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NOVEL OXYTOCIN RECEPTOR VARIANTS IN LABORING WOMEN REQUIRING HIGH DOSES OF OXYTOCIN

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Abstract:

To describe labor progression patterns with oxytocin for augmentation in women who achieve vaginal delivery; and to determine how long one should wait with effective uterine contraction before labor arrest can be diagnosed. It is a population-based retrospective cohort study.

Term, nulliparous women requiring oxytocin doses of ≤4 milliunits/minute (low-dose requiring, n=83) or ≥20 milliunits/minute (high-dose requiring, n=104) for labor augmentation or induction were consented to a post-partum blood draw. Targeted-amplicon sequencing (coverage > 30X) was performed to discover variants in the coding exons of the oxytocin receptor gene. Baseline relevant clinical history, outcomes, demographics, and oxytocin receptor gene sequence variants and their allele frequencies were compared between low-dose-requiring and high-dose-requiring women. A P-value < 0.05 was considered statistically significant. The HDR (high-dose-requiring) women had higher rates of obesity and diabetes and were more likely to have undergone labor induction and required prostaglandins. Targeted sequencing of the oxytocin receptor gene in the total cohort (n=187). One novel variant was found in both the low- and high-dose-requiring groups. Three novel variants resulting in an amino acid substitution, loss of 9 amino acids, and a frame-shift stop mutation, respectively, were identified only in LDR (low-dose-requiring) women. Nine non-synonymous variants were unique to the high-dose-requiring group. There was no statistically significant association between the numbers of synonymous and non-synonymous substitutions in the patient groups.

When oxytocin is just started for labor augmentation in early first stage, it may take up to 10 hours for the cervix to dilate by 1 cm. Labor induction and obesity were associated with the requirement for high doses of oxytocin. We did not identify significant differences in the prevalence of oxytocin receptor variants between low-dose-requiring and high-dose-requiring women, but novel oxytocin receptor variants were enriched in the high-dose-requiring women. Additionally, we found three oxytocin receptor variants (two novel, one known) that were predicted to damage oxytocin receptor function and would likely increase an individual's risk for requiring a high oxytocin dose.

Key Words: *oxytocin receptor variant; requirement of oxytocin doses; labouring women.*

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INTRODUCTION:

Labor arrest remains the dominant indication for primary caesarean sections in most countries. Oxytocin is frequently employed to address dystocia. However, our basic knowledge on how labor progresses when it is augmented by oxytocin is still very limited. Oxytocin is commonly used to induce or augment labor and is one of the most frequently used medications in obstetrics. This nonapeptide hormone, which is secreted by the pituitary gland, corpus luteum, placenta, amnion, and decidua, acts on uterine smooth muscle to initiate, enhance, and pace uterine contractions. On average, nearly half of women who give birth in the United States are administered synthetic oxytocin to induce or augment labour. However, the effectiveness of a given oxytocin dose is variable among women; consequently, oxytocin has a relatively wide therapeutic window between 1 mU/min and higher than 40 mU/min [1] [2].

According to the guidelines on dystocia management recommend that a minimum of 4 hours of effective uterine contractions with oxytocin augmentation should be allowed before labour arrest is declared and caesarean delivery performed. Guideline advises the woman to have a vaginal examination 4 hours after starting oxytocin in established labour, if cervical dilatation has increased by less than 2 cm after 4 hours of oxytocin; further obstetric review is required to assess the need for caesarean section. As labour progression accelerates in the active phase, does the 4-hour rule apply to the early as well as late active phase? The purpose of this study was to use contemporary labour data in a large number of parturients receiving oxytocin to describe labour progression patterns with oxytocin for augmentation [3] [4].

MATERIAL AND METHODS:

This prospective study included nulliparous women at or beyond 37 weeks of gestation with singleton, non-anomalous pregnancies who received oxytocin for labor or induction of labor at Murshid Hospital and Health Care Centre Karachi.

Study Duration: January-2017 to December-2017.

Inclusion Criteria: Patients meeting inclusion criteria were enrolled and consented postpartum by trained research nurses.

