Role of circulatory Telomere length, Oxidative stress markers, and inflammatory markers with Increasing Blood pressure in PREECLAMPSIA - A Study in western Uttar Pradesh, India

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DOI: 10.47750/pnr.2022.13.S01.243

Abstract

Introduction: PE is a pregnancy-related syndrome with Gestational hypertension and proteinuria. It is caused by improper maternal spiral artery remodelling leading to reduced perfusion to the placenta, promoting exaggerated inflammatory markers CRP, IL 6, TNF alpha, and oxidative stress (OS) tags (MDA) into circulation. Therefore, this study was planned to explore any association between telomere length, inflammatory markers, and oxidative stress markers with change in BP (increase every ten mmHg). To the best of our knowledge, it is the first study exploring this association in preeclamptic patients. **Methods:** A case-control study included PE cases (n=90) and controls (n=70). RT-PCR measured relative telomere length (RTL) as a T/S ratio. Serum levels of Malondialdehyde (MDA) and C-reactive protein (CRP) by using competitive ELISA and uric acid (UA) levels by autoanalyzer. **Results:** RTL is significantly(p=.000) reduced in PE compared to control. MDA, CRP, and Uric acid levels significantly(p=.000) increased in cases than in control. Telomere length decreased with the rise in BP, and CRP, MDA, and UA levels increased significantly with an increase in BP. Therefore, we planned to explore the association between telomere length, inflammation, and oxidative stress with every ten mmHg increase in BP. **Conclusion:** Increasing BP by ten mmHg led to shorter Telomere Length and increased MDA, CRP, and UA levels. Therefore, increasing BP is associated with shorter telomere length, oxidative stress, and inflammation in PE.

Keywords: Telomere length, CRP, inflammation, oxidative stress, Preeclampsia.

INTRODUCTION

Preeclampsia (PE) is a pregnancy-related syndrome with Gestational hypertension and proteinuria [1]. The risk factors for PE are genetic predispositions, environmental changes, obesity, gestational diabetes mellitus, multifetal gestations, and hydatidiform mole [2,3]. PE complications are reduced after the separation of the placenta. Pathogenesis of PE can occur by different mechanisms such as utero-placental origin, immunogenic origin, genetic predispositions, as well as the angiogenic and antiangiogenic origin, which may explain the imbalance between angiogenic (Vascular Endothelial growth factor (VEGF), placenta growth factor (PIGF) and Antiangiogenic factors like soluble fms-related tyrosine kinase 1(sFlt-1), soluble endoglin(sEng)) [4,5]. Initial stages of healthy pregnancy require fetal trophoblasts to invade a maternal spiral artery of the uterine wall before the tenth week of gestation. The spiral artery can transform into wide vessels to accommodate increased blood flow

to intervillous space and thereby perfusion to the fetus during embryonic development [6]. However, in PE, improper maternal spiral artery remodeling leads to decreased/intermittent perfusion to the fetus. Thus, severe maternal and fetal complications. The hypoxic placental organ may release microvesicles (apoptotic cell/atheromatous plaques), Hypoxia-inducible factor 1-alpha (HIF-1α) and oxidative stress(OS) altogether, which promote exaggerated inflammation causing increased CRP (C-reactive protein), Interleukin 6 (IL-6), TNF-alpha (Tumor Necrosis Factor-alpha), and OS markers (MDA-Malondialdehyde) into circulation [7,8].

TELOMERE LENGTH IN PE

Telomers are essential for genome stability. Telomere length may reduce by different disease conditions like OS, PE, Intrauterine Growth Restriction (IUGR) [9], gestational diabetes, aging, and cancer [10]. Normal pregnancy also has some OS due to higher demand for metabolites of the growing fetus [11]. Therefore, further alteration of placental oxidants, antioxidants, and inflammation markers may show poor pregnancy outcomes such as PE and IUGR [12].

Therefore, this study was planned to explore any association between telomere length, inflammatory markers, and oxidative stress markers with change in BP (increase every ten mmHg).

MATERIALS AND METHODS

A case-control study included 90 PE cases and 70 controls (≥ 140/90 mmHg). PE cases were classified into three (A. 140/90 – 149/99 mmHg, B. 150/100 – 159/109, and C. ≥ 160/110 mmHg) sub-groups based on every ten-mmHg increasing BP. After obtaining written informed consent and meeting inclusion and exclusion criteria, the study was from the Department of Obstructive and Gynecology, Government institute of Medical Science (GIMS), Greater Noida, Utter Pradesh. Institutional Ethics Committee (GIMS, Greater Noida (GIMS/IEC/2019/11) & Santhosh Medical college, Ghaziabad (SU/2018/1456(8)) approved the study. Diagnostic criteria of PE as per ACOG (American College of Obstetricians and Gynaecologists) recommendations are ≥ 20 weeks of gestational age, new-onset hypertension (BP ≥ 140/90 mmHg) along with proteinuria. Two BP readings were recorded on different occasions with four hours gap between the two readings.

