

HIF-1 Alpha and Placental Growth Factor in Pregnancies Complicated With Preeclampsia: A Qualitative and Quantitative Analysis

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Background: The pathophysiology of preeclampsia is not clearly understood worldwide. Hypoxia inducible factor 1 α (HIF-1 α) is thought to be the preliminary factor for the hypoxic conditions prevailing in preeclampsia, which causes imbalance in the expression of angiogenic proteins. A proangiogenic protein, placental growth factor (PIGF), is reported to be dysregulated in preeclampsia. Therefore, this study focuses on the investigation of HIF-1 α and PIGF in preeclamptic conditions and a possible molecular association between them. **Methods:** Placental tissue ($n = 45 + 45$) and serum samples ($n = 80 + 80$) of preeclamptic patients and healthy control were collected and processed for the analysis of HIF-1 α and PIGF by immunohistochemistry and enzyme-linked immunosorbent assay (ELISA). **Results:** In preeclamptic group, the significant nuclear and cytoplas-

mic expression of HIF-1 α was noticed in syncytiotrophoblast ($P = 0.0001$) but in control placenta, it was localized to cytoplasm ($P = 0.0001$). The intensity of PIGF expression was lower in syncytiotrophoblast cytoplasm ($P = 0.0001$) in preeclamptic cases as compared with control. Also, the significant upregulated concentration of HIF-1 α and downregulated PIGF was observed in serum samples of preeclamptic woman ($P = 0.0001$). Thus, there was a significant direct negative correlation between HIF-1 α and PIGF both at tissue and serum level ($P < 0.01$). **Conclusion:** The direct inverse association between HIF-1 α and PIGF in serum and placental tissues may be responsible for the low oxidative stress and endothelial dysfunction, leading to the pathogenesis of preeclampsia. J. Clin. Lab. Anal. 30:75–83, 2016. © 2014 Wiley Periodicals, Inc.

Key words: preeclampsia; hypoxia; HIF-1 α ; PIGF

INTRODUCTION

Preeclampsia is a heterogeneous pregnancy disorder that is characterized by hypertension and proteinuria mostly developing in late pregnancy. It affects 2–8% of pregnancies worldwide (1, 2). The incident rate of preeclampsia is 2.8% higher in developing countries like India as compared to that of developed nations (3). In spite of extensive research in the pathophysiology of this disease, the etiology is still poorly understood. It may be due to insufficient adaptation of decidual and intramyometrial portion of spiral arterioles or due to shallow trophoblastic invasion, resulting in reduced uteroplacental blood flow leading to placental hypoxia (4). Placental hypoxia results in the release of several mediators into mater-

nal circulation, which causes endothelial dysfunction prevailing in preeclampsia. The key mediator of the hypoxic condition is the hypoxia inducible factor 1 (HIF-1). HIF-1 is involved in transcription of many oxygen-dependent genes that encode for proteins associated with

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angiogenesis and cell metabolism. HIF-1 consists of two dimeric subunits α and β . HIF-1 α is hypoxia inducible, while HIF-1 β is constitutively expressed (5). During low oxygen conditions, HIF-1 α is highly expressed and helps in development of placenta in early gestation. Overexpression of HIF-1 α has been observed in many inflammatory disorders, including cancer and preeclampsia (6). Hypoxia induces angiogenesis by regulating angiogenic proteins such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and FLT-1. The change in concentration of these proteins causes angiogenic imbalance, leading to endothelial damage and the onset of preeclampsia. PlGF is a proangiogenic protein and member of the vascular endothelial growth factor (VEGF) family. It is one of the key molecules in angiogenesis and vasculogenesis especially during embryogenesis and placental trophoblast is the main source of PlGF throughout the gestational period of pregnancy (7). It shares structural as well as amino acid sequence similarity with VEGF, but PlGF has binding affinity only for VEGF receptor 1 (VEGFR-1; (8)). The inter- and intramolecular cross-talk between the VEGFR-1 and VEGFR-2 is regulated by PlGF. It binds to VEGFR-1 and displaces VEGF from this receptor, which results in activation and intermolecular transphosphorylation of VEGFR-2 thereby amplify the VEGF-induced angiogenesis (9). But soluble form of VEGFR-1 (known as sFLT-1) inhibit the interaction of PlGF and its receptor that causes endothelial dysfunction, a manifestation of preeclampsia (10). Although extensive studies have been conducted to investigate the expression of these two proteins in preeclampsia, but hardly any report is available regarding the serum analysis of HIF-1 α in preeclamptic patients as well as the association of HIF-1 α with the angiogenic protein PlGF at tissue and serum level. Thus, this study focused on the quantification of serum levels of HIF-1 α and finding the possible molecular link between the expression of HIF-1 α and PlGF in preeclamptic Indian women compared with normotensive pregnancy.

