JCHR (2022) 12(1), 1-6

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Evaluation of Salivary Oxytocin Level in Pregnant Women during Pregnancy in Fatemiyeh Hospital of Hamadan, Iran

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(Received: 11 January 2021 Accepted: 22 August 2021)

KEYWORDS

Oxytocin;
Saliva;
Pregnancy;
Hamadan;
Iran

ABSTRACT: We undertook this study to propose a noninvasive method for measuring oxytocin (OT) level in pregnant women. Most of previous studies have focused on the measurement of OT levels in blood plasma through invasive method. Due to the important role of OT hormone level during pregnancy, in this study, the level of salivary OT during pregnancy was measured to investigate its applicability as an alternative to invasive conventional methods. In this case-control study, 126 individuals (63 pregnant women as case group and 63 non-pregnant women as control group) referred to in Fatemiyeh Hospital of Hamadan, Iran, were selected and evaluated. After obtaining written consent, saliva sampling was performed by stripping, and the samples were transferred to a laboratory at -4°C and then stored at -22°C. ELISA technique was applied for measuring the level of salivary OT. Data were analyzed using SPSS software. The results indicated that the mean level of salivary OT in the control group was 98.40±362.92 pg mL^{-1}. The mean level of salivary OT in the case group was 1.016±403.75 pg mL^{-1} with the maximum and minimum concentrations of 628.60 and 169.60 pg mL^{-1}, respectively. The results of this study implied that the mean level of salivary OT in pregnant women was significantly higher than that in non-pregnant women (P = 0.024). Also, OT levels were not significantly different in trimesters of pregnancy (P1 = 0.941, P2 = 0.844, P3 = 0.552). Our findings depicted that measuring salivary OT in pregnant women can be used as a noninvasive and accurate method instead of blood test.

INTRODUCTION

Oxytocin (OT) as a 9-amino-acid neuropeptide is secreted from the hypothalamus by the magnocellular neurons of the paraviticular and supra-optic nuclei and stored in the posterior pituitary [1-3]. It is produced in peripheral tissues, including placenta, uterus, heart, pancreas, thymus, kidney, and neoplastic tissues [2]. In general, OT plays a role in childbirth, lactation, maternal behaviors, sexual behaviors, parental care, social bonds, stress reduction, trust, kindness, forgiveness, cooperation, induction, empathy and dependency [5,6] It can also act as a neuro-modulotar transmitter with central receptors in the brain [2]. OT is produced in the body at the beginning of the pregnancy and reached the highest level during childbirth [7]. Increasing the OT level during labor is associated with relaxation, better absorption of nutrients, better maternal sleep and maintaining energy level of mothers [8]. Stopping OT hormone secretion causes failure to progress, delay in timely delivery of the placenta and postpartum hemorrhage [7]. Nowadays, noninvasive methods for

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DOI: 10.22034/jchr.2021.1920489.1244

measuring OT hormone have received much attention from researchers especially in pregnant women. OT is released into saliva, plasma, cerebrospinal fluid, and urine, and measuring OT via cerebrospinal fluid is an invasive procedure that requires special care and skills [5]. Measuring urinary OT as a noninvasive procedure does not consider a valid method [9]. Plasma OT measurement as an invasive procedure is routinely performed for determining OT levels especially during labor [5, 6]. The levels of OT is about 1-2 pg mL^{-1} in saliva [6] and 30 pg mL^{-1} in plasma [5]. Previous studies have reported that the measurement of salivary OT as a noninvasive procedure can be used for the determination of the OT in body of pregnant women [1, 5]. In this regard, De Jong et al reported that salivary OT level can be used as a sensitive and reliable indicator for measuring this hormone in body [6]. On the other hand, Gordon et al., have reported contrary results regarding the use of salivary OT with immunoassay technique. They demonstrated that saliva does not contain detectable amounts of OT hormone and its trace levels in saliva are mainly due to nonspecific antigen-antibody binding [10]. Therefore, this study aimed at evaluating the OT hormone levels in biological fluid of saliva to be used as a noninvasive method in pregnant women as the study or case group and non-pregnant women as control group. For this purpose, salivary OT levels were measured during the first, second and third trimesters of pregnancy by using human OT ELISA Kit. Eventually, the measurement results of salivary OT levels in each trimester were compared.

