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Expert Opinion Article

Clinical Significance Of Salivary Siga, MMPS, Streptococcus Mutans and Streptococcus Sobrinus In Children With Dental Caries

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[Abstract]
OBJECTIVE To investigate the clinical significance of secretory immunoglobulin A (sIgA), matrix metalloproteinases (MMPs), Streptococcus mutans and Streptococcus sobrinus in saliva of children with dental caries.
METHODS From August 2021 to May 2022, 120 children with severe caries diagnosed in our hospital's oral outpatient department were selected. They were divided into severe group (60 cases) and mild group (60 cases) according to the severity of the disease. In addition, 60 children without caries were selected as the control group. Oral examination was carried out for all children and caries loss compensation index was calculated; The levels of sIgA and MMPs in saliva were detected by ELISA, and the levels of Streptococcus mutans and Streptococcus sobrinus in saliva were detected by qRT PCR. The correlation between sIgA and MMPs and Streptococcus mutans and Streptococcus sobrinus was analyzed.
RESULTS (1) Caries loss and filling index: Compared with the control group, the caries loss and filling index of children in severe group and mild group increased significantly (P<0.05). (2) SIgA, MMP-2, MMP-9: Compared with the control group, the salivary sIgA, MMP-9 levels in the mild group were significantly higher (all P<0.05), and the salivary sIgA, MMP-2, MMP-9 levels in the severe group were significantly higher (all P<0.05); Compared with the mild group, the salivary sIgA and MMP-2 levels in the severe group were significantly higher (all P<0.05). (3) Streptococcus mutans and Streptococcus sobrinus: compared with the control group, the levels of Streptococcus mutans and Streptococcus sobrinus in saliva of mild group and severe group increased (P<0.05); Compared with the mild group, the levels of Streptococcus mutans and Streptococcus sobrinus in the saliva of the severe group were significantly higher (P<0.05). (4) Correlation analysis: The levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2, MMP-9 in saliva of children with dental caries were positively correlated with caries loss index (P<0.05). (5) Multivariate linear regression analysis: The levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva had a significant predictive effect on children with dental caries, and their increased levels would increase the risk of children with dental caries (P<0.05).
CONCLUSIONS The increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva are related to the caries of children, and are positively related to the degree of caries.

[Keywords] Caries; Secretory immunoglobulin A; Matrix metalloproteinases; Streptococcus mutans; Streptococcus sobrinus

Caries is a hard tissue infectious diseases of teeth, and it is also a frequent and common disease of children in China [1,2]. The serious degree of caries and the high incidence rate of caries are important characteristics of caries, which may be related to the tissue structure of deciduous teeth such as easy demineralization and thin hard tissue. Children like sweet food and have poor ability to maintain oral hygiene [3,4]. Up to now, the etiology and pathogenesis of dental caries are still controversial, which is also an important direction of clinical research. Streptococcus mutans and Streptococcus sobrinus are considered as the main cariogenic bacteria due to their strong acid production and acid resistance [5]. Matrix metalloproteinases (MMPs) are the major cariogenic proteases in saliva, which can reshape normal tissues and degrade extracellular matrix, and participate in the proliferation of periodontal disease, caries, tumors and other diseases [6]. Secretory immunoglobulin A (sIgA) is the first line of defense against most pathogens in the mouth, and also the main antibody component in saliva. Literature [7] suggests that sIgA antibodies produced in saliva can mediate the specific immune response of Streptococcus mutans caries. This study explored the correlation between the changes of sIgA and MMPs in saliva of children with dental caries and the detection levels of

Streptococcus mutans and Streptococcus sobrinus in saliva, in order to provide a theoretical basis for the treatment and prevention of dental caries. The report is as follows.

1 Object and method

1.1 Research object

From August 2021 to May 2022, 120 children with dental caries diagnosed in our oral outpatient department were selected. They were divided into severe group (60 cases) and mild group (60 cases) according to the severity of the disease. In addition, 60 children without dental caries were selected as the control group. 60 patients in severe group (caries loss compensation index ≥ 5), 33 males and 27 females; The average age was (4.4 ± 1.0) years, ranging from 3 to 6 years old. 60 cases in mild group (caries loss index range 0~5), 30 males and 30 females; The average age was (4.5 ± 0.7) years. The control group consisted of 60 cases, 36 males and 24 females; The average age was (4.4 ± 0.9) years. There was no significant difference in general data among the three groups (all P>0.05).

1.2 Diagnostic criteria

(1) Diagnostic criteria: The diagnosis and disease classification of children with dental caries refer to the relevant standards in the International Caries Detection and Assessment System (ICDAS): Comprehensive System for Measuring Caries [8].

