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Total Antioxidant Capacity of Venous Blood, Blood Plasma, and Serum of Patients With Periodontitis, and the Effect of Traumeel S on These Characteristics

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Key words: *blood count; antioxidant capacity; nitroblue tetrazolium; periodontitis; Traumeel S.*

Summary. Introduction. Periodontal diseases are among the most common chronic infections in humans. Chronic low-level bacteremia and a septicemic inflammatory response have been suggested as a pathogenetic link between periodontal disease and atherosclerosis, diabetes and other systemic diseases. All this significantly increases the relevance of the search for the means for treatment and prevention of periodontal diseases. The aim of the present study was to evaluate blood count and the antioxidant capacity of venous blood, blood plasma, and serum in patients with periodontitis and control subjects with healthy periodontal tissues, and to investigate the effect of the homeopathic medication Traumeel S on the antioxidant capacity of venous blood, plasma, and serum.

Material and Methods. The study was performed using venous blood of 21 individuals with chronic periodontitis and 22 healthy subjects. Reduction properties of venous blood, blood plasma, and serum were investigated using the method of reduction of nitroblue tetrazolium, proposed by Demehin et al.

Results. The data showed that there was no significant difference in venous blood hemoglobin levels or erythrocyte counts between the groups, while significantly higher leukocyte counts were observed in the periodontitis group ($P<0.05$). The antioxidant capacity of blood plasma was significantly higher in the periodontitis group than it was in the controls ($P<0.05$). Meanwhile, the antioxidant capacity of serum was significantly lower in the periodontitis group as compared with controls ($P<0.05$). The preparation Traumeel S had no effect on the antioxidant capacity of venous blood or blood plasma in the studied groups.

Conclusions. Compared to healthy individuals, the antioxidant capacity of blood plasma in patients with periodontitis was higher, while the antioxidant capacity of serum was lower. The homeopathic medication Traumeel S had no effect on the antioxidant capacity of venous blood, blood plasma, or serum. Our findings concerning the elevated leukocyte counts in venous blood of patients with periodontitis confirm the presumption that periodontal diseases cause low-grade systemic inflammation induced by the host response to periodontal bacteria.

Introduction

Periodontal diseases are among the most common chronic infections in humans (1). Gingivitis and periodontitis are initiated by microbial plaque that accumulates in the gingival crevice region and induces an inflammatory response in the supporting tissues of the teeth, characterized by gradual loss of periodontal attachment and alveolar bone (2). The etiology of the disease is strongly related to the colonization of the periodontal tissues by a complex mix of anaerobic (gram-negative) bacteria. However, gram-positive bacteria also constitute a significant component of subgingival biofilm (3). Although bacteria are essential for the induction of the inflammatory response, it is insufficient to cure the disease (4). In conjunction with the bacterial challenge, the host immune response plays an important role in the onset and progression of periodontitis (5). Variability in host response may be a component of a genetic predisposition to periodontal diseases (6). It is possible that genetically determined differences in immune regulation or in homeostatic bone remodeling are also important for the outcome of periodontal disease (7). Studies on infectious diseases other than periodontal diseases provide convincing evidence that host genetic factors are important in determining who will succumb to the pathogen and who will not (8, 9). Suscep-

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tibility or resistance to many infectious diseases is dependent on genetically controlled differences in inflammatory responses (8, 10).

Neutrophils are a critical component of the local inflammatory response in periodontal disease (11). The mature neutrophils are short-lived, and thus hematopoiesis of neutrophils is a continuous process, producing a large pool of granulocyte progenitors (approximately 1.8×10^{12} cells per day) (12).

Neutrophils are terminally differentiated effector cells recruited to site of infection where they can carry out phagocytosis and destroy many potentially dangerous microorganisms. The powerful microbicidal properties of neutrophils depend on processes, such as the rapid formation of reactive oxygen species (ROS) and nitrogen intermediates in the respiratory burst (13–16) and the generation of destructive proteases contained within numerous cytoplasmic granules. ROS are thought to be produced at both the plasma membrane and the phagolysosomal membrane, and are consequently released into phagolysosomes and extracellular environment (17), which contributes to killing of the bacteria (18). Extracellular bacteria can also be killed, but principally by O_2-dependent mechanisms (19).

