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Systemic Inflammation after third molar removal: a case-control study

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Keywords:	Systemic health/disease, Surgery, Oxidative stress
Abstract:	Third molar extraction is one of the most frequent intervention in dentistry. Nevertheless, there is scarce evidence on the host response of individuals with impacted or semi-impacted third molars and the possible effects of surgical removal. A case-control study of 40 patients was designed to evaluate 1) the differences in biomarkers of systemic inflammation, vascular function and metabolism (high sensitive C-reactive protein, lipids, fibrinogen, oxidative stress and endothelial function analysis) and 2) the acute and short-term effects of surgical removal between patients with bilateral impacted or semi-impacted third molars compared to controls with no third molars. Patients undergoing third molar extraction exhibited greater levels of systemic inflammation, oxidative stress and triglycerides than controls. Raised white cell counts as well as peaks of serum levels of C-reactive protein and fibrinogen were noticed in the first post-operative week. Three months after the extraction, all markers returned to baseline values. Malondialdehyde, an indicator of oxidative stress indicator, was

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	significantly reduced after third molar removal. Semi-impacted or impacted third molars are associated with higher systemic inflammation and their removal may represent a useful human model to study acute inflammation and determine beneficial systemic effects. (NCT03048175)

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Systemic Inflammation after third molar removal: a case-control study

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Abstract

Third molar extraction is one of the most frequent intervention in dentistry. Nevertheless, there is scarce evidence on the host response of individuals with impacted or semi-impacted third molars and the possible effects of surgical removal. A case-control study of 40 patients was designed to evaluate 1) the differences in biomarkers of systemic inflammation, vascular function and metabolism (high sensitive C-reactive protein, lipids, fibrinogen, oxidative stress and endothelial function analysis) and 2) the acute and short-term effects of surgical removal between patients with bilateral impacted or semi-impacted third molars compared to controls with no third molars.

Patients undergoing third molar extraction exhibited greater levels of systemic inflammation, oxidative stress and triglycerides than controls. Raised white cell counts as well as peaks of serum levels of C-reactive protein and fibrinogen were noticed in the first post-operative week. Three months after the extraction, all markers returned to baseline values. Malondialdehyde, an indicator of oxidative stress indicator, was significantly reduced after third molar removal.

Semi-impacted or impacted third molars are associated with higher systemic inflammation and their removal may represent a useful human model to study acute inflammation and determine beneficial systemic effects. (NCT03048175)

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Key words:

Wisdom Tooth, C-reactive protein, Endothelium, Third Molar, Systemic Inflammation, Oxidative Stress

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Introduction

Surgical extraction of lower third molars is the most frequent intervention in oral surgery (Shepherd and Brickley 1994). This procedure it is often associated with important post-surgical sequelae mainly of inflammatory nature (Mercier and Precious 1992). Nonetheless, little is known about the potential impact of surgical extraction of lower third molars on systemic inflammation.

Systemic inflammation has been implicated in the pathogenesis of many chronic conditions including cardiovascular disease and diabetes: a bulk of evidence links small changes of inflammatory biomarkers with increased future risk of vascular disease and mortality (Emerging Risk Factors Collaboration et al. 2010). Further, even transient increase of systemic inflammation may result in an increased risk of vascular events (Smeeth et al. 2004). Interestingly, simple medical procedures are associated with increased risk of acute vascular events in the post-operative period and the perturbation of the body inflammatory level is thought to be a plausible triggering mechanism (Mamode et al. 1995).

Recently, our group preliminary assessed the effects of lower third molars removal on systemic inflammation measured by serum levels of inflammatory biomarkers (Cei et al. 2012). Our findings indicated a mild inflammatory response of one-week duration characterized by increase in serum C-reactive protein, fibrinogen and leukocyte counts after third molar extraction. This offered the opportunity to study the third molar removal as a model of human inflammation and vascular dysfunction. Nevertheless, the preliminary study lacked a control group and raised several questions including whether the changes observed were due to a Hawthorne effect.

The aims of this study were: 1) to characterize the host response differences between individuals with or without lower third molar impaction and 2) to evaluate the effects of their surgical removal on biomarkers of systemic inflammation and endothelial function.

Materials and Methods

Experimental design and patients selection

This was a single-center case-control clinical trial with a 3-months follow-up designed according to STROBE guidelines. The study was approved by the local ethical committee, registered

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(NCT03048175) and it was conducted according to the declaration of Helsinki on experimentation involving humans.

