ORIGINAL ARTICLE

Effect of Post COVID-19 hypoxia on Placenta of Pregnant Women: A possible Role of Hypoxia Inducible Factor-1α

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ABSTRACT

Background: *The hypoxia-inducible factors (HIF) are considered master regulators of oxygen homeostasis and are oxygen level sensitive. Currently, there is no information regarding the expression of HIF-1α in pregnant women with COVID-19 infection and its potential involvement in the placentation of this condition. Objective: The current study aims to detect the expression of HIF-1α and possible molecular link between the expression of HIF-1α and PLGF.* ***Methodology:*** *A case-control study was conducted in a tertiary university hospital between January and September 2022. Placental tissue of post COVID-19 infected pregnant women (n= 34) and healthy control (n=16) were collected and processed for gene expression of HIF-1α and PLGF by Quantitative real-time PCR.* ***Results:*** *There was a statistically significant higher median level of HIF-1α among cases compared to controls (P<0.001). Moreover, there was a statistically significant higher median level of PLGF among cases compared to controls (P<0.001). There was statistically significant moderate positive correlation between HIF-1α and PLGF gene (r=0.636, P <0.001) among studied samples, however no statistically significant correlation between the two genes among cases and controls groups separately.* ***Conclusion:*** *the higher levels of HIF-1α and PLGF in the placentas of post COVID-infected pregnant women compared to normal pregnant women indicate that COVID-19 hypoxia did not affect the process of placentation. This is confirmed also by the positive correlation between HIF-1α and PIGF in placental tissues among total studied samples.*

INTRODUCTION

In December 2019, a new outbreak of a lethal disease causing severe acute respiratory syndrome (SARS) first occurred in Wuhan, a major city in central China. This highly contagious disease was identified to be caused by a novel coronavirus, which was termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the World Health Organization, whereas the disease was termed as coronavirus disease 2019 (COVID-19). Due to the remarkably high person-to person transmission rate of SARS-CoV-2, COVID-19 has rapidly spread in the first quarter of 2020 [1].

Hypoxemia is an ominous sign of COVID-19, and it is usually an indicator of disease severity [2]. An oxygen saturation above 90% is associated with better outcomes [3]. Hypoxia indicates an imbalance of oxygen delivery to tissues and leads to compromised function. Hypoxic environment is essential for the proliferation and differentiation of cytotrophoblast for maintenance of maternofetal circulation at early periods of pregnancy. However, its prevalence in later stages of pregnancy causes several complications that may lead to maternal and fetal morbidity and mortality [4, 5].

The hypoxia-inducible factors (HIF) are considered master regulators of oxygen homeostasis and are oxygen level sensitive [6]. HIF-1α is a heterodimeric transcription factor that bind to hypoxia response elements, which participates through the regulation of the expression of several genes in numerous cellular events such as O2 sensing, glucose metabolism, lipid metabolism, angiogenesis, and other aspects of endothelial biology [7].

Placental growth factor (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. There are trophoblastic and non-trophoblastic sources of PLGF, but placental trophoblast is the main source of PLGF throughout the gestational

period of pregnancy [8]. It shares structural as well as amino acid sequence similarity with VEGF, but PLGF has binding affinity only for VEGF receptor1(VEGFR-1) [9]. The inter-and intramolecular cross-talk between the VEGFR-1 and VEGFR-2 is regulated by PLGF. It binds toVEGFR-1 and displaces VEGF from this receptor, which results in activation and intermolecular trans phosphorylation of VEGFR-2 thereby amplify the VEGF-induced angiogenesis [10].

Currently, there is no information regarding the expression of HIF-1α in normal pregnant women with COVID-19 infection and its potential involvement in the placentation of this condition. Therefore, the current study aims to detect the expression of HIF-1α and possible molecular link between its expression and PLGF.

METHODOLOGY

A case-control study was conducted in a tertiary university hospital between January and September 2022. The study protocol was approved by the Ethical Committee at Faculty of Medicine, Assiut University and all participants signed an informed consent **(**approval no: IRB17101597).

Study subjects: Fifty women were be enrolled in this study. The women were divided into two group:

- Post COVID-19 infected group: normal pregnant women infected by COVID during the third trimester (n = 34)
- Control group: normal pregnant women (n = 16).

