p53-mediated apoptosis as a molecular link between oxidative stress and bad obstetric history

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Abstract. Bad obstetric history (BOH), characterised by recurrent pregnancy loss and other adverse pregnancy outcomes, affects ~5% of women worldwide and often causes significant psychological distress to affected women and their families. Increasing evidence suggests that oxidative stress and dysregulated apoptosis play crucial roles in its pathogenesis. The p53 gene is a key regulator of apoptosis during normal pregnancy; however, its specific involvement in BOH and its association with oxidative stress are not yet clearly understood. Therefore, the present study aimed to assess oxidative stress and apoptotic markers along with p53 gene expression in women with BOH in order to explore their interrelationship. For this purpose, a case-control study was conducted with 100 BOH cases and 100 healthy controls. Malondialdehyde (MDA) and caspase-3 levels were measured from serum using ELISA in order to assess oxidative stress and apoptosis, respectively, while p53 gene expression ($2^{-\Delta\Delta Cq}$ method) was quantified using reverse transcription-quantitative PCR. Data were analysed for group differences and correlations using Jamovi software. Compared with the controls, women with BOH exhibited significantly elevated MDA (4.1±1.6 vs. 1.9±0.8 nmol/ml), caspase-3 (3.3±2.3 vs. 1.9±1.0 ng/ml) and p53 gene expression levels (1.4±0.7 vs. 1.0±0.2; all P<0.01). A moderate positive correlation was observed between MDA and p53 (r=0.4584, P<0.01), whereas a strong correlation was observed between caspase-3 and p53 (r=0.7136, P<0.01). On the whole, the present study demonstrates that oxidative stress and p53-mediated apoptosis exhibit a significant association with BOH, suggesting their potential relevance in the condition. An enhanced p53 expression and caspase-3 activity contribute to impaired placental function and pregnancy loss, highlighting p53 as a potential molecular marker for early risk identification.

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Introduction

Bad obstetric history (BOH) refers to the occurrence of repeated adverse pregnancy outcomes, including two or more spontaneous abortions, intrauterine foetal deaths, preterm births, stillbirths, congenital anomalies, or early neonatal deaths. It remains a critical reproductive health issue due to its profound emotional, medical and social impact on affected women and their families. Although BOH has multiple causes, its underlying molecular mechanisms are not yet fully understood (1). The present study is therefore relevant, as it focuses on oxidative stress and apoptosis, which are key factors in placental dysfunction, and may aid in the identification of early biomarkers and potential targets for the better prediction and management of BOH.

Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the capacity of antioxidant defence systems. During pregnancy, balanced and physiological levels of ROS are essential for normal placental functions, including trophoblast proliferation, differentiation, invasion and angiogenesis (2). Elevated levels of malondialdehyde (MDA), a widely recognised marker of oxidative stress in maternal blood, reflect increased lipid peroxidation and cellular damage, and have been consistently associated with BOH (3). Excessive oxidative stress can damage cellular components, such as lipids, proteins and DNA, ultimately disrupting normal cell function. At the materno-foetal interface, this oxidative injury can trigger apoptotic pathways, leading to increased trophoblast cell death, impaired placental development and a higher risk of recurrent pregnancy loss (4,5).

Apoptosis is a tightly regulated physiological process that is essential for maintaining tissue balance, particularly at the materno-foetal interface, where controlled trophoblast turnover supports normal placental development. However, when this process becomes dysregulated, it can contribute to pregnancy complications, such as implantation failure and recurrent pregnancy loss. Excessive oxidative stress is a major trigger for apoptosis, as elevated levels of ROS can damage cellular lipids, proteins and DNA, disrupt mitochondrial integrity, and activate intrinsic apoptotic pathways. At the placental level, this oxidative injury promotes trophoblast cell death, the increased shedding of syncytiotrophoblast fragments and heightened inflammatory responses, ultimately impairing placental function and increasing the risk of adverse pregnancy outcomes (6).

Caspase-3, the executioner of the cascade, is recruited during the terminal steps of both the intrinsic and extrinsic apoptotic pathways (7). Previous research has demonstrated that caspase-3 expression is increased in placental tissues from women with BOH, suggesting a role in the abnormal turnover of trophoblasts and trophectodermal dysfunction (8). Caspase-3 is not only a surrogate marker of apoptosis, but also a key functional component in understanding the development of BOH. As further knowledge about its role in pregnancy loss is acquired, it may be possible to identify women at risk earlier and this knowledge may aid in the development of potential biomarkers for its detection.

