CORRELATION DEPENDING ON AGE BETWEEN SALIVA AND PLASMA PARAMETERS

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Abstract

Introduction. *Because of the increasing interest in the study of saliva, many methods have become available that can allow us to perform multiple and rapid analyzes of the composition of salivary secretion using advanced techniques of nanotechnology and fluid micro-engineering. The objectives of our study were to identify immunoglobulin levels variations with age.*

Materials and methods. *We conducted an analysis to check the correlation between saliva and plasma parameters on 24 patients. The study population was divided into 2 groups based on age, with a cut-off at 35 years. The differences on the variables between two groups were evaluated by TTEST and CORREL.*

Results. *Total plasma calcium, as well as ionic calcium have increased values in group 2, the increase being statistically significant (p =0.04) only for ionic calcium. Significant correlation of plasma and salivary values in the case of IgA / IgG ratio, and also a significant correlation of salivary and plasma concentrations for IgG were identified in our study.*

Conclusions. *The correlation between plasma and salivary parameters is closely related to age and these can be used as markers for diagnosis and evaluation of various pathologies. Also, regarding women, salivary calcium and phosphate concentrations increase with age showing peak values around menopause.*

Keywords: *saliva, plasma, immunoglobulin, age.*



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Abstract

Introducere. *Datorită interesului din ce în ce mai mare pentru studiul salivei, au devenit disponibile numeroase metode care ne permit să efectuăm analize multiple și rapide ale compoziției secreției salivare, folosind tehnici avansate de nanotehnologie și microinginerie fluidă. Obiectivele studiului nostru au fost identificarea variațiilor nivelurilor de imunoglobuline în funcție de vârstă.*

Material și metodă. *Am efectuat o analiză pe 24 de pacienți pentru a verifica corelația dintre parametrii salivari și cei din plasmă. Populația studiată a fost împărțită în două grupuri în funcție de vârstă, cu un cut-off la 35 de ani. Diferențele variabilelor din cele două grupuri au fost evaluate prin TTEST și CORREL.*

Rezultate. *Calciul plasmatic total, precum și calciul ionic au valori crescute în grupa 2, creșterea fiind semnificativă statistic (p =0,04) doar pentru calciul ionic. În studiul nostru au fost identificate corelații semnificative ale valorilor plasmatice și salivare în cazul raportului IgA/IgG, precum și o corelație semnificativă a concentrațiilor salivare și plasmatice pentru IgG.*

Concluzii. *Corelația dintre parametrii plasmatici și salivari este strâns legată de vârstă și aceștia pot fi utilizați ca markeri pentru diagnosticul și evaluarea diferitelor patologii. De asemenea, în ceea ce privește femeile, concentrațiile salivare de calciu și fosfat cresc odată cu vârsta, prezentând valori de vârf în preajma menopauzei.*

Cuvinte cheie: *salivă, plasmă, imunoglobulină, vârstă.*

Introduction

Total saliva consists of a mixture of secretions produced by large and small salivary glands, the crevicular fluid that comes from the gingival groove, to which is added the transudate. Saliva also contains secretions from the cells of the nasal and pharyngeal mucosa, fungi, viruses, many bacteria, food debris, desquamated epithelial cells, blood cells - especially leukocytes, but small amounts of drugs or various chemicals of exogenous origin can also be detected[1].

There are only a few substances, including electrolytes, proteins, and enzymes, for which the correspondence between serum and saliva levels is known[2]. Nevertheless, saliva is currently used as a research medium to diagnose systemic diseases or to determine the risk of certain diseases in which oral health is closely related to general health[3, 4]. Sialochemistry and sialometry

methods tend to replace the dosages in the blood or derivatives (plasma or serum) especially when researching parameters that vary concordantly in the blood and saliva[5].

The parameters of salivary secretion can influence osmolarity, viscosity, salivary flow, salivary pH and salivary constituents, these being variable depending on the age of the person. Methods that use saliva as research material have a number of advantages because in saliva are found mostly same molecules as in the systemic circulation and also the collection of saliva is easily achievable, non-invasive and can be repeated, the patient's availability is high and they can even do it themselves if they receive instructions and containers for collecting[6,7,8].

The purpose of our study was to monitor age-related changes in salivary secretion in subjects without systemic disorders, as well as their correlations with plasma values, for the following parameters: proteins, immunoglobulins, aminotransferases, urea, creatinine and electrolytes.