MATERIAL AND METHODOLOGY

Sample

Placental tissue as well as serum samples of preeclamptic patients and age-matched controls were retrieved from the department of Obstetrics and Gynaecology, Safdarjung Hospital, New Delhi, India. All the preeclamptic patients included in our study had hypertension (systolic BP > 140 mmHg and diastolic BP > 90 mmHg) and proteinuria exceeding 0.3 g/day after 20th week of gestation. Ethical approval was obtained from the institutional ethics committee of VMMC and Safdarjung Hospital, New Delhi, India and written informed consent was obtained

from all the patients prior to sample collection. The placental samples were rinsed with normal saline to remove excess blood followed by perfusion with buffered 10% formalin (pH 7.0) and then processed for paraffin embedding sectioning for Immunohistochemistry (IHC). The serum was separated from blood samples by centrifugation at 12,000 rpm for 15 min for enzyme-linked immunosorbent assay (ELISA) analysis and stored at -20°C . The clinicopathological parameters of patients are summarized in Table 1. All the placental samples ($n = 45 + 45$) and serum samples ($n = 80 + 80$) were processed for IHC and ELISA, respectively.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue sections of 5 μ thickness were collected and processed for conventional histological assessment by Haematoxylin and Eosin (H & E) staining. The immunoexpression of HIF-1 α and PlGF was analyzed by immunohistochemistry. In

TABLE 1. Clinicopathological Characteristics of the Study Groups

Clinicopathological parameters	Control ($n = 45$)	Preeclampsia ($n = 45$)	<i>P</i> -value
Maternal age (years; mean \pm SE)	21.53 \pm 0.410	22.29 \pm 0.446	0.216
Gestational age (weeks; mean \pm SE)	37.62 \pm 0.485	38.58 \pm 0.366	0.120
Gravidity (no. /%)			
1	22/48.9	23/51.1	0.891
2	18/40.0	16/35.6	
3	5/11.1	6/13.3	
Parity (no. /%)			
0	22/48.9	21/46.7	0.945
1	18/40.0	18/40.0	
2	5/11.1	6/13.3	
Systolic blood pressure (mmHg; mean \pm SE)	117.73 \pm 0.368	145.07 \pm 1.730	0.0001*
Diastolic blood pressure (mmHg; mean \pm SE)	77.40 \pm 0.520	100.13 \pm 1.247	0.0001*
Urine albumin (no. /%)			
0	45/100	0/0.0	0.0001*
1	0/0.0	15/33.3	
2	0/0.0	19/42.2	
3	0/0.0	11/24.4	
Edema (no. /%)			
0	45/100	31/68.9	0.0001*
1	0/0.0	14/31.1	
Baby weight (kg; mean \pm SE)	3.133 \pm 0.0360	2.016 \pm 0.0360	0.0001*
Mode of delivery (no. /%)	VD = 42/93.3 CS = 3/6.7	VD = 39/86.7 CS = 6/13.3	0.292

Data are represented as mean \pm standard error or number/percentage as per requirement. Mann-Whitney *U*-test and Wilcoxon *W* test (Asymp. sig. (two-tailed)).

**P* < 0.05 is considered significant.

brief, the sections were deparaffinized in xylene, dehydrated by graded alcohols followed by blocking of endogenous peroxidase activity by 0.03% H₂O₂ in methanol for 15 min. The slides were then retrieved for antigen in 10 mM citrate buffer (pH 6.0) by heating the sections at 900 W for 15 min and at 360 W for 10 min in microwave oven. Further, the sections were incubated overnight with primary antibodies for HIF-1 α (concentration 1:70 [mouse anti-human monoclonal HIF-1 α antibody ab1-250; Abcam, Inc., Cambridge, UK]) and PIGF (concentration 1:40 [rabbit anti-human polyclonal PIGF antibody ab97618; Abcam, Inc.) in humid chamber, at 4°C. Next day, after brief washing with TBS (Tris buffer saline, pH 7.4), the serial sections were incubated with Envision™ (dextran conjugated with peroxidase and incorporated with molecules of secondary antibody against both immunoglobulins of mouse and rabbit, Dako Cytomation, Glostrup, Denmark) for 1 hr at room temperature. The 3,3-diaminobenzidine hydrochloride (DAB) was used for the chromogenic visualization reaction, counterstained with Mayer's hematoxylin, and mounted. The serial sections were then examined under light microscope (Olympus BX-51, Japan). In the negative control, isotype-specific immunoglobulin G was used in place of primary antibody.

