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Original Article

OXTR Gene Polymorphism and Plasma Oxytocin Levels for Predicting Chronic Pain in Caesarean Delivery

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ABSTRACT

Background: To explore the role of oxytoc in receptor gene (OXTR gene) polymorphism and plasma oxytocin levels in predicting the incidence of chronic persistent post-surgical pain (CPSP) following caesarean delivery (CD).

Methods: This observational follow-up study was conducted following IEC-H approval, prospective CTRI registration and written informed consent from each participant.We included obstetric patients of ASA grade II or III undergoing CD under either SAB or GA and excluded if had chronic pain, neurological disorders or cognitive dysfunction. A base line venous blood sample for plasma oxytoc in levels and OXTR genepolymorphism study was withdrawn and conducted as per the standard protocol. The pain severity and neuropathic component were assessed at 6, 10 and 14th week, postoperatively. Statistical tests like unpaired student-test, Fisher-exact test, correlation tests and binary logistic regression were used.

Results: A total of 50 patients were included and24% (n=12) of them had CPSP. The risk score of alleles with respect to CPSP shows that T allele has OR of 0.43 (95%CI= 0.08-2.2, P value-0.3) and C allele has OR of 1.06 (95% CI=0.11-11.2, P-value 0.96).

A significant correlation between mean oxytocin levels and NRS at 6th (R-0.34, p-value: 0.015) and 10th week (R-0.26, p-value: 0.067) was observed; however, not at 14th week. The mean oxytocin level is significantly raised in patients undergoing emergency CD vs elective CD i.e. 2.22 ± 1.17 vs 1.02 ± 1.00; p-value=0.013. Amongst patients undergoing emergency CD, a significant negative correlation between oxytocin levels and NRS-R atall time points i.e.6th, 10th and 14th weeks and NRS-M at 6th week only were observed

Conclusion: We observed the protective effect of T allele of OXTR rs53576 and the antinociceptive effect of endogenous oxytocinin the pathogenesis of CPSP following CD.

Key Words: *Caesarean delivery, chronic persistent post-surgical pain, oxytocin hormone, OXTR gene polymorphism*

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INTRODUCTION

The chronic persistent postsurgical pain (CPSP) in obstetric population is lower than non-obstetric population i.e. 10% at 8 weeks [1] vis-à-vis 10-50% [2], respectively.

Oxytocin (OXT), an endogenous hormone and neurotransmitter has been implicated to be the potential cause of the comparative low incidence of CPSP following CD when compared to other surgeries.[3,4,5] In numerous animal models,

oxytocin has been shown to possess antinociceptive effects.[4,5]In an only human study evaluating the role of plasma oxytocin in the pathogenesis of CPSP, an inverse relationship between post-caesarean pain severity and oxytocin levels was observed.[6]

The oxytocin receptor (OXTR), is a protein which functions as a receptor for the oxytocin hormone. Oxytocin activities are accomplished through oxytocin receptor (OXTR), a G -protein coupled receptor present in neural tissue, uterus, and breast.The mRNA polymorphism of OXTR gene is associated with circulating oxytocin levels, altered brain activity, and parenting behaviours.[7]OXTR gene is responsible for production of OXTR receptor which is required for the functioning of oxytocin hormone during the peripartum period. [8, 9]Genetic variants, including single nucleotide polymorphisms (SNPs) in the OXTR have been shown to independently influence gene expression. [10]Recently, along with the oxytocin levels, the polymorphism of OXTR gene is also implicated in the pathogenesis of neuropathic pain in various animal models[11,12]

On PubMed and MEDLINE search, we could not retrieve any human study exploring the role of oxytocin levels and OXTR gene polymorphism on the incidence of CPSP following CD.We hypothesized a positive inverse correlation between mean oxytocin levels &numeric rating pain score (NRS-pain) score and presence of specific genomic allele of OXTR gene in patients developing CPSP,thus implicating their potential roles in the pathogenesis of CPSP following CD.

