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A Prospective Cohort Study Protocol on Trimester-Specific G6PD Activity and Oxidative Stress as Predictors of Adverse Fetomaternal Outcomes in Anemic Pregnancy

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ABSTRACT

Introduction: Pregnancy is a state of heightened oxidative stress, significantly exacerbated by anemia, which affects 40-60% of pregnancies in India. Glucose-6-phosphate dehydrogenase (G6PD) is critical for maintaining redox homeostasis. The longitudinal interplay between G6PD activity, oxidative stress biomarkers, and anemia across gestation, and its collective impact on pregnancy outcomes, remains poorly characterized and represents a significant research gap, particularly in the Indian population.

Methods and Analysis: This prospective observational cohort study will enroll 300 pregnant women (150 anemic, 150 non-anemic per WHO criteria) at ≤12 weeks gestation from a tertiary care hospital in Wardha, India. Trimester-wise assessments will include spectrophotometric measurement of G6PD activity, serum oxidative stress markers (Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx)), and urinary F2-isoprostanes (by ELISA, normalized to creatinine). Cord blood hemoglobin and bilirubin will be measured at delivery. Primary outcomes include preeclampsia, preterm birth, intrauterine growth restriction, and low birth weight. Statistical analyses will employ regression models and ROC curves to identify and validate predictive biochemical risk factors. Written informed consent will be obtained from all participants. Findings will be disseminated through peer-reviewed publications and national and international conferences.

KEYWORDS: GPx, MDA, SOD, CAT and ROS

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INTRODUCTION

Physiological adaptations in pregnancy increase metabolic demand and mitochondrial activity, leading to elevated generation of reactive oxygen species (ROS) [1]. Under normal conditions, this pro-oxidant state is counterbalanced by a robust antioxidant system. However, when this equilibrium is disrupted, oxidative stress ensues, contributing to endothelial dysfunction, impaired placentation, and a spectrum of adverse outcomes including preeclampsia, gestational diabetes mellitus (GDM), preterm birth, and intrauterine growth restriction (IUGR) [2, 3].

This risk is magnified by anemia, a highly prevalent condition defined by the World Health Organization (WHO) as hemoglobin <11 g/dL during pregnancy [4]. India bears a disproportionate global burden, with an estimated 50% of pregnant women being anemic [5]. Anemia potentiates oxidative stress through mechanisms like tissue hypoxia-reperfusion injury and compromised antioxidant capacity [6].

A pivotal defender against oxidative damage is Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the pentose phosphate pathway. G6PD generates NADPH, which is essential for regenerating reduced glutathione (GSH), the body's primary antioxidant [7]. Consequently, reduced G6PD activity predisposes cells to oxidative injury. The hypermetabolic state of pregnancy may unmask subclinical G6PD insufficiency, creating a vicious cycle of oxidative damage when coexisting with anemia [8].

Despite clear biological plausibility, there is a notable lack of comprehensive, longitudinal studies that simultaneously track trimester-wise progression of G6PD activity and a full panel of oxidative stress biomarkers in anemic versus non-anemic women. This study aims to address this critical gap by investigating this relationship and evaluating the combined utility of these biochemical parameters as early predictors of adverse fetomaternal outcomes.

METHODS AND ANALYSIS

Study Design: A prospective observational study.

Setting and Duration: The 36-month study will be conducted at the Department of Biochemistry in collaboration with the Department of Obstetrics &Gynaecology at Acharya Vinoba Bhave Rural Hospital (AVBRH), a tertiary care teaching hospital in Wardha, Maharashtra, India.

Participants: Pregnant women attending the antenatal clinic will be screened for eligibility.

Inclusion Criteria: (1) Singleton pregnancy with gestational age ≤12 weeks at recruitment, (2) Willingness to provide written informed consent and comply with follow-up schedules.

