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EVALUATION SALIVARY LEVELS OF LACTOPEROXIDASE AND LACTOFERRIN IN PRESCHOOL CHILDREN WITH DENTAL CARIES

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ABSTRACT

This study was conducted to determine the saliva levels of lactoperoxidase and lactoferrin between children who do not suffer from tooth decay and those who have early childhood caries. Eighty healthy children of kindergarten age (aged between 4-5 years) were enrolled in this study, according to the World Health Organization, in the city of Baghdad. An oral examination was performed and 40 kindergarten children were suffering from severe caries, which was detected. According to Wyne classification, it was considered a (study group), while 40 caries-free kindergarten children were considered a (control group) that matched by age, sex, and socioeconomic status. Salivary lactoperoxidase and lactoferrin levels were assessed using an ELISA device. The results of the statistical analysis showed that the levels of salivary lactoperoxidase and salivary lactoferrin increased significantly in the study group (those with caries) compared to the caries-free group (control group). The research found that lactoperoxidase and lactoferrin can be used as markers to predict the risk of tooth decay in children's saliva.

Keywords: Dental caries, Lactoperoxidase, Lactoferrin, Childhood, Saliva.

تقييم مستويات اللعاب من لاكتوبيروكسيديز واللاكتوفيرين في الأطفال في سن ما قبل المدرسة الذين لديهم تسوس الأسنان

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الخلاصة

اجريت هذه الدراسة لمعرفة مستويات اللعاب من اللاكتوبيروكسيديز واللاكتوفيرين بين الأطفال الذين لا يعانون من تسوس الأسنان وأولئك الذين لديهم تسوس في مرحلة الطفولة المبكرة. حيث تم تسجيل في هذه الدراسة ثمانين طفلاً يتمتعون بصحة جيدة بسن رياض الاطفال (تتراوح أعمارهم بين 4-5 سنة) حسب منظمة الصحة العالمية, في مدينة بغداد، وتم إجراء الفحص الفموي وكان 40 طفلاً من أطفال الروضة يعانون من حالة تسوس حاد وتم الكشف عنه وفقًا لتصنيف واين وتم اعتبارها (مجموعة دراسة), بينما كان 40 طفلًا خاليًا من التسوس في اطفال الروضة وتم اعتبارها (مجموعة ضابطة) التي تتطابق مع العمر والجنس والحالة الاجتماعية والاقتصادية. تم تقييم مستويات اللاكتوبروكسيديز اللعابي واللاكتوفيرين باستخدام جهاز الاليزا. حيث أظهر نتائج التحليل الإحصائي أن مستوى اللاكتوبروكسيديز اللعابي واللاكتوفيرين اللعابي ارتفع بشكل ملحوظ في مجموعة الدراسة (المصابين بالتسوس) مقارنة بالمجموعة الخالية من التسوس (المجموعة الضابطة). توصل البحث الى انه يمكن استخدام اللاكتوبيروكسيديز واللاكتوفيرين كعلامات للتنبؤ بمخاطر تسوس الأسنان في لعاب الأطفال.

الكلمات المفتاحية: تسوس الأسنان، لاكتوبيروكسيديز ، لاكتوفيرين ، الاطفال ، اللعاب.

*This article is taken from the first researcher's master's thesis.

INTRODUCTION

Dental caries is a multifactorial, locally damaging, microbial based illness that is mostly irreversible. The host, the microorganisms, and the diet each play a different role in the development and growth of tooth caries **(Najm & Al-Mizraqchi, 2019)**. Moreover dental caries is a multifactorial oral illness caused by a glucose induced plaque that is distinguished by the modal demineralization of the tooth's tough tissues **(Pitts *et al.*, 2017)**. therefore saliva play a crucial function as indicators in the identification of caries because saliva around both hard and soft tissue of the mouth contains some inorganic as well as organic substances that include specific elements that significantly contribute to decay in the patient's body **(Ahsan, 2019)**. Unbalanced cariogenic bacteria cause dental caries **(Chen *et al.*, 2020)**. Thus, The predominant cariogenic microorganisms in biofilm and salivary will produce further acids during the process of fermentation **(Al-zahraa & Aldhaher, 2017)**. As a result, the acid destroys the enamel layer form by causing loss of minerals within the hard tissues of the tooth **(Chen *et al.*, 2020)**.

