

Maternal blood and amnionic oxytocin receptor gene expression and serum oxytocin levels in preterm birth: a case-control study

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Purpose of investigation: The oxytocin (OXT)-oxytocin receptor (OXTR) system provides a promising candidate gene for studies of genetic contributions to prematurity. The author studies the quantification and comparison of oxytocin receptor (OXTR) gene expression and serum OXT levels in the blood and amnion of women delivering preterm and evaluation of the correlation between OXTR gene expression in blood and amnion with serum OXT levels in them. **Material and methods:** Seventy pregnant women in spontaneous labor delivering vaginally preterm i.e., < 37 weeks and an equal number of matched controls delivering spontaneously at term (37–42 weeks) were recruited. Maternal serum OXT levels were quantified by ELISA collected in the active stage of labor i.e., 4 cm cervical dilatation. Gene expression studies in the maternal blood and amnion were done by using real-time quantitative polymerase chain reaction (RT-qPCR). **Results:** The mean serum OXT level in preterm labor (PTL) was 48.56 ± 6.97 pg/mL; significantly higher than in controls (43.00 ± 3.96 pg/mL), $P < 0.001$. OXTR gene expression in maternal blood (2.5 times) as well as in amnion (3.5 times) was significantly higher in PTL. A significant positive correlation was observed between serum OXT levels and OXTR gene expression in amnion ($r = -0.190$, $P = 0.025$). **Conclusions:** The serum OXT levels and OXTR gene expression in amnion surge significantly in the active phase of PTL. Thus, amnion probably links OXT-PTGs (prostaglandins) autocrine paracrine circuit to facilitate PTL. Future studies are needed to devise better OXTR receptor antagonists preferably acting on amnionic OXTRs to prevent inflammatory pathways leading to PTL.

Keywords

Preterm birth; Preterm labor; Oxytocin; Oxytocin receptor; Placenta; Amnion

1. Introduction

An estimated 15 million babies are born too early every year i.e., 1 in 10 babies. Approximately 1 million children die each year due to complications of preterm birth (PTB) [1]. Globally, prematurity is the leading cause of death in children under the age of 5 years [1]. Many survivors face a lifetime of disability, including learning disabilities, visual

and hearing problems. PTB rates are increasing despite advancing knowledge of risk factors for preterm labor (PTL) and the introduction of public health and medical interventions [2–6].

Key treatments for PTL have focused on the prevention or inhibition of myometrial contractions, mainly to provide time to administer steroids to aid fetal lung maturation and transfer to a special neonatal care unit [7, 8].

Understanding more about the mechanisms of labor is essential to identify targets for novel and more effective therapies to stop or prevent PTL. The neurohypophysial hormone oxytocin (OXT) is named after the “quick birth” which it causes due to its uterotonic activity [9]. Oxytocin receptor (OXTR) gene expression is present in humans in the amnion, chorion, and decidua [10] OXT binding to OXTR significantly increases in human fetal membranes specially amnion with the onset of labor. Terzidou *et al.* [11] revealed that human OXTR expression increases spontaneously in post-labor amnion epithelial cells and that treatment with interleukin (IL) 1B stimulates OXTR expression in pre-labor amnion when cultured. Amnion is not a contractile tissue; therefore, the physiological role of the OXT/OXTR system in amnion must be in some respect different from its role in the myometrium. The amnion plays an important role in the onset of human labor. It is a major source of prostaglandins (PTGs) and inflammatory cytokines synthesis, which increase both before and during labor. Terzidou *et al.* [11] found that the release of PTGE2 by human amnion epithelial cells is significantly increased after OXT stimulation. This supports a complementary role for OXT/OXTR in the activation of the amnion that occurs at the time of labor. It is established that the PTGS2 (prostaglandin synthetase 2) enzyme, which mediates the committing and rate-limited step of PTG biosynthesis, generating a PTGH2 intermediate that is converted to the terminal PTGs, is central to increased PTGs synthesis in the human amnion at the time of labor [12–14].

