

STUDY OF PLACENTAL GROWTH FACTOR (PIGF) GENE EXPRESSION IN UNKNOWN EARLY PREGNANCY LOSS CASES: A CROSS SECTIONAL STUDY

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ABSTRACT

Introduction: The formation of new blood vessels represents a critical biological process essential for establishing functional placental circulation and supporting embryonic development. Multiple signaling molecules and growth factors regulate this vascular development. Defective angiogenesis and inadequate vascular network formation have been implicated in adverse pregnancy outcomes and significantly elevate the probability of pregnancy loss.

Objective: This investigation was designed to evaluate the hypothesis that impaired placental vascularization and compromised angiogenic capacity may contribute to early pregnancy loss. Specifically, we examined placental growth factor (PIGF) mRNA expression levels to determine whether altered PIGF expression represents a mechanistic factor underlying early pregnancy loss through impaired placental angiogenesis.

Materials and Method: We analyzed PIGF mRNA expression levels in maternal blood serum and villous tissue samples obtained from 30 women experiencing first-trimester pregnancy loss. Tissue samples were collected following institutional ethics approval and participant informed consent protocols.

Result: Quantitative analysis revealed significantly diminished PIGF mRNA transcript levels in placental tissue samples from women with early pregnancy loss compared to its expression in blood of same cases. This downregulation of PIGF was more pronounced in the placental tissue (0.27 fold) relative to maternal blood samples (1.24 fold), suggesting that compromised placental PIGF expression may have a pathological role.

Conclusion: The findings indicate that early pregnancy loss is associated with suppressed placental expression of the PIGF gene, suggesting that reduced PIGF availability negatively impacts placental vascular development and increases pregnancy loss risk. These results may establish PIGF as a potential molecular marker for identifying high-risk pregnancies in the first trimester and warrant further investigation into PIGF-targeted therapeutic interventions.

KEYWORDS: Angiogenesis, Placental growth factor, Early pregnancy loss, Gene expression, Vascular insufficiency, Pregnancy complications.

INTRODUCTION

Early pregnancy loss remains among the most frequent adverse maternal health outcomes, occurring in approximately 12-15% of clinically recognized pregnancies within the first 20 weeks of gestation [1]. Recurrent pregnancy loss impacts 1-3% of couples attempting conception. The etiology of approximately half of all first-trimester losses remains unidentified despite comprehensive diagnostic testing, pointing to the existence of under recognized biological mechanisms contributing to pregnancy failure[2-4].

Well-characterized risk factors for early pregnancy loss comprise advanced maternal age, previous recurrent losses, active smoking, alcohol use, and genetic or chromosomal abnormalities in the developing embryo[2,5]. However, many individuals presenting with pregnancy loss lack identifiable conventional risk factors. Additionally, a substantial proportion of cases lack obvious abnormalities in routine biochemical markers such as beta-human chorionic gonadotropin (β -hCG) levels, yet progress toward fetal demise.

Recent evidence has accumulated suggesting that molecular and biochemical derangements at the placental implantation site—including inflammatory processes, oxidative damage, and impaired endothelial cell function—may trigger early pregnancy failure. Oxidative stress within chorionic tissues appears to disrupt normal placental development and may precipitate placental dysfunction leading to preeclampsia and intrauterine growth restriction, particularly during first trimester when placental structure is being established[6-12].

Multiple angiogenic and angiostatic factors have emerged as critical regulators of pregnancy success. PIGF (placental growth factor) and VEGF (Vascular endothelial growth factor) represent key mediators of new blood vessel formation and endothelial cell maturation necessary for normal placental development [13-15]. Conversely, excessive expression of anti-angiogenic molecules, such as soluble fms-like tyrosine kinase-1 (sFlt-1), may inhibit angiogenic signaling and cause pregnancy complications and loss[16].

