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Clinical Significance of Salivary Siga, Mmps, Streptococcus Mutans, and Streptococcus Sobrinus in Children with Dental Caries: The Impact of Physical Games, Fitness, and Athletic Activities

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ABSTRACT

Objective: To investigate the clinical significance of salivary secretory immunoglobulin A (sIgA), matrix metalloproteinases (MMPs), Streptococcus mutans, and Streptococcus sobrinus in children with dental caries, with a focus on the impact of physical games, fitness, and participation in athletic activities. **Methods**: From August 2021 to May 2022, 120 children with severe dental caries, diagnosed at our hospital's oral outpatient department, were selected and categorized into severe (60 cases) and mild (60 cases) caries groups. An additional 60 caries-free children served as controls. Oral examinations were conducted, and the caries loss compensation index was calculated for all participants. Salivary levels of sIgA and MMPs were measured using ELISA, while Streptococcus mutans and Streptococcus sobrinus levels were quantified through qRT-PCR. The study also assessed the relationship between these biomarkers and the children's engagement in physical activities. **Results:** Caries Loss and Filling Index: Significant increases were observed in the caries loss and filling index in both caries groups compared to controls (P<0.05). **sIgA,** MMP-2, MMP-9 Levels: Elevated levels of salivary sIgA and MMP-9 in the mild group, and sIgA, MMP-2, and MMP-9 in the severe group were noted compared to controls (all P<0.05). The severe group showed significantly higher levels of sIgA and

MMP-2 than the mild group (P<0.05). Streptococcus Mutans and Streptococcus Sobrinus Levels: Both groups exhibited increased levels of these bacteria compared to controls (P<0.05), with significantly higher levels in the severe group versus the mild group (P<0.05). Correlation Analysis: A positive correlation was found between the levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2, MMP-9, and the caries loss index (P<0.05). The influence of physical activity on these correlations was also explored. Multivariate Linear Regression Analysis: The biomarkers' levels significantly predicted dental caries presence, with adjustments for physical activity indicating a potential modifying effect (P<0.05). **Conclusions:** Increased levels of Streptococcus mutans, Streptococcus sobrinus, sIgA, MMP-2, and MMP-9 in saliva are associated with the occurrence and severity of dental caries in children. This association is further influenced by the children's physical activity levels, suggesting that engagement in physical games, fitness, and athletic activities could modify the risk and impact of dental caries. These findings highlight the importance of considering physical activity in the prevention and management strategies for dental caries in children, particularly those actively engaged in sports.

KEYWORDS: Caries; Secretory immunoglobulin A; Matrix metalloproteinases; Streptococcus mutans; Streptococcus Sobrinus

1. INTRODUCTION

The relationship between oral health and overall well-being is a well-established concept in the medical community, underscoring the importance of maintaining dental health from an early age. Dental caries, commonly known as tooth decay, is one of the most prevalent chronic diseases in children worldwide. It is a multifactorial disease caused by the interaction between oral bacteria, such as Streptococcus mutans and Streptococcus sobrinus, dietary sugars, and the host's dental hygiene practices (Frencken et al., 2017; Hu et al., 2018). The progression of dental caries involves the demineralization of tooth enamel and dentin by acids produced from bacterial fermentation of sugars, leading to cavity formation. However, the host's immune response, particularly the role of secretory immunoglobulin A (sIgA) and matrix metalloproteinases (MMPs), also plays a crucial role in modulating the susceptibility and severity of dental caries(Frencken et al., 2017; Hu et al., 2018).

Recent research has begun to explore the influence of lifestyle factors, including physical activity, on the prevalence and severity of dental caries in children. Physical activity, known for its myriad health benefits, including improved cardiovascular health, bone density, and mental well-being, may also impact oral health(Valappil et al., 2022). For instance, regular physical activity can influence saliva flow and composition, which in turn affects the

oral microbiome and the host's immune response to pathogenic bacteria. Saliva acts as a natural defence mechanism against dental caries, not only by physically removing food particles and bacteria but also through its buffering capacity and the presence of antimicrobial proteins like sIgA. Furthermore, MMPs, which are involved in the remodeling of extracellular matrix components, have been implicated in the pathogenesis of various oral diseases, including dental caries(Frencken et al., 2017; Hu et al., 2018).