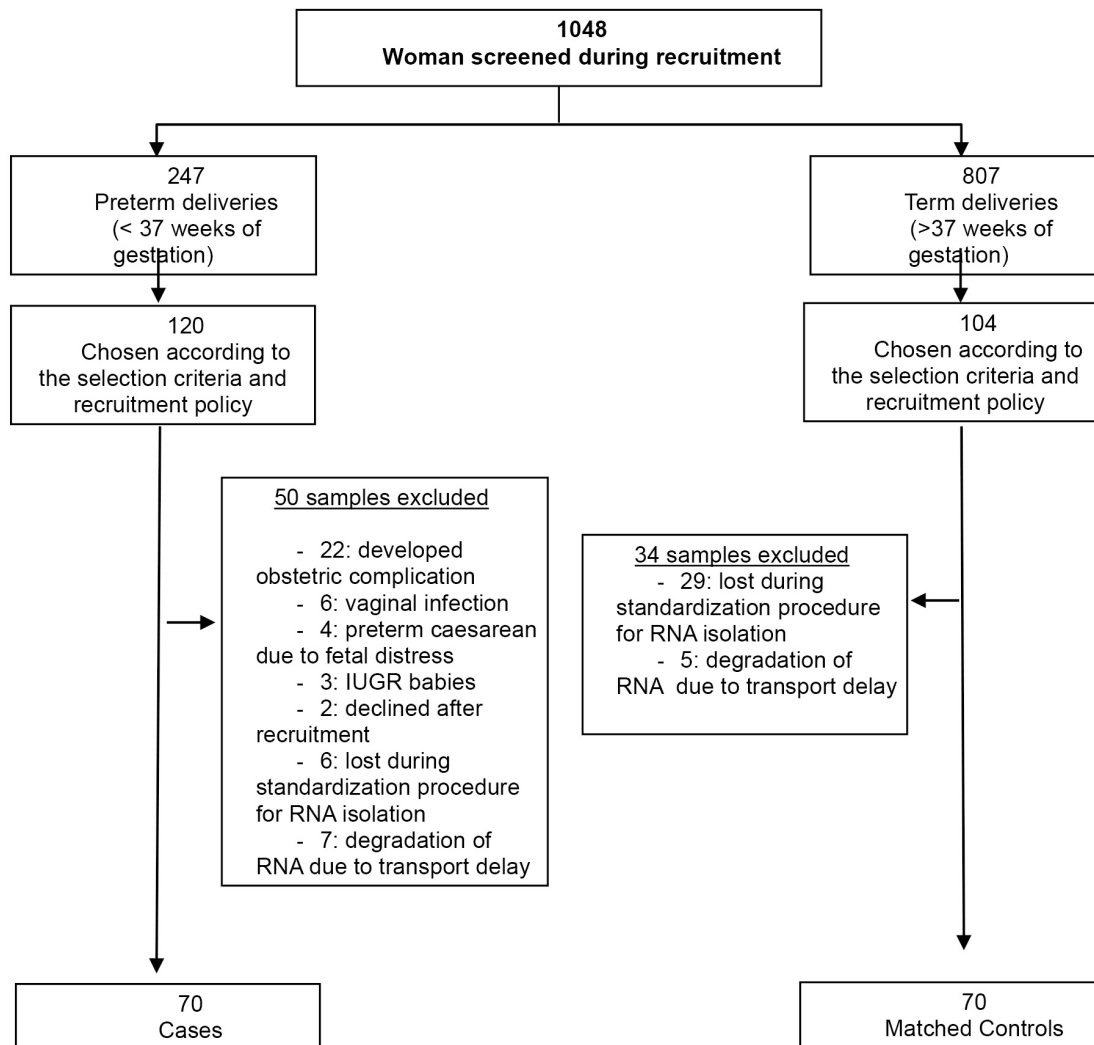


Fig. 1. Flow chart participant recruitment in the study.

OXT stimulates myometrial contractions through multiple signaling pathways. Binding of OXT to its receptor has been known to lead to G-protein coupling and subsequently, an increase in intracellular calcium levels to mediate the generation of force [15]. There is a role for the OXT-OXTR system in the onset of human labor, additional to the stimulation of myometrial contractions, that involves an increase in the expression of cyclooxygenase-2 (COX-2) and other inflammatory mediators known to be associated with the onset of labor [16, 17].

Kim *et al.* [16] in their study demonstrated that NF- κ B activation is required for OXT-induced COX-2 expression in human myometrium and amnion. They also showed that Cross-talk exists between the OXT induced activation of Mitogen-Activated Protein Kinases (MAPKs) and NF- κ B signaling cascades in the human amnion, but not myometrium. OXT-OXTR interaction leads to NF- κ B activation and subsequent upregulation of PTGs, inflammatory chemokines, and cytokines that are known to play roles in fe-

tal membrane remodeling, cervical ripening, and myometrial activation. Amnion is an important site of PTG production within the human uterus and its activation is critical for fetal membrane remodeling, cervical ripening, and the stimulation of myometrial contraction [16].

Therefore, OXTR is commonly used as a target for the development of tocolytics. Atosiban, an OXTR antagonist acting on both myometrial & decidual OXTRs has been used to arrest premature uterine contraction but has failed to reduce the incidence of PTB or improve the neonatal outcome compared with placebo [18].

The role of OXT in PTG pathway activation leading to the onset of labor suggests that potential clinical use of OXTR antagonists requires re-evaluation. The study of molecular genetics involving the OXT-OXTR system in gestational tissues may prove fruitful in deciphering these complex mechanisms leading to PTB. The present study gains insight into the OXT-OXTR system in PTB to build the ground for future research for better-targeted interventions.