Published research indicates that dysregulated expression of angiogenic factors (including low VEGF levels and elevated anti-angiogenic factor expression) characterizes women experiencing recurrent pregnancy loss, preeclampsia, and growth-restricted pregnancies[9,12]. Recent studies have demonstrated associations between altered VEGF family member expression and pregnancy complications. Specifically, data indicate that reduced PIGF availability may contribute to recurrent pregnancy loss and spontaneous abortion. Some investigations have identified differential PIGF expression in spontaneous abortion compared to intentional pregnancy termination, suggesting PIGF dysregulation may be specific to failed pregnancies[13-15].

Research Gap: Conventional screening approaches—including measurement of markers such as serum β -hCG—can assess pregnancy viability but do not elucidate the pathophysiological mechanisms underlying approximately 50% of first-trimester losses. Our laboratory investigation was conceived to explore whether novel molecular biomarkers at the angiogenic level might enhance identification of high-risk pregnancy status and clarify biological mechanisms of loss. PIGF was selected as a candidate marker because of its established role in placental vascular development and preliminary evidence of dysregulation in pregnancy complications.

MATERIALS AND METHODOLOGY

Study Design and Setting:

A cross-sectional comparative investigation has been carried out at two institutions: Department of Obstetrics and Gynecology (HSK Hospital & Medical Sciences Research Centre, Bagalkot) and the Department of Biochemistry (BLDE [Deemed to be University], Vijayapura, Karnataka, India). Data collection spanned April 2023 through December 2024.

Ethical Approval:

Study authorization was obtained from Institutional Ethics Committee of S. Nijalingappa Medical College, Bagalkot (Reference No. SNMC/IECHSR/2021-2022/A-102/1.0, dated 4 January 2023). Prior to enrollment, each study participant provided written informed consent.

Study Population:

Inclusion criteria:

Case Group (n=30): Women aged 19-45 years presenting at 9-20 weeks of gestation with clinical and ultrasound evidence of early pregnancy loss (absence of fetal cardiac activity on ultrasound) accompanied by vaginal bleeding and/or abdominal pain were enrolled as the case group.

Exclusion Criteria:

Participants were excluded if they had: evidence of intrauterine infections(TORCH); antiphospholipid antibody syndrome, thyroid dysfunction, related (consanguineous) spouses; known chromosomal disorders or significant malformations, congenital uterine anomalies, or other systemic medical conditions.

Sample Collection and Processing:

Peripheral venous blood (5 mL) was obtained using sterile phlebotomy technique with collection into PAX gene tubes for RNA stabilization. Villous placental tissue (approximately 0.5-1 cm³) was collected from aborted tissue specimens and immediately immersed in RNAlater solution for RNA preservation and subsequent analysis.

Total RNA extraction from placental tissue was performed utilising a commercial RNA isolation kit (RNeasy Mini Kit, Qiagen) based on manufacturer specifications. RNA quantity and purity were verified by spectrophotometry (Nanodrop, Thermo Scientific).

Primer Design and Sequence Validation:

Oligonucleotide primers for the PIGF target gene and the β -actin reference gene were custom synthesized following retrieval of cDNA sequences from NCBI (National Center for Biotechnology Information) GenBank database.

PIGF forward primer: 5'-GGCTGTTCCCCTTGCTTCC-3'

PIGF reverse primer: 5'-TACCACTTCCACTCTGACC-3'

β -actin forward primer: 5'-GCCTGCCGATCCGC-3'

β -actin reverse primer: 5'-GCCGTAGCCGTTTGCG-3' [17]

Primer sequences were aligned against known transcriptome databases (Clustal Omega) to confirm target specificity. Primer design validation was completed using Basic Local Alignment Search Tool (BLAST) and Primer Quest (Integrated DNA Technologies) searches via NCBI for verifying absence of off-target alignments.

cDNA Synthesis and qRT-PCR Protocol:

Reverse transcriptase and quantitative PCR amplification were used to create complementary DNA from extracted RNA (QuantiTect RT-PCR Kit, Qiagen). TaqMan chemistry (Applied Biosystems) was used for real-time quantitative PCR. 20 ng of cDNA template, 10 μ L of 2 $\times$ SYBR Green Master Mix (Takara), and 100 nM of forward and reverse oligonucleotides in a final reaction volume of 20 μ L have been included in each reaction.