Eligible study participants were identified among referrals for third molar extraction to the Unit of Dentistry and Oral Surgery of the University Hospital of Pisa (Italy). All participants gave written informed consent. Full medical and dental histories were recorded and the oral examination completed by experienced clinicians. A radiographic analysis was undertaken using an ortopantomogram.

Individuals were excluded if (a) younger than 18 years and older than 65 years; (b) females being pregnant or during lactation ; (c) females using contraceptive methods; (d) suffering from any reported systemic illness; (e) chronic use of any medication within 30 days prior to the study inclusion (f) affected by periodontitis (radiographic diagnosis of vertical bone defects or bone resorption equal to 20% of the root length); (g) periapical and periradicular radiolucencies were detected on X-rays; (h) unable to participate into the study.

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Control Group

Twenty consecutive patients, satisfying the inclusion criteria, referred to Unit of Dentistry and Oral Surgery, for caries, were invited to participate to the trial as a control group. Probands showed hypodontia/previous extraction or no pathologies affecting the lower third molars.

Tooth removal group

Twenty patients were enrolled in the tooth extraction group if presenting with a clinical indication for the bilateral surgical removal of mandibular third molars i.e. recurrent pericoronitis, caries, orthodontic reasons or pathological damage to the second molar. Surgery was performed by an experienced surgeon as previously described (Graziani et al. 2006). Briefly, after administering inferior dental and buccal nerve anesthesia, a triangular full thickness flap with a releasing incision on the mesio-buccal aspect of the second molar was designed. Ostectomy was then performed and the third molar was sectioned and gently elevated. Once all the tooth components were removed, the socket was carefully inspected and the flap sutured with single interrupted sutures. Duration of the surgery and ostectomy was recorded. Sutures were removed 7 days after surgical intervention. All patients were given post-operative instructions after the surgical intervention. A standard antibiotic therapy (amoxicillin and clavulanic acid, 2 g/die, 1 every 12h) for 5 days was prescribed. Penicillin-allergic participants

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were administered azithromycin 500mg/day for 3 days. Thirty days after the first surgical removal patients underwent the contralateral third molar extraction.

Examinations

Control group patients were examined at baseline and after 90 days (Fig. 1). At both visits blood, clinical and vascular parameters were collected. Tooth removal group patients were examined prior the first surgical procedure and all study parameters were collected. Thirty days after the first intervention, the contralateral third molar was extracted and new blood, clinical and vascular parameters were collected at 24 hours, and 7 and 90 days after the tooth removal.

Biomarkers analyses

Serum samples were collected from venipuncture of the antecubital fossa before 8.15 AM after an overnight fast for all patients. Blood samples were immediately processed and serum aliquots were then stored at -80C°. Serum markers of systemic inflammation assessed included: C-reactive protein (immuno-turbidimetric assay), fibrinogen (Clauss method) and White Cell Leucocyte Counts. All biomarkers were quantified using high-sensitivity assays. Oxidative stress was evaluated by measurement of plasma malondialdehyde by spectrophotometric assay (Esterbauer and Cheeseman 1990) and plasma lipoperoxides with a colorimetric method, as previously described (Jiang et al. 1992). Antioxidant capacity was measured as plasma total antioxidant capability by measuring ferric-reducing antioxidant power (spectrophotometric assay) (Benzie and Strain 1996).

Vascular Parameters

Participants were all examined at the Hypertension Unit after a 12-hour fasting period for arterial blood pressure measurement and vascular assessment. All measurements were taken in a quiet air-conditioned room (22-24°C). Patients were asked to refrain from smoking and drinking caffeine during the six hours prior to the assessment.

Averaged triplicate brachial blood pressure measurements at 2-min intervals with patients resting in supine position for at least 10 min were performed by using an automatic oscillometric device (OMRON-705IT, Omron, Kyoto, Japan).

Arterial stiffness was assessed as carotid to femoral pulse wave velocity and central augmentation index. Arterial tonometry (SphygmoCor, AtCor Medical, Sydney, NSW, Australia) was performed according to international recommendations (Laurent et al. 2006). Carotid to femoral pulse wave velocity was assessed sequentially recording pressure waveforms at the

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3 femoral and carotid site. Pulse wave velocity was calculated as the ratio of the surface distance between the two recording sites (subtracting the carotid-sternal notch distance from the femoral-sternal notch distance) and wave transit time. Transit time was estimated by the foot-to-foot (foot of the wave coincides with the systole beginning) method.

