

THIOBARBITURIC ACID REACTING SUBSTANCES IN SALIVA AND THEIR RELATION TO THE GINGIVAL INFLAMMATION

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REZUMAT

Introducere: Substanțele care reacționează cu acidul tiobarbituric (SRATB) sunt markeri ai stress-ului oxidativ și ale alterărilor lipidice. Concentrația de SRATB este în mod obișnuit măsurată în homogenate tisulare și în serul pacienților cu boli inflamatorii.

Obiectiv: Nivelurile de SRATB în salivă sunt corelate cu statusul periodontal al pacienților cu gingivită latentă.

Material și metode: Mostrele de salivă au fost colectate repetat de la 60 de pacienți cu vârste între 18 și 25 de ani, într-o perioadă de 2 luni. Nivelurile de SRATB au fost măsurate spectrofluorometric ($\lambda_{ex}=515\text{nm}$, $\lambda_{em}=553\text{nm}$). Testosteronul salivar a fost măsurat radioimunologic, iar parametrii hematologici au fost evaluați prin proceduri standard. Statusul periodontal a fost încadrat conform indexului de sângerare papilară (ISP).

Rezultate: Femeile cu grad mai mare de gingivită (ISP înalt) au prezentat un nivel crescut de testosteron ($p<0,03$), față de cele cu ISP scăzut. Rezultatele la bărbați au arătat o tendință opusă. SRATB salivare sunt corelate pozitiv cu testosteron salivar la femei ($p<0,02$) și negativ la bărbați. Efectul imunosupresiv al steroizilor androgeni a devenit aparent prin scăderea IgM ($p<0,01$), neutrofilelor ($p<0,02$) și printr-o creștere paradoxală a limfocitelor ($p<0,03$).

Concluzii: Măsurarea SRATB salivare ar putea deveni o metodă diagnostică simplă, neinvazivă și eficientă a statusului parodontal, dar interacțiunile cu alți factori ca sexul, imunitatea și statusul hormonal trebuie studiate mai detaliat. Originea SRATB salivare rămâne necunoscută. Sunt necesare studii suplimentare ale factorilor microbieni.

Cuvinte cheie: SRATB, malondialdehidă, testosteron salivar, sângerare periodontală, stress oxidativ

ABSTRACT

Background: Thiobarbituric acid reacting substances (TBARS) are markers of oxidative stress and lipids impairment. Concentrations of TBARS are usually measured in tissue homogenates and in the serum of patients with inflammatory diseases.

Objective: In this study the levels of TBARS in saliva were related to the periodontal status of subjects with latent gingivitis.

Subjects and Methods: Saliva samples were collected repeatedly from an ensemble of 60 subjects aged 18–25 years during a 2-months period. TBARS levels were measured by a spectrofluorometric method ($\lambda_{ex}=515\text{nm}$, $\lambda_{em}=553\text{nm}$), salivary testosterone was radioimmunoassayed and haematological parameters were gauged by standard procedures. Periodontal status was rated by the Papillary-Bleeding-Index (Papillen-Blutungs-Index) (PBI).

Results: Women with a higher grade of gingivitis (high PBI) had higher levels of testosterone ($p<0,03$) than those with low PBI. Results in men showed an opposite trend. Salivary TBARS levels were positively correlated with salivary testosterone levels in women ($p<0,02$) and negatively in men. Immunosuppressive effect of androgen steroids became apparent by decreased IgM ($p<0,01$), neutrophils ($p<0,02$) and by a paradoxically increased lymphocyte count ($p<0,03$).

Conclusion: Measurement of salivary TBARS could become a simple, non-invasive and cost-effective diagnostic parameter of periodontal status, but the interactions with factors like sex, immunity and the hormonal status must be described and considered. The origin of TBARS in saliva remains unknown. Studies observing the effect of microbial factors are needed.

Key Words: TBARS, malondialdehyde, salivary testosterone, periodontal bleeding, oxidative stress

INTRODUCTION

Nowadays it is hardly possible to manage with knowledge from only one field. To achieve valid results, information from the related fields is often needed, focusing on a specific problem and also for its solution form an interdisciplinary point of view. Such an approach was needed in this project in order to find connections between stomatology, biochemistry and physiology. The following parameters were assessed: levels of salivary testosterone (TST), differential number

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of lymphocytes and neutrophils, blood levels of immunoglobulins, and salivary levels of thiobarbituric acid reactive substances (TBARS) as markers of oxidative damage of biomembranes or other lipids by free radicals. The aim was to demonstrate the theoretical relationship between these parameters and the Papillary bleeding index (PBI – Papillen-Blutungs-Index).

