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Original Article

Confined Placental Mosaicism of chromosomal aneuploidy is not associated with preeclampsia: A pilot study

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Abstract

Background: Preeclampsia is a pregnancy-related complication characterized by increased blood pressure without any history of hypertension. Confined placental mosaicism is the presence of chromosomal abnormalities in the extra-embryonic tissue such as placenta. The association between confined placental mosaicism of chromosomal aneuploidy and preeclampsia in the Indian population is unknown.
Objective: To determine the association of confined placental mosaicism of chromosomal aneuploidy with preeclampsia.
Methods: Cross-sectional study was designed to find the association of confined placental mosaicism and preeclampsia. Placenta (trophoblasts) (n=17), cord blood (n=8), and both placenta (trophoblasts) and cord blood (n=8) samples were collected from preeclamptic women after delivery. The samples were then processed and analyzed to find the mosaicism in trisomy 13 (13q14) and 16 (D16Z2) using FISH and karyotyping.
Results: Both FISH and karyotyping analysis showed that there was no chromosome 13 (13q14) and 16 (D16Z2) mosaicism in both placenta (trophoblasts) and cord blood samples, collected from preeclamptic women after delivery. The results suggest that confined placental mosaicism was not observed in preeclamptic women.
Conclusion: The results showed that the confined placental mosaicism of chromosome 13 (13q14) and 16 (D16Z2) aneuploidy is not associated with preeclampsia.
Keywords: Preeclampsia, Confined Placental Mosaicism

Introduction

Preeclampsia (PE) is a pregnancy-related complication involving new-onset hypertension in a pregnant woman without any history of hypertension. It is classified based on the gestational age of onset as early-onset (≤33 weeks of gestation) and late-onset (≥34 weeks of gestation).[1] The clinical presentation of PE ranges from mild to severe. It is characterized by systolic blood pressure levels of 140–160 mmHg or diastolic pressure levels of 90–110 mmHg and often proteinuria (urinary albumin protein ≥300 mg/24 h) or other complications arising after 20 weeks of gestation. Other complications such as HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), renal insufficiency, pulmonary edema, and new onset of cerebral or visual disturbances are associated with preeclampsia.[2]

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The worldwide incidence of preeclampsia is about 3–10% of pregnancies, it is a major cause of maternal and perinatal morbidity/mortality.[3] The incidence of preeclampsia in India is about 8-10% of all the pregnancies.[4] The prevalence of preeclampsia varies in different populations and ethnic groups.[5]

Confined Placental Mosaicism (CPM) is defined as the presence of chromosomal abnormalities in the extra-embryonic tissue (placenta), which are absent in fetal tissue.[6] CPM involves aneuploidy (commonly trisomy) in the placenta and but not in the foetus. CPM affects placental function and is observed in loss of pregnancy and intrauterine growth restriction (IUGR) cases. The placental trisomy abnormality leads to preeclampsia.[7, 8] CPM of trisomy 13 and 16 showed an increased risk of preeclampsia in western populations.[9, 10]

There are no studies that have reported on the frequency of CPM in the Indian population. Therefore, we aimed to determine the association of CPM of chromosomal aneuploidy with preeclampsia in the Indian population.

Materials and Methods

We designed a cross-sectional study. The current study was approved by the Central Ethics Committee, Sri Devaraj Urs Academy of Higher Education and Research. India. Ethics No.SDUAHER/KLR/Dept. of R & I/68/2020-2021. Patients who were Preeclamptic were enrolled for the study from the Department of Obstetrics and Gynecology, R.L. Jalappa Hospital and Research Centre, Sri Devaraj Urs Medical College, after obtaining the prior informed consent.

Clinical methods

Diagnosis of the patients was done using the following criteria: blood pressure ≥140/90 mmHg on two occasions at least 4 hours apart and gestational age ≥20 weeks.[11] Inclusion criteria are (i) pregnant women with mild and severe preeclampsia. Exclusion criteria are patients with (i) history of chronic hypertension and (ii) urinary tract infection. Expelled placenta (trophoblasts) (n=17), cord blood (n=8), and both placenta (trophoblasts) and cord blood (n=8) samples were collected from preeclamptic women after delivery. Approximately 50 mg of a placental sample from the fetal side of the expelled placenta and cord blood of 1 ml were collected in sterile transport media and heparin vacutainer respectively. Placental cells were cultured and performed fluorescence in situ hybridization (FISH) to screen the aneuploidy for Chromosomes 13 locus specific in the region 13q14 (Kreatech probe, Leica Bio systems) and 16 region D16Z2 (Kreatech probe, Leica Bio systems). Cord blood was cultured and performed karyotype to find the mosaicism in Chromosome 13 and 16.

