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The usefulness of Spotchem® Analyser (Arkray) in determining the risk in oral diseases in adolescents – a pilot study

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Keywords

salivary tests, Spotchem® Analyser, caries, gingivitis, oral hygiene

Summary

Introduction. Saliva Check Buffer® has been long used for assessing saliva physical and chemical properties, while CRT Bacteria® is used for estimating the count of cariogenic bacteria. Spotchem® Analyser is a new device for the assessment of saliva properties.
Aim. To determine the consistency of results obtained using Spotchem®, Saliva Check Buffer® and CRT Bacteria® kits, as well as to evaluate the correlations between the obtained results and oral hygiene status, ICDAS II indices in the diagnosis of the risk of oral diseases in children.
Material and methods. Patients aged 12-17 years were evaluated for oral hygiene (% API, OHI), gingival inflammation (GI), and carious lesions (ICDAS II). Salivary tests using Saliva Check Buffer® (GC), CRT Bacteria® (Ivoclar Vivadent) and Spotchem® (Arkray) Analyser were conducted. The consent of the bioethical committee of Warsaw Medical University, as well as written consent from all the parents of all the subjects or legal guardians of all the subjects were obtained.
Results. The study included 25 patients (mean age 13.7 ± 2.2 years). The following mean index values were obtained: OHI-S – 0.93 ± 0.43; API% – 72 ± 0.26; GI – 0.83 ± 0.61; DMFt – 6.44 ± 4.12. Active white lesions were observed in 13 patients (mean number of lesions 2.2 ± 2.92). Spearman's rank correlation coefficient showed significant correlations between pH values according to Saliva Check Buffer® and salivary buffer capacity (r = 0.608) and acidity (r = -0.713) according to Spotchem®; as well as a negative correlation between salivary buffer capacity and pH values in Spotchem® (r = -0.845). High count of *S. mutans* (> 10^5 CFU/mL) assessed by CRT Bacteria® correlated significantly with high bacteria count estimated by the Spotchem® Analyser (r = 0.54). Significant correlations were found between OHI and high (r = 0.46) and average (r = -0.54) metabolic activity of *S. mutans*; GI and salivary protein levels (r = 0.42); carious lesions and salivary protein levels (r = 0.40); salivary blood levels (r = 0.47) (Spotchem®) and the levels of *S. mutans* and *Lactobacillus* spp. (CRT Bacteria®) (0.47 and 0.42, respectively).
Conclusions. The parameters estimated by the Spotchem® Analyser were correlated with the results obtained with the commonly known salivary kits and oral health indices. However, its clinical relevance should be confirmed by further studies.

Introduction

Contemporary dental treatment involves risk assessment and monitoring for oral diseases, dental caries and periodontal diseases in particular (1, 2). Risk assessment for these diseases requires the determination of the balance between pathogenic factors and body defences, including salivary physicochemical properties and oral microbial load (3-6).

Commercially available saliva tests with proven clinical usefulness, such as Saliva Check Buffer®, CRT Baceria® (Ivoclar Vivadent), Dentocult SM and Dentobuff (Orion Diagnostica), have been long used in the clinical practice. Saliva Buffer Check® allows an assessment of salivary pH and buffer capacity. CRT Bacteria® is used to determine the levels of *Streptococcus mutans* and *Lactobacillus* spp. following

stimulated saliva application in SM and LAB-AGAR culture medium and incubation at 37°C for 48 hours. Patients with bacterial count > 10^5 CFU/mL are considered to be at an increased risk of dental caries (5, 7, 8).

Spotchem® (Arkray) is a novel test that allows an assessment of salivary *S. mutans* count, acidity, and buffer capacity, as well as protein, blood, leukocyte and ammonia levels. The scale ranges between 0 and 100 units for each test. The analyser measures the following parameters: *S. mutans* levels (10^6-10^8 CFU/mL); acidity (pH 6.0-8.0); salivary buffer capacity (pH 2.8-6.0); salivary blood levels (0-0.50 mg/dL); salivary leukocyte count (0-200 U/L); salivary protein levels (0-6 mg/dL); and salivary ammonia levels (0-10 000 N-µg/dL). Mean values are as follows: 28-47 units for *S. mutans* levels; 35-52 for acidity; 28-47 for salivary buffer capacity; 14-29 for salivary blood levels; 37-60 for salivary leukocyte count; 36-53 for salivary protein levels; 43-83 for salivary ammonia levels.