2. Materials and methods

2.1 Sample size

To detect a mean change of 0.5 $\mu\text{U/mL}$ oxytocin levels between preterm labor and term labor controls in the active phase of labor, the sample size of 68 delivering preterm (cases) and 68 low-risk pregnant females delivering at term (controls) was sufficient for 80% power of the study and 5% level of significance with SD of oxytocin level in the active phase of term labor taken as 1.00 [19]. We enrolled 70 women having preterm birth and 70 women with term delivery in the present study (Fig. 1).

2.2 Subjects

In this case-control study, one hundred forty study subjects ($n = 140$) in spontaneous labor were recruited at University College of Medical Sciences (UCMS) & Guru Teg Bahadur Hospital, Delhi, India from November 2014 to April 2016 (Fig. 1). This study has been designed according to the STROBE Statement. All the participants gave informed written consent for participation in the study. They also gave consent for public dissemination and future use of the data generated from the study for the welfare of mankind and research purposes. Ethical approval was taken from the Institutional Ethics Committee for Human Research (IEC-HR), UCMS & GTB hospital, University of Delhi, India. Seventy women aged 18 to 35 years with BMI 19 to 26 kg/m^2 in spontaneous PTL with intact membranes who delivered vaginally giving birth to appropriate for gestational age neonates were recruited as cases. Women both in preterm and term labor did not receive exogenous oxytocin infusion for labor augmentation during the course of labor till delivery. Women having a history of one or more spontaneous PTB or late second-trimester spontaneous abortion or cervical cerclage were excluded from the study. Women with a multiple pregnancy, fetal growth restriction, stillbirth, anemia, diabetes, hypertension, chronic diseases, urinary tract infections, chorioamnionitis, recent intake of anti-inflammatory drugs/steroids, history of smoking or any complications during pregnancy and/or in labor were excluded from the study groups. For each case, an age, weight and parity matched, low-risk women ($n = 70$) in spontaneous labor delivering vaginally at term (37–42 weeks) appropriate for gestational age neonate were recruited as controls.

2.3 Sample collection and storage

Maternal blood (2 mL) was collected in an EDTA vial during the active phase of labor (≥ 4 cm cervical dilatation) and 250 μL of it was fixed in 750 μL of TRIzol LS (Ambion, USA) reagent and stored at -80°C . Maternal serum (2 mL) was separated by centrifugation and stored at -20°C . Serum OXT levels were estimated by ELISA using OXT Enzyme Immune Assay Kit (K048-H1) by Arbor Assays (Ann Arbor, Michigan, US) with declared sensitivity as 17.0 pg/mL with a limit of detection as 22.9 pg/mL as per manufacturer's protocol.

Amnion was carefully separated from chorion just after delivery of the placenta. A $5 \times 5 \text{ cm}^2$ of amnion was col-

lected, cut into small strips, and washed in sterile phosphate-buffered saline (PBS). 0.1 gm of homogenized amnion was fixed in 1 mL of TRIzol reagent & stored at -80°C . Ribonucleic acid (RNA) was isolated from maternal blood & amnionic tissue and used for complimentary deoxy nucleic acid (cDNA) synthesis using Maxima first-strand cDNA synthesis kit (Fermentas, USA). It was used to study OXTR gene expression in maternal blood and amnionic tissue by Real-Time quantitative polymerase chain reaction (RT-qPCR) using CFX Connect (Biorad, USA) RT-qPCR machine, and corresponding ΔCq (Quantification cycle) values were determined by using gene-specific primers. The primers sequences used for the amplification of human OXTR and β -actin genes were following: forward 5'ACT TTA GGT TCG CCT GCG G 3' and reverse 5'CTC CTC TGA GCC ACT GCA AA3' (Primer for OXTR, amplicon size-126 bp) and forward 5'TCA CAA GGA TTC CTA TGT GG3'5'CTC ATT GTA GAA GGT GTG G 3' (Primer for β -actin, amplicon size-136 bp).

The qPCR amplification master mix for a sample of a gene was made, 20 μL which contained 1 μL of cDNA, 10 μL SSO Fast Evagreen SuperMix, 1 μL each of forward and reverse specific primer pairs (conc. 10 $\text{pmol}/\mu\text{L}$), 7 μL of nuclease-free water. All reactions were set in duplicate along with no template control. The PCR was carried out for initial denaturation (95°C for 5 min) followed by 40 cycles consisting of template denaturation (10 sec at 94°C), primer annealing, and extension (40 sec at 60°C) and run under appropriate cycling conditions.