Early childhood caries is the condition in which a kid under the age of five has a number of primary teeth that are missing, decaying, or filled. Medically, ECC has several of distinctive traits, such as fast decay development which impacts multiple teeth shortly following their appear in the mouth **(Feldens *et al.*, 2010)**. The maxillary incisors' labial surface and the lingual and buccal surfaces of the mandibular and maxillary molars' surface are among the dental sites where it typically occurs **(Anil & Anand, 2017)**. Salivary biochemical and microbiological changes may have an impact on the happening and riskiness of tooth decay **(Al-Khayoun & Diab, 2015)**. In another hand dental caries may be made more likely by poor oral hygiene practices **(Misbah, 2005)**. Children's oral and dental health depends on a healthy diet **(Chaloob, & Qasim, 2013)**.

Dental decay can be caused by food or substrate in addition to other variables, and the etiology of early childhood caries is complex. The chance of tooth decay increases when sweets are added to milk or juice. In comparison with kids who don't have these dietary habits, kids who drink sugary drinks at least twice at night are more prone to early childhood caries **(Bachtiar *et al.*, 2019)**.

The salivary glands in the mouth produce saliva, which is a watery secretion. Saliva is crucial for the development of caries and for preventing it **(Munther, 2020)**. It is composed of water, electrolytes, mucus, enzymes, and antibacterial agents. Saliva may be used to diagnose systemic diseases or as indicators of exposure to many toxic or harmful substances due to the existence of hundreds of various components **(Arunkumar *et al.*, 2014)**.

Salivary enzymes such as lactoperoxidase are keeping important functions of maintaining dental health. It is considered as necessary for the host's initial line of defense against infections including *Streptococcus mutans, Streptococcus sobrinus*, and *Streptococcus sanguinis*. Salivary materials may offer a lot of medical data. Additionally, this salivary proteomics can result in the identification of clinical indications **(Si *et al.*, 2015)**. Salivary lactoperoxidase is a type of protein that has distinctive enzyme properties and guards against microbial breakdown of saliva. It functions together with hydrogen peroxide and thiocyanate.

This produces lead to oxidize bacterial sulfhydryl bonds and impede microbial digestion of glucose **(Gornowicz *et al.*, 2014)**. The lactoperoxidase enzyme functions as a catalyst in this reaction. hydrogen peroxide (H2O2) and thiocyanate ion undergo an oxidation process and that results hypothiocyanate ions which these substances are in charge of eliminating microorganisms **(Jyoti *et al.*, 2009)**, One more potential aid dentin affected by

decay, due to its antimicrobial properties, is a combination of the enzymes lactoferrin, and lactoperoxidase are among the various antimicrobial agents associated with the immune function of saliva **(Jyoti *et al.*, 2009)**.

The lactoferrin is non-enzymatic microbial protein. It has been found to be extensively distributed in bodily fluids including saliva, tears, and even white blood cells. The main and minor salivary glands' serous cells release it. The microbes are deprived of this protein crucial due to iron-chelating his activity. Lactoferrin, also known as apolactoferrin when it is iron-free, has the ability to act as an antimicrobial substance by directly interacting with organisms and agglutinating *S. mutans*. This makes it simple to remove the agglutinated organisms from the mouth using the physical forces of saliva and gulping the agglutinated microbes. Additionally, a wide range of types have shown lactoferrin to have significant antimicrobial and antiviral action **(Aziz, 2023)**. These substances may have a bactericidal or potentially inhibiting impact on dental infections **(Shimada *et al.*, 2008)**. The aim of the investigation was to compare the salivary levels of lactoferrin and lactoperoxidase between children without dental decay and those who had early childhood caries.

MATERIALS AND METHODS

This case control study was conducted from December 2022 to May 2023 in eighty healthy looking Kindergartens children with an age of 4-5 years old according to **(World Health Organization, 2013)**, in Baghdad city and oral examination was done and 40 Kindergartens children suffering a severe form of early childhood caries were detected according to **(Wyne, 1999)**, class and were considered as study group and 40 Kindergartens caries free children were the control group matching age and gender and socioeconomic status. The Shapiro-Wilk test was run to determine whether the data's distribution was normally distributed.

EXAMINATION OF THE ORAL CAVITY

Oral evaluation was performed using a decay, missing, and filled index in accordance with **(World Health Organization, 2013)**. And 40 children suffering a severe form of early childhood caries were selected as study group according to **(Wyne, 1999).**

SALIVA COLLECTION

After the oral examination, make sure the childhood did not eat or drink for at least one hour before saliva collection. Four milliliters of unstimulated saliva were collected by drooling method in plain tube, saliva were obtained collected in the morning between 9-11am. **(Al-Musawi & Ali, 2023)**. After this, each tubes was transported in an ice box to the laboratory. To preserve salivary proteins from hydrolysis, saliva was separated in lab from 10 to 15 minutes at (3000) rpm at 28°C. The supernatant was transported to an Eppendorf tube, numbering and freezing under -20°C for identification of markers**.**

BIOMARKERS DETECTION

The level of lactoperoxidase and lactoferrin were measured as instructed in the brochure. By using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit that is commercially available (Cloud-Clone Corp; USA).