Central blood pressure values were obtained by tonomery from radial pressure waveform analysis based on a validated transfer function. Two consecutive measurements were recorded and averaged.

Endothelium-dependent response was assessed as flow-mediated dilation of the brachial artery using high resolution ultrasound as previously described (Ghiadoni et al. 2008; Bruno et al. 2012). Briefly, a B-mode scan of the right brachial artery was obtained in the longitudinal section between 5 cm and 10 cm above the elbow, with the probe held by a stereotactic clamp to ensure steady recordings. A pediatric cuff was placed around the forearm below the elbow, inflated for 5 min at 300mmHg, and then deflated to induce reactive hyperemia. Endothelium-independent was obtained by sublingual administration of a 25 µg of glyceryl trinitrate. Vessel diameter was measured using a real-time computerized edge detection system. Flow-mediated dilation and response to glyceryl trinitrate were calculated as maximal percentage increase in diameter above baseline. The intra-observer coefficient of variation for repeated flow-mediated dilation measurements was 14%. Arterial blood flow velocity was determined by pulsed Doppler signal at 70° angle, with the range gate in the center of the artery and measured at baseline and within 15s after cuff release (peak local shear stress calculated as 8*blood viscosity*mean flow velocity/brachial artery diameter, assuming that blood viscosity was 0.0035 Pa*s) (Mitchell et al. 2004). Vascular parameters were performed at baseline and 90 days in both study groups. Tooth removal group patients had these measurements taken at 31,37 days.

Clinical Oral Parameters

A single calibrated examiner recorded: probing pocket depth at the distal surface of the second lower molar, facial edema, trismus, and pain. Facial edema was evaluated by measuring the distance from the corner of the of the mouth to the attachment of the ear lobe following the bulge of the cheek, and the distance from the outer canthus of the eye to the angle of the mandible as previously described (Graziani et al. 2006). Trismus was the difference in inter-incisal distance at maximum opening (Ustün et al. 2003). Patients' pain perception was assessed via a simplified visual analogue scale of 100mm in length with "0" representing "no pain" up to "100"

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considered as the "most severe pain imaginable". This scale was further subdivided into four intervals (0-25 mm=no pain, 26-50= mild pain, 51-75=moderate pain, 76-100=maximum pain).

Statistical analysis

Data was entered, checked for errors and imported in a statistical software package (SPSS, IBM, Ver 23). Data is presented as mean and standard errors unless specified. Case-control comparisons were performed between study groups using Anova or Chi square statistics. If data was not normally distributed (even after log-transformations) equivalent comparative tests were used. Multivariate models were constructed using linear regression when statistical differences were observed (all models were adjusted for age, gender, BMI and smoking differences).

Comparison of inflammatory and vascular function parameters between study groups at baseline and 3 months were performed using a two-way ANOVA analysis (group and time), covariate in the models included age, gender, smoking and BMI values at baseline. Relative change differences were calculated subtracting follow-up values from the baseline/baseline and multiplied by 100 [example on C-reactive protein levels at day 1 after surgery: ((C-reactive protein day 1 - C-reactive protein at baseline)*100)/ C-reactive protein baseline].

Acute changes in inflammatory and vascular function biomarkers were compared between study visits in the test group by repeated ANOVA (baseline, day1, day7 and 3 months) as previously described (D'Aiuto et al. 2005). Correlation analyses between continuous outcomes were performed by Spearman rank tests. A p value less than 0.05 was deemed statistically significant.

The sample size calculation was based a pilot study (Cei et al. 2012) on acute increases of serum C-reactive protein following third molar removal. A minimum of 17 patients were needed to detect a 3.9 mg/l difference in serum C-reactive protein levels after 24 h (90% power, a 0.05, standard deviation of 4mg/l). A final sample of 20 participants per group was planned including a 10% drop-out rate.

Results

Population characteristics at baseline

Cases were balanced for age, smoking and BMI differences compared to controls with a slight imbalance for gender distribution (Table 1). When local inflammation was recorded at the

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gingival tissue surrounding semi-impacted third molars, it was also associated with a state of low-grade systemic inflammation and dyslipidemia. Indeed, statistically significant higher systemic concentrations in triglycerides, C-reactive protein, fibrinogen and malondialdehyde were observed in tooth removal group patients versus controls. No substantial differences were however found for all measures of vascular function and arterial distensibility. All patients completed the study with no loss to follow-up.