The diagnosis of COVID-19 was confirmed by history from patients, nasopharyngeal swab, and oxygen saturation level of blood < 90% by pulse oximeter. We excluded women with Diabetes mellitus, Chronic hypertension, Preeclampsia, Acute or chronic infectious diseases or other chronic illness and multiple pregnancy.

Sampling:

After delivery a villus tissue (2.5 X 2.5 X 2.5 cm) was cut off immediately from the center of placenta, avoiding the area of infarction, bleeding, or calcification. The tissue was washed with normal saline and preserved in ThermoFisher RNA stabilizing reagent (Cat.no. AM7022). Total RNA was then extracted from the placental tissues using the Quick-RNA™ MiniPrep kit (Cat.no. R1054). The extracted RNA was stored at -80°C for later use.

RNA concentrations of each sample were measured using (BioTek Epoch NanoDrop, USA) to determine the starting amount of RNA. Reverse transcription into cDNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Ca.no 4374966).

Quantitative real-time PCR (qRT-PCR) was performed on 7500 fast real time PCR (Applied Biosystems, USA)) Maxima SYBR Green qPCR Master Mix (Cat.no. k0251) was used under the following conditions, Hot start step at 95°C for 7 minutes, initial denaturation for 20 sec at 95º c , annealing and extension for 60 sec at 59 º c , in 40 cycles . The relative transcription levels of Hif-1 and PLGF were calculated using the equation of fold change = $2^{-\Delta\Delta ct}$ method as described by Livak & Schmittgen [11]. B Actin acted as an internal control, and all primers were synthesized by Thermo Fisher Scientific (USA).

Primers and control sequences:

Reverse	Forward	
AACCATCCAAGGCTTTCAAATAA	TCTCGGCGAAGTAAAGAATC	Hif α
CTGCATGGTGACATTGGC	AAGATGCCGGTCATGAGGC	PIGF
CTCCTGCTTGCTGATCCACATC	GATTACTGCCCTGGCTCCTAGC	B-actin

Statistical analysis:

Data was analyzed using Statistical Package for Social Science (SPSS), version 26.0 for Windows. Quantitative data tested for normality by Shapiro-Wilk test data, expressed as mean ± SD or median and range according to normality of data. Independent Sample T-test/ Mann Whitney U test was used to compare mean/median difference between two cases and controls. Spearman correlation was used to explore the correlation between HIF-1α and PLGF. The level of significance was considered at P value < 0.05.

RESULTS

I- Basic characteristics of the studied groups:

Table.1 shows that there was no statistically significant difference between mean age of cases and controls, P value=0.339, mean age of cases was 28.29±4.13 and in controls was 29.50±4.16, moreover, no statistically significant difference regarding mean BMI of cases and controls, P value=0.342, mean BMI of cases was 24.34±3.44 and in controls was 25.22±3.38

Table 1: Characteristics and clinical data of the study participants

Variables	Cases (n=34)	Controls (n=16)	P-Value*
Age (years):			
▪ Mean± SD	28.29±4.13	29.50±4.16	0.339
BMI			
▪ Mean± SD	24.34±3.44	25.22±3.38	0.342

Data were expressed as mean ± SD.
*Independent Sample T test

II- Relative expression of HIF-1α and PLGF between cases and controls in the placenta:

Table 2 and figure 1, 2 show statistically significant higher median level of **HIF-1α** among cases compared to controls; 34.16 and ranged from 13.58 to 71.69 among cases, 1.01 and ranged from 0.73 to 1.27 among controls, P value <0.001.

Moreover, there was a statistically significant higher median level of **PLGF** among cases compared to controls; 14.87 and ranged from 5.31 to 23.58.69 among

cases, 0.98 and ranged from 0.71 to 1.74 among controls, P value <0.001.

