DOI:
10.1111/iej.13979

Document Version
Publisher's PDF, also known as Version of record

Received: 12 March 2023 | Accepted: 18 September 2023

DOI: 10.1111/iej.13979

INTERNATIONAL ENDODONTIC JOURNAL | WILEY

ORIGINAL ARTICLE

Successful endodontic treatment reduces serum levels of cardiovascular disease risk biomarkers—high-sensitivity C-reactive protein, asymmetric dimethylarginine, and matrix metalloprotease-2

Noor Al-Abdulla[1] | **Abdulaziz Bakhsh**[1,2] | **Francesco Mannocci**[1] | **Gordon Proctor**[3] | **David Moyes**[3] | **Sadia Ambreen Niazi**[1]

[1]Department of Endodontics, Centre of Oral Clinical & Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, Guy's Dental Hospital, King's College London, London, UK

[2]Department of Restorative Dentistry, Division of Endodontics, Faculty of Dental Medicine, Umm Al-Qura University, Makkah, Kingdom of Saudi Arabia

[3]Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, Guy's Dental Hospital, King's College London, London, UK

Correspondence
Sadia Ambreen Niazi, Department of Endodontics, Centre of Oral Clinical & Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, Floor 17, Tower Wing, Guy's Dental Hospital, King's College London, London SE1 9RT, UK.
Email: sadia.niazi@kcl.ac.uk

Funding information
British Endodontic Society; European society of endodontology

Abstract

Aim: To investigate serum biomarkers of inflammation 2 years following non-surgical root canal re-treatment (Re-RCT) and peri-apical surgery (PS). The results were correlated with signs and symptoms, treatment outcome, metabolic syndrome factors, infection with severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 (COVID-19) infection and COVID-19 vaccination.

Methodology: Subjects from our previous study were recalled for 2 years post-treatment follow-up. Changes to the patient's history (medical, dental, social) were noted. Periapical health of the treated teeth was examined both clinically and radiographically. Blood pressure, fasting HbA1C and low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and total cholesterol (TC) levels were measured. Serum inflammatory marker levels were assayed using a Bio-Rad Bio-Plex 200 analyser and values at different time points within the same group were compared using a Wilcoxon signed-rank test and differences between groups with a Mann–Whitney test. Linear associations were tested using Pearson's correlations.

Results: The recall percentage at 2 years was 56.9% ($n = 37$), with a 100% radiographic success rate using periapical radiographs. In total, 21 cases (56.8%) were completely healed, and 16 cases (43.2%) were healing. Higher matrix metalloprotease 2 (MMP2) levels were present in the healing group compared to the healed group. Serum levels of high-sensitivity C-reactive protein (hs-CRP), asymmetric dimethylarginine (ADMA) and MMP-2 were significantly reduced ($p \leq .001$) whereas other biomarkers showed significant increases at 2 year compared to pre-operative levels, while FGF-23 and ICAM-1 were not significantly increased. HbA1C ($p = .015$), TC ($p = .003$), LDL ($p = .003$) and HDL ($p = .003$) reduced significantly at 2 years post-treatment compared to their preoperative levels. COVID infection showed a significant association with MMP-9 ($p = .048$).

Conclusions: hs-CRP, ADMA and MMP-2 can be regarded as prognostic biomarkers of successful Re-RCT and PS as they reduced at 2 year recall in cases which showed evidence of clinical and radiographic success. The successful treatment of chronic apical periodontitis is correlated with improvements in metabolic syndrome indicators, better glycemic control, and reduction at 2 year of some systemic inflammatory markers which are related to risks of cardiovascular disease events.

KEYWORDS

apical periodontitis, asymmetric dimethylarginine (ADMA), cardiovascular diseases, high-sensitivity C-reactive protein (hs-CRP), matrix metalloprotease-2 (MMP-2), metabolic syndrome enzyme-linked immunosorbent assay

INTRODUCTION

Apical periodontitis (AP) is a chronic inflammatory disease of the periradicular tissues (Figdor, 2002). It is caused by complex interactions between root canal microbiota, microbial virulence factors such as toxins, and the host immune response (Kakehashi et al., 1965; Márton et al., 1988; Marton & Kiss, 2014). AP manifests itself in different ways, ranging from completely asymptomatic and detected as a periapical radiolucency on a radiograph, to being symptomatic presenting with pain and abscess (Nair, 2004, 2006). The global prevalence of AP and root canal treatments in adults is high (one periapical lesion per patient and two root canal treatments per patient) (Dos Santos Tiburcio-Machado et al., 2021). Moreover, by contributing to a chronic systemic inflammation AP might have a negative impact on a patient's overall health (Georgiou et al., 2019; Khalighinejad et al., 2016; Niazi & Bakhsh, 2022).

Many studies have investigated the associations between AP and conditions such as diabetes and cardiovascular diseases (CVDs) (Berlin-Broner et al., 2017; Garrido et al., 2019; Jakovljevic et al., 2020; Jimenez-Sanchez et al., 2020; Nagendrababu et al., 2020; Pérez-Losada et al., 2020). CVDs are a complex group of disorders (Myers & Mendis, 2014) that are the primary cause of death worldwide, accounting for 30% of total global mortality (Bhatnagar et al., 2015; Vasan & Benjamin, 2016) and affecting more than 17.9 million people annually (Mendis et al., 2011). Most CVDs are caused by atherosclerosis, a chronic inflammatory condition characterized by the formation of a lipid-rich plaque in the innermost layers of arteries (Milutinović et al., 2020; Wolf & Ley, 2019).

There are several potential pathways by which AP can impact the initiation and advancement of atherosclerosis. For example, locally produced inflammatory mediators associated with AP can potentiate endothelial dysfunction leading to the progression of atherosclerosis (Jimenez-Sanchez et al., 2020). Inflammatory markers induced in AP are similar to those involved in atherosclerosis. Georgiou et al. (2019) in a systematic review and meta-analysis reported that AP can increase the levels of high-sensitivity C-reactive protein (hs-CRP), Interleukin-6 (IL-6), asymmetric dimethylarginine (ADMA), and complement-C3 (Georgiou et al., 2019). However, the authors indicated the need for further longitudinal studies to provide critical evidence to support the proposed link with atherosclerosis. Furthermore, some interventional studies showed reductions in levels of inflammatory marker including hs-CRP, C3 and ADMA, between baseline and follow-up (Cotti et al., 2015; Poornima et al., 2021).

In our longitudinal interventional cohort study, we showed that the pre-operative serum levels of Interleukin-1β (IL-1β), high-sensitivity C-reactive protein (hs-CRP), fibroblast growth factor (FGF-23), and ADMA were significantly raised in AP subjects compared to controls, indicating an increased systemic inflammatory burden in AP (Bakhsh et al., 2022). Furthermore, 1-year post-treatment, the levels of most of the investigated markers generally reduced, indicating the effectiveness of non-surgical root canal re-treatment (Re-RCT) and peri-apical surgery (PS) in reducing this systemic inflammation (Bakhsh et al., 2022).

A large number of periapical radiolucencies (54%) were still in the healing phase at 1-year post-treatment, so the aim of the present longitudinal study was to examine serum inflammatory biomarkers at a 2 years follow-up. The findings of the present study were correlated with clinical signs and symptoms, outcome of treatment, metabolic syndrome factors, infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19) infection and COVID-19 vaccination.

MATERIALS AND METHODS

This longitudinal cohort study complied with the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines (von Elm et al., 2014) (Figure 1).

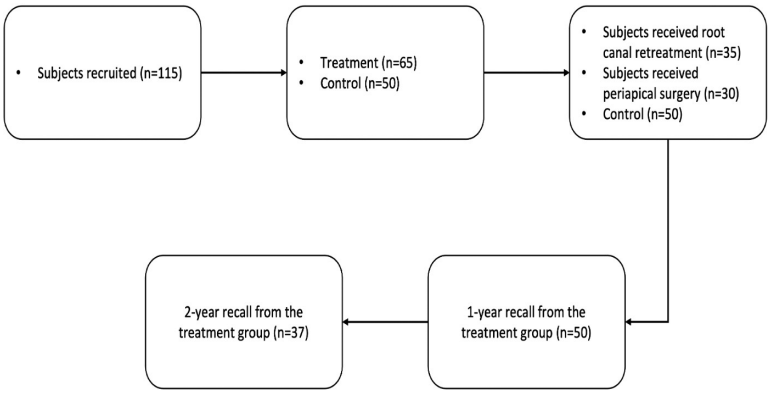

FIGURE 1 STROBE flow diagram for recruited subjects.

Ethical approval

This study was approved by the London-Hampstead Research Ethics Committee (IRAS project ID 207795). Written consent was obtained from the participants in agreement with the Declaration of Helsinki.

Patient recruitment

Subjects who had been treated with non-surgical root canal re-treatment (Re-RCT) and peri-apical surgery (PS) in our previous prospective longitudinal cohort study (Bakhsh et al., 2022) were reviewed 2 years post-treatment.

Clinical examination

Any changes to the patient's medical, dental, and social histories including SARS-CoV-2 infection and vaccination status were noted. Extraoral and Intraoral examinations were performed. Basic Periodontal Examination (BPE) was undertaken in all recalled subjects. The periapical health of the endodontically treated tooth was investigated by tenderness to palpation/percussion, mobility and presence of swelling or sinus tracts. Periapical radiograph of the teeth under investigation was also taken using a beam-aiming device x-ray unit at 65 kV and 7 mAs (Heliodent; Sirona) and photostimulable phosphor plates (Digora Optime, Soredex). Two readings of subjects' blood pressure were recorded using an upper arm blood pressure monitor (A&D Medical). The average reading was recorded according to previous recommendations (Chobanian et al., 2003).

Subjects were advised to have an overnight fast prior to the 2-year review appointment. Their HbA1C (A1cNow + BHR Pharmaceuticals Ltd.); low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TRG), total cholesterol (TC) and TC/HDL levels were measured with CardioChek PA blood analyser (BHR Pharmaceuticals Ltd.) using testing strips (CHO + HDL strips, BHR Pharmaceuticals Ltd.).

Radiographic outcome analysis

Radiographic outcomes were classified as described by Patel et al. (2012). Two endodontic specialists evaluated the periapical radiographs. Following consensus agreement between the two examiners, the outcome scores were recorded with a six-point categorization according to Patel et al. (2012). Cases were classified as successful if they showed incomplete healing (reduction in the size of the periapical radiolucency) or complete healing (resolved or absence of periapical radiolucency) using periapical radiographs.