TBARS are a group of substances of which the better known is malondialdehyde (MDA).¹ In serum and tissue homogenates, where TBARS levels are measured, MDA is the main component of this mixture. That cannot be stated for saliva, because we could not find the quantification of the separate TBARS components in saliva in literature. That is why the levels of TBARS and not MDA were considered. The origin of TBARS in saliva is unknown, but one of the possible explanations could be its source from damaged gingiva as a result of inflammatory reactions and the release of free radicals.^{2,3} In this phase, we looked closer to the conditions of the humoral (antibodies) and cellular (neutrophils, lymphocytes) components of immune system. As known, hormonal changes have effect on the immune system. In saliva, there are two hormones standardly measured – cortisol and testosterone.⁴ Testosterone was chosen because it affects the immune system, and its levels in saliva are relatively constant in time, whereas levels of cortisol are unstable, easily and quickly influenced by exogenous factors. Examinations were undertaken on a group of volunteers with a complete set of teeth without any clinical signs of advanced periodontal disease. It was expected that the hormonal changes taking place during puberty would be overcome and the hormone levels would remain relatively constant.

The purpose of the research was to describe the relationship between PBI and TBARS, and the immunity status influence (induced by the changes of endogenous levels of TST) on this relationship.

SUBJECTS AND METHODS

Stomatologic examination – Subjects were 31 young healthy male volunteers and 29 young healthy female volunteers with an average age of $21,5 \pm 1,4$ yr. The ensemble was characterized by age, stomatological examination and teeth-care knowledge homogeneity. Oral cavity soft and hard tissues inspection took place in the IIIrd Stomatological Clinic of the Faculty of Medicine in Comenius University Bratislava, between 20.10. – 20.12.2000. Explorations were performed by inspection and probing. PBI index was rated on all papillae from oral and vestibular side, except on the papillae between the 7 and the 8. Mark 0 was considered as no bleeding, mark 1 indicated just point bleeding,

joining bleeding was marked 2, when the blood filled the space between teeth – mark 3, and mark 4 corresponded to massive bleeding.⁵ A questionnaire of basic anamnesis and other factors directly concerning the oral cavity condition was part of the examination.

Biochemical analysis – Samples of saliva were collected from 60 volunteers, where the samples were collected every second (1st month) and every third day (next 1,5 month), before professional cleaning was undertaken. The samples were deep-frozen (-20°C) where they remained till the measurement. Samples of the same time (samples from different subjects but with the same ordinal number or date) were measured at once. Salivary thiobarbituric acid reactive substances (TBARS) were gauged by spectrofluorometric method ($\lambda_{\text{ex}} = 515\text{nm}$, $\lambda_{\text{em}} = 553\text{nm}$) after derivatization with 0,6% thiobarbituric acid in acid medium of acetic acid (1:1, 100°C , 45 min.). Before derivatization, the samples were separated by centrifugation (3000 rpm, 10 min.), after derivatization the colored product was extracted to n-butanol, separated (3000 rpm, 10 min.) and measured. TBARS concentration was expressed in mmol/l on the basis of the calibration curve. 1,1,3,3-tetramethoxypropan was taken as standard. Testosterone was radio-immunoassayed at the Institute of Endocrinology in Lubochna and the haematological parameters were measured by standard methods in the Department of Clinical Biochemistry.

Statistical analysis – The data were analyzed using Microsoft Excel 2000 by Pearson coefficient mutual correlation, and level $\alpha = 0,05$ was chosen as a limit level of significance. No data were excluded.

RESULTS

The relationship between the average PBI and TST is negatively associated in women (Fig. 1), which means that in female volunteers with high TST, lower gingival bleeding (expressed as PBI; $p < 0,03$) was found. On the other hand, in men, we have seen more positive correlation between TST and PBI, which was, however, statistically insignificant ($p > 0,05$; Fig. 2).

