Differential regional fatty acid distribution in normotensive and preeclampsia placenta

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ABSTRACT

Background: Long chain polyunsaturated fatty acids (LCPUFAs) are biologically active fatty acids which regulate placental angiogenesis, inflammation, and oxidative stress. Abnormalities in these aspects have been associated with preeclampsia (PE). Further, placenta has a heterogeneous structure with differential vascularization across different regions. We therefore hypothesize that the distribution of fatty acids in various regions of the placenta is altered in PE leading to poor fetal outcome.
Methods: In this cross-sectional study we recruited 69 normotensive control (NC) and 44 women with PE. PE women were further classified as those delivered preterm (PTPE, n = 24) and at term (TPE, n = 20). Fatty acid levels were analyzed from placental samples from four different regions (CF—central fetal, PF—peripheral fetal, CM—central maternal and PM—peripheral maternal).
Results: In the NC placenta, AA levels were lower (p < 0.05) in CM as compared with CF region. However, such differences were not seen in the TPE and PTPE. In contrast, the DHA levels varied between regions only in the PTPE placenta. Between groups, DHA levels were lower (p < 0.05 for both) in the CM and CF regions of the PTPE as compared with NC. The levels of DHA in TPE placenta were similar to NC. AA levels were lower (p < 0.05 for both) in CF region of TPE and PF region of PTPE placenta than NC.
Conclusions: There is differential pattern of LCPUFA distribution across various regions of the NC, TPE and PTPE placenta. This may have implications for placental growth and development as well as transfer of LCPUFA to the fetus.

1. Introduction

Preeclampsia (PE) is a pregnancy induced hypertensive disorder which manifests after 20 weeks of gestation and till date its etiology is not well understood [1]. A number of placental abnormalities have been associated with PE pregnancies, such as insufficient spiral artery remodeling, shallow invasion, and reduced villous number, diameter and surface area. Placental function gets affected by these abnormalities, and ultimately deprives the developing fetus of the nutrients required for optimal growth [2,3]. Nutrients like fatty acids are not only required by the developing fetus but also metabolized by the placenta for its growth and development [4].

Fatty acids especially long chain polyunsaturated fatty acids (LCPUFAs) are biologically active fatty acids which have been found to be associated with a number of developmental and functional aspects of the placenta. LCPUFAs are required by the placenta for membrane synthesis and to maintain fluidity for intercellular signaling [5]. Further,

LCPUFAs act as ligands for transcription factors which regulate genes involved in trophoblast proliferation and differentiation [6]. Our earlier studies on women with PE report altered placental LCPUFA levels, disturbed angiogenesis and fatty acid transport in the maternal region of the placenta [7,8]. It is well known that the morphological heterogeneity of the placenta comprises of both fetal and maternal tissues [9–11]. Differences in vascularisation across the placenta resulting in differences of oxygen availability are also reported within the placenta [12, 13]. Several studies have reported gradients in protein levels, gene expression and enzyme activities across the normal placenta [14–18]. Therefore, there could be functional specialization of different regions of the placenta which requires extensive research.

PE has also been associated with increased oxidative stress and inflammation originating from the placenta and affecting both mother and the fetus [19,20]. LCPUFA can form active metabolites like eicosanoids, resolvins and protectins which regulates inflammation and it can also reduce oxidative damage in the trophoblast cells [21]. In the presence of reactive oxygen species, LCPUFA get per-oxidized into malondialdehyde (MDA) which is an oxidative stress marker [22]. Recently we have shown increased levels of MDA and decreased levels of catalase an antioxidant enzyme in a region specific manner in the

PE placenta [23]. Further, studies have also reported regionwise alterations in the inflammatory cytokines, nitric oxide synthase enzyme and heat shock proteins in the PE placenta [20,24–26].

It is likely that the LCPUFA metabolism may differ in the placenta depending upon the requirement by the trophoblast cells for different physiological processes. We therefore hypothesize that the regional distribution of fatty acids in the placenta is altered in preeclampsia leading to poor birth outcome. In the current study we examined the levels of fatty acid in various regions of the PE and normotensive control (NC) placenta and compared the levels within and between groups. Further, the associations of LCPUFA levels with birth weight and blood pressure were studied.

2. Materials and methods

2.1. Study participants

This is a cross-sectional study where pregnant women were recruited at Department of Obstetrics and Gynecology of Bharati Hospital, Pune, India. This study was ethically approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee, Pune, India. Written informed consents were taken from each participant. Maternal weight and height were taken at the time of recruitment for the calculate body mass index (BMI). A total of 69 normotensive pregnant women delivering at term (gestation ≥ 37 weeks, baby weight ≥ 2.5 kg) and 44 pregnant women with PE were recruited for the study. PE was defined by systolic BP and diastolic BP >140 and 90 mm Hg (repeated measures by Mercury sphygmomanometer) respectively and the presence of proteinuria (>1 + or 300 mg per 24 h, by Dipstick test). The detailed exclusion and inclusion criteria were as described by us earlier [23]. The PE cases were further divided into 24 preterm PE (PTPE) and 20 term PE (TPE) after delivery. Baby birth weight was measured using a digital weighing scale (Zeal Medical Private Limited, India) with 10 g accuracy. Baby length was measured using a portable infantometer.

