

ASSOCIATION BETWEEN ADVANCED GLYCATION END PRODUCTS AND INFLAMMATION IN ROOT CANAL INFECTIONS: A CROSS-SECTIONAL OBSERVATIONAL STUDY

Boca Antonia^{1,2}, Cosmina Ioana Bondor³, Sanda Ileana Cîmpean^{2*}, Ioana Bâldea⁵, Sebastian Candrea^{1,6}, SALIVAGES Project group*, Aranka Ilea¹

1“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Dentistry, Department of Oral Rehabilitation, Cluj-Napoca, Romania

2“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Dentistry, Department of Conservative Dentistry, Cluj-Napoca, Romania

3“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Medicine, Department of Medical Informatics and Biostatistics, Cluj-Napoca, Romania

4“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Dentistry, Department of Oral Health, Cluj-Napoca, Romania

5“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Medicine, Department of Physiology, Cluj-Napoca, Romania

6“Iuliu Hațieganu” University of Medicine and Pharmacy, Faculty of Dentistry, Department of Paediatric Dentistry, Cluj-Napoca, Romania

*SALIVAGES Project group: Anida-Maria Băbșan, Anca Ionel, Claudia Nicoleta Feurdean, Willi Andrei Uriciuc, Nausica Bianca Petrescu, Bianca Adina Boșca, Maria Crișan, Elena Alina Pârvu, Ana Uifălean, Marian Tăulescu, Anca Ștefania Mesaros, Friederike Manig, Michael Hellwig, Thomas Henle, Vlad Andrei, Manuela Tăut.

*Corresponding author: Sanda Ileana Cîmpean *e-mail* sandacimpean@gmail.com

ABSTRACT

Background: This study aimed to assess the relationship between the levels of advanced glycation end products (AGEs), specifically Nε-carboxymethyl-lysine (CML), in the pulpar and periapical tissues of patients with chronic apical periodontitis (CAP) and their association with oxidative stress markers and other clinical parameters.

Methods: Seventy-three patients diagnosed with Chronic apical periodontitis (CAP) were recruited from the Oral Rehabilitation and Prosthodontic Departments, Faculty of Dentistry, and the Regional Diabetes Center in Cluj-Napoca, Romania, between 2018 and 2019. Fifteen patients required tooth extractions due to severe CAP-related root damage. The extracted teeth were sectioned, and tissue samples were collected from both the pulp and periapical regions. Blood samples were drawn to measure various biochemical parameters and inflammatory markers. Stimulated saliva was also collected to assess oxidative stress markers. CML and other AGEs were quantified in plasma and saliva using liquid chromatography-mass spectrometry (LC-MS/MS).

Results: The study revealed significant positive correlations between CML levels in pulpal and periapical tissues and oxidative stress markers in saliva, including total oxidative status (TOS) and oxidative stress index (OSI).

Conclusions: The findings suggest that CML levels in dental tissues are linked to oxidative stress, as reflected in saliva biomarkers. These results support the potential use of salivary markers as non-invasive indicators of oxidative stress and CAP progression. Further studies with larger sample sizes are needed to confirm these findings and explore their clinical implications.

Key words: oxidative stress; advanced glycation end products (AGEs); Chronic apical periodontitis; meta-inflammation; metabolic diseases;

INTRODUCTION

Root canal infections are typically caused by factors such as physical trauma, untreated dental caries, or the gradual wear of tooth surfaces, all of which can result in exposure of the pulp tissue. Once the pulp is exposed, it

becomes vulnerable to microbial invasion, which, if left untreated, leads to pulp necrosis. The microorganisms responsible for the infection, including bacteria and fungi, along with their byproducts, play a critical role in both initiating and advancing the infection.

These microbial agents release toxic substances that contribute to the ongoing damage of the pulp and surrounding tissues. When pulp necrosis sets in, the lack of an adequate blood supply to the infected area further complicates the situation. Without a functional blood flow, the body's immune response is significantly hindered. The impaired circulation means that immune cells, which are essential for fighting off the infection, cannot reach the affected tissues in sufficient numbers, preventing the body from effectively combating the bacteria. This creates a vicious cycle in which the infection continues to spread, exacerbating tissue damage and increasing the risk of complications, such as abscess formation or even tooth loss¹⁻⁴.

Meta-inflammation is a systemic chronic low-grade inflammation, which involves increased basal immune activation and declining immune response. It is marked by elevated levels of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α)⁴⁻⁶.

Advanced glycation end products (AGEs) are a class of molecules that form through a nonenzymatic chemical process known as glycation, wherein sugars react with proteins, lipids, or nucleic acids. This process leads to the formation of covalent cross-links between these biomolecules, significantly altering their structure and function. AGEs are primarily formed through the Maillard reaction, a multi-step chemical pathway that begins with the reaction of reducing sugars with amino groups on proteins or other macromolecules. Over time, this reaction progresses through intermediate stages to produce advanced glycation end products⁷.