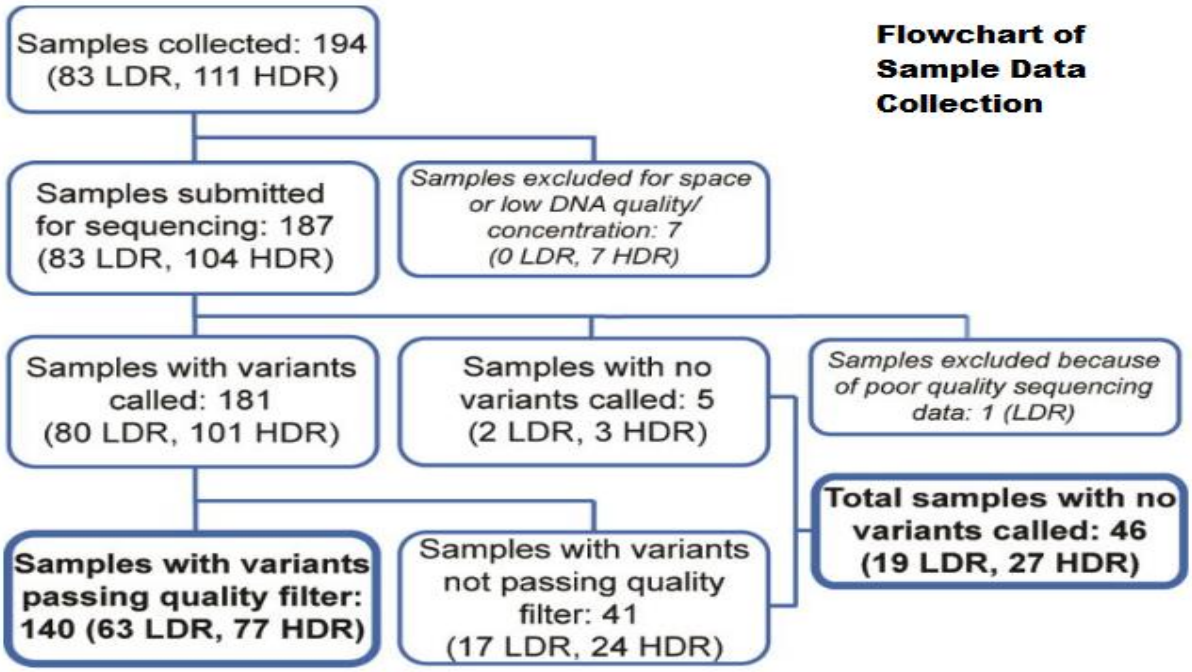
Exclusion Criteria: Exclusion criteria included a history of HIV or hepatitis, multiple pregnancy, ages < 13 or > 48 years old, or received magnesium.

Statistical Analysis: Baseline characteristics were compared between LDR and HDR groups and were statistically assessed by using Fisher's exact or chi-squared tests for categorical variables and Student *t*-tests or Wilcoxon rank sum test for continuous variables. A *P*-value < 0.05 was considered significant.

Filtering and Identification of Variants: Sequence data were analysed with a custom pipeline. Raw sequence reads were trimmed to remove primer sequences and aligned to human reference sequence hg19. Variants were called with "FreeBayes" and then filtered with "SnpSift" for quality scores of 1000 or greater, depth of 40 or more reads, and percent of paired reads ≥ 20%.

RESULTS:

Targeted sequencing of the *OXTR* gene was performed and obtained from consented women who were treated with oxytocin for labor induction or augmentation as mentioned in below Figure 1. Of 187 women, 104 (56%) were defined as HDR, and 83 (44%) were LDR. One LDR sample failed to yield quality sequence data and was eliminated from any further analysis.



Source: [5]

Compared to those in the LDR group, women in the HDR group were more likely to be obese, have diabetes, undergo induction of labor, and require prostaglandins for induction as mentioned in below Tables 1a, 1b and 1c.

Table 1a

Baseline demographics of cohort

Variable	LDR n=82	HDR n=104	*P*
Maternal Age (yrs)	25±5.6	26±6.0	0.27

Table 1b

BMI (kg/m^2)	31.7±6.1	35.0±7.4	**<0.01**
Obesity	45 (54.9)	73 (70.2)	**0.03**
Gestational Age (days)	273±19.6	274±8.9	0.54
Infant Birthweight (g)	3245±482	3319±540	0.34
Smoking	4 (4.9)	7 (6.7)	0.60
Illicit drug use	7 (8.5)	6 (5.8)	0.46
Chronic hypertension	2 (2.4)	5 (4.8)	0.40
Hypertensive disorder of pregnancy	13 (15.9)	29 (27.9)	0.05
Diabetes	0 (0)	10 (9.6)	**<0.01**