The p53 gene, known as the 'guardian of the genome', is crucial for regulating cell cycle arrest, DNA repair and apoptosis under stressful conditions. During pregnancy, high levels of oxidative stress can lead to trophoblastic apoptosis and affect placental function, which may result in negative outcomes, such as BOH. p53 functions as a molecular connector by responding to oxidative stress and activating pro-apoptotic pathways, such as caspase-3 and BAX, influencing both implantation and foetal survival (9,10). Changes in p53 expression have been noted in women with recurrent pregnancy loss, suggesting that it may serve as a potential biomarker for identifying the condition (11).

Oxidative stress and apoptosis are key biological processes involved in normal pregnancy, and their imbalance has been implicated in the pathogenesis of BOH. Increased oxidative stress can damage placental cells and trigger excessive apoptosis, leading to adverse pregnancy outcomes. The p53 gene plays a central role in linking oxidative stress to apoptosis by regulating cell cycle arrest and programmed cell death. Although p53 is essential for normal pregnancy, its dysregulated expression may contribute to pathological apoptosis in patients with BOH. Therefore, the present study aimed to evaluate the oxidative stress markers, apoptotic markers, and p53 gene expression in women with BOH and explore their interrelationships.

Materials and methods

Study participants. The present case-control study was conducted at Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala, India, between September, 2023 and July, 2025. A total of 200 participants were enrolled, including 100 women with BOH and 100 age-matched controls. The case group consisted of women aged 20-45 years with a history of two or more spontaneous abortions, intrauterine foetal deaths, stillbirths or early neonatal deaths. Participants were recruited from obstetrics and gynaecology outpatient departments across South Kerala and referred to the the Genetika laboratory for an advanced genetic evaluation. Only women with documented obstetric records confirming previous adverse outcomes were included. The control group comprised women aged 20-45 years with at least two successful pregnancies and no history of pregnancy loss. Women with consanguineous marriages, prolonged medication use, or severe systemic illnesses were excluded from the study. Ethical approval was obtained from the Institutional Ethics Committee of Genetika, Centre for Advanced Genetic Studies (Reference no. 17/2023/IECG).

After obtaining written informed consent, relevant clinical and demographic data were collected using a pre-structured questionnaire. A total of 7 ml fasting venous blood was collected from each participant. Of this, 4 ml was transferred to a clot activator tube for serum separation, and 3 ml was collected in a sodium heparinised vacutainer for gene expression analysis.

Assessment of oxidative stress and apoptosis. Oxidative stress was assessed by estimating serum MDA levels using a competitive inhibition ELISA kit (cat. no. OPK8428; Origin). Apoptosis was evaluated by measuring serum caspase-3 levels using a sandwich ELISA kit (cat. no. OPK1527; Origin). All ELISA procedures were carried out according to the manufacturer's instructions.

Gene expression analysis. For gene expression analysis, total RNA was isolated from heparinised blood samples using an RNA extraction kit (cat. no. ODP419; Origin). Complementary DNA (cDNA) was synthesised using a cDNA synthesis kit (cat. no. ODR41; Origin). Reverse transcription-quantitative PCR (RT-qPCR) was then performed using a 2X SYBR-Green Master Mix (cat. no. ODQ383-01; Origin). The thermal cycling conditions included an initial denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation at 94˚C for 1 min, annealing at 54˚C for 1 min, and extension at 72˚C for 1 min, with a final extension at 72˚C for 10 min. The primer sequences used for p53 amplification were 5'-CCTCAGCAT CTTATCCGAGTGG-3' (forward) and 5'-TGGATGGTGGTA CAGTCAGAGC-3' (reverse), with an annealing temperature of 54˚C. Gene expression levels were normalized using GAPDH as the internal control, with primer sequences 5'-CCATGG AGAAGGCTGGGG-3' (forward) and 5'-CAAAGTTGTCAT GGATGACC-3' (reverse). Relative p53 gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (12). The expression levels were categorized based on fold-change values as normal (1) or upregulated (>1.0).