ELISA

The serum samples of control ($n = 80$) and preeclampsia ($n = 80$) were quantitatively analyzed for both HIF-1 α and PIGF by sandwich ELISA. For HIF-1 α , Cusabio ELISA kit was used and manufacturer's protocol was followed. The minimum detectable dose of human HIF-1 α was typically less than 15.6 pg/ml. For PIGF, the ELISA kit of R&D System (Minneapolis, Minnesota) was used and manufacturer's protocol was followed. The minimum detectable dose of human PIGF was typically less than 7 pg/ml.

Scoring of Immunohistochemical Staining

Evaluation of immunohistochemistry for all proteins was performed on two arbitrarily chosen slide of each case (both preeclamptic and control). Result of immunohistochemistry was evaluated by two observers and followed the scoring criterion given by Tripathi et al. (11). Protein expression was semiquantified with regard to the intensity of cell staining graded as 0–3 ("0" for negative staining if there is total absence, "1" for mild staining, "2" for moderate, "3" for intense staining). *H*-score was calculated for both preeclampsia and control using the formula, $H\text{-score} = P(S + 1)$, where *P* represent the aggregate percentage of stained cells and *S* represents the intensity of the cell.

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 statistics software (SPSS, Inc., Chicago, IL). These data are represented as mean \pm standard error. Chi-square test was carried out to determine the significance of protein expression among control and preeclamptic placenta. The significance of clinicopathological parameters of preeclamptic patients and control was determined with Wilcoxon's *W*-test and Mann–Whitney's *U*-test (Asymp. sig. [two-tailed]). The associations between proteins HIF-1 α and PIGF was explored using Pearson correlation test (two-tailed). The *P* values less than 0.05 were regarded as significant for Mann–Whitney's *U*-test (Asymp. sig. [two-tailed]) and less than 0.01 was considered significant for Pearson correlation test [two-tailed].

The receiver operating characteristic (ROC) analysis was also carried out to find the significance of immunohistochemistry result. For ELISA results, Wilcoxon's *W*-test and Mann–Whitney's *U*-test (Asymp. sig. [two-tailed]) as well as box plot were performed. Results were considered significant, when *P* was less than 0.05.

RESULTS

The clinicopathological characteristics, such as baby weight, urine albumin, edema, systolic and diastolic blood pressure of preeclamptic mother, were found to be statistically significant ($P = 0.000$), while there was no significant difference between control and preeclamptic group in terms of maternal age, gestational age, gravidity, and parity ($P = 0.216$, $P = 0.120$, $P = 0.0.891$, $P = 0.945$, respectively; Table 1).

Immunohistochemistry

The IHC analysis revealed that HIF-1 α was localized both in nucleus and cytoplasm of syncytiotrophoblast in preeclamptic placental tissue. The nuclear expression was intense in 64.4% (29/45), moderate in 22.2% (10/45), and mild in 13.3% (6/45) cases. However, the intensity of HIF-1 α in cytoplasm was moderate in 8.9% (4/45) and mild in 91.1% (41/45) of preeclamptic group. While in the control group, cytoplasmic expression was noticed. The expression was intense in 42.2% (19/45), moderate in 40.0% (18/45), and mild in 17.8% (8/45). And only 6.6% (3/45) cases showed the nuclear expression of HIF-1 α in control. Both nuclear and cytoplasmic expression was found to be significant in preeclampsia as well as in control ones ($P = 0.0001$; Fig. 1A to C; Table 2).

The immunoreactivity of PIGF revealed a significant downregulation in the cytoplasm of syncytiotrophoblast of preeclamptic placenta. The intensity was mild in 95.6% (43/45) and moderate in 4.4% (2/45) of the study group.