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids, and proteins (20) and can contribute directly to the development of oxidative stress. Oxidative stress is an imbalance between the production of ROS and antioxidant defense, leading to tissue damage. The produced ROS, such as superoxide anion, hydroxyl radical, and peroxyl radical, results in damage to many biological molecules including DNA, lipids, and proteins, and prolonged existence of these ROS promotes severe tissue damage and cell death (21, 22). ROS are products of normal cellular metabolism. Overproduction of them – most frequently either by excessive stimulation of NADPH by cytokines or by the mitochondrial electron transport chain and xanthine oxidase – results in oxidative stress (20).

Exposure to ROS from a variety of sources has led organisms to develop a series of defense mechanisms (23). These mechanisms against ROS-induced oxidative stress include preventive mechanisms, repair mechanisms, physical defense, and antioxidant defense.

The first line of the defense mechanism against ROS-induced injury involves several antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. Biological compounds with antioxidant properties may contribute to tissue protection against ROS. One of the natural molecules known to prevent or retard oxidative stress is lipoic acid (LA) (24, 25). LA is specified for ROS-quenching and metal-chelating activities. It also interacts with and regenerates other cellular antioxidants (26).

During the recent years, the efforts of many researchers have been focused on the free radical-scavenging activity of plants (27–32), which are important components in many Chinese traditional medicines as well as in homeopathic medicine.

The cardinal principle, on which the theory of homeopathic medicine is based, is that of "similarity" according to which a homeopathic remedy in a healthy subject will produce certain sets of symptoms, while the same remedy will cure similar sets of symptoms in unhealthy (sick) subjects (33). Hahnemann's theory withstands the test of time and is supported by scientific findings in an array of fields (34, 35). Homeopaths treat people based on genetic and personal health history, type of body composition, and current physical, emotional, and mental symptoms. Treatments are individualized or tailored to each person. Homeopathic remedies are derived from natural substances that come from plants, minerals, or animals.

A homeopathic complex medication, Traumeel S, has been sold over the counter in German, Austrian, and Swiss pharmacies for more than 50 years. It contains extracts from the following plants and minerals, all of them highly diluted (10^{-9}–10^{-1} of the stock solution): *Arnica montana*, *Calendula officinalis*, *Achillea millefolium*, *Matricaria chamomilla*, *Symphytum officinale*, *Atropa belladonna*, *Aconitum napellus*, *Bellis perennis*, *Hypericum perforatum*, *Echinacea angustifolia*, *Echinacea purpurea*, *Hamamelis virginiana*, *Mercurius solubilis Hahnemanni*, and *Hepar sulfuris*. The preparation has been found to be beneficial to humans suffering from a wide spectrum of pathological conditions, including trauma, inflammation, and degenerative processes (30, 31). Traumeel S has a wide range of indications, whereas its mode of action has been insufficiently studied (36). There have been little data on the role of antioxidants in the pathogenesis of periodontal diseases (37).

The aim of the present study was to evaluate blood count and the antioxidant capacity of venous blood, plasma, and serum of patients with periodontitis and control subjects with healthy periodontal tissues and to investigate the effect of homeopathic medication Traumeel S on the antioxidant capacity of venous blood, plasma, and serum.

Material and Methods

Selection of Patients. Patients (n=21) for the study were selected from a large number of individuals treated at the Faculty of Odontology, Lithuanian University of Health Sciences (former Kaunas University of Medicine). They were examined clinically and radiographically and were diagnosed with

advanced periodontitis. For periodontal status evaluation, the Russell's periodontal index (PI) was used (38). Patients with periodontitis had to meet the following inclusion criteria: minimum 18 remaining teeth; periodontal pocket ⩾6 mm in depth on at least 2–3 teeth in a quadrant; vertical and horizontal bone resorption visible on x-ray; and missing teeth removed due to complications of periodontitis, as indicated in the medical history. Only patients with very marked signs of periodontitis (PI, >6) were included in our study (Table 1).

Table 1. Demographic and Clinical Characteristics of the Studied Groups

Characteristic	Control Group n=22	Periodontitis Group, n=21
Age, years	31.6 (4.2)	35.8 (4.5)
Sex, men/women, n	10/12	9/12
PI index, points	0.02 (0.01)	6.91 (0.32)

Values are mean (SD) unless otherwise indicated.

The control group consisted of 22 subjects with healthy periodontal tissues (no periodontal pockets and no bleeding on probing; sulcus gingivalis, 1–2 mm); 2–3 teeth might be missing, but not due to complications of periodontitis.

Both groups were medically healthy, nonsmokers not pregnant, and had used no medications (including anti-inflammatory drugs or vitamins) for at least two months before the study. The age of the studied subjects ranged from 18 to 50 years.

