

Impact of High Altitude on Maternal Serum Leptin Level and Its Correlation with Oxidative Stress and Endothelial Inflammatory Markers in Preeclamptic Women

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Abstract

Involvement of leptin in the pathogenesis of preeclampsia (PE) is still a controversy subject. Several researches reported the changes in serum leptin in high altitude (HA) residents. The aim of the present work was to investigate the impact of oxidative stress (OS) induced by HA residence on maternal serum leptin in PE and if there was a significant correlation between the serum leptin with either OS or endothelial inflammatory markers. One hundred fifty eight pregnant women were included in this study, divided into: low altitude normal pregnancies (NL), HA normal pregnancies (NH), low altitude preeclamptic (PL), and HA preeclamptic (PH) who presented to the obstetrics and gynecology outpatient clinic in both Muhayl (500 m over sea level) and Abha General Hospitals (all of them resident at Alsoda district with the average altitude 2700 m over sea level). Serum leptin, superoxide dismutase (SOD) activity, malondialdehyde (MDA), plasma nitrite/nitrate (NOx), serum tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), blood urea nitrogen (BUN) and creatinine were determined. Both NH and PL groups showed significant increases in leptin ($P < 0.01$), SOD ($P < 0.01$), MDA ($P < 0.001$), NOx ($P < 0.001$), TNF- α ($P < 0.001$) and IL-6 ($P < 0.001$) compared with the NL group without any significant changes between both groups. The PH group showed significant accentuation of the previously measured parameters ($P < 0.001$ for all) compared with all other groups (NL, NH and PL groups). We can conclude that the combination of PE and HA residence resulted in significantly elevated maternal serum leptin suggesting involvement of leptin in the pathogenesis of PE accentuated by HA residence.

Key Words: high altitude, inflammatory markers, leptin, oxidative stress, preeclampsia

Introduction

Preeclampsia (PE) is a common disorder of human pregnancy complicating about 10% of all pregnancies (15) and about 10-12% of the primipara (16). In spite that the etiology and pathophysiology of the disease is still a subject of extensive investigations; growing evidence from animal and human studies implicate placental ischemia with subsequent hypoxia in the etiology of this maternal syndrome (20). One of the more widely accepted etiological pathways is failure of spiral artery remodeling, leading to placental ischemia with subsequent placental hypoxia. This in turn stimulates the production of placental factors that damage or alter the function of systemic endothelial cells (23). The ischemic placental hypoxia stimulates the release of different dysfunctional factors, particularly from the renal tissue eliciting maternal hypertension and proteinuria in PE. Severe oxidative stress (OS) and the release of various maternal and placental factors are the main players inducing endothelial dysfunction which is the crucial event in pathophysiology of PE (12). Addition of severe OS induced by PE to its already elevated level due to a high altitude (HA) residence could accentuate the release of these placental and maternal factors causing more severe maternal endothelial dysfunction that increases the prevalence of this condition in HA resident pregnant women.

Several inflammatory and angiogenic factors seem to have major impact in the pathogenesis of PE (22). Leptin is a protein hormone expressed predominantly by adipocytes. Smaller amounts of leptin are also secreted by cells of the placenta, T lymphocytes and vascular endothelial cells (6). It plays an important role in control of food intake, reproduction, and angiogenesis and also has a crucial role in immune-regulatory mechanisms and inflammatory disorders (21). Although the role of leptin in fetal growth is still not yet documented, some investigators like Mantzoros *et al.* reported that, in normal pregnancy, leptin produced by placental and fetal tissues, acts through certain leptin receptors (LEPRs) to regulate fetal growth and development (13). It has been reported that, hypoxia was associated with an increase expression of a group of leptin genes in trophoblastic cells (4). HA associated hypoxia increases the Ob (Lep) gene expression and the LEPR gene expression in preeclamptic mothers (24). Another study reported that serum leptin is a significant biomarker for the severity of PE (15).

