

# The Capacity of a Specific Anti-Streptococcus Mutans Monoclonal Antibodies Test to Identify the Diabetic Patients with Increased Risk of Periodontal Disease

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*By means of a specific anti-Streptococcus mutans monoclonal antibodies test we want to identify the diabetic patients which have an increased risk to develop the periodontal disease. The highest percentage, of 88.1% of all patients included in this study represents the subjects with a level greater than 500,000 cfu / mL of streptococcus mutans. The Kruskal-Wallis test reveals a value of  $p = 0.283$  resulted from the status of diabetes in patients and the level of streptococcus mutans in saliva. In conclusion, the status of diabetes in patients seems not to influence the salivary level of mutans streptococci determined with the method used in our study.*

*Keywords: streptococcus mutans, periodontal disease, diabetes, saliva check mutans detection kit*

Periodontal disease in advanced or incipient forms appears today in most people regardless of age and geographic location [1].

Children represent a population group on which we focus in particular, the impact on their health being later on when they become adults [2]. They often suffer from aggressive forms of the periodontal disease. If undiagnosed in time and without proper treatment, the disease can lead to important destructions of the supporting tissues of the teeth. In time these patients become adults with serious periodontal problems [3].

Risk factors such as diabetes, may worsen the periodontal problems [1]. Diabetes is a metabolic disease, often genetically determined, with a chronic stage course, influenced by various etiological factors. It is characterized by abnormal glucose metabolism and may cause disorders of the lipid metabolism, angiopathy and neuropathy [4-6].

Diabetes in childhood and adolescence is of type 1 in almost all cases being produced by the destruction of insulin producing pancreatic beta cells [7-9]. Diabetes can cause changes in the salivary clinical parameters, such as spontaneous salivary flow and buffering capacity of saliva [10].

Diabetes in children and adolescents increases the risk to develop periodontal tissue [11-13]. El-Tekeya believes that the value of streptococcus mutans is higher in children with diabetes than in healthy ones [14].

Dental offices identify/test the streptococcus mutans level by means of saliva tests based on epidemiological methods. The salivary test could be a useful help for the dentist in achieving a prevention plan and in determining the prognosis of each patient [15].

## Experimental part

The evaluation of the oral hygiene and the periodontal indices of inflammation help the dentist make a staging of gingivitis and periodontitis. We wanted to see if there is a relationship between the amount of streptococcus that

can be identified in saliva and the markers of periodontal disease, hygiene and diabetic condition.

The first null hypothesis is, that there are no differences in the amount of streptococcus mutans between healthy patients and those with diabetes.

The second null hypothesis is that there are no differences in the oral hygiene and gingival inflammation indices between patients who have increased or reduced amounts of streptococcus mutans in saliva.

By means of clinical examination and specific tests we determined following parameters:

- Oral hygiene index IHB
- Gingival bleeding index
- Inflammation of periodontal index RUSSELL
- Community periodontal index of treatment needs (CPITN) index, OMS advised
- The excess of streptococcus mutans of 500,000 cfu / mL by means of the Saliva Check Mutans test kit

The study group consisted of 143 patients aged between 5 and 18 years old. Among them were 68 diabetic patients and 75 healthy patients (without diabetes). The diabetic patients were patients during clinical follow-up at the Medical Center for evaluation and rehabilitation for children and youth Cristian Serban Buzias and the patients without systemic disease were the patients who come for dental check-ups.

The oral hygiene index (OHI) has two components: the plaque index and the index of tartar. The total value of the oral hygiene index results from summing the plaque and tartar index value.

The clinical criteria for plaque/ tartar index are:

- absence of plaque / tartar;
- supragingival plaque / tartar tooth-third of the package;
- plaque / tartar in the middle third of the crown;
- plaque / tartar in incisal or occlusal third of the crown.

The clinical criteria for Gingival Bleeding Index or index of gingival bleeding or GBI:

- absence of bleeding;

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- punctate bleeding, isolated, single;
- multiple bleeding points or a small area;
- bleeding that fills the interdental space;
- bleeding exceeding the free gingival margin.

The clinical criteria for periodontal index (RUSSELL)

- absence of gingival inflammation and deep periodontium;

- moderate gingivitis, does not circumscribe the tooth;
- advanced gingivitis which circumscribes the tooth, without apparent epithelial injury;

- gingival pocket with epithelial damage. The tooth has been implanted, chewing is performed normally. Radiologically an alveolar bone loss is observed extending upto half of the root length.

- advanced periodontal bone destruction, severe disorders of mastication, dull sound at the percussion of the teeth with a metal instrument, axial mobility of the teeth.

The clinical criteria for CPITN or Community Periodontal Index of Treatment Needs (Ainamo et al 1982)

- no sign of illness
- gingival bleeding achieved at probe
- subgingival or supragingival tartar
- 4 to 5.5 mm deep pockets
- deep pockets of 6 mm or more [1].