So far, no studies using this device have been published.

Aim

The aim of the study was to determine the consistency of results using Spotchem®, Saliva Check Buffer® and CRT Bacteria® kits, as well as to assess correlations between the obtained results and oral hygiene status (including gingival and dental health status) in the diagnosis of the risk of oral diseases in paediatric patients.

Material and methods

Generally healthy patients with permanent dentition aged between 12 and 17 years, who reported for dental visits to the Department of Paediatric Dentistry of the Medical University of Warsaw, were included in the study. A written consent to participate in the study and patient's cooperation were inclusion criteria. Patients using orthodontic appliances, as well as patients with a medical history of chronic diseases or pharmacotherapy affecting salivary properties were excluded from the study. This pilot study has been approved by the Bioethical Committee of the Medical University of Warsaw, no. of approval KB/6/2017. Written consent of the parents or guardians of the patients were obtained. The study involved clinical evaluation and assessment of salivary parameters using commercially available tests. Clinical evaluation was performed in a dental office setting to assess oral health (Approximal Plaque Index %API according to Lange et al., the Simplified Oral Hygiene Index OHI-S) (9, 10), gingival health (GI by Silness & Löe) (11, 12), and dental health – the presence of carious lesions (International Caries Detection and Assessment System II – ICDAS II) (13). Salivary properties were assessed using commercially available tests, such as Saliva Check Buffer® (GC) and CRT Bacteria® (Ivoclar Vivadent), in accordance with manufacturers' instructions, as well as Spotchem® Analyser (Arkray). Saliva Check Buffer® (GC) and CRT Bacteria® (Ivoclar Vivadent) were used to assess parameters measured by Spotchem®. The tests were performed 30 minutes before the use of analyser. Both stimulated and unstimulated saliva (according to manufacturer's instructions) was tested in the morning hours, at least 2 hours after last meal or tooth brushing. During the test with the Spotchem® Analyser, the patient received a rinse solution for a 10-second mouthwash, after which all of the liquid was spat into a disposable cup. Using a pipette, the spat liquid was applied on a test strip, which was placed in the analyser connected to the computer. After 260 seconds, the computer programme produced results for the metabolic activity of *S. mutans*, acidity, salivary buffer capacity, protein, blood, leukocyte and ammonia levels (fig. 1).

Saliva Check Buffer® was used to assess unstimulated salivary pH and stimulated salivary buffer capacity. Unstimulated salivary pH was considered normal for 6.8-7.8; moderately acidic for 6.0-6.6, and acidic for 5.0-5.8. Salivary buffer capacity was assessed after stimulated saliva collection by the patient during a 5-minute paraffin chewing. Using a colour score (green – 4 points, green/blue – 3 points, blue – 2 points, blue/red – 1 point and red – 0 points), the parameter was ranked as high (10-12 points), average (6-9 points) or low (0-5). CRT Bacteria® was used to evaluate *S. mutans* counts (the result was recorded after 48 hours of incubation at 37°C). The assessment was performed based on the number of bacterial colonies on the agar. Patients with bacterial counts > 10^5 CFU/mL were considered to be at a high risk of dental caries.

Correlations between the analysed variables were assessed based on Spearman's rank correlation coefficient. Additionally, a simple linear regression analysis or logistic regression was used to evaluate selected correlations. Statistica 8 used for statistical analysis and a $p \leq 0.05$ was considered statistically significant.

Results

The study included 25 patients aged between 12 and 17 years (mean age 13.7 ± 2.2 years). The results for oral, gingival and dental health status are shown in table 1. Salivary assessment using the Saliva Check Buffer® (GC) and Spotchem® (Arkray) are summarised in table 2. CRT Bacteria® (Ivoclar Vivadent) detected high *S. mutans* counts in 8 (32.0%) patients and high *Lactobacillus* spp. counts in 17 (68.0%) patients.