Several studies reported that leptin levels do not change in PE and thus it has no role in its pathogenesis (3, 7). However some recent studies suggest a highly significant role for leptin in pathogenesis of PE (1). They claim that the increase in

leptin in PE is an adaptive response by the fetoplacental unit to impaired placental perfusion (4).

Thus, it is logical to postulate that, exposure of the preeclamptic women to chronic hypoxia due to residence at HA could provoke more increases in serum leptin and OS levels that adding to their already existing higher levels. Also, we can suggest that, disturbance in leptin level in PE could be the link between the increased OS and the higher inflammatory response. So, the current study was undertaken to investigate the impact of OS, induced by HA residence, on the maternal serum leptin level in preeclamptic women and their possible link to the higher inflammatory reaction. Also, we investigated whether there was a significant correlation between the maternal serum leptin level and either OS markers or endothelial inflammatory mediators incriminated in the pathophysiology of PE.

Materials and Methods

Study Design and Subjects

This study followed the instructions for the ethical committee on human experimentation and was adherent to the Helsinki Declaration of 1975, revised in 2000 (available at http://www.wma.net/e/policy/17-c_e.html). Also, it was approved by the Ethical Committee of the College of Medicine, King Khalid University, Saudi Arabia. This study was conducted among 158 pregnant women who presented to the outpatient obstetrics and gynecology clinic (between Sept, 2014 and Oct 2015) in both Muhayl General Hospital (75 pregnant women) (altitude 500 m over sea level) and Abha General Hospital (83 pregnant women, all of them resident at Alsoda district with the average altitude 2700 m over sea level) in southwestern Saudi Arabia. All the participants were borne and lived where we selected them and they never change their area of living especially at the time of pregnancy. An informed consent was taken from each pregnant woman included in the study. All the participants were selected with a pre-partum body mass index (BMI) less than 28 kg/m² including both primipara and multipara (para 2 to 3) to exclude the effect overweight or obesity on leptin level. Also, they matched by age (mean age 26.95 ± 2.98) with the gestational age (GA) between 28-35 weeks (Table 1). We had two main groups; the first main group who attended the outpatient clinic of Muhayl General Hospital, and included: the healthy pregnant control group (low altitude normal pregnancies, NL) (n = 41) and the low altitude preeclamptic (PL) group (n = 34). The second main group (all of them resident at Alsoda district) who attended the outpatient clinic of Abha General Hospital, and included:

Table 1. Showed mean \pm standard deviation (SD) for the age, GA, hematocrit value, BMI (Pre-partum), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), BUN and creatinine levels in different study groups.

Group	NL	NH	PL	PH
Age (years)	24.20 \pm 3.1	27.30 \pm 2.7 (<i>P</i> = 0.06)	26.10 \pm 2.4 (<i>P</i> = 0.18)	30.20 \pm 3.7 (<i>P</i> = 0.07)
GA (weeks)	28.40 \pm 2.3	30.20 \pm 3.1 (<i>P</i> = 0.16)	34.90 \pm 3.5 (<i>P</i> = 0.08)	35.20 \pm 5.1 (<i>P</i> = 0.478)
Hematocrit value	52.10 \pm 2.5	62.20 \pm 4.1 ^{ac}	53.10 \pm 1.3	64.30 \pm 3.2 ^{ac}
BMI (Pre-partum)	23.10 \pm 2.6	24.10 \pm 1.3	23.20 \pm 1.4	22.30 \pm 2.1
SBP (mmHg)	98.21 \pm 14.18	107.30 \pm 13.62	145.31 \pm 16.12 ^{ab}	157.05 \pm 18.11 ^{ab}
DBP (mmHg)	67.55 \pm 5.31	71.91 \pm 8.57	97.95 \pm 4.13 ^{ab}	118.20 \pm 7.47 ^{ab}
BUN (mg/dl)	24.72 \pm 2.79	28.31 \pm 4.81	30.65 \pm 3.83 ^a	30.29 \pm 3.12 ^a
Serum creatinine (mg/dl)	0.65 \pm 0.07	0.61 \pm 0.08	0.75 \pm 0.07	0.89 \pm 0.09

Results are expressed as mean \pm SD. Significance level is at *P* < 0.05. ^a Significantly different from NL group, ^b Significantly different from NH group. ^c Significantly different from PL group.

the healthy pregnant control group (high altitude normal pregnancies, NH) (n = 46) and the high altitude preeclamptic (PH) group (n = 37).