Materials and methods

We ruled the statistical significance of the differences in the values determined and their plasma-saliva correlations. In order to reduce the variables as much as possible, we excluded smokers and chronic alcohol users from the study. The results we gathered show that saliva could be a good plasma substitute for determining some of the parameters of diagnostic interest.

In the study we managed we included a number of 24 healthy, clinically normal subjects, 18 men and 6 women, with a mean age of 34.5 (±13.6) years, from whom we obtained the consent for blood sampling and saliva and for the processing and use of the research results.

We divided the 24 participants into 2 groups: group 1 - consisting of 12 male subjects, aged 18 to 35 years and group 2 - consisting of 12 subjects, 6 males and 6 females, aged 36 to 55 years,

Blood and saliva samples were collected in the morning, on an empty stomach, after a total fast of 12 hours, between 8:30 AM and 9:30 AM. Except for patients with mandatory medication, participants did not take medication for 24 hours before collecting; those on chronic treatment took their morning medication after collecting blood and saliva. The blood was collected aseptically by venous puncture, and the treatment was performed using standard sample preparation techniques for clinical laboratory tests.

Immediately after collection, the saliva samples were placed in ice boxes and transported in an isothermal shell, then centrifuged for 20 minutes at 6000 rpm with the ALC Centrifugette 4206 centrifuge. The supernatant of each sample was divided into smaller, stored volumes in the freezer, at a temperature of - 20°C. From the time of collecting to the time of spinning, the time interval was a maximum of 3 hours.

The following determinations were taken from saliva and blood: total protein levels; Immunoglobulin (Ig) G and IgA levels, salivary and plasma IgA / IgG ratio; aminotransferase levels of aspartate amino-transferase (AST, glutamate-oxaloacetic transaminase or TGO), alanine amino-transferase (ALT, glutamate-pyruvate transaminase or TGP), AST / ALT ratio; salivary and plasma urea levels; salivary and plasma creatinine levels; ion levels: chlorine, calcium, potassium.

Biochemical determinations, AST, ALT, urea, creatinine, electrolytes, were performed by the method of dry biochemistry on Vitros devices, which perform colorimetric and

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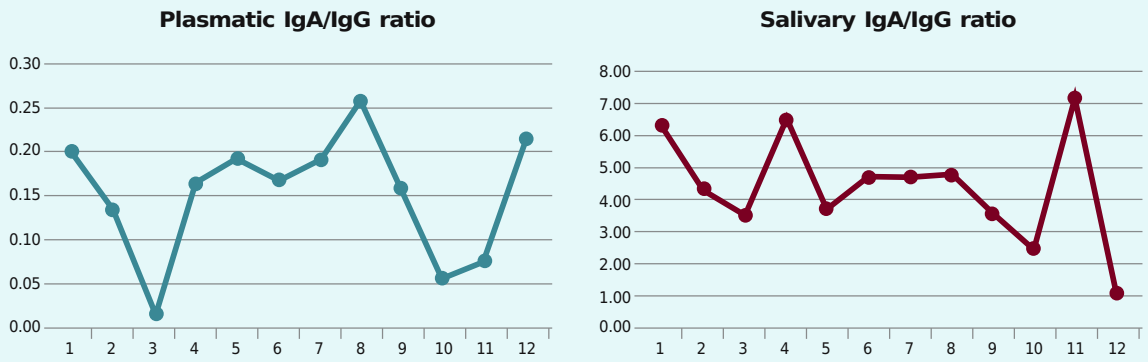


Figure 1. *Statistically significant correlation between the determined values of the plasma and salivary IgA / IgG ratio in group 1 (18-35 years)*

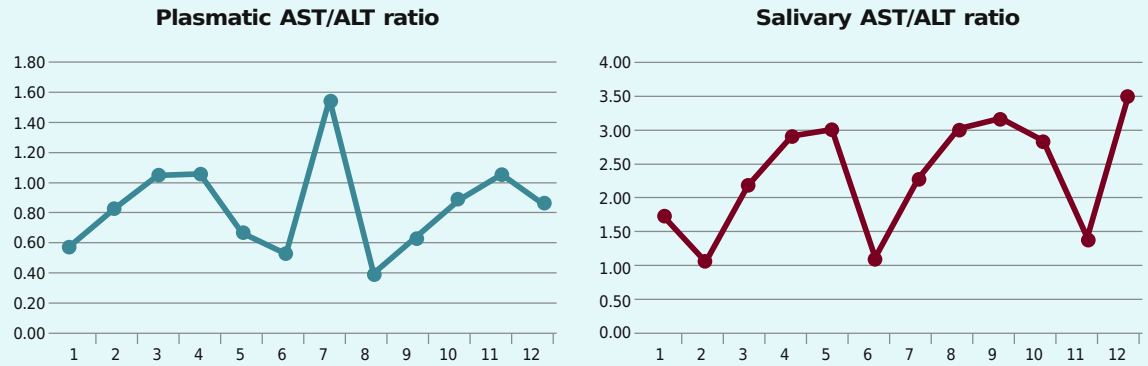


Figure 2. *Statistically significant correlation between the determined values of the plasma and salivary IgA / IgG ratio in group 1 (18-35 years)*