Inflammatory markers, Oxidative Stress and Cardiovascular Parameters

In the control group, no changes of systemic parameters/biomarkers were noted (Fig. 2 & Tab.2). Three months after the removal of the last impacted third molar, there were however reductions in C-reactive protein, fibrinogen and plasma lipoperoxides in tooth removal group patients versus controls but they did not reach statistical significance. Nevertheless, patients in the tooth removal group exhibited a statistically significant reduction (-17% versus 66%, $p=0.035$) of serum malondialdehyde levels when compared to controls (Appendix 1).

Following the extraction of third molars a mild systemic inflammation was observed during the first week. Statistically significant changes were observed for white blood cells ($p<0.001$), C-reactive protein ($p=0.004$), fibrinogen ($p<0.001$), plasma lipoperoxides ($p=0.001$) and ferric-reducing antioxidant power ($p=0.002$). An acute increase in Neutrophil counts and reduction in Lymphocytes were observed 24 hours following the surgical intervention (Fig. 2). Similarly, acute increases in C-reactive protein and fibrinogen were observed over the first week (with a peak one week after) following the dental extraction (Fig. 2). At 3 months, C-reactive protein levels were higher than baseline whilst fibrinogen levels were lower than baseline (Appendix 2). A strong positive linear correlation was confirmed for C-reactive protein with both fibrinogen ($R=0.4$, $p=0.007$) and white blood cells ($R=0.4$, $p=0.035$).

An altered oxidative status was observed in patients especially with regards to the ferric-reducing antioxidant power activity. Indeed, a statistically significant reduction was observed 24hrs and 1 week following the dental procedure. A strong positive correlation was found between fibrinogen and plasma lipoperoxides ($R=0.4$, $p=0.011$). No substantial correlation was observed between the serum levels, relative increase from baseline of acute phase and oxidative markers with intra-surgical measurements (ostectomy and surgery time, socket dimension, distance from CEJ and alveolar bone) (data not shown). The only exception was a strong negative

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correlation between recorded facial edema 7 days after third molar removal and relative increase of fibrinogen levels (R=-0.7, p=0.02).

A non-statistically significant reduction in flow-mediated dilation was observed during the acute inflammatory response. Flow-mediated dilation tended to be lower than baseline at one week follow-up after the second third molar removal when post-hoc multiple comparisons were made. A different trend was observed for response to glyceryl trinitrate (p=0.07). GTN was significantly reduced in comparison to baseline, 1 day after the second tooth extraction. Pulse wave velocity and central augmentation index remained unchanged throughout the study.

Flow-mediated dilation and GTN response in tooth removal group patients and controls were not different at baseline and after three months (p for interaction time per group 0.17 for flow-mediated dilation and 0.87 for glyceryl trinitrate). No differences were found for the other vascular parameters (data not shown). A moderate negative correlation was observed between ferric-reducing antioxidant power and flow-mediated dilation (R=0.3, p=0.038).

Oral Clinical Parameters

All third molars examined were partially impacted except for two cases with osteo-mucosal inclusion and no clinical communication with the oral cavity. At baseline patients presented with spontaneous (N=12) or triggered (N=9) pain, pus discharge (N=5), local inflammation (N=14) and some lympho-adenopathy (N=10). During the first post-operative period, ostectomy time correlated with development of edema on the first day after surgery (R=0.6, p=0.03). Patients presenting with pre-existing local inflammation around third molars experienced greater pain scores the day after the extraction (visual analogue scale scores from 52±15 to 29±20 and an average difference of 24, 95%CI 1-47, p=0.04). A statistically significant reduction in pocket probing depth between baseline and 3 months was detected on the distal aspect of the second molar in the tooth removal group (from 3.5±0.6mm to 2.9±0.5mm, with an average 0.6 mm average reduction, 95%CI 0.3-0.8, p<0.0001).

Discussion

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This case-control study indicated that patients in need of third molar extraction exhibited higher levels of systemic inflammation, oxidative stress and lower lipid fractions when compared to controls. Further, following the extraction of third molar a modest inflammatory response is mounted and sustained up to 1 week. Three months after tooth removal all biomarkers returned to values similar to baseline. No changes in endothelial function were observed. A modest impact of the post-surgical inflammation was observed for both endothelium-dependent and -independent vasodilation.

Third molar presence/pathology may be associated with systemic inflammation and an alteration of the overall patients' wellbeing. Indeed, the presence of third molars is associated with higher levels of C-reactive protein and IL-6 compared to patients with no visible third molars (Offenbacher et al. 2012). Moreover, young pregnant females with the presence of third molars exhibit more periodontitis and this may ultimately account for the higher level of systemic inflammation (Moss et al. 2007).