The production of AGEs is particularly pronounced under conditions that promote non-enzymatic glycation (NEG). Hyperglycemia and elevated levels of

oxidative stress create an environment that accelerates this process. As a result, tissues and organs exposed to these conditions may accumulate AGEs more rapidly, contributing to the structural and functional impairments associated with chronic diseases such as diabetes, cardiovascular disorders, and neurodegenerative diseases^{8,9}.

AGEs exhibit proinflammatory properties and include over 20 identified variants, with carboxymethyllysine (CML), pyrraline (Pyr), and pentosidine being notable examples. AGEs induce dysfunction by modifying protein function or by binding to the receptor for AGE (RAGE), found on cell surfaces in tissues such as the heart, lung, skin, bone and skeletal muscle¹⁰⁻¹².

N-carboxymethyl-lysine (CML) was the first dietary advanced glycation end product (dAGE) identified in food, human tissues, and urine. It plays a critical role in understanding the metabolic pathways and biological impacts of dAGEs due to its frequent formation in lipoxidation and glycooxidation processes. CML has become widely recognized as a general marker of oxidative stress and chronic protein damage, reflecting the damage inflicted on biomolecules over time. In dental tissues, CML accumulates in necrotic pulp, where it significantly alters collagen structures and activates receptor for advanced glycation end products (RAGE) signaling, contributing to local inflammation and tissue damage. Within periodontal tissues, CML further exacerbates the inflammatory response by enhancing the release of pro-inflammatory cytokines, promoting osteoclastic activity, and disrupting the normal microvascular function, ultimately leading to tissue breakdown and bone loss. Elevated levels of CML in both saliva and plasma are associated with periodontal disease, which is also linked to systemic conditions such as diabetes, illustrating the broader implications of its role

in metabolic dysfunction. Targeting CML and its underlying pathways, through strategies like antioxidants and RAGE inhibitors, may present promising therapeutic potential for improving both dental and periodontal health, potentially reducing inflammation and preventing further tissue damage ^{8,13}.

The study objective was to investigate possible correlations between the soft tissue (pulp chamber and the periapical region) level of CML and plasmatic, and salivary levels of CML. A secondary objective was comparing tissular CML levels with oxidative stress markers and other AGEs.

MATERIALS AND METHODS

In the study conducted by Băbțan et al.¹⁴, an analytical, observational, and transversal approach was employed, involving the recruitment of 200 patients from the Oral Rehabilitation and Prosthodontic Departments, Faculty of Dentistry, and the Regional Diabetes Center in Cluj-Napoca, Romania, between 2018 and 2019. The inclusion criteria required participants to be 18 years or older, while the exclusion criteria disqualified individuals weighing more than 150 kg. Only participants diagnosed with chronic apical periodontitis (CAP) were included in the study. All participants provided written consent, with the study receiving ethical approval from the Ethics Committee for the human cohort SALIVAGES (Approval no. 93/8.03.2017).

The diagnosis of chronic apical periodontitis (CAP) was confirmed in 73 patients through orthopantomography images by two independent evaluators (A.B and S.C). These evaluators were calibrated to ensure consistency in their decision-making by confronting the radiographs where discrepancies in the number of affected teeth occurred. The positive diagnostic criteria for CAP, based on orthopantomography, included

periapical radiolucency, disruption of the lamina dura, and widening of the periodontal space. These radiographic findings were correlated with the coronal status of each dental unit, assessed using the Decayed, Missing, Filled Teeth (DMFT) index. Exclusion criteria for periapical chronic inflammatory pathology included the integrity of the lamina dura, absence of alveolar bone demineralization, and the relationship with various anatomical structures (such as the maxillary sinus, mandibular canal, and nasal fossae), as well as edentulous patients.

Out of the 73 patients diagnosed with chronic apical periodontitis (CAP) affecting one or more dental units, 15 patients had non-restorable root remnants exhibiting this pathology. Following the extractions, the dental units were sectioned, and the infected tissue was debrided from both the pulp chamber and the periapical region. The collected samples were subsequently subjected to immunohistochemical analysis to quantify the amount of CML present within the lesions. To ensure accuracy and consistency across all tests, each sample was standardized to a protein concentration of 1 mg/ml.

DMFT (Decayed, Missing, Filled Teeth)-index notation

The carious experience of the patients was assessed using the DMFT (Decayed, Missing, Filled Teeth) index, which was calculated for each patient by a dental practitioner based on their dental records. For partially edentulous patients, the cause of missing teeth was determined through clinical examination and orthopantomography images, corroborated with anamnesis data. Patients with multiple carious lesions were identified as having undergone extractions due to complications from carious disease, whereas those with multiple periodontal lesions were considered to have lost teeth due to periodontal disease

progression. In cases of mixed pathology, missing teeth were attributed to carious disease.

Demographic, clinical, and anthropometric data, along with general pathologies, were recorded for each participant, including age, gender, BMI (Body Mass Index), and waist circumference. Venous blood samples were collected from the upper arm into sterile osmotic tubes. These samples were then centrifuged at 4000 rpm for 15 minutes, and the resulting plasma was divided into sterile cryotubes in samples of 100, 200, and 500 μ l. The following parameters were measured in the blood samples: complete blood count, erythrocyte sedimentation rate (ESR), fasting blood glucose, high-sensitivity C-reactive protein (hsCRP), and lipid profile.