Therefore, we undertake the present study with the aim to explore the role of OXTR genes polymorphism and plasma oxytocin levels in the pathogenesis of CPSP following CD and thus their potential role in predicting the patients who are predisposed to develop CPSP in future.The primary outcome was the distribution&correlation of OXTR gene polymorphism with CPSP. The relationship of plasma oxytocin levels with pain severity formed the secondary exploratory analysis.

METHODS

The study was undertaken following approval from the Institutional ethics committee - Human research, UCMS, Delhi, India. The trial was prospectively registered at the Clinical Trial Registry of India(CTRI) (Ref/2019/12/029867) and was approved on 16/01/2020. A written informed consent was obtained from each participant. Patients of ASA grade I or II undergoing elective lower segment caesarean section with pfannenstiel incision were included between the period 15.07.21 till 31.07.21 following resumption of routine electivesurgeries after the second wave of COVID pandemic in the capital of Delhi, India. Patients were excluded if had chronic pain of any etiology, cognitive dysfunction or inability to comprehend various questionnaires, chronic neurological disorders/substance abuse or body mass index (BMI) > 40 kg/m^2.All patients were assessed for their predisposition to development of chronic pain by assessing them for generalized pain syndrome like headache, low back pain, musculoskeletal pain, tendency for exaggerated pain at other than operated site.

Standard anesthetic techniques for subarachnoid block (SAB)&general anesthesia (GA)were adopted in all patients undergoing CD. In SAB group, injbupivacaine 1.8-2.2 ml was administered intrathecally. In SAB group, patients with height <150 cm and >150 cm, received 2.0 ml and 2.2 ml of inj. hyperbaric bupivacaine hydrochloride (5mg/ml) intrathecally, respectively.

Baseline venous blood sample for estimation of plasma oxytocin levels and OXTR gene polymorphism study was withdrawn.Soon after baby delivery 1 U bolus oxytocin was administered followed by oxytocin infusion @ 2.5-7.5U/hr was started . [13]The surgical incision was infiltrated using 20 ml of 0.375% ropivacaine in all the patients. Following surgery, inj. paracetamol 1 gm was administered 6 hourly for first 24 hours and then 12 hourly, the next day. The breakthrough pain in between was treated using inj. diclofenac 75 mg intravenously. If not relieved, inj. tramadol 1 mg/kg was administered intravenously following intravenous inj. ondansetron 0.1mg/kg. After two days, oralparacetamol 650 mg wasadministered for pain relief as and when required

A detailed evaluation for intensity and quality of pain was done by using various different questionnaires at varying intervals i.e., at the end of post-operative day 1, 6^{th} week, 10^{th} week, and 14^{th} week. All the patients were oriented to the details of the various questions and scales to which they were subjected for assessment at repeated intervals postoperatively.The aforesaid intervals correlate with national immunization guidelines of the baby in India for better patient compliance.The pain assessment was done by numeric rating scale (NRS)at rest and with active movement, the neuropathic component of pain was assessed by usingNeuropathic Pain Symptoms Inventory (NPSI).[14]The aggravating factors and relieving factors for pain were also recorded.

The pain experienced or perceived by the patients was labelled as chronic persistent postsurgical pain (CPSP) provided the following criteria are met i.e. if the pain has developed following CD, the total duration of pain is at least three months ortill the end of 14 weeks and whenrest of the causes of pain e.g. chronic infection, continuing malignancy etc. were excluded. [15]

Immunoassay for oxytocine:

Oxytocine immunoassay was performed in blood samples withdrawn before CD (baseline). The serum was separated and oxytocine levels were measured by quantitative standard sandwich ELISA technique using a 96 well plate pre- coated

with specific monoclonal antibody kit according to manufacturer's instructions, supplied by Bioassay Technology Laboratory (USA).

Genotyping of OXTR polymorphism:

Isolation of genomic DNA: Two ml venous blood sample was collected from the study subjects in EDTA coated collection vialsand stored at 40C until analysis. The genomic DNA was extracted from whole blood using commercially available DNA extraction kit (Qiagen, UK) following manufacturer's protocol. The isolated DNA was quantified using Nano drop 2000 spectrophotometer (Thermo Scientific, USA). The DNA was stored at -200C until genotyping study.