Blood samples processing

Subjects' blood samples were collected in the BD Vacutainer SST II Advance 8.5 mL tube (Becton Dickinson).

For serum collection, the tubes were centrifuged at 2000 × g for 10 min at 4°C using Eppendorf centrifuge 5810R (Eppendorf). The serum samples collected were aliquoted and kept in a −80°C freezer.

Multiplex microbead assay

The Magnetic Assay human premixed multi-analyte kit (R&D Systems, Bio-Techne) was used to analyse the serum according to manufacturer's instructions to determine the inflammatory mediators' levels (tumour necrosis factor [TNF-α], IL-1β, IL-8, IL-6, hs-CRP, ADMA, pentraxin-3, vascular cell adhesion molecule [VCAM-1], E-selectin, intercellular adhesion molecule [ICAM-1], matrix metalloprotease [MMP-2], MMP-8, MMP-9) using a Bio-Rad Bio-Plex 200 analyser (Bio-rad).

Elisa

Enzyme-linked immunosorbent assay (ELISA) and iMark microplate absorbance reader (Bio-Rad) were used to detect serum levels of ADMA in accordance with the manufacturer's instructions.

Statistical analysis

The results were statistically analysed using IBM® SPSS® (Version 15.0). Normality was tested using histogram, Kolmogorov–Smirnov and Shapiro–Wilk's tests. Since the data were not normally distributed, therefore, the comparison of the difference in distributions between different time points within the same group was carried out using the Wilcoxon signed-rank test. Whereas, to identify the significant difference between groups, the Mann–Whitney test was used. The level of significance was agreed to $p < .05$ and 95% confidence interval was made using a one-sample *t*-test.

The values were converted to a logarithmic scale due to the lack of normality in biomarker levels and the difference between the means of the groups was determined using a two-sample *t*-test. Linear associations were calculated using Pearson's correlations. Moreover, regression models were conducted to estimate beta coefficients involved in those significant correlations.

Multiple linear regression models were conducted to assess the correlation between each (log) biomarker and independent factors and covariates (age, gender, COVID, HbA1c, TC, HDL, LDL, TRG and TC/HDL). In order to avoid multicollinearity, a mixed method of entry of variables into the model was considered (fixed and stepwise). Beta coefficients and 95% CI were provided for significant findings.

Inter-examiner reliability for periapical radiographs (PA) was determined using linearly weighted Kappa's index to assess the concordance.

RESULTS

The recall percentage at 2 years (R4) (T0) was 56.9% ($n = 37$). Out of these 37 subjects (18 males, mean age: 48.2 [27–69]; 19 females, mean age: 48.15 [28–76]), 20 subjects were from the root canal retreatment (Re-RCT) group and 17 subjects from the periapical surgery (PS) group.

None of the subjects had symptoms from the treated tooth. The treated teeth were functional with intact coronal restorations.

Of the 37 subjects reviewed at 2-years (R4), only three reported changes in medical history since their 1-year post-operative review (R3). One subject reported a history of lung fibrosis, taking a budesonide inhaler; another subject reported neurological pain in finger, taking pregabalin; and the third subject had been diagnosed with glaucoma and was taking Timolol eye drops. Median levels of metabolic syndrome indicators were; Systolic blood pressure (mmHg) (120 [99–160]), Diastolic blood pressure (mmHg) (82 [60–100]), HbA1C (%) (4.7 [3.5–6.4]), TC (mmol/L) (5.10 [0.20–8.38]), HDL (mmol/L) (1.65 [0.50–3.01]), LDL (mmol/L) (2.93 [0.60–5.31]) and TRG (mmol/L) (1.00 [0.55–4.15]). Four subjects showed an increase in the BPE score of 3 or 4 in one of the sextants with a diagnosis of localized periodontitis with periodontal pockets >4 mm, as compared with those recorded at baseline and 1 year review. There was no change in the smoking history and all reviewed subjects were non-smokers.

Radiographic treatment outcome

Linear Kappa (κ) index of inter-examiner reliability of the outcome showed a good agreement between the readings of examiners for PA (0.72 [0.50–0.94]).

Comparison of the outcome based on periapical radiographs in total subjects (Re-RCT and PS groups) between 1-year post-treatment (R3) and 2-year post-treatment follow-up (R4)

Out of 37 subjects, at 2-year follow-up (R4), based on PA radiographs, 21 cases (56.8%) had completely healed, and 16 cases (43.2%) were healing. When the same cases were reviewed at 1-year post-treatment (R3), only 13 of them (35%) were completely healed, 23 cases (62%) were still healing and 1 case (2.7%) had failed. Out of the 23 cases that were healing at R3, when reviewed at R4, 14 cases (60.9%) were still healing whereas 9 cases (39.1%) had completely healed. Moreover, out of 13 healed cases at R3, 1 case (7.7%) was now healing at R4. Moreover, the one failed case at R3 was now classified as 'healing' at R4 (Table 1).

Comparison of the outcome based on periapical radiographs in Re-RCT group between 1-year post-treatment (R3) and 2 years post-treatment follow-up (R4)

Out of 20 subjects at R4, based on PA radiographs, 10 cases (50%) had completely healed, and 10 cases (50%) were still

TABLE 1 Comparison of the outcome based on PA radiographs in total subjects, Re-RCT and PS groups at 1-year (R3) and-2-year follow-up (R4).

	Total		Failure		Healing		Healed	
PA outcome R4	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
PA outcome total subjects AT R3								
Total	37	100.0	1	100.0	23	100.0	13	100.0
Healing	16	43.2	1	100.0	14	60.9	1	7.7
Healed	21	56.8	0	.0	9	39.1	12	92.3
PA outcome Re-RCT AT R3								
Total	20	100.0	1	100.0	12	100.0	7	100.0
Healing	10	50.0	1	100.0	8	66.7	1	14.3
Healed	10	50.0	0	0.0	4	33.3	6	85.7
PA outcome PS at R3								
Total	17	100.0	11	100.0	6	100.0		
Healing	6	35.3	6	54.5	0	0.0		
Healed	11	64.7	5	45.5	6	100.0		

healing. Comparing the same subjects' outcome at R3, 7 cases (35%) were classified as healed, 12 cases (60%) were healing and 1 case (5%) had failed. When reviewed at R4, out of the 12 cases that were healing at R3, 8 cases (66.7%) were still healing at R4, and 4 cases (33.3%) had healed. However, out of the 7 cases that had healed at R3, 1 case (14.3%) was classified as healing at R4. The 1 failed case at R3 was now classified as healing at R4 (Table 1).

Comparison of the outcome based on PA radiographs in the PS group between 1-year post-treatment (R3) and 2-year post-treatment review follow-up (R4)

Out of 17 cases at R4, 11 cases (64.7%) had healed, 6 cases (35.3%) were healing whereas at R3 6 cases (35.2%) had healed, and 11 cases (55%) were healing. At R3, out of the 11 cases that were healing, 6 cases (54.5%) continued to heal, and 5 cases (45.5%) had completely healed at R4 (Table 1).

SERUM INFLAMMATORY BIOMARKERS LEVELS

Comparison between serum inflammatory biomarker levels at T0 (pre-operative) and R4 (2-year post-treatment) based on PA radiographic outcome ('healed'/'healing')

The inflammatory markers level comparison between pre-operative (T0) and 2-year review (R4) in all samples showed that there was a significant reduction in the levels of hs-CRP, ADMA and MMP-2 at R4 compared to T0 ($p = .001$; $p < .001$; $p < .001$ respectively). All other biomarkers except FGF-23 and ICAM-1 showed a significant increase in their levels from T0 to R4 (Table 2).

In the Re-RCT group, R4 serum levels of hs-CRP ($p = .036$) and MMP-2 ($p = .012$) decreased significantly more in healed cases compared to the healing ones ($p = .043$; $p = .017$, respectively) whereas there was more reduction of ADMA ($p = .005$) in healing as compared to healed Re-RCT cases ($p = .036$). In Re-RCT group, the ICAM-1 levels were also reduced at R4, however, this reduction was not significant (Table S1–S4). In the PS group, the reduction of ADMA and MMP-2 was more evident in healed ($p = .012$; $p = .018$ respectively) than in healing cases ($p = .028$; $p = .068$, respectively) (Table S2). However, contrary to the findings of the Re-RCT group ICAM-1 levels at R4 were increased compared to T0 levels, (Figure 2).

Comparison between serum inflammatory biomarker levels at R3 (1-year post-treatment) and R4 (2-year post-treatment) based on PA radiographic outcome ('healed'/'healing')

Levels of the inflammatory markers hs-CRP, ADMA and MMP-2 were significantly reduced at R4 compared to R3 ($p < .001$; $p < .001$; $p < .001$, respectively). In contrast, there were significant increases in levels of all other biomarkers (Table 3). MMP-2 was significantly higher in the healing compared to the healed group ($p = .041$) (Figure 3). In the Re-RCT group from R3 to R4, hs-CRP, ADMA and MMP-2 levels significantly reduced ($p = .001$; $p = .002$; $p < .001$, respectively), with more hs-CRP and ADMA reduction seen in the healed cases in ($p = .002$,

TABLE 2 Comparison of serum inflammatory biomarker levels between pre-operative (T0) and 2-year post-treatment follow-up (R4) based on PA radiographic outcome.

	Total			Healing
	Median (min–max)			Median (min–max)
	T0	R4	*p*-Value	T0
hs-CRP	54725.76 (1330.48–470330.34)	24283.22 (6394.72–79057.88)	.001**	65869.14 (1330.48–470330.34)
ADMA	6850.46 (104.19–555164.61)	1073.94 (138.91–2446.04)	<.001***	6836.71 (1467.78–555164.61)
MMP-2	17167.21 (7465.01–40088.94)	7585.02 (2289.15–11637.36)	<.001***	14182.18 (7465.01–40088.94)
FGF-23	49.49 (23.32–2325.92)	75.10 (16.03–106.33)	.213	40.26 (23.32–358.44)
ICAM-1	369245.19 (600.76–1288559.50)	370422.42 (21853.65–2978502.73)	.050	347645.91 (99434.69–1077122.50)
IL-8	9.94 (4.27–59.47)	34.38 (10.17–225.91)	<.001***	11.21 (4.27–42.79)
Pentraxin 3	1220.05 (170.36–8838.34)	2480.43 (1025.58–7054.81)	<.001***	1291.44 (579.48–6160.28)
TNF-α	13.46 (4.90–72.59)	35.17 (8.19–55.58)	<.001***	10.52 (5.40–44.50)
IL-1β	13.21 (1.47–44.60)	54.82 (19.83–72.31)	<.001***	14.89 (1.47–44.60)
IL-6	5.68 (1.93–54.76)	28.46 (8.24–34.69)	<.001***	5.59 (1.93–54.76)
MMP-8	1665.84 (180.50–14879.36)	5517.70 (2161.64–23805.90)	<.001***	1806.50 (681.11–14879.36)
E-Selectin	24935.13 (9784.72–60580.21)	30583.12 (13574.41–94895.98)	.034*	23094.21 (10797.25-59282.24)
VCAM-1	635225.96 (131680.40–1614700.00)	1702051.78 (278043.62–3668931.12)	<.001***	717514.80 (131.680.40–1614700.00)
MMP-9	15590.07 (2529.10 -51066.38)	24717.51 (11512.12–33950.52)	.009**	14817.26 (2960.72–51066.38)

Note: The values are given as median (minimum–maximum); *p*-value—difference between Median of pre-operative (T0) and 2-year post-treatment follow-up (R4) using Wilcoxon's test; *($p < .05$); **($p < .01$); ***($p < .001$).