In the initial cycles of PCR, there is a little change in fluorescence signal (produced from double-stranded DNA). This defines the baseline for the amplification plot. An increase in fluorescence above the baseline indicates the detection of the accumulated target. The parameter Cq is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. Cq levels are inversely proportional to the amount of target nucleic acid in the sample i.e., lower the value of Cq, the higher the amount of target nucleic acid in the sample. Expression normalization was done by β -actin gene to correct for the sample to sample variations in qPCR efficiency and errors in sample quantification. ΔCq was evaluated which is the difference between average Cq of the target gene (OXTR gene) and reference gene (β -actin gene).

$$\Delta\text{Cq} = \text{Average Cq}_{\text{OXTR}} - \text{Average Cq}_{\beta\text{-actin gene}}$$
Again, the difference between mean Cq values of control and cases was determined, which is $\Delta\Delta\text{Cq}$.

$$\Delta\Delta\text{Cq} = \Delta\text{Cq}_{\text{case}} - \Delta\text{Cq}_{\text{control}}$$

After this, true Fold change (FC) was represented to compare the expression of genes between cases and controls by the following formula: $\text{FC} = 2^{-\Delta\Delta\text{Cq}}$.

2.4 Statistical analysis

Microsoft Excel (version 2007) and statistical software SPSS for windows (version 17.0) was used for data presentation and statistical analysis. Unpaired Student's *t*-test and Chi-square/Fisher's exact test was applied to compare

Table 1. Serum oxytocin levels, Δ Cq OXTR in maternal blood and amnion in the two study groups (cases and controls) and two subgroups (early and late preterm).

Parameter	Serum Oxytocin levels (pg/mL)	OXTR Δ Cq (maternal blood)	OXTR Δ Cq (Amnion)
	(mean \pm SD)	(mean \pm SD)	(mean \pm SD)
Group 1 Cases (preterm); n = 70	48.56 \pm 6.97	6.40 \pm 1.42	10.88 \pm 1.38
Group 2 Controls (term); n = 70	43.00 \pm 3.96	7.69 \pm 1.47	12.66 \pm 2.17
P-value	< 0.001	< 0.001	< 0.001
Early preterm (< 34 weeks); n = 37	48.79 \pm 6.68	6.26 \pm 1.38	10.702 \pm 1.46
Late preterm (34–36 ⁺⁶ weeks); n = 33	48.29 \pm 7.38	6.55 \pm 1.48	11.088 \pm 1.28
P-value	0.764	0.404	0.249

*P value < 0.05 is significant.

all socio-demographic characteristics, clinical profile, OXTR gene expression in cases and controls. Pearson coefficient of correlation was used for various correlation studies.

3. Results

Various socio-demographic features in the two groups were comparable. The mean serum OXT level in active labor in PTB cases was 48.56 \pm 6.2 pg/mL which was significantly higher ($P < 0.001$) than those with term labor (43.00 \pm 3.96 pg/mL) (Table 1). In the subgroup analysis of early v/s late preterm, the serum OXT levels continued to be higher in preterm than term but there was no statistically significant difference. The values of OXTR Δ Cq in maternal blood of cases and controls are compared in Table 1.

The maternal blood OXTR Δ Cq value in preterm cases was significantly lower (i.e., expression of OXTR gene in maternal blood was significantly higher) in comparison to controls ($P < 0.001$). Also, the Fold change (FC) was > 2.44 which means its expression was 2.44 times in PTL than that in term labor (Fig. 2). OXTR gene expression in amnion was significantly higher in PTL (FC > 3.44) as compared to term labor ($P < 0.001$) (Fig. 2). However, the value of OXTR Δ Cq in maternal blood as well in amnion was not statistically significantly different in early and late preterm subgroups.

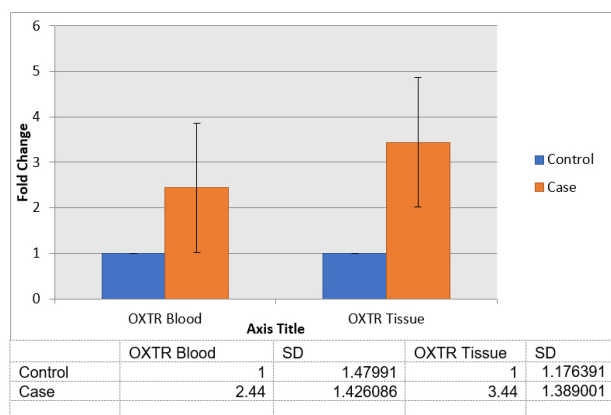


Fig. 2. OXTR gene expression in maternal blood and Amnion expressed in terms of Fold Change.

In correlation studies, a negative correlation was observed between maternal serum OXT levels and OXTR Δ Cq in maternal blood (i.e., positive correlation between maternal serum OXT levels and OXTR gene expression in maternal blood) in PTL (Table 2). Similarly, a negative correlation was seen between maternal serum OXT levels and OXTR Δ Cq in amnion in PTL (i.e., positive correlation between maternal serum OXT levels and OXTR gene expression in amnion) (Table 2).

A negative correlation was observed between maternal serum OXT levels and amnionic OXTR Δ Cq (i.e., positive correlation between maternal serum OXT levels and amnionic OXTRs) when all the study subjects (preterm & term) were combined into a single group (Table 3 and Fig. 3). There was a significant proportionate rise in maternal serum OXT levels with increased OXTR gene expression in amnion ($P < 0.025$) in all laboring subjects irrespective of the period of gestation [POG].