Spearman's analysis showed statistically significant correlations between pH values measured by Saliva Check Buffer® (GC) and salivary buffer capacity (r = 0.608) and acidity (r = -0.713) in Spotchem® (Arkray). Spotchem® salivary buffer capacity negatively correlated with salivary pH value (r = -0.845). Although the correlation coefficient between the results for acid buffer capacity was positive for both tests, it was not statistically

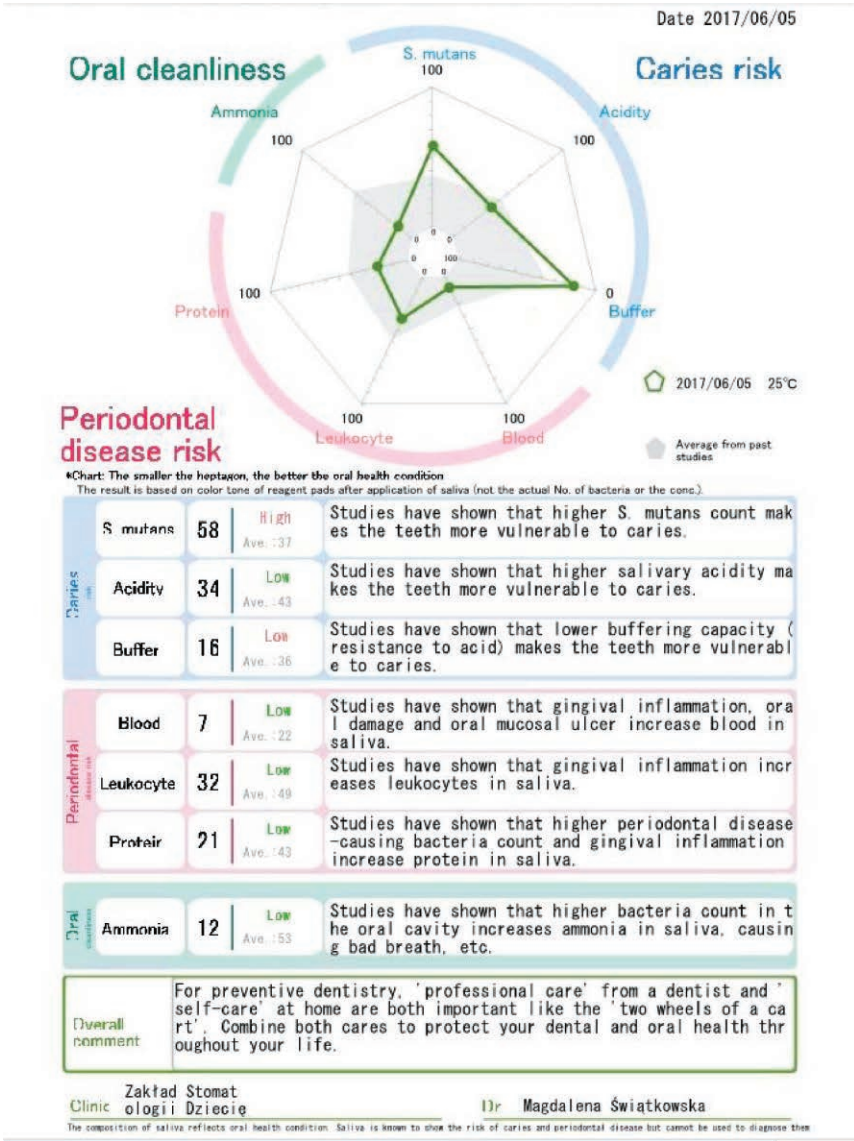

Fig. 1. An exemplary form with results obtained in the Spotchem® Analyser

Tab. 1. Clinical findings in patients included in the study

Clinical evaluation parameters			Value
Hygiene status	OHI-S	mean ± SD	0.93 ± 0.43
	API%		72 ± 0.26
Gingival status	GI		0.83 ± 0.61
	GI > 0.1	n/%	21/84.0
Dental status	active white lesions	n/%	13/52.0
		mean number ± SD	2.2 ± 2.92
	DMFT > 0	n/%	23/92.0
	DT > 0		14/56.0
	DMFT	mean ± SD	6.44 ± 4.12
	DMFS		9.0 ± 6.84

significant. There was a statistically significant correlation between high *S. mutans* counts (> 10^5 CFU/mL) according to CRT Bacteria® and high bacterial metabolic activity in Spotchem® (Arkray) (r = 0.54). Both results indicate high oral *S. mutans* burden and are positively correlated with salivary ammonia levels (r = 0.41 for CRT Bacteria® and r = 0.45 for analyser, respectively). Also, statistically significant correlations were observed between salivary erythrocyte count and salivary leukocyte count (r = 0.57) and salivary protein levels (r = 0.43).