Statistical analysis. The sample size was determined using the formula Z^2pq/d^2. A 95% confidence level was used, with an assumed prevalence of 5.27% and an allowable error of 10%. Based on this calculation, the minimum required sample size was determined. To ensure adequate statistical power and account for possible variability, the sample size was increased in each group (cases and controls). The Mann-Whitney U test was used to compare non-parametric variables between groups. Pearson's correlation analysis was performed to assess

Table I. Comparison of oxidative stress, apoptosis and p53 gene expression in cases vs. controls.

Variable	Mean ± SD		P-value[a]
	Cases	Controls	
MDA (nmol/ml)	4.1±1.6	1.9±0.8	<0.01
Caspase-3 (ng/ml)	3.3±2.3	1.9±1.0	<0.01
p53 gene ($2^{-\Delta\Delta Cq}$ method)	1.4±0.7	1.0±0.2	<0.01

[a]P-values were obtained using the Mann-Whitney U test. MDA, malondialdehyde.

the association between p53 expression and the molecular biomarkers (MDA and caspase-3). A two-tailed P-value <0.01 was considered to indicate a statistically significant difference. Data management and statistical analyses were carried out using Microsoft Excel and Jamovi software (version 2.5.3).

Results

MDA levels (nmol/ml), a marker of oxidative stress, were significantly higher in the cases than in the controls (Fig. 1). The cases exhibited both a higher median value and greater variability, indicating increased oxidative stress in this group. By contrast, the control group exhibited lower and more consistent values, although there were some outliers.

The box plot depicted in Fig. 2 demonstrates the comparison between the caspase-3 levels between the cases and controls. The case group exhibited a higher median and greater variability, with one extreme outlier on the upper end, suggesting increased apoptotic activity in the cells. By contrast, the controls exhibited generally lower values and a more compact distribution, although a few mild outliers were present.

The analysis of p53 expression patterns (Fig. 3) demonstrated a clear difference between the cases and controls. A normal expression was predominantly observed in the controls (78%) compared with the cases (22%). By contrast, upregulation was more frequent in the cases (58%) than in the controls (15%), while downregulation was observed in a smaller proportion in both groups. This indicates that the dysregulation of the p53 gene, particularly its upregulation, is closely associated with the case group, suggesting its key role in disease development.

The mean levels of MDA and caspase-3 were significantly elevated in the cases compared with the controls, suggesting increased oxidative stress and apoptotic activity in the affected group (Table I). Likewise, p53 gene expression, assessed using the $2^{-\Delta\Delta Cq}$ method, exhibited a significant increase in the cases. All these variances were statistically significant (P<0.01; Table I).

A significant positive correlation was found between MDA levels (nmol/ml), an indicator of oxidative stress, and p53 gene expression ($2^{-\Delta\Delta Cq}$ method). As illustrated in Fig. 4, the correlation coefficient was r=0.4584 (P<0.01), indicating a moderate positive correlation. This suggests that elevated oxidative stress is linked to the increased expression of p53. The regression line also illustrated the trend of rising p53 expression with increasing MDA concentration.

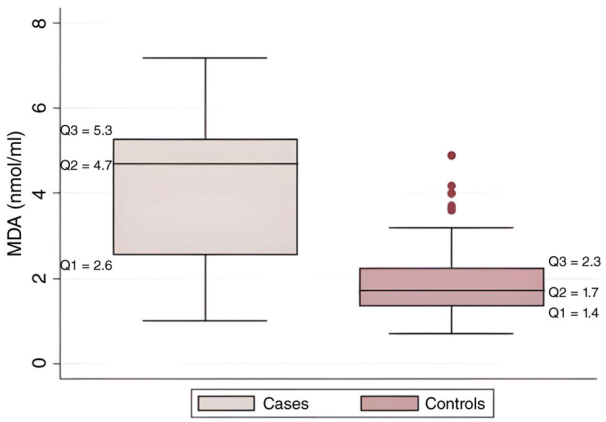

Figure 1. MDA levels the among cases and controls. MDA, malondialdehyde.

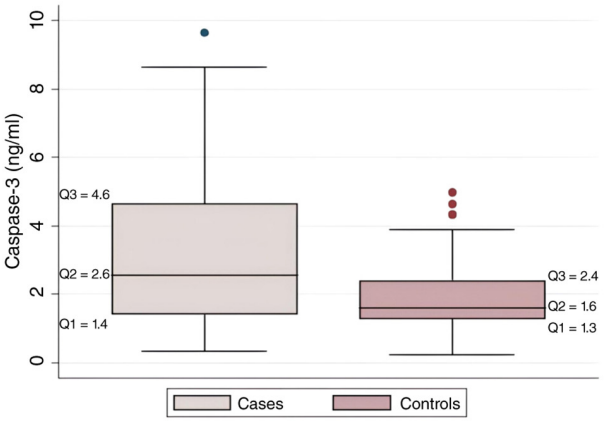

Figure 2. Caspase-3 levels among the cases and controls.