The baseline level of C-reactive protein in the tooth removal group was higher than the expected values reported in non-smokers healthy controls of same age and similar to those observed in patients with periodontitis (Tonetti et al. 2007). This host response linked to third molar was characterized by the high level of fibrinogen and malondialdehyde, indicating a higher systemic oxidative stress. Nevertheless, in this study we noted no impairment of vascular function between tooth removal patients and controls. One possible explanation for this discrepancy could be that the exposure to inflammation and oxidative stress is too short to determine a significant endothelial dysfunction especially as patients were all young individuals with a low cardiovascular risk profile. Alternatively, the inflammatory response associated with the impaction of third molar might be of insufficient magnitude to trigger endothelial dysfunction.

The first week following tooth removal was characterized by peaks of blood cells count, C-reactive protein and fibrinogen. This had been noted in the immediate post-surgical days in patients with high levels of systemic inflammation at baseline (Chander et al. 2013). Our data are also in agreement with previous evidences of the systemic impact of periodontal treatment. Indeed, periodontal treatment, both non-surgical and surgical, determine a moderate response of the systemic inflammation as shown by an increase of C-reactive protein and other systemic inflammatory bio-markers 24 hours after treatment (D'Aiuto et al. 2004; Graziani et al. 2010; Graziani et al. 2015). However the host response following tooth removal is still detected at day 7 whereas periodontal treatment triggers a faster but greater systemic inflammatory response.

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3 We can only speculate on the possible reasons behind these differences. Following periodontal treatment, the systemic inflammatory response is likely to be determined by both bacteremia and local tissue trauma which ultimately may determine a release of inflammatory mediators and acute-phase proteins (Birkedal-Hansen 1993; Gabay and Kushner 1999). In the periodontal model, non-surgical periodontal treatment determines a higher level of post-operative inflammation compared to surgical. This might be because periodontal surgery is usually performed in a previously decontaminated area. Conversely, third molar surgery is usually performed in a non-decontaminated wound (Rajasuo et al. 2004). It is also true that using not specific markers of systemic inflammation in both models does not allow interpretation of how much the local inflammation would contribute to the overall inflammatory burden and how much this is produced by the surgical trauma. Further research using more specific biomarkers linked to the local infectious inflammatory burden should be performed.

An association between oral and atherosclerotic inflammation has been reported (Subramanian et al. 2013). In this study the low-magnitude acute inflammatory response following the surgical procedure confirms the modest reduction of endothelium-dependent vasodilation observed when compared to that observed following whole mouth non-surgical periodontal treatment (Tonetti et al. 2007). A trend to reduction in endothelium-independent vasodilation in the brachial artery was noted. Although a time-dependent reduction in glyceryl trinitrate was reported, this finding did not reach statistical significance. It is conceivable that the methodology used (low-dose nitrates) produced a degree of vasodilation similar to that of flow-mediated dilation, did not allow detection of subtle differences in the acute phase following tooth removal. This study findings suggest that smooth muscle function may be compromised by acute inflammation and this should be further investigated.

Removal of third molar might have also a mid-term systemic beneficial effect as assessed by a reduction of malondialdehyde. Conversely, endothelial function did not improve at the final evaluation. This apparent discrepancy with other models of oral inflammation may be explained with the apparent better health status and younger age of our study population, lesser duration of exposure to chronic inflammation and shorter follow – up period for vascular assessment.

Lastly, the removal of the third molar determines consistent post-surgical sequelae affecting the oral cavity and facial tissues in the immediate days following the surgery. The time spent intrasurgically to remove bone correlates with the amount of post-surgical edema and this is consistent with previous data reported by our group (Graziani et al. 2006). Interestingly, the

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facial edema observed following third molar removal was also correlated with fibrinogen serum levels post tooth removal as further confirmation of an important relationship between local and systemic inflammation.

The authors are aware of the strengths and limitations of the study. On the one hand information on the bacteremia following the tooth removal might have helped in clarifying possible sources of the host response. A non-randomized design and limited follow-up are recognized as limitations of the study. On the other hand, a larger array of biomarkers of systemic inflammation and vascular parameters used in the study provides evidence for a novel model to study systemic inflammation in humans.

In conclusion, third molar removal is associated with a transient systemic inflammatory response of one-week duration. Three months after third molars removal, a reduction in oxidative stress is observed. Conversely, endothelial function was not affected by the presence and removal of third molars. The potential systemic benefits of third molars removal merits further investigation.