Stimulated saliva samples were collected 15 minutes after tooth brushing by having participants chew sterile cotton pellets soaked in citric acid for 2 minutes (Salivette® Sarstedt AG & Co., Nümbrecht, Germany). These saliva samples were centrifuged at 1450 rpm for 7 minutes and then divided into sterile cryotubes in aliquots of 100, 200, and 500 μ l. All biological fluids, including saliva and plasma, were stored at -20 °C until analysis.

2.2. The assessment of the nitro-oxidative stress

The markers of the nitro-oxidative stress were assessed in the plasma and saliva. Saliva was collected in Salivette® Sarstedt devices after tooth brushing. The following markers were evaluated: the nitric oxide (μ mol nitrites/l), total oxidative status (μ mol H₂O₂ Equiv./l), total antioxidant capacity (mmol Trolox Equiv./l), the oxidative stress index (Arbitrary Unit), malondialdehyde (nmol / ml) and total thiols (mmol/ml).

2.2.1. Assessment of nitric oxide levels (NO)

The Griess reaction was used to indirectly determine NO synthesis by measuring nitrites

(NO₂-) and nitrates (NO₃-). The concentration of plasma NO_x was determined using a sodium nitrite-based standard curve, and expressed as nitrite μ mol/L.

2.2.2. Assessment of the Total Oxidative Status (TOS)

Total Oxidative Status (TOS) of the plasma was assessed using a colorimetric assay, the results are expressed in μ mol H₂O₂ Equiv./L.

2.2.3. Assessment of the Total Antioxidant Capacity (TAC)

The total antioxidant response (TAR) was measured in plasma using a colorimetric assay. The results are expressed as mmol Trolox Equiv/L. The ratio of the TOS to the TAR represents the oxidative stress index (OSI), an indicator of the degree of oxidative stress: OSI (Arbitrary Unit) = TOS (μ mol H₂O₂ Equiv/L) / TAR (mmol Trolox Equiv/L).

2.2.4. Assessment of malondialdehyde (MDA)

Malondialdehyde (MDA) was used as a marker of the lipidic peroxidation using the thiobarbituric acid according to the previously described method. The supernatant absorbance was read at 532 nm. A standard curve was performed with 1,1,3,3- tetra-ethoxypropane (0.3-10 nmol/ml). Plasma concentration of MDA was expressed in nmol/ml plasma.

2.2.5. Assessment of Total Thiols (tSH)

Total thiols (tSH) were estimated using the Ellman reagent. Plasma concentration of thiols was expressed in mmol/ml.

2.3. Advanced glycation end-products (AGEs) assessment in biofluids

We identified and characterized key glycation agents, including CML, FruLys, Pyr, MG-H1, CEL, Lys, and Arg, highlighting their roles in protein modification and their potential implications in metabolic disorders. The concentration of AGEs as free glycated amino acids in saliva was performed according to Manig *et al.* description: 500 μ l saliva was mixed with 10 μ l internal standard solution and

490 µl of ice-cold acetonitril/methanol (70/30, v/v) [37]. After 10 min at 4 °C, the tubes were centrifuged (10000 g, 10 min), the supernatant was evaporated under nitrogen to dryness and the remnant was redissolved in 90 µL 20 mM NFPA. Double distilled water was used as basis for blanks.

For the analysis of plasma, 10 µl of the internal standard solution, 25 µl of the plasma sample, and 400 µl of ice-cold acetonitrile/methanol (70/30 v/v) were mixed for deproteinization. After overnight incubation at -18 °C, samples were centrifuged and a defined amount of the supernatant was dried under nitrogen to dryness. The sample remnant was resolved in with 20 mM NFPA in bidist. Water was used for re-constitution and the samples were analyzed with liquid chromatography coupled to mass spectrometry (LC-MS/MS).

Statistical analysis

Data were presented using the

absolute/relative frequency, arithmetic mean and standard deviation, or median and interquartile range, depending on the type of variable: qualitative or quantitative with normal/non-normal distribution.

To explore the relationship between two quantitative variables, both normally distributed coefficients of correlations Pearson were computed, coefficients of correlations Spearman were computed in the other cases.

A multivariate linear regression analysis was conducted. The levels of CML at pulpar and periapical tissues, as well as the number of dental units with chronic apical periodontitis were considered as dependent variables. Any quantitative variables that showed statistically significance or moderate correlation in the univariate analysis were taken as independent variables.

The significance level was set at $\alpha=0.05$. The analysis was performed using SPSS 25.0.

RESULTS AND DISCUSSIONS

In Table 1 demographic and anthropometric characteristics of the subjects were presented in total number.

Table 1. Demographic anthropometric characteristics and biochemical evaluation of the subjects.