PE cases(n=90) were selected as follows; Inclusion Criteria: ≥ 20 weeks of gestational age, new-onset hypertension (BP ≥ 140/90 mmHg), and proteinuria. **Exclusion Criteria:** Family history of preeclampsia, previous history of preeclampsia, tobacco consumption, alcohol consumption, irrespective of parity.

5 ml of blood samples was collected in the appropriate vial and stored at – 80◦C deep refrigerator until the analysis.

1. Estimation of Relative Telomere Length by Modified (Richard M. Cawthon et al.) RT- PCR

DNA isolation: Genomic DNA was isolated from whole blood per the manufacturer (Nucleo-pore, Genetix Biotech Asia Pvt. Ltd., India) protocol. Quality and quantity of DNA checked by Nanodrop- Spectrophotometer (Genetix Biotech Asia Pvt. Ltd., India).

The reaction mix was prepared as follows: Syber green master mix 10 ul, Telomere primers F -Telg, R-Telc 0.9 ul each, β globin primers F-Hbgu, R- Hbgd 0.5 ul each, NFW 3.2 ul DNA template 4 ul.

PCR primers were designed as follows: Single Copy Gene(scg) are Hbgu Hbgd and Telomer primers

are set as per Richard M. Cawthon et al. Primers are as follows

Hbgd(β-globinR) GCCCGGCCCGCCGCGCCCGTCCCGCCGGAGGAGAAGTCTGCCGTT,

Hbgu(β-globinF) CGGCGGCGGGCGGCGCGGGCTGGGCGGCTTCATCCACGTTCACCTTG

Telc(TelomereR)TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA,

Telg(TelomereF) ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT.

RTPCR incubation conditions were explained in 3 stages, Stage 1: 07 min at 95◦C, Stage 2: 2 cycles of 15 seconds at 94◦C, 15 s at 49◦C; and stage 3: 39 cycles of 15 seconds at 94◦C, 10 seconds at 62◦C, 15 seconds at 74◦C with a signal acquisition, 10 seconds 84◦C, 15 seconds at 87◦C with signal acquisition. RTPCR signals were recorded as a Ct value at 74◦C for Telomer copy and scg at 87◦C. Data were normalized by β globin (Hbgu Hbgd). δ Ct values are the difference between T and S signals from which T/S(Telomere/SCG) values are generated. These δCt values are inversely proportional to Relative Telomere Length[13].

2. Estimation of MDA in serum: Serum MDA levels were measured by Competitive ELISA kit (Puregene, Genetix Biotech Asia Pvt. Ltd., India) as per kit insert. Standards with known concentrations of 2000, 1000, 500, 250, 125, 62.5,31.25,0 ng/mL were used to find unknown concentrations of MDA present in the serum. All samples and standards were processed on ImmunoWashTM 1575 Microplate Washer, iMark Microplate Reader (BIO-RAD, USA).

3. Quantification of serum CRP: Serum CRP level was quantified by turbidimetric analysis as per manufacturer (LAB KIT, CHEMELEX, SA) protocol and daily internal quality control was within ±2SD. Samples were run by a Fully automated analyzer (Selectra Pro XL, ELITECH Group Solutions).

4. Estimation of serum UA: Serum UA level was analyzed by uricase method as per manufacturer (Q-Line S+ Clinical Systems) protocol, and daily internal quality control were within ±2SD. Samples were run by a Fully automated analyzer (Selectra Pro XL, ELITECH Group Solutions).

STATISTICAL ANALYSIS

The Shapiro–Wilk test was used to check distribution of the data. One-way analysis of variance (ANOVA) tests was performed to compare the demographic and biochemical tests between the case and control. Tukey Post Hoc Test determines the significant difference between the case subgroups (A, B, and C) and the control group. The level of significance considered was < 0.05. Demographic, RTL, CRP, and UA were expressed as Mean ± Standard deviation. MDA was expressed as median and inter quartiles (Q1(25%, Q3(75%)). The Kruskal-Wallis H test compared MDA data between the case subgroup and control group and was considered significant if the p-value was < 0.05. Odds ratio (OR) with 95 % confidence intervals was analyzed for all parameters by Multinominal Logistic regression analysis.

RESULT

The descriptive analysis of the demographic data (shown in table number 1) was not statistically significant (p-value >0.05) between cases and control groups.

