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ORIGINAL RESEARCH

Evaluation of the Effect of Diacetyl Morphine on Salivary Factors and their Changes after Methadone Therapy

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ABSTRACT

Objectives: Psychoactive drugs are responsible for pathological changes in the mouth including dental caries, which most troublesome. The aim of the present study was to evaluate the effect of heroin on several salivary factors which are involved in the oral health and their changes after methadone maintenance therapy (MMT).

Materials and methods: Forty patients with heroin abuse history, who referred to Imam Reza Hospital for MMT were included.

Saliva sampling was carried out two times; at the first visit (time 1) and repeated 1 month after MMT (time 2). The saliva was analyzed immediately to evaluate the total volume, Ph, CPR, the Uric acid concentration, nitric oxide and antioxidant capacity.

Results: The mean values for saliva volume, pH, CRP, Uric acid, antioxidant and nitric oxide were 0.38 ± 0.14, 7.63 ± 1.22, 5.2 ± 2.3, 1.47 ± 0.8, 0.80 ± 0.23, and 0.26 ± 0.03, respectively at first visit and 0.34 ± 0.22, 7.37 ± 1.01, 6.1 ± 2.6, 2.18 ± 0.9, 0.74 ± 0.3 and 0.29 ± 0.08 after 1 month of MMT.

These values are below the normal ranges; however, there was no significant difference between two times in term of saliva volume, pH and saliva component ($p > 0.05$).

Conclusion: Heroin addiction changed the effective salivary factors and therefore could negatively contribute to oral health. These factors were not return to the normal range after 1 month of MMT. Physicians should be informed about focusing on oral health in patients under MMT.

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Clinical significance: Heroin addiction changed the effective salivary factors and these factors were not return to the normal range after 1 month of MMT.

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INTRODUCTION

Drug addiction is a worldwide problem. Opioids are widely available in Iran. This country has the highest number of opiate addicts in the world, with a reported rate of 2.8% among people aged 15 years and over.[1] It is officially estimated that of around 2 million of the entire population are addicted[2] Heroin is a semi-synthetic opiate which abused in the United States. Heroin is prepared by extraction from the poppy (Papaver somniferum) as morphine, then acetylated to form diacetylmorphine (heroin).[3]

Methadone maintenance therapy (MMT) is a widely accepted treatment method for opioid abused patients over the past decade in Iran. Over 800000 patients are under MMT scheme in this country; however, oral health of these patients has never been investigated.

Drug abuse is linked with a number of oral diseases and disorders. The major adverse health effects of long-term heroin use is severe dental caries. Several studies have shown that the oral health of the drug addicts is poorer than that of the general population.[4,5] For former heroin users who have received or are receiving methadone treatment in withdrawal period, the prevalence rates of caries and periodontal diseases are greater than that in the general population. The etiology of dental caries secondary to long-term use of the drug has been attributed to salivary glands hypofunction, depression of the body's immune system, high frequency consumption of carbohydrates and poor oral hygiene.[6] Methadone maintenance treatment that used for the management of opioid dependence also is associated with some degree of xerostomia.[7]

Saliva plays an important role in oral homeostasis.[8] So, quantitative and/or qualitative alterations in salivary secretion may caused local adverse effects.[9]

The buffer capacity of saliva plays a crucial role in the salivary pH, and in dental re-mineralization. This capacity of saliva basically correlates with the salivary flow rate,[10] so any factor which decrease salivary flow rate tends to decrease buffering capacity and increasing the risk of caries development.[11]

In addition, saliva possesses a variety of defence mechanisms responsible for the protection of the oral cavity from oxidative attacks. Uric acid and peroxides are among the major antioxidants in the saliva.[12] The other important function of salivary antioxidants is to control the oral bacteria that form dental plaque, it causes imbalance in the ecosystem leading to dental caries and periodontal diseases.[13]

Other saliva components that play a significant role in oral and periodontal pathogenesis are nitric oxide (NO) and complex reactive protein (CRP). Higher levels of NO have been found in inflamed periodontal tissue and excessive amount of NO may contribute to tissue destruction in patients with periodontitis.[14] CRP is an immune marker and an indicator of general inflammation that can be detected in saliva.[15]

Although, the harmful effects of drug abuse on general and mental health have been well-established, little evidences have been mentioned in the literature on qualitative and quantitative biological systemic changes produced in saliva of drug addicts.[16] Knowing the possible effects of the drug and substitute materials on the salivary gland function as an important part in promoting oral health, at the time of withdrawal, may help to prevent further problems in the oral health. In the present study we have measured the volume and pH of saliva, the total concentration of the salivary peroxidase, nitric oxide, uric acid and CRP in heroin drug abusers. In addition, we have evaluated their changes 1 month after methadone therapy. The null hypothesis was that there were no differences in the volume and composition of saliva in the heroin drug abuser and after drug withdrawal with methadone therapy. To the best of knowledge, this is the first scientific report with regard to salivary factors in patients under MMT.