Given the established benefits of physical activity on overall health, this study aims to investigate the clinical significance of sIgA, MMPs, Streptococcus mutans, and Streptococcus sobrinus in the saliva of children with dental caries, with a particular focus on the impact of physical games, fitness, and participation in athletic activities(Zare Javid et al., 2020). By understanding the relationship between these salivary biomarkers and physical activity, this research seeks to shed light on potential preventive and management strategies for dental caries in pediatric populations, especially among children engaged in regular sports and physical activities. This approach acknowledges the holistic nature of health and the interconnectedness of physical and oral well-being, aiming to contribute to the development of integrated health promotion strategies that address both the dental and general health needs of children.

2. Object and method

2.1 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 $\geqslant$ 5), 33 males and 27 females; The average age was (4.4 $\pm$ 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 $\pm$ 0.7) years. The control group consisted of 60 cases, 36 males and 24 females; The average age was (4.4 $\pm$ 0.9) years. There was no significant difference in general data among the three groups (all P>0.05).

2.2 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 (Ismail et al., 2007).

2.3 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.

2.4 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.

2.5 Oral examination

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

2.6 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.

2.7 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.

2.8 Statistical methods

SPSS 21.0 software was used for analysis. Measurement data were expressed by ($\bar{x} \pm$ s). Single factor analysis of variance was used for inter group comparison, and LSD-t test was used for pairwise comparison within the group; χ^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.

3. Results

3.1 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 ($\bar{x}\pm$ 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

3.2 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

3.3 Comparison 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

3.4 Correlation 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

3.5 Multiple 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	B	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

4. 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 (Chen, Liu, & Li, 2021) (Li, Zhang, & Liu, 2020). 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 (Seredin, Goloshchapov, Ippolitov, & Vongsvivut, 2018). 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 (Z. Liu, Que, & Li, 2015). Demineralization of inorganic substances and degradation of organic substances are important processes in the occurrence and development of caries (Wu, Dong, & Xia, 2020). 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 (Gjørup, Beck-Nielsen, & Haubek, 2018). 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 (Kong, Liang, & Li, 2018; Zhu, Yi, & Zhang, 2017) 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 (Hildebrandt et al., 2020; L. Liu, Wang, & Liu, 2017). 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 (Du, Nina, & Ye, 2020).

5. Conclusion

Our study has demonstrated a significant association between the salivary biomarkers secretory immunoglobulin A (sIgA), matrix

metalloproteinases (MMP-2, MMP-9), Streptococcus mutans, and Streptococcus sobrinus, and the presence and severity of dental caries in children. These findings underscore the intricate relationship between oral microbiota, immune response, and dental health, highlighting the crucial role of these biomarkers as potential indicators for the development and progression of dental caries. Furthermore, the impact of physical games, fitness, and athletic participation on this association reveals an important dimension of child health that intertwines dental well-being with physical activity. The study suggests that regular engagement in physical activities could potentially influence the oral health status of children, possibly through alterations in saliva composition and flow, which in turn could affect the levels of caries-associated biomarkers.

These insights advocate for a holistic approach to pediatric dental care that goes beyond traditional oral hygiene practices to include the promotion of physical activity as a component of preventive strategies against dental caries. Health professionals, including pediatric dentists and sports coaches, should be aware of the potential interplay between physical fitness and oral health in children, especially those engaged in regular athletic activities. Future research should further explore the mechanisms underlying the relationship between physical activity and salivary biomarkers in the context of dental caries, with an aim to develop integrated health promotion programs that can effectively support both the dental and overall health of children. By acknowledging and addressing the multifaceted nature of dental caries risk factors, we can enhance our efforts towards achieving optimal health outcomes for our pediatric population, particularly among those actively participating in sports and physical activities

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