase 3 could represent a new potential osteoimmunological biomarker of pathology (Menale *et al*. 2019).

When comparing the results of this study with those recently presented by Loureiro *et al*. (2021), it is notable that there are common proteins in diabetic patients with asymptomatic AP (this study) and in healthy patients with symptomatic AP (Loureiro *et al*., 2021). These proteins appear to be primarily related to the inflammatory response and oxidative processes. This is an important comparison, as both conditions are associated with an imbalance in the host's inflammatory response (Lima *et al*. 2013; Gomes & Herrera, 2018).

Proteins with high levels of expression were shown in both conditions (diabetic/symptomatic AP), such as Serine/threonine-protein kinase 31, Myeloperoxidase and Peroxiredoxin-1 and -2. Proteins with low levels of expression were also found in both conditions, including Protein S100-A9, Prolactin-inducible protein and Neutrophil defensin 1 and 3. Furthermore, Glyceraldehyde-3-phosphate dehydrogenase showed increased expression in the asymptomatic diabetic group, and decreased expression in the symptomatic healthy group.

Regarding the use of medications, it should be noted that all patients in the diabetic group were on metformin, and those with arterial hypertension were taking an antihypertensive drug, such as captopril, losartan or olmesartan. During treatment planning and execution, it is important to account for medications used to control systemic conditions, as the execution of endodontic treatment in systemically uncontrolled patients can represent a risk to the patient's health. Uncontrolled diabetic patients are more likely to have crises of hyper- or hypoglycaemia and to experience excessive bleeding (Lalla & D'Ambrosio 2001), while patients with hypertension may suffer hypertensive crises or orthostatic hypotension (Southerland *et al.* 2016). Endodontic treatment is only indicated for uncontrolled patients in urgent cases, and elective procedures must be postponed until the systemic condition stabilises.

The proteins identified in patients with DM were frequently associated with the presence of AGEs, as the protein glycation process induces the expression of specific proteins (NGAL) and oxidative and apoptotic processes. In this

context, proteomic analysis can be applied to study the proteins produced by the glycation process (Chiu et al. 2018; Thornalley et al. 2003). Although this is a technique that commercially requires rigorous analytical standards, liquid chromatography-mass spectrometer is a method with high sensitivity and specificity for detecting products formed by the glycation of proteins. This approach is also known as AGEomics (Rabbani & Thornalley 2020). Future studies on AGEomics should be performed to identify the types of AGEs that influence the immunoinflammatory modulation of periapical tissues, and could potentially affect the prognosis of AP in diabetic patients.

5. Conclusions

In conclusion, this study presents novel data on the proteomic profile of root canal infections in patients with T2DM, and on the complex interplay of identified proteins that are particularly involved in the immune response, oxidative stress, apoptotic and proteolytic processes. Most common differentially expressed proteins are up regulated in the diabetic group, and are largely associated with defence mechanisms. These findings reveal biological pathways that provide the basis for understanding the clinical relationship between AP and T2DM.

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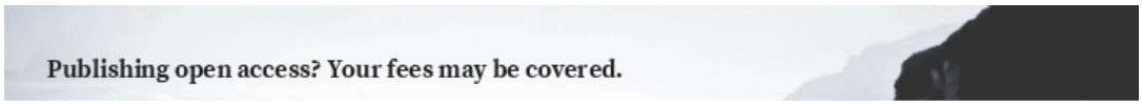

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Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based

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Vandenbroucke JP, von Elm E, Altman DG, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M, STROBE Initiative. (2014) Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. International Journal of Surgery 12, 1500–24

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When submitting manuscripts that have been written using the PRILE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines.

A PRILE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material The PRILE 2021 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/prile/

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRILE 2021 guidelines and their importance:

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, J Camilleri, C Boutsioukis, G Rossi-Fedele, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: a consensus-based development. International Endodontic Journal May 3. doi: 10.1111/iej.13542. (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13542)

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, N Suresh, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: Explanation and elaboration. International Endodontic Journal (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13565)

2.2.7 Systematic reviews

The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively (http://www.prisma-statement.org/). Authors submitting a systematic review must register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in the "Registration" section in the abstract and 'Methods' section in the main body of the text.

A PRISMA checklist and flow diagram as a Figure (to be included in the manuscript for readers) should also be included in the submission material. Source of funding (grant number, if available) should be added in the 'Acknowledgements' section.

It is recommended that authors consult the following papers, which help in the production of high quality reviews:

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLOS Medicine 6, e1000097.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Journal of Clinical Epidemiology 62, e1-34.

Nagendrababu V, Duncan HF, Tsesis I, Sathorn C, Pulikkotil SJ, Dharmarajan L, Dummer PMH (2019) PRISMA for abstracts: best practice for reporting abstracts of systematic reviews in Endodontology. International Endodontic Journal 52, 1096-1107. (https://onlinelibrary.wiley.com/doi/10.1111/iej.13118)

Nagendrababu V, Dilokthornsakul P, Jinatongthai P, Veettil SK, Pulikkotil SJ, Duncan HF, Dummer PMH (2020) Glossary for systematic reviews and meta-analyses. International Endodontic Journal 53, 232-249. (https://onlinelibrary.wiley.com/doi/full/10.1111/iej.13217)

2.2.8 Scoping reviews

Reviews should be reported using the PRISMA guidelines. A checklist for scoping reviews should also be included in the submission material - see: http://www.prisma-statement.org/Extensions/ScopingReviews.

