



Evaluation of Mutation in Oxytocin Receptor Gene in rs35062132 Variant in Women with a History of Preterm Delivery and Women without a History of Preterm Delivery

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Received: May 03, 2021

Published: June 25, 2021

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Abstract

Background and Objective: Oxytocin is a neuropeptide made in the ventral and supraoptical adjacent nuclei of hypothalamus. Oxytocin is a hormone regarded for activity of the neuropeptides establishing sexual behaviors, but, first of all, it is known for a role played in establishment of contraction of uterus while delivering.

Receptor of oxytocin is OXTR and availability of its receptor depends on the maternal serum level which increase of serum plays an important role in progression of pregnancy. This research aims to study a relationship between rs35062132 (R376G) polymorphism of OXTR gene and/with premature labor.

Studying Method: In this study conducted by case control method in 2018, 152 women divided into two groups of those with premature labor history (from 22 to 36 weeks who referred to the Shahid Rajaei hospital of Tonekabon) and those without premature labor history were studied. After collection of information using questionnaire, sampling and performance of the rest steps, study of polymorphism was carried out by PCR-sequencing technique, and results of sequencing determination were analyzed by the Chromas software.

Finding: Out of collection of 152 studied cases, there existed rs35062132 polymorphism of OXTR gene in 53 individuals (34.8%) while presenting results of their sequencing determination and 99 individuals (65.1%) lacked this polymorphism.

Conclusion: Results of this research show that there is a relationship between rs35062132 polymorphism of OXTR gene with premature labor.

Keywords: Oxytocin; Premature Labor; Polymorphism; PCR-sequencing

Introduction

Oxytocin is a neurohypophyseal hormone which exists in the mammals. This hormone is produced under receptacle naturally and reserved in the posterior hypophysis and plays a basic role in amorous relationship, sexual reproduction and before and after labor. In a lot of behaviors, including orgasm, social cognition, bonding placenta, anxiety and maternal behaviors, oxytocin contributes [1]. Mechanism of effect of oxytocin occurs through increase

of permeability of the uterus's myofibrils into sodium and stimulation of contraction of the womb's flat muscles indirectly. Stimulant influence of milk is higher through contraction of myoepithelial cells surrounding the breast's alveoli and direction of milk from alveoli to the lacteal tracts [2].

Oxytocin receptor has been identified by abbreviated name of OXTR, and this receptor is present in the peripheral tissues such as thymus, heart and reproductive organs and has a high disper-

sion in the central members related to maternal, reproductive and adaptation-to-environment behaviors.

This receptor is placed on the short arm of chromosome 3, band 2, sub band 5 and 3(3p25.3) chromosomal zone. Oxytocin receptor is of protein type, and availability of oxytocin receptor depends on level of LNPEP maternal serum which is a hydrolysis maker and inactivator enzyme and plays an important role in increase of maternal serum during labor and progression of pregnancy through regulation of activity and level of oxytocin [3].

Several dozen single nucleotide polymorphisms (SNPs) have been identified within the OXTR gene. These SNPs are indicated in the National center for Biotechnology Information database [19,22,23,26].

Oxytocin is crucially involved in the onset and maintenance of labor. It is a nine amino acid neuropeptide which stimulates uterine contraction and action and modulates behavior and cognition [19-23]. The human oxytocin receptor (OXTR) is a polypeptide with seven transmembrane domains belonging to the class I G protein-coupled receptor family [19,21]. Oxytocin Activates the OXTR, which is expressed in both the myometrium and the endometrium, as well as in diverse peripheral tissues and the central nervous system [19,21]. In the myometrium oxytocin activates phospholipase C, which increases the intracellular calcium concentration and thus intensifies uterine contractions. Mediated by activation of the OXTR, oxytocin additionally seems of prostaglandins in the chorion, deciduous and amnion [19,24,25]. Parturition in most mammalian species is preceded by a rise in estrogen and a decline in progesterone concentrations in maternal plasma [33,34]. These events occur over several days or hours before parturition and lead to changes that increase myometrial contractility. These changes include an increase in myometrial oxytocin receptors and gap junction formation [33,35,36]. Immediately prior to labor onset, neurohypophyseal oxytocin is released into the maternal circulation [33,37]. This release is accompanied by increased production of prostaglandins from intrauterine tissues. The end result is coordinated uterine contractions resulting in delivery of the fetus [33,38]. Despite the widespread use of oxytocin to induce or augment human labor, there is considerable doubt the physiologic role of oxytocin in normal human parturition. This doubts is based on the following findings: most investigators have been unable to detect an increase in plasma oxytocin before labor onset [33,39,40]. There is no apparent correlation between plasma oxytocin levels and myo-