History taking and clinical examination were done for all cases of the study. Thorough obstetric history, complete general and obstetric examinations were done. A specialized obstetrician performed ultra-sonography for all participants to confirm the fetal viability, evaluate GA, and to assess fetal growth and development and exclude any gynecological or obstetrical abnormalities or twin pregnancy. Twenty-four hour urine collections were done to detect proteinuria. Full laboratory investigations including hematocrit value were performed to assess the general condition of the participants. Abdominal ultrasound was done for all the participants to assess any abdominal or pelvic abnormalities or ascites. Exclusion criteria among the participants included any serious medical problems or risk factors as preexisting diabetes mellitus, gestational diabetes, essential hypertension, chronic renal or hepatic or cardiac diseases or collagen vascular diseases such as systemic lupus erythematosus or antiphospholipid syndrome or other chronic illness. Women with a prior past history of PE, obesity, and smoking or under treatment were excluded. We applied The American College of Obstetricians and Gynecologists (Ref Vol. 122, NO. 5, November 2013) diagnostic criteria for PE which specified: SBP \geq 140 or DBP \geq 90 on two separate readings taken at least four to six hours apart after 20 weeks gestation in an individual with previously normal blood pressure together with a proteinuria

\geq 300 mg/24 h urine sample (or this amount extrapolated from timed collection). Three readings for the blood pressure were taken using the ordinary sphygmomanometer technique.

Blood Samples

After an overnight fasting, three venous blood samples (2 ml/each) were collected from participants in three separate visits. The mean value of three readings for all measured parameters was taken for each participant.

Determination of Blood Urea Nitrogen (BUN), Serum Creatinine and Leptin Levels

BUN and serum creatinine levels were determined by the enzymatic colorimetric method using a specialized kit (Boehringer Mannheim, Germany). Serum levels of total leptin were assessed using commercially available enzyme-linked immunosorbent assay method following the manufacturer's instructions (Avibionhuman leptin enzyme-linked immunosorbent assay (ELISA) kit, Ani Biotech Oy, Tillitie-3, Vantaa, Finland).

Determination of Serum Superoxide Dismutase (SOD) Activity, Serum Malondialdehyde (MDA) and Plasma Nitrite/Nitrate (NOx) Levels

Serum SOD activity level was determined using commercial kit (Cayman Chemical, Michigan, USA).

The xanthine oxidase method and the thiobarbituric salt method were applied to measure serum MDA level; the kit were purchased from Nanjing Jiancheng Bioengineering Institute, and the procedures were performed in accordance with manufacturer instructions. Plasma NOx levels were measured and the results were expressed as micromole per liter.

Determination of Serum Tumor Necrosis Factor-Alpha (TNF- α) and Interleukin 6 (IL-6) Levels

Determination of serum TNF- α level was assayed by the commercial TNF- α ELISA kit and expressed in picogram per milliliter. Also, human IL-6 ELISA kit were used for the assay of serum IL-6 and expressed in picogram per milliliter.

Statistical Analysis

The data were expressed as means \pm SD. The Kolmogorov-Smirnov test was used to ensure the normality of the distribution of data. Testing significance was performed using χ^2 test and the one-way analysis of variance (ANOVA) using SPSS version 20 (SPSS Inc., Chicago, IL, USA). The *post hoc* Scheffe's test was applied to identify the source of statistical significance. *P* values less than 0.05 were considered statistically significant. Pearson's correlation statistical analysis was performed for detection of a probable significance correlation between two parameters.