Parameters	Total (n=15)
Male, no (%)	5 (33.3)
Age* (years)	50.93±11.79
BMI** (kg/m ²)	28.07±7.39
HbA1c* (%)	5.36 (5.14; 5.65)
HDL* (mg/dL)	48.2 (42.3; 52.9)
TC* (mg/dL)	149.4 (137.9; 186.6)
TG* (mg/dL)	109.77±46.39
hsCRP* (mg/L)	3.85±3.5
ESR* (mm/h)	15.85±6.68
L* (thousands/µL)	7.44±1.32
Diabetes mellitus, (no, %)	2 (13.3)
Hypertension, (no, %)	6 (40.0)
Cardiovascular diseases, (no, %)	6 (40.0)
* median (25th – 75th percentile), ** arithmetic mean ± standard deviation, BMI – Body mass	

index, HbA1c – Glycated hemoglobin, HDL – High-density lipoprotein cholesterol, TC – Total Cholesterol, TG – Tryglicerides, hsCRP – high sensitivity C-reactive protein, ESR – erythrocyte sedimentation rate, L – total leucocyte count.

There were no statistically significantly correlation between levels of CML in pulpar and periapical tissues with demographic anthropometric characteristics, and biochemical parameters. However, there was a moderate non-significantly negative correlation between levels of CML in pulpar and periapical tissues with HDL (Table 2).

There were no statistically significantly correlation between number of units with CAP with demographic anthropometric characteristics, and biochemical parameters. However, there was a moderate non-significantly positive correlation between number of units with CAP with total cholesterol (Table 2).

Table 2. Correlation for levels of CML at pulpar and periapical tissues, number of units with CAP and demographic anthropometric characteristics, biochemical parameters.

Parameters in plasma	Levels of CML in pulpar and periapical tissues (ng/ml)				Number of units with CAP			
	Pearson correlation coefficient		Spearman correlation coefficient		Pearson correlation coefficient		Spearman correlation coefficient	
	r	p	ρ	p	r	p	ρ	p
Age* (years)	-	-	0.06	0.840	-0.19	0.517	-	-
BMI** (kg/m ²)	-	-	0.23	0.436	0.13	0.649	-	-
HbA1c* (%)	-	-	-0.01	0.986	-	-	0.02	0.943
HDL* (mg/dL)	-	-	-0.25	0.383	-	-	0.21	0.470
TC* (mg/dL)	-	-	-0.10	0.737	-	-	0.26	0.363
TG* (mg/dL)	-	-	-0.15	0.605	0.10	0.728	-	-
hsCRP* (mg/L)	-	-	0.16	0.596	0.13	0.671	-	-
ESR* (mm/h)	-	-	0.20	0.516	0.19	0.536	-	-
L* (thousands/ μ L)	-	-	-0.11	0.721	-0.15	0.614	-	-

BMI – Body mass index, HbA1c – Glycated hemoglobin, HDL – High-density lipoprotein cholesterol, TC – Total Cholesterol, TG – Tryglicerides, hsCRP – high sensitivity C-reactive protein, ESR – erythrocyte sedimentation rate, L – total leucocyte count

3.2. Chronic apical periodontitis and CML level in tissues

In Table 3 we presented the disease characteristics of our patients. The average number of total teeth was 18 (missing 14 teeth) from which about 11 teeth in median were with decay.

Table 3. Subjects condition in terms of CAP and DMFT index.

Parameters	Total (n=15)
Total number of dental units*	18.4±8.93

Decay*	10.73±8.19
Missing*	13.6±8.93
Filled**	0 (0; 0.5)
DMFT index*	24.67±6.62
Number of teeth affected by CAP*	5.87±3.09
CML in pulpar and periapical tissues (ng/ml)	10.93 (7.51; 17.75)
* median (25th – 75th percentile), ** arithmetic mean ± standard deviation, CAP - chronic apical periodontitis, DMFT - Decayed, Missing, Filled Teeth	

There were no statistically significant correlation between levels of CML in pulpar and periapical tissues with parameters indicating CAP and DMFT index. There was a moderate non-significantly positive correlation between levels of CML in pulpar and periapical tissues with filled number of dental teeth (Table 4).

There were statistically significantly

positive correlations between number of units with CAP with total number of dental units, and number of decay. There were statistically significantly negative correlations between number of units with CAP with number of missing teeth. There was a moderate non-significantly negative correlation between number of units with CAP with number of filled (Table 4).

Table 4. Correlation for levels of CML at pulpar and periapical tissues, number of units with CAP with demographic anthropometric characteristics, biochemical parameters.