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Figure Legend

Figure 1 Study Design

Figure 2. Mean (SE) changes in white blood cells (WBC) (Fig. 2a), Neutrophils (Fig. 2b), Lymphocytes counts (Fig. 2c), C-reactive protein (CRP) (Fig. 2d), fibrinogen (Fig. 2e), flow-mediated dilation (FMD) (Fig. 2f), malondialdehyde (MDA) (Fig. 2g), plasma lipoperoxides (LOOH)(Fig. 2h), ferric-reducing antioxidant power (FRAP) (Fig. 2i) in both tooth extraction and control group. Stars indicated statistically significant differences from baseline.

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Table 1.

Case control comparison at baseline

	GROUP		
	Tooth Removal (N=20)	Control (N=20)	*P value*
Age, years	25±5	26±4	0.480
Gender, Male (%)	7(35)	14(70%)	0.06
Smoking, current (%)	7(35%)	8(40%)	1.0
BMI, kg/m^2	22.4±4.4	21.8±2.3	0.559
WBC,10^3 cells/ml	6.94±2.53	6.23±1.16	0.725
Systolic BP, mmHg	120.90±14.97	125.15±12.39	0.311
Diastolic BP, mmHg	67.80±8.62	66.90±9.34	0.690
Cholesterol, mmol/l	178.50±33.10	174.60±39.91	0.603
HDL, mmol/l	53.85±12.42	60.10±18.64	0.354
Triglycerides, mmol/l	105.15±30.80	81.45±36.19	**0.016**
Glucose, mmol/l	81.35±12.21	67.55±30.25	0.104
AIx @75 bpm	-5.4±13.4	4.1±17.3	0.979
PWV (carotid_femoral),%	5.60±0.80	5.57±0.81	0.748
FMD, %	8.25±3.15	7.36±3.09	0.297
RH, %	660.65±374.92	504.76±411.65	0.096
GTN, %	8.30±3.68	7.16±2.16	0.255
CRP, mg/l	3.42±5.62	0.93±1.05	**0.028**
Fibrinogen, g/dl	255.70±64.86	226.65±46.28	**0.038**
MDA, μmol/l	2.68±1.13	1.49±0.91	**<0.001**
LOOH, μmol/l	2.00±2.20	1.47±4.67	0.547
FRAP, mmol/l	673±181	644±156	0.642

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Table 2

Mean values (± SD) of study parameters in the control (first line) and tooth removal (second line) groups at Baseline and 3 months. P-value refers to the difference between study groups after 3 months.

Variable	**Baseline**	**3 Months**	***p value***
Red cells, 10^6 cells/ml	4.91±0.43	4.81±.52	0.968
	4.83±0.45	4.69±.54	
White cells, 10^3 cells/ml	6.23±1.16	6.48±2.91	0.402
	6.03±1.37	6.14±2.03	
Platelets, 10^3 cells/ml	271.95±59.83	278.35±66.56	0.499
	266.75±59.58	251.94±66.35	
Fibrinogen, g/dl	226.65±46.28	255.70±64.86	0.304
	227.35±54.38	237.15±61.72	
Cholesterol, mmol/l	174.60±39.91	178.50±33.10	0.894
	174.65±36.85	178.12±37.18	
HDL, mmol/l	60.10±18.64	53.85±12.42	0.053
	54.80±12.83	58.23±11.34	
Triglycerides, mmol/l	81.45±36.19	105.15±30.80	0.624
	107.60±31.92	91.06±58.50	
Glucose, mmol/l	67.55±30.25	81.35±12.21	0.311
	64.90±36.21	83.00±8.03	
Ca	85.25±7.24	9.55±.57	0.885
	84.90±6.27	9.56±.44	
CL	11.73±9.25	104.82±3.24	0.511
	9.63±0.30	103.18±4.02	
Na	141.30±1.78	140.00±2.08	0.621

	141.00±2.13	139.59±1.91	
K	4.00±.21	3.97±.29	0.852
	4.05±0.31	3.97±.25	
Systolic BP, mmHg	125.15±12.39	120.90±14.97	0.918
	121.60±7.84	117.83±13.01	
Dyastolic BP, mmHg	66.90±9.34	67.80±8.62	0.624
	65.25±7.76	65.61±7.44	
PWV (carotid_femoral),%	5.57±0.81	5.60±0.80	0.405
	5.62±0.57	5.74±1.80	
FMD, %	7.36±3.09	8.25±3.15	0.323
	7.03±2.39	7.03±2.74	
RH, %	504.76±411.65	660.65±374.92	0.221
	339.87±247.62	603.01±427.85	
GTN, %	7.16±2.16	8.30±3.68	0.729
	7.15±2.34	7.97±4.08	
CRP, mg/l	0.93±1.05	0.68±0.76	0.021
	3.42±5.62	2.53±3.06	