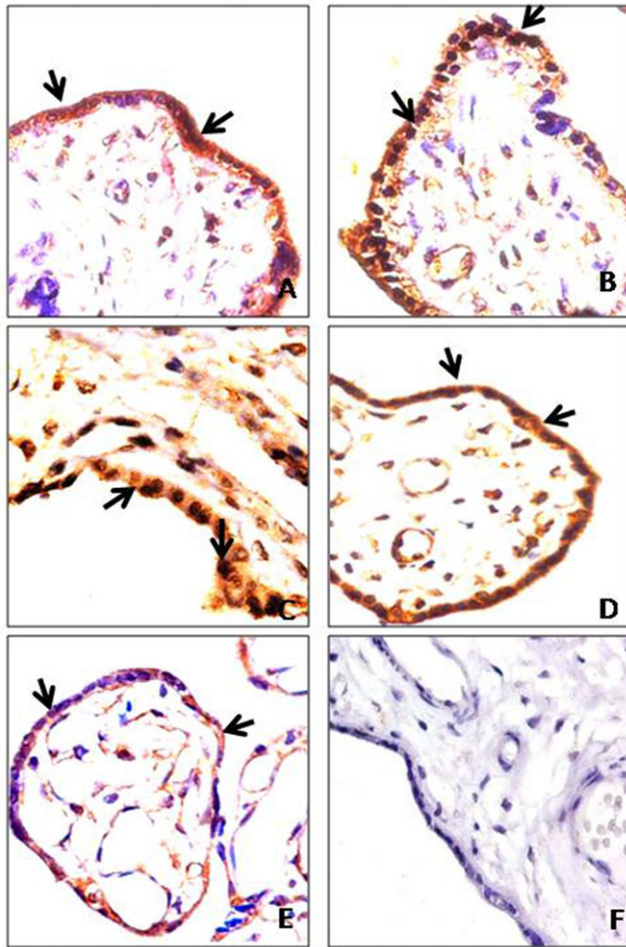


Fig. 1. (A) Control placenta (40 weeks) showing moderate cytoplasmic expression of HIF-1 α in syncytiotrophoblast; (B and C) preeclamptic placenta (38 and 36 weeks) showing nuclear accumulation of HIF-1 α in syncytiotrophoblast; (D) control placenta (32 weeks) showing moderate cytoplasmic expression of PIGF in syncytiotrophoblast; (E) preeclamptic placenta (32 weeks) showing mild cytoplasmic expression of PIGF in syncytiotrophoblast; (F) negative control incubated with IgG showing placental villi. Arrow shows the expression of protein. Magnification: 400 \times .

However, the cytoplasmic expression was moderate in 44.4% (20/45) and intense in 55.6% (25/45) of the control placenta. In both control and preeclamptic cases, PIGF expression was found to be significant ($P = 0.0001$; Fig. 1D and E; Table 2). Although cytotrophoblast was present in some of the studied preeclamptic cases, the immunorexpression of HIF-1 α and PIGF was not significant.

The *H*-score analysis was carried out for control as well as experimental group to assess the intensity of HIF-1 α and PIGF at nuclear and cytoplasmic level. The mean and standard error value of HIF-1 α was higher (312.22 ± 11.132) for nuclear expression in preeclamptic group, but in control the mean and standard error

TABLE 2. Assessment of IHC for HIF-1 α and PIGF in Control and Preeclamptic Placenta

Protein	Expression	Preeclampsia	Control	<i>P</i> values
		(<i>N</i> = 45) n (%)	(<i>N</i> = 45) n (%)	
HIF-1 α nuclear	No staining	0 (0.0)	42(93.3)	0.0001*
	Mild	06 (13.3)	3 (6.6)	0.0001*
	Moderate	10 (22.2)	0 (0.0)	0.0001*
	Intense	29 (64.4)	0 (0.0)	0.0001*
HIF-1 α cytoplasmic	No staining	4(8.9)	0(0.0)	0.0001*
	Mild	41 (91.1)	8 (17.8)	0.0001*
	Moderate	0 (0.0)	18 (40.0)	0.0001*
PIGF	No staining	0 (0.0)	19(42.2)	0.0001*
	Mild	43(95.6)	0 (0.0)	0.0001*
	Moderate	2 (4.4)	20(44.4)	0.0001*
	Intense	0 (0.0)	25 (55.6)	0.0001*

Pearson chi-square test (Asymp. sig. [two-tailed]).

* $P < 0.05$ is considered significant.

N, no. of cases.

TABLE 3. *H*-Score Assessment of HIF-1 α and PIGF in Control and Preeclamptic Placenta

Study group	HIF-1 α		PIGF
	Nucleus (mean \pm SD)	Cytoplasm (mean \pm SD)	Cytoplasm (mean \pm SD)
Control (<i>N</i> = 45)	102.13 \pm 4.875	275.69 \pm 11.872	314.20 \pm 7.305
Preeclampsia (<i>N</i> = 45)	312.22 \pm 11.132	167.62 \pm 4.353	177.51 \pm 3.275
<i>P</i> -value	0.0001	0.0001	0.0001

Mann–Whitney *U*-test and Wilcoxon *W* test (Asymp. sig. [two-tailed]).
 $P < 0.05$ is considered significant.