Similarly, a negative correlation was seen between maternal serum OXT levels with the period of gestation, birth weight, and placental weight in the whole study population (Table 3, Fig. 4 and Fig. 5). So, as maternal serum OXT levels increased; there is a corresponding significant reduction in POG, birth weight, placental weight.

4. Discussion

The mean serum OXT level in PTB cases was 48.56 \pm 6.97 pg/mL; significantly higher than in controls (43.00 \pm 3.96 pg/mL), $P < 0.001$ similar to earlier studies. OXTR gene expression in maternal blood was significantly higher (2.44 folds) in the PTB cases as compared to term controls ($P < 0.001$). There is also an increase in OXTR mRNA in human amniotic cells up to 3.44 folds in preterm. OXTR mRNA concentrations increased significantly after the initiation of the active phase of labor both in blood and amnion which was significant. Chibbar *et al.* [20] compared prelabour and post-labor tissues which revealed OXT mRNA levels were three to fourfold higher after the spontaneous labor. The second change is that the cervical connective tissue and smooth muscle must be capable of dilatation to allow the passage of the fetus from the uterus. These changes are accompanied by a shift from progesterone to estrogen dominance, in-

Table 2. Correlation between maternal serum oxytocin levels with OXTR gene expression in maternal blood and amnion and with period of gestation (POG), birth weight (B.W), and placental weight in preterm birth cases (n = 70).

Parameter		Maternal Serum OXT levels	OXTR blood Δ Cq	OXTR Amnion Δ Cq	POG	B.WT. (Kg)	Placenta WT (gms)
Maternal serum OXT levels	r	1	-0.074	0.027	-0.008	0.063	0.027
	P	-	0.544	0.824	0.945	0.605	0.827
OXTR blood Δ Cq	r	-0.074	1	-0.116	0.048	-0.130	-0.145
	P	0.544	-	0.338	0.695	0.282	0.231
OXTR Amnion Δ Cq	r	0.027	-0.116	1	0.074	0.001	-0.022
	P	0.824	0.338	-	0.543	0.995	0.857

*P value < 0.05 is significant, r = Pearson correlation coefficient.

Table 3. Correlation between maternal serum oxytocin levels with OXTR gene expression in maternal blood and amnion and with period of gestation (POG), birth weight (B.W), and placental weight in preterm birth cases & term controls combined (n = 140).

Parameter		Maternal Serum OXT levels	OXTR blood Δ Cq	OXTR Amnion Δ Cq	POG	B.WT. (Kg)	Placenta WT (gms)
Maternal serum OXT levels	r	1	-0.078	-0.190	-0.387	-0.304	-0.312
	P	-	0.361	0.025	< 0.001	< 0.001	< 0.001
OXTR blood Δ Cq	r	-0.078	1	0.113	0.365	0.316	0.340
	P	0.361	-	0.183	< 0.001	< 0.001	< 0.001
OXTR Amnion Δ Cq	r	-0.190	0.113	1	0.418	0.371	0.374
	P	0.025	0.183	-	< 0.001	< 0.001	< 0.001

*P value < 0.05 is significant, r = Pearson correlation coefficient.