Abbreviations: ADMA, asymmetric dimethylarginine; FGF, fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IL, Interleukin; MMP, matrix metalloprotease; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule.

$p = .002$, respectively) than in the healing ones ($p = .080$, $p = .037$ respectively) (Table S3). Similarly in the PS group, the reduction of hs-CRP, ADMA, and MMP-2 was also significant ($p = .036$; $p = .001$; $p = .005$ respectively) with higher reduction in ADMA and MMP-2 levels in healed cases ($p = .018$; $p = .028$, respectively) than healing ones ($p = .028$; $p = .068$, respectively) (Figure 2, Table 4, Table S4). None of the teeth reviewed at R4 was classified as failed radiographically.

Comparison between serum inflammatory biomarker levels at R3 (1-year post-treatment) and R4 (2-year post-treatment) in relation to COVID-19 infection

At R4, out of the 37 subjects, 9 subjects had a history of COVID-19 infection and 28 reported no known COVID-19 infection. Thirty-three subjects had COVID-19 vaccinations (21 had three doses, 12 had two doses), three subjects did not receive the vaccine, while one patient did not report data about the vaccination status.

All inflammatory markers showed significant changes from the R3 to R4 timepoints in both COVID-19 and non-COVID-19 groups. Although the median of difference of FGF-23, IL-1β, IL-8, Pentraxin 3, IL-6, MMP-8, and E-selectin in the COVID-19 group was slightly higher compared to non-COVID-19 group, the difference was not statistically significant ($p = .643$, $p = .476$, $p = .798$, $p = .316$, $p = .806$, 0.629 and $p = .977$ respectively). Only ICAM-1 showed a weak tendency ($p = .097$), where the median of difference was stronger in the Covid group (median 709.3) compared to non-covid group (median 19.0). Overall, the biomarker level variations were similar between subjects with and without a history of COVID-19 infection (Figure S1).

Comparison of HbA1C, LDL, HDL, TRG, TC, TC/HDL levels between pre-operative and 2-year post-treatment

Median levels of metabolic syndrome indicators including HbA1C, TC, LDL, TRG and HDL were lower at 2 years post-treatment (R4) compared to (T0), with statistically significant differences in levels of HbA1C ($p = .015$), TC ($p = .003$), LDL ($p = .003$) and HDL ($p = .003$). A significant reduction was seen in the levels of HbA1C ($p = .004$), TC ($p = .006$), HDL ($p = .039$) and LDL ($p = .001$) in the Re-RCT group from T0 to R4, whereas in PS group, there was a significant reduction in the levels of HDL ($p = .019$) (Figure 4).

Furthermore, a significant reduction was also found in the levels of HbA1c ($p = .009$), TRG ($p = .011$) and

		Healed		
		Median (min–max)		
R4	*p*-Value	T0	R4	*p*-Value
27057.99 (16101.72–52252.55)	.017*	50164.40 (5868.02–254.419.38)	22320.41 (6394.72–79057.88)	.019*
1109.00 (138.91–2446.04)	<.001***	6876.93 (104.19–7101.90)	1061.22 (435.47–1709.52)	.001**
8731.74 (4648.39–11637.36)	.003*	18686.72 (8293.51–29434.75)	6735.65 (2289.15–10556.08)	.001**
76.75 (27.10–106.33)	.279	52.43 (30.16–2325.92)	74.44 (16.03–94.35)	.609
316663.11 (100764.53–2467275.77)	.117	402029.97 (600.76–1288559.50)	426926.04 (21853.65–2978502.73)	.158
34.38 (10.17–157.94)	.008**	9.10 (4.39–59.47)	33.82 (18.12–225.91)	.002**
2196.21 (1025.58–5066.43)	.028*	1098.19 (170.36–8838.34)	2495.09 (1526.93–7054.81)	.011*
36.17 (10.09–48.84)	.004**	15.33 (4.90–72.59)	34.95 (8.19–55.58)	.006**
58.85 (19.83–72.31)	.005**	12.41 (2.68–41.08)	52.47 (21.85–63.72)	.001**
28.54 (8.24–32.69)	.028*	5.78 (2.81–14.22)	27.53 (11.03–34.69)	.001**
4825.86 (3453.37–16211.99)	.008**	1665.84 (180.50–9737.18)	5933.85 (2161.64–23805.90)	.003**
35166.10 (13574.41–94895.98)	.060	25370.12 (9784.72–60580.21)	27849.65 (17733.91–54734.28)	.221
1603026.69 (278043.62–3668931.12)	.002**	626333.02 (284815.96–1446300.00)	1724710.31 (599472.16–2188442.82)	.001**
20886.00 (12444.24–30400.26)	.272	16395.44 (2529.10–50787.52)	27539.68 (11512.12–33950.52)	.056

TC/HDL ($p = .022$) from the 1-year post-treatment review (R3) to R4. Following Re-RCT treatment, HbA1C, LDL, TRG, TC, and TC/HDL were further reduced from R3 to R4 with a significant reduction in HbA1C levels ($p = .017$) whilst there was an increase in HDL levels. In the PS group, there was a significant reduction in TRG ($p = .018$) whilst HDL levels increased between R3 and R4.

With respect to radiographic outcome clustering, in the healing group, there was a significant reduction between T0 and R4 in the levels of HbA1C and HDL ($p \leq .001$; $p = .030$, respectively), whereas in the healed group, TC and LDL levels ($p = .016$; $p = .008$, respectively) were significantly reduced.

HbA1C levels at 2 years (R4) were positively correlated with IL-8 ($r = 0.392$, $p = .039$) and MMP-8 levels ($r = 0.388$, $p = .041$) (Figure 5). Correlations were weak in magnitude but reached statistical significance.

Relationship between biomarkers at R4 and independent factors and covariates.

After adjusting the data for age, gender, HbA1C, LDL, HDL, TRG, TC, TC/HDL, and COVID-19 status using a multiple linear regression model, the results showed that higher HbA1C values increased mean levels of IL-8 ($\beta = 0.61$, $t = 3.01$, $p = .007$), whereas higher HDL values reduced mean levels of hs-CRP ($\beta = -0.53$, $t = -2.30$, $p = .035$) and higher TRG values reduced mean levels of VCAM-1 ($\beta = -0.48$, $t = -2.23$, $p = .038$). It was also found that COVID-19 infection increased MMP-9 levels ($\beta = 0.42$, $t = 2.09$, $p = .048$) since subjects with a previous COVID-19 infection had higher serum levels of MMP-9. Moreover, there was a reduction in ADMA levels in older subjects ($\beta = -0.47$, $t = -2.68$, $p = .013$) (Table 5). Medical history/medication and periodontitis history were irrelevant because of very few positive cases.

DISCUSSION

In the follow-up analysis of this longitudinal cohort study on serum biomarkers level in subjects with AP treated with root canal retreatment and surgical endodontics, the comparison between pre-operative and 2 years follow-up values showed a significant reduction in the levels of hs-CRP, ADMA and MMP-2, whereas all other biomarkers except FGF-23 and ICAM-1 showed a significant increase. Considering that more cases radiographically healed at 2-year compared to the 1-year follow-up, as expected from previous outcome studies (Molven et al., 2002; Orstavik, 1996), it is possible that the increased levels of IL-8, Pentraxin 3, TNF-α,