N, no. of cases.

was more for cytoplasmic expression (275.69 ± 11.872 ; Table 3, Fig. 2A). In case of PIGF, the preeclamptic cytoplasmic expression was lower (177.51 ± 3.275) than the control group (314.20 ± 7.305 ; Table 3, Fig. 2B).

The ROC curve analysis revealed the significant nuclear expression of HIF-1 α in preeclamptic group as compared with controls ($P = 0.0001$, Fig. 3A) with area under curve of 0.989, specificity 88.9%, and sensitivity 88.9%. However, PIGF was significantly downregulated in preeclamptic group as compared with controls ($P = 0.0001$, Fig. 3B) with area under curve of 0.962, specificity 82.2%, and sensitivity 82.2%.

ELISA

The Mann–Whitney test showed that the serum concentration of HIF-1 α was higher (mean = 6.581 pg/ml) in preeclamptic cases than that of control group (mean

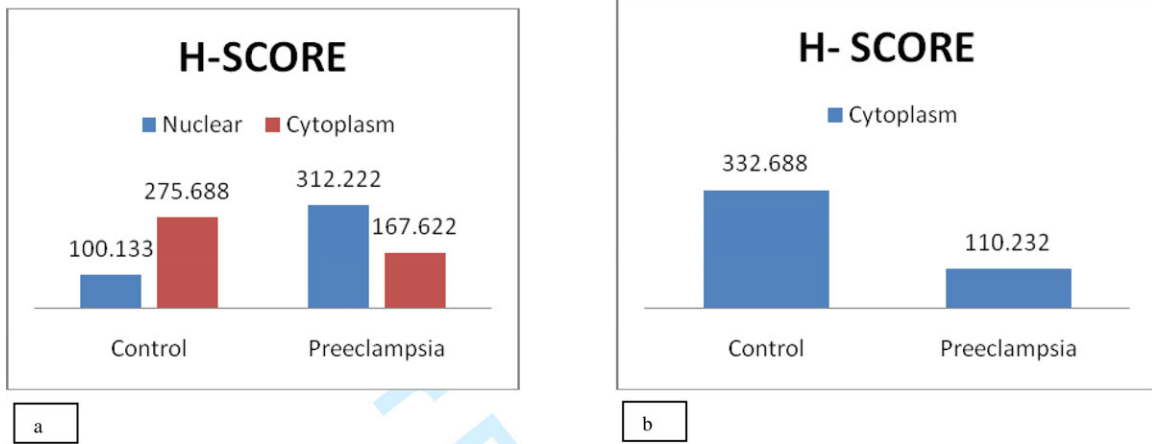


Fig. 2. Comparison of immunohistochemical *H*-score analysis. (A) HIF-1 α expression in cytoplasm and nucleus of syncytiotrophoblast cells of control and preeclamptic patients. (B) PIGF expression in cytoplasm of syncytiotrophoblast cells of control and preeclamptic group.

= 4.947 pg/ml). For PIGF, the serum concentration of control was increased (mean = 6.333 pg/ml), while that of preeclampsia was lower (mean = 3.939 pg/ml). The concentration of HIF-1 α and PIGF in serum of control and preeclamptic patients was represented as mean \pm standard error in Table 4.

According to Box plot analysis, the serum levels of HIF-1 α were higher (median 6.640 pg/ml) in preeclamptic cases as compared with the control ones (median = 4.945 pg/ml; Fig. 4A); however, for PIGF, the serum levels were found to be downregulated significantly (median = 3.434 pg/ml) in preeclamptic group than that of control (median = 7.462 pg/ml; Fig. 4B).

Correlation Between HIF-1 α and PIGF

The significant negative association was noted between HIF-1 α nuclear and PIGF cytoplasmic expression in patients suffering from preeclampsia ($r = -0.196, P < 0.05$; Table 5). There was also a significant inverse correlation between the serum levels of HIF-1 α and PIGF in preeclamptic group ($r = -0.220, P < 0.05$; Table 6).

