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Salivary Cortisol Associated with Increasing *Mutans streptococci* Count in Drug Abuser

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ABSTRACT

Background and Aim: The aim of this study was to evaluate the relationship between salivary cortisol levels and the viable count of *mutans streptococci* in drug abusers.

Materials and Methods: The study was conducted from March 2022 to May 2022 and included 86 participants aged between 17 to 30 years, who were equally divided into two groups: cases (n= 43) and controls (n= 43). All participants underwent a urine drug line test to confirm their drug use status. Saliva was collected, and centrifuged at 3000 for 10 min, and the supernatant was stored at -20°C for later estimation of salivary cortisol levels using a Cobas device. The viable count of *Mutans streptococci* was determined using an identification and culturing method to calculate the colony-forming units per milliliter (CFU/ml).

Results: The drug abusers exhibited a significantly higher concentration of salivary cortisol as compared to the control group. In addition, the cases showed a positive correlation between salivary cortisol levels and the count of *Mutans Streptococci*. In contrast, the control group demonstrated a negative and non-significant relationship.

Conclusion: The data from the current study indicated a higher concentration of salivary cortisol associated with an increasing count of *Mutans streptococci* compared to the control group.

Keywords: Mutans Streptococci, Salivary Cortisol, Drug Abuser

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1. Introduction

Addiction is a chronic brain disease that can have negative effects on both physical and social well-being, as individuals cannot control their need for drugs. The brains of addicted individuals have altered responses compared to non-addicted individuals **(1, 2).** Addiction is considered as a brain disorder because it involves functional changes to brain circuits involved in reward, stress, and self-control. These changes can persist for a long time, even after withdrawal **(3)**. Addiction is similar to other life-threatening diseases, such as heart disease, by disrupting organs' normal, healthy functioning with harmful severe effects. However, in many cases, addiction can be prevented and treated; otherwise, if left untreated, it can result in fatal consequences **(4, 5)**.

The cortisol hormone is normally secreted in response to increased stress. The biological response to stress includes the interaction of two systems: the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system (SNS) or sympathetic adreno-medullary system (SAM). The latter is linked with the production of epinephrine and norepinephrine, which rapidly initiate a fight or flight response **(6, 7)**. A cascade of neurochemicals is produced by the HPA, which is activated more slowly. When a stressful event is experienced, the hypothalamus is stimulated to produce Corticotropin-

releasing hormone (CRH; previously known as corticotropin-releasing factor), which stimulates the release of adrenocorticotropic hormone (ACTH) into the circulation from the pituitary gland. Circulating ACTH stimulates the adrenal cortex to secrete the glucocorticoid hormone i.e., cortisol **(8, 9)**. Unbound serum cortisol enters the saliva via intracellular mechanisms. Due to the partial conversion of cortisol to cortisone during passage through the salivary glands, the absolute level of free cortisol in the saliva is estimated between 10% to 35% lower than the level in the blood **(10, 11)**. The use of salivary biomarkers has gained increased popularity over the past decade in psychological and biomedical research since collecting salivary samples is non-invasive and convenient. Nowadays, salivary cortisol measurement is widely used as an alternative to measuring cortisol levels in plasma or serum. In addition, the adrenal cortex is expected to increase plasma glucocorticoid levels in response to the stress of venipuncture for blood collection **(10, 12)**, which may bias the results.

Mutans streptococci are classified into five biotypes and nine serotypes (a-h and k). Oral *streptococcus mutans* (serotype c, e, f, k) and *streptococcus sorbinus* (serotype d, g) are the predominant species isolated from human saliva and dental plaque **(13, 14)**. The oral cavity of an individual can be colonized by one or by multiple clonal types of *Mutans streptococci* **(15, 16)**. *Mutans streptococci* have all the requirements for being a caries-inducing bacteria. Therefore, oral cavity of individuals heavily colonized by *Mutans streptococci* were thought to be at high risk for caries (17) automatically.

Saliva is one of the innate immune system components that counteracts pathogenic microorganisms both mechanically by its flow and immunologically by range of immunoglobulins (Ig) and enzymes. Increasing levels of unbound salivary cortisol may reduce the level of these protective proteins. Even when drug abusers stop consuming addictive substances, They may persistently experience elevated cortisol levels. Thus, we hypothesized that increasing systemic cortisol might compromise immune elements of saliva e.g., IgA; hence, paving the way for increasing *Mutans streptococci* count. Therefore, this study aimed to investigate this potential relationship in drug abusers.