Correlations between salivary findings and clinical oral health parameters are shown in table 3. Mean GI values in patients with high/average and low salivary protein levels were 1.08 ± 0.44 and 0.75 ± 0.64, respectively. DMFT in

Tab. 2. Salivary findings obtained with Saliva Check Buffer® and Spotchem®

Salivary properties		n/% of patients	Saliva Check Buffer®	Spotchem®
Salivary pH	acidic	n/%	1/4.0	6/24.0
	moderately acidic		10/40.0	5/20.0
	normal		14/56.0	14/56.0
	mean score ± SD		6.87 ± 0.59	51.08 ± 20.29
Buffer capacity	low	n/%	4/16.0	16/64.0
	average		18/72.0	5/20.0
	high		3/12.0	4/16.0
	mean score ± SD		7.12 ± 1.96	25.36 ± 22.34
S. mutans metabolic activity	low	n/%	parameters are not evaluated in the test	10/40.0
	average		parameters are not evaluated in the test	12/48.0
	high		parameters are not evaluated in the test	3/12.0
	mean score ± SD		parameters are not evaluated in the test	24.88 ± 20.79
Ammonia levels	low	n/%	parameters are not evaluated in the test	19/76.9
	average		parameters are not evaluated in the test	3/12.0
	normal		parameters are not evaluated in the test	3/12.0
	mean score ± SD		parameters are not evaluated in the test	25.84 ± 28.42
Erythrocyte levels	low	n/%	parameters are not evaluated in the test	7/28.0
	average		parameters are not evaluated in the test	4/16.0
	normal		parameters are not evaluated in the test	14/56.0
	mean score ± SD		parameters are not evaluated in the test	37.56 ± 29.46
Leukocyte levels	low	n/%	parameters are not evaluated in the test	14/56.0
	average		parameters are not evaluated in the test	6/24.0
	normal		parameters are not evaluated in the test	5/20.0
	mean score ± SD		parameters are not evaluated in the test	35.08 ± 24.20
Protein levels	low		parameters are not evaluated in the test	19/76.0
	average		parameters are not evaluated in the test	5/20.0
	normal		parameters are not evaluated in the test	1/4.0
	mean score ± SD		parameters are not evaluated in the test	27.28 ± 12.89

Tab. 3. Statistically significant correlations between oral health parameters and salivary findings based on Spearman's rank correlation analysis

Oral health indicator	Salivary parameter	Test	Spearman's correlation coefficient
OHI-S	high bacterial metabolic activity	Spotchem®	0.46
OHI-S	average bacterial metabolic activity	Spotchem®	-0.54
API%	average bacterial metabolic activity	Spotchem®	-0.43
GI > 0	average bacterial metabolic activity	Spotchem®	-0.45
GI	protein levels	Spotchem®	0.42
Carious lesions (DT > 0)	colony count of *S. mutans* > 10^5 CFU/mL	CRT Bacteria®	0.47
	colony count of *Lactobacillus* spp. > 10^5 CFU/mL	CRT Bacteria®	0.42
	protein levels	Spotchem®	0.40
	erythrocyte levels	Spotchem®	0.47
DMFT	colony count of *S. mutans* > 10^5 CFU/mL	CRT Bacteria®	0.44

patients with high and low *S. mutans* counts in CRT Bacteria® was 8.89 ± 3.98 and 5.06 ± 3.62, respectively. The results for pH and acid buffer capacity were not statistically significantly correlated with the evaluated clinical parameters. No significant correlations were observed between salivary findings and the presence and number of active white lesions. Compared to patients with present/absent active white lesions, Saliva Check Buffer® more often detected lower unstimulated salivary pH and lower stimulated salivary buffer capacity (fig. 2). However, these differences were statistically insignificant. Also, higher GI values were reported in patients with acidic saliva compared to normal and/or moderately acidic saliva (0.74 ± 0.51 vs. 0.95 ± 0.73). However, this difference was statistically insignificant.