Results

Hematocrit Value, Systolic and Diastolic, BUN, and Serum Creatinine Levels

Both HA groups (NH and PH groups) showed significantly higher hematocrit values (62.20 ± 4.1 and 64.30 ± 3.2 respectively) when compared with NL and PL groups (52.10 ± 2.5 and 53.10 ± 1.3 , respectively) without any significant changes when compared with each other (Table 1).

PL group showed a significant elevation of both SBP and DBP pressures (145.31 ± 16.12 mmHg, 97.95 ± 4.131 mmHg respectively) compared with NL group (98.21 ± 14.18 mmHg, 67.55 ± 5.31 mmHg respectively). On the other hand, PH group showed a significant elevation of their both SBP and DBP (157.05 ± 18.11 mmHg, 118.20 ± 7.47 mmHg respectively) compared with both NH (107.30 ± 13.62) and PL groups (Table 1).

PL and PH groups had positive proteinuria test (more than 0.3 gm protein/24 h), while both NL and NH groups had not. BUN was insignificantly changed in NH group (28.31 ± 4.81) compared with

NL group (24.72 ± 2.79). While both PL (30.65 ± 3.83) and PH (30.29 ± 3012) groups showed significant increases in BUN compared with NL group and insignificantly changed compared with either NH group or with each other. Serum creatinine was insignificantly changed in NH, PL, and PH groups (0.61 ± 0.08 , 0.75 ± 0.07 and 0.89 ± 0.09 , mg/dl respectively) compared with NL group (0.65 ± 0.07) (Table 1).

Maternal Serum Leptin Levels

Maternal serum leptin levels in both NH and PL group showed significant increases (17.63 ± 2.95 ng/ml, 22.86 ± 4.67 ng/ml, respectively) (*P* < 0.01 for both) compared with the NL group (9.42 ± 2.15 ng/ml), without any significant changes between both groups. While the preeclamptic women resident at HA showed significant increase in their serum leptin level (36.72 ± 7.42 ng/ml) (*P* < 0.001) compared with all other groups (NL, NH and PL groups) (Fig. 1A).

Serum SOD Activity, Serum MDA and Plasma NOx Levels

Serum SOD activity level, serum MDA and plasma NOx levels in both NH group [25.16 ± 2.30 ng/ml (*P* < 0.01), 17.51 ± 1.87 nmol/ml (*P* < 0.001), 60.24 ± 6.17 μ M (*P* < 0.001) respectively] and PL group (23.75 ± 2.41 ng/ml (*P* < 0.01), 16.41 ± 1.76 (*P* < 0.001), 56.77 ± 6.13 μ M (*P* < 0.001) respectively) showed significant elevation compared with the NL group (16.72 ± 1.87 ng/ml, 13.84 ± 1.55 nmol/ml, 31.53 ± 3.75 μ M, respectively) without any significant changes between both groups. On the other hand, preeclamptic pregnant women of the PH group showed significant increases in their serum SOD activity level, serum MDA and plasma NOx levels [34.14 ± 3.82 ng/ml (*P* < 0.001), 23.99 ± 2.53 nmol/ml (*P* < 0.001), 85.16 ± 8.91 μ M (*P* < 0.001) respectively] compared with all other groups (NL, NH and PL groups) (Figs. 1, B-D).