All experiments were conducted in accordance with the rules and regulations approved by the Kaunas Regional Bioethics Committee (approval No. BE-2-21). All subjects involved in this study signed written informed consent approved by the Kaunas Regional Bioethics Committee.

Reagents. Hank's balanced salt solution and nitroblue tetrazolium (NBT) were obtained from the Sigma Chemical Co. (St. Louis, Missouri, USA). Plastic vials and other disposable pieces of plastic ware were obtained from the Carl Rot GmbH & Co KG (Karlsruhe, Germany). Traumeel S (aqueous solution for injections) was obtained from the Biologische Heilmittel Heel GmbH (Baden-Baden, Germany).

Fresh working solution of Traumeel S (1×10^{-2}) in Hank's balanced salt solution was prepared every day. The final concentration of Traumeel S in the medium was set at 10^{-4}, with respect to the recommendations provided in literature (31, 36).

Blood preparation. Twenty milliliters of venous blood were collected from the subjects by venipuncture, and the samples were anticoagulated with heparin (20 U/mL). Cells were counted and morphologically evaluated using a hematologic blood analyzer ADVIA 2120 (Siemens Healthcare Diagnostics, Dublin, Ireland).

Determination of the Effect of the Preparation Traumeel S on the Reduction Properties of Venous Blood. The reduction of nitroblue tetrazolium (NBT test) was performed following the technique proposed by Demehin et al. (39). Six test tubes were filled with 1.6 mL of the blood each; then Traumeel S solution (at the final dilution of 10^{-4}) was added into 3 test tubes, while respective volumes of phosphate buffer were added into other 3 test tubes. The resulting specimens were incubated for 10 minutes. Subsequently, NBT (at the final concentration of 100 μM) was added. The test tubes were incubated in a water thermostat for 20 minutes at 37°C. Following this, the test tubes were centrifuged for 10 minutes (at 1500*g*), and the supernatant was examined under a spectrophotometer (wavelength, 570 nm; cuvette thickness, 2 mm). The obtained data were expressed in percentage of translucence change.

NBT Reduction Reaction of Blood Plasma. The test tube with the remaining blood was placed into a refrigerator and stored for 60 minutes at 4°C. Subsequently, leukocyte-containing plasma was extracted, and leukocyte counts and morphological composition was determined. Using serum obtained by centrifuging the remaining blood (at 2500*g*), neutrophilic leukocyte counts in the studied plasma was considered as 1×10^{6} cells/mL. Such prepared plasma was poured into 6 plastic test tubes (0.8 mL each). NBT reduction reaction was performed by applying the previously described technique.

NBT Reduction Reaction of Blood Serum. The remaining serum was poured into 6 plastic test tubes (0.8 mL each), and the testing was performed by applying the aforementioned technique. In this case, the final NBT concentration was 40 μM.

Calibration of the spectrophotometer was performed using serum.

The results shown are the mean value of three identical experiments.

Statistical Analysis. Experimental results are presented as mean (SD). Data analysis was performed by applying the statistical software PASW Statistics 18 and PASS 11. Differences between the groups were established by applying nonparametric one-way analysis of variance – the exact Kruskal-Wallis test. The power of the test was evaluated via modeling (PASS 11 procedure "One-Way analysis of Variance [Simulations]"). Multiple pair-wise comparisons (post hoc) were conducted using the Dunn's test, whose power was also evaluated by modeling (PASS 11 procedure "Pair-Wise Multiple Comparisons [Simulations]").

Results

Data of Laboratory Tests. Table 2 presents data on the venous blood hemoglobin levels as well as erythrocyte and leukocyte counts of studied sub-

Table 2. Laboratory Data of Venous Blood in the Studied Groups

	Control Group n=22	Periodontitis Group n=21	*P*
Hemoglobin level, g/L	132.3 (1.5)	130.1 (2.3)	NS
Erythrocyte count, $\times 10^{12}$/L	4.4 (0.3)	4.3 (0.2)	NS
Leukocyte count, $\times 10^{9}$/L	5.6 (0.3)	6.7 (0.4)	<0.05

Values are mean (SD). NS, not significant.

jects. The data showed that there was no significant difference in venous blood hemoglobin levels or erythrocyte counts between the groups ($P>0.05$), while the difference in leukocyte count was statistically significant ($P<0.05$). The highest leukocyte counts – 6.7×10^9/L (SD, 0.4) – were observed in the periodontitis group, while in controls they reached 5.6×10^9/L (SD, 0.3).