Genotyping: Allele specific PCR method was used for determining the OXTR rs53576 T/C polymorphism (Reference).

The PCR for rs53576 T/C was performed using a common forward primer (F1:5'……3') and two sequence specific reverse primers (R1:5'……..3' and R2:5'……3'). Two PCR reactions per sample weresetup and both created a 224 bp band for both T and C allele. A total volume of 25µL PCR reaction mixture contained 1X PCR reaction buffer, 1.5mM MgCl2, 0.2 mM dNTPs, 10µM each primer, 1 U DNA Taq polymerase and 50ng genomic DNA. The PCR amplification was performed by initial denaturation at 950C for 3 min, followed by 35 repeated cycles of denaturation at 950C for 30 sec, primers annealing at 550C for 30 sec, extension at 720C for 45 sec, and a final extension at 720C for 1 min. The amplification product was resolved on electrophoresis system using 1.5% agarose gel and visualized on UV transilluminato

Immunoassay for oxytocine:

Oxytocine immunoassay was performed in blood samples withdrawn before CD (baseline). The serum was separated and oxytocine levels were measured by quantitative standard sandwich ELISA technique using a 96 wellplate pre-coated with specific monoclonal antibody kit according to manufacturer's instructions, supplied by Bioassay Technology Laboratory (USA). Genotyping of OXTR polymorphism: Isolation of genomic DNA: Two ml venous blood sample was collected from the study subjects in EDTA coated collectionvialsandstoredat4 0C until analysis. The genomic DNA was extracted from whole blood using commercially available DNA extraction kit (Qiagen, UK) following manufacturer's protocol. The isolated D NA was quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The DNA was stored at -200C until genotyping study.

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Immunoassay for oxytocin:

Oxytocin immunoassay was performed in blood samples withdrawn before CD (baseline). The serum was separated and oxytocin levels were measured by quantitative standard sandwich ELISA technique using a 96 well plate pre-coated with specific monoclonal antibody kit according to manufacturer's instructions, supplied by Bioassay Technology Laboratory (USA).

Genotyping of OXTR polymorphism:

Isolation of genomic DNA: Two ml venous blood sample was collected from the study subjects in EDTA coated collection vials and stored at 4^0C until analysis. The genomic DNA was extracted from whole blood using commercially available DNA extraction kit (Qiagen, UK) following manufacturer's protocol. The isolated DNA was quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The DNA was stored at -20^0C until genotyping study.

Genotyping: Allele specific PCR method was used for determining the OXTR rs53576 T/C polymorphism. (15) The PCR for rs53576 T/C was performed using a common forward primer (F:TGTGATTTGTACCCAGAAGG) and two sequence specific reverse primers (R1:CCTGTTTCTGTGTGACTGAGGTT) andR2: CCTGTTTCTGTGTGACTGAGGTC). Two PCR reactions per sample were setup and both created a 224 bp band for both T and C allele. A total volume of 25µL PCR reaction mixture contained 1X PCR reaction buffer, 1.5mM $MgCl_2$, 0.2 mM dNTPs, 10µM each primer, 1 U DNA Taq polymerase and 50ng genomic DNA. The PCR amplification was performed by initial denaturation at 95^0C for 3 min, followed by 35 repeated cycles of denaturation at 95^0C for 30 sec, primers annealing at 55^0C for 30 sec, extension at 72^0C for 45 sec, and a final extension at 72^0C for 1 min. The amplification product was resolved on electrophoresis system using 1.5% agarose gel and visualized on UV transilluminator.

Statistical analysis:

On PubMed & Medline search, we could not retrieve any human study evaluating the role of OXTR gene polymorphism and plasma oxytocin levels in the pathogenesis of CPSP following CD. Therefore, we considered it as a pilot study and included 50 patients.