2. Materials and Methods

The subjects

The study design was a case-control consisting of a total of 86 male subjects, aged between 17 and 30 years. The study was conducted between March 2022, and May 2022, after obtaining ethical approval from the Ethics Committee, College of Dentistry, University of Baghdad (Ref# 508, Project# 580822). Samples were collected from patients who were admitted to Ibn Rushd psychiatric hospital, Iraq. The participants were divided into two groups: the cases group (n= 43), which included withdrawn drug abusers and the control group (n= 43), who were clinically healthy and non-drug users. Drug abusers were defined as previously described **(18)**. Before conducting the study, all participants or their guardians for those < 17 years, had to sign a consent form.

Inclusion Criteria

The participant included in this study should have no signs and symptoms of any chronic diseases or immune deficiency diseases. All participants should not take any medication on the day of sample collection that might alter the biomarker or microorganisms under study. Each volunteer smokes less than 10 cigarettes per day and does not drink alcohol. In addition, patients who were previously consuming cocaine, amphetamine and other derivative were also included.

Exclusion Criteria

Exclusion criteria included participants aged less than 17 years, uncooperative, fearful, anxious, or unconscious patients, participants smoking more than 10 cigarettes or another type of smoking such as vape, and participants using other types of drugs not consistent with our study criteria.

Each patient was confirmed as a drug abuser by the laboratory of Ibn Rushd psychiatric hospital by taking a urine sample and analyzing it with a multidrug line cup urine test, which was also conducted for controls to confirm the negative result. Consent was obtained from all participants before conducting the study.

Salivary sample collection

Unstimulated saliva was collected in the morning between 8 to 10 am. Each participant was asked to refrain from eating or drinking for one hour before saliva collection. The participant was then instructed to put the collection tube near the lip and drool the saliva by tilting the head forward.

After collecting about 3 to 5 ml of saliva, collection tubes were numbered and placed in a cool box with ice to transfer it to the laboratory to be cultured in less than one hour. Salivary samples were centrifuged at 3000 rpm for 10 min, and the supernatant was isolated in Eppendorf tubes, which were stored in a deep freeze at -20ºC for chemical analysis later **(19)**.

Identification of *Mutans streptococci*

Culturing, isolation and identification of bacteria including colony morphology, Gram stain, catalase

and fermentation test were done according to methods previously described **(20, 21)**.

Determination of salivary cortisol level

In the first incubation step, 10 µL of the sample was incubated with a cortisol-specific biotinylated antibody and a ruthenium complex labeled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with the sample analyte and in part with the ruth enylated hapten.

In the second incubation step, streptavidin-coated microparticles were added, and the complex was bound to the solid phase via the interaction of biotin and streptavidin.

The reaction mixture was then aspirated into the measuring cell, where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were removed with ProCell/ProCell M. Application of a voltage to the electrode then induced chemiluminescent emission which is measured by a photomultiplier.

Results were determined using a calibration curve generated by 2-point calibration and a master curve provided via the reagent barcode Or e-barcod.

Statistical analysis

Data analysis was performed using SPSS software (version 26). The data were normally distributed, as tested by Shapiro-Wilk test. Independent t-test were used to compare the means of variables between the study groups. Liner regression was used to estimate the relation between salivary levels of cortisol and Mutans in the study sample.

3. Results

Salivary Cortisol

Salivary levels of cortisol in drug abusers and control groups are shown in Table 1. The mean of salivary cortisol level among drug abusers (24.61 ±6.77 nmol/L) was significantly higher (P = 0.0001) than in the control group (10.56 ±5.19 nmol/L). Higher levels of salivary Mutans were also detected in drug abusers (101.47 ± 27.49) than in the control group (30.69 ±10.49), as shown in Table 1.

Table 1. Mean of salivary levels of cortisol and Mutans in the study sample groups.

