Determination of salivary IgA (sIgA) levels in saliva of patient with dental caries

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Abstract:
Using the ELISA test, the content of saliva IgA (sIgA) in the saliva of the patient with dental caries was determined, as 85 samples were collected for the current study from patients aged (20-40) years out of (60) clinical samples of saliva. 25 saliva samples were collected from healthy individuals as a control group and the results were 60 (70.59)% patients with severe dental caries and 25 (29.41)% of healthy subjects. Their ages ranged from (20-50) years with a mean age of (33.75 ± 7.501) for dental caries patients while the mean age of health was (28.16 ± 8.05). found significant differences (P ≤ 0.05) in patients with dental caries between female 42 (70)% while 18 (30)% in males. The Immunological study to determination (sIgA) concentration in saliva of dental caries patients By using enzyme-linked immunosorbent assay (ELISA) technique, The result shows significant decrease the concentration in Three markers in dental caries cases than control ones, P≤0.001. It indicates that biomarkers sIgA, have correlation with age groups of dental caries patients with a reverse relationship between level of sIgA in saliva with progress of age group .
Keywords: salivary IgA (sIgA) , ELISA Assay, Dental carries, age ,Gender

Introduction

Dental caries is a chronic infectious illness that develops as a result of a reaction between dental plaque bacteria, diet, and a variety of host variables, including sociocultural, genetic, chemical, and immunologic responses. Dental caries is one of the most frequent chronic infectious disorders in the world, despite the different preventative methods available. It's a multifactorial, dynamic biofilm-related, sugar-driven disease that causes demineralization and remineralization of tooth hard tissues. (1). Caries refers to the acidic by-products of bacterial fermentation of dietary carbohydrates that cause localized damage of vulnerable tooth hard tissues. It is a chronic condition that affects the majority of people and is caused by an ecological imbalance in the balance between tooth minerals and oral biofilms (plaque). Microbial activity characterizes the biofilm, resulting in ph fluctuations in the plaque. This is due to bacterial acid production as well as the buffering effect of saliva and the surrounding tooth structure. As a result, the tooth surface is in a state of dynamic balance with its surroundings. As the pH falls below a critical value, the demineralization of enamel, dentine or cementum occurs, while a gain of mineral (remineralisation) occurs as the pH increases. (2). One of the elements that causes a childhood illness is the child's age. Caries has to be seen as a life-long condition, because caries experience is severe, the disease appears to begin early, and it is common in young people (3). Variations in the prevalence of dental caries affect people of all ages, especially youngsters, who are seeing an increase in caries disease. Dental caries was observed to continue rising in the primary dentition until the age of six, after which there was a decline in illness. (4) Gender is another element that influences and causes women to have greater caries rates than males. According to statistics, a variety in salivary content and flow rate, hormonal fluctuation, dietary choices, genetic diversity, and various social roles among family members are all risk factors for caries in women. (5). In study by (6) Females are frequently found to have higher prevalence rates than males when dental caries rates are broken down by gender. This applies to all civilizations, subsistence systems, and historical periods. Female caries rates are generally twice as high as male caries rates, with a few exceptions. Lactobacilli make up about 1% of the oral microbiota that can be cultivated. They are seen in carious lesions because they prefer or generate an acidic environment that destroys tooth enamel. Lactobacilli are linked to two harmful properties: acid generation and oral epithelial cell adhesion. (7)., Saliva is a distinct bodily fluid produced by the salivary glands. It is secreted by three main pairs of salivary glands: the parotid, submandibular, and sublingual. The oral and buccal mucosa also contains a slew of other tiny glands. Saliva is mostly water (94–99 percent), with organic and inorganic molecules accounting for almost 0.5 percent and 0.2 percent, respectively (8). The biomarker salivary immunoglobulin A is vital for the mouth's local defense. The organism's overall health and immunity have an impact on their secretion. (9). It's often described as a part of the immune system's first line of protection against dangerous bacteria. These antibodies react to the formation of dental biofilms, interfering with the defense of plaque-related oral

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illnesses such as "caries and periodontal disorders" by limiting microbe adhesion. (10) Secretory immunoglobulin A is secreted into the saliva even when there is no stimulation. The quantity of antibodies supplied by these pathways can be affected by gingival and mucosal inflammation, as well as the quality of the mucosal and acinar epithelial barrier. (11).

The main purpose of the current study was to determine the salivary IgA (sIgA) content in saliva of patient with dental caries by using ELISA Assay.

Materials and methods:

85 specimens were taken for the current study from persons ranging in age from 20 to 40 years old, out of a total of (60) clinical specimens from saliva. 25 saliva samples from healthy people were utilized as a control, and these samples were used to analyze salivary IgA (sIgA) levels in the saliva of dental caries patients using an ELISA technique.