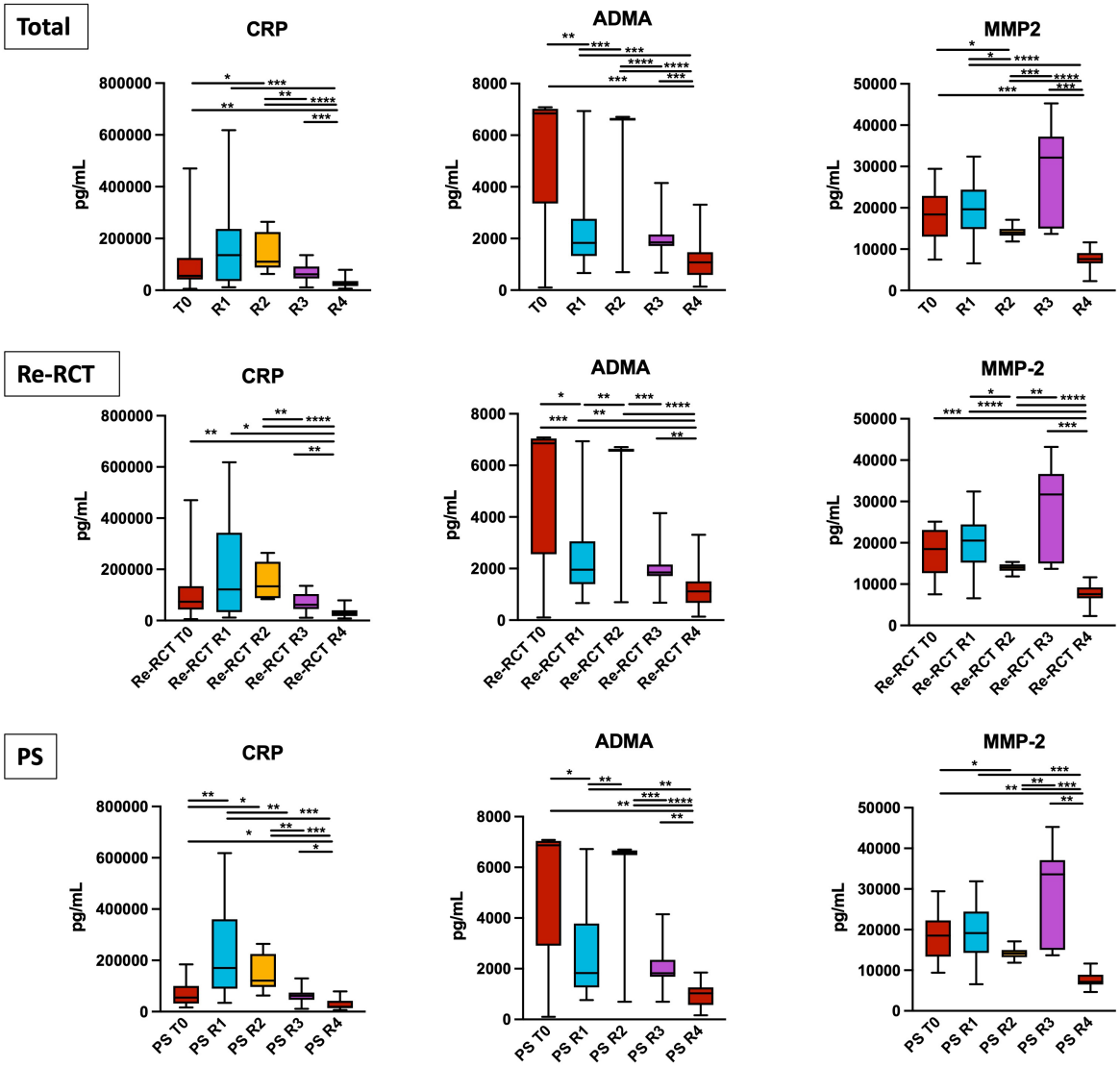

FIGURE 2 Serum levels of hs-CRP, MMP-2, ADMA and ICAM-1 in total, non-surgical root canal retreatment group (Re-RCT) and periapical surgery group (PS) of 37 subjects who were recalled at 2 year. Wilcoxon Signed-Rank test was used to compare the difference between Medians between preoperative (T0); 3 months post-treatment follow-up (R1); 6 months post-treatment follow-up; 1-year post-treatment follow-up (1 year) and 2-year post-treatment follow-up (2 year). *($p < .05$); **($p < .01$); ***($p < .001$); ****($p < .0001$).

IL-1β, IL-6 reflect the progression of this healing processes which entails complex host immune mechanisms (Maruyama et al., 2020). Our overall radiographic success rate was 100%, however, we still had 43.2% of cases ($n = 16$) that were healing. In the outcome study by Ng et al. (2011) which showed 80% success based on periapical health following re-RCT, the follow-up was for 2–4 years (Ng et al., 2011). Following ESE quality guidelines for endodontic treatment (European Society of Endodontology, 2006) we will be reviewing these cases annually up to 4 years.

Local immune response associated with AP can increase systemic inflammatory burden by activating the chronic systemic inflammatory response (Bakhsh et al., 2022; Gomes et al., 2013; Kimak et al., 2015; Márton et al., 1988; Torabinejad et al., 1983). Our previous study showed higher serum levels of IL-1β, hs-CRP, FGF-23 and ADMA in AP subjects compared with control cases (Bakhsh et al., 2022). However, Georgiou et al. (2023), indicated that at the baseline, AP cases with one asymptomatic tooth had lower levels of blood inflammatory mediators including CRP and IL-1β compared to the control group, which is inconsistent with our previous findings (Bakhsh et al., 2022). The effect on these serum inflammatory mediators is dose-dependent and can be affected by the increase in the size of periapical lesions as shown in our previous study (Bakhsh et al., 2022), and/or due to the

presence/absence of symptoms. Although both studies included subjects with only one AP tooth, the inconsistency could be attributed to the difference in the case selection, since in Georgiou et al. (2023) the 27 cases included were asymptomatic AP cases whereas in our study, the 65 AP subjects also included symptomatic AP cases. Such chronic low-grade inflammation can increase the risk of initiating endothelial dysfunction which can influence the onset, development, and systemic consequences of atherosclerotic plaques (Libby et al., 2002), a primary cause of mortality and morbidity in many developed countries (Tibaut et al., 2019).

CRP is a non-specific systemic inflammatory marker that can rise up to 1000-fold following tissue damage, infections and inflammation (Devaraj et al., 2009). In follow-up studies of apparently healthy individuals, elevated CRP levels have been associated with atherosclerosis leading to vascular events including acute myocardial infarction (MI), stroke, and peripheral artery disease (Ridker et al., 2002). For this reason, serum hs-CRP levels have been suggested as a screening marker for coronary heart disease risk (Luan & Yao, 2018). In our longitudinal study, hs-CRP levels were significantly reduced 2 years following Re-RCT and PS compared to pre-treatment and 1-year post-treatment levels, suggesting a long-term reduction of the systemic inflammatory burden associated with this mediator. At 2 years, more cases healed compared to 1-year post-treatment, which highlights hs-CRP as a potential biomarker of AP healing and indicates the potential significance of Re-RCT and PS in reducing CVD risks. Poornima et al. (2021), showed that the hs-CRP levels were significantly reduced at 6 months following the root canal treatment in subjects with AP (Poornima et al., 2021), whereas in our study reduction was slower and more significant at 2-year follow-up. Furthermore, our study included only AP cases with failed root canal treatments. This might be associated with a slower healing and hence slower hs-CRP reduction as compared with Poornima's primary treatments.

Raised serum levels of ADMA, an L-arginine analogue that inhibits nitric oxide (NO) production, have emerged as a significant risk predictor in cases of atherosclerosis, CVD and renal disorders (Cooke, 2000; Dückelmann et al., 2007; Fukuchi & Giaid, 1999; Kuvin et al., 2002; Leone et al., 1992; McDermott, 1976; Moncada & Higgs, 2006; Perticone et al., 2005; Schnabel et al., 2005; Wetzel et al., 2020). High serum ADMA levels are associated with renal dysfunction which reduces ADMA renal excretion leading to higher CVD risk in cases of kidney failure (Lajer et al., 2008; Sapa et al., 2022). In our previous study, the pre-operative levels of ADMA were significantly higher in AP subjects as compared to the controls (Bakhsh et al., 2022), which is consistent with other studies (Cotti et al., 2011; Georgiou et al., 2019). ADMA inhibition of NOS enzymes decreased the number of osteoblasts, rate of mineralization and bone production (Afzal et al., 2004; Aguirre et al., 2001). ADMA remains stable until a hydrolytic enzyme expressed by the osteoblasts—dimethylarginine dimethylamine hydrolase (DDAH) hydrolysed it (Lin et al., 2002). Recent research indicates a substantial correlation between plasma ADMA levels and DDAH1 Single Nucleotide Polymorphisms. In AP, it is likely that chronic inflammation reduced osteoblast DDAH1 activity resulting in increased ADMA levels which subsequently increases the risk of atherosclerosis, CVD and thrombotic stroke (Ding et al., 2010; Schulze et al., 2006; van der Zwan et al., 2011). Our previous study showed that the post-Re-RCT and PS, serum levels of ADMA were decreased at 3 months, but increased significantly from 3 to 6 months. However, at 1-year post-treatment, the ADMA levels were significantly reduced below baseline (Bakhsh et al., 2022). In the current study, at 2-year post-treatment follow-up, ADMA levels significantly reduced further, reaching levels which were 6 times lower than those at baseline. Rapone et al. (2022) did not see any effect of intensive periodontal treatment on the plasma ADMA levels in subjects without any risk factor for CVD (Rapone et al., 2022). In our longitudinal study, reduced serum concentration of ADMA after treatment indicates the clinical relevance and long-term impact of endodontic treatment (Re-RCT and PS) in reducing inflammatory biomarker burden associated with high ADMA serum levels and potential risk of CVD.

MMP-2 is a gelatinase that is involved in the physiological and pathophysiological processes of extracellular matrix degeneration, remodelling and tissue repair (Page-McCaw et al., 2007). In endodontic lesions, pulp fibroblasts produce MMP-2 which in turn degrades the extracellular matrix protein components of the pulp and aids in the pulpal inflammation, tissue destruction and periapical pathosis (Corotti et al., 2009; O'Boskey Jr. & Panagakos, 1998; Orsini et al., 2011; Shin et al., 2002). Pattamapun et al. (2017) demonstrated that MMP-2 levels in root canal exudates were reduced after root canal treatment, suggesting its role in the periapical healing (Pattamapun et al., 2017). Furthermore, MMPs play a crucial role in developing cardiovascular events including atherosclerosis, increased blood pressure, vascular dysfunction, arterial aneurysmal dilation and failure of vein grafts (Galis & Khatri, 2002; Prado et al., 2021). Increased MMP-2 proteolytic activity plays a significant role in the rupture of atherosclerotic plaques resulting

TABLE 3 Comparison of serum inflammatory biomarker levels between 1-year post-treatment (R3) and 2-year post-treatment follow-up (R4) based on PA radiographic outcome.

	Total			Healing
	Median (min–max)			Median (min–max)
	T3	R4	*p*-Value	T3
hs-CRP	61853.60 (11181.97–135433.96)	24283.22 (6394.72–79057.88)	<.001***	56547.88 (19293.46–135433.09)
ADMA	1851.36 (698.33–4152.19)	1073.94 (138.91–2446.04)	<.001***	1847.06 (1135.47–3519.09)
MMP-2	33490.66 (9907.22–45284.73)	7585.02 (2289.15–11637.36)	<.001***	33081.48 (13635.34–43553.09)
FGF-23	24.83 (25.99–45.48)	75.10 (16.03–106.33)	<.001***	25.30 (16.14–37.38)
IL-1β	17.05 (6.51–33.08)	54.82 (19.83–72.31)	<.001***	19.03 (8.53–29.62)
IL-8	12.58 (7.45–52.39)	34.38 (10.17–225.91)	<.001***	13.92 (9.11–52.39)
Pentraxin3	913.87 (35.28–3124.63)	2480.43 (1025.58–7054.81)	<.001***	975.62 (70.95–2361.16)
TNF-α	10.74 (4.91–19.80)	35.17 (8.19–55.58)	<.001***	9.75 (4.91–19.80)
ICAM-1	340761.89 (8331.98–1553783.89)	370422.42 (21853.65–2978502.73)	<.001***	296828.90 (213522.78–1171427.91)
IL-6	5.43 (2.12–64.52)	28.46 (8.24–34.69)	<.001***	5.39 (2.12–64.52)
MMP-8	2028.16 (592.24–9796.51)	5517.70 (2161.64–23805.90)	<.001***	2060.85 (990/07–4339.06)
E-selectin	20840.14 (8839.09–53907.74)	30583.12 (13574.41–94895.98)	<.001***	21758.78 (11316.07–37751.49)
VCAM-1	542040.52 (208772.28–990571.40)	1702051.78 (278043.62–3668931.12)	<.001***	531997.97 (208772.28–694208.14)
MMP-9	17181.87 (4565.60–53604.39)	24717.51 (11512.12–33950.52)	.008**	17181.87 (7518.58–53604.39)

Note: The values are given as Median (Minimum–Maximum); *p*-value—Difference between Median of 1-year post-treatment (R3) and 2-year post-treatment follow-up (R4) using Wilcoxon's test; *($p < .05$); **($p < .01$); ***($p < .001$).