Patients with active white lesions tested with Spotchem® presented with higher salivary acidity, leukocyte count, protein and ammonia levels, as well as metabolic *S. mutans* activity, and lower buffer capacity (fig. 3). High *S. mutans* and *Lactobacillus* spp. counts were recorded more frequently in CRT Bacteria® (fig. 4). However, the observed differences were not statistically significant.

Also, statistically significant correlations were observed between gingival inflammation and OHI-S (r = 0.61) and API% (r = 0.52), as well as between GI and hygiene status indicators (r = 0.57 and 0.48, respectively). No statistically significant correlations were reported between gingival and oral health status and the presence of carious lesions and DMFT and DMFS.

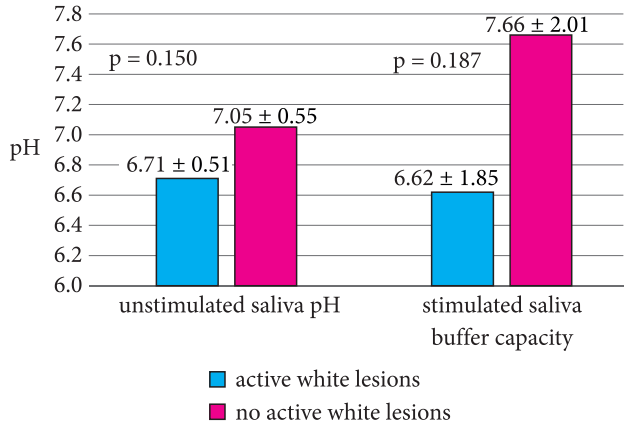

Fig. 2. Salivary properties assessed by Saliva Check Buffer® in patients with and without white lesions

Discussion

The relationship between salivary parameters assessed using commercially available tests and dental caries is well documented in literature (5, 14-16). However, the knowledge of the usefulness of Spotchem® Analyser is insufficient.

Our study confirmed that there is a moderately strong correlation between physicochemical salivary findings according to Saliva Check Buffer® and Spotchem®, as well as a slightly weaker correlation between oral microbiological burden evaluated by CRT Bacteria® and analyser. Due to the small sample size it is difficult to statistically assess the compliance of salivary evaluation results obtained using different methods. Differences in the scale and assessment categories used in these tests are an additional difficulty. Similar difficulties may be found in the comparison of results for oral microbiological burden. Spotchem® classifies SM metabolic activity as low, average and high,

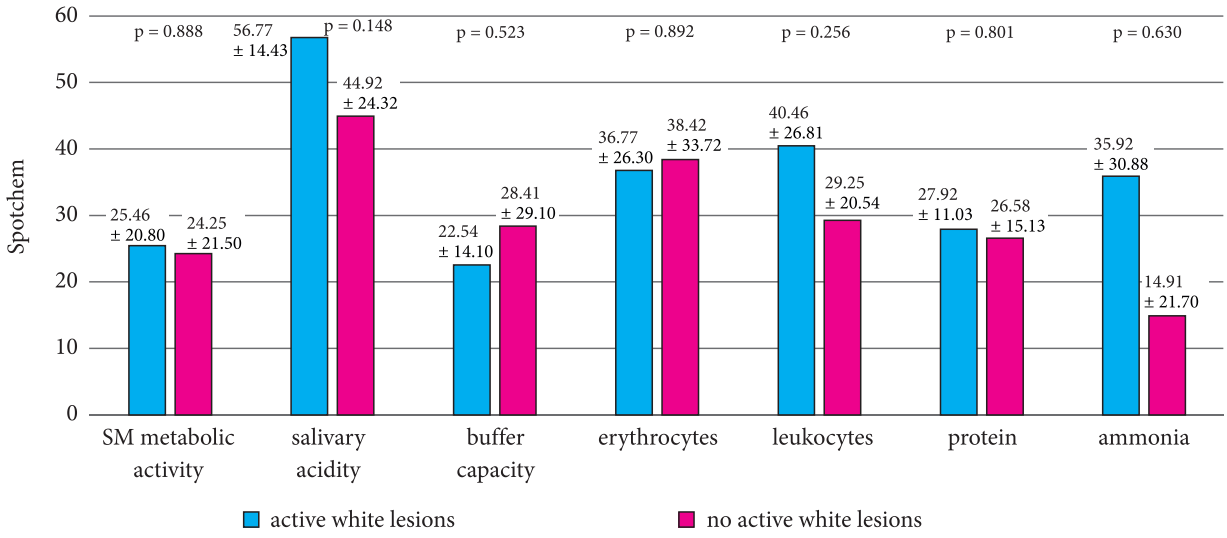

Fig. 3. Salivary properties assessed by Spotchem® in patients with and without white lesions

