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PERIODONTITIS-INDUCED CHANGES TO VITAMIN E LEVELS AND THEIR EFFECTS ON THE HEART, LIVER AND BRAIN IN AN EXPERIMENTAL RAT MODEL

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ABSTRACT

Background: In our study, we observed how periodontitis changes the vitamin E levels of the heart, liver and brain due to free radicals and their effects. The main problem of periodontitis is the increasing of free radicals and their subsequent metastatic impact on multiple organs via the bloodstream from the periodontium .

Materials and methods: Two randomized groups were prepared between 18 Wistar albino rats. The rats weighed 200 ± 20 g and were male. Group C was the control group (n = 8) and group P was the periodontitis group (n = 10), induced using a 3-0 silk ligature at 14 days. The heart, liver and brain were dissected from the body to assess how they were affected by periodontitis. As such, we investigated the vitamin E levels and malondialdehyde (MDA) in the heart, liver and brain affected by experimental periodontitis .

Results: Serious alveolar bone resorption was observed in the periodontitis group when compared to the control group. As an oxidative stressor, MDA levels were very high in the heart, liver and brain tissues of the periodontitis group. The antioxidant vitamin E showed decreasing potential, conversely to MDA. All of the results significantly differed ($p < 0.001$).

Conclusion: Periodontitis stimulates an oxidative stress attack in different organs in an experimental periodontitis model. The balance between oxidants and antioxidants is very important for health, though this balance might diminish in oxidants due to the oxidative attacks triggered by periodontitis . However, other scientific studies must be conducted to confirm this.

INTRODUCTION

Periodontitis is a chronic inflammatory disease that induces free oxidative radicals and causes spreading damage in periodontal tissues. The primary aetiology of periodontitis is an oral bacterial infection. Periodontal infections and free radicals can affect multiple organs and be the bad hero of several diseases and conditions, such as diabetes mellitus, cardiovascular disease, pre-term or low birth, kidney diseases and hepatic diseases (1). The relationship between periodontitis and systemic diseases is well-known in the literature. Although periodontitis's real mechanism is still unclear, the realistic approach is that bacterial pathogens invade tissues, then host inflammatory responses to bacterial attacks and their products, such as lipopolysaccharide (LPS) and proteases (2). In this long-term challenge, the first issue of the immune system is starting polymorphonuclear leukocyte (PMNL) accumulation and producing reactive oxygen species (ROS) (3). The longer an inflammatory reaction takes, the more ROS are released. This triggers an imbalance between antioxidants and oxidants. In addition, increasing ROS levels occur not only because of an inflammatory or systemic attack, but also because of antioxidant reserves melting. This then increases lipid peroxidation and produces lipid peroxides (LPO) and malondialdehyde (MDA) (4). LPO is the key element in this reaction chain. Many studies have reported that high LPO production and metastatic move to other organs by way of blood in periodontitis cases (1). The toxic LPO attacks in this cycle, as active in

periodontitis, gradually affect multiple organs, such as the brain (5), heart (6), kidneys (7) and liver (8).

Vitamin E is a non-enzymatic, fat-soluble antioxidant (9) that protects cellular structures against harmful free radicals and LPO (10). A previous study showed that vitamin E has inhibitory effects on oxidative stress in the heart, liver and kidneys (11). Vitamin E strongly supports humoral immune responses by causing antibody-producing cells to proliferate and develop. As known from previous studies, periodontitis suppresses plasma's total antioxidant status (12). Accordingly, some authors suggest the intake of additive nutrients to decrease gingival inflammation (13). However, this treatment alternative requires further investigation.

The purpose of this study is to determine how periodontitis alters vitamin E levels in the heart, liver and brain.