2.2. Placental sampling

Placenta was collected in 1X phosphate buffer saline (PBS) immediately following delivery. Small pieces of fresh tissues were collected from four different sites of the placenta after removing the fetal membrane as described in our recent study [23]. Velamentous cord insertion cases were excluded from the study. Briefly, four sampling sites were selected 1) CM (central maternal) — small pieces (~1 cm^2) of basal plate villous tissue were cut out from the normal appearing cotyledons around the cord insertion avoiding blood clots and infarcts, 2) PM (peripheral maternal) — from peripheral cotyledons of the basal plate farthest from the cord insertion and 1 cm away from the lateral edge of the placental disk, similarly 3) CF (central fetal) — from central cotyledons of the chorionic plate and 4) PF (peripheral fetal) — from peripheral cotyledons of the chorionic plate (Fig. 1). Excess blood was washed off with 1X PBS and then the samples were stored at −80 °C till further analyzed.

2.3. Fatty acid analysis

The fatty acid estimation procedure followed for analysis was as reported in series of our earlier studies [7,27]. Briefly, placental tissue was homogenized with chilled lysis buffer and ultra-centrifuged. Transesterification of fatty acids present in the cell membrane fraction was done using methanolic–hydrochloric acid. These fatty acid methyl esters (FAMEs) were analyzed using a Perkin Elmer Gas Chromatograph at our standardized settings (SP 2330, 30 m capillary Supelco column). A mixture of standard fatty acid methyl esters (Sigma) was used to identify 15 important fatty acids by retention time. The relative FAME amounts were expressed as g/100 g fatty acid (% total fatty acids). Fatty acids were categorized into total omega 6 fatty acids (omega 6):
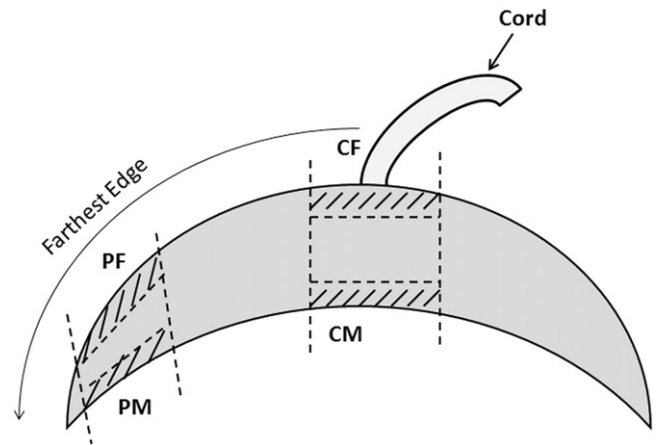

Fig. 1. Sampling sites of the placenta. CM — central maternal region, PM — peripheral maternal region, CF — central fetal region, PF — peripheral fetal region.

summation of linoleic acid (LA), gamma linolenic acid, dihomo gamma linolenic acid, docosapentaenoic acid and arachidonic acid (AA); total omega 3 fatty acids (omega 3): summation of alpha linolenic acid (ALA), eicosapentaenoic acid and docosahexaenoic acid (DHA); saturated fatty acids (SFAs): summation of myristic acid, palmitic acid and stearic acid; and monounsaturated fatty acids (MUFAs): summation of myristoleic acid, palmitoleic acid, oleic acid and nervonic acid.

2.4. Statistical analysis

The sample size was calculated based on our earlier study taking the mean difference of placental DHA levels between the case and the control [7]. It was done using PS sample size calculator software (ver. 3.0.43) with 80% power of the study, 0.05 type I error probability and 3 control subjects per case. Hence, 69 controls and around 23 cases in each PE group were included in this study.

The data was analyzed using the SPSS/PC + package (Version 20, Chicago, IL, USA). Values are reported as Mean ± Standard Deviation (S.D.). Skewed variables were normalized using the log_{10} transformation. Means were compared using one way ANOVA. Associations with birth weight and BP were performed using Pearson's partial correlation after adjusting for confounder i.e. gestational age, maternal age and BMI in NC and PE as separate groups. It was also done for whole cohort after adjusting for groups. Results were considered significant when $p < 0.05$.

3. Results

3.1. Maternal and neonatal characteristics

The systolic BP and diastolic BP of both PTPE and TPE women were higher ($p < 0.01$ for all) as compared with NC women. PTPE women had even higher systolic BP and diastolic BP ($p < 0.01$ for both) as compared with TPE women. BMIs of both the TPE ($p < 0.01$) and PTPE ($p < 0.05$) women were higher as compared with NC women.

In PTPE and TPE groups, birth weight and baby length were lower ($p < 0.01$ for all) as compared with NC group. These birth measures were even lower ($p < 0.01$ for both) in PTPE as compared with TPE group (Table 1).

3.2. Comparison of fatty acid levels across different placental regions within normotensive control, preterm-preeclampsia and term-preeclampsia

In the NC group, no differences were observed in the levels of LA within the placenta. However, AA levels were higher ($p < 0.05$ for