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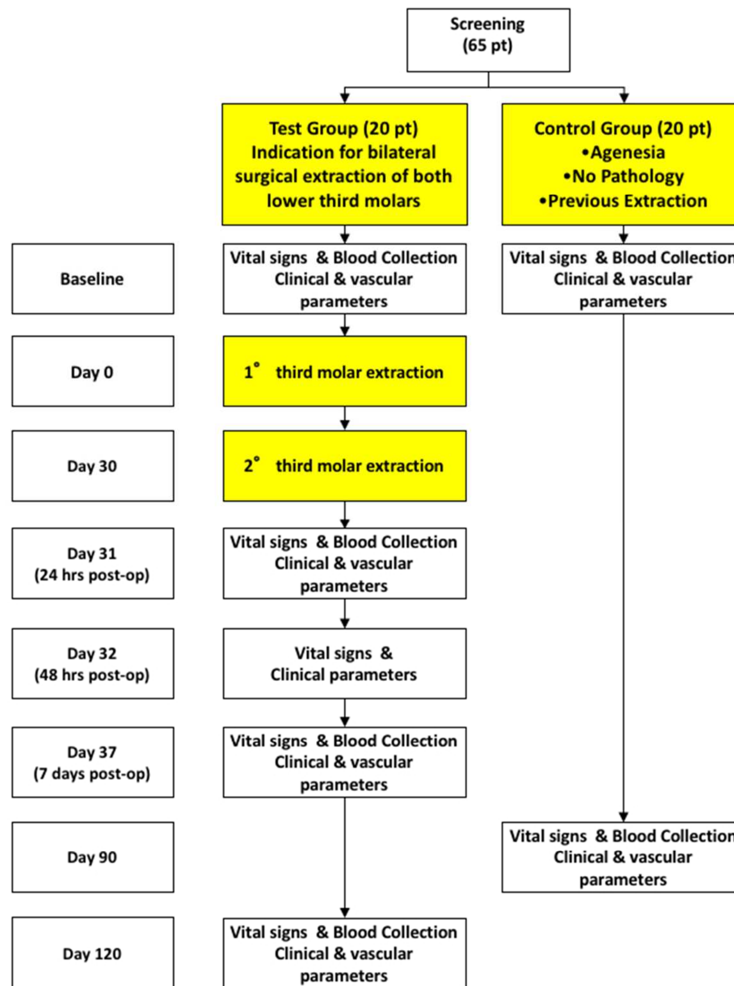

Study Design

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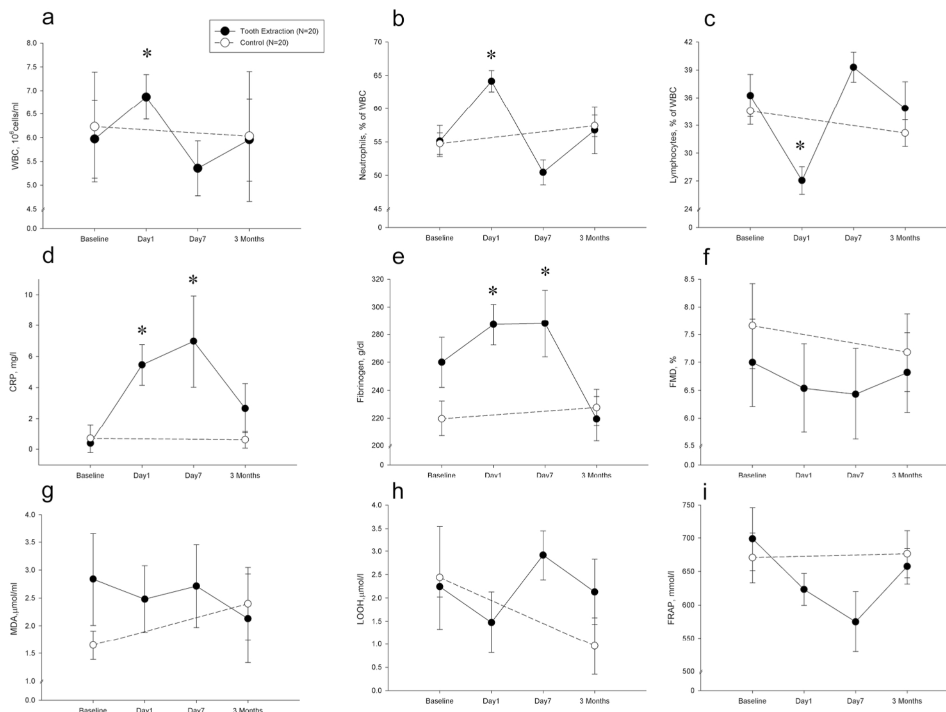