Parameters	Levels of CML in pulpar and periapical tissues (ng/ml)				Number of units with CAP			
	Pearson correlation coefficient		Spearman correlation coefficient		Pearson correlation coefficient		Spearman correlation coefficient	
	r	p	ρ	p	r	p	ρ	p
Total number of dental units	-	-	-0.19	0.498	0.63	0.012		
Decay	-	-	-0.01	0.960	0.57	0.025		
Missing	-	-	0.19	0.498	-0.63	0.012		
Filled	-	-	0.29	0.294			-0.33	0.234
DMFT index	-	-	0.23	0.400	-0.17	0.537		
Number of teeth affected by CAP	-	-	-0.19	0.500	-	-	-	-
BMI – Body mass index, HbA1c – Glycated hemoglobin, HDL – High-density lipoprotein cholesterol, TC – Total Cholesterol, TG – Triglycerides, hsCRP – high sensitivity C-reactive protein, ESR – erythrocyte sedimentation rate, L – total leucocyte count								

3.3. Saliva AGE and oxidant/antioxidant parameters

Parameters of the oxidant/antioxidant balance from saliva for the total number of 15 patients were presented in Table 5.

Table 5. Parameters of the oxidant/antioxidant balance and AGEs compounds in saliva

Parameters in saliva	Total (n=15)
FruLys (ng/ml)	20.98 (20.63; 22.8)
Pyr (ng/ml)	1.69 (1.69; 1.72)
MG-H1 (ng/ml)	2.69 (2.34; 3.96)
CEL (ng/ml)	1.05 (1.02; 1.26)
CML (ng/ml)	3.8 (2.74; 4.56)
Arg (μg/ml)	0.33 (0.06; 0.81)
Lys (μg/ml)	0.3 (0.14; 0.73)
TAC (Mmol trolox equiv./L)	1.09±0.005
TOS (μmol H ₂ O ₂ equiv./L)	8.7 (8.05; 10.8)
OSI	8.03 (7.34; 9.94)
NOx (μmol/L)	13.02±5.72
MDA (nmol/L)	2.91±1.14
tSH (μmol/L)	110.2±33.72
FruLys – Nε-fructosyllysine, Pyr – Pyrraline, MG-H1 – methylglyoxal-derived hydroimidazolone 1, CEL – Nε-carboxyethyllysine, CML – Nε-carboxymethyllysine, Lys – Lysine, Arg – Arginine, TAC – Total antioxidant capacity, TOS – Total oxidative status, OSI – oxidative stress index, MDA – Malondialdehyde, NOx – nitric oxide levels, tSH – Total thiols	

There were statistically significantly positive correlations between levels of CML in pulpar and periapical tissues with TOS, and OSI from saliva. There were moderate non-significantly positive correlations between levels of CML in pulpar and periapical tissues with Pyr, CEL, Lys from saliva; and a moderate non-significantly negative correlation between levels of CML in pulpar and periapical tissues with tSH (Table 6).

In multivariate analysis with levels of CML in pulpar and periapical tissues as dependent variable, CEL ($b=45.63$; $p<0.001$), Pyr ($b=-268.47$; $p=0.014$) and OSI ($b=3.61$; $p=0.001$) were statistical significant related with levels of CML in pulpar and periapical tissues.

There were statistically significantly negative correlations between number of units with CAP with MG-H1, Arg, and TAC from saliva. There was a moderate non-significantly positive correlation between number of units with CAP with MDA. There were moderate statistically non-significantly negative correlations between number of units with CAP with FruLys, CML, Lys, and NO from saliva (Table 6).

In multivariate analysis with number of units with CAP as dependent variable, TAC determined in saliva ($b=-404.39$; $p=0.037$) were statistical significant related with number of units with CAP.

There were moderate non-significantly positive correlations between levels of CML in pulpar and periapical tissues with TAC,

TOS, OSI, NO from saliva; and a moderate non-significantly negative correlation between levels of CML in pulpar and periapical tissues with TAC from saliva (Table 6).

There were statistically significantly positive correlations between number of units with CAP with TAC, and MDA from saliva.

There were moderate non-significantly positive correlations between number of units with CAP with FruLys, and CML from saliva. There were moderate statistically non-significantly negative correlations between number of units with CAP with TOS, and OSI from saliva (Table 6).

Table 6. Correlation for levels of CML at pulpar and periapical tissues, number of units with CAP with AGEs, oxidative stress markers determined in saliva.

Parameters in saliva	Levels of CML in pulpar and periapical tissues (ng/ml)				Number of units with CAP			
	Pearson correlation coefficient		Spearman correlation coefficient		Pearson correlation coefficient		Spearman correlation coefficient	
	r	p	r	p	r	p	r	p
FruLys (ng/ml)			0.11	0.703			-0.47	0.086
Pyr (ng/ml)			0.43	0.126			-0.10	0.746
MG-H1 (ng/ml)			-0.01	0.970			-0.58	0.030
CEL (ng/ml)			0.41	0.144			-0.05	0.857
CML (ng/ml)			0.02	0.958			-0.48	0.083
Arg (μg/ml)			0.04	0.899			-0.59	0.026
Lys (μg/ml)			0.31	0.274			-0.44	0.118
TAC (mmol trolox equiv./L)			-0.23	0.454	-0.58	0.037		
TOS (μmol H ₂ O ₂ equiv./L)			0.59	0.033			0.13	0.661
OSI			0.60	0.029			0.16	0.600
NO (μmol/L)			0.18	0.627	-0.26	0.473		
MDA (nmol /L)			-0.15	0.629	0.35	0.238		
tSH (μmol/L)			-0.47	0.108	-0.21	0.484		
FruLys – Nε-fructosyllysine, Pyr – Pyrraline, MG-H1 – methylglyoxal-derived hydroimidazolone 1, CEL – Nε-carboxyethyllysine, CML – Nε-carboxymethyllysine, Lys – Lysine, Arg – Arginine, TAC – Total antioxidant capacity, TOS – Total oxidative status, OSI – oxidative stress index, MDA – Malondialdehyde, NOx – nitric oxide levels, tSH – Total thiols								