Abbreviations: ADMA, asymmetric dimethylarginine; FGF, fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IL, Interleukin; MMP, matrix metalloprotease; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule.

in cardiovascular events (Brown et al., 1995; Kramsch et al., 1971; Newby, 2005; Rekhter, 1999). In our previous study, we found that MMP-2 levels fluctuated during the initial 1-year follow-up period (Bakhsh et al., 2022). Since there was an increased number of cases which were still in the healing category at 1-year follow-up, we found higher MMP-2 levels related to periapical tissue repair and remodelling. However, at 2-year recall, most of the cases healed completely, resulting in a significant reduction in MMP2 levels compared to 1-year post-treatment. The role of MMP-2 in periapical healing is further supported by the significantly higher levels found in the healing group as compared to the healed group at 2-year post-treatment.

The findings of our previous study (Bakhsh et al., 2022) suggested that AP potentiates metabolic syndrome (MetS) indicators, including waist circumference/BMI, triglycerides, HDL, HbA1C, and blood pressure. MetS is a known risk factor for CVD development (Expert Panel on Detection & Adults, 2001). At baseline, although within the normal range, the AP subjects had increased levels of these MetS indicators' compared to the control group (Bakhsh et al., 2022). Our current study clearly demonstrated a significant improvement in MetS indicators' levels at 2 years follow-up compared to both, their preoperative and 1-year recall levels, especially in Re-RCT cases. Previous studies have positively correlated AP with higher HbA1C levels (Arya et al., 2017; González Navarro et al., 2017; Segura-Egea et al., 2012, 2015). To our knowledge, this study is the first longitudinal study with a 2-year recall, which has demonstrated the positive impact of endodontic treatment (Re-RCT and PS) on MetS factors, and potential reduction of the risk of CVD development.

During the 2-year recall period, some of our subjects reported a history of SARS-CoV-2 infections. In both the COVID-19 and non-COVID-19 groups, all inflammatory markers showed significant variations between 1-year and 2-year recall. The COVID-19 group had a smaller number of subjects ($n = 9$) compared to non-COVID-19 group ($n = 28$). Although the median of difference of some inflammatory markers was slightly higher in subjects who had COVID-19 infection, there was no significant difference present in the median of difference between the COVID-19 and non-COVID-19 groups. Recent studies have shown that COVID-19 can lead to inflammation, contributing to COVID-19-associated endothelial dysfunction and inflammatory vasculopathy (Jud et al., 2021), which might explain the slightly higher median of difference seen in our study COVID-19 groups. Moreover, although not statistically significant there was a more obvious median of difference of ICAM-1 in

		Healed		
		Median (min–max)		
R4	*p*-Value	T3	R4	*p*-Value
27057.99 (16101.72–52252.55)	.017*	65655.91 (11181.97–129544.44)	22320.41 (6394.72–79057.88)	.003**
1109.00 (138.91–2446.04)	.002**	1908.79 (698.33–4152.19)	1061.22 (435.47–1709.52)	.001**
8731.74 (4648.39–11637.36)	.002**	33490.66 (9907.22–45284.73)	6735.65 (2289.15–10556.08)	.001**
76.75 (27.10–106.33)	.001**	24.57 (15.99–45.48)	74.44 (16.03–94.35)	.001**
58.85 (19.83–72.31)	.002**	15.26 (6.51–33.08)	52.47 (21.85–63.72)	.001**
34.38 (10.17–157.94)	.003**	11.65 (7.45–25.94)	33.82 (18.12–225.91)	.001**
2196.21 (1025.58–5066.43)	.002**	908.04 (35.28–3124.63)	2495.09 (1526.93–7054.81)	.001**
36.17 (10.09–48.84)	.003**	11.28 (6.36–19.34)	34.95 (8.19–55.58)	.001**
316663.11 (100764.53–2467275.77)	.182	477715.82 (8331.98–1553783.89)	426926.04 (21853.65–2978502.73)	.035*
28.54 (8.24–32.69)	.034*	5.43 (3.06–10.88)	27.53 (11.03–34.69)	.001**
4825.86 (3453.37–16211.99)	.002**	1960.28 (592.24–9796.51)	5933.85 (2161.64–23805.90)	.004**
35166.10 (13574.41–94895.98)	.003**	20244.44 (8839.09–53907.74)	27849.65 (17733.91–54734.28)	.048*
1603026.69 (278043.62–3668931.12)	.002**	585457.62 (214593.00–990571.40)	1724710.31 (599472.16–2188442.82)	.001**
20886.00 (12444.24–30400.26)	.084	16542.05 (4565.60–41542.30)	27539.68 (11512.12–33950.52)	.074

the COVID-19 group. This is consistent with a previous study, where raised serum soluble ICAM-1 and E-selectin were found to be linked with COVID-19 illness severity (Shi et al., 2022). Another finding was that the COVID-19 infection showed a significant positive association with serum levels of MMP-9. Studies have associated elevated serum MMP-9 levels with asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, respiratory failure and lung pneumonia (Atkinson & Senior, 2003; Ueland et al., 2020). Furthermore, MMP-9 was shown to have a positive and strong correlation with the severity of COVID-19 condition and mortality of the subjects (Gelzo et al., 2022; Ueland et al., 2020). Therefore, our finding of higher MMP-9 levels in the COVID-19 group is consistent with previous studies showing an overexpression of MMP-9 in COVID-19 subjects where it is involved in the processes leading to the intensive destruction of lung tissue and state of COVID-19 pneumonia (da Silva-Neto et al., 2022).

Although in our study, a higher recall at 2-year review would have been ideal, the recall of 56.9% ($n = 37$) was acceptable, especially during the COVID-19 pandemic. The total ($n = 37$) and Re-RCT recall ($n = 20$) were above our initial power calculation of 19 (Bakhsh et al., 2022). PS recall ($n = 17$) although lower than the power calculation, still showed significant results. The recall numbers are higher than other similar studies like Poornima et al., 2021 which showed power calculation and recall of 15 subjects (Poornima et al., 2021). Most subjects who did not attend the 2-year review appointment were those who could not be contacted or had relocated and could not justify or afford the cost and time of attending; a problem highlighted in the literature on recalling patients in clinical studies (Ross et al., 2009; Sprague et al., 2003).

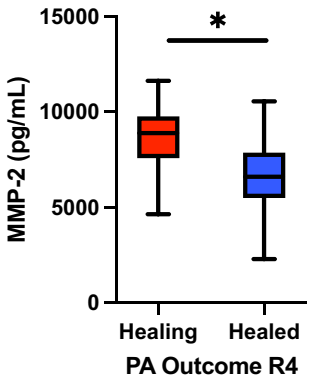

FIGURE 3 Comparison of the serum levels of MMP-2 at 2-year post-treatment follow-up (R4) based on PA radiographic outcome between healing group and healed group using a Wilcoxon Signed-Rank test; *($p < .05$).

TABLE 4 Comparison of the mean and median of differences in Inflammatory marker levels between R3 and R4 in relation to COVID-19 infection.

	COVID-19 R4				
	NO		Yes		
	Mean (SD)	Median (min–max)	Mean (SD)	Median (min–max)	*p*-Value
hs-CRP	−42479.01 ± 33006.19	−35680.28 (−116543.81 to 6236.23)	−33186.55 ± 31920.15	−34297.94 (−82257.15 to 8232.74)	.731
ADMA	−944.73 ± 885.10	−890.67 (−3399.74 to 43.03)	−918.13 ± 879.19	−954.73 (−2136.20 to 739.78)	.781
MMP-2	−20763.81 ± 12123.18	−24873.21 (−38239.15 to 4806.01)	−21008.29 ± 12365.46	−25163.69 (−35808.31 to 5747.08)	.932
IL-6	16.32 ± 16.70	19.56 (−50.61 to 31.02)	19.75 ± 8.61	21.87 (5.11 to 30.05)	.806
FGF-23	42.69 ± 20.40	45.21 (−17.43 to 69.56)	49.25 ± 7.81	50.73 (35.17 to 56.53)	.643
IL-1β	33.91 ± 12.52	35.36 (2.03 to 49.51)	38.66 ± 5.43	39.18 (30.64 to 45.20)	.476
IL-8	49.98 ± 60.97	20.38 (−3.44 to 215.45)	44.16 ± 64.37	21.60 (4.70 to 173.97)	.798
Pentraxin 3	2028.18 ± 1329.85	1506.17 (588.50 to 4584.05)	2374.69 ± 1047.64	2239.73 (1257.52 to 3930.18)	.316
TNF-α	22.90 ± 12.25	24.90 (−7.69 to 43.29)	24.04 ± 5.73	24.40 (16.61 to 30.00)	.712
ICAM-1	188913.62 ± 487151.54	19040.18 (−321976.53 to 1700091.65)	722602.89 ± 735810.60	709326.72 (−7145.94 to 1451502.09)	.097
MMP-8	4870.81 ± 3983.50	3201.30 (−377.68 to 13238.83)	5685.18 ± 5327.10	5143.06 (−1629.47 to 14009.40)	.629
E-selectin	10960.39 ± 16133.57	6968.02 (−16920.61 to 57144.49)	7435.95 ± 3812.30	7731.00 (1157.88 to 12323.03)	.977
VCAM-1	1100300.89 ± 718480.03	1115219.75 (−74375.94 to 3127442.65)	1013593.89 ± 395004.64	1007221.63 (500313.02 to 1555234.42)	.977
MMP-9	4856.77 ± 11813.69	6457.66 (−35871.85 to 22487.07)	5528.27 ± 11060.88	6203.49 (−8582.34 to 20525.30)	.932

Abbreviations: ADMA, asymmetric dimethylarginine; FGF, fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IL, Interleukin; MMP, matrix metalloprotease; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule.