metrial activity [33,41]; human labor occurs normally in cases of maternal posterior pituitary dysfunction and in the absence of oxytocin from the fetal circulation (as in fetal anencephaly) [33,42].

Naturally, time of neonate birth is from 37th week of pregnancy on. Premature labor is referred to as labor prior to 37 weeks of pregnancy [4]. The earlier birth of neonate occurs in the appointed time, the higher possibility of occurrence of serious problems for neonate. The neonates born before 24th week of pregnancy survive very less possibly, and, in case of survival, a lot of difficulties, including learning, motor skills and...etc will be occurred. In the first week of birth, probability of death in the neonates born earlier before appointed time ranged from one to three weeks will be six times as much as higher than those neonates born on time and three times as much as higher than those neonates born in the first year of birth [5] preterm birth is a major health problem. It is the leading cause of perinatal mortality as well as neonatal and long-term morbidity in the industrialized world. In developed countries up to 11% of children and born preterm and several studies have reported increasing trends in preterm labor [19,27]. The mechanisms leading to preterm labor are complex and still not fully understood beside known risk factors, such as demographic characteristics, lifestyle and infection, genetic factors seem to play a crucial role in preterm labor [19,28-32].

In study of Dencker, *et al.* conducted on 630 first-born women in 2009, by spontaneous beginning of labor whom they had been gotten involved in stoppage of dilatation of the uterus's orifice in the active. Phase of labor for 2 hours, prescription of oxytocin was led to average 85minute decrease of labor timespan immediately and three hours later [6].

Study of Faraz, *et al.* in 1998 showed that initial interventions with amniotomy and oxytocin are accompanied by negligible decrease in rate of caesarean section [7].

In a study conducted by Marazziti, *et al.* in 2000, it was specified that there is a report showing existence of a positive relationship between plasma level of oxytocin and rate of anxiety in the mothers [8] Premature time of labor has not a certain treatment. None of its controlling methods is effective completely, and each one has potential complications for mother and fetus [9].

Tocolysis sermin has been a prevalent factor of respiratory distress and death in the pregnant women [10]. Controlling factor/blocker of prostaglandin synthase has undesirable effects on the

embryo as well. This case prevents from extensive consumption of these drugs [11]. Considering high outbreak of these factors and lack of ideal treatment in the premature labor, it's appearance can be inhibited by prevention. One of the factors related to labor strongly is oxytocin hormone. Rs35062132 polymorphism is placed in the OXTR gene promoter area, and it appears that it is correlated with change of its expression level. In this study, our objective is to investigate rs35062132 polymorphism in the oxytocin receptor (OXTR) gene and its relationship with premature labor. Perhaps through study of this factor, one background, to prevent from relapse of premature labor, is to be provided.

Studying Method

In this study carried out by case-control method in 2018, 152 women who had necessary conditions of entering into the study and divided into two groups of those with premature labor history (from 22 to 36 weeks whom they referred to the Shahid Rajaei hospital of Tonekabon) and those without premature labor history were studied. Criteria of entering into the study included: Age of pregnancy (from 22 to 36 week), lack of affliction with medical diseases (cardiac, pulmonary and renal diseases as well as increase of blood pressure/hypertension), lack of cigarette addiction or becoming addicted to another special substance and satisfaction with participation in the research. Sampling of this study was conducted in the being-available sampling form. After presentation of some descriptions to the individuals participated in the research and getting their satisfaction regarding how to research and its objectives, the interview form, including individual information such as age of individuals, age at time of pregnancy, age of menstrual period, age of menopause and type of labor was completed. The patients' study checklist was regulated following study of the book and articles and confirmed by a few university professors. Genomic DNA was extracted from environmental blood leukocyte by use of BIO BASIC kit. For the purpose of qualitative study of appropriate DNA extraction, the samples were electrophoresized on the 1% Agarose gel and, for the purpose of quantitative study of the extracted DNA purity, absorption ratio ranged from 260 to 280 was used.