3.4. Plasma AGE and oxidant/antioxidant parameters

Parameters of the oxidant/antioxidant balance from plasma for the total number of 15 patients were presented in Table 7.

Table 7. Parameters of the oxidant/antioxidant balance and AGEs compounds in plasma

Parameters in plasma	Total
----------------------	-------

	(n=15)
FruLys (ng/ml)	471.22±198.12
Pyr (ng/ml)	25.24 (24.86; 32.53)
MG-H1 (ng/ml)	33.67±3.52
CEL (ng/ml)	12.98 (12.78; 13.35)
CML (ng/ml)	50.47±3.77
Arg (μg/ml)	3.12 (2.84; 4.08)
Lys (μg/ml)	5.8±1.19
TAC (Mmol trolox equiv./L)	1.09 (1.09; 1.11)
TOS (μmol H ₂ O ₂ equiv./L)	56.58±15.98
OSI	51.56±14.68
NOx (μmol/L)	45.82±13.35
MDA (nmol/L)	5.42±1.65
tSH (μmol/L)	472.77±139.86
* median (25th – 75th percentile), ** arithmetic mean ± standard deviation, FruLys – Nε-fructosyllysine, Pyr – Pyrraline, MG-H1 – methylglyoxal-derived hydroimidazolone 1, CEL – Nε-carboxyethyllysine, CML – Nε-carboxymethyllysine, Lys – Lysine, Arg – Arginine, TAC – Total antioxidant capacity, TOS – Total oxidative status, OSI – oxidative stress index, MDA – Malondialdehyde, NOx – nitric oxide levels, tSH – Total thiols.	

There were no statistically significantly correlation between levels of CML in pulpar and periapical tissues with AGEs, oxidative stress markers determined in plasma (Table 8).

Table 8. Correlation for levels of CML at pulpar and periapical tissues, number of units with CAP with AGEs, oxidative stress markers determined in plasma.

Parameters in plasma	Levels of CML in pulpar and periapical tissues (ng/ml)				Number of units with CAP			
	Pearson correlation coefficient		Spearman correlation coefficient		Pearson correlation coefficient		Spearman correlation coefficient	
	r	p	r	p	r	p	r	p
FruLys (ng/ml)			-0.21	0.464	0.31	0.285		
Pyr (ng/ml)			0.18	0.527			0.05	0.856
MG-H1 (ng/ml)			0.20	0.493	0.21	0.470		
CEL (ng/ml)			-0.02	0.946			-0.06	0.839
CML (ng/ml)			-0.19	0.513	0.26	0.373		
Arg (μg/ml)			-0.20	0.483			-0.21	0.465
Lys (μg/ml)			-0.40	0.159	-0.19	0.507		
TAC (mmol trolox equiv./L)			0.49	0.091			0.57	0.040
TOS (μmol H ₂ O ₂ equiv./L)			0.38	0.194	-0.40	0.179		
OSI			0.37	0.209	-0.41	0.170		
NO (μmol/L)			0.44	0.133	0.00	0.992		

MDA (nmol /L)			0.17	0.578	0.56	0.048		
tSH (μmol/L)			0.18	0.563	0.04	0.891		
FruLys – Nε-fructosyllysine, Pyr – Pyrraline, MG-H1 – methylglyoxal-derived hydroimidazolone 1, CEL – Nε-carboxyethyllysine, CML – Nε-carboxymethyllysine, Lys – Lysine, Arg – Arginine, TAC – Total antioxidant capacity, TOS – Total oxidative status, OSI – oxidative stress index, MDA – Malondialdehyde, NOx – nitric oxide levels, tSH – Total thiols								

4. DISCUSSION

This study assessed the correlation between tissular CML and its presence in plasma and saliva, along with oxidative stress markers and advanced glycation end products (AGEs). The findings revealed a significant correlation between CML levels in pulp and periapical tissues and the total oxidative status (TOS) and oxidative stress index (OSI) from saliva. These results indicate that local oxidative stress in these tissues is mirrored salivary biomarkers. This suggests that oxidative stress plays some role in the development of chronic apical periodontitis (CAP) and supports the potential of salivary markers as non-invasive indicators of tissue damage.

The results showed no statistically significant correlations between CML levels in pulp and periapical tissues and AGEs or oxidative stress markers in plasma. However, moderate, non-significant positive correlations were observed between CML levels in tissues and several salivary markers, including TAC, TOS, OSI, and NO, suggesting a potential relationship between CML accumulation in dental tissues and oxidative stress in saliva. Additionally, a moderate, non-significant negative correlation was found between CML levels in tissues and TAC in saliva.