MATERIALS AND METHODS

Sampling

This case-control study was performed in Fatemieh Hospital of Hamadan University of Medical Sciences, Hamadan, Iran, during 2017 to 2018. The experiments were conducted in the Reference Laboratory in Hamadan University of Medical Sciences, Hamadan, Iran. In the present study, totally 126 individuals including 63 pregnant women were selected in the first, second and third trimesters who had first pregnancy, no history of preterm labor or abortion, and it was expected to have a normal birth (normal vaginal delivery) based on their medical records. The average age of these participants was in the range 18-38 years. Moreover, 63 non-pregnant women with the same age range referred to Fatemieh Hospital in Hamadan were considered as the control group. The cases were informed about the study objectives and asked to sign the written consent. In this study, the sample size was obtained based on the White-Traut et al. study, in which the standard deviation of the oxytocin level in the both pregnant women and controls (non-pregnant women) were considered to be 3.09. In this regard, with a 95% confidence interval (α=0.5), 80% power of the test, and a minimum significant difference of 10, and 20% loss in the group samples, the number of samples in each group was calculated to be 63 for each group [1].

Cases and controls

Inclusion criteria included women aged from 18 to 38 years who had a blood CBC test in the last two months without any problem. These individuals had no systemic, periodontal disease, salivary gland secretion disorders such as sub-mandibular duct obstruction, diabetes mellitus and asthma. They also had no history of medication, tobacco, and specific diet. Exclusion criteria included having a history of systemic and periodontal disease, drug use (especially antipyretic drugs, bronchodilators and multivitamin supplements), tobacco use, abnormal CBC test results, experiencing childbirth, abortion, preterm deliveries, possibility of cesarean section, and non-primary deliveries [11]. After obtaining written consent from all of the participants, unstimulated saliva samples were taken from them between 8 am and 11 am.

Experiments and procedures

The participants were asked to refrain from eating, drinking and brushing for 90 min before sampling. The participants were seated with the head slightly bent forward and were asked to pour 5 mL of saliva into the graduated Falcon tubes for 5 min, and then the saliva samples were rapidly frozen at -4°C. The collected samples were stored and transported to the laboratory within 10 min. The samples were centrifuged at 2 rpm for 10 min and then stored at -22 ° C in the laboratory at Fatemiyeh Hospital until subsequent tests to measure

salivary OT levels. Before analysis, the frozen specimens were allowed to thaw at room temperature (25°C) for 30 min. These kits were from EASTBIOPHARM Co. (CK-E 11373 made in China) under US license number of E20180207 with expiration date. 2019/02/06.

ELISA Technique

In this study, EASTBIOPHARM kit was used to measure OT salivary levels. This kit is based on non-competitive Sandwich ELISA Protocol for measuring OT level. This type of ELISA method has a very high sensitivity for detection of antigen. In the sandwich ELISA protocol, antigen is placed between two groups of antibodies (receptor and identifier antibodies). The antigen measured must have at least two epitope regions to be capable of binding to the antibodies. Monoclonal and polyclonal antibodies can be used in the sandwich ELISA protocol. Monoclonal antibodies identify only one epitope region, and thereby lead to the specific identification of antigen. While the polyclonal antibodies are usually used as receptor antibodies to identify the highest amount of antigen. The measurable concentration range of the kit was between 2 and 600 pg mL^{-1}, and therefore, the used kit was not able to measure the samples with higher concentration than 640 pg mL^{-1}.

Data analysis

Data were analyzed by SPSS software and the OT concentrations were analyzed using multiple regression and adjusting data based on age.

RESULTS AND DISCUSSION

According to the results, the mean of OT in pregnant women was significantly higher than non-pregnant women (P = 0.024). Also, multiple linear regression analysis showed that OT levels were not significantly different in the trimesters of pregnancy (P1 = 0.941, P2 = 0.844 and P3 = 0.552). Table 1 compares the OT levels in pregnant and non-pregnant women using linear regression model.

Table 1. Comparison of OT levels in pregnant and non-pregnant women using linear regression model

Variables	Not standardized coefficients		Standardized coefficients	T	Significance level
	B	Beta	Beta		
Constant	362.920	12.594		28.809	0.000
Year	-0.044	-0.499	0.618	-0.045	0.983
First trimester	-0.034	-0.429	0.669	-0.039	P1= 0.941
Second trimester	-0.012	-0.127	0.899	-0.011	P2= 0.888
Third trimester	0.047	0.394	0.695	0.035	P3= 0.552
	40.827	17.816	0.202	2.292	P= 0.024