After extraction of the samples' DNA, specific primers were designed by use of Oligo 7 software, and blast was conducted in the on-line form in NCBI site. Sequence of the primers used in this research has been presented in table 1.

Then, duplication of the considered fragments was carried out, using primers, with the aid of PCR technique. PCR plan was

Primer	Sequences	Tm	Pcr product
Forward	ATGTCCAGGACAAGGAGGGA	60.5	560 bp
Reverse	CCAAGGAGGGGAGGGATACA	63.5	560 bp

Table 1: Sequence of the primers used in this research has been presented in table.

Frequency of people's age In two groups with preterm labor history and no history of preterm labor.

conducted as the following form: Initial denaturation in 95°C for 5 minutes; then, 35 cycles of denaturation in 95°C for 30 seconds, connection of primers in 60°C for 30 seconds; Elongation 72°C for 20 seconds and, at the end, final elongation in 72°C for 5 minutes.

Then, for the purpose of successful duplication of the desired fragment, 10 Ml of the PCR product was tested on the 1.5% Agarose gel. Later, in order to study more accurately, PCR product was sequenced. Sequences achieved from determining the sequence were analyzed in the Chromas software.

Findings

In the results of sequence determination, out of total 152 women who possessed the criterion for entering into study, there were 84 ones (55.2%) among which rs35062132 polymorphism existed in their OXTR gene and 99 ones (65.1%) lacked this polymorphism. Of total studied 152 individuals, 76 individuals were those with history of premature labor. Out of this 76 individuals, there were 41 individuals (53.9%) among which rs35062132 polymorphism existed in their OXTR gene, and 35 individuals (46%) out of these 76 individuals lacked this polymorphism, showing a significant relationship between premature labor and rs35062132 polymorphism of the OXTR. In study of 76 individuals lacking premature labor history, 12 individuals (15.7%) had this polymorphism and 64 individuals (84.2%) lacked this polymorphism.

Table 2 shows the information related to age of individuals in the two groups. After DNA extraction and preparation for the performance of PCR process, PCR product, in order to determine sequence and study more accurately, was sent to the Novin-Genetic domestic company for the purpose of sequencing, and results of sequencing were analyzed by Choromas software. Figure 1 shows RS SNP in NCBI. Figure 2 shows rs35062132 OXTR gene and amino acid changes. As you can see, at position 376 the type of mutation is missense. And nucleotide changes in this position cause amino acid

changes. If the nucleotide C changes to G the amino acid of Arginine is converted to glycine and the nucleotide C changes to T the amino acid of arginine is converted to cysteine.

Groups	Age > 18 years	18-35 years	35 years < Age
Preterm labor	22 person	44 person	8 person
Without preterm labor	27 person	41 person	6 person

Table 2: Shows the information related to age of individuals in the two groups.

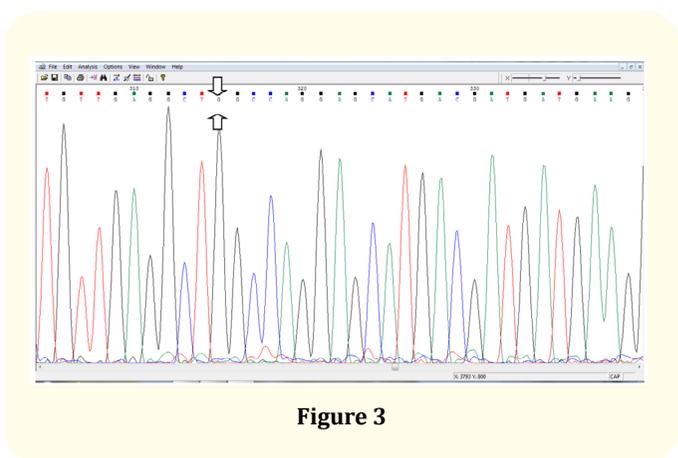


Figure 3

As it is observed in the results of DNA sequencing, nucleotide C has been changed into nucleotide G in this individual with the premature labor history and rs35062132 polymorphism of the OXTR gene is observed in it and the amino acid of Arginine has become Glycine.