The following cycling parameters were used for amplification on a real-time PCR system (Applied Biosystems, Step One): 40 cycles of denaturation (95°C, 5 secs) and combined annealing-extension (58°C, 30 secs) after the initial enzyme activation at 95°C for 30 seconds. To verify amplicon specificity, a final dissociation analysis has been conducted at 95°C for 15 secs, 60°C for 30 secs, 95°C for 15 secs [18] .

Statistical Analysis:

Relative PIGF mRNA expression was calculated utilizing $2^{-\Delta\Delta Ct}$ methodology (Livak method), with β -actin serving as the internal reference gene. We used a paired two-tailed Student's t-test to compare case and control groups, with $\alpha = 0.05$ as the significance level. SPSS statistical software (version 19.0) has been utilised for the analyzes. Statistical significance has been defined as a p-value<0.05.

RESULTS

Study Population Characteristics:

Participant demographics are displayed in Table 1. Control group's mean age was 22.8 ± 2.08 years, while the mean age of women who experienced pregnancy loss was 23.1 ± 2.11 years ($p = 0.531$, not significant). Gestational age at enrollment was comparable between groups: 12.61 ± 1.27 weeks in cases versus 11.21 ± 1.01 weeks in controls ($p = 0.418$, not significant).

Table 1. Demographic Profile of Study Participants

	Cases (N=30)
Age (years)	23.1 ± 2.11
Gestational (weeks)	12.61 ± 1.27

N = number of participants

PIGF Gene Expression Results

Quantitative real time PCR analysis was performed to measure relative PIGF mRNA abundance in maternal blood samples and placental tissue obtained from cases blood and aborted placenta. Results are presented in Table 2.

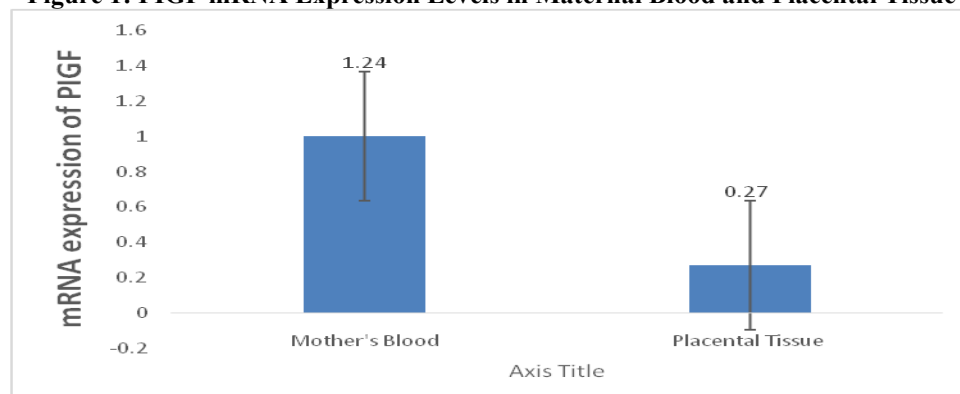
Table 2: Fold change expression values in blood and placental tissue of cases

Gene	ΔCt	$\Delta\Delta Ct$	$2^{-\Delta\Delta Ct}$	SD	SE	p value
PIGF gene expression in blood (N=30)	8.67	0.31	1.24	0.37	0.21	<0.001
PIGF gene expression in placental tissue (N=30)	8.36	-1.91	0.27	0.31	0.19	

N = number of participants, SD= Standard Deviation, SE = Standard Error,* $p < 0.05$ considered significant

Our analysis demonstrated markedly reduced PIGF mRNA transcript abundance in placental tissue derived from women with early pregnancy loss in comparison to blood sample of same cases. Specifically, PIGF expression was suppressed approximately 32-fold in the loss group (0.27 fold change, $p < 0.001$) relative to the blood expression pregnancy group. Maternal serum PIGF expression showed less pronounced but still significant suppression in the loss group.