RTL: There was a significant difference between cases and control in the RTL, as shown by one-way ANOVA F (3, 126) = 6.653, p= .000. A Tukey Post Hoc Test displays that the telomere length was significantly reduced in Group A with 333.4±46.6 T/S ratio, p=.000 and Group-B 259.7±108 T/S ratio, p=.032 telomere length in comparison to Group-C 105±32.7 T/S ratio, p=.000. Further, Group-C displays significantly reduced telomere length (105±32.7) in comparison to reference control (290±185), p=0.001. At the same time, no statistically significant difference was found between Group- A (p= .516) and Group B (p=.829) regarding control. Calculated OR indicates significantly reduced telomere length in Group-C than in control (1.985, p=.003) (table 1). The OR is more than 1.000, indicating the reduced telomere length might be a risk factor for preeclampsia (Table 2).

MDA: MDA levels were significantly different in the cases than control, as shown by Kruskal-Wallis H (X2=3, 43.46, p =.000). Increasing Serum MDA levels were found compared to the control group, Group-A 152.3 (73 – 841) p=.000, Group-B 276.1 (138 – 540) p = .000, Group-C 1180 (527 – 2065), p = .000 and Control Group 79.5 (50.9 – 437.9). Calculated OR was two times higher among all the case subgroups (A, B & C) than control (A 1.998, p=.001, B 1.998, p = 0.002, and C 1.998, p =

.022) (Table 1). The OR is more than 1.000, and MDA might be a risk factor for preeclampsia (Table 2).

CRP: CRP levels were significantly different in the cases and control, as shown by one-way ANOVA (F (3, 126) =171.315, p .000). A Tukey Post Hoc Test displayed a significant difference in CRP levels in Group-A 86±46.6 mg/dl in comparison to group C, 255.3±74.4 mg/dl (p=.000); similarly, increased levels were seen in group C 255.3±74.4 mg/dl in comparison to Group-B 118.6±44.2, p=.007. Therefore A, B, and C Groups have a significant increase in CRP levels compared to the control (Table 1). The respective OR are Group-A 1.157, p=.000, Group-B 1.174, p = 0.000, and Group-C 1.215, p = .000, indicating levels to be two times higher in the case of groups than in the Control group.

The odds ratio is more than 1.000; CRP might be a risk factor for preeclampsia (Table 2).

UA: UA levels have a significant difference in the cases than in the control group, as shown by one-way ANOVA (F (3, 126) =171.315, p .000) (Table 1). Serum UA was significantly ($p < 0.05$) increased in preeclampsia. Calculated OR was three times higher among case group than control group (A 1.737, p=.002, B is 2.405, p = 0.000, and C 2.795, p = .000). The OR is more than 1.000; Uric acid might be a risk factor for preeclampsia (Table 2).

DISCUSSION

The study of clinical parameters such as age, gestational age, and BMI are comparable in both groups.

Our study reports significantly reduced TL in PE compared to control, and the finding was similar to those of previous studies [11,14]. We also investigated reduced telomere length in groups A and B compared to C-Group, and a similar finding was there for Group C compared to the Control group.

The OR is lower than the control; it might be a risk factor for PE. Furthermore, we found significantly increased MDA levels in PE compared to control, and the findings were similar to those of previous studies [15,16]. We also found increased MDA levels in subgroups A, B & C compared to the Control group. The OR was two times higher than the control group, which might be a risk factor for PE.

PE is a state of oxidative stress because of hypoxic placental tissue, which may enhance the excessive production of oxidative markers like MDA [16,17]. Hypoxia is an inducer of stress and inflammation. OS and inflammation will lead to endothelial dysfunction. Telomere sequences rich in GGG can attack by reactive oxygen species (ROS) [18]. Further, the imbalance between oxidants and antioxidants may lead to DNA damage, shortening telomere length. When it reaches critically short, it may lead to cell arrest and cellular death. A significant increase in serum CRP levels was also seen in preeclamptic patients compared to control. The finding is similar to those of previous studies [19,20,21,22]. We also found an increase in CRP levels in subgroups of PE compared to control. Also, group C has higher CRP levels than groups A and B. The OR is two times higher in the case group than in the control group, so it might also be a risk factor for PE [23]. Thus, this shows an association between telomere length, oxidative stress, and inflammation in PE [15, 19, 24].

Hypoxic placental tissue can induce inflammation by different cytokines and CRP [25]. Furthermore, as per Mendelian randomization tests, there is an association between CRP with increased BP [23]. CRP values also increased in the first trimester and may later have PE and IUGR [21]. Our results contrast with the study of Hilary S. Gammill et al. [26], where no change in CRP values was reported.

Furthermore, our study found a significant increase in Uric acid levels in PE vs. Control. The finding was similar to those of previous studies [27,28]. The OR of UA was three times higher than control; it might be a risk factor for PE. The finding was similar to those of previous studies [29]. UA is increased if there is a defect in renal clearance. Oxidative stress, hypoxic placental tissue, and endothelial dysfunction [30] may further enhance xanthine oxidase levels, thereby causing an increase in serum UA levels. Therefore, it may be used as an early prognostic indicator of the pathophysiology of PE [27].