Figure 4 shows results of rs35062132 sequence determination of OXTR gene in an individual without premature labor history. In this individual who lacks premature labor history, nucleotide C has not been changed at all, and rs35062132 polymorphism of OXTR gene is not observed in it.



Figure 1: RS SNP in NCBI.

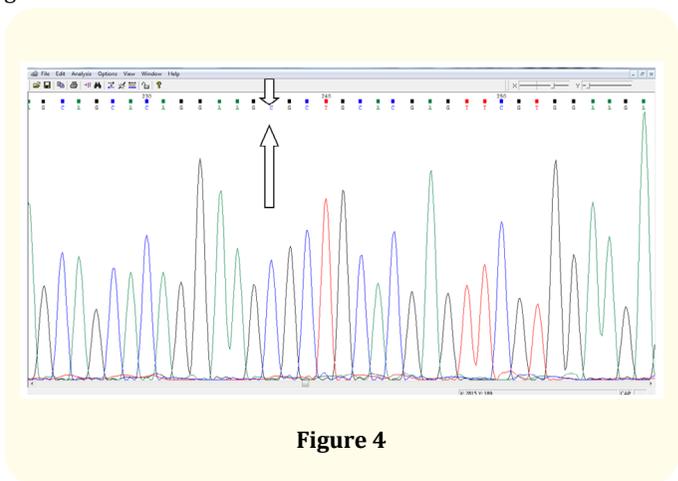


Figure 4

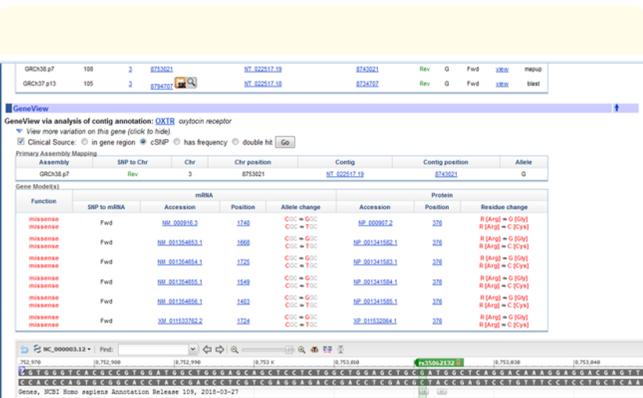


Figure 2: Shows rs35062132 OXTR gene and amino acid changes.

Figure 3 shows results of sequence determination of rs35062132 polymorphism of the OXTR gene in an individual with premature labor history.

Figure 5 indicates results of rs35062132 sequence determination of OXTR gene in an individual with premature labor history.

In this individual who has premature labor history, nucleotide C has been changed into nucleotide T, and rs35062132 polymor-

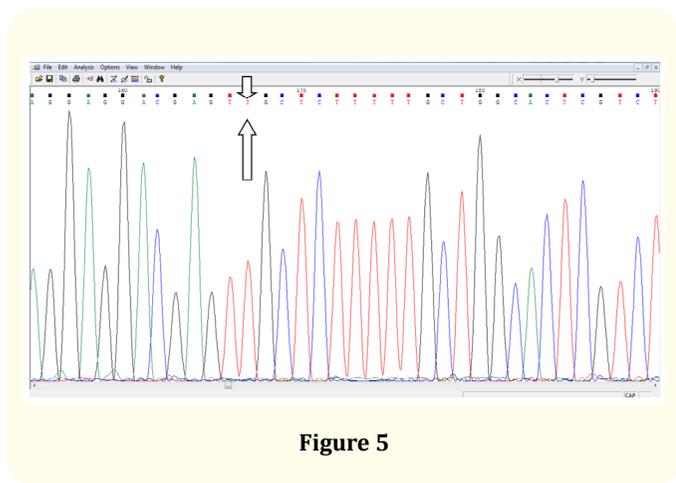


Figure 5

phism of the OXTR gene is observed in this individual and the amino acid of Arginine has become cysteine.