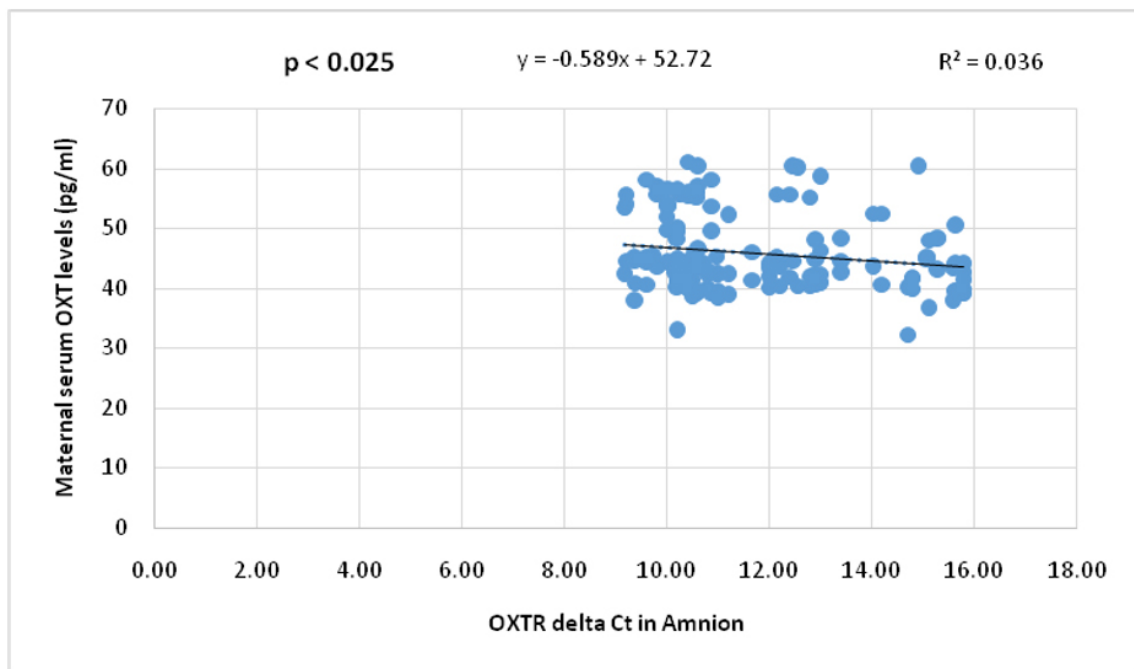


Fig. 3. Scatter dot plot graph showing a negative correlation between OXTR Δ Cq amnion and maternal serum OXT levels in the whole study population.

creased responsiveness to oxytocin through up-regulation of the myometrial oxytocin receptor, increased PTG synthesis in the uterus, increased myometrial gap junction formation, decreased nitric oxide (NO) activity, and an increased influx of calcium into myocytes [21, 22] with ATP dependent binding of myosin to actin [23], increased endothelin leading to augmented uterine blood flow and myometrial activity [24].

The local increase in the estrogen/progesterone ratio in late pregnancy may activate OXT gene transcription and increase the number of OXTRs in the myometrium and other gestational tissues. The latter may sensitize the myometrium to any local OXT, thereby leading to increased contractile activity and production of stimulatory PTGs. The increased PTGs would directly stimulate the myometrium and poten-

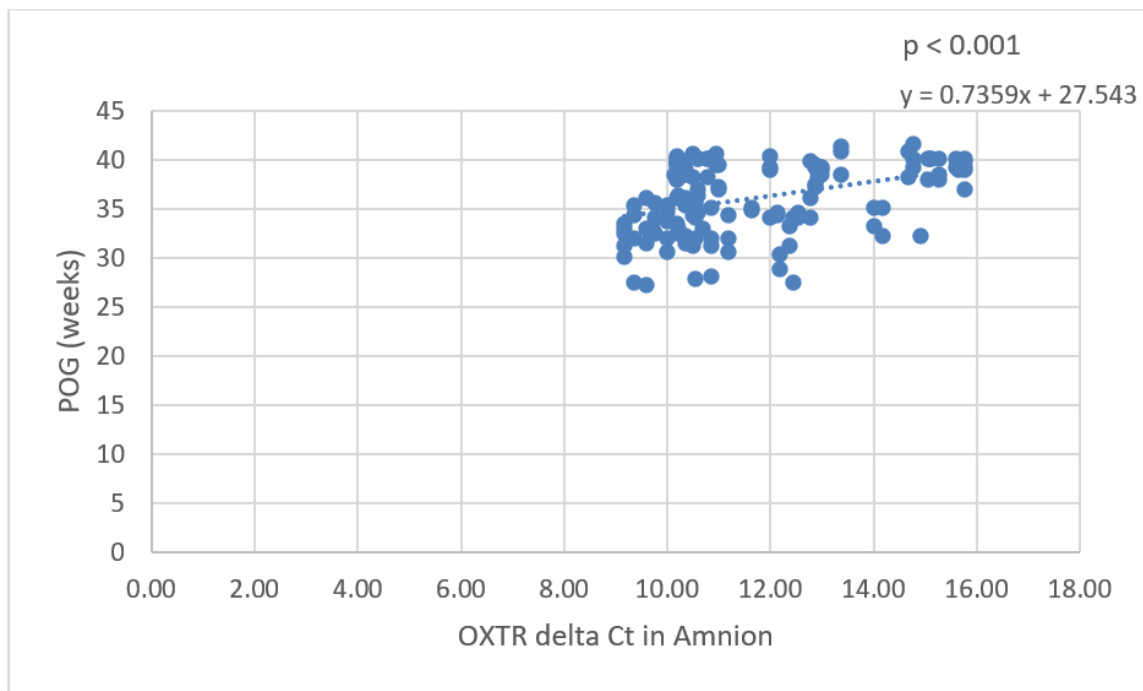


Fig. 4. Scatter dot plot graph showing a positive correlation between OXTR delta Ct in Amnion and period of gestation in preterm & term subjects combined.

tially lead to a further increase in the production of OXT and its receptor. OXT binding to OXTR significantly increases in human fetal membranes specially amnion with the onset of labor [25]. Studies in several species have shown OXT to stimulate uterine prostaglandin (PTG) synthesis when administered near term. Amnion is a major site of PTG production in human pregnancy and its activation is critical for cervical ripening and the stimulation of myometrial contractions. Just prior to the onset of labor, there is an increase in inflammatory cytokine release from the amnion [26, 27] as well as increased PTG synthesis, particularly PTGE2 [28, 29]. Collectively these changes promote cervical ripening, lower uterine segment remodeling, and initiation of myometrial contractions [29–33].