In this study, age range and mean age of the case and control groups were considered equal to increase the accuracy of the study. Moreover, at the time of sampling and testing, the available standard facilities were applied to reduce possible errors. According to the results of the multiple regression analysis, the mean of OT levels in pregnant women was significantly higher than non-pregnant women. This finding is in agreement with the results of previous studies on the measurement of OT levels in blood samples of pregnant women [12]. Because, plasma OT level is slowly increased during pregnancy until delivery [13] In this regard, a previous study showed that the level of OT increased in blood samples of pregnant women with a maximum at term [13]. In another study, it was found that the levels of OT increased slowly until delivery and then started to up to 8 weeks postpartum [14]. A similar study evaluated the concentration of in saliva of pregnant women reported that salivary OT is not measurable and cannot a valid biomarker by immunoassay [10]. This may be due to a weakness in the application of laboratory techniques for measuring OT. In line with our results, a study on the determination of salivary OT levels before, during, and after lactation, and showed that salivary OT measurement is a reliable noninvasive method for monitoring its level during pregnancy [1]. This finding

could be due to the development of laboratory techniques over the years, which confirmed our findings.

Moreover, in a study to determine the levels of salivary OT in response to running, sex, and lactation with the aim of validating salivary OT as a reliable and sensitive biomarker, it was found that OT in oral salivary fluid can be used as a valuable diagnostic tool [6]. This result is consistent with the our findings, however, further study are required to determine accuracy and precision of the proposed method in comparison with blood OT level.

The results of this study showed that the OT hormone level increased during pregnancy especially in the last trimester of the pregnancy. According a previous study in 2007, increased OT was observed from the beginning of the pregnancy to one month after postpartum, which is closely attributed to the fetal–maternal contact, and this information can be used to provide a standard level for OT proportional to the contact between mother-fetus and mother-infant at different times of pregnancy and postpartum [15]. According to the results of this study, the levels of salivary OT showed an increasing trend in the pregnancy trimesters (Table 2).

Table 2. Mean levels of salivary OT in pregnant women by Trimester

Trimester of pregnancy	Mean levels of salivary OT	SD	Maximum level	Minimum level
First trimester of pregnancy	388.43	84.69	805.28	290.10
Second trimester of pregnancy	401.10	103.34	621.70	263.40
Third trimester of pregnancy	407.65	105.54	628.60	169.60

Therefore, it can be suggested that as the plasma OT level increases, salivary OT levels rises as well. Further studies with more statistical population are required to use salivary OT level as a reliable laboratory criterion in paraclinical diagnostic tests, especially when repeated sampling is necessary to monitor the level of OT in pregnant women. Also, in a previous study, the plasma OT levels were measured during pregnancy and delivery, a gradual increase in plasma OT level was observed over pregnancy period [16]. This results are consistent with the findings of the present study. In a previous study, it has been reported that during the milk-ejection reflex, pulsatile waves of OT are released from the posterior pituitary and cause milk to be released from alveoli into ducts that open into nipple pores [17, 18]. This justify our findings regarding the higher level of OT in pregnant women compared to those were not pregnant.

CONCLUSIONS

In the present study, a noninvasive method was tested for monitoring the levels of OT in pregnant women. For this reason, saliva samples were taken from 63 pregnant women (as case group) and 63 non-pregnant women (as control group). The results implied that the mean level of salivary OT in pregnant women was significantly higher than that in non-pregnant women (P = 0.024). Also, OT levels were not significantly different in trimesters of pregnancy (P1 = 0.941, P2 = 0.844, P3 = 0.552). The results depicted that there was a significant difference in the salivary OT levels between pregnant and non-pregnant women, and its levels in the pregnant women were higher than non-pregnant women. According to the results, an increasing trend was observed in OT levels in the trimesters of pregnancy. The results of this study implied that the salivary OT can be measured as a reliable and during pregnancy instead of blood OT test that is invasive and may pose risk to a risk to the both mother and fetus.

ACKNOWLEDGEMENTS

The authors would like to thank the Hamadan University of Medical Sciences for financial support of this research. This manuscript was extracted from a dissertation of general dentistry (No. 9609145718).

ETHICAL CONSIDRATION

The protocol of the present study was approved by the ethical committee of the Hamadan University of medical sciences, Hamadan Iran (No. IR.UMSHA.REC.562).

Conflict of interest

The authors declare no conflict of interest regarding this papers.

Authors' contributions

ZK conducted the experiments and taking samples. HRA has designed the study and supervised the findings of this work. ARS contributed the analysis of the data and verified the analytical methods. Other authors have contributed in the manuscript preparation and discussed the results and contributed to the final manuscript. All authors have confirmed the final version of the manuscript**.**

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