Sample collection

Saliva samples were carefully collected in a contamination-free environment, and all participants' lips were washed for 30– 60 seconds with pure water (10mL) to ensure that any debris was removed. To guarantee viability, non-stimulated clean saliva was collected in sterile laboratory cups and maintained in a cool box with ice bags until it was delivered to the lab for testing. After that, each sample was centrifuged at 3000 rpm for 10 minutes.

Results and Discussion

Distribution of dental caries cases according to the Age and Gender :

85 samples have been collected from subjects, with 60 (70.59) percent of patients suffering from severe dental caries and 25 (29.41%) of healthy subjects serving as a control group in the study. The age range between (20 – 50) years with mean age (33.75 ±7.501) for patients while the age mean of healthy was (28.16 ± 8.05). Table (1,2) and figure (1) show most of the patients were females 42 (70)% while 18 (30)% were males with significant differences P≤ 0.005

Table (1): Distribution of dental caries cases according to the Age			
Characteristic	Number (%)	Age Mean ± S.D	P Value
Patients	60 (70.59)	33.75 ±7.501	≤ 0.05*
Control	25(29.41)	28.16 ± 8.05	

Table (2): Distribution of dental caries cases according to Gender				
Gender	Number	Percent	x^2	P
Male	18	30%	8.138	≤ 0.05*
Female	42	70%		
Total	60	100%		

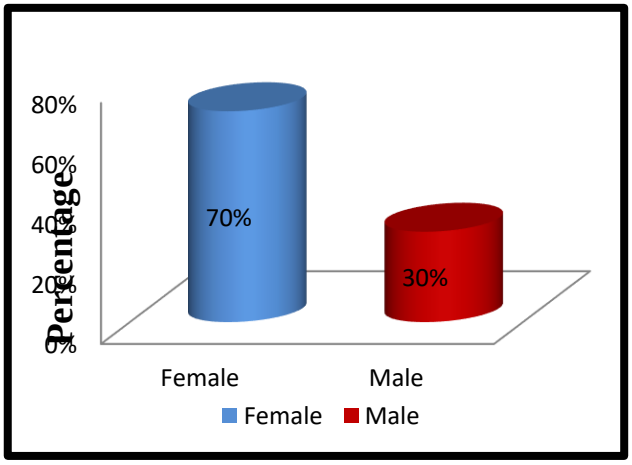

Fig. (1) :Distribution of patient against gender

Because caries risk factors differ in men and women, including as salivary composition and flow rate, hormonal swings, dietary habits, genetic variances, and specific social roles within their family, women's caries rates are higher than men's. The feminine gender has been linked to systemic disorders linked to caries (12)

Caries is more common in females and at a younger age. The incidence of dental plaque grew during childhood and adolescence, according to the findings. These findings were consistent with those published by (5 ; 13), who found that the incidence of dental plaque increased during childhood and adolescence. The former is due to the fact that dental caries develops over time, and teenagers have a high sugar consumption habit, which includes sweets and other sticky sugar-rich foods. The fact that tooth caries is a cumulative process could explain the latter circumstance.This study is accordant with the study (14) who found that The former can be attributed to the fact that dental caries develops slowly, while sugar consumption in the form of sweets and other sticky sugar-rich foods is rather common among teenagers.

Because dental caries is a cumulative process that increases with age, the progression of untreated caries in high-risk people is likely to be faster than in younger people, resulting in tooth extraction, as explained by (15). Another factor to consider is that people who are older experience higher dental pain.

Patients of this age group were more likely to wear fixed orthodontic appliances for cosmetic and functional reasons, which resulted in a higher rate of tooth caries formation. The current study's findings revealed that the length of fixed orthodontic treatment had a substantial impact on plaque formation (16).

(16) are inagreement with the present study showed that most of the patients were females 42 (70)% while 18 (30)% was males with significant differences

Estrogen levels change in females throughout pregnancy, menstruation, and adolescence. (17) discovered a link between the rate of caries and estrogen levels. Elevated estrogen levels can change the ecology of the oral cavity dramatically. According

to clinical studies, pregnancy is the most severe of all hormonal fluctuation events.

Females with a lower salivary flow rate are more likely to acquire caries because they lack the mechanical cleansing, buffering, and remineralization actions of saliva. This finding is corroborated by the findings of (18), who found that females' salivary flow rates are lower than men's.

This research supports the findings of (19), who found that more women are becoming single parents, placing them under pressure to care for their children while also dealing with financial restrictions. Women are more affected by domestic violence and eating disorders than men. Their drug use is on the rise, as is the occurrence of systemic diseases. All of these factors have the potential to alter host defense responses in the oral cavity, perhaps assisting in the fight against disease.

