

## Role of altered Notch signalling in the pathogenesis of recurrent pregnancy loss (RPL): A northeast Indian cohort based pilot study

*N Kashyap (IN) [1], A Begum (IN) [2], C R Das (IN) [3], R Dutta (IN) [4], D Tiwari (IN) [5], S Bose (IN) [6], P D Bose (IN) [7]*

**Context:** RPL affects 1-2% of women globally, but is a major issue in India (7.46%) including its north-eastern part. The mechanism underlying the pathology is largely unknown and needs elucidation. Notch signalling plays an important role in the regulation of embryonic and placental development, and defects in the Notch receptor-ligand system had adverse impact on placentation.

**Objective:** A transcript based analysis to evaluate the role of the differential placental Notch receptors and ligands expression in RPL pathogenesis.

**Methods:** Realtime PCR based evaluation of differential mRNA expression was performed for all the notch receptors (notch1 to 4) and its ligands (dll1, dll3, dll4, jagged1 and jagged2) using the cDNA as template prepared from total RNA extracted from product of conceptions (POCs) of RPL patients with that of placenta of term delivery (TD) cases.

**Patient(s):** (i) RPL-Women who had undergone 3 or more spontaneous miscarriages (N=16) and (ii) TD cases (n=25) as the control group.

**Intervention(s):** None

**Main Outcome Measure(s):** Pregnancy outcome.

**Result:** The results showed a sharp downregulation in the expression of all the four Notch receptors viz., notch1 ( $0.117 \pm 0.098$  folds) associated with promotion of the development of the invasive extravillous trophoblast lineage; notch2 ( $0.0216 \pm 0.0026$  folds) associated with placental circulatory system, notch3 ( $0.228 \pm 0.198$  folds) and notch4 ( $0.063 \pm 0.0374$  folds) in the RPL cases as compared to the TD cases. Except dll3 mRNA expression ( $1.239 \pm 1.047$  folds), the mRNA expression of all the other notch ligands dll1 ( $0.3913 \pm 0.168$  folds), dll4 ( $0.684 \pm 0.435$  folds), jagged1 ( $0.1056 \pm 0.0525$  folds) and jagged2 ( $0.325 \pm 0.2035$  folds) were found to be downregulated in RPL cases compared to TD cases. Correlation analysis showed a significant positive correlation between downregulated (i) notch3-jagged1 ( $p=0.036$ ) associated with survival, proliferation, and invasion of placenta; (ii) notch4-dll4 ( $p=0.025$ ) associated with placental angiogenesis; and (iii) notch4-jagged1 ( $p=0.043$ ) which regulates microvessel differentiation.

**Conclusion:** Given the background of importance of notch receptor-ligand based signalling during embryogenesis, the present transcript level pilot study data clearly underlines the importance of the downregulation of notch receptors and ligands expression, and their positive correlation in the pathogenesis of RPL. The study also emphasizes on the prognostic significance of the pathway in RPL.