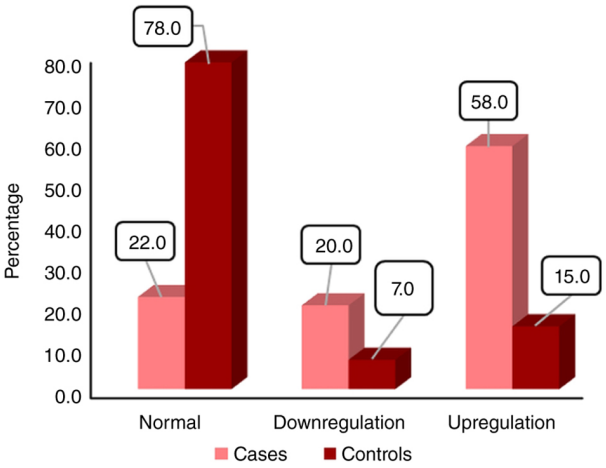

Figure 3. p53 gene expression among the cases and controls.

The scatter plot presented in Fig. 5 demonstrated a strong positive correlation (r=0.7136, P<0.01) between p53 gene expression and caspase-3 levels. As p53 is a key regulator of apoptosis and caspase-3 is an executioner caspase, this correlation indicates that the upregulation of p53 directly enhances caspase-3-mediated apoptotic activity.

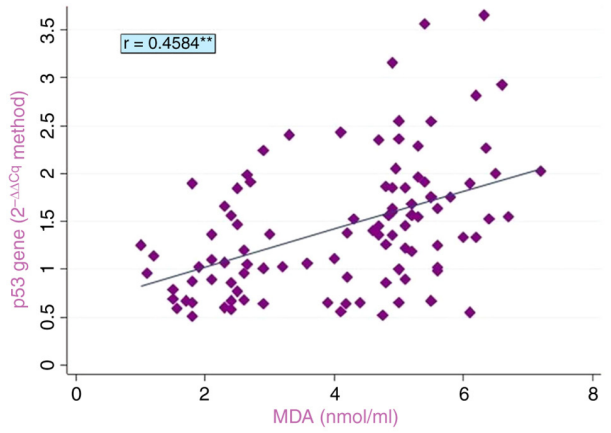

Figure 4. Correlation between MDA and p53 gene expression in women with bad obstetric history. **P<0.01, indicates that the correlation coefficient (r) is statistically significant. MDA, malondialdehyde.

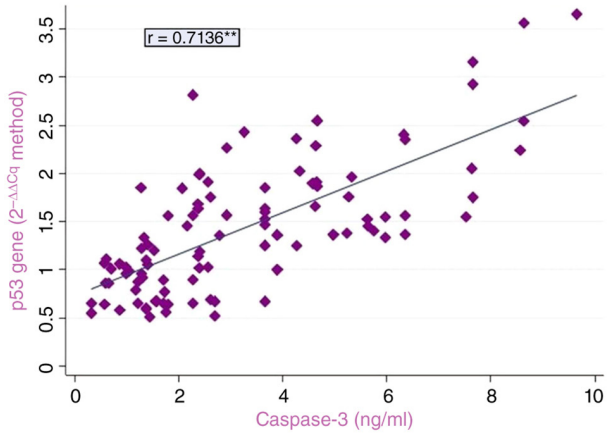

Figure 5. Correlation between caspase-3 and p53 gene expression in women with bad obstetric history.**P<0.01, indicates that the correlation coefficient (r) is statistically significant.

Discussion

The present study demonstrates that oxidative stress-induced, p53-mediated apoptosis plays a crucial role in the pathogenesis of BOH. The significantly elevated serum MDA levels observed in women with BOH indicate increase lipid peroxidation and excessive ROS generation, reflecting a sustained oxidative burden. Such an altered intrauterine redox environment is known to disrupt placental homeostasis and initiate cellular stress responses that are essential for maintaining normal pregnancy. Consistent with previous studies linking oxidative stress to recurrent pregnancy loss and placental dysfunction, the findings of the present study suggest that oxidative injury functions as an early and key pathogenic event in BOH rather than merely a secondary consequence (13,14).