MATERIALS AND METHODS

Animals: The study involved 18 male Wistar albino rats. The rats weighed 200 ± 20 g and were systematically and periodontally healthy. The rats were checked according to the 'Süleyman Demirel University Care and Use of Laboratory Animals' guide. Study groups were randomly designated into group C (control, periodontally healthy, n = 8) or group P (periodontitis, n = 10). The rats were kept in standard environmental conditions, ate standard rat food and drank standard tap water. The cage temperature was set at 24.0 ± 0.6°C and the cages were kept in the dark for half the study time (the lights were turned on at 07.30). These were the conditions provided during the study.

Induction of experimental periodontitis: Periodontitis was induced by fixing sterile 3-0 silk ligatures* subgingivally around the maxillary second molars under general anaesthesia. The rats were euthanized after 14 days. Then, the periodontitis findings were observed in all of the rats that belonged to the experimental group.

Measurement of alveolar bone loss: Bone resorption levels were measured at three buccal points between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC) using a stereomicroscope (×4 magnification). CEJ signs were present after 24 hr in 3% H_2O_2 and after 1 min with 1% methylene blue bedding (14, 15). The data were recorded with standardized digital photographs** and analyses were completed using a software programme ¶. The study began after the confirmation of an ethical council at Süleyman Demirel University. The serum materials were collected and the maxillae separated from the heads and divided into two parts; the right sides were examined to observe the alveolar bone resorption performance via histomorphometric analysis, and the left sides were used to confirm periodontitis's effects on the organs via histopathological analysis.

Histomorphometric and histopathological analyses: For 2 weeks, 0.1 M EDTA and 10% buffered formalin solution were used to decalcify the left maxillae. The samples were embedded in paraffin after washing and dehydration. The paraffin parts were divided into 5 µm-thick sections in the mesiodistal direction along the long axis of the tooth, then stained with haematoxylin and eosin (H&E). Histopathological values were scored as a modification of Leitão et al.'s technique (16). The Manual Cell Sens Life Science Imaging Software System 7 was used for the morphometric analyses of alveolar bone loss and PMNL infiltration. The number of PMNLs in the junctional epithelium and connective tissue (in a 0.05 mm × 0.05 mm area) were evaluated and counted under ×40 magnification.

LPO and MDA levels: The serum samples' LPO levels were analysed via the Placer method (17). The quantification of thiobarbituric acid (TBA) reactive substances was determined by comparing their absorption to the standard curve of MDA equivalents generated by acid-catalysed hydrolysis of 1,1,3,3 tetramethoxypropane. A pink colour was the key step in determining what was produced to form a coloured MDA–TBA adduct in the interaction of TBA with MDA. Lipid peroxidation levels, as indicated by MDA, were determined spectrophotometrically (Shimadzu UV-1800, Shimadzu Corp., Kyoto, Japan) at a wavelength of 532 nm.

Quantification of vitamin E: Reverse-phase high performance liquid chromatography (HPLC) was used to measure the vitamin E (alpha- and gamma-tocopherol) found in the plasma or tissues. Exactly 100 mL of the plasma or homogenized tissue samples were mixed with the same level of ethanol; after vortexing, the tocopherols were extracted into 500 mL hexane containing 0.002% butylated hydroxyl toluene (BHT; Sigma Chemical Co., St Louis, MO). Tocol (a gift from Hoffmann-La Roche, Nutley, NJ) was added to the mixture at an internal standard. The samples were centrifuged at 800 rpm for 5 min at 48°C. The supernatant was then collected and dried under a stream of nitrogen gas, and reconstituted in 100 mL methanol. Eluted peaks were detected at an applied potential of 10.6 V by an LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN). The tocopherols were eluted at well-separated peaks with a retention time of 2–6 min. The peaks were integrated with a ChemStation (Hewlett Packard), and tocopherol concentration was expressed in pmol/mg protein (18). Protein was measured following Lowry et al.'s method (19).

The antioxidant parameters were determined in tissues of the heart, liver and brain.