Exclusion Criteria: (1) Pre-existing chronic diseases (e.g., diabetes, hypertension, renal, thyroid, or hepatic disorders), (2) Multiple pregnancies, (3) Consumption of antioxidant supplements beyond routine iron-folic acid, (4) Acute infections at the time of recruitment.

Sample Size Calculation: The sample size is calculated for the primary objective of comparing adverse outcome rates. Assuming a 30% incidence in the anemic group versus 15% in the non-anemic group [9], with 80% power and a 5% significance level, 112 participants per group are required. Inflating by 20% for potential attrition, the final sample size is 150 per group (300 total).

Grouping: Based on first-trimester hemoglobin levels, participants will be classified as anemic (Hb <11 g/dL) or non-anemic (Hb ≥11 g/dL) as per WHO criteria.

DATA COLLECTION AND LABORATORY PROCEDURES

Schedule: Participants will be assessed once per trimester: T1 (≤13 weeks), T2 (14-27 weeks), and T3 (≥28 weeks). At each visit, 5 mL of venous blood and a spot urine sample will be collected. Cord blood will be collected at delivery.

Primary Exposure Variables:

1. G6PD Activity: Measured spectrophotometrically from erythrocyte lysate by monitoring the rate of NADPH production at 340 nm [10].

2. Oxidative Stress Biomarkers:

Lipid Peroxidation: Serum Malondialdehyde (MDA) will be quantified using the Thiobarbituric Acid Reactive Substances (TBARS) assay [11].

Antioxidant Enzymes: Serum Superoxide Dismutase (SOD) will be assayed by inhibition of pyrogallol autoxidation, Catalase (CAT) by monitoring H_2O_2 decomposition at 240 nm, and Glutathione Peroxidase (GPx) via a coupled assay with glutathione reductase measuring NADPH oxidation at 340 nm [12-14].

In Vivo Oxidative Stress: Urinary F2-isoprostanes will be measured using a competitive ELISA kit (Cayman Chemical, USA), with concentrations normalized to urinary creatinine to account for dilution.

3. Cord Blood Analysis: Hemoglobin will be measured by the cyanmethemoglobin method, and total bilirubin by the diazo method.

EXPECTED OUTCOME

Primary Maternal Outcomes: (1) Preeclampsia, (2) Preterm birth (<37 weeks), (3) Preterm premature rupture of membranes (PPROM).

Primary Neonatal Outcomes: (1) Low birth weight (LBW; <2500 g), (2) Intrauterine growth restriction (IUGR; birth weight <10th percentile for gestational age), (3) Admission to the neonatal intensive care unit (NICU) for >24 hours.

STATISTICAL ANALYSIS

Descriptive statistics will summarise baseline characteristics. Continuous variables will be compared using the Student's t-test or Mann-Whitney U test, and categorical variables with the Chi-square test. The correlation between G6PD activity and oxidative stress markers will be assessed using Pearson's or Spearman's correlation coefficient.

DISCUSSION

This protocol describes a comprehensive investigation into the trimester-wise dynamics of G6PD activity and oxidative stress in anemic pregnancy. Its major strength is the prospective, longitudinal design with a well-defined control group, allowing for the establishment of temporal relationships and causal inference. The use of F2-isoprostanes, a gold-standard marker of in vivo oxidative stress, adds significant validity to the findings [15].

A potential limitation is the single-centre design, which may affect the generalisability of the results. However, the study setting is representative of a tertiary care centre in a region with a high prevalence of anemia. Furthermore, participant attrition over three trimesters is a risk, which we aim to mitigate through diligent follow-up and patient engagement.

This research is expected to yield two key contributions: Firstly, it will generate novel, population-specific data on the biochemical interrelationship between anemia, G6PD, and oxidative stress throughout pregnancy. Secondly, and more importantly, it aims to develop a predictive model using first and second-trimester biomarkers to identify women at high risk for complications. If successful, this model could inform the development of a cost-effective screening tool for integration into routine antenatal care in resource-limited settings, enabling targeted interventions and ultimately improving fetomaternal health outcomes.

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