Determination of the Effect of the Preparation Traumeel S on the Reduction Properties of Venous Blood, Blood Plasma, and Serum. The data on the effect of the preparation Traumeel S on the antioxidant capacity of venous blood and blood plasma in the studied groups are presented in Table 3.

Data presented in this table show that the antioxidant capacity of blood plasma was significantly ($P<0.05$) higher in the periodontitis group than in the control group. Meanwhile, the antioxidant capacity of serum was significantly ($P<0.05$) lower in the periodontitis group as compared with the controls.

The preparation Traumeel S had no effect on the antioxidant capacity of venous blood or blood plasma in the studied groups.

Discussion

The findings of our study showed that there was no significant difference in hemoglobin levels and erythrocyte counts in venous blood of patients with periodontitis and healthy controls ($P>0.05$). Literature data concerning this issue are scarce (40, 41) and indicate that hemoglobin and erythrocyte levels should decrease in venous blood of patients with periodontitis.

Our findings showed that leukocyte counts in venous blood of periodontitis patients significantly exceeded those in venous blood of healthy controls, which is comparable to the findings of other studies (42, 43). Literature indicates that elevated leukocyte counts in venous blood of periodontitis patients is a sign of systemic inflammation provoked by microbes that cause periodontitis (44).

Human erythrocytes are continuously exposed to oxidative stress (39). During their relatively short life (during which no protein synthesis occurs), these cells, whose principal role is to carry oxygen to the tissues and organs, are exposed to ROS from various sources. The high levels of cytoplasmic antioxidants present in erythrocytes, including superoxide dismutase, catalase, glutathione, and ascorbic acid, minimize oxidative damage to the cytoplasmic components of the erythrocyte (45). This indicates that erythrocytes play an important role in the antioxidant systems of blood. Currently, there are no gold standard methods for measuring antioxidant capacity or ROS-mediated tissue damage in humans. All the systems utilize different measurement indices, and the specificity of the biomarkers employed dictates the measurement obtained, which differs between assays and between different biological samples and their components (46).

Free radicals and other reactive species have extremely short half-lives in vivo (10^{-9}–10^{-6} s) and simply cannot be measured directly (47). A majority of clinical studies employ biomarkers of oxidative stress or tissue damage to vital macromolecules, rather than spin traps. All these assays are sufficiently sophisticated. Demehin et al. (39) suggested rather a simple method for assessment of total blood

Table 3. The Effect of the Preparation Traumeel S on the Antioxidant Capacity of Venous Blood, Blood Plasma, and Serum in the Studied Groups

Reduction Potential (Translucence %)	Control Group n=22		Periodontitis Group n=21		*P*
	Group 1a With Traumeel S	Group 1b With Buffer	Group 2a With Traumeel S	Group 2b With Buffer	
Venous blood	73.1 (2.7)	72.2 (2.0)	67.5 (2.6)	66.9 (2.2)	1a vs. 1b, NS 2a vs. 2b, NS 1b vs. 2b, NS 1a vs. 2a, NS
Blood plasma	75.9 (2.2)	76.1 (2.3)	69.1 (2.1)	68.4 (2.2)	1a vs. 1b, NS 2a vs. 2b, NS 1b vs. 2b, <0.05 1a vs. 2a, <0.05
Serum	78.2 (1.4)	77.8 (1.5)	82.6 (1.7)	84.1 (1.6)	1a vs. 1b, NS 2a vs. 2b, NS 1b vs. 2b, <0.05 1a vs. 2a, <0.05

Values are mean (SD). NS, not significant.

antioxidant capacity – the reduction of nitroblue tetrazolium. The use of tetrazolium salts in different branches of the biological sciences is mostly based on one characteristic – their reducibility to formazans. The change in color that occurs during formazan formation allows for taking the advantage of the visualization of biological redox processes (48). Our study using the NBT test showed (Table 3) no significant difference in the total antioxidant capacity of venous blood between patients with periodontitis and healthy controls ($P>0.05$).

We did not find any data in the medical and biological literature on total blood antioxidant capacity in patients with periodontitis. Our findings also showed that the total blood antioxidant capacity significantly exceeded that of blood plasma ($P<0.05$) and especially that of blood serum ($P<0.05$). Most probably, this is due to marked antioxidant capacity of erythrocytes.