Given the discovery that a vegetarian diet promotes to caries production, it's reasonable to assume that more women are vegetarians than men, which could explain their higher caries prevalence. (20) reported that more of the women were vegetarian than men (71% versus 56%) .

Laboratory culture results

From culturing (60) Dental caries swabs , 45 (75%) isolates were gram-positive rustle of (*Lactobacillus sp*), were 15(25%) isolates were negative rustle. These results were shown in Figure (2)

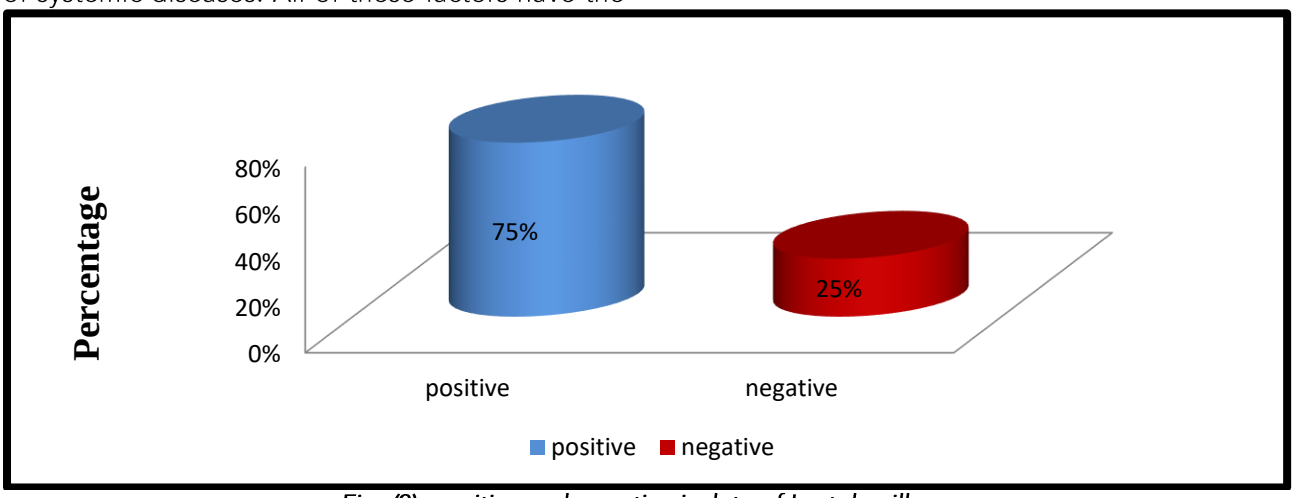

Fig. (2): positive and negative isolate of Lactobacillus sp

Lactobacillus was shown to be related with 62.8 percent of dental caries cases (21;22). While Lamont et al., (2018) discovered that 30 percent of dental caries cases were caused by bacteria, with Lactobacillus accounting for 65 percent of the cases and other species accounting for 35 percent.

Lactobacillus are the first germs implicated in the development of dental caries, according to (20, who investigated the ecology of lactobacilli in the oral cavity.

The percentage of lactobacillus increased before the commencement of carious lesions in a study by (23); the results of this study also revealed that lactobacillus played an essential part in the formation of dental plaque, which might lead to the development of dental caries.

Poor academic performance, being a primary cycle student, a sugary diet, poor teeth cleaning practices, a history of dental aches, and salivary pH were all determinant variables for the burden of dental caries. (24) On the other hand, this could be attributed to a decrease in salivary self-clearance, a change in oral flora composition, an increase in the amount of oral biofilm produced, and the colonization of oral surfaces by cariogenic bacteria (25).

Distribution of positive isolate of Lactobacillus sp. against age group.

Table (3) shows the distribution of the positive and negative cases against the age group and it is clear that the age group (30-40) years give significant differences with positive cases more than others age groups

Table(3): distribution of positive and negative cases of *Lactobacillus sp.* against age

P-value Pos.	p-value Neg.	(%)	No.	Negative cases	Positive cases	Age group
0.020	0,051	31.66	19	8	11	20 – 30
0.016	0.066	35	21	2	19	30 – 40
0.029	0. 050	33.34	20	5	15	40 – 50
		100	60	15(25)	45 (75)	Total

The study found the distribution of Lactobacillus among the various age groups (was highest in group of 30 to 40 years as compared to other ages). The group of people aged 18 to 29 had the highest percentage of dental caries (92.7%), while the group of people aged 50 to 59 had the lowest rate (79.1 percent) The group of people aged 18 to 29 had the highest percentage of dental caries (92.7%), while the group of people aged 50 to 59 had the lowest rate (79.1 percent) The group of people aged 18 to

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Streptococcus.mutans and Lactobacillus are the primary pathogenic bacteria, according to (26) As a result, Lactobacillus is regarded as the oral flora's second most cariogenic bacteria.
(27) report that the Most Lactobacillus species found in caries lesions in both adult and childhood caries include *Lactobacillus fermentum* Less common species included Lactobacillus mucosae.