Data were analysed using SPSS-20 statistical software (IBM corp., Armonk, NY, USA). Data was presented as mean (±SD) and/or median (IQR) for all continuous variables. Categorical variables (Age, ASA physical status, anaesthetic technique) were expressed using Fisher-exact test and Chi-square test. Unpaired student-test was used to compare various demographic parameters and SF-12 score. For sub-group analysis between patients with or without CPSP and between SAB and GA group, independent t-test was applied. P-value significance level was set at ≤0.05 for all the test. Pearson correlation was used to correlate mean oxytocin level and NRS scores at various designated time intervals. Student-t test was applied to correlate association between mean oxytocin levels amongst patients undergoing elective or emergency CD.Spearman correlation was used to derive association between oxytocin levels and NRS day 1 and NRS 14^{th} week in patients with and without CPSP.The correlation between the OXTR rs53576 genotypes (CC,TT,CT) and the two alleles i.e. C and T with NRS score at 14^{th} week was estimated by applying binary logistic regression.

RESULTS

The demographic details and obstetric details are depicted in table 1. Female subjects (n=50) had a mean age of 27 years, a mean gestational age of38 weeks and were scheduled for CD at a tertiary care institute (Table 1). Out of 50 patients, 30 patients (60%) had ASA physical status II and 20 patients (40%) were ASA grade III. None of the patients reported preoperative pain before the current CD.66% of patients received SAB and 34% received GA. Out of 50, 43 patients underwent emergency CD and only 7 underwent elective CD.

OXTR gene polymorphism

Distribution&Correlation of OXTR polymorphism rs53576 (T/C) with CPSP

The distribution of OXTR genotype is shown in table 3. In patients with CPSP, 87.5%% patients had wild allele (CC); whereas, 12.5% had mutant allele i.e. CT+TT. However, in patients without CPSP, 72% had wild and 28% had mutant allele. The difference was not significant between wild and mutant group in patients with CPSP and without CPSP which may be due to the small sample size. [Table 2]

On analyzing the risk score of alleles with respect to CPSP using bimodal logistic regression, we found that T allele shows OR of 0.43 (95%CI= 0.08-2.2; P=0.3) and C allele shows OR of 1.06 (95% CI=0.11-11.2; P= 0.96) which implies to the protective in nature of T allele with respect to CPSP in the study population. [Table 3]

Mean oxytocin levels and NRS score at post-operative day 1 and NRS at 14^{th} week amongst various OXTR genotypes -The mean plasma oxytocin levels were comparable between the two genotypes. NRS at postoperative day 1 and 14^{th} week werefound to be reduced with the mutant alleles; however, not significant. A larger sample size is recommended to attain the level of significance.[Table 4]

PLASMA OXYTOCIN HORMONE LEVELS

Correlation between the plasma oxytocin level and NRS-M at various time points in the total sample size (n=50)-Correlation between mean plasma oxytocin levels and NRS-M was observed to be significant at 6^{th} week (pearson p,0.34;P= 0.015) and 10^{th} week (pearson p, 0.261;P= 0.067) post-operatively; however, not significant at the end of 14^{th} week(pearson p, 0.221;P= 0.123). (Table 5)

Correlation between oxytocin levels and NRS-M onpostoperative day 1 and NRS-M on 14th week in patients with and without CPSP-Spearman correlation was used to assess the relationship between plasma oxytocin levels and NRS levels at postoperative day 1 and 14th weekboth amongst the patients who developed CPSP and not developed CPSP. No significant correlation was observed at any time points between thetwo groups.

Mean oxytocin levels between patients undergoing elective or emergency caesarean delivery- The mean plasma oxytocin level is significantly raised in patients undergoing emergency CD when compared to elective CD i.e. 2.22 ± 1.17 vs 1.02 ± 1.00; p-value=0.013.