Group	Cortisol (nmol/L)	Mutans (CFUml)*10^{-5}
	Mean ± SD	Mean ± SD
Drug abuser	24.61 ± 6.77	101.47 ± 27.49
Control	10.56 ±5.19	30.69 ±10.49
P-value*	0.0001	0.0001

* Independent T test; significance was considered at P < 0.05

A positive association was found between the salivary levels of cortisol and Mutans in the study sample (R = 0.801). The increase in the salivary cortisol level could be accounted for by an elevation of salivary Mutans in approximately 64% (R2= 0.64.2) of the sample, with a prediction value of 3.863, as shown in Table 2.

Table 2. Liner regression between salivary levels of cortisol (predictor) and mutans (dependent variable)

	Mean ±SD	R[c]	R^2	Unstandardized B		P value
				constant	predictor	
Cortisol[a]	16.57 ±8.54	0.801	0.642	1.961	3.863	< 0.0001
Mutans[b]	66.08 ±41.17					

a: predictor; b: dependent variable; c: Pearson correlation test

4. Discussion

Salivary cortisol can be used as a biomarker of adrenocortical function, even though its levels correspond to only 50-60% of plasma cortisol concentrations **(22-24)**. The saliva collection procedure is considered non-invasive and comfortable; therefore, salivary cortisol levels were selected as the biomarker of choice for the present study **(25, 26)**.

The results of the present study showed that the mean of the salivary cortisol level among the drug-abuser was higher than that of the control group, with a significant difference between the two groups. This finding is consistent with results reported by other studies **(27, 28)**. The salivary cortisol concentration significantly reduced when patient received therapy in contrast to untreated patients. Psychomotor stimulants, such as cocaine and amphetamine, as well as natural rewards, such as sex and food, cause a release of dopamine in the nucleus accumbens, regardless of their mechanism of action **(29, 30)**.

The current study also demonstrated a positive and significant relation between the salivary cortisol level and the viable count of *Mutans streptococci*. Notably, no available data was found from previous studies concerning the relationship between the salivary cortisol level and the viable count of *Mutans streptococci* in drug-abuser except one study **(19)** that highlighted such a relationship in children suffering from early childhood caries. The relation between salivary cortisol level and *Mutans streptococci* in this study might be explained through several possible reasons. For instance, higher cortisol levels might suppress oral immunity and induce the proliferation of cariogenic bacteria, leading to an increased incidence of dental caries **(31, 32)**. Additionally, an increase in salivary cortisol may affect microbial colonization processes such as adhesion and co-adhesion **(33, 34)**. Basal salivary cortisol secretion was positively associated with dental caries; from a theoretical perspective, salivary cortisol could suppress mucosal immunity against cariogenic bacteria **(35)**. Further, during stress, the circulating cortisol level increases the production of acid which provide a suitable medium for these bacteria. Furthermore, atrophic changes in the major salivary glands caused by corticosteroids may lead to a decrease in the quantity, volume, and composition of the saliva. Meanwhile, decreasing the salivary secretion contributes to reducing the mechanical clearance of cariogenic bacteria **(36, 37)**.

Limitations of the current study included the lack of measuring oral hygiene/periodontal and caries activity indices. The addictive substances used by the subjects were not specified, and all subjects who had withdrawn from drug addiction were included. Measuring plasma cortisol levels would give a better picture of this hormone for the participants. Investigating the viable count of individual serotype is more precise than measuring *Mutans streptococci* group. Furthermore, *Lactobacilli*, another important food-fermenting lactic acid bacteria, was not included. Despite these limitations, to the best of our knowledge, this study is one of the few that investigated the relationship between *Mutans streptococci* and salivary cortisol in patients withdrawn from addictive substances. However, further larger-scale studies are advised to confirm the current findings, which is derived from this pilot study, before generalizing the results.

5. Conclusion

The data from the current study indicate that a higher concentration of salivary cortisol is associated with an increasing count of *Mutans streptococci* in patients who have quit consuming addictive drugs compared to control subjects. These results suggest including oral hygiene care as a part of rehabilitation programs of drug addiction.

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Author Contribution

ASA conceived and designed the study, conducted research, provided research materials, and collected and organized data. BAY analyzed and interpreted data. BAY wrote the initial and final draft of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Ethical approval

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Conflict of Interest

No conflicts of interest.