potentiometric determinations. A slide is used for each biochemical test; several slides (50-60) are provided as a cartridge. After use, the cartridge is removed and replaced with a new set of determinations; no other conventional chemical reagents are used, other than those in the standardized kits.

The determination of salivary immunoglobulins was performed by the Mancini simple radial immunodiffusion method adapted to saliva, on immunoplates, containing agar gel, in which a different colored monospecific serum is incorporated: red - for IgG, blue - for IgA, yellow - for IgM. Reading is performed after incubation for 48 hours at 37°C. The diameter measured in mm corresponds to a certain concentration according to the table, which accompanies the immunoplates. In order to dose very small amounts of salivary immunoglobulins (inflammation and gingival retraction, increased tooth mobility), in cases where no precipitation was obtained even after repeated addition of the sample, saliva was concentrated 2, 3 or 4 times by dialysis bags Serva Visking Dialysis tubing, also making the correction to determine the concentration.

The determination of total protein concentration in saliva was achieved with the Lovry method: 0.5 ml of saliva was used, over which 1.5 ml of distilled water and 1.5 ml of 4% Na2CO3 were added. After stirring, 0.5 ml of Folin-Ciocâlteu reagent (CuSO4, KI, Na tartrate in 2N Na2CO3 buffer) diluted 1: 3 was added. The mixture was stirred, left to stand for 30 minutes in the dark, developing a blue color. It was read in Spekol RA-50 at λ =620 nm. The optical density was converted to protein concentration (g/l) by the standard curve.

We processed the obtained data and expressed the results as average values ±standard deviation, for each batch. We analyzed the differences of the variables between two groups by the Student's test (TTEST). The values of $p < 0.05$ are considered statistically significant, and if the values of $p < 0.01$ are high significantly. For the correlations between the variables we used the CORREl test, the regression analysis (r determination), the correlations being significant if $p < 0.05$.

Results

Following the study, we determined the correlations of the following plasma and salivary parameters: IgG, IgA, IgA / IgG ratio, aminotransferases and AST/ALT ratio, urea and creatinine, calcium and potassium managing the results to be presented.

Regarding the analysis of the correlations between plasma and salivary concentrations of total proteins, IgG, IgA, IgA / IgG ratio, we obtained a significant correlation of plasma and salivary values in the case of IgA / IgG ratio, and also a significant correlation of salivary and plasma concentrations for IgG (Figure 1).

For the analysis of the correlations between the plasma and salivary concentrations of AST, ALT and the AST/ALT ratio, we determined the values of p, which proved to have a significant correlation between the plasma and salivary values of the AST/ALT ratio (Figure 2).

Regarding the correlations of calcium and potassium determined in plasma and saliva in group 1 (18-35 years), the p value shows that there are significant serum-plasma correlations of plasma ionic calcium with salivary calcium. In group 2 (36-55 years), total plasma calcium is correlated with salivary calcium, but also plasma potassium with salivary calcium.

Total plasma calcium, as well as ionic calcium have increased values in group 2, the



increase being statistically significant (p =0.04) only for ionic calcium: 4.23±0.35 mg/dl in group 2 compared to the average value of 3.97±0.36 mg/dl in the first group.

Discussions

In the elderly, there is a reduction in unstimulated salivary flow, total saliva has a lower overall flow, higher stimulated total salivary flow, reduced salivary amylase, antifungal defense factors, lactoferrin and neutrophil function are lower, while in children secretory flux and Ig A concentration are higher.

The study of Kiran SA et al[9] is the first comprehensive, high-throughput proteomic study which identifies and quantifies the global changes in the abundance of salivary proteins associated with normal aging in healthy females. More than 900 proteins were recently identified in parotid saliva by mass spectrometry, 349 of which overlapped with the 532 proteins detected in the present study. Although many proteins were common to both age groups, a surprisingly large number of proteins (50%) were uniquely expressed in an individual age group, the highest number of proteins being identified in the older age group. The abundance of antimicrobial proteins such as proline rich proteins, mucin-7 and bacterial permeability membrane proteins did not change with age, while lysozyme, lactoferrin, histatin-1, serotransferrin, mucin-5 and NGAL abundance were increased in the older age group. In contrast, other antimicrobial proteins such as granulins and jacalin-like lectin decreased with age (group 1>2)[10].