5. Level of salivary IgA (sIgA) in saliva

The result of the study show that the mean ±S.D of sIgA value of dental caries control group more than in patients groups in probability (P≤0.001) with significant differences as shown in Table(4),figure (3).

Table(4): concentration of salivary IgA (sIgA) in saliva				
Character	Patients group	Control group	t -test	P
SIgA	402.88 ±82.32 mg/dl	431.75 ± 245.87 mg/dl	37.591	0.001**

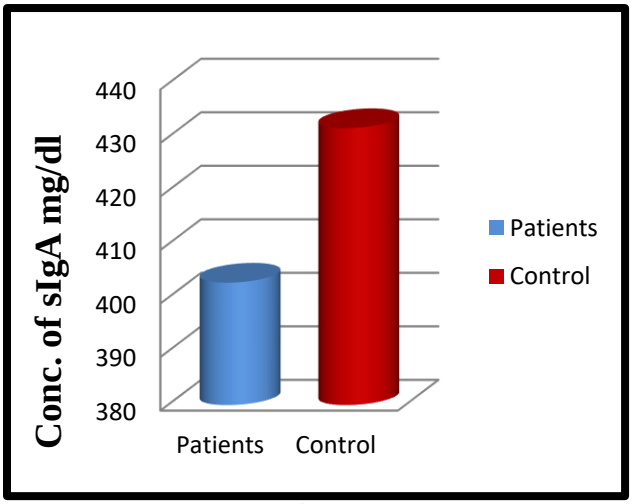
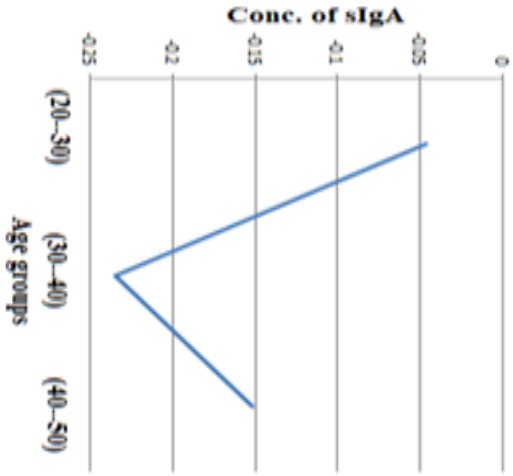


Fig. (3) :Level of salivary IgA (sIgA)

Secretory immunoglobulin A is the most abundant immunoglobulin in all of saliva and is thought to be the mouth's principal defense mechanism. It helps to prevent dental cavities by limiting bacterial adhesion, lowering hydrophobicity, agglutinating bacteria, and inactivating bacterial enzymes and toxins (28).
This research supports the findings of (29) who found that low sIgA levels in the oral cavity are associated with a high caries risk, whereas high sIgA levels are associated with a low caries risk.
The sIgA proteins are thought to operate as antibacterial defenses in the mouth, according to (30). These salivary antibacterial mechanisms protect both hard and soft tissues from oral bacteria that cause tissue deterioration.
Saliva contains a variety of secreted proteins and chemicals that help people to buffer and inhibit the spread of caries-causing bacteria, hence preventing sickness, according to (31).
(32). It was discovered that there was a substantial difference in Secretory IgA levels between dental caries patients and healthy groups, implying that the salivary concentration of sIgA in low caries children is significantly higher than in caries-active children. The presence of dental caries and the level of sIgA have a negative connection..

6. Correlation of sIgA concentration with age groups

Figure (4) demonstrates a substantial association between immunological markers and the patient's age groups. There is a substantial inverse link between sIgA concentration and age groups, and this has an effect on caries progression.



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When it comes to the mouth, Changes in salivary protein content with age were found to be strongly associated to the occurrence of dental caries. (33).
(34) discovered that sIgA secretion appears to decrease by two-thirds as people become older, which has implications for immune defense in the elderly. Mucin secretion appears to be decreasing with age, according to further investigation of mucous gland saliva (submandibular, sublingual, and small salivary glands). The secretion of several organic components, such as sIgA, MG-1, and MG-2, is reduced, which may have an impact on oral health. When an individual's immunological and non-immune defense systems degrade with age, the oral soft tissues may become more vulnerable to environmental stimuli.

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