Reference

1. Angres DH, Bettinardi-Angres K. The Disease of Addiction: Origins, Treatment, and Recovery. Dis Mon. 2008;54(10):696-721. [DOI:10.1016/j.disamonth.2008.07.002] [PMID]
2. Cleck JN, Blendy JA. Making a bad thing worse: adverse effects of stress on drug addiction. J Clin Investig. 2008;118(2):454-61. [DOI:10.1172/JCI33946] [PMID] [PMCID]
3. Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. Addiction. 2006;101(S1):23-30. [DOI:10.1111/j.1360-0443.2006.01586.x] [PMID]
4. Volkow ND. Drugs, brains, and behavior: The science of addiction. 2010. Contract No.: 2011.
5. Johnson BA. Addiction Medicine. Science and Practice. Publisher: Springer. 2011. [DOI:10.1007/978-1-4419-0338-9]
6. Pervanidou P, Chrousos GP. Early-Life Stress: From Neuroendocrine Mechanisms to Stress-Related Disorders. Horm Res Paediatr. 2018;89(5):372-9. [DOI:10.1159/000488468] [PMID]
7. Kazakou P, Nicolaides NC, Chrousos GP. Basic Concepts and Hormonal Regulators of the Stress System. Horm Res Paediatr. 2023;96(1):8-16. [DOI:10.1159/000523975] [PMID]
8. Kodavanti UP. Stretching the stress boundary: Linking air pollution health effects to a neurohormonal stress response. Biochim Biophys Acta - Gen. 2016;1860(12):2880-90. [DOI:10.1016/j.bbagen.2016.05.010] [PMID]
9. Vodička M. The effect of stress on regulation and regeneration of glucocorticoids in animal models differing in response of hypothalamo-pituitary-adrenal axis. Univerzita Karlova, Přírodovědecká fakulta; 2021.
10. Raja Hadi BDS. Assessment of cortisol as salivary psychological stress marker in relation to temporomandibular disorders among a sample of dental students. Dentistry. 2015;27(2):86-92. [DOI:10.12816/0015300]
11. Miller WL, Flück CE, Breault DT, Feldman BJ. The adrenal cortex and its disorders. Sperling Pediatric Endocrinology: Elsevier; 2021. p. 425-90. [DOI:10.1016/B978-0-323-62520-3.00014-2]
12. Ahmed JN. Determination of the effect of stress on the salivary cortisol level among sample of university students having myofacial pain. 2013.
13. Theilade E. Factors controlling the microflora of the healthy mouth. Human Microbial Ecology1990. p. 1-56. [DOI:10.1201/9781003068020-1]
14. Marsh P, Martin M, Marsh P, Martin M. The resident oral microflora. Oral Microbiol. 1992;1: 27-55. [DOI:10.1007/978-1-4615-7556-6_3]
15. Seki M, Yamashita Y, Shibata Y, Torigoe H, Tsuda H, Maeno M. Effect of mixed mutans streptococci colonization on caries development. Oral microbiol immunol. 2006;21(1):47-52. [DOI:10.1111/j.1399-302X.2005.00253.x] [PMID]
16. Hatim FA, Diajil AR, Al-Mizraqchi AS. The Study of Oral Microbiological Changes in non-Hodgkin Lymphoma Patients Receiving Chemotherapy. Scopus Ijphrd Citation Score. 2019;10(01):1107. [DOI:10.5958/0976-5506.2019.00210.9]
17. Al-Ubaidi A. The prevalence of streptococcus mutans biotypes among preschool children 1993.
18. Moeller KE, Lee KC, Kissack JC. Urine Drug Screening: Practical Guide for Clinicians. Mayo Clin Proc. 2008;83(1):66-76. [DOI:10.4065/83.1.66] [PMID]
19. Yahya AA, Al-Haidar AHMJ, Al-Mizraqchi AS. The Role of Salivary Cortisol and Mutans Streptococci in the Development of Early Childhood Caries. Indian J Public Health Res Dev. 2019;10(10). [DOI:10.5958/0976-5506.2019.03350.3]
20. Guthof O, Fridrich J. The genus Streptococcus and dental disease. Procaryotes Hand Book of Habitats, isolation and identification of bacteria" Mortimer, PS (edt), Berlin, New York1981. p. 1598-613.
21. Horton WA. On the Cariogenicity of Streptococci: The University of Manchester (United Kingdom); 1986.
22. Lewis JG. Steroid Analysis in Saliva: An overview. Clin Biochem Rev. 2006;27(3):139-46.
23. Tahara Y, Sakurai K, Ando T. Influence of Chewing and Clenching on Salivary Cortisol Levels as an Indicator of Stress. J Prosthodont. 2007;16(2):129-35. [DOI:10.1111/j.1532-849X.2007.00178.x] [PMID]
24. Tammayan M, Jantaratnotai N, Pachimsawat P. Differential responses of salivary cortisol, amylase, and chromogranin A to academic stress. PLoS One. 2021;16(8):e0256172.[PMID] [PMCID] [DOI:10.1371/journal.pone.0256172]
25. Padilla GA, Calvi JL, Taylor MK, Granger DA. Saliva Collection, Handling, Transport, and Storage: Special Considerations and Best Practices for Interdisciplinary Salivary Bioscience Research. Salivary bioscience: Foundations of interdisciplinary saliva research and applications: Springer; 2020. p. 21-47. [DOI:10.1007/978-3-030-35784-9_3]