Table 1c

Indication for induction of labor			
IUGR	4 (10.5)	10 (12.4)	0.71
Hypertension	13 (34.2)	24 (29.6)	
PROM	2 (5.3)	11 (13.6)	
Fetal anomaly	2 (5.3)	3 (3.7)	
Elective	5 (13.2)	7 (8.6)	
Diabetes	1 (2.6)	6 (7.4)	
Other	11 (29.0)	20 (24.7)	
Prostaglandin	25 (30.5)	47 (45.2)	**0.04**

There was no difference in age, gestational age, or indication for induction between groups. After adjusting for confounders, HDR was associated with an increased risk of cesarean delivery for first stage arrest (aOR 5.4, 95% CI 1.8–16.7) and a decreased risk of cesarean delivery for non-reassuring fetal status (aOR 0.3, 95% CI 0.1–0.8). There were no differences in rates of vaginal delivery, cesarean section, or completion of first stage of labor between the two groups as mentioned in Table 2 below:

Table 2

Labor outcomes.

Variable	LDR n=82	HDR n=104	RR (95%CI)	aOR* (95% CI)
Cesarean Section	32 (39.0)	53 (51.0)	1.3 (0.94–1.82)	1.4 (0.7–2.6)
Indication for cesarean				
First stage arrest	4 (4.9)	27 (26.0)	**5.3 (1.9–14.6)**	**5.4 (1.8–16.7)**
Second stage arrest	5 (6.1)	9 (8.7)	1.4 (0.5–4.1)	1.9 (0.6–6.5)
Non-reassuring fetal status	15 (18.3)	8 (7.7)	**0.4 (0.2–0.9)**	**0.3 (0.1–0.8)**
Other	7 (8.5)	9 (8.7)	1.2 (0.5–3.1)	1.1 (0.4–3.3)
Spontaneous Vaginal Delivery	48 (58.5)	49 (47.1)	0.8 (0.6–1.1)	0.7 (0.4–1.4)
Operative Vaginal Delivery	2 (2.4)	2 (1.9)	0.8 (0.1–5.5)	0.9 (0.1–7.4)
Completion of 1st stage	55 (67.1)	63 (60.6)	0.9 (0.7–1.1)	1.0 (0.5–1.9)

aOR, adjusted odds ratio; LDR, low dose requiring; HDR, high dose requiring; RR, relative risk

*Adjusted for induction of labor and obesity

Data represents n (%). RR calculated by chi square test. aOR estimated by multivariable logistic regression.

Source: [6]

Accordingly, we identified 30 *OXTR* variants, of which17 were non-synonymous substitutions (NSS), 11 were synonymous substitutions (SS), and two were small structural variations. Moreover, 10 variants were novel. Of these, eight were NSS (Y106H, R150L, M133V, H173R, A243T, A248V, G253R, and I266V), and two were deletions (A240_A249del, and P197delfs*206).

Because NSS variants were more likely to affect the structure and function of OXTR than SS variants, we sought to determine whether the HDR women had a higher burden of NSS *OXTR* variants than the LDR women. We identified 14 NSS and 6 SS variants in HDR women and 8 NSS and 7 SS in LDR women. Twelve NSS variants were each identified in only one individual (either HDR or LDR) (minor allele frequency < 1%). Relative risk assessment determined that HDR women were 1.31 (0.78 – 2.44) times more likely than LDR women to have a NSS, but this was not statistically significant ($P = 0.48$). Both groups had a higher representation of SS variants than NSS variants (mean 59.6% versus 33.2%). A higher percentage of HDR than LDR women had NSS alleles (34.6% vs. 31.7%), but this difference was not statistically significant ($P = 0.68$). These data show no significant difference between overall *OXTR* variant loads in oxytocin HDR vs. LDR women.

DISCUSSION:

Our study identified ten novel variants of *OXTR*, and six of these were unique to women requiring high doses of oxytocin. Both HDR and LDR women had more NSS variants than SS variants of *OXTR*, and HDR women had more potentially damaging variants. Although these findings were not statistically significant, they suggest a genetic component to oxytocin sensitivity in women undergoing labor induction or augmentation. Four of the NSS variants were predicted by SIFT to be damaging (R150L, R151C, H173R, and W195R). A variant in R150, R150S, was previously identified in an individual with autism spectrum disorder [7] [8].