2.2.9 Guidelines for reporting of microarray and next-generation sequencing data

Submission will be assessed according to MIAME and MINSEQE standards. The complete current guidelines are available at

Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), and the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

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Appendix

Abbreviations:

The International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

Additional Guidelines for Cover Pictures, Visual Abstracts, Frontispieces and Table of Contents Graphics

- Concepts illustrated in graphical material must clearly fit with the research discussed in the accompanying text.
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This journal has the option for authors to embed rich media (i.e. video and audio) within their final article. These files should be submitted with the manuscript files online, using either the "Embedded Video" or "Embedded Audio" file designation. If the video/audio includes dialogue, a transcript should be included as a separate file. **The combined manuscript files, including video, audio, tables, figures, and text must not exceed 350 MB.** For full guidance on accepted file types and resolution please see here.

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Examples of correct forms of reference

Standard journal article

Jakovljevic, A., Duncan, H.F., Nagendrababu, V., Jacimovic, J., Milasin, J. & Dummer, P.M.H. (2020) Association between cardiovascular diseases and apical periodontitis: an umbrella review. *International Endodontic Journal,* 53, 1374–1386.

Selman, P. (2016) The global decline of intercountry adoption: what lies ahead? Social Policy and Society, 11(3), 381–397.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* 16, 192-5.

Department of Health. (2009) Living well with dementia: a national dementia strategy.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Holding, M.Y., Saulino, M.F., Overton, E.A., Kornbluth, I.D. & Freedman, M.K. (2008) Interventions in chronic pain management. 1. Update on important definitions in pain management. Archives of Physical Medicine and Rehabilitation, 89 (3, Supplement 1), S38–S40.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Barnes, R. (1995) Successful study for degrees, 2nd edition, London: Routledge.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Partridge, H. & Hallam, G. (2007) Evidence-based practice and information literacy. In: Lipu, S., Williamson, K. & Lloyd, A. (Eds.) Exploring methods in information literacy research. Wagga Wagga, Australia: Centre for Information Studies, pp. 149–170.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

The University Encyclopedia (1985) London: Roydon.

Anexo 2 – Comitê de Ética em Pesquisa em Humanos

UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Perfil proteômico das infecções endodônticas em pacientes diabéticos

Pesquisador: Rogério de Castilho Jacinto

Área Temática:

Versão: 1

CAAE: 34694620.7.0000.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Universidade Estadual Paulista Júlio de Mesquita Filho
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 4.161.055

Apresentação do Projeto:

Serão selecionados 18 pacientes para tratamento endodôntico na Faculdade de Odontologia de Araçatuba - UNESP. Todos pacientes que concordarem em participar da pesquisa assinarão o Termo de Consentimento Livre e Esclarecido. A seleção dos pacientes será determinada pelo histórico odontológico, bem como pelo exame clínico e radiográfico. As seguintes características serão observadas para cada paciente: idade, gênero, estado dentário e pulpar, natureza da dor, história de dor prévia, dor espontânea sensibilidade à percussão, dor à palpação, mobilidade, presença de fístula e sua origem, presença de inchaço dos tecidos periodontais, e profundidade da bolsa periodontal. Após o diagnóstico baseado nos critérios clínicos e radiográficos acima os pacientes serão divididos em dois grupos: controle (n= 9) e diabéticos (n= 9). Serão incluídos pacientes maiores de 18 anos de idade, de ambos os sexos. Apenas dentes com infecção endodôntica primária e presença de lesão periapical visível na radiografia serão incluídos. Para o grupo de pacientes diabéticos, somente os pacientes portadores de Diabetes Mellitus tipo II com exame de hemoglobina glicada entre 6,5 e 8 participarão da pesquisa. Serão excluídos do estudo pacientes que receberam antibioticoterapia nos últimos 3 meses, com patologia associada à doença periodontal, pacientes com diagnóstico de outras doenças sistêmicas associadas ao Diabetes, pacientes com dentes cuja coroa esteja destruída impedindo o isolamento absoluto,

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dentes cujo canal radicular esteja exposto na cavidade oral, dentes com canais atrésicos que não permitam a coleta de material. Pode ou não haver uma sensação alterada ou uma leve sensibilidade à percussão ou palpação, mas não haverá respostas positivas extremas para qualquer teste, incluindo teste de sensibilidade ao frio. Ausência de edema e fístula. Também serão excluídos pacientes com sinais de disseminação da infecção endodôntica como febre, linfadenopatia, trismo e mal-estar geral. Durante o tratamento endodôntico, serão realizadas coletas do canal radicular para posterior análise proteômica das amostras.

Objetivo da Pesquisa:

Objetivo Primário:

Analisar qualitativa e quantitativamente o perfil proteômico das infecções endodônticas de pacientes diabéticos comparados a pacientes saudáveis.

Objetivo Secundário:

Correlacionar a expressão das proteínas humanas e bacterianas detectadas nos canais radiculares e suas funções biológicas nos dois grupos do estudo.

Avaliação dos Riscos e Benefícios:

Riscos:

O risco é mínimo, a participação nesta pesquisa não infringe as normas legais e ética (A coleta da amostra é indolor, pois o dente envolvido será anestesiado. Caso sinta dor, esta não será por causa da coleta de amostra e sim pela realização da anestesia, limpeza do dente ou causada pela infecção no canal radicular). Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 466/12 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos à sua dignidade.

Benefícios:

Ao participar desta pesquisa o paciente não terá nenhum benefício direto. Entretanto, esperamos que o conhecimento construído a partir desta pesquisa possa tornar o tratamento endodôntico mais efetivo, onde pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o sigilo das informações coletadas.

Comentários e Considerações sobre a Pesquisa:

Pesquisa Apresenta-se apta para a sua realização.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram adicionados de acordo com a resolução 466/12 do CNS.

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ARACATUBA, 17 de Julho de 2020

Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))

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