Excessive oxidative stress can damage cellular lipids, proteins and DNA, thereby triggering intrinsic apoptotic pathways. In the present study, significantly higher caspase-3 levels in the BOH cases indicate the active execution of apoptosis, confirming that trophoblastic cell death is a prominent feature in these patients (15). Increased apoptotic activity at the materno-foetal interface can impair trophoblast invasion, placental vascular remodelling and nutrient exchange, ultimately compromising placental function and contributing directly to pregnancy failure (16). Thus, oxidative stress-induced apoptosis appears to be a central mechanism linking cellular injury to adverse pregnancy outcomes in BOH.

A key finding of the present study was the marked upregulation in p53 gene expression in women with BOH. The expression pattern analysis revealed that upregulation was significantly more frequent in the cases than the controls, highlighting the role of p53 dysregulation in the development of disease. Notably, the positive correlation observed between MDA levels and p53 expression indicates that increased oxidative stress is closely associated with activation of the p53 pathway (17). This supports the well-established role of p53 as a stress-responsive regulator that becomes activated in response to oxidative DNA damage.

Furthermore, the strong positive correlation between p53 expression and caspase-3 levels provides clear evidence that p53 activation directly promotes apoptotic execution in BOH. Since p53 functions as a transcriptional regulator of pro-apoptotic genes, its overexpression likely shifts the balance from cellular survival toward programmed cell death (18). The coordinated alterations in oxidative stress, p53 signalling and caspase-3-mediated apoptosis indicate a potential molecular axis associated with trophoblastic injury and placental dysfunction. These markers may serve as a biomarker panel for early risk stratification and clinical monitoring in BOH, enabling targeted surveillance and supportive management. However, these applications remain preliminary and require validation through prospective and interventional studies. Notably, p53 expression, in conjunction with related markers, may aid in early risk detection, although causality cannot be inferred from this case-control study.

The present study had certain limitations, which should be mentioned. The case-control design may be prone to selection bias, as participants were recruited from a single geographic region in South Kerala, India. The single-centre setting and lack of multivariate adjustment for potential confounders, such as lifestyle variables and associated clinical conditions may also affect the generalisability and interpretation of the findings. The cross-sectional nature of the study limits the ability to establish causality, making it unclear whether p53 upregulation is a cause or a consequence of oxidative stress in BOH. The present study also relied on biomarker measurements without functional or mechanistic experiments, which limits direct confirmation of the causal role of the oxidative stress-p53-apoptosis pathway. Larger, multicentre longitudinal studies with experimental validation are thus required to strengthen these findings.

In conclusion, the findings of the present study support an association between oxidative stress, p53-mediated apoptosis, and BOH. Elevated levels of oxidative stress are associated with an increased expression of p53, which is associated with enhanced trophoblastic apoptosis, potentially contributing to impaired placental function. The coordinated upregulation of oxidative stress markers, apoptotic indicators, and p53 expression suggests a possible molecular link underlying BOH. Collectively, these results indicate that p53 may function as a potential mediator linking oxidative stress and apoptosis in BOH. The assessment of p53 expression, along with oxidative

stress and apoptosis markers, may provide insight for early risk identification and clinical monitoring in susceptible women.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

RPR was involved in data curation, formal analysis, data investigation, project administration, in the provision of resources (including the purchase of laboratory reagents and consumables), visualization (figure preparation), and in the writing of the original draft of the manuscript. DC was involved in the conceptualization of the study, in the study methodology, data investigation, project administration, validation, study supervision, and in the writing, reviewing and editing of the manuscript. DRD contributed to the conceptualization and design of the study, study supervision, software support, and formal analysis. NM provided resources (supported subject recruitment to Genetika through collaboration with various hospitals) and was involved in data validation. SM was involved in data validation, and in the writing, reviewing and editing of the manuscript. RGM was involved in the conceptualization of the study, in the study methodology, data validation, in project administration, and in the writing, reviewing and editing of the manuscript. DC and DRD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Ethics Committee of Genetika, Centre for Advanced Genetic Studies (Reference No. 17/2023/IECG) on 03.08.2023. All procedures adhered to the Declaration of Helsinki of 1975, as revised in 2000. Written informed consent was obtained from all participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools (QuillBot and Grammarly) were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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