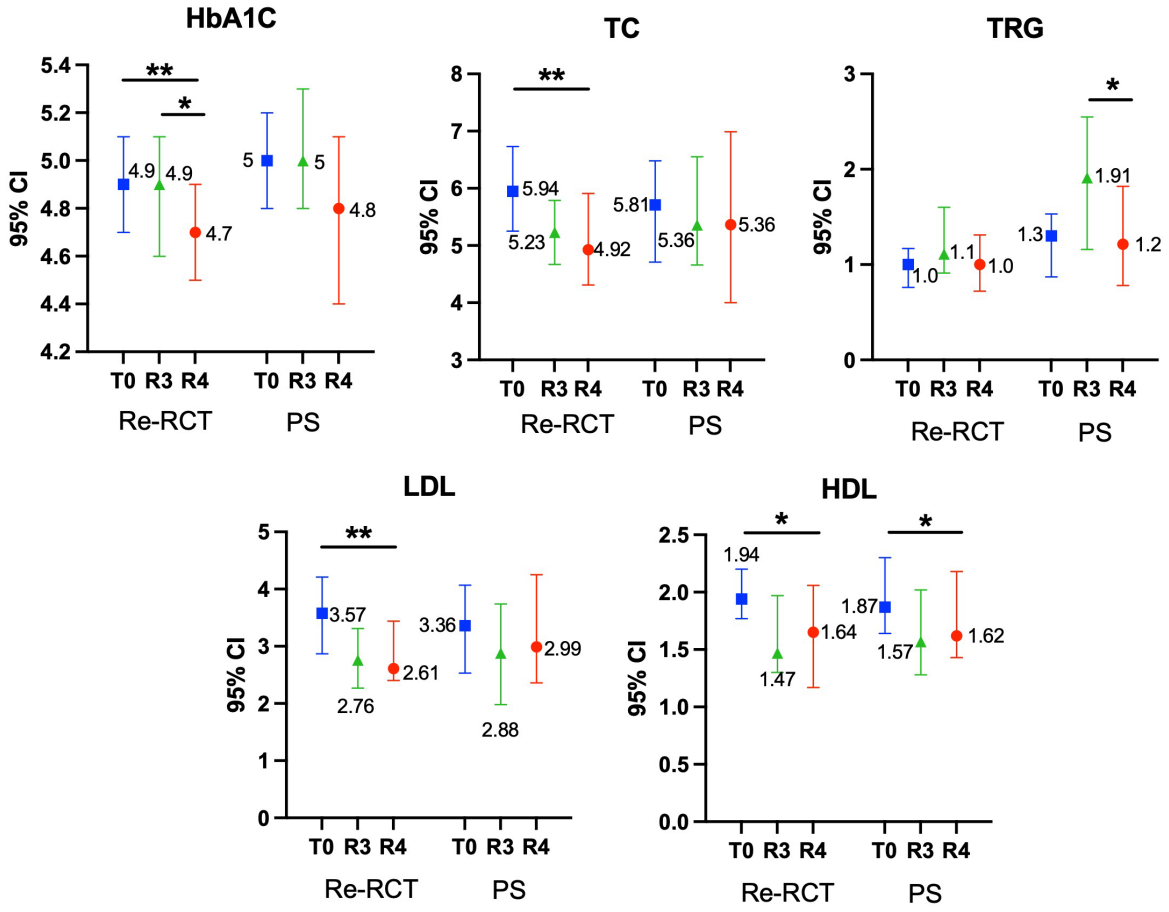

FIGURE 4 Mean values and 95% confidence interval for MetS indicators (HbA1C, TC, TRG, LDL and HDL) measured at pre-operative time (T0), 1-year post-treatment follow-up (R3) and 2-year post-treatment follow-up (R4) timepoint using a paired *t*-test. Re-RCT, non-surgical root canal re-treatment; PS periapical surgery; TC, total cholesterol; TRG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *($p < 05$); **($p < .01$).

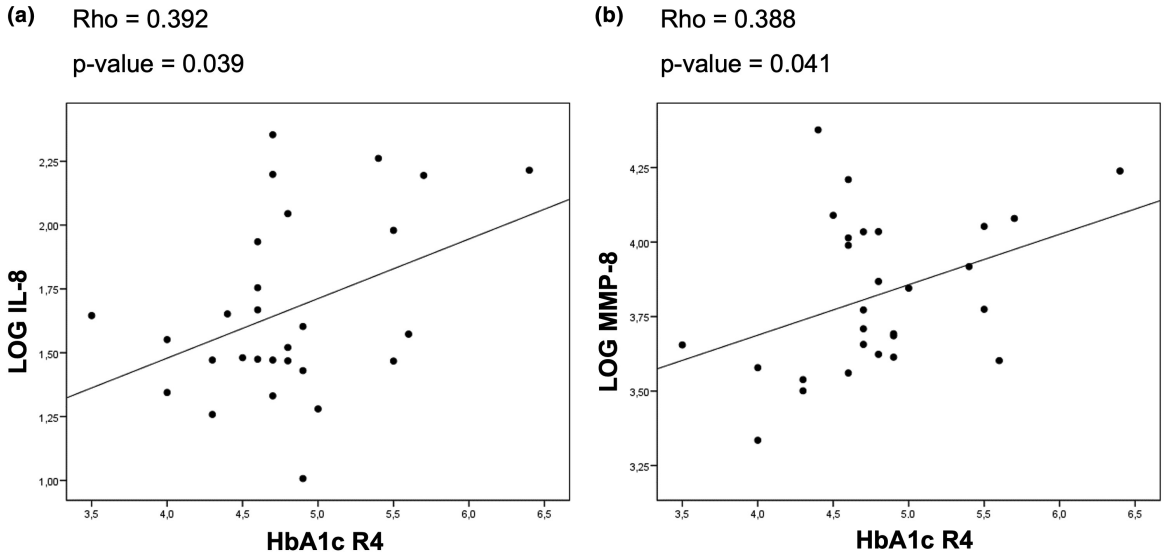

FIGURE 5 Correlation plots; (a) correlation between 2-year post-treatment follow-up (R4) HbA1c levels with levels of IL-8; (b) correlation between 2-year post-treatment follow-up (R4) HbA1c levels with levels of MMP-8. Increased HbA1C is associated with a significant increase in the level of IL-8 and MMP-8.

TABLE 5 Relationship between biomarkers at 2-year post-treatment follow-up (R4) and independent factors and covariates.

	Age	Gender	COVID R4	HbA1c R4	TC/HDL R4	TRG R4	HDL R4
hs-CRP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	B = -0.27 (−0.52 to 0.02) β = −0.53 t = −2.30 p = .035*
ADMA	B = −0.01 (−0.02 0.00) β = −0.47 t = −2.68, p = .013*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MMP-2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ICAM-1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FGF-23	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-8	n.s.	n.s.	n.s.	B = 0.37 (0.11 0.63) β = 0.61 t = 3.01 p = .007**	B = −0.15 (−0.29 to 0.01) β = −0.42 t = −2.25 p = .036*	n.s.	n.s.
Pentraxin 3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TNF-α	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-1β	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MMP-8	n.s.	n.s.	n.s.	B = 0.17 (−0.03 0.37) β = 0.388 t = 1.80 p = .086	n.s.	n.s.	n.s.
E-selectin	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
VCAM-1	B = 0.01 (0.00 0.02) β = 0.40 t = 2.06 p = .053	n.s.	n.s.	n.s.	n.s.	B = −0.14 (−0.28 to 0.01) β = −0.48 t = −2.23 p = .038*	n.s.
MMP-9	n.s.	n.s.	B = 0.14 (−0.12 0.11) β = 0.42 t = 2.09 p = .048*	n.s.	n.s.	n.s.	n.s.

Note: Results of multiple linear regression models for each log-transformed biomarker. For significant ($p < .05$) or marginally significant ($p < .1$) results, unstandardized beta coefficients (B) (95% CI), standardized beta coefficients (β), t-value and p-value are shown. Non-significant results are expressed as 'n.s.'.

Abbreviations: ADMA, asymmetric dimethylarginine; HDL, high-density lipoprotein; FGF, fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IL, Interleukin; MMP, matrix metalloprotease; TC, total cholesterol; TNF, tumour necrosis factor; TRG, triglycerides; VCAM-1, vascular cell adhesion molecule.

CONCLUSION

The reduction in levels of hs-CRP, ADMA and MMP-2 seen at 2-year post-treatment is associated with findings of higher radiographic success found at 2-year follow-up compared to 1-year post-treatment. Therefore, hs-CRP, ADMA and MMP-2 may be used as prognostic biomarkers of apical periodontitis healing following endodontic treatment (Re-RCT and PS). Furthermore, there was a significant improvement in MetS factors at 2 years post-treatment. Therefore, abrogation of apical periodontitis associated with endodontic infection not only leads to apical healing but might have clinical relevance in improving MetS factors and potentially reduce CVD risks.

AUTHOR CONTRIBUTIONS

Noor Al-Abdulla: data curation, project administration, investigation, validation, writing—original draft preparation, and writing—review and editing; Abdulaziz Bakhsh: data curation, formal analysis, investigation, validation, writing—original draft preparation, and writing—review and editing; Francesco Mannocci: methodology and writing—review and editing; Gordon Proctor: resources and writing—review and editing; David Moyes: methodology and writing—review and editing; Sadia Ambreen Niazi: conceptualization, methodology, data curation, formal analysis, funding acquisition, investigation, project administration, supervision, resources, validation, visualization, writing—original draft preparation, and writing—review and editing.

ACKNOWLEDGEMENTS

We would like to acknowledge Juan Luis Gómez Martínez for his service in the data statistical analysis.

FUNDING INFORMATION

Funding was received by the British Endodontic Society (Grant for Research Work) and European Society of Endodontology (Annual Research Grant). Sadia Ambreen Niazi is the principal grant holder of these grants.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The protocol of the study was approved by the London – Hampstead Research Ethics Committee (IRAS project ID 207795). The participants were given a detailed verbal and written information regarding the purpose of the study, and written consent was obtained in accordance with the Declaration of Helsinki.