DISCUSSION

Hypoxic environment is essential for the invasion and infiltration of cytotrophoblast into the maternal decidua

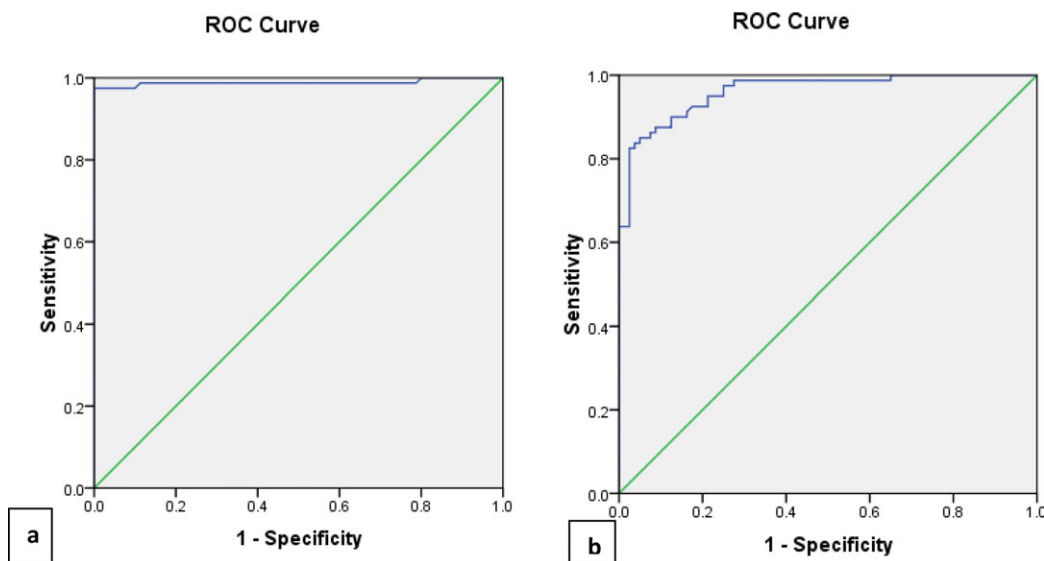


Fig. 3. ROCs curve showing the expression of HIF-1 α and PIGF to differentiate preeclamptic group from control. (A) HIF-1 α (AUC = 0.989, sensitivity = 88.9%, specificity = 88.9%). (B) PIGF (AUC = 0.962, Sensitivity = 82.2%, specificity = 82.2%).

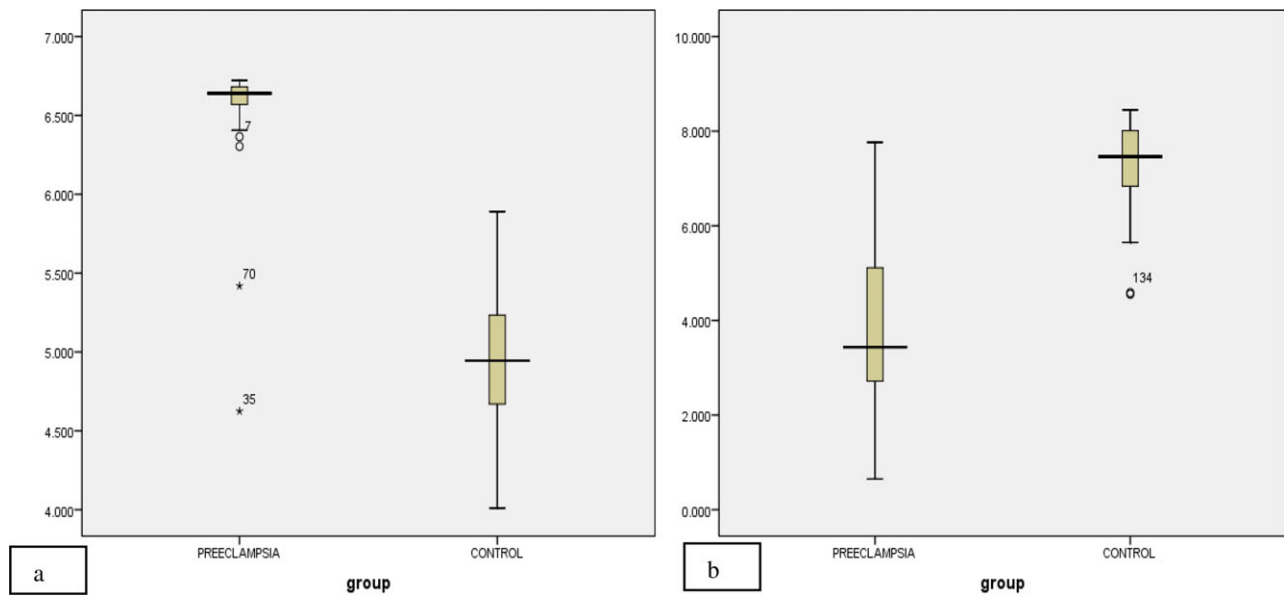


Fig. 4. Box plot showing the serum concentration (pg/ml) of (A) HIF-1 α and (B) PIGF in control and preeclamptic patients. The solid bar indicates median, upper, and lower limits of box, 75th and 25th percentiles; upper and lower bars, maximum and minimum values ($P < 0.05$).