This suggests a role for OXT/OXTR in the activation of the amnion that occurs at the time of labor. Present study results have confirmed the findings of previous studies where OXTR mRNA or OXT binding sites were identified in Amnion [11]. Our data have extended these findings by systematic determination of gestation and labor-associated effects on OXTR mRNA expression both in maternal blood and amniotic tissue samples. There was a significant effect of the labor process, with both blood and amnion OXTR mRNA concentrations increasing after the initiation of the active phase of labor. It indicates upregulation of OXTR concentrations seen at parturition is primarily regulated at the transcriptional level. This supports previous data from binding studies [25] and Northern blot analyses [34]. This coordinated interaction involving OXT, OXTRs, and PTGs in human fe-

tal membranes could ultimately result in the onset of parturition. OXTRs are present in blood & amnion and show major regulatory changes at the onset of labor whether at term or preterm with increased expression in PTB cases.

Terzidou *et al.* [11] showed the increased synthesis of PTGs in human amnion between 2 and 6 hours following OXT treatment and was associated with increased prostaglandin synthetase 2 (PTGS2) expression. The increased ability of human amnion to produce PTGE2 in response to OXT treatment suggests a complementary role of the OXT/OXTR system in the activation of human amnion and the onset of labor. This suggests OXT-PTGs autocrine paracrine circuit system in decidua, amnion, and myometrium induce and facilitate labor *in situ*. It makes sense that plasma OXT levels do not alter before the onset of labor if this system is mainly regulated within decidua and fetal membranes *in situ* [20].

Terzidou V *et al.* [11], in their study, revealed that human OXTR expression increases in post-labor amnion epithelial cells and that treatment with interleukin IL1B stimulates OXTR expression before the onset of labor. The increased ability of human amnion to produce PTGE in response to OXT treatment suggests a complementary role of the OXT/OXTR system in the activation of the PTG pathway in the human amnion resulting in the onset of labor. In addition to mediating contractions, the role of OXT in biochemical processes i.e., PTG pathway that leads to the onset of labor suggests that the potential clinical use of OXTR antagonists requires re-evaluation. Szukiewicz *et al.* [35] concluded that

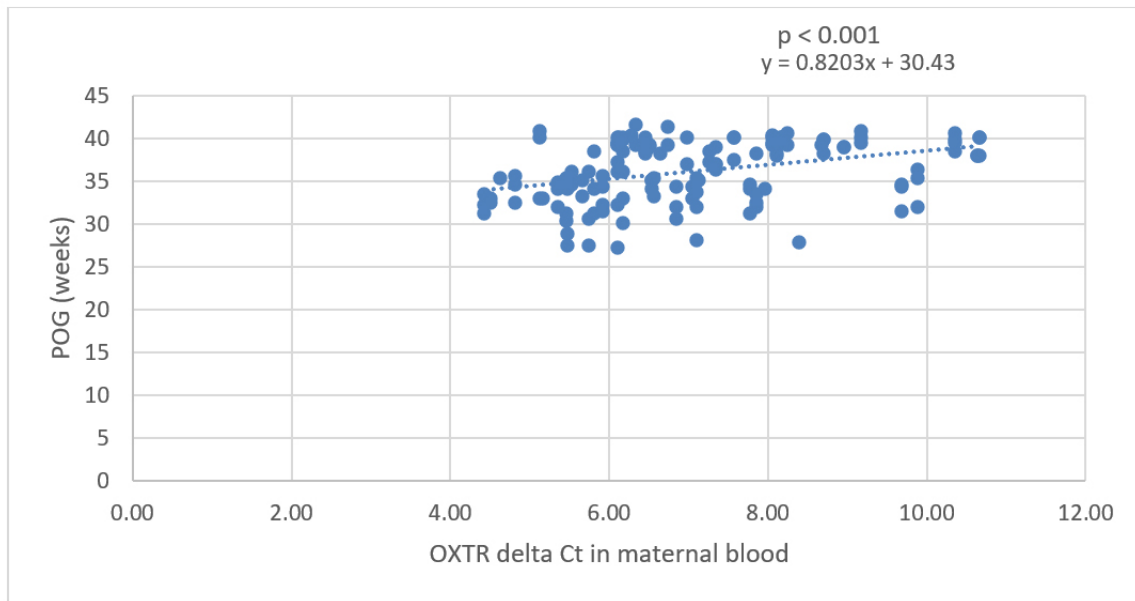


Fig. 5. Scatter dot plot graph showing a positive correlation between OXTR delta Ct in maternal blood and period of gestation in preterm & term subjects combined.

upregulation of OXTR within placental trophoblast cells localized close or adherent to the uterine wall may play a crucial role in labor with efficient contractile activity leading to vaginal delivery. The present study further supports the concept that OXTR expression is regulated in a paracrine as well as endocrine fashion and OXTR expression is a limiting factor of parturition, which was proposed in the previous reports [25, 36]. They also explain how OXT may play a pivotal role in the onset of labor in the absence of significant changes in maternal plasma concentration.