Discussion and Conclusion

Premature labor is the main cause of the affliction, prior-to-labor death and principal factor of undesirable consequences of pregnancy which, despite the entire interventions conducted in this regard, some births have been occurred yet [12]. Also, other studies have shown that, almost, 75% of the death cases occur while the immature neonates are born, and this rate in the neonates born prior to 32th week of pregnancy is higher up to 30% to 40% [13]. In the present study, out of 152 studied individuals, there were 53 individuals (34.8%) among which polymorphism was observed in the results of their sequence determination in order to study rs35062132 polymorphism of oxytocin receptor gene, and this polymorphism was not found in 99 individuals. (65.1%) Of total 76 individuals with the premature labor history, this polymorphism was not found in 41 individuals (53.1%) while investigating their sequence determination, and 35 individuals (46%) lacked this polymorphism. Of 76 individuals without premature labor history, 12 individuals (15.7%) had this polymorphism and 64 individuals (64.2%) lacked it. In a meta-analysis study conducted in 5 continents from 1987 to 2004, 10 studies have investigated work trail and concluded in such a way that oxytocin with high dose for the purpose of labor reinforcement has been accompanied by reduction of rate of caesarean section labors as well as timed decrease of labor without it is accompanied by maternal unwanted consequences [14]. Also, in this research, rate of significance of oxytocin and it's effect on the process of labor has been shown:

There was a significant relationship between the individuals which rs35062132 polymorphism of OXTR exists in them and premature labor. In a study conducted on the comparison of effect of oxytocin dose on induction of labor in Tehran Vali-E-Asr hospital by Nahid Ghanbarzadeh and Mohammad Najafi- Semnani., *et al.* in 2013, it was specified that high dose of oxytocin for induction of labor can be led to decrease of labor time and increase of rate of satisfaction with labor process in patient without leaving undesirable impact on neonate [15]. In the present study, it has been, also, shown that existence of rs35062132 polymorphism in the oxytocin receptor and its untimely secretion accelerate induction of labor. In a study carried out on 200 patients by Jamal., *et al.* in Tehran, it was shown that, in the women who received high dose of oxytocin, time from hospitalization to labor decreases obviously [16].

In 2010, Nachum., *et al.* studied on the stimulation of labor by a combination of aminotomy and oxytocin in the first-labor women with a long hidden phase and term pregnancy. He., *et al.* expressed that usage of oxytocin with a high dose for reinforcement of labor is accompanied by decrease of rate of rate of the Caesarean section and shortening labor time [17]. Also, they expressed that initial usage of oxytocin aiming at reinforcement of labor in the cases of diagnosis of labor dystocia is accompanied by increase of spontaneous labor [14]. In 2008, Masoumi., *et al.* conducted a research on the study of oxytocin's role in rate of anxiety during a natural labor.

This study specified that role of oxytocin hormone in decrease of anxiety rate is a situation whose analysis puts emphasis on importance and useful effects of promotion of natural labor regarding mental health of mother. By stimulation of its own special receptor in the central nervous system, oxytocin hormone will be led to decrease of anxiety and, subsequently, progression and acceleration of labor [18].

Other studies and present study suggest significance of oxytocin in progression of labor. However, occurrence of premature labor is prevalent and plentiful in the society relatively, but it's appearance and intensity can be reduced extensively by preventive and controlling measures. Results of the present study showed that there exists a significant relationship between rs35062132 (R376G) (R376C) polymorphism of OXTR gene with premature labor, and existence of this polymorphism in the OXTR gene can be led to earlier induction of labor compared to it's own natural time. However, more researches and studies in the greater populations and other regions are required.

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Volume 3 Issue 7 July 2021

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