Regarding the number of CAP-affected units, significant positive correlations were found with salivary TAC and MDA, indicating that higher oxidative stress may be linked to more extensive CAP.

Moderate, non-significant positive correlations were also observed between the number of CAP units and FruLys and CML levels in saliva. Conversely, moderate, non-significant negative correlations were found between the number of CAP units and TOS and OSI in saliva. A limitation of the study that may have contributed to these results is the small patient sample size. A larger sample size could potentially reveal more statistically significant results.

A meta-analysis revealed the possible role of chronic periodontitis in systemic diseases. The study showed a significant association between periodontal disease and blood levels of oxidative stress biomarkers, namely TAC, MDA and NO¹⁵. The literature contains only a limited number of studies that have investigated this relationship. However, there is a growing body of evidence showing the relationship between periodontal disease, namely periodontitis (P) and oxidative stress¹⁶.

A study by Baltacıoğlu et al.¹⁷ showed that TAS and TAC were closely related to periodontal status. It also suggested the potential of using OSI as a marker for periodontal disease. Another study by the same author revealed that some oxidative stress markers are locally increased in periodontitis, which suggests that these markers are closely associated with the severity of the disease and with bone resorption. Given this connection to bone resorption, we considered the possibility that oxidative stress markers might also be

associated with chronic apical periodontitis (CAP). This consideration arises from the fact that severe CAP also results in varying levels of bone loss, albeit at the periapical level¹⁸.

Another research indicates that young obese women, despite being otherwise healthy, exhibit signs of early periodontal disease, such as gingival inflammation, when compared to age-matched healthy lean women. This suggests that localized oxidative stress associated with obesity may play a role in the development of periodontal disease¹⁹. These findings underscore a strong correlation between obesity and meta-inflammation, revealing how excess adiposity drives chronic low-grade inflammation that contributes to the development of metabolic dysfunction and related disorders^{20–22}.

A prospective cross-sectional study aimed to investigate the relationship between temporomandibular disorders (TMD) and oxidative stress markers. This research was designed to compare salivary and serum oxidative stress and inflammation markers in individuals with TMD and healthy controls. While existing hypotheses suggest a potential link between pain, inflammation, oxidative stress markers, and temporomandibular pathology, the results of this study did not demonstrate any significant positive correlations in the saliva samples analyzed²³.

In this study, we conducted a comprehensive evaluation of various parameters, comparing tissue levels of CML with its levels in plasma and saliva. Furthermore, we investigated the associations between CML levels, oxidative stress markers,

and the presence of additional glycating agents. The results demonstrated a significant correlation between CML levels in pulp and periapical tissues and both TOS and the oxidative stress index OSI.

This study has several limitations. Chronic apical periodontitis (CAP) was assessed using bi-dimensional radiographs (orthopantomograms, OPG) only. As a result, the analysis focused on the frequency (number of dental units affected by CAP) rather than the size of the lesions. Evaluating lesion size would have required complementary cone-beam computed tomography (CBCT) examinations, which were not available for the patients included in this study. Additionally, given the limited inflammatory response typically observed in chronic conditions, it may be more appropriate to focus future research on acute apical periodontitis. Another limitation was the small sample size, which may have influenced the findings. A larger sample could potentially yield more statistically significant results.

CONCLUSIONS

In conclusion, while a range of parameters were assessed, the only significant correlations identified were between CML levels in pulp and periapical tissues and both total oxidative status TOS and the oxidative stress index OSI from saliva. Considering the literature on other pathologies, especially systemic conditions, we find that this topic is of considerable interest in the context of endodontic pathology and requires further investigation.

Acknowledgements

This study was supported partially by the COFUND-ERA-HDHL ERANET Project, European and International Cooperation - Subprogram 3.2 – Horizon 2020, PNCDI III Program - Biomarkers for Nutrition and Health –“Innovative technological approaches for validation of salivary AGEs as novel biomarkers in evaluation of risk factors in diet-related diseases”, grant no 25/1.09.2017.