Serum TNF- α and IL-6 Levels

Serum levels of both TNF- α and IL-6 increased significantly in NH (98.36 ± 10.24 pg/ml (*P* < 0.001), 10.76 ± 1.12 pg/ml (*P* < 0.001) respectively) compared with the NL (72.41 ± 7.12 pg/ml, 6.10 ± 1.03 pg/ml respectively). PE women resident at low altitude showed significant increased in their serum TNF- α (131.62 ± 12.97 pg/ml) (*P* < 0.001) without any significant changes in serum IL-6 levels (12.06 ± 1.31 pg/ml) (*P* > 0.05) compared with the NH group. PE women resident at HA showed a sig-

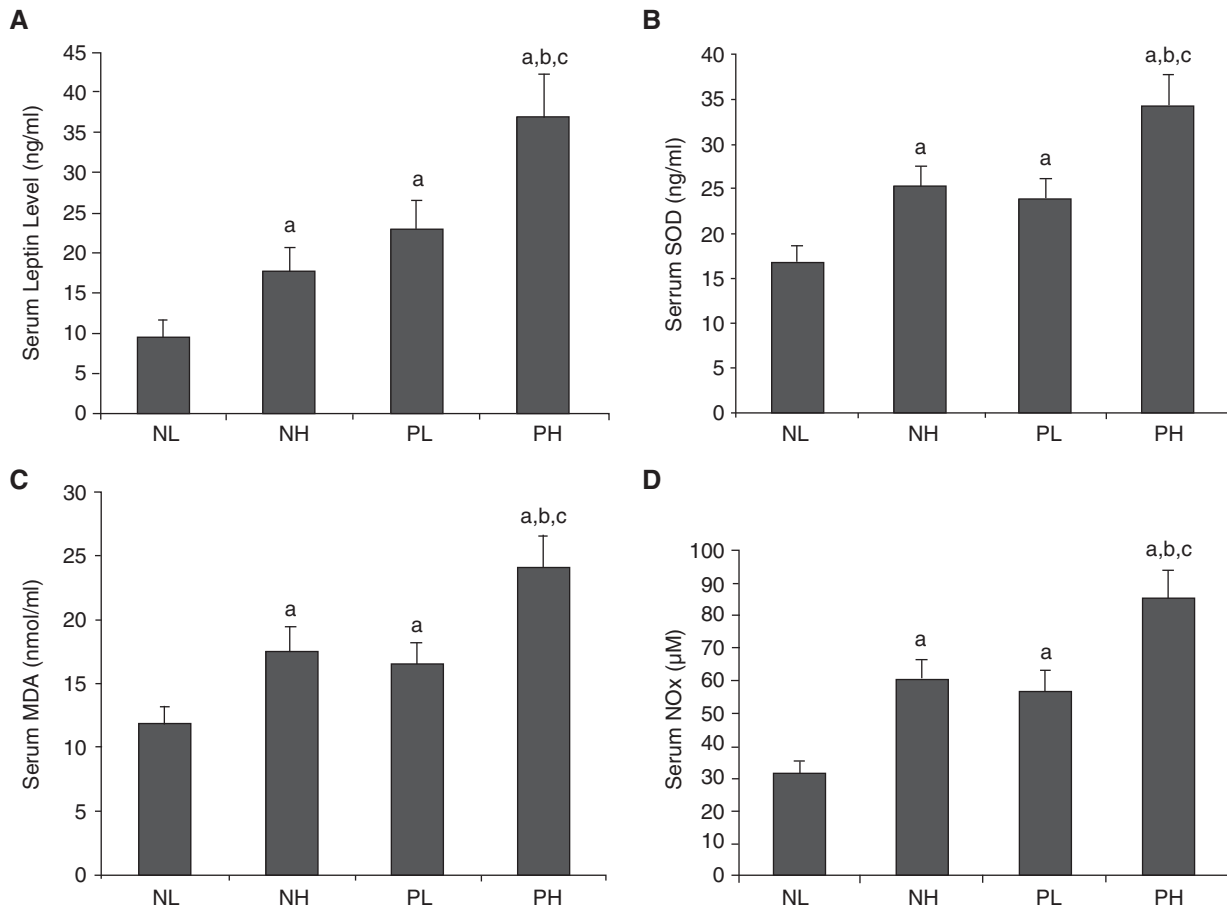


Fig. 1. High and low altitude effect on (A) serum leptin, (B) serum SOD enzyme activity, (C) serum MDA and (D) NOx in normal and preeclamptic pregnant. NL: Normal pregnant women at low altitude (n = 41); NH: Normal pregnant women of at HA (n = 46); PL: preeclamptic patients at low altitude (n = 34); PH: preeclamptic patients at HA (n = 37). SOD: superoxidase dismutase; MDA: malondialdehyde; NOx: nitrite/nitrate. Results are expressed as means \pm SD. Significance was considered when P value was <0.05 . ^a Significantly different from NL group. ^b Significantly different from NH group. ^c Significantly different from PL group.