Table 2: comparison of HIF-1α and PLGF between cases and controls

Variables	Cases (n=34)	Controls (n=16)	P-Value*
HIF-1α			
Median (range)	34.16 (13.58-71.69)	1.01 (0.73-1.27)	**<0.001**
PLGF			
Median (range)	14.87 (5.31-23.58)	0.98 (0.71-1.74)	**<0.001**

Data were expressed as median (range)
* Mann Whitney U test

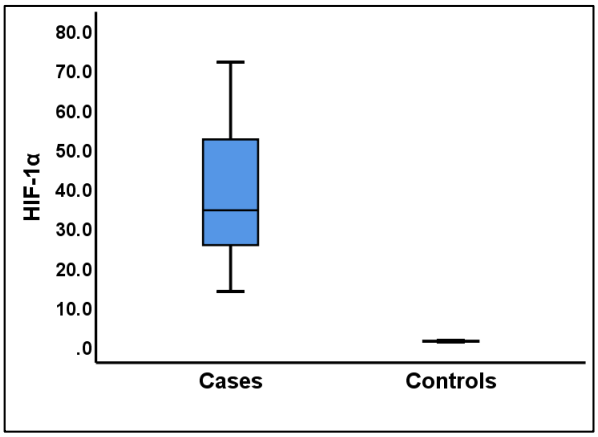

Fig. 1: Boxplot for distribution of HIF-1α between cases and controls

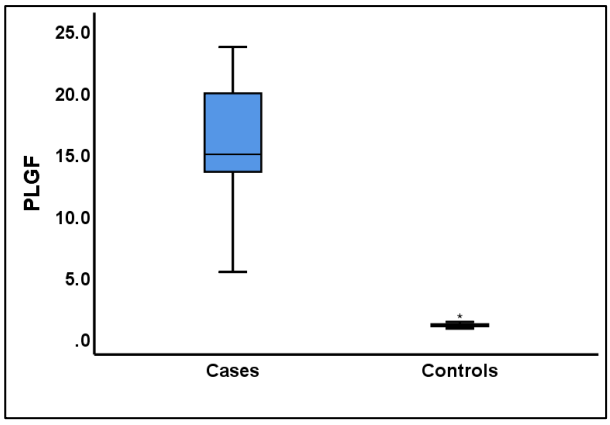

Fig. 2: Boxplot for distribution of PLGF between cases and controls

III-Correlation between Hif-1 and PLGF

Table 3 and figure 3 shows statistically significant moderate positive correlation between HIF-1α and PLGF gene (r=0.636, P value<0.001) among total studied samples, however no statistically significant correlation between the two gene among cases and controls groups separately**.**

Table 3: Correlation between HIF-1α and PLGF genes

	HIF-1α	
	r	**P-Value***
PLGF		
▪ **Total participants**	**0.636**	**<0.001**
▪ **Cases**	0.077	0.665
▪ **Controls**	0.205	0.447

r (correlation coefficient), *spearman correlation

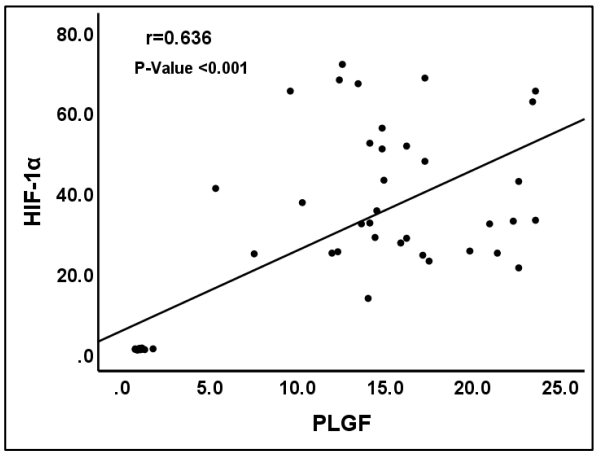

Fig. 3: Scatter diagram for correlation between HIF-1α and PLGF among all studied participants

DISCUSSION

In the present study we have demonstrated that HIF-1α is significantly higher in the placentas of post COVID-19 infected women compared to controls. This increase could be explained by the COVID-19 induced hypoxia.

HIF-1α is oxygen sensing and under hypoxic conditions, the HIF-1a protein accumulates and translocate to the nucleus where it heterodimerizes with HIF-1b to form an active transcription factor that bind to hypoxia response elements (HREs), which participates through the regulation of the expression of several genes in numerous cellular events that counteract hypoxia . The induction of HIF-1 by hypoxia takes place at the protein level because HIF-1a messenger RNA expression remains unchanged [12].