Saliva Check Mutans Kit

Patients with high levels of Streptococcus mutans can be quickly and easily identified within 15 min without any specialized equipment and without cultivation of the bacteria. Saliva Check Mutans Kit detects Streptococcus mutans in saliva using a highly specific immunochromatography process and does not rely on the bacterial growth. Therefore problems such as contamination by other bacteria or pathogens are avoided. The test result is shown visually as a line, so the outcomes can be easily determined.

Open the foil package and take out the test device, paraffin gum, mixing container and pipette. Instruct the patient to chew the gum provided for 1 min to stimulate their secretion of saliva. Gum should not be swallowed. Collect the stimulated saliva sample in the mixing container. The volume obtained must reach line A. Remove any excess above this.

Hold the bottle of reagent 1 vertically, add 1 drop of reagent 1 to the saliva. Hold the opening of the mixing container tightly to avoid spilling the saliva. Tap the mixing container 15 times over a period of 10 s with a finger to mix the saliva and reagent 1 thoroughly.

Add 4 drops of reagent 2 (yellow) to the mixing container. Shake the device for several seconds to mix. Check that the saliva sample has changed to light green (alkaline to neutral pH).

Using the graduated pipette, take sufficient saliva from the mixing container to fill line 3 on the pipette and dispense into the sample window at the end of the test device.

Store container at room temperature for 15 min. A red thick line should be observed in the control C window of the test device, indicating that the test is working properly. At the same time, check the test T window. The result is

positive if a thin red line appears in the T window, indicating that salivary levels of Streptococcus mutans are higher than 500.000cfu/mL and thus the patient has a potential high risk of future caries activity. If no line can be observed after 15 min, this indicates a low salivary level of Streptococcus mutans and a low potential risk of caries at this time.

## Results and discussions

Of the 143 patients included in the study the highest percentage, of 88.1%, represent the subjects with a higher level of 500.000 cfu / mL mutans streptococcus. Patients with less than 500.000 cfu / mL represent 11.9% of the total group studied.

Considering the first null hypothesis, we compared the results based on the Kruskal-Wallis statistical test to see whether there is a connection between the diabetic status of the patient and the level of mutans streptococci in saliva. Since the statistical test calculated a value of  $p = 0.283$  (Asymp. Sig., Chi-Square 1.154, df 1) we believe that the first null hypothesis is valid and that there are no differences in the amount of streptococcus mutans between healthy patients and those with diabetes.

The graphical representation of the percentage distribution of the hygiene indices (fig. 1) indicates a clear difference between the patients who have the level of streptococcus mutans under 500.000 cfu / mL (blue) and the patients with levels of streptococcus mutans higher

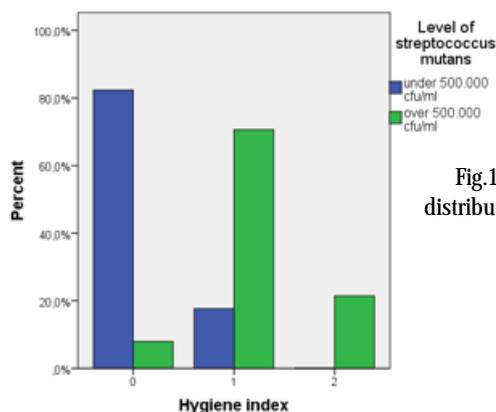


Fig.1 Percentage distribution of hygiene indices

than 500.000 cfu / mL (green). The Kruskal-Wallis test results,  $p = 0.000$  (Asymp. Sig., Chi-Square 37.780, df 1) confirm that there is a statistically significant difference between the two groups of patients.

Regarding the periodontal indices we found significant differences in the level of streptococcus mutans. There were patients with values higher than 500,000 cfu / mL and patients with values lower than 500,000 cfu / mL. The values resulted from the Kruskal-Wallis test are presented in table 1. The graphical representation of the percentage distribution of the periodontal indices (fig. 2, 3, 4) suggests that those patients with a low level of streptococcus mutans fit, in percentage of over 80%, into the group of absence of signs of periodontal disease.