REFERENCES

1. Karamifar K, Tondari A, Saghiri MA. Endodontic Periapical Lesion: An Overview on the Etiology, Diagnosis and Current Treatment Modalities. *Eur Endod J* 2020; 5: 54–67.
2. Siqueira JF, Rôças IN. Clinical Implications and Microbiology of Bacterial Persistence after Treatment Procedures. *J Endod* 2008; 34: 1291-1301.e3.
3. Pinto KP, Barbosa AFA, Silva EJNL, Santos APP, Sassone LM. What Is the Microbial Profile in Persistent Endodontic Infections? A Scoping Review. *J Endod* 2023; 49: 786-798.e7.
4. Gardiyanoglu E, Ünsal G, Akkaya N, Aksoy S, Orhan K. Automatic Segmentation of Teeth, Crown–Bridge Restorations, Dental Implants, Restorative Fillings, Dental Caries, Residual Roots, and Root Canal Fillings on Orthopantomographs: Convenience and Pitfalls. *Diagnostics* 2023; 13: 1487.
5. Qu L, Matz AJ, Karlinsey K, Cao Z, Vella AT, Zhou B. Macrophages at the Crossroad of Meta-Inflammation and Inflammaging. *Genes* 2022; 13: 2074.
6. Verma D, Fekri SZ, Sigurdardottir G, Bivik Eding C, Sandin C, Enerbäck C. Enhanced Inflammasome Activity in Patients with Psoriasis Promotes Systemic Inflammation. *J Invest Dermatol* 2021; 141: 586-595.e5.
7. Norlin A-K, Walter S, Icenhour A, Keita ÅV, Elsenbruch S, Bednarska O, Jones MP, Simon R, Engström M. Fatigue in irritable bowel syndrome is associated with plasma levels of TNF- α and mesocorticolimbic connectivity. *Brain Behav Immun* 2021; 92: 211–20.
8. Teodorowicz M, Hendriks WH, Wichers HJ, Savelkoul HFJ. Immunomodulation by Processed Animal Feed: The Role of Maillard Reaction Products and Advanced Glycation End-Products (AGEs). *Front Immunol* 2018 Sep 13
9. Thomas CJ, Cleland TP, Sroga GE, Vashishth D. Accumulation of carboxymethyl-lysine (CML) in human cortical bone. *Bone* 2018; 110: 128.
10. Kosmopoulos M, Drekolias D, Zavras PD, Piperi C, Papavassiliou AG. Impact of advanced glycation end products (AGEs) signaling in coronary artery disease. *Biochim Biophys Acta BBA - Mol Basis Dis* 2019; 1865: 611–9.
11. Van Puyvelde K, Mets T, Njemini R, Beyer I, Bautmans I. Effect of advanced glycation end product intake on inflammation and aging: a systematic review. *Nutr Rev* 2014; 72: 638–50.
12. MacLean M, Derk J, Ruiz HH, Juranek JK, Ramasamy R, Schmidt AM. The Receptor for Advanced Glycation End Products (RAGE) and DIAPH1: Implications for vascular and neuroinflammatory dysfunction in disorders of the central nervous system. *Neurochem Int* 2019; 126: 154–64.
13. Shen C-Y, Lu C-H, Cheng C-F, Li K-J, Kuo Y-M, Wu C-H, Liu C-H, Hsieh S-C, Tsai C-Y, Yu C-L. Advanced Glycation End-Products Acting as Immunomodulators for Chronic Inflammation, Inflammaging and Carcinogenesis in Patients with Diabetes and Immune-Related Diseases. *Biomedicines* 2024; 12: 1699.
14. Stanislovaitienė D, Žaliūnienė D, Steponavičiūtė R, Žemaitienė R, Gustienė O, Žaliūnas R. N-carboxymethyllysine as a biomarker for coronary artery disease and age-related macular degeneration. *Med Kaunas Lith* 2016; 52: 99–103.
15. Băbțan A, Aranka I, Boșca A, Crisan M, Petrescu N, Collino M, Sainz R, Gerlach J, Câmpian R. Advanced glycation end products as biomarkers in systemic diseases: premises and perspectives of salivary advanced glycation end products. *Biomark Med* 2019 Apr 10
16. Liu Z, Liu Y, Song Y, Zhang X, Wang S, Wang Z. Systemic Oxidative Stress Biomarkers in Chronic Periodontitis: A Meta-Analysis. *Dis Markers* 2014; 2014: 931083.
17. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol* 2018; 45 Suppl 20: S162–70.
18. Baltacıoğlu E, Yuva P, Aydın G, Alver A, Kahraman C, Karabulut E, Akalın FA. Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease? *J*

- Periodontol 2014; 85: 1432–41.
19. Baltacıoğlu E, Kehribar MA, Yuva P, Alver A, Atagün OS, Karabulut E, Akalın FA. Total oxidant status and bone resorption biomarkers in serum and gingival crevicular fluid of patients with periodontitis. *J Periodontol* 2014; 85: 317–26.
 20. Dursun E, Akalın FA, Genc T, Cinar N, Erel O, Yildiz BO. Oxidative Stress and Periodontal Disease in Obesity. *Medicine (Baltimore)* 2016; 95: e3136.
 21. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* 2006; 83: 461S-465S.
 22. Lee J. Adipose tissue macrophages in the development of obesity-induced inflammation, insulin resistance and type 2 Diabetes. *Arch Pharm Res* 2013; 36: 208–22.
 23. Khanna D, Khanna S, Khanna P, Kahar P, Patel BM, Khanna D, Khanna S, Khanna P, Kahar P, Patel BM. Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus* 2022 Feb 28
 24. Kazan D, Baş Akkor B, Aksoy A, Atmaca E. The evaluation of oxidative stress and inflammation markers in serum and saliva of the patients with temporomandibular disorders. *Turk J Med Sci* 2023; 53: 1690–6.