TABLE 4. HIF-1 α and PIGF Concentration (pg/ml) in Serum of Control and Preeclamptic Cases

Study group	HIF-1 α (mean \pm SE)	PIGF (mean \pm SE)
Control ($N = 80$)	4.947 \pm 0.045	6.335 \pm 0.093
Preeclampsia ($N = 80$)	6.581 \pm 0.030	3.939 \pm 0.179
<i>P</i> -value	0.0001*	0.0001*

Mann-Whitney *U*-test and Wilcoxon *W* test (Asymp. sig. [two-tailed]).

*Significant.

$P < 0.05$ is considered significant.

N, no. of cases.

TABLE 5. Correlation of HIF-1 α and PIGF in Placental Tissue of Preeclamptic Patients

Protein	No. of cases	HIF-1 α nuclear	PIGF cytoplasm
HIF-1 α nuclear	45	1	$r = -0.752^*$ $P = 0.0001$
PIGF cytoplasm	45	$r = -0.752^*$ $P = 0.0001$	1

Pearson correlation test (two-tailed); *r*, correlation coefficient.

*Correlation is highly significant at 0.01 level.

for maintenance of materno-fetal circulation at early periods of pregnancy. But its prevalence in later stages of pregnancy causes several complications that may lead to maternal and fetal morbidity and mortality (12, 13). Under hypoxic conditions, HIF-1 α plays a vital role in multiple physiological responses, such as erythropoiesis, glycolysis, and also affects the transcription of VEGF

TABLE 6. Correlation of HIF-1 α and PIGF in Serum Samples of Preeclamptic Group

Protein	No. of cases	HIF-1 α	PIGF
HIF-1 α	80	1	$r = -0.220^*$ $P = 0.05$
PIGF	80	$r = -0.220^*$ $P = 0.05$	1

Pearson correlation test (two-tailed); *r*, correlation coefficient.

*Correlation is highly significant at 0.01 level.

gene that counteract angiogenesis (14). It also has an important role in vascularization and survival of embryos, pulmonary vascular remodeling, and vascularization of tumors (15). Caniggia and Winter noticed the increased expression of HIF-1 α mRNA and protein in placental tissues of preeclamptic patients (16). Several researchers also showed the overexpression of HIF-1 α in preeclamptic human placenta that modulates the pathogenesis of preeclampsia (17–19). It has been observed that during hypoxic conditions, HIF-1 α gets stabilized and translocates to nucleus from cytoplasm (20, 21). In the present study, the immunoreactivity of HIF-1 α in preeclamptic placental tissue showed significant nuclear expression ($P = 0.0001$) in syncytiotrophoblast. However, cytoplasmic expression of HIF-1 α with low intensity was also observed in preeclamptic tissues ($P = 0.0001$). While in the control group, significant cytoplasmic immunoreactivity was noticed in syncytiotrophoblast ($P = 0.0001$). Thus, there is possibility of significant translocation of HIF-1 α

from cytoplasm to nucleus, leading to low oxidative stress prevailing in preeclampsia.

Many researchers have worked on serum concentration of HIF-1 α in different pathological conditions, such as cancer. Zhang et al. observed the overexpression of HIF-1 α in serum of primary hepatocellular carcinoma (PHC) (22). To the best of our knowledge, no investigator has evaluated the expression of HIF-1 α in serum samples of preeclamptic patients. Therefore, in this study quantification of the serum concentration of HIF-1 α in maternal circulation of both control and preeclamptic patients was carried out. The ELISA analysis showed that the mean serum concentration of HIF-1 α was higher (mean = 6.581 pg/ml) in preeclamptic cases than that of control group (mean = 4.947 pg/ml), which infer that the maternal serum levels of HIF-1 α also get significantly elevated during pathogenesis of preeclampsia ($P < 0.05$).