Placental cell culture

The placental tissue (trophoblasts) was cleaned using phosphate-buffered saline and digested with Collagenase-II. The culture was set up in T25 flask with amniomax media. Once the cells were attached and transformed, media was replaced by a fresh media on every alternate day. Sufficient numbers of well-grown colonies with doublets were observed, the cells were harvested. All samples were performed karyotype, but we didn't get well speard metaphase chromosome due to culture failure. Therefore, from uncultured placenta (trophoblasts) samples Fluorescence in-situ hybridization (FISH) was carried out to identify aneuploidy in chromosome 13 (13q14) and 16 (D16Z2).

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed using chromosome 13 (13q14) and 16 (D16Z2) specific probes. One day aged slides were dehydrated in alcohol and air-dried. Probes were added on the slide and co-denatured at 73˚C for 2 min and hybridized at 37˚C for 16 h in the hybridization chamber. After hybridization, slides were washed at 73˚C water bath with NP40 and counterstained with DAPI. Slides were analyzed for interphase and metaphase cells using FISH software (ISIS) (Carl Zeiss, Axio Imager A2). Minimum of 50 cells was analysed and about five representative images were captured for each sample.[20]

Karyotype of cord blood samples

Cord blood culture was set up by adding 0.5 ml of blood, 5 ml of RPMI 1640 medium, 1 ml of fetal bovine serum and 0.3 ml of PHA in a sterile culture vial. The cultures were incubated for 72 h at 37˚ C by adding the colchicine at 68 ½ h. The samples were harvested and performed by GTG banding. Around 50 good spreads and well-banded metaphases were analysed and captured using Karyotyping Software (Carl Zeiss, Axio Imager A2).[12] The karyotype abnormalities in chromosome 13 and 16 were analysed and designated as per ISCN (2013) nomenclature.

Statistical analysis

The data was processed using SPSS software (version-22.0, IBM, USA) and the mean and standard deviation were calculated for age of the preeclamptic women. The percentages for subgroups were calculated to compare between groups.

Results

Mean ± SD age of the preeclamptic women is 24.8 ± 2.81 years. The percentage of Primigravida is 64.0% and Multigravida is 35.29%. The clinical

presentation ranged from mild PE to severe PE (35.2-64.0%). The percentage of Early-onset gestational age was 23.5%, and late-onset gestational age was 76.4%. The mean ± SD of Systolic Blood pressure (144 ± 10.8 mmHg) and Diastolic blood pressure (90.7 ± 21.0 mmHg). Dipstick proteinuria 1+, 2+ is 17.64% and 5.88%. Co-Morbidities' such as Eclampsia (5.88%), IUGR (11.76%), HELLP syndrome (5.88%). The demographic details of the study subjects are given in Table 1.

Association of Confined Placental Mosaicism with preeclampsia

Aneuploidy screening for Chromosome 13 and 16 using FISH in all preeclamptic women placental sample (trophoblasts) showed no abnormalities. No trisomies were observed in all the cells of interphase and metaphase. Representative images of FISH were given in Figure-1a and 1b. Similar to placental samples cord blood karyotype also did not show any abnormalities in chromosome 13 and 16. The representative karyotype taken from the cord blood samples is provided in Figure-2.