RESULT

There are no significant differences between male and female in age in study and control group as illustrated in table (1).

Table (1): Age distribution of study and control group.

Age	Study group	Control group	
	Mean ± SD	Mean ± SD	p value
Male	4.755 ± 0.393	4.435 ± 0.427	0.007*
Female	4.715 ± 0.394	4.615 ± 0.450	0.174 ns
p value	0.762 ns	0.126 ns	

*= significant ns= non-significant

There are no significant differences in gender distribution between study and control group as demonstrated in table (2).

Table (2): Gender distribution of study and control group.

Gender		Control group No=40	Study group No=40	Total
Male	No	20	20	40
	% within group	50	50	50
Female	No	20	20	40
	% within group	50	50	50
Total	No	40	40	80
	% within type	100	100	100

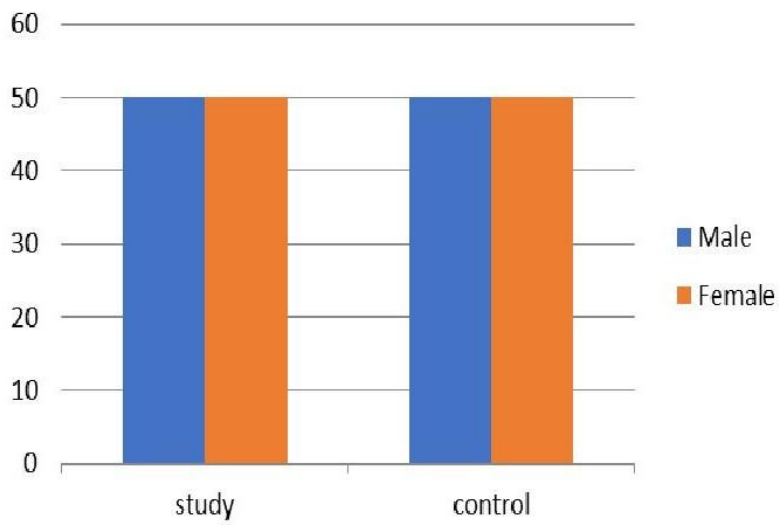

Figure (1): Gender distribution of study and control group.

DETECTION OF SALIVARY LACTOPEROXIDEASE LEVELS

The results in Tab. (3) and Fig. (2) Revealed increase in salivary lactoperoxidease level in study group (644.6±122) in comparison to control groups (245.9±36.27) having differences that are statistically significant (P<0.05).

Table (3): Descriptive criteria of lactoperoxidease marker of study and control groups.

Descriptive criteria	lactoperoxidase concentration (ng/ml) (n= 40)	
	Control group	Study group
Minimum	137.2	507.4
Maximum	306.8	927.4
Median	231.8	593.2
Range	169.5	419.9
±Std. Deviation	36.27	122
Std. Error of Mean	5.735	19.29
P value	<0.0001[a]*	

* Highly significant, a Mann Whitney

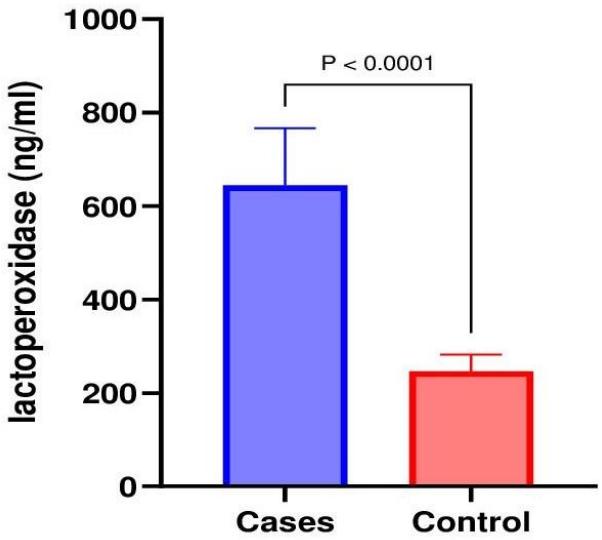

Figure (2): Descriptive criteria of lactoperoxidase marker.

DETECTION OF SALIVARY LACTOFERRIN LEVELS

The results in Tab. (4) and Fig. (3) Revealed increase in salivary Lactoferrin level in study group (15.42±2.208) in comparison to control groups (6.812±1.963) having differences that are statistically significant (P<0.05).