PIGF, an angiogenic protein, plays an important role in placental development and is expressed in villous cytotrophoblast and syncytiotrophoblasts in placenta (23). Various researchers had noticed the increased expression of PIGF gene in normal trophoblast, while the expression was reduced in preeclampsia (24). Torry et al. found in normal pregnancy PIGF is highly expressed in trophoblasts and its expression is significantly downregulated in preeclampsia by low oxygen tension (25). The same group in their another study observed the decreased PIGF mRNA expression in preeclamptic trophoblast (26). In the present study, the immunoreactivity of PIGF revealed a significant downregulation of PIGF ($P = 0.0001$) in the cytoplasm of syncytiotrophoblast of preeclamptic placenta. However, the intensity of cytoplasmic expression in the control placenta was lower in comparison to preeclamptic cases ($P = 0.0001$). Therefore, this study coincides with the reports provided by previous authors (24–26) regarding the decreased expression of PIGF in the preeclamptic conditions. Furthermore, maternal serum levels of PIGF were quantified to assess the quantity of PIGF in the preeclamptic conditions. Many researchers had suggested that the abnormal serum levels of PIGF in preeclampsia result in improper trophoblast invasion and the generalized maternal endothelial dysfunction, which leads to preeclampsia (27,28). Some authors also noticed the increased serum concentration of PIGF in normal pregnancy and decreased concentration in preeclampsia (29,30). Livingston et al. also observed that the median concentrations of PIGF were significantly lower in pregnancies complicated by severe preeclampsia than in control (31). Torry et al. also reported the significantly reduced concentration of maternal serum placental growth factor ($P < 0.0001$) in women with preeclampsia than normotensive controls (32). In this study, the significant low concentration of serum PIGF was noticed in preeclamptic group as compared to normal pregnant

woman ($P = 0.0001$), which suggests that the decrease in the serum concentration of PIGF may be responsible for the improper trophoblast invasion, leading to endothelial dysfunction in preeclampsia.

Several researchers have proved that the overexpression of HIF-1 α is associated with the increased maternal serum concentration of soluble Fms-like tyrosine kinase 1 (sFlt1) during hypoxic conditions (33). High circulating levels of sFlt1 exerts an antiangiogenic state that is associated with low levels of proangiogenic factors, such as PIGF, and inhibition of PIGF with its receptor VEGFR-1, causing endothelial dysfunction in preeclampsia (34). Other researchers proved that VEGF and PIGF are dysregulated in preeclampsia due to high levels of sVEGFR-1, which leads to impaired placental angiogenesis (35). The downregulation of trophoblast PIGF gene expression was mediated by hypoxia, which is differentially regulated by HIF-1, and had been observed in previous studies (36,37). HIF-1 α affects the expression of PIGF gene that is dependent on the type of cell and the conditions prevailing in a cell (38). Gobble et al. reported that hypoxia decreases PIGF gene transcription that results in the decreased value of PIGF via mechanisms independent of HIF-1 (24). In this study, the significant negative association was noted between HIF-1 α nuclear and PIGF cytoplasmic expression in patients suffering from preeclampsia ($r = -0.752$, $P = 0.0001$). Also, the statistical analysis of the serum levels of HIF-1 α and PIGF showed a significant negative correlation between the proteins in preeclampsia ($r = -0.220$, $P < 0.05$). The expression of VEGFR-1 (sFLT-1) was also studied in another study and it was upregulated both at tissue and serum level. There was no statistical association found between VEGFR-1 and PIGF (data not shown in this report). Therefore, we hypothesized that the upregulation of HIF-1 α and downregulation of PIGF in serum and placental tissues may be directly associated with the pathogenesis of preeclampsia. Under pathological conditions, due to failure of replacement of vascular smooth muscle and endothelial cells of spiral artery by cytotrophoblast, the spiral artery remodeling do not occur, leading to the prevalence of hypoxic conditions in late gestation of pregnancy. During this period, HIF-1 α may be responsible for the molecular modification of several mediators involved in pathophysiology of pregnancy disorders. In addition, the abnormal expression of angiogenic factor, such as PIGF, may be associated with the hypoxic conditions prevailing in preeclampsia via activation of HIF signaling pathway.

In light of the present study on preeclampsia, the inverse correlation of PIGF and HIF-1 α may offer tremendous promise in screening of preeclamptic patients. Several treatment strategies may be aimed at preventing endothelial dysfunction by using PIGF. Further studies on different angiogenic factors and

hypoxic proteins have also been designed on larger cohorts to understand the exact molecular mechanisms regulating the pathogenesis of preeclampsia. This may allow the development of new preventive strategies for pregnancy complications, such as preeclampsia.

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