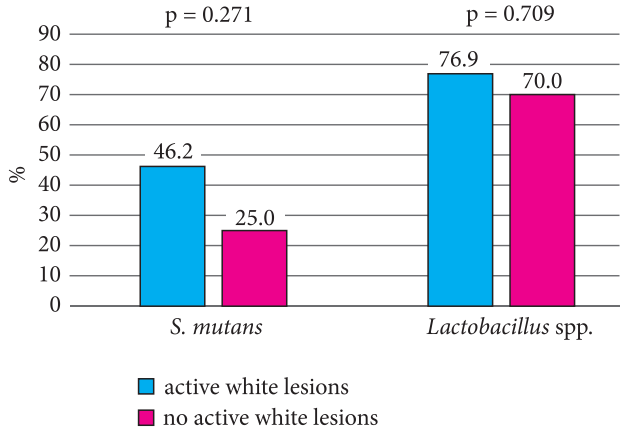

Fig. 4. The rates of *S. mutans* and *Lactobacillus* spp. > 10^5 CFU/mL colony counts in CRT Bacteria® in patients with and without white lesions

while CRT Bacteria® allows for a quantitative assessment of *S. mutans* and *Lactobacillus* spp. colonies. The number is then subjectively evaluated by the investigator using two categories, i.e. above and below 10^5 CFU/mL.

The analysis of relationships between the evaluated physicochemical salivary parameters and clinical oral health assessment parameters showed no statistically significant correlations. However, higher salivary pH values were observed in patients with no active white lesions in each test, and higher GI values in patients with low salivary acidity according to Saliva Check Buffer®. Similar conclusions were drawn by other authors. Animireddy et al. (14) observed no statistically significant differences in salivary pH values according to Buffer Saliva Check Kit® in two groups of paediatric patients – with minor caries intensity and early childhood caries (formerly known as nursing bottle caries). However, the authors observed significantly higher salivary pH values in patients with dmft = 0 compared to patients with carious lesions. Similar results were obtained by other authors (15-19). An evaluation using a pH meter showed higher salivary pH values in caries-free patients compared to those with early childhood caries (ECC). Prabhakar et al. (20) and Preethi et al. (21), who used the same method, observed only a minor difference in salivary pH between the groups. According to the authors, the difference in caries activity indicators is due to other risk factors for dental caries, which dominated the initiation of the process. Piróg et al. (22) showed no significant correlation between salivary pH and permanent dental caries in their studies assessing the effects of physicochemical salivary parameters on dental, gingival and oral mucosal status in children. However, a negative statistically significant correlation was found between unstimulated salivary pH and gingival inflammation (r = -0.529), which supports the relationship reported by the authors and the effects of salivary buffer capacity on the intensity of permanent dental caries (22). An increase in buffer capacity by 1 unit resulted in a decrease in DMFt/dmft by about 1.26 and a decrease in DMFs/dmfs by 1.43. Our study did not show such correlations. However, lower salivary buffer capacity was reported for both evaluated tests (Saliva Check Buffer®, Spotchem®) in patients with active white lesions. Similar, yet statistically insignificant findings were presented by Prabhakar et al. (20), Preethi et al. (21) and Malekipour et al. (23). Singh et al. (16), on the other hand, obtained statistically significantly higher mean salivary buffer capacity levels in patients without active carious foci compared to patients with carious lesions (10.92 vs. 7.46). Animireddy et al. (14) observed significantly higher salivary buffer capacity in children with DMFs/dmfs = 0 compared to DMFs/dmfs ≤ 0. Sakeenabi

and Hiremath (24) also reported that lower salivary buffer capacity was accompanied by higher dmfs, dmft, DMFs and DMFt. Piróg et al. (22) also showed statistically significant effects of lower salivary buffer capacity levels on increased DMFt/dmft and DMFS/dmfs (20). Different findings related to salivary buffer capacity were presented by Zabokova-Bilibilova et al. (6). Higher salivary carbohydrate levels were detected in caries-free patients.