ORCID

Noor Al-Abdulla https://orcid.org/0009-0003-2436-9646
Abdulaziz Bakhsh https://orcid.org/0000-0003-3228-2025
Francesco Mannocci https://orcid.org/0000-0002-0560-1054
Gordon Proctor https://orcid.org/0000-0002-5684-841X
David Moyes https://orcid.org/0000-0002-1657-918X
Sadia Ambreen Niazi https://orcid.org/0000-0003-0334-4119

REFERENCES

Afzal, F., Polak, J. & Buttery, L. (2004) Endothelial nitric oxide synthase in the control of osteoblastic mineralizing activity and bone integrity. *Journal of Pathology*, 202(4), 503–510.

Aguirre, J., Buttery, L., O'Shaughnessy, M., Afzal, F., Fernandez de Marticorena, I., Hukkanen, M. et al. (2001) Endothelial nitric oxide synthase gene-deficient mice demonstrate marked retardation in postnatal bone formation, reduced bone volume, and defects in osteoblast maturation and activity. *American Journal of Pathology*, 158(1), 247–257.

Arya, S., Duhan, J., Tewari, S., Sangwan, P., Ghalaut, V. & Aggarwal, S. (2017) Healing of apical periodontitis after nonsurgical treatment in patients with type 2 diabetes. *Journal of Endodontics*, 43(10), 1623–1627.

Atkinson, J.J. & Senior, R.M. (2003) Matrix metalloproteinase-9 in lung remodeling. *American Journal of Respiratory Cell and Molecular Biology*, 28(1), 12–24.

Bakhsh, A., Moyes, D., Proctor, G., Mannocci, F. & Niazi, S.A. (2022) The impact of apical periodontitis, non-surgical root canal retreatment and periapical surgery on serum inflammatory biomarkers. *International Endodontic Journal*, 55(9), 923–937.

Berlin-Broner, Y., Febbraio, M. & Levin, L. (2017) Apical periodontitis and atherosclerosis: is there a link? Review of the literature and potential mechanism of linkage. *Quintessence International*, 48(7), 527–534.

Bhatnagar, P., Wickramasinghe, K., Williams, J., Rayner, M. & Townsend, N. (2015) The epidemiology of cardiovascular disease in the UK 2014. *Heart*, 101(15), 1182–1189.

Brown, D.L., Hibbs, M.S., Kearney, M., Loushin, C. & Isner, J.M. (1995) Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation*, 91(8), 2125–2131.

Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L., Jr. et al. (2003) The seventh report of the joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA*, 289(19), 2560–2572.

Cooke, J.P. (2000) Does ADMA cause endothelial dysfunction? *Arteriosclerosis, Thrombosis, and Vascular Biology*, 20(9), 2032–2037.

Corotti, M.V., Zambuzzi, W.F., Paiva, K.B.S., Menezes, R., Pinto, L.C., Lara, V.S. et al. (2009) Immunolocalization of matrix metalloproteinases-2 and -9 during apical periodontitis development. *Archives of Oral Biology*, 54(8), 764–771.

Cotti, E., Dessì, C., Piras, A., Flore, G., Deidda, M., Madeddu, C. et al. (2011) Association of Endodontic Infection with detection of an initial lesion to the cardiovascular system. *Journal of Endodontics*, 37(12), 1624–1629.

Cotti, E., Zedda, A., Deidda, M., Piras, A., Flore, G., Ideo, F. et al. (2015) Endodontic infection and endothelial dysfunction are associated with different mechanisms in men and women. *Journal of Endodontics*, 41(5), 594–600.

da Silva-Neto, P.V., do Valle, V.B., Fuzo, C.A., Fernandes, T.M., Toro, D.M., Fraga-Silva, T.F.C. et al. (2022) Matrix metalloproteinases on severe COVID-19 lung disease pathogenesis: cooperative actions of MMP-8/MMP-2 Axis on immune response through HLA-G shedding and oxidative stress. *Biomolecules*, 12(5), 604.

Devaraj, S., Yun, J.M., Adamson, G., Galvez, J. & Jialal, I. (2009) C-reactive protein impairs the endothelial glycocalyx resulting in endothelial dysfunction. *Cardiovascular Research*, 84(3), 479–484.

Ding, H., Wu, B., Wang, H., Lu, Z., Yan, J., Wang, X. et al. (2010) A novel loss-of-function DDAH1 promoter polymorphism is associated with increased susceptibility to thrombosis stroke and coronary heart disease. *Circulation Research*, 106(6), 1145–1152.

Dos Santos Tiburcio-Machado, C., Batistin Zanatta, F., Schunke Gomes, M. & Souza Bier, C.A. (2021) Response to the letter to the editor regarding "the global prevalence of apical periodontitis: a systematic review and meta-analysis". *International Endodontic Journal*, 54(9), 1678–1681.

Dückelmann, C., Mittermayer, F., Haider, D.G., Altenberger, J., Eichinger, J. & Wolzt, M. (2007) Asymmetric dimethylarginine enhances cardiovascular risk prediction in patients with chronic heart failure. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(9), 2037–2042.

European Society of Endodontology. (2006) Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. *International Endodontic Journal*, 39(12), 921–930.

Expert Panel on Detection E, Adults ToHBCi. (2001) Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA*, 285(19), 2486–2497.

Figdor, D. (2002) Apical periodontitis: a very prevalent problem. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 94(6), 651–652.

Fukuchi, M. & Giaid, A. (1999) Endothelial expression of endothelial nitric oxide synthase and endothelin-1 in human coronary artery disease. Specific reference to underlying lesion. *Laboratory Investigation*, 79(6), 659–670.

Galis, Z.S. & Khatri, J.J. (2002) Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circulation Research*, 90(3), 251–262.

Garrido, M., Cardenas, A.M., Astorga, J., Quinlan, F., Valdes, M., Chaparro, A. et al. (2019) Elevated systemic inflammatory burden and cardiovascular risk in young adults with endodontic apical lesions. *Journal of Endodontics*, 45(2), 111–115.

Gelzo, M., Cacciapuoti, S., Pinchera, B., De Rosa, A., Cernera, G., Scialò, F. et al. (2022) Matrix metalloproteinases (MMP) 3 and 9 as biomarkers of severity in COVID-19 patients. *Scientific Reports*, 12(1), 1212.

Georgiou, A.C., Crielaard, W., Armenis, I., de Vries, R. & van der Waal, S.V. (2019) Apical periodontitis is associated with elevated concentrations of inflammatory mediators in peripheral blood: a systematic review and meta-analysis. *Journal of Endodontics*, 45(11), 1279–1295.e1273.

Georgiou, A.C., Twisk, J.W.R., Crielaard, W., Ouwerling, P., Schoneveld, A.H. & van der Waal, S.V. (2023) The influence of apical periodontitis on circulatory inflammatory mediators in peripheral blood: a prospective case-control study. *International Endodontic Journal*, 56(2), 130–145.

Gomes, M.S., Blattner, T.C., Sant'Ana Filho, M., Grecca, F.S., Hugo, F.N., Fouad, A.F. et al. (2013) Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. *Journal of Endodontics*, 39(10), 1205–1217.

González Navarro, B., Pintó Sala, X. & Jané Salas, E. (2017) Relationship between cardiovascular disease and dental pathology. Systematic review. *Medicina Clínica*, 149(5), 211–216.

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(10), 1374–1386.

Jimenez-Sanchez, M.C., Cabanillas-Balsera, D., Areal-Quecuty, V., Velasco-Ortega, E., Martin-Gonzalez, J. & Segura-Egea, J.J. (2020) Cardiovascular diseases and apical periodontitis: association not always implies causality. *Medicina Oral, Patología Oral y Cirugía Bucal*, 25(5), e652–e659.

Jud, P., Gressenberger, P., Muster, V., Avian, A., Meinitzer, A., Strohmaier, H. et al. (2021) Evaluation of endothelial dysfunction and inflammatory vasculopathy after SARS-CoV-2 infection—a cross-sectional study. *Frontiers in Cardiovascular Medicine*, 8, 750887.

Kakehashi, S., Stanley, H.R. & Fitzgerald, R.J. (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery, Oral Medicine, Oral Pathology*, 20, 340–349.

Khalighinejad, N., Aminoshariae, M.R., Aminoshariae, A., Kulild, J.C., Mickel, A. & Fouad, A.F. (2016) Association between systemic diseases and apical periodontitis. *Journal of Endodontics*, 42(10), 1427–1434.

Kimak, A., Strycharz-Dudziak, M., Bachanek, T. & Kimak, E. (2015) Lipids and lipoproteins and inflammatory markers in patients with chronic apical periodontitis. *Lipids in Health and Disease*, 14, 162.

Kramsch, D.M., Franzblau, C. & Hollander, W. (1971) The protein and lipid composition of arterial elastin and its relationship to lipid accumulation in the atherosclerotic plaque. *Journal of Clinical Investigation*, 50(8), 1666–1677.

Kuvin, J.T., Rämet, M.E., Patel, A.R., Pandian, N.G., Mendelsohn, M.E. & Karas, R.H. (2002) A novel mechanism for the beneficial vascular effects of high-density lipoprotein cholesterol: enhanced vasorelaxation and increased endothelial nitric oxide synthase expression. *American Heart Journal*, 144(1), 165–172.

Lajer, M., Tarnow, L., Jorsal, A., Teerlink, T., Parving, H.H. & Rossing, P. (2008) Plasma concentration of asymmetric dimethylarginine (ADMA) predicts cardiovascular morbidity and mortality

in type 1 diabetic patients with diabetic nephropathy. *Diabetes Care*, 31(4), 747–752.

Leone, A., Moncada, S., Vallance, P., Calver, A. & Collier, J. (1992) Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *The Lancet*, 339(8793), 572–575.

Libby, P., Ridker, P.M. & Maseri, A. (2002) Inflammation and atherosclerosis. *Circulation*, 105(9), 1135–1143.

Lin, K.Y., Ito, A., Asagami, T., Tsao, P.S., Adimoolam, S., Kimoto, M. et al. (2002) Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation*, 106(8), 987–992.

Luan, Y.-Y. & Yao, Y.-M. (2018) The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. *Frontiers in Immunology*, 9, 1302.

Márton, I., Kiss, C., Balla, G., Szabó, T. & Karmazsin, L. (1988) Acute phase proteins in patients with chronic periapical granuloma before and after surgical treatment. *Oral Microbiology and Immunology*, 3(2), 95–96.

Marton, I.J. & Kiss, C. (2014) Overlapping protective and destructive regulatory pathways in apical periodontitis. *Journal of Endodontics*, 40(2), 155–163.

Maruyama, M., Rhee, C., Utsunomiya, T., Zhang, N., Ueno, M., Yao, Z. et al. (2020) Modulation of the inflammatory response and bone healing. *Frontiers in Endocrinology*, 11, 386.