In patients who underwent emergency CD there was significant negative correlation between plasma oxytocinlevels and NRS-R at all time points i.e.6th week (spearman p= - 0.37, P=0.013),10th week (spearman p, -0.39; P=0.009) and at 14th week (spearman p, -0.29; P=0.053). Similarly, there was significant inverse correlation between plasma oxytocin level and NRS-M at 6th week (spearman p,-0.33; P=0.02); however, no statistical difference was observed at other time points but similar inverse trend was observed.

Ancillary observation

Post-operative pain assessment was done for allat the end of post-operative day 1, 6th week, 10th week and 14th week using NRS-R, NRS-M, NPSI and PDQ.Table 6 depicts the NRS-R & M scores at aforementioned timepoints.

Incidence of CPSP and its relation to ASA physical status, anesthetic technique and type of surgery (elective/emergency)- From the sample size of 50 patients, 12 patients (24%) had pain at the end of 14th week and thus labelled to have CPSP. As far as the influence of anesthetic technique on occurrence of CPSPis concerned, 33 (66%) and 17 (34%) patients had received SAB and GA, respectively.

Out of 50 patients 30 patients (60%) had grade II ASA physical status, out of which 7 (23.33%) patients developed CPSP. Similarly, 20 patients (40%) had ASA grade III and 5 (25%) patients out of these 20 developed.As far as anesthetic technique is concerned, 33 (66%) and 17 (34%) patients received SAB and GA, respectively. 41.6% patients in SAB and 58.3% in GA developed CPSP. No significant difference was observed between the two groups (p-value:0.07). Out of 50 patients,7 patients underwent elective CD and 43 underwent emergency CD.14.28% of elective CD patients and 25.5% of emergency CD developed CPSP. No significant difference was observed between the two groups (p-value:1.00).

Prevalence of the various types of pain (as per NPSI) at the end of 14th week in patients with CPSP- Out of the 24% (n=12/50) patients who had pain at the end of 14th week,"burning pain" was present in 58.33% (n=7) patients, "Tingling" in 58.33%(n=7) patients, "electric shock like" in 75%(n=9) patients, "pressure" in 8.3%(n=1), "stabbing" in 33.33%(n=4), "Squeezing" in 33.33%(n=4), "pins & needles in 16.66% (n=2), and "allodynia" in 41.66%(n=5) patients.

DISCUSSION

The presentpilot study is the first human study evaluating the influence of OXTR gene polymorphism and plasma oxytocin level on the pathogenesis of chronic pain following CD. It showed a significant protective role of the T allele of OXTR rs53576 polymorphism on the occurrence of CPSP and significant inverse correlation between oxytocin levels and persistence of pain till 10th week following CD.

The most commonly reported incidence of CPSP following CD ranges between 6-18%.5.[16, 17]CPSP in these young women may exert a negative effect on activities of daily living and quality of life, thus representing an important clinical problem. [16] This lower incidence of CPSP following CD when compared to other surgeries has been attributed to the shorter duration of CD and the release of endogenous oxytocin in the peripartum period. [5, 18]

Oxytocin has proven antinociceptive effects in various animal studies and the levels has been found to be upregulated in the intrapartum period whichis responsible for modulation in pain experience thus implicating a lower incidence of CPSP following CD.[5, 19, 20] Most of the studies evaluating oxytocin levels during pregnancy have shown that the level rises till delivery and then decline slowly, thereafter. Various animal studies suggest that oxytocin levels rise as delivery approaches. In addition, the number of oxytocin receptors increases dramatically in late pregnancy.[21]

In a human study, levels of oxytocin were found to increase slowly until delivery and then decrease up to 8 weeks postpartum.[21]In contrast, two studies observed no variations of oxytocin levels between the trimesters, and the first month postpartum.[22, 23]

However, in an only human study evaluating the role of endogenous oxytocin in the pathogenesis of CPSP, an inverse relationship between post-caesarean pain severity and oxytocin levels was observed.Ende et al in this observational pilot study investigated the association of plasma oxytocin and post-caesarean incisional pain. Plasma samples from 18 patients undergoing elective caesarean delivery were drawn at 1 hour preoperatively and 1 and 24 hours postoperatively. Pain was assessed at various designated intervals i.e. 1 day, 8 weeks, 3 months, and 6 months postoperatively. Incisional pain at 24 hours was inversely correlated with oxytocin levels at 1 hour and 24 houri.e. higher

plasma oxytocin level is associated with lower pain (ρ, −0.52 and −0.66; P < .05).However, this study did not evaluate the influence of oxytocin levels on the incidence of CPSP at the end of 3rd month.

