A NEW INDICATOR FOR EVALUATION OF ORGANIC COMPOUNDS IN SALIVA

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Abstract

Introduction. The environment of oral cavity is an integral part of the body, dependent on systemic metabolism, but at the same time a separate ecosystem which affects the whole organism. Many studies on etiology of systemic diseases confirm the role of oral cavity diseases in their course. For a number of parameters, the importance of the reciprocal relationship between serum and saliva (e.g. toxicological studies, assessment of metabolites) has been confirmed. Concentrations of parameters in serum used for assessing metabolism do not show a normal distribution, whereas concentrations of the same parameters in saliva are n-fold lower than the values in serum. Consequently, lack of linear serum-saliva relationship has led to creation of the saliva/serum index that allows to evaluate oral health in relation to systemic processes.

Aim. The aim of this study is to assess the author's Saliva Serum Index (SaS Index) which analyses parameters in saliva in conjunction with the metabolism of the body.

Material and methods. The new SaS Index was used in this study. The index is based on the assumption that the most objective way of presenting the clinical significance of selected saliva parameters is to determine them as log% of values in serum. The usefulness of the SaS Index was evaluated using serum and saliva lipid's parameters obtained by the photometric method on the Cobas Mira analyzer.

Results. There were no statistically significant correlations of serum total cholesterol and triglycerides values with the presence of dental caries. However, statistically significant higher values of triglycerides were found in saliva of people with dental caries compared to saliva of people without caries ($p<0.05$). The results have shown a statistically significant link between dental caries and the value of the SaS Index for triglycerides ($p<0.05$) and for total cholesterol ($p<0.05$).

Conclusions. Saliva Serum Index (SaS Index) allows an objective assessment of the parameters in saliva. The relative biochemical markers of serum and saliva may be used to assess and monitor the condition of the oral cavity.

Key words: saliva, serum, dental caries, cholesterol, triglycerides.

Introduction

Saliva is secreted from six major and many minor salivary glands. Most of it (about 90%) is secreted by the major salivary glands. A variety of cells are responsible

for the secretion of saliva including acinar cells, striated duct cells, intercalated duct cells and main excretory duct cells. Saliva contains a large amount of water and inorganic and organic ingredients constituting about 0.6%. One of the major organic constituents next to proteins and urea are lipids. According to the international lipid classification, the LIPID MAPS, the following lipids are present in saliva: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids [1]. Saliva comprises cholesterol, cholesteryl esters, mono-, di- and triglycerides and free fatty acids [2]. The total fatty acids of parotid and submaxillary secretions may be particularly unique in their high concentrations of long chain, polyunsaturated fatty acids. It is thought that lipids of saliva may have a role in the binding of salivary proteins, adsorption of bacteria to apatite, and the aggregation of bacteria in plaque.

Dental caries is a multifactorial microbial disease dependent on the quantity and quality of saliva. Lipids' analysis in parotid's saliva among two groups of female subjects susceptible to- and resistant to dental caries showed higher total lipids' concentration in caries susceptible group [3].

The environment of oral cavity is an integral part of the body, dependent on systemic metabolism and processes, but at the same time a separate ecosystem which affects the whole organism. Many studies on etiology of systemic diseases confirm the role of oral cavity in systemic diseases detection. More recently, the advances in biotechnology, salivary diagnostics, genomics, and proteomics have extended the range of salivary diagnostics to systemic diseases, monitoring in cancer, autoimmune diseases and microbial systemic infections [4]. A correlative study of serum and saliva performed in about 100 healthy individuals showed moderate correlations of total cholesterol, triglycerides, high density and very low density lipoprotein cholesterol between the serum and saliva, emphasizing the use of saliva as a non-invasive diagnostic fluid for lipids' analysis [5]. A study by Karjalainen *et al.* showed that the salivary concentration of cholesterol reflects the serum concentration in hypercholesterolemic individuals [6].

Concentrations of lipids parameters in serum and saliva do not show a normal distribution. Consequently, lack of linear serum-saliva relationship has led to creation of the saliva/serum index that allows to evaluate oral health in relation to systemic processes.

Aim

The aim of this study is to assess the author's Saliva Serum Index (SaS Index) which analyzes parameters in saliva in conjunction with the metabolism of the body.

Material and methods

This study includes 160 patients of the University Children's Hospital. Patients between 10 and 16 year's old (mean 11.16±1.89) were qualified for examination, whose blood had been taken for treatment or control tests during hospitalization. The necessary consent was acquired from bioethical commission and from patients and their families. For the lipids' fraction analysis the unstimulated total saliva

was taken in a passive method with saliva free flowing into a plastic disposable vial or actively spit directly into the vial [7-9]. The oral examination was done after collecting serum and saliva. The condition of the teeth was assessed using the DMFT index. In the analysis, the D component of the DMFT index was used, illustrating the number of teeth with carious cavities or caries around dental fillings found during examinations. The study group was divided into a group of patients without dental caries (D=0) (23 participants) and with dental caries (D>0) (137 participants).