1.3 Inclusion criteria

(1) The age of the children ranged from 3 to 6 years; (2) No fluoride preparation has been used in recent 6 months; (3) This study was in line with the relevant medical ethics standards of the Helsinki Declaration and obtained the informed consent of the children's parents.

1.4 Exclusion criteria

(1) Other types of oral diseases, such as oral ulcer, periodontal disease, periapical periodontitis, pulpitis, etc;
(2) Dental fluorosis; (3) Thyroid dysfunction; (4) Concomitant with other types of diseases.

1.5 Oral examination

With reference to the methods in the International Caries Detection and Assessment System (ICDAS): A Comprehensive System for Measuring Caries [8], children were examined for caries using routine examination methods, including the color, shape and quality of teeth, and the caries loss index was calculated.

1.6 Detection of salivary sIgA and MMPs

In the morning, after the children brushed their teeth to remove the oral residue, they waited for 5 minutes and then collected 2 mL of non stimulated saliva from all patients by drip method. After centrifugation, the supernatant was separated. The levels of sIgA and MMPs in saliva of the three groups of children were detected by enzyme-linked immunosorbent assay (ELISA). The experimental operation was carried out in strict accordance with the instructions of the kit.

1.7 Detection of salivary streptococcus mutans and streptococcus sobrinus by qRT PCR

The saliva of 3 groups of children was collected, primers were designed for Streptococcus mutans and Streptococcus sobrinus, the detection level of real-time fluorescent quantitative PCR (qRT-PCR) was determined, and the product concentration was determined by ultraviolet spectrophotometer.

1.8 Statistical methods

SPSS 21.0 software was used for analysis. Measurement data were expressed by ($\bar{x}$ ± s). Single factor analysis of variance was used for inter group comparison, and LSD-t test was used for pairwise comparison within the group; $\chi 2$ test was used for counting data; Pearson linear correlation analysis was used for correlation analysis; Multivariate linear regression analysis was used for multivariate analysis; The difference was statistically significant with P<0.05.

2 Results

2.1 Comparison of caries loss compensation index

Compared with the control group, the caries loss compensation index of the severe group and the mild group increased significantly (P<0.05); There was no significant difference in caries loss index between severe group and mild group (P>0.05).

Table 1 Comparison of caries loss compensation index ($\bar{x}$ ± s)

Group	Number of cases	dmf index
Severe group	60	4.65±2.63*
Mild group	60	3.81±1.88*
Control group	60	1.64±1.20
F value		36.517
P value		0.000

Note: Compared with the control group,*P<0.05

Comparison of salivary sIgA, MMP-2 and MMP-9 levels

Compared with the control group, the salivary sIgA and MMP-9 levels of children in the mild group were significantly higher (all P<0.05), while the level of MMP-2 had no significant difference (P>0.05); Salivary sIgA, MMP-2 and MMP-9 in severe group were significantly increased (P<0.05). Compared with the mild group, the salivary sIgA and MMP-2 levels in the severe group were significantly higher (all P<0.05), and the differences were statistically significant, as shown in Table 2.

Table 2 Comparison of salivary sIgA, MMP-2 and MMP-9 levels（$\bar{x} \pm s$）

Group	Number of cases	sIgA（μg/mL）	MMP-2（μg/L）	MMP-9（μg/L）
Severe group	60	24.28±8.60*#	139.81±22.17*#	448.91±61.05*
Mild group	60	16.81±5.73*	108.34±18.33	429.44±70.34*
Control group	60	11.05±4.21	103.02±15.84	377.87±40.82
F value		61.609	66.395	23.456
P value		0.000	0.000	0.000

Note: Compared with the control group, * P<0.05; Compared with mild group,#*P*<0.05

2.2Comparison of levels of Streptococcus mutans and Streptococcus sobrinus in saliva

Compared with the control group, the levels of Streptococcus mutans and Streptococcus sobrinus in saliva of mild group and severe group increased (P<0.05); Compared with the mild group, the levels of Streptococcus mutans and Streptococcus sobrinus in the saliva of the severe group increased (P<0.05), and the difference was statistically significant, as shown in Table 3.

Table 3 Comparison of levels of Streptococcus mutans and Streptococcus sobrinus in saliva（$\bar{x} \pm s$）

Group	Number of cases	streptococcus mutans（×103 copyis/mL）	Streptococcus sobrinus（×103 copyis/mL）
Severe group	60	2861.87±619.52*#	120.67±32.55*#
Mild group	60	572.28±186.28*	9.12±3.85*
Control group	60	13.84±0.53	0.004±0.001
F value		979.582	756.385
P value		0.000	0.000

2.3Correlation analysis

By linear correlation analysis, the levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2, MMP-9 in saliva of children with caries were positively correlated with caries loss index (all P<0.05), as shown in Table 4.