Table 1: Demographic details of the study subjects

Parameter	Preeclamptic pregnant women (n=17)
Age (years) (Mean ± SD)	24.8 ± 2.81
Gravida • Primigravida • Multigravida	 11 (64.0%) 06 (35.29)
Severity • Mild • severe	 11(64.0%) 06 (35.2%)
Gestational age of Onset • Early-onset(≤33weeks) • Late-onset (≥34 weeks)	 04(23.5%) 13(76.4%)
Blood pressure • Systolic blood pressure • Diastolic blood pressure	 144 ± 10.8 90.7± 21.0
Dipstick Proteinuria • 1+ • 2+	 3 (17.64%) 1(5.88%)
Co-Morbidities • Eclampsia • IUGR • HELLP syndrome	 1 (5.88%) 2 (11.76%) 1 (5.88%)

IUGR=Intrauterine growth restriction,
HELLP= (Hemolysis, elevated liver enzymes, and low platelet count)

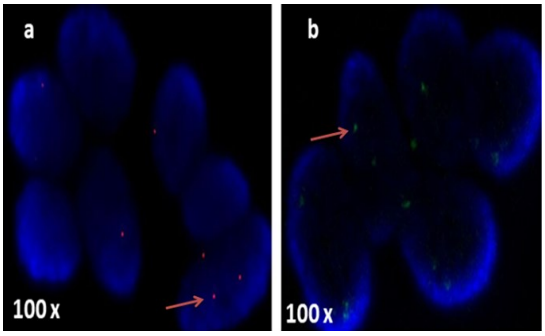

Figure 1a and 1b: Representative images of chromosome 16 (a: Red signal in DAPI stained nucleus) and 13 (b: Green signal in DAPI stained nucleus) obtained using FISH technique performed on placental tissue samples of preeclamptic women. Presence of two signals represents two copies of chromosome 13 (13q14) and 16 (D16Z2).

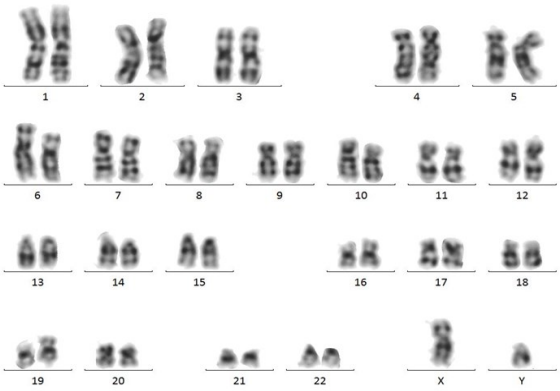

Figure 2: Representative image of karyotype obtained from cord blood collected from preeclamptic women after delivery.

Discussion

This study aimed to determine the association of confined placental mosaicism in chromosomes 13 and 16 with preeclampsia. As far as we are aware, this is the first study to describe the association between confined placental mosaicism and preeclampsia in the Indian population. The major finding of our study is that aneuploidy of chromosomes 13 and 16 was absent in the placental and cord blood samples collected from preeclamptic women. Our results indicate that mosaicism of chromosomes 13 and 16 is not associated with preeclampsia.

Previous studies conducted in the western population indicated that the IUGR or preeclampsia

can be caused by placental trisomy.[7,8,13] Especially confined placental mosaicism of trisomy 13 has been reported as having an increased risk of preeclampsia.[14-17] This is assumed to be due to the localisation of FLT1 gene on 13q12.3.[18] sFLT1 protein levels in maternal serum of pregnancies with a trisomy 13 confined to the placenta, were 35% higher compared to normal pregnancies.[16] sFLT1 is elevated in preeclampsia and is now being increasingly used as a diagnostic biomarker. Additionally, in non-pregnant rats, the administration of sFLT1 evokes preeclampsia like symptoms.[19] Available evidence point to a gene-dosage effect of sFLT1 in preeclampsia.

Trisomy 16 is the most common aneuploidy observed in spontaneous pregnancy losses, which ranges between 1 to 1.5% of all pregnancies.[20] Placental trisomy 16 showed 3–4 times increased risk in preeclamptic women compared to a control population.[21-23] all newborns with normal IUGR showed term placentas with less frequency of trisomic cells.[22] Frequency of trisomy 16 was higher in the placenta and is associated with preeclampsia. Despite the existed evidence, our study did not find the association of placental mosaicism in preeclamptic women in Indian population. The lack of association might be due to the lower sample size. Further studies need to be carried out with a higher sample size to confirm our findings to determine the association of confined placental mosaicism in 13 and 16 with preeclampsia in the Indian population.

Conclusion

Our results suggest that there is no association between confined placental mosaicism and preeclamptic women.

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