In the present study, significant correlation between the total mean oxytocin levels and pain score at 6th and 10th week was observed; however, no significant difference was observed in 14th week in the total sample of 50 patients. However, no significant correlation was observed between the oxytocin levels and NRS at various designated time points between patients with and without CPSP; this could be attributed to the limited sample size and the inclusion of patients undergoing both elective or emergency CD. On comparing our study findings with the only human study be Ende et al, wherein the acute pain was found to be inversely correlated with oxytocin level, in our study the acute pain did not correlate with plasma oxytocin levels i.e. at day 1 time point. However, we observed inverse correlation between plasma oxytocin levels and NRS at 6th and 10th week. On the contrary, Ende et al did not assess this correlation beyond postoperative day 1. [6]

In the present study, the mean plasma oxytocin level is significantly raised in patients undergoing emergency CD when compared to elective CD, the reason could be the administration of exogenous oxytocin in the patients scheduled for emergency CD. This finding is in concordance to the study by Prevost et al. which highlighted the fact that the quantity of intrapartum exogenous oxytocin administered during labour predicted the plasma oxytocin levels till two months postpartum implicating the influence of exogenous oxytocin on the plasma oxytocin levels. [20]In this study, a significant negative correlation between oxytocin hormone levels and NRS-R at all time points i.e. 6th,10thand 14th week amongst the patients undergoing emergency CD was observed.

Oxytocin shows its effects via binding and activating the oxytocin receptor (OXTR). The OXTR gene is located on chromosome 3, composed of 4 exons and 3 introns and is responsible for the production of oxytocin receptors. Thus the various polymorphism of OXTR gene could be implicated to be the cause of variation in various physiologic effect of oxytocin.[7, 8]

Genetic variants, including single nucleotide polymorphisms (SNPs), in the OXTR have been shown to independently influence gene expression and has been associated with human emotional responsiveness and social behaviour[9]. Currently, more than 20 SNPs have been identified within the OXTR gene. [18]Two SNPs rs2254298 and rs53576 have been most investigated regarding the OXTR (Intron 3). [19, 24, ,25]

Oxytocin receptor gene polymorphisms are associated with circulating oxytocin levels, altered brain activity, and parenting behaviours.The SNP polymorphism of rs53576 has been most widely evaluated out of all OXTR polymorphisms. OXTR SNP Rs53576 (A > G) has been associated with reduced positivity, reduced empathy and decreased parental sensitivity. Recently two studies have evaluated the OXTR rs53576 T/C in genotype TT and T allele, emphasizing the protective role this receptor has in occurrence of type-II diabetes mellitus.[24, 25] Both these studies have asserted the protective role of genotype TT rs53576 OXTR in diabetes mellitus.[24, 25] Despite the fact that the oxytocin has a known antinociceptive action, none of the study has evaluated the OXTR rs53576 polymorphism to predict the predisposition for pain development. In the present study, the risk score of alleles with respect to CPSP shows that T allele has OR of 0.43 (95%CI= 0.08-2.2, P value-0.3) and C allele has OR of 1.06 (95% CI=0.11-11.2, P-value 0.96). This shows that T allele is protective in nature with respect to CPSP in our study population.

The study dealt with few limitations. Firstly, the sample size was small. Secondly, in addition to the baseline plasma oxytocin levels, the plasma oxytocin levels at other time points could have been useful in correlating it with NRS scores at other time points e.g. 6th, 10th or 14th week. This would have helped in observing the trends further. Thirdly, the pain assessment at subsequent time points was done telephonically due to the outbreak of the second wave of COVID pandemic. Fourthly, although the intraoperative oxytocin administration was standardized, but the dose administered was not recorded and not correlated with the blood oxytocin levels.