Given these results, we believe that the second null hypothesis is rejected. There are differences in the oral

	CPITN	Russel Periodontal Index	Papillary Bleeding Index
Chi-Square	41.486	27.939	11.828
df	1	1	1
Asymp. Sig.	0.000	0.000	0.001

**Table 1**  
THE RESULTS OF THE STATISTICAL KRUSKAL-WALLIS TEST FOR THE RUSSEL PERIODONTAL INDEX, PAPILLARY BLEEDING INDEX AND CPITN

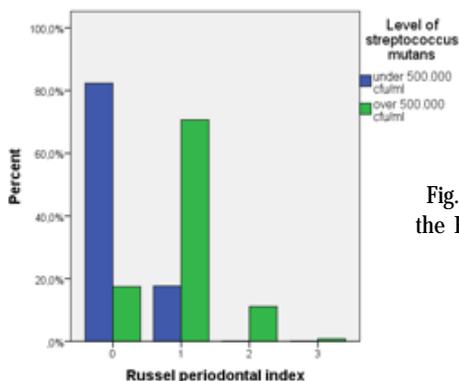


Fig. 2 Distribution of the Russel Periodontal Index

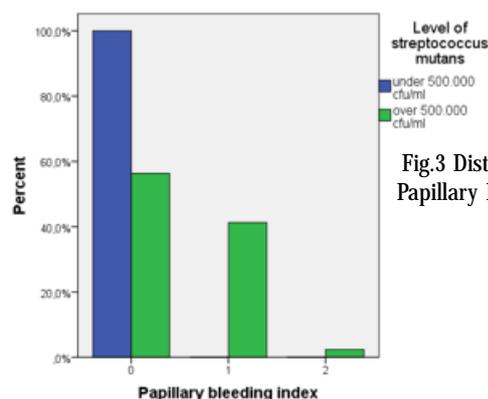


Fig.3 Distribution of the Papillary Bleeding Index

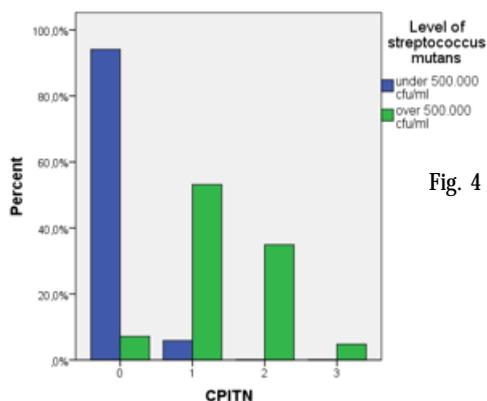


Fig. 4 Distribution of CPITN

hygiene indices and the gingival inflammation indices between patients who have an increased or reduced level of salivary mutans streptococci.

The main reasons for the early loss of teeth are the dental caries and the periodontal diseases. The most probable cause of developing caries and periodontal diseases, leading to premature removal of teeth, is the bad hygiene of the oral cavities [16]. Bacterial plaque is the causative factor of the periodontal disease [1]. The pathogenic microflora in dental plaque along with other factors intervenes in the pathogenesis of tooth decay [17].

Many bacterial species were detected in the microflora of the mouth [18, 19]. Possible nonpathogenic periodontal bacteria are considered to be the actinomyces viscosus in gingivitis, and in periodontitis the actinobacillus actinimycetemcomitans, porphyromonas gingivalis, fusobacterium nucleatum, prevotella intermedia, capnocytophaga, spirochete. But in the bacterial pathogenicity plaque special importance has the streptococcus mutans [1]. In patients with diabetes periodontal structures produce an altered response to plaque. In gingivitis associated with diabetes, in the gingival crevice, the main microorganisms are the streptococcus mutans, actinomices, veillonella parvula, fusobacterium [1].

Streptococcus mutans are gram-positive bacteria involved in biofilm production on the surfaces of teeth [20]. There are studies that have found a relationship between the quantities of streptococcus mutans in plaque and saliva [21, 22]. As saliva is continuously in contact with all the teeth, it provides a better reflection of the colonization of streptococcus mutans on all dentition [23]. Streptococcus mutans is considered as the main causative agent of both primary and secondary caries [24, 25] and also of the root caries [26, 27]. Streptococcus mutans produces an increased acidity exceeding the pH of caries [17]. The acid saliva pH level is higher in patients with chronic gingivitis and generalized chronic periodontitis compared to healthy patients [28].

A severe periodontal disease is related with a high level of streptococcus mutans [29]. Our study shows similar results when comparing the indices of oral hygiene assessment and periodontal disease status and the amount of streptococcus mutans.

The test used by us to identify the amount of streptococcus mutans is the Saliva Check mutans kit, a test with a sensitivity of 88 and 90% specificity [30].

It has been used so far only to determine an increased risk of caries [31]. Our study may open a new research regarding the possibility to use the specific streptococcus mutans test in conjunction with other specific periodontal disease indexes when monitoring patients.

The prevalence of streptococcus mutans is significant in children [32]. Also, the amount of streptococcus mutans is increased in diabetic patients [14]. Thus we cannot speak about a link between the amount of streptococcus mutans and health.

## Conclusions

The diabetic status of the patient does not seem to influence the salivary level of streptococcus mutans determined with the method we used in our study. Within the limits of our study we conclude, that the determined salivary level of streptococcus mutans can be related to periodontal problems.

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