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DETERMINATION OF MUC5B AND MUC7 LEVELS IN SALIVA OF PATIENT WITH DENTAL CARIES

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ABSTRACT

Human saliva is a complex secretion that contains a mixture of inorganic and organic molecules, it plays an essential role in the maintenance of oral health. Mucins are the major macromolecular component of this secretion and are considered as the first line of defense for epithelial tissues. To determine the MUC5B and MUC7 levels in saliva of patient with dental caries by using ELISA Assay. The current study included 85 samples which were collected from patients with dental caries attending the Conservative Dentistry teaching dental clinics of Babylon University -College of Dentistry and private dental clinics with age from (20 to 40) years. The results showed that the levels of MUC5B in control (23.32 ± 10.39) were more than in patient's groups (19.11 ± 10.69) with high significant differences. And there was significant difference (p≤ 0001) represented by increase in MUC7 levels in control group (0.93 ± 1.34) compared with patients groups (0.83 ±1.34).

***Keywords*:** Muc5B, Muc7, ELISA Assay.

Introduction

Dental caries is a multifactorial infectious microbiologic disease of the teeth that is characterized by a localized dissolution of inorganic tooth content followed by the destruction of the organic content and progress to form a cavity (Turgeon, 2019). Human saliva is a complex secretion that contains a mixture of inorganic and organic molecules, plays an essential role in the maintenance of oral health (Van *et al.,* 2004). The tooth surface is protected by a film of salivary mucins and prolinerich glycoprotein (Patil and Patil, 2011). Whole saliva contains several antimicrobial components that mediateselective adhesion and colonization of bacteria on the tooth surfaces. Agglutinins include mucins, glycoproteins, fibronectin, lysozyme and salivary Immunoglobulin A (SIgA) promote agglutination of bacteria and enhance bacteria removal. This may inhibit bacterial adherence to saliva-coated hydroxyapatite and epithelial surfaces and neutralize bacterial enzymes and virulence factors (Maddu, 2019). Salivary mucins are very large and are strongly glycosylated. In addition, salivary mucins are primarily developed by the submandibular salivary gland and the small labial and palatinalsalivary glands (Fábián *et al.,* 2012). Two major mucin subtypes exist, namely the membrane associated and the secreted form. In the oral cavity, mucins form membrane-associated (i.e., MUC-1) are primarily secreted by oral mucosal epithelial cells. After secretion, these proteins mainly stay on the cell surface (in the mucous layer) and primarily require defense of the epithelial surfaces (Carvalho and Lussi, 2017). A highest concentration of salivary mucins was observed in sublingual (MG1and MG2) saliva and palatine minor salivary (high molecular weight mucins) glands. MUC5b has a molecular weight above (1000) kDa and consists of related subunits of disulphide, in contrast to MUC7 which is approximately monomer of (180–200) kDa. In addition, (MUC5b) type mucins form "low concentration" viscoelastic hydrophilic gel that causes high saliva matrix viscosity. The salivary mucins, particularly MUC7, have a high affinity with microorganisms, and agglutinate and trap bacteria, viral and fungal particles (Agha *et al.,* 2017). Mucins, as part of the enamel pellicle, help to initiate the bacterialcolonization by encouraging the development of benign oral commensalflora to form a protective barrier and lubricate against excessive wear, toprovide a diffusion barrier against acid penetration and to restrict themineral egress from the tooth surface. High concentration of secreted mucin MUC5B predominates in people with severe caries. During caries development, a 14-fold increase inMUC5B concentration was determined in adolescents with DMF>11 and MUC7 mucin concentration was slightly decreased (Gabryel *et al.,* 2014).

The main purpose of the current study was to determine the salivary MUC5B, MUC7 content in saliva of patient with dental caries by using ELISA Assay.

Materials and Methods

Eighty-five samples were collected for the current study from patients with age from (20 to 40) years, out of (60) clinical specimens from saliva (according to (DMF) index more than 10) (WHO, 2017). 25 saliva sample were collected from healthy individuals as control (according to (DMF) index less than 10), these samples were utilized to detect the Muc5B and Muc7 levels in saliva of patient with dental caries by using ELISA Assay.

Sample collection

Saliva samples were collected properly in contamination-free environment, the mouth of all subjects was rinsed with distill water (10mL) for 30– 60 seconds to ensure the removal of any debris. Non-stimulated clean saliva was collected in sterile laboratory cup and stored in a cool box containing ice bags to maintain its viability till they were taken to the laboratory examination, then each sample was centrifuged at (3000 rpm)for 10 min. to make separation for the undesired free salivary particles.

Detection of MUC5B, MUC7 by ELISA technique

The ELISA plate provided in this kit has been pre-coated with an antibody specific to MUC5B, MUC7. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody, then a biotinylated detection antibody and Avidin-Horseradish Peroxidaes (HRP) conjugate was added to each microplate well successively and incubated. Free components were washed away; the substrate solution is added to each well. Biotinylated detection antibody and Avidin –HRP conjugate will appear blue in color. The enzyme –substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow.