Serum and saliva samples

The samples were collected fasting in the early morning between 8:00 and 10:00 am. Saliva was taken as the first sample and blood as the second. Each participant was instructed to refrain from brushing teeth for at least 2 hours prior to saliva collection. At the beginning of session, participants were requested to provide unstimulated saliva according to the study protocol [10]. The patient was comfortably seated, with their head bent forward for collecting the secreted saliva for 10 minutes into a sterile disposable tube [9]. The saliva was centrifuged to eliminate bacterial contamination and stored at -80°C until used [11].

The collected blood was coagulated at room temperature, and then the serum was separated by centrifugation for 10-15 minutes in a centrifuge at 2000-3000 revolutions per minute and frozen in -80°C.

After being thawed serum and salivary lipids analysis were done on La Roche Cobas MiraS colorimetric analyzer in the laboratory at the Medical University in Lublin using the Cormay diagnostics (PZ Cormay S.A. Poland) reagents. For determination of triglycerides (TG) a colorimetric enzymatic method with glycerophosphate oxidase was used, and for total cholesterol (CH) with esterase and cholesterol oxidase (CHD/PAP). To ensure reading accuracy, the instrument was calibrated on standardized samples. The calibrators and Cormay serum HP control material were diluted 100 times to obtain values μmol/l.

The new Saliva Serum Index (SaS Index) has been developed by the authors of this article, to assess the importance of the lipid fraction of the body in oral diseases. The index is based on the assumption that the most objective way of presenting the clinical significance of selected saliva parameters is to determine them as log% values in serum. It presents the examined parameter as a number, which value reflects the concentration of the examined parameter in samples taken at the same time in serum and saliva assessed by the same methods. The formula for calculating the SaS Index is:

$$SaS = \{(\text{saliva concentration}/\text{serum concentration}) \times 100\%\}\log$$

This study aims at evaluating the usefulness of the SaS Index using serum and saliva lipid's parameters.

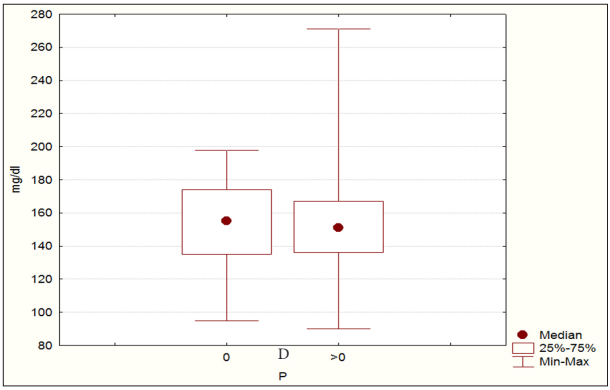
Determined parameters were subjected to statistical analysis with the use of ANOVA analysis of variance. Statistical significant differences were established at $p<0.05$. The statistical analysis included the SaS Index.

Results and discussion

The results of the dental caries examination are presented using the DMFT index. To analyze the results, a D – caries component was used to show the number of teeth with caries during sampling. The adolescent subjects were divided into two groups, with (D>0) and without (D=0) signs of active dental caries.

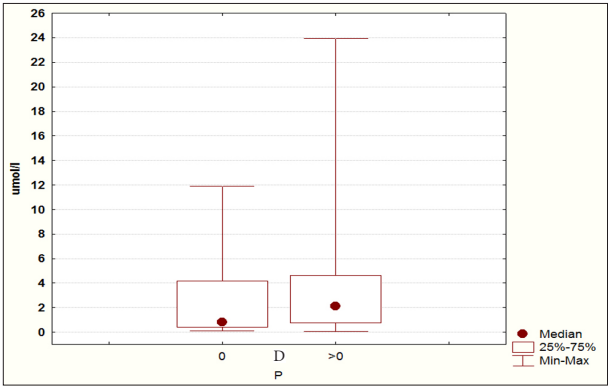
Total cholesterol in serum and unstimulated saliva and dental caries

Figure 1 shows the concentrations of serum cholesterol for subjects with and without dental caries. There were no statistically significant correlations between the serum total cholesterol value and the presence or absence of dental caries in investigated adolescents (p=0.7372). Figure 2 shows the concentrations of saliva



p=0.7372

Figure 1. Concentration of serum total cholesterol in the absence (D=0) or presence (D>0) of teeth with caries (D – active caries)