CONCLUSION:

We conclude thatT allele of OXTR rs53576 polymorphism has been found to have significant protective role on the occurrence of CPSP following CD; in addition, a significant inverse correlation was observed between oxytocin levels and persistence of pain till 10th week following CD. Further multicentre trials with a larger sample size and different ethnic groups is warranted to confirm the findings of the present study. In future, this research background shall help in formulating individual pain management plans based on the individual's predisposition to develop chronic pain following CD.

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Conflicts of interest: None

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Table 1: Demographic and surgical variables (n=50)

Demographic parameter	MEAN ±SD / %(N)
Maternal age (y)	27.01 ± 4.74 y(19-38 y)
Gestational age	38.2 weeks
ASA Grade II Grade III	60% (30/50) 40% (20/50) (p value= 1.0)
Chronic pain of any etiology Previous CD	None 27/50
Duration of surgery (mins)	96.61 ± 4.01
Anesthetic technique	33 (66%) SAB 17 (34%) GA (p value=0.09)
Elective or Emergency CD	7 -elective CD and 43-emergency CD (p value=1.0)

N=number of patients; SAB=subarachnoid block; GA- general anesthesia; CD=cesarean delivery

Table 2: Correlation of OXTR genotype with CPSP

	CPSP(N=12)		Without CPSP(N=38)		P-value*
Genotype	N	P	N	P	
CC	10	84%	26	68%	0.88
CT	1	8%	3	8%	0.96
TT	1	8%	9	24%	0.57

N= number of patients P= proportion of patients *P value ≤0.05 = significant

Table 3: Correlation of alleles with CPSP

	CPSP (N=12)		Without CPSP (N=38)		P-Value*	OR	CI	P-Value*
Alleles	N	P	N	P				
C (Wild CC)	21	87.5%	55	72%	0.70	1.06	0.11-11.2	0.96
T (mutant CT+TT)	3	12.5%	21	28%	0.90	0.43	0.08 -2.2	0.30

Test applied- Bimodal logistic regression.: N= number of patients P= proportion of patients OR= odds ratio CI= 95% confidence
Interval *P value ≤0.05 = significant

Table 4: Mean plasma oxytocin levels and Numeric rating score (NRS) for pain amongst different OXTR genotypes

	OXTR Genotype	N	Mean±SD	P-value (2-tailed)*
Baseline Oxytocin levels	Wild homozygous (CC)	36	2.31 ± 1.32	0.489
	Mutant Homozygous (TT)+ Heterozygous (CT)	14	2.05 ± 0.86	
NRS day 1	Wild homozygous (CC)	36	6.08 ± 2.00	0.230
	Mutant Homozygous (TT)+ Heterozygous (CT)	14	5.50 ± 1.45	
NRS 14th week	Wild homozygous (CC)	36	1.78 ± 2.16	0.328
	Mutant Homozygous (TT)+ Heterozygous (CT)	14	1.00 ± 1.01	

*P value ≤0.05 = significant N= number of patients

Table5: Correlation between mean oxytocin level and NRS-M at various time points

Mean plasma oxytocin levels	NRS day 1	NRS 6th week	NRS 10th week	NRS 14th week
Pearson correlation	0.223	0.342	0.261	0.221
P-value*	0.120	0.015*	0.067*	0.123

test applied – Pearson correlation *P value ≤0.05 = significant

Table 6: Mean NRS-R and NRS-M at various designated intervals

Time interval	NRS-R (MEAN ± SD)	NRS-M (MEAN±SD)	Level of significance (P value)
Day 1	4.36 ± 2.038	5.92 ±1.871	Ref
6th week	2.76 ± 1.927	4.78 ± 2.092	<0.001*
10th week	2.18 ± 2.022	2.18 ± 2.022	<0.001*
14th week	1.10 ± 2.022	1.56 ± 2.843	<0.001*

*P value ≤0.05 = significant