nificant increase in both serum levels of TNF- α and IL-6 [176.72 ± 18.55 pg/ml ($P < 0.001$), 16.82 ± 1.92 pg/ml ($P < 0.001$) respectively] compared with the NL, NH and PL groups (Fig. 2, A and B).

A Correlation Study of Serum Leptin with Each of Serum SOD, MDA, TNF- α , IL-6, Plasma NOx, Levels in Preeclamptic Low Altitude and HA Groups

The PL group showed no correlation between serum leptin with the serum SOD ($r^2 = 0.024$) ($P = 0.51$), MDA ($r^2 = 0.018$) ($P = 0.58$), TNF- α ($r^2 = 0.044$) ($P = 0.39$), IL-6 ($r^2 = 0.060$) ($P = 0.30$) and plasma NOx ($r^2 = 0.001$) ($P = 0.90$) levels. Also, no correlation was observed in the PH group between the serum leptin level with the serum SOD ($r^2 = 0.044$) ($P = 0.37$), MDA ($r^2 = 0.066$) ($P = 0.27$), TNF- α ($r^2 = 0.068$) ($P = 0.29$), IL-6 ($r^2 = 0.007$) ($P = 0.72$) and plasma NOx ($r^2 = 0.003$) ($P = 0.82$) levels.

Discussion

The effect of HA residence on serum leptin levels is controversial. Some studies indicate that prolonged exposure to HA result in decreased leptin levels (26). However, a large body of research found that HA is associated with increased serum leptin levels (14). A similar contradiction exists in the studies examining serum leptin levels in PE. Some investigators reported hyperleptinemia in preeclamptic women suggesting a significant role for leptin in the pathogenesis of PE (19), whereas others reported no change and assumed that leptin has no role (9). Our results showed that HA per se caused a significant increase in NH group compared with the NL group. While PL group showed a comparable increase in their serum leptin to that of the normal pregnant women resident at HA (NH group). The combination of HA and PE in the PH group resulted