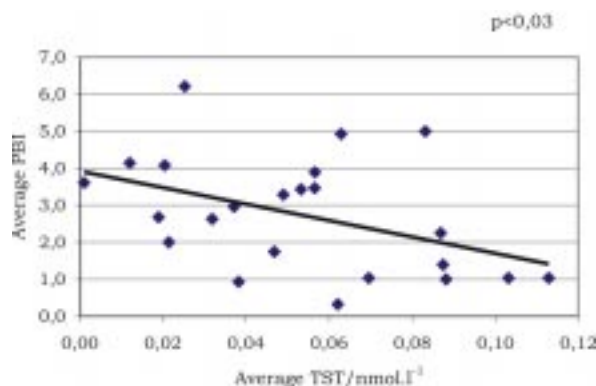


Figure 1. PBI versus TST in women

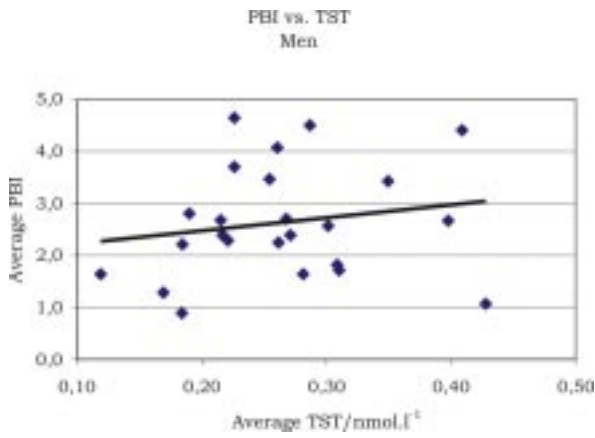


Figure 2. PBI versus TST in men

No statistical correlation between PBI and TBARS (Fig. 3) was found in women, but significant positive correlation ($p < 0,02$) between the values of average TST and TBARS (Fig. 4) was observed. On the contrary, this relationship (TST and TBARS) had an insignificant negative trend in men (Fig. 5).

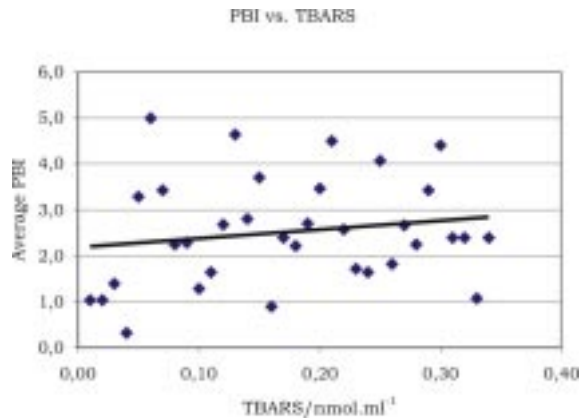


Figure 3. PBI versus TBARS

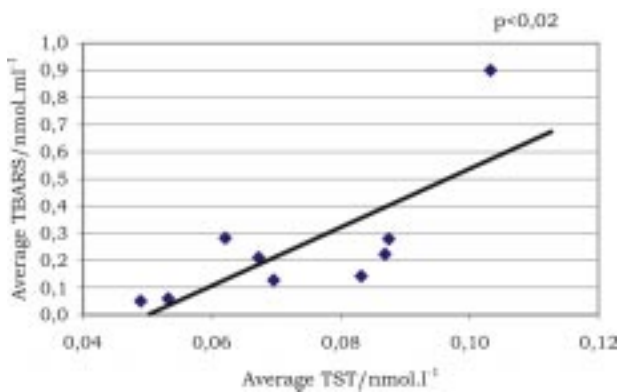


Figure 4. TBARS versus TST in women

When comparing the data of average TST levels and number of neutrophiles (Fig. 6) and lymphocytes (Fig. 7), a decreased number of neutrophiles ($p < 0,02$) and increased number of lymphocytes ($p < 0,03$) in the subjects with higher TST levels were found.