Mean (SE) changes in white blood cells (WBC) (Fig. 2a), Neutrophils (Fig. 2b), Lymphocytes counts (Fig. 2c), C-reactive protein (CRP) (Fig. 2d), fibrinogen (Fig. 2e), flow-mediated dilation (FMD) (Fig. 2f), malondialdehyde (MDA) (Fig. 2g), plasma lipoperoxides (LOOH)(Fig. 2h), ferric-reducing antioxidant power (FRAP) (Fig. 2i) in both tooth extraction and control group. Stars indicated statistically significant differences from baseline. ‼ ┼

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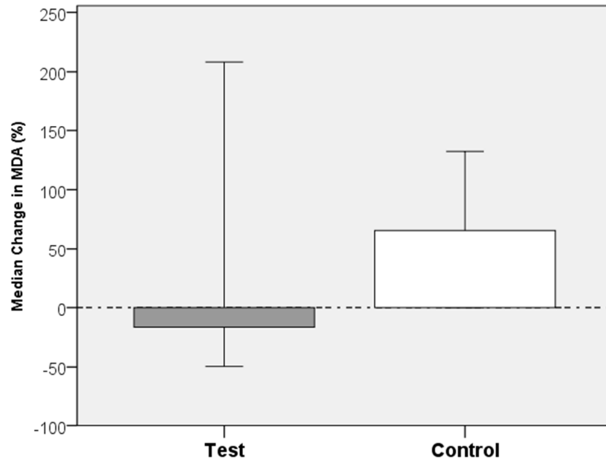
Appendix 1



Relative changes of malondialdehyde among two groups in 90 days.

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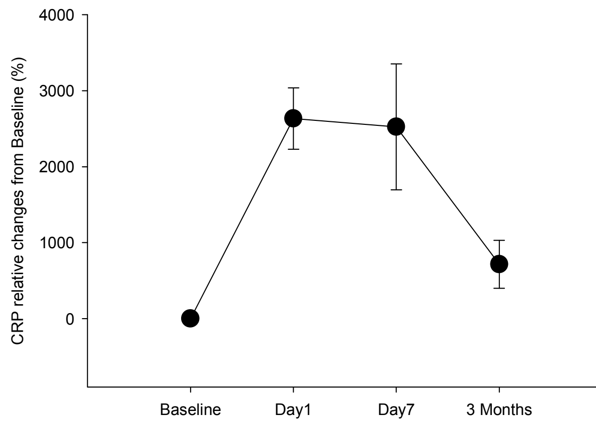
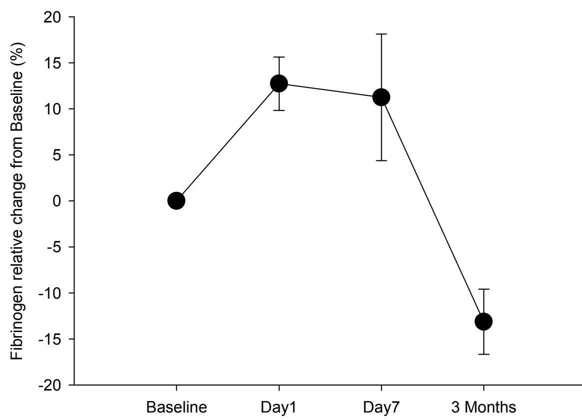
Appendix 2



Relative changes of CRP within the tooth extraction group



Relative changes of fibrinogen within the tooth extraction group

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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	**Recommendation**	**Page**
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	3-4
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-6*
Bias	9	Describe any efforts to address potential sources of bias	4-7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how matching of cases and controls was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7 – Appendix 1
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Appendix 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable	NA

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		of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	7-9, Fig. 1-4
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-9
		(*b*) Report category boundaries when continuous variables were categorized	7-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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