We applied the NBT test for the evaluation of the antioxidant capacity of blood plasma and serum. It is noteworthy that the antioxidant capacity of blood plasma in patients with periodontitis significantly exceeded that of healthy controls ($P<0.05$). This was most probably due to excess amounts of superoxide anions produced by neutrophils in venous blood of patients with periodontitis (49), which resulted in NBT reduction (50).

However, the NBT test showed significantly reduced antioxidant capacity of blood serum in patients with periodontitis ($P<0.05$). Our findings were in accordance with those obtained by other researchers (51, 52) who also noted the reduced antioxidant capacity of blood serum in patients with periodontitis. Brock et al. (52) found that the reduced serum total antioxidant capacity in periodontitis did not completely reach significance, whereas the differences in plasma levels did, which may reflect the differences in serum and plasma preparation methods (serum is prepared at higher centrifugal forces and is more prone to oxidation) or the effects of clotting factor removal or too small number of samples. The reduced total antioxidant capacity of venous blood serum could result from low-grade septicemic inflammation induced by the host response to periodontal bacteria or may be an innate feature of patients with periodontitis (53).

The data of our study also showed that Traumeel S had no effect on the reduction properties of the subjects' blood or blood plasma. We did not find any similar research data in literature.

Conclusions

Leukocyte counts in venous blood of patients with periodontitis significantly exceeded those in venous blood of healthy controls. Compared to healthy individuals, the antioxidant capacity of blood plasma in patients with periodontitis was greater, while the antioxidant capacity of serum was lower. The homeopathic medication Traumeel S had no effect on the antioxidant capacity of venous blood, blood plasma, or blood serum in both the subjects with periodontitis or those with healthy periodontal tissues.

Statement of Conflict of Interest

The authors state no conflict of interest.

Homeopatinio preparato *Traumeel S* poveikis redukcinėms periferinio veninio kraujo, plazmos ir serumo savybėms

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Raktažodžiai: kraujo sudėtis, redukcinis potencialas, nitromėlio tetrazolis, periodontitas, *Traumeel S*.

Santrauka. *Įvadas.* Periodontitas yra viena labiausiai paplitusių ligų pasaulyje. Nustatyta, kad priedančio audinių destrukcijos laipsnis stipriai koreliuoja su širdies ligų, cukrinio diabeto ir kitų sisteminių ligų sunkumu. Visa tai didina periodontito gydymo ir profilaktikos priemonių paieškų aktualumą.

Tyrimo tikslas – ištirti ir palyginti sveikų asmenų ir sergančiųjų periodontitu kraujo sudėtį bei veninio kraujo, plazmos ir serumo redukcinį potencialą, taip pat nustatyti homeopatinio preparato *Traumeel S* poveikį redukcinėms periferinio kraujo, plazmos ir serumo savybėms *in vitro*.

Tirtųjų kontingentas ir tyrimo metodai. Tyrime dalyvavo 21 asmuo, sergantis periodontitu, ir 22 asmenys, kurių priedančio audiniai sveiki. Redukcinėms kraujo, plazmos ir serumo savybėms tirti buvo naudojama nitromėlio tetrazolio redukcijos reakcija, kuri atlikta pagal A. A. Demehin ir kt. pasiūlytą metodiką.

Rezultatai. Tiriamų grupių asmenų periferinio kraujo hemoglobino kiekis ir eritrocitų skaičius reikšmingai nesiskyrė ($p>0,05$), tačiau leukocitų skaičius statistiškai patikimai ($p<0,05$) buvo didesnis periodontitu sergančiųjų kraujyje. Sergančiųjų periodontitu kraujo plazmos redukcinis potencialas iš esmės

buvo didesnis ($p<0,01$) nei sveikų asmenų. Tuo tarpu sergančiųjų priedančio audinių uždegimu kraujo serumo redukcinis potencialas buvo statistiškai patikimai ($p<0,05$) mažesnis už sveikų asmenų. Preparatas *Traumeel S in vitro* neturėjo įtakos tiriamųjų grupių periferinio kraujo ir plazmos redukciniam potencialui.

Išvados. Lyginant su sveikais asmenimis sergančiųjų priedančio audinių uždegimu serumo redukcinis potencialas yra mažesnis, o plazmos redukcinės savybės yra stipresnės. Homeopatinis preparatas *Traumeel S* neturi įtakos sergančiųjų periodontitu ir asmenų, kurių priedančio audiniai sveiki, periferinio kraujo, plazmos ir serumo redukciniam potencialui *in vitro*.

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