MATERIALS AND METHODS

Forty healthy individuals with heroin abuse history that had been referred to Imam Reza Hospital for maintenance therapy were included in the study population. The participants comprised drug users that were entered into the MMT plan at this center in 2012. All participants were given a brief explanation about the investigation and all consented to participate and signed a consent form approved by the Ethics Research Committee of the, Mashhad University of Medical Sciences.

The study of salivary secretion was done without any stimulus in the morning (9-11 am), under standard temperature and humidity conditions. All the subjects refrained completely from eating, drinking or smoking for a minimum of 2 hours before the saliva collection.

Subjects were seated for 5 minutes in a relaxed position and they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced for 10 minutes into a graduated test tube, through a glass funnel. The whole volume of the salvia produced in 10 minutes was then measured. Saliva sampling carried out two times for each participant, one at the time of first refer (time 1) and the other one, 1 month after withdrawal (time 2) and saliva composition analysing was performed as follows.

In order to perform the laboratory tests, samples were prepared through a mucolysis step. After freezing saliva for 2-3 days at –20°C, samples were thawed to room temperature, centrifuged at 3000 rpm for 15 minutes in order to remove mucins.

To determine salivary pH, a Cyberscan pH 110 pH meter (Eutech Instruments) was utilized. In order to reduce the error-probability, each sample was analyzed three times, and the mean value was recorded.

Total antioxidant capacity' (TAC) of the saliva has been measured as previously described (Ref: PMID:23935266 [PubMed]). The method is based on the ability of the total antioxidants to inhibit the oxidation of 2,2'-azinobis (3-ethylbenthiazoline)-6-sulfonic acid (ABTS) by metmyoglobin. The reaction was followed spectrophotometrically at 405 nm; at which, the suppression of absorbance is proportional to the concentration of antioxidants. Antioxidant concentration was quantified as millimolar trolox equivalents. The uric acid (UA) concentration was measured in the saliva using a spectrophotometric kit (Parsazmun Co, Tehran, Iran), in which the absorbance was read at 546 nm.

The concentration of saliva nitric oxide was measured using a modified Griess reaction method (Green LC, et al). Analysis of nitrate, nitrite, and [< sup> 15</sup> N] nitrate in biological fluids. Analytical biochemistry, 1982. 126(1): p. 131-138). Briefly, the prepared saliva was mixed with an equal volume of modified Griess reagent for the colorimetric assay. After 10 minutes incubation at room temperature, the concentration of the resultant chromophore was spectrophotometrically measured at 550 nm after enzymatic conversion of the nitrate to nitrite by nitrate reductase. The nitrite concentration in samples was calculated from nitrite standard curves made from sodium nitrite employing the same sera. All samples were assayed for CRP using a commercially available immunoassay kit without any modification to the manufacturer's

Table 1: Mean ± SD of the volume, pH and concentration of other salivary factors before and after withdrawal

	Time 1	*Time 2*	*Normal range**	*P (T1 & T2)*
Salivary flow rate (ml/min)	0.38 ± 0.14	0.34 ± 0.22	0.48	0.205
pH	7.63 ± 1.22	7.37 ± 1.01	5.8-7.5	0.303
CRP (mg/l)	5.2 ± 2.3	6.1 ± 2.6	0	0.329
Uric acid (mg/dl)	1.47 ± 0.8	2.18 ± 0.9	3	0.237
Antioxidant (mmol)	0.80 ± 0.23	0.74 ± 0.3	0.68	0.591
Nitric oxide (µmol)	0.26 ± 0.03	0.29 ± 0.08	0.1	0.658

The normal values were derived from different studies that was cited in the discussion. CRP: C-reactive protein; SD: Standard deviation

recommended protocol. The test volume was 15 µl, with a range of standards from 93.75 to 3000 pg/ml and the assay had a lower sensitivity limit of 10 pg/ml.

STATISTICAL ANALYSIS

All samples were assayed in duplicate, and the average of the duplicates was used in the statistical analyses. Intra- and inter-assay coefficients of variation were less than 10 and 15%, respectively. In order to address the normality of the data obtained with a Koromogorov Smirnov test, Paired t-test was used in SPSS software (Ver 11.5) to compare the two groups for saliva volume and composition. The level of significance (p-value) was set less than 0.05.

RESULTS

Forty individuals included in this study were consisted of 24 males and 16 females with ages ranged between 18 and 55 years with the previous history of heroin abuse.