Table (4): Descriptive criteria of lactoferrin marker of study groups.

Descriptive criterion	Lactoferrin concentration (ng/ml) (n= 40)	
	Control group	Study group
Minimum	1.831	13
Maximum	9.57	24.85
Median	7.129	15.16
Range	7.739	11.85
±Std. Deviation	1.963	2.208
Std. Error of Mean	0.3103	0.3492
P value	<0.0001[a]*	

* Highly significant, a Mann Whitney

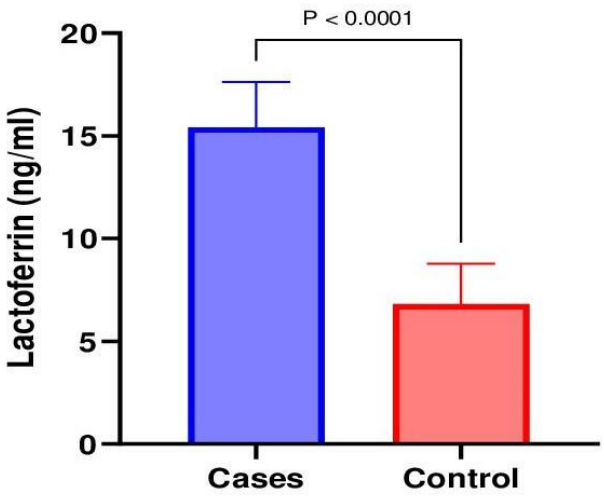

Figure (3): Descriptive criteria of lactoferrin marker.

DISCUSSION

Saliva has a variety of roles in defending the teeth toward abrasion, wear, erosion, and tooth decay **(Dawes *et al.*, 2015)**, where it consists of 99% water, One of the salivary glands' primary jobs is to continuously release saliva into the mouth in order to keep the oral tissue and made it moist and less prone to abrasion as well as to facilitate the removal of microbes , desquamated cells of the epithelium, white blood cell, and food particles during process of swallowing **(Dawes *et al.*, 2015; Alobadi, 2020)**. Several studies have sought in previous years to link certain characteristics of salivary production and content to risk of tooth decay. Furthermore salivary samples are diagnostic tools because saliva is easily collected and analysed, also many of salivary indicators may be able to distinguish between periodontal wellness and illness with accuracy **(Abdullameer & Abdulkareem, 2023; Kazem *et al.*, 2023)**.

Forty children with dental caries (severe type) ECC and forty free caries children participated in the current study and no significant age differences and gender were recorded.

The results also revealed that the study group had higher salivary levels of lactoperoxidase and lactoferrin as compared to the control groups. Regarding lactoperoxidase the findings of this research were in accordance with others someone uncovered that high-intensity caries is linked with increased levels of lactoperoxidase **(Gornowicz *et al.*, 2014)**, another study done by Ruan and et.al. Also came in agreement with the results of the current study **(Ruan *et al.*, 2021)**. These outcomes might be attributed to the fact that the lactoperoxidase enzyme functions as a catalyst in this reaction. Hydrogen peroxide (H2O2) and thiocyanate ion undergo an oxidation process and that results hypothiocyanate ions which these substances are in charge of eliminating microorganisms **(Al-Baarri *et al.*, 2011)**.

The components of the lactoperoxidase structure like lactoperoxidase, hydrogen peroxide, and thiocyanate ions exert an inhibitory effect on cariogenic oral microbiota. The amounts of thiocyanate ions may be raised in vivo by adding more natural enzymes, such as lactoperoxidase, to the saliva and therefore the quantity of cariogenic microbiota in children with Early Childhood Caries shall be decreased by this elevated level of thiocyanate **(Jyoti *et al.*, 2009)**. All these findings support the increased level of lactoperoxidase in saliva of children with dental caries in this study.

In this research also revealed increased level of saliva lactoferrin in children with dental caries these results are supported with the results of researchers who found that lactoferrin was significantly lower in the caries-free group than in the dental caries group **(Ruan *et al.*, 2021)**. Also Felizardo and et.al. Found Lactoferrin was discovered to be positively linked with Dacay Massing and Filling (DMFT) **(Felizardo *et al.*, 2010)**.

Another study noticed was a connection between the DMFT index and lactoferrin expression and declared that dental caries tend to be more likely to arise when lactoferrin is present, additionally a correlation between decay risk /activities and lactoferrin genetic polymorphism discover **(Vitorino *et al.*, 2006).**

CONCLUSION

Lactoperoxidase and Lactoferrin may be used as markers for dental caries risk prediction in saliva of children.

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