

Study protocol Histomorphometric study of human umbilical cord and its correlation with oxidative stress markers in normal and intrauterine growth retarded newborn in Maharashtra, India

Paras Thapa¹, Dr. Raindra Ved Pathak², Dr. Ranjit Ambad³, Dr. Roshan Kumar Jha⁴, Dr Shyambabu Prasad Rauniyar⁵

¹PhD Scholar, Department of Anatomy, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha

²Professor, Department of Anatomy, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha

³ Professor, Department of Biochemistry, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha

⁴ Assistant Professor, Department of Biochemistry, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha

⁵ Tutor, Department of Anatomy, Datta Meghe Medical College, Wanadongri, Datta Meghe institute of Higher Education and Research, Sawangi (M), Wardha, Email:Id : shyambaburauniyar@gmail.com

Corresponding author:

Paras Thapa,

PhD Scholar, Department of Anatomy,

Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha

Email ID: thapas13@gmail.com

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INTRODUCTION

The umbilical cord is essential for fetal growth, ensuring exchange of oxygen, nutrients, and waste between mother and fetus¹. Its histological and morphometric features reflect fetal well-being, and variations are linked to outcomes like intrauterine growth restriction (IUGR), caused by impaired umbilical blood flow². IUGR arises from multiple maternal and fetal factors such as malnutrition, anemia, hypertension, infections, and cord abnormalities³, contributing significantly to perinatal morbidity and mortality. Despite its impact, effective treatment remains difficult due to its multifactorial nature⁴.

Oxidative stress, resulting from imbalance between reactive oxygen species (ROS) and antioxidant defenses, plays a key role in IUGR pathophysiology⁵. It can cause endothelial dysfunction, placental insufficiency, and cord vasoconstriction, restricting fetal growth⁶. Evaluating umbilical cord parameters like vessel dimensions, Wharton's jelly, and wall thickness alongside oxidative stress markers may clarify underlying mechanisms.

In India, socioeconomic, nutritional, and environmental influences make this research especially relevant. By comparing cord histometry and oxidative stress in normal and IUGR newborns, this study aims to improve early detection of at-risk pregnancies and guide targeted clinical interventions, enhancing neonatal outcomes in affected regions.

Review of literature:

Morphometric changes of umbilical cord in IUGR and normal babies

Vivek Singh Malik et al. reported reduced total umbilical area, cord jelly area, and vessel area in IUGR neonates, though luminal area and wall thickness showed no difference⁷. Similarly, Sangeeta S. et al. found significantly reduced vessel, wall, and lumen areas in IUGR cases, with no sex-based differences⁴. In pre-eclampsia, Barnwal et al. noted increased vessel and vein areas, arterial wall thickness, but reduced jelly and vein wall thickness⁸. Blanco et al. and Chillakuru S. also observed marked cord alterations in hypertensive pregnancies⁹.

Together, these studies provide compelling evidence that oxidative stress plays a significant role in the pathophysiology of IUGR, highlighting the disruption of antioxidant defences and increased oxidative damage in affected neonates.

Research Gap:

There is limited region-specific data from Wardha, Maharashtra, on how umbilical cord histomorphometry and oxidative stress markers relate to intrauterine growth restriction (IUGR). While oxidative stress in IUGR has been studied, correlations with cord vessel morphology in the Indian context remain unclear. This study aims to fill this gap by providing localized insights, enhancing understanding of IUGR pathophysiology, and contributing to improved diagnosis and management.

Research Question:

What is the correlation between histomorphometric alterations of the human umbilical cord and oxidative stress marker levels in intrauterine growth-restricted (IUGR) newborns compared to healthy controls in the Wardha region of Maharashtra, India?

Hypothesis:

Null Hypothesis (H₀):

There is no significant correlation between histomorphometric changes in the human umbilical cord and oxidative stress markers in normal and intrauterine growth-retarded (IUGR) newborns.

Alternative Hypothesis (H₁):

Histomorphometric changes in the human umbilical cord are significantly correlated with oxidative stress markers in IUGR newborns, indicating that oxidative stress contributes to structural alterations in the umbilical cord.

Aim:

To investigate the correlation between umbilical cord histomorphometric parameters and oxidative stress markers in normal and IUGR newborns in Maharashtra, India

Objectives:

To evaluate histomorphometric and morphological changes in the umbilical cord of normal and IUGR newborns.

To assess oxidative stress markers and develop predictive models by integrating morphometric and biochemical parameters for IUGR diagnosis.

Study Type/Design- Case control cross-sectional study

Study Site:

Datta Meghe Institute of Higher Education & Research Sawangi (Meghe), Wardha-442107, Maharashtra, India

Inclusion criteria:

Gestational age between 36 and 41 completed weeks.

Singleton live birth.

Delivered either by normal vaginal delivery or caesarean section.

Maternal age between 18–35 years at the time of delivery.

Exclusion Criteria:

Multiple pregnancies.

Congenital malformations in the newborn.

Chromosomal abnormalities in the fetus/newborn.

Maternal chronic illnesses.

Maternal history of smoking, alcohol consumption, or substance abuse during pregnancy.

Maternal infections during pregnancy.

Sample collection and storage:

Umbilical cord blood and tissue samples will be collected from normal and IUGR neonates under aseptic precautions, with blood processed for oxidative stress marker estimation and tissue preserved in formalin for histomorphometric analysis.

Histomorphometric Study:

The tissues will be fixed in 10% buffered formalin for 48 hours and further processed for paraffin blocks. Sections of 5µm thickness will be produced from the tissue blocks using a microtome. The paraffin sections will be cut and stained with Haematoxylin and Eosin for general histological study and Masson's Trichrome stain to differentiate collagen from smooth muscles. Morphometric analysis of umbilical cord includes:

Total cord cross-sectional area

Wharton's jelly area

Umbilical vessel diameters (arteries and vein)

Vessel wall thickness

Wall-to-lumen ratios

Collagen content and distribution

All these parameters will be measured by using ImageJ Software.

Sample Size Calculation:

The sample size is calculated by referring the similar study conducted for Indian population.

Using the formula,

$$\begin{aligned} N &= \frac{Z^2 \times P \times (1-P)}{d^2} \\ &= \frac{(1.96)^2 \times 0.28 \times (1-0.28)}{0.005^2} \\ &= \frac{3.84 \times 0.28 \times (1-0.28)}{0.005^2} \\ &= \frac{3.84 \times 0.28 \times 0.72}{0.0025} \\ &= 310 \end{aligned}$$

Where:

n = Sample size

Z = Z-score (standard normal variate corresponding to the desired confidence level); For 95% confidence: Z = 1.96

P = Estimated prevalence (in proportion) is 28.3

d = Margin of error (precision) in proportion (e.g., 0.05 for ±5%)

Study Participants: Study participants will be selected from the Obstetric Department and Gynaecology, consisting of two groups:

Group I: Normal healthy pregnancies (control group) and

Group II: Pregnancies with IUGR neonates (IUGR group).

Translatory Component (Conceptualized):

The study's translatory potential lies in identifying oxidative stress biomarkers that may enable early detection of pregnancies at risk for IUGR, supporting timely interventions. By correlating histomorphometric changes with biochemical markers, it could improve diagnostic accuracy and fetal monitoring. The findings may further guide clinical strategies to reduce oxidative stress through lifestyle, dietary, or therapeutic measures, and inform preventive approaches that promote healthier maternal and fetal outcomes.

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