Figure 1: PIGF mRNA Expression Levels in Maternal Blood and Placental Tissue



[Bar chart showing fold change in PIGF expression comparing Mother's Blood and Placental Tissue in pregnancy loss cases]

DISCUSSION

Angiogenesis and Placental Development:

The formation and maturation of the placental vascular network represents an essential developmental process enabling fetal oxygenation and nutrient exchange necessary for embryonic growth. Placental angiogenesis

depends upon coordinated signaling through multiple growth factors and cytokine cascades. Key factors regulating this process include VEGF family members and placental growth factor, which promote endothelial cell proliferation, migration, and tube formation [13].

Role of PlGF in Implantation and Early Development:

The endometrial tissue shows elevated expression of placental growth factor during the peri-implantation period, providing evidence that PlGF signaling facilitates trophoblast invasion and spiral artery remodeling necessary for pregnancy establishment. Fms-like tyrosine kinase-1 (Flt-1) represents the cognate receptor for PlGF in the receptor tyrosine kinase family. VEGF and PlGF demonstrate overlapping but distinct binding affinities for Flt-1, creating a competitive signaling environment where relative abundance of each ligand determines downstream biological effects [19].

The placental trophoblast comprises the principal endogenous source of PlGF during pregnancy, with expression showing a characteristic increase during the first and second trimester, peaking around 28-32 weeks of gestation. Early elevations in trophoblastic PlGF are thought to support placental vascular remodeling [20].

PlGF in Complicated Pregnancies:

Observations from studies of pregnancies with poor outcomes—including ectopic implantation, arrested development, and termination—have documented reduced PlGF levels in both maternal serum and trophoblastic tissue. A fundamental biochemical principle holds that approximately one-half of early pregnancy losses lack identifiable etiological factors despite exhaustive investigation, suggesting operation of molecular mechanisms beyond current diagnostic capabilities [21].

Circulating PlGF typically reaches peak concentrations during late second trimester. Women with preeclampsia and other placental-mediated disorders characteristically exhibit reduced PlGF concentrations relative to healthy pregnancy, establishing PlGF as a potential prognostic marker for high-risk pregnancies [18,22,23].

Interpretation of Current Findings:

The current investigation documents a marked (32-fold) reduction in PlGF mRNA expression within placental tissue from pregnancies ending in early loss compared to viable pregnancies. This substantial differential suggests that suppressed PlGF transcription may represent a pathophysiological mechanism underlying loss in a subset of cases. The tissue-specific nature of this finding (greater change in placenta than in blood) supports a local placental origin for the dysregulation.

In contrast, a 2021 investigation by Galaziou and colleagues examining VEGF and PlGF expression in spontaneous versus induced abortion found minimal differences between abortion types for PlGF, with a slight increase observed in spontaneous abortion. However, their work focused on a different gestational age window and examined different molecular endpoints, limiting direct comparison [16].

More recent work by Ezter Toth et al. (2024) demonstrated significant downregulation of PlGF in recurrent pregnancy loss cases relative to controls—findings concordant with the present results and supporting the hypothesis that PlGF deficiency characterizes at least a subset of pregnancies with unexplained loss [24].

The observation that peripheral blood PlGF shows less dramatic suppression (approximately 3.4-fold reduction) than placental PlGF (32-fold reduction) merits consideration. Maternal circulating PlGF may derive from sources beyond the placenta, including endothelial cells and other peripheral tissues. Therefore, placental PlGF measurement may provide superior diagnostic specificity for identifying placental insufficiency compared to blood-based assessment alone.

CONCLUSION

This investigation provides evidence that early pregnancy loss is related to significantly diminished placental expression of the PlGF gene. The marked suppression of PlGF transcription suggests that compromised angiogenic signaling through the PlGF pathway may represent a contributing pathophysiological mechanism in a subset of unexplained early pregnancy losses. These findings support the emerging concept that impaired placental vascular development, mediated through dysregulation of pro-angiogenic factors including PlGF, constitutes material biological risk factor for early pregnancy failure.

Further research elucidating the upstream regulators of PlGF expression in pregnancy and investigation of PlGF-targeted therapeutic interventions may provide novel avenues for identifying at-risk pregnancies and developing preventive strategies to reduce the burden of early pregnancy loss. The application of PlGF assessment as a biomarker in first-trimester risk stratification warrants exploration in future prospective investigations.

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