The mean values of each group for saliva volume, pH and composition are shown in Table 1.

Statistical analysis showed no significant difference between two groups in term of saliva volume (p = 0.205), pH (p = 0.303), CRP (p = 0.329), Uric acid (0.237), antioxidant (p = 0.591) and nitric oxide (p = 0.658).

DISCUSSION

This study revealed for the first time that saliva factors in patients under MMT are similar to those of Heroin addicted individuals. A consistent similar pattern was found for the volume and pH of the whole saliva, the total concentration of salivary peroxidase, nitric oxide, uric acid and CRP in MMT. These factors were below the normal ranges in both addicted individuals and 1 month after the MMT plan.

The poor oral health status in heroin addicts have been reported previously.[4] The novel finding of the present study is measuring the salivary factors that were important in oral health and that important measured factors stay at the same levels at least 1 month after the MMT.

This is biologically accepted as methadone, similar to other opioids, could limit salivary glands secretion.

Oral hygiene is important for all addicts, as they are susceptible to oral diseases. However, this is the personal responsibility of each patient. After changing to Methadone, impairment of oral hygiene could be considered as a side-effect. Therefore, educating patients and designing a prophylactic regimen should be ethically considered as an essential part of the treatment. Physicians should be educated about the extent of the oral health problem in patients under MMT, including regular oral hygienist visit and prophylactic treatment such as fluoride therapy.

The mean salivary flow rate of a drug abuser in this study was 0.38 which was reduced to 0.34 ml/minutes. However, this reduction did not reach statistical significance, although it was much less than previously reported normal salivary flow rate (i.e. 0.48 ml/min).[17] Therefore, the illicit drug abuse and methadone treatment both could lead to some degree of hyposalivation, which could be associated with dental caries.[7] Xerostomia is a condition in which the salivary flow is reduced to less than 0.1 mm/min[18] so, the values obtained in this study did not show the drug induced xerostomia. While it is well-established that opioids, including methadone, reduce pancreatic, biliary and gastric secretion, Odeh stated that it is not widely accepted that opioids can also result in xerostomia.[19]

The normal value of pH was reported in the range of 5.8 to 7.5.[17] Our data on the pH of saliva showed that this parameter did not change in illicit drug abuser and 1 month after methadone therapy (7.63 and 7.37, respectively). Methadone, used in the rehabilitation of drug-users, is available as a sugar-free preparation; however, the sugar-based version is also available.[20] It has been reported that the pH of a 1% water solution of sugar based methadone is 4.5 to 5.5, suggesting that taking methadone could increase oral acidity, thus increasing the rate of caries.[7] In this study, we used the sugar free version of methadone; therefore, the pH remained in the normal range.

Recently, it has been proposed that the imbalances in reactive oxygen species with antioxidants and levels of free radicals play an important role in the onset and

progression of several inflammatory oral pathologies.[21,22] Uric acid is the main antioxidant in saliva, than consist 85% of the total antioxidant activity of whole saliva in healthy and periodontally-compromised subjects.[23] Normal salivary urate concentration is estimated approximately 3 μm.[24] The UA concentration in this study is much less than the normal range and may be one of the factors leading to dental carries and periodontal disease in these patients.

On the other hand, in this study the total antioxidant values increased from normal range[25] in illicit drug buser and did not change 1 month after methadone therapy. The poor oral health status in illicit drug abuser could be one of the factors for the increased levels of total antioxidant of saliva. The authors reported that the total antioxidant increased with increase in caries activity.[26,27]

In the present study, the concentration of NO was not changed after 1 month of methadone therapy instead of heroin, although it was more than the normal range (0.1 ± 0.03). Ohashi et al[28] showed that production of salivary nitric oxide elevated in oral mucosal diseases. Moncada et al,[29] illustrated that excess generation of, nitric oxide is a key mediator of cell damage, tissue injury and organ failure. Therefore, the high level of NO in this study in both groups could be an indicator of periodontal damage.

Physicians should be aware of dental needs of the patients. Under-estimation of these patients may be result in being stigmatized and marginalized and prevent them to be integrated with the society and increases the risk of treatment relapse.

The results of present study indicate that methadone could not change the quantity and quality of salivary secretion when used as a succeeder of heroin for 1 month. However, this hypofunction of salivary glands may be as a long-term effect of heroin abuse that should be a matter of investigation in further studies.

Further, studied should be performed with a longer period of follow-up time to find out when and how the capacity of salivary gland return to the normal condition.

CONCLUSION

The results of the present study suggest that the methadone therapy and heroin addiction both have the same effect on salivary factors, therefore, the dental management should be continued in withdrawal period.

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