Results and Discussion

Evaluation salivary MUC5B levels.

Table (1), Fig. (1) shows the mean of concentration MUC5B in control (23.32 ± 10.39) more than in patient's groups (19.11 ± 10.69) with high significant differences.

Table 1 : Mean and Standard deviation of MUC5B in dental caries patients and control groups

Character	Patients group (N = 60)	Control group (N = 25)	*t* test	P	Sig.
	Mean ±S.D	Mean ± S.D			
MUC5B	19.11± 10.6	23.32 ± 10.39	13.849	0.0001	HS.

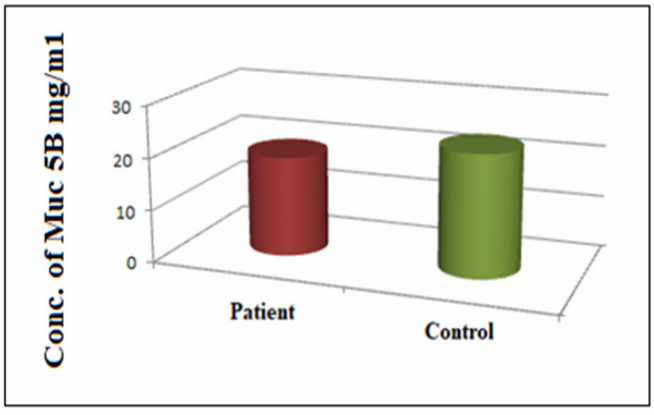

Fig. 1 : The concentration of MUC5B in patients and control groups

Evaluation salivary MUC7 levels

Figure (2) and table (2), show there was significant difference (p≤0001 0) represented by increase in MUC7 levels in control group (0.93 ± 1.34). compared with patient's groups (0.83 ±1.34).

Table 2 : Mean and standard deviation of MUC7 in dental caries patients and control groups

Character	Patients group No. = 60	Control group No. = 25	t test	P	Sig.
	Mean ±S.D	Mean ± S.D			
MUC7	0.83 ± 1.34	0.93 ±1.34	13.849	0.0001	HS.

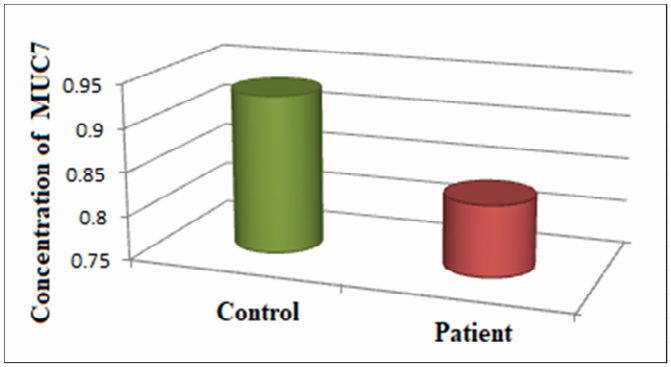

Fig. 2 : The concentration of MUC7 in patients and control groups

Discussion

The results obtained in this study were analyzed in two distinct groups of patients. Group1 included healthy andrelativly caries-free individuals. In turn, group 2 included patients with more severe caries.

This study is accordant with the study of (Buzalaf *et al.,* 2020) who found that the reduced levels of salivary MUC5B and MUC7 can contribute to the development of caries in adults.

Saliva contains salivary mucins (MUC7 and MUC5B) that cover and protect the mucosa and tooth surfaces. They also maintain the viscoelastic, hydrophobic, and lubricities properties of saliva. There are two genetically distinct types of mucins: high molecular weight (MG1) and low molecular weight (MG2), The MG1 mucin is encoded by the MUC5B gene and the MG2 mucin is encoded by the MUC7 gene (Frenkel and Ribbeck, 2015).

Salivary MUC5B was found to significantly inhibit bacterial attachment and biofilm formation on hydroxyapatite surfaces . The unique effect of mucin action is thought to result from specific interaction between glycans on MUC5B with bacteria (Osahon, 2020).This result is supported by the earlier studies of (Bhattarai *et al.,* 2018). who demonstrated, in patients with dental caries, a significant reduction of high molecularweight mucins(MUC5B) and also lowmolecularweight mucins (MUC7) as compared to subjects with control groups.

In addition, (Iyengar *et al.,* 2014) showed the role of membrane bound MUC7 and gel forming MUC5B, which would strengthen epithelial protective barrier and stabilizes mucin in oral cavity for longtime

Thestudy disagree with the result of (Gabryel *et al.,* 2014) who found that no significant differences were disclosed between salivary levels of MUC5B, and also MUC7 between patients with very low (control group) and moderate (research group) intensities of dental caries

Conclusions

From this study, it was found that, there was elevationof MUC5B and MUC7level in control groupswith significant differences compared with patient's groups.

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