PLGF is a proangiogenic factor that shares structural as well as amino acid sequence similarity with VEGF. In the human placenta VEGF and PLGF are differentially expressed throughout gestation; VEGF is higher during early gestation and decline as pregnancy advances [13-15], while PLGF increases towards term [16, 17] suggesting that VEGF is involved in the first two trimesters of pregnancy in the establishment of the richly branched capillary beds of the mesenchymal and immature intermediate villi, while PLGF is more likely

to be involved in the formation of the long, poorly branched, terminal capillary loops in the last trimester.

The development of the placental vasculature is the effect of the local oxygen environment during gestation. Oxygen is thought to be a major regulator of the balance between VEGF and PLGF function; early placental development (first trimester) occurs in an environment of relative hypoxia which is known to stimulate cytotrophoblast proliferation [18].

True intervillous blood flow is established at about 10–12 weeks of gestation and as the placenta progresses towards the second trimester, maternal blood flow starts and the PO_2 increases [19]. This striking rise in PO_2 may be the trigger for the trophoblast to change from the proliferative state within 'hypoxic' cell columns to an invasive extravillous trophoblast that is responsible for the secondary wave of trophoblast invasion of the maternal spiral arterioles to establish a high flow, low impedance uteroplacental circulation [20]. Thus, hypoxia has been demonstrated to upregulate VEGF and downregulate PLGF expression.

Various researchers had noticed the increased expression of PLGF gene in normal trophoblast, while the expression was reduced in preeclampsia [21-23]. Many researchers had suggested that the abnormal serum levels of PLGF in preeclampsia result in improper trophoblast invasion and generalized maternal endothelial dysfunction, which leads to preeclampsia [24, 25].

In contrast to the previous studies, our study demonstrated that there was a statistically significant higher median level of PLGF among cases compared to controls which is the first study proved that covid-19 induced hypoxia doesn't affect the PLGF expression and even the expression was higher in covid-19 group than control group. This could be explained by that the type of hypoxia in our study is pre placental hypoxia (covid-19 induced hypoxia) and in the previous studies the hypoxia is uteroplacental (preeclampsia). On the other hand, hypoxia increases PLGF expression in non-trophoblastic cells [26-29].

Regarding the correlation between HIF-1α and PLGF, our study shows statistically significant moderate positive correlation between HIF-1α and PLGF gene among total studied samples, however no statistically significant correlation between the two genes among cases and controls groups separately.

In contrast to our study in Rath et al. [23] showed that there was a significant negative association between HIF-1α nuclear and PLGF cytoplasmic expression in patients suffering from preeclampsia. Also, the statistical analysis of the serum levels of HIF-1α and PLGF showed a significant negative correlation between the proteins in preeclampsia. Therefore, they hypothesized that the upregulation of HIF-1α and downregulation of PLGF in serum and placental tissues may be directly associated with the pathogenesis of preeclampsia. The overexpression of HIF-1α is associated with the increased maternal serum concentration of soluble Fms-like tyrosine kinase 1 (sFlt1) during hypoxic conditions [30]. High circulating levels of sFlt1 exerts an antiangiogenic state that is associated with low levels of proangiogenic factors, such as PLGF, and inhibition of PLGF with its receptor VEGFR-1 [31,32].

The explanation of the difference between our study and the previous studies is that the effect of HIF-1α on the expression of PLGF gene is dependent on the type of cell and the conditions prevailing in a cell [33]. In addition, Gobble et al. [21] reported that hypoxia decreases PLGF gene transcription that results in the decreased value of PLGF via mechanisms independent of HIF-1.

Also, down regulation of PLGF transcription in hypoxic trophoblast could be that other transcription factors binding to sequences adjacent to the PLGF HREs may be sequestering the hypoxic induction of PLGF [34].

CONCLUSION

The higher levels of HIF-1α and PLGF in the placentas of post COVID-infected pregnant women compared to normal pregnant women indicate that COVID-19 hypoxia did not affect the process of placentation. This is confirmed also by the positive correlation between HIF-1α and PIGF in placental tissues among total studied samples.

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Conflicts of interest/Competing interests
The authors declare that they have no conflict of interest.

Ethics approval
The study protocol was approved by the Ethical Committee at Faculty of Medicine, Assiut University **(**approval no: IRB17101597).

Consent to participate.
All participants signed an informed consent before participation in the study.

Consent for publication
Participants have consented to the submission of data.

Availability of data and material
All related data and materials are available from the corresponding author upon request.

Code availability
Not applicable

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