We could not find any studies showing the association of study parameters (RTL, MDA, and CRP) with an increase in BP (10 mmHg) in PE. Our study found reduced telomere length and increased MDA, CRP, and UA levels with every 10mmHg rise in BP in case groups.

CONCLUSION:

The Telomere length, MDA, CRP, and Uric acid levels are associated in PE patients with an increase in BP. A positive association was seen between MDA, CRP, and Uric acid levels with increasing BP. A negative association was seen in Telomere length with increasing BP. Therefore, this study may link oxidative stress and inflammation with a reduction in telomere length that leads to the pathophysiology of PE.

- Reduced Telomere length may predict preeclampsia(PE) severity and increase blood pressure.
- Hypoxic placental tissue enhances oxidative stress and inflammation. The imbalance between oxidants, antioxidants, and inflammation reduces Telomere length.
- The CRP, MDA, and Uric acid levels indicate the severity of PE as every ten mmHg increases BP.
- Preeclamptic women's inflammatory status and stress may reduce if we can maintain blood pressure within the normal.

ACKNOWLEDGEMENT:

I thank my colleagues for their valuable suggestions and input.

GRANT:

Government Institute of Medical Sciences (GIMS), Greater Noida, UP, provided funding **(letter number: GIMS/2022/098).**

DECLARATIONS:

Compliance with ethical standards

CONFLICT OF INTEREST:

We declare no conflict of interest.

ETHICAL:

The Institutional Ethics Committee approved the study (GIMS, Greater Noida & Santhosh Medical college, Ghaziabad).

The study subjects were invalued after obtaining **a written informed consent** form.

Our manuscript has not been published and is not under consideration for publication in any other journal. All authors have approved our manuscript and its submission to the journal.

Tab. 1. Demographic and Biochemical data of study subjects.

Characteristics	**Normal pregnant women (n=70)**	**Preeclampsia (A, B, and C n=30 each)**	**One way ANOVA p-value**
Age (years)	24.9±3.9	24.2±3.3 27.2±5.7 24.3±3.4	.065
Gestational Age (weeks)	33±5.6	35.4±4.2 34.5±4.7 35±4.5	.156
BMI	24±3.5	26±3.2 26±3.4 24±2.3	.071
Proteinuria (Dipstick)	*Nil*	+3	.000*
RTL (T/S ratio)	290±185	333.4±46.6 259.7±108 105±32.7	.000*
MDA** (ng/mL)	79.5 (50.9 – 437.9)	152.3 (73 – 841) 276(138 – 540) 1180(527.8 – 2065)	.000*
CRP (mg/L)	10.7±5.7	85±46.8 118.6±44.2 255.3±74.4	.000*
Uric Acid(mg/dl)	4.5±1.2	5.4±1.6 6.3±2.2 7±0.8	.000*

Data represented for Normally distributed data as mean ± SD , for MDA as median(Q_1-Q_3) for non-normally distributed data, RTL: relative telomere length, MDA; malondialdehyde matrix, CRP`; c- reactive protein. Kruskal-Wallis H Post Hoc, Analysis of variance (ANOVA) and followed by Tukey Post Hoc Tests used according data distribution *

statistical significance if $p < 0.05$; study groups: normal pregnant women(control) and preeclamptic women (three sub-groups a. BP 140/90 – 149/99, b. BP 150/100 – 159/109, c. BP ≥160/110 mmHg).

Tab. 2 Results of multinominal Logistic regression analysis.

Parameters	df	Unadjusted OR	95 % CI	p- value
Relative Telomer Length (T/S ratio)	1^A	1.002	0.9-1	0.186
	1^B	0.998	0.9-1	0.398
	1^C	1.985	1.6-2	0.003*
MDA (ng/mL)	1^A	1.998	1.9-1.9	0.001*
	1^B	1.998	1.9.1.9	0.002*
	1^C	1.998	1.9-2	0.022*
CRP (mg/L)	1^A	1.157	1.1-1.2	0.000*
	1^B	1.174	1-1.3	0.000*
	1^C	1.215	1.2-1.4	0.000*
Uric Acid(mg/dl)	1^A	1.737	1.226	0.002*
	1^B	2.405	2.405	0.000*
	1^C	2.795	2.795	0.000*

Data represented as Df: Degrees of freedom, Unadjusted Odds ratio (OR), 95% confidence interval (CI), RTL; Relative Telomer Length, MDA; Malondialdehyde, CRP; C- reactive protein, UA; Uric acid. * For statistical significance ($p < 0.05$); study groups: normal pregnant women and preeclamptic women (three sub-groups are marked as superscript A. BP 140/90 – 149/99, B. BP 150/100 – 159/109, C. BP ≥160/110 mmHg).

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