Oral mucosal infections are common and are associated with a number of changes in saliva's ability to defend itself, such as significant reductions in the level of IgA and IgM in total saliva and the concentration of IgA secretor and mucin in the saliva produced by the small salivary glands[11, 12]. Age influences salivary immunoglobulins, but there are no significant differences between men and women. Salivary IgA increases with age up to 60 years, then there is a slight decrease in the concentration of this immunoglobulin. IgE also increases with age up to 40, then the level remains constant[13].

The data obtained in the study of Bayram RO et al.[14] show that plasma immunoglobulin levels are strongly influenced by age, for example IgG is increased in newborns, then decreases and increases again at the age of 18 years. IgA is very low in the newborn and increases with age, thing we also observed in the groups I studied. IgM has minimal values in newborns and high values at 18 years.

According to a study led by Manishkumar DS et al.[15] of healthy subjects aged 1-70 years old, the results showed that the salivary IgA levels exhibit age-related changes. Oral

immunization may be considered to improve oral immunity when the salivary concentrations of IgA begin to decrease during lifetime. The salivary IgA levels increased with increasing age up to 60 years and then decreased. Moreover, the detectable rates and levels of salivary IgA increased from age 1 to 40 years and then decreased.

Eliasson et al.[16] investigated the IgA concentrations in secretions of palatal, buccal, and labial salivary glands in individuals aged 18-72 years. They have observed that the salivary IgA concentrations in these saliva samples were higher in elderly individuals (≥65 years) as compared to individuals aged 18-64 years. On the other hand, in the study of Weemaes et al.[17] he reported that the salivary IgA secretion rate increased during infancy and childhood period at age 1-12 years[18]. Diabetes and predisposing factors also interfere the protective role of saliva[19,20].

Serum determination of the two aminotransferases, AST and ALT, is part of the usual assay for detecting and tracing patients with chronic liver diseases. Although it has some limitations, such as lack of specificity and inconsistent growth, AST and ALT dosing is the most widely used of serum enzyme assays for the detection of hepatocellular damage or liver necrosis. Data emerging from certain studies, such asWillems JL et al. and Bohnen N. et al show that AST activity decreases with age, and ALT does not change with age[21,22].

With age, there has been an increase in plasma parathyroid hormone and a decrease in ionized calcium only in men, possibly by reducing intestinal absorption[23].

In a study of Liisi Sevón et al.[24] conducted on women, it was observed that salivary calcium and phosphate concentrations increase with age showing peak values around menopause. Therefore we suggest that menopause is reflected in saliva as elevated levels of calcium and phosphate. Salivary calcium and phosphate concentrations showed a clear increase with increasing age. Peak values occurred at around 50-54 years of age. Calcium and phosphate increased about 12 % at the age group of 50-54 years as compared to the younger age group from 45 to 49 years. Alongside other serological individual characteristics, saliva is the major predictive and prognostic factor for most oral cavity pathologies[25].

Conclusions

There are various changes in plasma and salivary parameters depending on age. Regarding the concentration of immunoglobulins: in patients aged 36-55 years old, there is a statistically significant increase for plasma and salivary IgA and salivary IgG, respectively.

Thus, if there are no quantitative changes in plasma proteins, as age increases, qualitative and ratio changes between different types of plasma proteins remain possible and very likely. Evaluating liver enzymes AST and ALT showed higher levels in both saliva and plasma for the patients between 39 to 55 years. The use of aminotransferase dosing in saliva can be a non-invasive test to help diagnose liver and local inflammatory conditions.

Among the electrolytes studied, potassium and calcium have higher levels in the group of older subjects, while urea and creatinine in plasma and saliva have no significant variations depending on age. Parameters whose plasma and salivary concentrations correlate significantly in subjects aged 18-27 years are the IgA/IgG ratio, the ASL/ALT ratio



and plasma ionic calcium with salivary calcium, and in those aged 39-55 years are: IgG, ASL/ALT ratio, potassium and total plasma calcium with salivary calcium. Thus, we can conclude that the correlation between plasma and salivary parameters is closely related to age and these can be markers for diagnosis and evaluation of various pathologies.

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