Heart tissue homogenization: Homogenization was completed at 16,000 rpm. The samples were placed in a refrigerated centrifuge (at 3,220 rpm for 30 min, +6° C). The supernatant 1/1 (v/v) mixture of chloroform and ethanol (3/5, v/v) was carried in glass tubes, vortexed and then centrifuged at 3,220 rpm for 40 min at +4°C.

Liver and brain tissue homogenization: The tissues were homogenized in 5 mL cold buffer/g tissue using a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 10,000 x g for 20 min at 4°C to estimate the enzymatic assays, centrifuged at 2,500 rpm to confirm MDA level and the resultant supernatant transferred into Eppendorf tubes preserved in deep freeze until their use (10).

Statistical analysis: Group significance levels were determined via a one-way analysis of variance (ANOVA) test. The results were outlined as means and standard deviations. The mean values were detected using a Tukey multiple comparison test. Histopathological scores were recorded using a Bonferoni-Dunn test. All statistical analyses were done using the SPSS 17.0 software.¶

RESULTS

Periodontitis was achieved in group P using a ligature in the interproximal areas of the molars. The first record of alveolar bone level was 0.218 ± 0.040, with the score reaching 0.828 ± 0.242 in the periodontitis group (Table 1). The base limit of the serum MDA levels in the control group was 1.82 ± 0.11 microm/g prot and increased significantly to 2.56 ± 0.28 microm/g prot by periodontitis induction. The same results were seen in the heart (21.34 ± 4.0 to 31.80 ± 1.38 microm/g prot), liver (29.34 ± 5.71 to 31.80 ± 1.38 microm/g prot) and brain (13.78 ± 3.5 to 25.54 ± 4.840 microm/g prot) of the rats in the periodontitis group ($p<0.001$; Table 1). Interestingly, the biggest distinction was observed in the liver between the control and periodontitis groups. The possible results of periodontal infection occurred as well.

Table 1: MDA Levels

MDA (microm/g prot)	C	P
Serum	1.802 ± 0.117	2.562 ± 0.280
HEART	21.341 ± 4.014	31.807 ± 1.385
LIVER	29.347 ± 5.710	77.315 ± 11.807
BRAIN	13.784 ± 3.588	25.54 ± 4.849

$p < 0.001$ vs Control

Vitamin E levels were decreased in the assayed tissues affected by periodontitis. This means that periodontal infection impaired the balance between oxidant MDA and antioxidant vitamin E. The values decreased from 9.73 ± 1.26 to 6.39 ± 1.04 in the heart, from 58.54 ± 10.30 to 54.29 ± 12.36 in the liver and from 30.08 ± 8.40 to 24.701 ± 9.58 in the brain (Table 2). The differences in vitamin E were statistically significant ($p<0.001$). As such, periodontitis is a strong stimulator of reactive oxygen radicals, thereby affecting multiple organs. Periodontitis also showed its oxidative performance in our study results.

Table 2: Vitamin E levels

VIT E (microm/g tissue)	C	P
HEART	9.735 ± 1.266	6.395 ± 1.043
LIVER	58.548 ± 10.305	54.291 ± 12.362
BRAIN	30.081 ± 8.400	24.701 ± 9.587

DISCUSSION

In the present study, we compared the effects of periodontitis on the heart, liver and brain. We determined the potential oxidative damage of periodontitis using MDA assessments in serums of heart, liver and brain tissues. Additionally, we assessed vitamin E levels as an antioxidant marker in the studied organs. We observed that periodontitis decreased vitamin E levels in different tissues, as discussed in previous studies (13). We preferred to evaluate vitamin E as an antioxidant, because the absence or insufficient amount of vitamin E can easily be rectified through nutrient supplements and other methods.

The relationship between ROS and antioxidants, and also their impacts on pathological events in periodontitis, have been discussed many times (20,21). The increasing potential of protein oxidation due to decreasing antioxidants was the most interesting figure (21). Infectious activity in periodontitis stimulates a gingival response, which can cause excess lipid peroxidation. Thus, the metastatic impaction of lipid peroxidation in systemic health can be witnessed in periodontal diseases (22). DNA damage in tissues, such as those of the kidneys, liver, heart and brain, also present due to periodontitis (22).