Table 4 Correlation Analysis Results

Test index	dmf index	
	r value	*P* value
streptococcus mutans	0.538	0.000
Streptococcus sobrinus	0.462	0.000
sIgA	0.668	0.000
MMP-2	0.611	0.000
MMP-9	0.582	0.000

2.4Multiple linear regression analysis

Through multiple linear regression analysis, the levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva have a significant predictive effect on children's caries. The increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva will increase the risk of children's caries (P<0.05), as shown in Table 5.

Table 5 Analysis Results of Multiple Linear Regression

parameter	β	SE	Walds	*P*
streptococcus mutans	66.381	33.291	3.976	0.000
Streptococcus sobrinus	47.293	24.008	3.880	0.000
sIgA	81.491	43.882	3.449	0.001
MMP-2	63.408	41.763	2.305	0.032
MMP-9	39.772	24.481	2.639	0.021
Constant term	-11.842	7.552	2.459	0.029

3 Discussion

Caries is the most common and serious disease endangering oral health, and its essence is a chronic bacterial disease occurring in enamel and dentin [9, 10]. The hard tissues of the teeth have a high degree of mineralization and hardness, but the ability of self repair and defense is weak, and the perception is also poor. Therefore, when the dentin is not damaged by caries, most patients are unaware of discomfort, while when the caries causes discomfort, most of the lesions develop to a serious degree [11]. Therefore, early diagnosis of caries is difficult.

sIgA is an antibody that plays an important role in oral health, and is an important component of the mucosal immune system of the body. It is formed by lymphocytes and plasma cells in human salivary glands, and can better inhibit the decomposition and digestion of oral protease aggregation, thus preventing the adhesion and proliferation of pathogenic organisms [12]. Demineralization of inorganic substances and degradation of organic substances are important processes in the occurrence and development of caries [13]. Inorganic demineralization is reversible, mainly induced by acid production of oral cariogenic bacteria; The degradation of organic substances is an irreversible process, in which salivary protease plays an important role [14]. In this study, the levels of sIgA, MMP-2 and MMP-9 in saliva of three groups of children were compared and analyzed. The results showed that the levels of sIgA, MMP-2 and MMP-9 in saliva of children in severe group were higher than those in control group on average, the levels of sIgA and MMP-9 in saliva of children in mild group were higher than those in control group, and the levels of sIgA and MMP-2 in saliva of children in severe group were higher than those in light group. The above results indicate that sIgA and MMPs are involved in the formation of children's dental caries, and the content of sIgA and MMPs gradually increases with the increase of disease degree.

Microbial studies [15,16] suggest that cariogenic bacteria play an important role in the occurrence and development of caries, among which Streptococcus mutans and Streptococcus sobrinus are considered as the main cariogenic bacteria due to their strong acid production and acid resistance. The results of this study showed that the levels of Streptococcus mutans and Streptococcus sobrinus in the saliva of the severe group were higher than those of the mild group and the control group, and the levels of Streptococcus mutans and Streptococcus sobrinus in the saliva of the mild group were higher than those of the control group. The above results indicate that Streptococcus mutans and Streptococcus sobrinus participate in the occurrence and development of dental caries. Streptococcus mutans can adsorb sucrose on the surface of teeth, which is closely related to the formation of smooth surface caries and pit and fissure caries; Streptococcus sobrinus has stronger acid production and acid resistance than Streptococcus mutans, which is closely related to smooth surface caries in children. Linear correlation analysis showed that the levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2, MMP-9 in saliva of children with dental caries were positively correlated with caries loss index. By multiple linear regression analysis, the levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva had a significant predictive effect on children with dental caries. The increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva would increase the risk of children with dental caries. The results showed that the increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva had a predictive effect on children's caries. The higher the level of the above indicators, the higher the possibility of children's caries.

The positive correlation between children's caries loss index and salivary sIgA secretion level indicates that sIgA has an anti caries effect in the occurrence and development of caries, and can play an immune response role, thus providing an important line of defense for inhibiting the contact between mucosal epithelial cells and bacteria. Passive and active immunity can induce and promote the production of specific SIgA antibodies

in saliva and serum and increase the production, while the adhesion of Streptococcus mutans and Streptococcus sobrinus to saliva coated apatite will be inhibited by SIgA [17, 18]. The acid production of some bacteria in carious lesions causes demineralization of dentin and release of phosphorylated proteins, which in turn activates the host MMPs and promotes the degradation of organic substances; The acidic pH value caused by demineralization of dentin caused by acid production of bacteria alternates with the neutral pH value caused by saliva buffering, which
greatly improves the activity of MMPs, degrades exposed dentin matrix, and promotes the development of caries [19].

To sum up, the increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2 and MMP-9 in saliva are related to children's caries, and are positively related to the degree of caries.

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