Determination of cariogenic bacteria levels is an important element in the risk assessment of dental caries. Saakenabi and Hiremath (24) detected *S. mutans* in 98.47% of patients, including CFU > 10^5 in 29.59%, and *Lactobacillus* spp. (37.75% with CFU > 10^5) detected in 87.24% of patients. A statistically significant relationship was observed between the levels of these bacteria and DMFT/DMFS and dmft/dmfs. We observed a correlation between carious lesions and high *Streptococcus mutans* and *Lactobacillus* spp. counts in CRT Bacteria®. It should be also emphasised that patients with active white lesions presented with higher *S. mutans* colony counts in CRT Bacteria® and higher metabolic activity of these bacteria in Spotchem® Analyser (statistically insignificant differences). Gamboa et al. (25) demonstrated that culture on MSB agar allowed to detect *S. mutans* in only 62% of patients; however, no correlation with carious activity was found. Similar *Streptococcus* count was reported in caries-free patients and patients with active caries by Xu et al. (26), Tao et al. (27) and Jiang et al. (28). The authors explain this fact by the presence of many species of this genus, which may play different roles in the initiation of carious processes. Gilbert et al. (7) showed in their study in patients with severe early childhood caries that only isolated *S. mutans* colonies were found both in cavities and white lesions. However, the authors point to the diversified virulence of bacteria, which may cause rapid formation of carious lesions. However, Hallet and O'Rourke (29) observed no relationship between increased carious lesions and the presence of *S. mutans* colonies in their study. The author points to the important role of the already existing carious lesions, reduced salivary flow and the presence of orthodontic appliances in this process.

According to our analysis, the metabolic activity of *S. mutans* measured by Spotchem® Analyser was correlated with oral hygiene indicators and gingival inflammation. Spotchem® further allows for an assessment of salivary leukocytes, protein, blood and ammonia levels. Increased salivary protein, leukocyte and erythrocyte levels indicate oral inflammation. It is believed that gingival inflammation may indicate an increased risk of carious disease (30). Despite small sample size, a correlation was observed between protein levels and GI value. Also, a relationship was observed between the presence of active carious foci and salivary protein levels as well as salivary erythrocytes. This confirms that gingival inflammation is one of dental caries risk indicators. It should be also noted that bleeding from inflamed gums initiates the process of local salivary protein accumulation (immunoglobulins, albumins, glycoproteins), which act as first the line of defence against oral pathogens, which explains the relationship with high protein levels (31). A study conducted by Roa et al. (32) using the Bradford protein assay showed no relationship between salivary protein levels and DMFT. A study using protein electrophoresis showed a significant difference in protein levels between patients with active caries and caries-free individuals (33).

There are also studies assessing the relationship between salivary ammonia levels (based on urease levels) and dental caries activity. Shu et al. (34) observed significantly increased levels of urease, an enzyme degrading urea into ammonia, in caries-free patients compared to patients with active caries (< 0.0001). The risk of caries in patients with urease levels below the threshold (< 0.9-> 3.6 units/mg of protein) is 10-17-fold higher compared to patients with urease levels above this value. Similar conclusions were drawn by Nascimento et al. (35), who observed a reverse correlation between urease activity and carious activity ($p < 0.0001$). Morou-Bermudez et al. (36), on the other hand, showed higher salivary urease activity in patients with active caries (mean DMFS > 2) and high *S. mutans* count (a mean ≥ 10^4 CFU/mL). Other authors showed no statistically significant relationship between the levels of salivary urease and dental caries activity as well as the level of cariogenic bacteria (37). Our study also demonstrated no correlation between salivary ammonia levels and carious activity; however, a relationship was observed between ammonia levels and high *S. mutans* count.

The presence of red blood cells in the saliva is another salivary parameter measured by Spotchem®. Statistical analysis showed a significant correlation been its levels and salivary levels of leukocytes and proteins as well as caries. However, no other current studies analysing this relationship using other methodologies were found.

Conclusions

The obtained results do not allow to draw final conclusions. The correlations between salivary findings obtained using the Spotchem® Analyser and those obtained using well-known salivary tests as well as oral health parameters point to the usefulness of the device in risk assessment of dental caries and gingival inflammation in adolescents. This, however, requires confirmation by further research.

Conflict of interest

None

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