Kim *et al.* [37] showed that OXT increases the expression of COX-2 and other inflammatory mediators known to be associated with the onset of labor in both the myometrium and amnion via activation of NF- κ B and MAPKs. OXT-OXTR interaction leads to Nuclear factor (NF- κ B) activation and subsequent upregulation of PTGs, inflammatory chemokines, and cytokines that are known to play a key role in fetal membrane remodeling, cervical ripening, and myometrial activation. It is well established that IL1B concentrations within the uterus increase at the time of both term and PTL, as does the expression of several “labor-associated proteins”, such as PTGS2 and IL8, each of which is up-regulated by IL1B [12, 38–41]. OXTR expression is also up-regulated at term, at a time when IL1B concentrations are high, and PTGS2 and IL8 expression increases. Increased mRNA levels encoding OXTR and other uterine activation proteins such as COX-2 or PTGF2 α receptor in decidua after the onset of labor were observed in the uterine tissues during term and PTL [42], suggesting that the basic regulation for uterine activation was similar between term and PTL.

Loudon *et al.* [39] have previously shown that PTGE2 production is lower in prelabour than in post-labor cells and

that PTGE2 production in prelabour cells can be stimulated by IL1B to levels similar to that found in post-labor cells. This shows that the pathways for PTG synthesis are activated in post-labor cells, and our present study reinforces that upregulation of OXTR is a feature of amnion activation and is sensitive to inflammatory cytokines.

Szukiewicz D *et al.* [35] in their recent study concluded that upregulation of OXTR within placental trophoblast cells localized close or adherent to the uterine wall may play a crucial role in labor with efficient contractile activity leading to vaginal delivery.

There is some evidence for a premature activation of OXT secretion in PTB, suggesting a pathogenic role for it in PTL. Our study reinforces this fact as there is a significant rise in OXT levels in PTB cases with *P* value of 0.001.

OXTR antagonists are one class of treatments that have been developed as tocolytic agents for women in PTL. Kim SH *et al.* [37], in their study, found that Atosiban, a mixed OTR/V1a antagonist widely used as a tocolytic drug, acts as a biased ligand in amnion cells. It fails to inhibit the activation of pro-inflammatory mediators stimulated by oxytocin, and, in the absence of OXT, it activates the same pro-labor inflammatory pathways in amnion in a way similar to OXT and increases cytokine/chemokine and PTG secretion, which may have detrimental effects upon the fetus also in PTL at early gestational ages.

Amnion is a major site of prostaglandin and proinflammatory cytokine and chemokine release leading to cervical ripening and fetal membrane remodeling [16]. Persistent activation of pro-inflammatory pathways in human amnion including activation of NF- κ B, appears to precede the onset of labor. Atosiban has a low affinity for the OXTR and its an-

tagonistic activities at the $V_{1a}R$ [37], has ignited interest in the development of other peptide and nonpeptide antagonists with greater specificity for the OXTR. It appears from the present study that a molecule with targeted intervention at amniotic OXTR gene may be a promising drug prospect to prevent inflammatory pathway cascade leading to PTL. The significant reduction in gestational length (PTL) with over-expression of the OXTR gene in amnion & blood could be used as a landmark for future studies on formulating preventive strategies for preterm birth.

5. Conclusions

Significantly higher maternal serum OXT levels seen in PTB cases suggest that the basic regulation for uterine activation was similar between term and PTL. The OXTR expression in blood & amnion is 2.44 folds & 3.44 folds higher respectively in women having PTB. Upregulation of OXTR expression in amnion seems to be crucial in the mechanisms involved in PTL leading to contractile activity and PTB. Amnion thus may be acting as a major site of prostaglandins production on stimulation of OXTRs and linking OXT-PTGs autocrine paracrine circuit system to facilitate PTL. This gives a new insight into the OXT/OXTR system in human parturition involving amnion and suggests that its therapeutic modulation could be a strategy for regulating both contractile and inflammatory pathways in the clinical context of PTB.

Future studies may be planned to unveil these local OXT/OXTR signaling to devise better OXTR antagonists preferably targeting amniotic OXTR receptors to prevent PTB.

Author contributions

All authors were involved in designing the research study. All authors contributed to the performance of the experimental work and analysis of data generated. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the participants gave informed written consent for participation in the study and also for public dissemination and future use of the data generated from the study for the welfare of mankind and research purposes. Ethical approval was taken from the Institutional Ethics Committee for Human Research (IEC-HR-3472), UCMS & GTB hospital, University of Delhi, India.

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Conflict of interest

The authors declare no conflict of interest.

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