McDermott, J.R. (1976) Studies on the catabolism of NG-methylarginine, NG, NG-dimethylarginine and NG, NG-Dimethylarginine in the Rabbit. *Biochemical Journal*, 154(1), 179–184.

Mendis, S., Puska, P., Norrving, B., World Health, O., World Heart, F. & World Stroke, O. (2011) In: Mendis, S., Puska, P. & Norrving, B. (Eds.) *Global atlas on cardiovascular disease prevention and control*. Geneva: World Health Organization.

Milutinović, A., Šuput, D. & Zorc-Pleskovič, R. (2020) Pathogenesis of atherosclerosis in the tunica intima, media, and adventitia of coronary arteries: an updated review. *Bosnian Journal of Basic Medical Sciences*, 20(1), 21–30.

Molven, O., Halse, A., Fristad, I. & MacDonald-Jankowski, D. (2002) Periapical changes following root-canal treatment observed 20-27 years postoperatively. *International Endodontic Journal*, 35(9), 784–790.

Moncada, S. & Higgs, E.A. (2006) The discovery of nitric oxide and its role in vascular biology. *British Journal of Pharmacology*, 147(Suppl 1), S193–S201.

Myers, L. & Mendis, S. (2014) Cardiovascular disease research output in WHO priority areas between 2002 and 2011. *Journal of Epidemiology and Global Health*, 4(1), 23–28.

Nagendrababu, V., Segura-Egea, J.J., Fouad, A.F., Pulikkotil, S.J. & Dummer, P.M.H. (2020) Association between diabetes and the outcome of root canal treatment in adults: an umbrella review. *International Endodontic Journal*, 53(4), 455–466.

Nair, P.N. (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology and Medicine*, 15(6), 348–381.

Nair, P.N.R. (2006) On the causes of persistent apical periodontitis: a review. *International Endodontic Journal*, 39(4), 249–281.

Newby, A.C. (2005) Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiological Reviews*, 85(1), 1–31.

Ng, Y.L., Mann, V. & Gulabivala, K. (2011) A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. *International Endodontic Journal*, 44(7), 583–609.

Niazi, S.A. & Bakhsh, A. (2022) Association between endodontic infection, its treatment and systemic Health: a narrative review. *Medicina*, 58(7), 931.

O'Boskey, F.J., Jr. & Panagakos, F.S. (1998) Cytokines stimulate matrix metalloproteinase production by human pulp cells during long-term culture. *Journal of Endodontics*, 24(1), 7–10.

Orsini, G., Mazzoni, A., Orciani, M., Putignano, A., Procaccini, M., Falconi, M. et al. (2011) Matrix Metalloproteinase-2 expression induced by two different adhesive systems on human pulp fibroblasts. *Journal of Endodontics*, 37(12), 1663–1667.

Orstavik, D. (1996) Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. *International Endodontic Journal*, 29(3), 150–155.

Page-McCaw, A., Ewald, A.J. & Werb, Z. (2007) Matrix metalloproteinases and the regulation of tissue remodelling. *Nature Reviews: Molecular Cell Biology*, 8(3), 221–233.

Patel, S., Wilson, R., Dawood, A., Foschi, F. & Mannocci, F. (2012) The detection of periapical pathosis using digital periapical radiography and cone beam computed tomography – part 2: a 1-year post-treatment follow-up. *International Endodontic Journal*, 45(8), 711–723.

Pattamapun, K., Handagoon, S., Sastraruji, T., Gutmann, J.L., Pavasant, P. & Krisanaprakornkit, S. (2017) Decreased levels of matrix metalloproteinase-2 in root-canal exudates during root canal treatment. *Archives of Oral Biology*, 82, 27–32.

Pérez-Losada, F.L., Estrugo-Devesa, A., Castellanos-Cosano, L., Segura-Egea, J.J., López-López, J. & Velasco-Ortega, E. (2020) Apical periodontitis and diabetes mellitus type 2: a systematic review and meta-analysis. *Journal of Clinical Medicine*, 9(2), 540.

Perticone, F., Sciacqua, A., Maio, R., Perticone, M., Maas, R., Boger, R.H. et al. (2005) Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *Journal of the American College of Cardiology*, 46(3), 518–523.

Poornima, L., Ravishankar, P., Abbott, P.V., Subbiya, A. & PradeepKumar, A.R. (2021) Impact of root canal treatment on high-sensitivity C-reactive protein levels in systemically healthy adults with apical periodontitis – a preliminary prospective, longitudinal interventional study. *International Endodontic Journal*, 54(4), 501–508.

Prado, A.F., Batista, R.I.M., Tanus-Santos, J.E. & Gerlach, R.F. (2021) Matrix metalloproteinases and arterial hypertension: role of oxidative stress and nitric oxide in vascular functional and structural alterations. *Biomolecules*, 11(4), 585.

Rapone, B., Ferrara, E., Qorri, E., Dipalma, G., Mancini, A., Corsalini, M. et al. (2022) The impact of periodontal inflammation on endothelial function assessed by circulating levels of asymmetric dimethylarginine: a single-blinded randomized clinical trial. *Journal of Clinical Medicine*, 11(14), 4173.

Rekhter, M.D. (1999) Collagen synthesis in atherosclerosis: too much and not enough. *Cardiovascular Research*, 41(2), 376–384.

Ridker, P.M., Rifai, N., Rose, L., Buring, J.E. & Cook, N.R. (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *New England Journal of Medicine*, 347(20), 1557–1565.

Ross, C., Scheetz, J., Crim, G., Caicedo, R., Morelli, J. & Clark, S. (2009) Variables affecting endodontic recall. *International Endodontic Journal*, 42(3), 214–219.

Sapa, H., Gutiérrez, O.M., Shlipak, M.G., Katz, R., Ix, J.H., Sarnak, M.J. et al. (2022) Association of Uremic Solutes with Cardiovascular Death in diabetic kidney disease. *American Journal of Kidney Diseases*, 80(4), 502–512.e501.

Schnabel, R., Blankenberg, S., Lubos, E., Lackner, K.J., Rupprecht, H.J., Espinola-Klein, C. et al. (2005) Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene study. *Circulation Research*, 97(5), e53–e59.

Schulze, F., Lenzen, H., Hanefeld, C., Bartling, A., Osterziel, K.J., Goudeva, L. et al. (2006) Asymmetric dimethylarginine is an independent risk factor for coronary heart disease: results from the multicenter coronary artery risk determination investigating the influence of ADMA concentration (CARDIAC) study. *American Heart Journal*, 152(3), 493.e491–493.e498.

Segura-Egea, J.J., Castellanos-Cosano, L., Machuca, G., Lopez-Lopez, J., Martin-Gonzalez, J., Velasco-Ortega, E. et al. (2012) Diabetes mellitus, periapical inflammation and endodontic treatment outcome. *Medicina Oral, Patología Oral y Cirugía Bucal*, 17(2), e356–e361.

Segura-Egea, J.J., Martín-González, J. & Castellanos-Cosano, L. (2015) Endodontic medicine: connections between apical periodontitis and systemic diseases. *International Endodontic Journal*, 48(10), 933–951.

Shi H, Zuo Y, Navaz S, Harbaugh A, Hoy CK, Gandhi AA, Sule G, Yalavarthi S, Gockman K, Madison JA, Wang J, Zuo M, Shi Y, Maile MD, Knight JS, Kanthi Y (2022) Endothelial Cell–Activating Antibodies in COVID-19. *Arthritis & Rheumatology* 74(7), 1132–1138, Endothelial cell–activating antibodies in COVID-19.

Shin, S.-J., Lee, J., II, Baek, S.-H. & Lim, S.-S. (2002) Tissue levels of matrix metalloproteinases in pulps and periapical lesions. *Journal of Endodontics*, 28(4), 313–315.

Sprague, S., Leece, P., Bhandari, M., Tornetta, P., III, Schemitsch, E. & Swiontkowski, M.F. (2003) Limiting loss to follow-up in a multicenter randomized trial in orthopedic surgery. *Controlled Clinical Trials*, 24(6), 719–725.

Tibaut, M., Caprnda, M., Kubatka, P., Sinkovič, A., Valentova, V., Filipova, S. et al. (2019) Markers of atherosclerosis: part 1 – serological markers. *Heart, Lung & Circulation*, 28(5), 667–677.

Torabinejad, M., Theofilopoulos, A.N., Ketering, J.D. & Bakland, L.K. (1983) Quantitation of circulating immune complexes, immunoglobulins G and M, and C3 complement component in patients with large periapical lesions. *Oral Surgery, Oral Medicine, Oral Pathology*, 55(2), 186–190.

Ueland, T., Holter, J.C., Holten, A.R., Müller, K.E., Lind, A., Bekken, G.K. et al. (2020) Distinct and early increase in circulating MMP-9 in COVID-19 patients with respiratory failure. *Journal of Infection*, 81(3), e41–e43.

van der Zwan, L.P., Scheffer Peter, G., Dekker Jacqueline, M., Stehouwer Coen, D.A., Heine Robert, J. & Teerlink, T. (2011) Systemic inflammation is linked to low arginine and high ADMA plasma levels resulting in an unfavourable NOS substrate-to-inhibitor ratio: the Hoorn study. *Clinical Science*, 121(2), 71–78.

Vasan, R.S. & Benjamin, E.J. (2016) The future of cardiovascular epidemiology. *Circulation*, 133(25), 2626–2633.

von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gotzsche, P.C., Vandenbroucke, J.P. et al. (2014) The Strengthening the reporting of observational studies in epidemiology (STROBE) Statement: guidelines for reporting observational studies. *International Journal of Surgery*, 12(12), 1495–1499.

Wetzel, M.D., Gao, T., Stanley, K., Cooper, T.K., Morris, S.M., Jr. & Awad, A.S. (2020) Enhancing kidney DDAH-1 expression by adenovirus delivery reduces ADMA and ameliorates diabetic nephropathy. *American Journal of Physiology: Renal Physiology*, 318(2), F509–F517.

Wolf, D. & Ley, K. (2019) Immunity and inflammation in atherosclerosis. *Herz*, 44(2), 107–120.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Al-Abdulla, N., Bakhsh, A., Mannocci, F., Proctor, G., Moyes, D. & Niazi, S.A. (2023) Successful endodontic treatment reduces serum levels of cardiovascular disease risk biomarkers—high-sensitivity C-reactive protein, asymmetric dimethylarginine, and matrix metalloprotease-2. *International Endodontic Journal*, 00, 1–18. Available from: https://doi.org/10.1111/iej.13979