This residue is part of the oxytocin receptor 'polar pocket', which changes conformation during receptor activation and is involved in G-protein interaction. R151C was previously annotated in the Ensembl database (rs772841181), but its functional impact is not known. Given its polarity and proximity to R150, R151 may contribute to the stability of the polar pocket. H173 and W195 are located within the regions of the OXTR binding pocket that recognize the cyclic and linear, respectively, components of oxytocin. The cyclic binding region is thought to confer more specificity for oxytocin binding than the region recognizing the linear portion of oxytocin, so the H173R and W195R mutations may differentially disrupt oxytocin binding strength or specificity [9].

The other *OXTR* variants we identified in HDR women, G221S, W228C, V172A, L206V, A218T, and A238T, were all in the Ensembl and ExAC databases, but their phenotypic effects are unknown. Neither these nor the other nine *OXTR* variants we found were predicted by SIFT to be damaging, but this does not preclude them from having unpredictable damaging effects on OXTR function. Future *in vitro* studies should reveal the effects of these variants on OXTR function [10].

Previously, Kim et al. (2013) investigated the link between *OXTR* variants and preterm birth but did not

find any variants that were statistically associated with preterm birth. Kim et al. identified some novel and damaging variants, including the more common variants in our study (V172A, L206V, A218T, and A238T), but we note no overlap in our studies between the extremely low-frequency (0.003) variants we found or the predicted damaging variants they found. By using a heterologous expression system, Kim et al. showed that some of their variants reduced oxytocin binding and IP_3 production capability. Taken together, our studies suggest that oxytocin sensitivity is, at least in part, genetically determined [11].

Strength of our study is our use of high-throughput targeted amplicon sequencing, which enabled us to discover and individually genotype the variants across the entire patient cohort. In contrast, several previous studies in search of genetic variation in disease and patient outcomes have relied on SNP micro-arrays and subsequent Sanger sequencing for validation. [12].

Our study has some potential limitations to consider. By only sequencing exons, we may have missed variants in the promoter affecting gene expression, enhancers affecting allele-specific expression, or splice acceptor site mutations in introns. Variants in these regions could affect overall *OXTR* expression levels, which could also have a profound effect on oxytocin responsiveness, as myometrial OXTR levels rise in preparation for labor. Additionally, the highly guanine rich region in the 5' end of prevented us from designing robust primers for PCR amplification and thus precluded our abilities to successfully sequence this region in any of the samples. We anticipate future high-throughput sequencing strategies to resolve the 5' end of *OXTR* in women of interest, especially those with no other risk factors for decreased oxytocin sensitivity. Although we discovered 14 *OXTR* variants in HDR women, our study was not powered to investigate whether any of the variants contributed to LDR or HDR status. However, the results provide novel information about unique *OXTR* variants that have not been previously identified or described [13].

This study corroborated the findings by Frey et al. demonstrating that maternal body mass index (BMI), maternal diabetes, and labor induction were risk factors for requiring high doses of oxytocin to achieve delivery. We found that HDR patients were more likely than LDR patients to have cesarean sections for first stage arrest and less likely to have cesarean sections for non-reassuring fetal status. We suspect that those with non-reassuring fetal status were likely to proceed to cesarean section sooner, thereby limiting duration of oxytocin exposure and titration. There was no difference in second stage arrest between HDR and LDR women, suggesting that the genetic aspect of oxytocin sensitivity is most applicable for first stage management. The HDR women with NSS variants predicted to be damaging (H173R, R151C, and R150L) had the additional risk factor of labor induction. The subject with the R151C variant also had a high BMI (35.7 kg/m^2), and the subject with R150L had a high BMI (45.5 kg/m^2), pre-gestational diabetes, and large birthweight (4125 g). Thus, although these women's requirements for a high dose of oxytocin cannot be attributed to the variants alone, the variants may add additional risk [14].

CONCLUSION:

This study suggests that a genetic factor, variation in *OXTR*, is associated with oxytocin responsiveness during labor. Although these results are insufficient to make absolute conclusions about the effect of the *OXTR* variants we identified on uterine contractions and labor dystocia, they introduce novel genetic variants that will serve as a foundation for future work. Future studies should include a larger sample size of women to more fully assess the association between specific *OXTR* variants, requirement for high and low oxytocin doses, and labor arrest diagnoses. Additionally, functional studies evaluating oxytocin binding, downstream signaling, and myometrial contractility should be performed to assess consequences of the variants we identified. In combination with our study, such experiments will improve our knowledge about genetic contributions to oxytocin responsiveness and may, in the long term, contribute to precision medicine in managing labor.

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