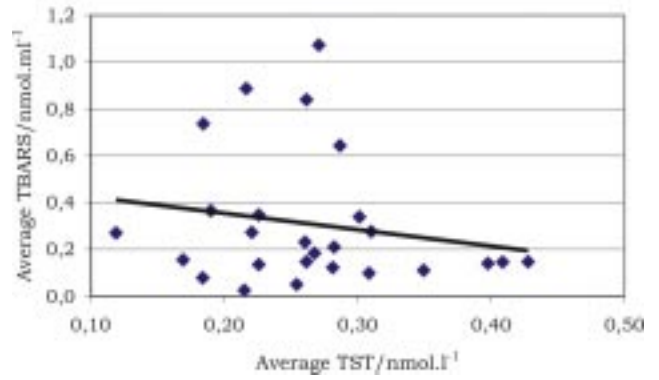


Figure 5. TBARS versus TST in men

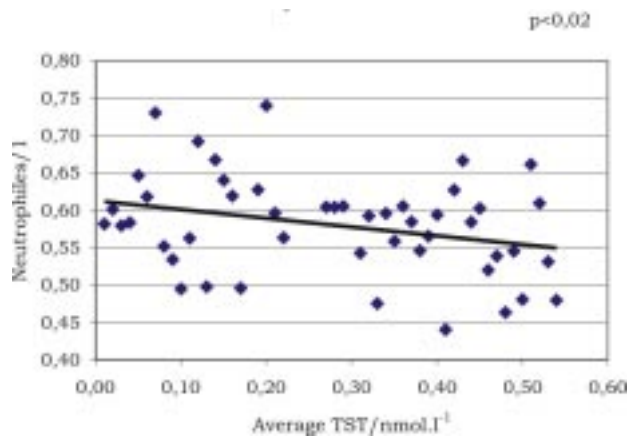


Figure 6. Neutrophiles versus TST

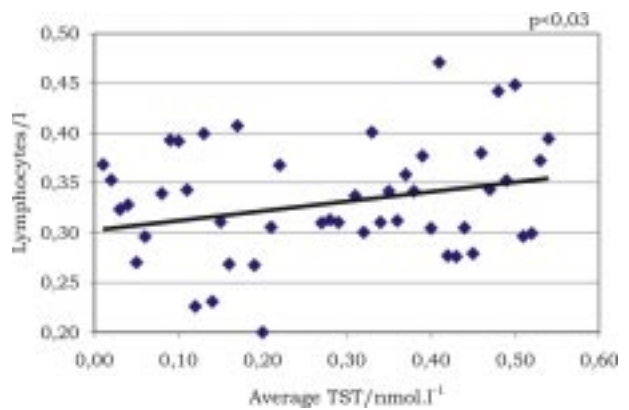


Figure 7. Lymphocytes versus TST

As statistically significant and negatively correlated occurred TST and IgM relationship (Fig. 8). In table 1, there are correlation coefficients among TST, TBARS, PBI and other classes of immunoglobulins.

DISCUSSIONS

Negative relationship between TST and PBI in women could be explained by androgen immunity alternation.⁶ Enlarged relative number of neutrophiles

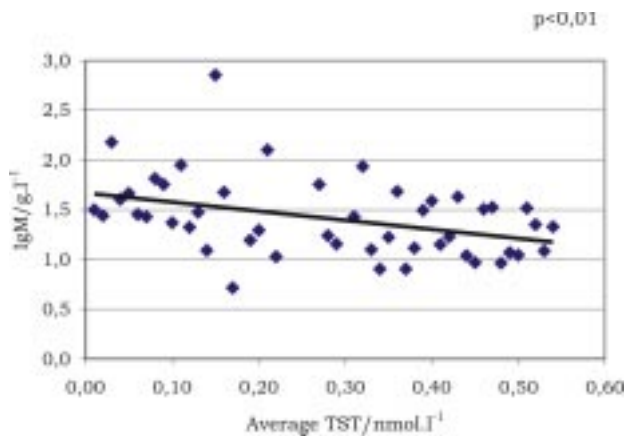


Figure 8. IgM versus TST

in subjects with low levels of salivary TST could lead to gingival inflammation. TST may influence neutrophiles not only quantitatively but also qualitatively by impeding the penetration into tissues⁷ as a consequence of decreased expression of immuno-competent cells (including neutrophiles), adhesive molecules, which results in their extravasation.⁸ Increased relative number of lymphocytes, related to increased TST levels, did not correspond with the changes of antibody levels in serum. It appears that this effect is of a qualitative – functional – character. As it is known, androgens decrease expression of adhesive molecules that are needed for information exchange between lymphocytes and antigen presenting cells,⁹ what of course interferes with the acute antibody response on infection, and the result is the reduced production of IgM. That can also be the reason for the lack in significant dependence among hormonal levels and other types of antibodies. (Table 1)