Another approach to periodontal infections' effects on bodily organs was defined by Gendron et al. (23). According to this study, bacteraemia spreads via the bloodstream to the lymphatic system, which causes metastatic and immunologic injury. This circulation stimulates specific antibodies to form macromolecular complexes.

Vitamin E is one of the natural defence mechanisms against oxidative pressure and infection. Vitamin E behaves as a chain breaker antioxidant that prevents the proliferation of free radicals (24). It also serves as a main lipid solver antioxidant, despite its low concentration in cell membranes. In addition, it may have not just antioxidant functions, but also become a signalling molecule, regulator of gene expression and help prevent cancer and atherosclerosis (24). Specifically, in oral cancers, lipid peroxidation and MDA levels increase, though some supplemental agents, such as vitamins E and C, can downregulate the resulting increase in free radicals (25). In a recent experimental rat study, vitamin E proved to decrease inflammatory responses and prevent MDA formation in periodontitis; however, it did not decrease alveolar bone loss (13). In our study, group P's MDA levels increased with periodontitis, and the differences were significant when compared with group C ($p<0.001$).

Carvalho et al. (13) correlated vitamin E supplementation with bone loss in periodontitis. In this study, vitamin E showed preventive effects in alveolar bone loss, but the results were not statistically significant (13). In another study, the relationship between insufficient nutrition and periodontitis were investigated by controlling vitamin A, B, C, D and E levels. This study also reported the importance of vitamin levels in periodontal disease severity (26). Further, Ebersole et al. (27) emphasized that aging increases periodontitis progression: when vitamin E decreases, aging accelerates, as does periodontitis severity. Similarly, Muniz et al. (28) evaluated the impact of antioxidants on oxidative stress in periodontal tissues, and told that vitamin E has statistically significant effects on periodontal parameters. Vitamin E's effect on bone formation has also been analysed in implantology by coating implant surfaces with vitamin E; the results demonstrated that vitamin E showed extra cellular matrix (ECM) destruction and lessened inflammatory responses (29).

Another study was conducted to evaluate the protective effect of only vitamin E or vitamin E with another antioxidant in multiple organs and systems, such as the blood, liver, heart and kidneys, irrespective of periodontitis. Vitamin E proved a good antioxidant for different organs (30). Almost the same results in an experimental study that investigated vitamin E's antioxidative effects against oxidative damage

induced by sodium azide in the liver, testes, kidneys and heart. The researchers observed that vitamin E decreased LPO levels in several organs (30). And in another study, vitamin E deficit correlated with the downregulation of brain functions and Alzheimer's diseases, and neuroinflammation in the brain. However, vitamin E deficiency's main effect on the brain is still uncertain (31). Meanwhile, Violet et al. analysed the position of vitamin E in obese human livers. Obesity causes hepatosteatosis, which can dysregulate vitamin E. They found that the dysregulation and deficiency of vitamin E stimulates different liver diseases (32). As shown in previous studies, we also discovered that vitamin E levels go down in brain, liver and heart tissues affected by periodontitis, and MDA levels increase. All the parameters in the two groups also significantly differed ($p<0.001$).

In general, vitamin E can modulate gene expression and interact with cell regulatory signals (33). As such, suppressing vitamin E can affect the activity of other antioxidants in the body.

CONCLUSION

We conclude that vitamin E is an important antioxidant against MDA, which destroys periodontal heart, liver and brain tissues. While vitamin E alone is insufficient for a healthy body, it is easy to maintain through either supplemental agents or nutrients. Indeed, if vitamin E levels reach their optimum limits, many different tissues might be better regulated.

Footnotes

*3.0, Doğsan, Istanbul, Turkey.

** ImageJ 1.46r, Bethesda MD, USA.

¶SPSS (version 17.0 software) Inc. Chicago IL, USA.

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