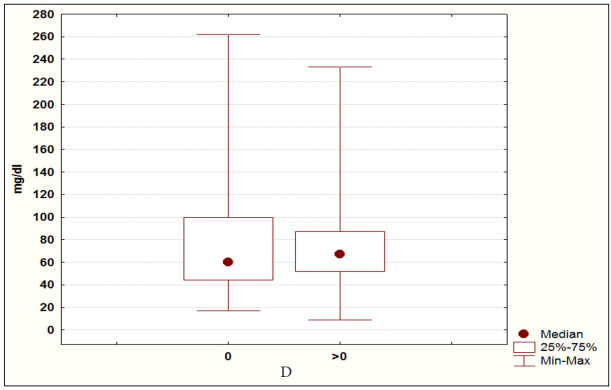
p=0.0829

Figure 2. Concentration of saliva total cholesterol in the absence (D=0) or presence (D>0) of teeth with caries (D – active caries)

cholesterol for subjects with and without dental caries. There was no statistically significant relationship between the total cholesterol in saliva and dental caries (p=0.0829).

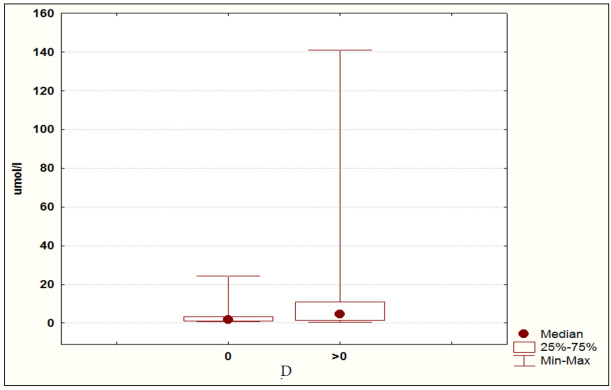
Triglycerides in serum and unstimulated saliva in relation to dental caries

There were no statistically significant correlations between serum triglycerides' value and the presence or absence of dental caries p=0.4509 (Figure 3), statistically significant correlations occurred between the triglycerides' values in saliva and dental caries (p<0.05) (Figure 4). A few studies have conducted research to investigate the correlation between the lipid values in saliva and dental caries, indicating that



p=0.4509

Figure 3. Concentration of serum triglycerides in the absence (D=0) or presence (D>0) of teeth with caries (D – active caries)



p=0.0421

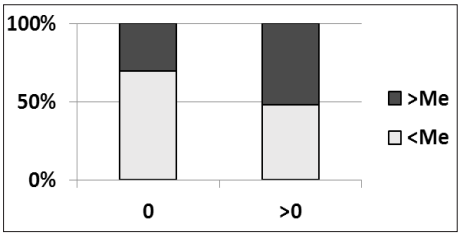
Figure 4. Concentration of saliva triglycerides in the absence (D=0) or presence (D>0) of teeth with caries (D – active caries)

saliva of people susceptible to development of caries contains more neutral lipids than of people resistant to development of caries [3,12].

SaS Index and dental caries

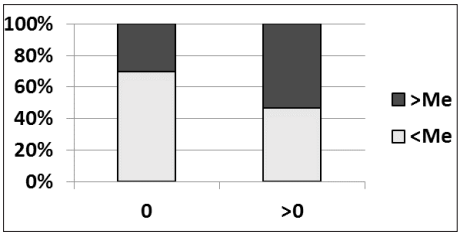
Figure 5 and 6 show the correlation between SaS Index and the presence or absence of dental caries. The SaS Index values for total cholesterol and for triglycerides were calculated and divided into values higher or lower than the median (Me) for each of these parameters. Higher values of SaS Index for cholesterol were significantly correlated with the presence of teeth with caries $p<0.05$ (Figure 5). Similar results were obtained for triglycerides. Higher values of triglycerides' SaS Index significantly correlated with the presence of teeth with caries $p<0.05$ (Figure 6).

The analysis of salivary results is more difficult than analyzing the same parameters in serum or urine as there are no established ranges of concentrations for compounds found in saliva in healthy people. The use of the indicator requires the determination of the serum and saliva parameters according to generally accepted principles. Normalized serum parameters and variable salivary parameters were introduced in the index so that individual variability in saliva parameters is stabilized by normalized serum parameters. Therefore, in these analyzes, the use of parameters with norms differentiating health and disease seems to be necessary. The new SaS Index provides this possibility for lipids as well as other parameters tested in serum and saliva.



p<0.05

Figure 5. SaS Index for total cholesterol, in the group with dental caries (D>0) or without (D=0)



p<0.05

Figure 6. SaS Index for triglycerides, in the group with dental caries (D>0) or without (D=0)

Conclusions

Lipids' higher content in saliva might be a marker of active carious processes in the oral cavity. The biochemical parameters of serum and saliva may be used to assess and monitor the condition of the oral cavity using SaS Index. The SaS Index could be used in the analysis of other organic compounds in saliva by analogy.

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