26. Cui Y, Yang M, Zhu J, Zhang H, Duan Z, Wang S, et al. Developments in diagnostic applications of saliva in human organ diseases. Med Nov Technol Device. 2022;13:100115. [DOI:10.1016/j.medntd.2022.100115]

27. Maddox-Rooper TR, Sklioutouskaya-Lopez K, Sturgill T, Fresch C, Clements CW, Lamichhane R, et al. Intake assessments of salivary cortisol, survey responses, and adverse childhood experiences are associated with recovery success in an abstinence-based treatment program for substance use disorders. Alcohol Clin Exp Res. 2022;46(10):1865-74. [DOI:10.1111/acer.14913] [PMID]

28. Ligabue KP, Schuch JB, Scherer JN, Ornell F, Roglio VS, Assunção V, et al. Increased cortisol levels are associated with low treatment retention in crack cocaine users. Addict Behav. 2020;103:106260. [DOI:10.1016/j.addbeh.2019.106260] [PMID]

29. Kalivas PW, Duffy P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse. 1990;5(1):48-58. [DOI:10.1002/syn.890050104] [PMID]

30. Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI. Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology. 1997;133:7-16. [DOI:10.1007/s002130050365] [PMID]

31. Boyce WT, Den Besten PK, Stamperdahl J, Zhan L, Jiang Y, Adler NE, et al. Social inequalities in childhood dental caries: The convergent roles of stress, bacteria and disadvantage. Soc Sci Med. 2010;71(9):1644-52. [PMID] [PMCID] [DOI:10.1016/j.socscimed.2010.07.045]

32. Bright MA, Alford SM, Hinojosa MS, Knapp C, Fernandez-Baca DE. Adverse childhood experiences and dental health in children and adolescents. Community Dent Oral Epidemiol. 2015;43(3):193-9. [DOI:10.1111/cdoe.12137] [PMID]

33. Tikhonova S, Booij L, D'Souza V, Crosara KTB, Siqueira WL, Emami E. Investigating the association between stress, saliva and dental caries: a scoping review. BMC Oral Health. 2018;18(1):1-9. [DOI:10.1186/s12903-018-0500-z] [PMID] [PMCID]

34. Vacaru R-P, Didilescu AC, Sfeatcu R, Tănase M, Munteanu A, Miricescu D, et al. The Effect of Dental Treatments in Caries Management on Stress and Salivary Protein Levels. J Clin Med. 2022; 11(15):4350. [DOI:10.3390/jcm11154350] [PMID] [PMCID]

35. Abdul-Ameer AK, Radhi NJ, Abdul-Ghani HJ. Stressful life events in relation to dental caries and selected salivary constituents among secondary school students in Baghdad city. J Baghdad Coll Dent. 2017;325(4203):1-9. [DOI:10.12816/0038655]

36. Jain M, Singh A, Sharma A. Relationship of Perceived Stress and Dental Caries among Pre University Students in Bangalore City. J Clin Diagn Res. 2014;8(11):Zc131-4. [PMID] [PMCID] [DOI:10.7860/JCDR/2014/11664.5213]

37. Enad HH, Al-Mizraqchi AS. Salivary Cortisol as a Stress Biomarker and Total Viable Count of Salivary Bacterial Microbiome among COVID-19 Patients. J Baghdad Coll Dent. 2021;33(4):6-10. [DOI:10.26477/jbcd.v33i4.3013]