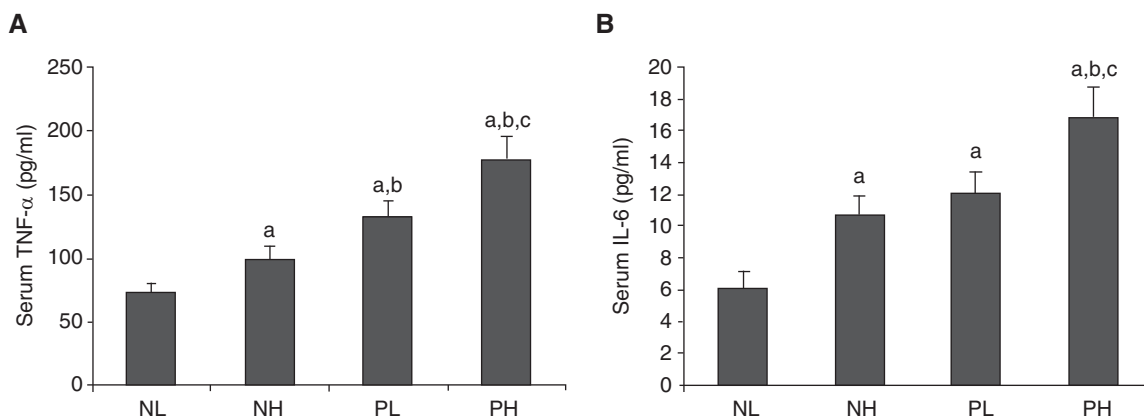


Fig. 2. High and low altitude effect on (A) serum TNF- α and (B) serum IL-6 in normal and preeclamptic pregnant women. Normal pregnant women at low altitude (n = 41); NH: Normal pregnant women of at HA (n = 46); PL: preeclamptic patients at low altitude (n = 34); PH: preeclamptic patients at HA (n = 37). TNF- α : serum tumor necrosis factor and IL-6: interleukin 6. Results are expressed as means \pm SD. Significance was considered when *P* value was <0.05 . ^a Significantly different from NL group. ^b Significantly different from NH group. ^c Significantly different from PL group.

in the highest observed levels of leptin compared with all other groups (Fig. 1A). It has been suggested that placental hypoxia induces hyperleptinemia in PE and it assumed that it is a general response of trophoblastic cells to hypoxia. Also, it has been reported that hypoxia was associated with an increase expression of a group of leptin genes in trophoblastic cells (4). HA associated hypoxia increases the Ob (Lep) gene expression and the LEPR gene expression in preeclamptic mothers (24).

Involvement of leptin in pathogenesis of PE has been previously investigated. A study reported that, PE is considered an exaggerated inflammatory condition in which leptin inhibits the generation and proliferation of T-regulatory cells and induces the Th-17 cells responsible for activation of the inflammatory reaction (12). Leptin also, mediates the production of pro-inflammatory cytokines like TNF- α and IL-6 in PE inducing maternal vascular and renal pathology that provoke hypertension and proteinuria (25). Also higher inflammatory mediators TNF- α and IL-6 in PE encourage renin-angiotensin system upset and stimulate angiotensin II type-1 receptor's auto-antibodies *via* reactive oxygen species (ROS) causing hypertension (8). Interestingly, a recent study showed that, administration of leptin in rodents increased SBP, endothelial vasoconstrictor mediators and produced proteinuria during pregnancy (10). Another evidence for leptin involvement in the pathogenesis of PE could be that, injection of leptin into the nucleus of the solitary tract elicits sympatho-excitatory responses causing elevation of the blood pressure (5).

Our results showed that the PL group has similar high levels of OS markers (SOD activity, MDA and

NOx) and inflammatory mediators (TNF- α and IL-6) compared with the NH group. This could point towards that, the high OS and inflammatory responses induced by HA acts as a risk factor for the development of PE in the pregnant women resident at HA. Jarvie *et al.* reported that, severe OS in PE impairs angiogenesis, inhibits trophoblastic invasion and provokes endothelial dysfunction (11). The accumulation of oxidant-antioxidant imbalance of both HA and PE could be behind the higher incidence of PE.

Ambrosini *et al.* suggested that leptin could be one of the mediators of the deleterious effects of chronic hypoxia caused by residency at HA (2). In PE, marked OS induced by placental hypoxia triggers the release of leptin and other pro-inflammatory mediators as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) (17, 18). It is assumed that, hyperleptinemia in PE is produced by severe OS in human endothelial cells through the accumulation of reactive oxygen species (18). However, in our study we did not find any positive correlation between the maternal serum leptin level and OS marker's, SOD activity, serum MDA, plasma NOx and the inflammatory mediators TNF- α and IL-6 in preeclamptic women. The lack of direct correlation with leptin could be attributed to the involvement of multiple factors in the genesis of the oxidative changes.

In summary, our results support a possible significant role of leptin in PE. The significant increase in leptin levels resulting from exposure of the pregnant women to chronic hypoxia at HA could induce the development or aggravate the already existing cases of PE with subsequent complications. The results also suggest that women with high risk

of PE resident at HA may benefit from a move to a lower altitude till parturition. Further studies are needed on leptin to investigate its exact role in the pathogenesis of PE.

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Conflict of Interests

The authors declare that they have no competing interests.

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