Table 1. Pearson correlation coefficients between TST, TBARS, PBI and different types of antibodies (*- $p < 0,05$; ** - $p < 0,01$)

| | | IgG | IgA | IgM | IgE |
|-------|----------|-------|--------|---------|--------|
| TST | women | 0,15 | -0,05 | -0,16 | 0,11 |
| | men | -0,15 | 0,02 | -0,02 | -0,01 |
| | together | -0,12 | 0,21 | -0,37** | -0,04 |
| TBARS | women | -0,46 | -0,22 | 0,81** | 0,82** |
| | men | -0,11 | 0,01 | -0,43* | 0,31 |
| | together | -0,21 | -0,01 | -0,19 | 0,37* |
| PBI | women | 0,12 | 0,40* | 0,01 | 0,24 |
| | men | -0,37 | 0,52** | 0,01 | 0,11 |
| | together | -0,09 | 0,40** | 0,00 | 0,18 |

The incidence of periodontal disease is well-known in chronic stress affliction. Considering that TST is produced in suprarenal glands and in ovaries in the same amount in pre-menopausal women, its level must be regulated by hypothalamic- hypophyseal products.¹⁰ Except FSH and LH levels that change regularly, it is ACTH, which stimulates all three morphological zones of suprarenal cortex.^{11,12} This stimulation leads primarily

to cortisol augmentation. In long-term stress affliction the cortisol levels are permanently increased resulting in secondary ACTH suppression. When there is an insufficient ACTH stimulation of suprarenal glands in women, decreased excretion of androgen and mineral corticoids of suprarenal origin usually develops.¹³ Serum TST decreases, which indicates the reduction of measured salivary TST. Acute stress results in an increased amount of ACTH, which heads to increased TST levels. This should cause the dropping of neutrophiles penetration and thus decrease of local inflammatory reaction. However, increased levels of salivary TBARS in our statistical ensemble were found. We know that the relationship between TBARS and TST could be secondary in women, while the primary hormonal factor may be the immunosuppressive effect of glucocorticoids. Alternatively, TST levels affect calcium storage into bones and teeth,¹⁴ where higher TST levels induce this process, which could result in reduced teeth impairment and decreased gingival marginal inflammation. TST with its proteoanabolic effect could stimulate the production of collagen, which is an important part of periodontal tissue, and might help the local mechanic defense against the development of the periodontal disease. Reduced PBI values correlated with increased TST levels in women could be explained by the mechanisms mentioned above, but the quantification of single mechanism's participation on this relationship is difficult to estimate. However, a combination of these mechanisms in the risk of periodontal disease development is more likely to be implicated.

TBARS are the products of lipoperoxidation (LPX), a reaction that takes place in every living organism in nature, almost everywhere.^{1,15} It was not possible to differentiate the origin of LPX products using the available methods. These products could come from increased LPX that took place somewhere else in the organism (not in the oral cavity) and they enter saliva by penetration or diffusion from plasma. Other possible reasons could be found in microorganisms, which are the part of plaque, or the TBARS could be of direct origin from damaged/undamaged gingivae.^{2,16,17} TBARS and PBI statistical dependence can be explained by several divergent ways; e.g. if TBARS could come from microorganisms present in normal flora, then higher PBI could indicate greater number of pathogenic microorganisms with the possibility of lowered TBARS production – endanger of environment for original colonizers. Decreased number of original colonizers would naturally insinuate to lowered TBARS production. In contrast, it is likely that TBARS penetrate directly from blood by diffusion, which indicates that they are produced somewhere out

of oral cavity, during pathological processes in the organism. This impedes the interpretation of the results. On the other hand, that would give the possibility of future utilization of the salivary TBARS as markers of different pathological conditions with no invasion into the organism, unlike nowadays with the measurement of plasmatic levels. TST increases the levels of LDL,^{10,18} which is oxidable, and if oxidable, then LPX takes place and the TBARS levels rise as well. Mouth hygiene and type of toothpaste, its ingredients and the fluoride content probably play an important role in TBARS production, levels etc. Other important factors could be: body temperature and its effect on hormonal changes and immunity, genetic factors and, last but not least, food composition, where vitamins decrease the TBARS production¹⁷ or phytoestrogens in soy products that increase the free TST levels.^{19,20}

In conclusion, we can say that the relationship between TST, TBARS and periodontal status is unclear in men, probably due to the high intraindividual and interindividual variability of salivary TST levels. On the other hand, interesting and significant positive correlations between TST vs. TBARS and TST vs. PBI were found in women. These results might be explained by the effects of TST on immunological parameters that are related to both, the periodontal status and the oxidative stress. The usefulness of salivary TBARS measurement should be proved in further studies.

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