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Maternal Flt-1 and endoglin expression by circulating monocyte subtype and polarization in preeclampsia and fetal growth restriction

Thushari Indika Alahakoon*, Heather Medbury, Helen Williams, Nicole Fewings, Xin Wang, Vincent Lee

Hawkesbury Road, Westmead, Australia

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ABSTRACT

Objective: Circulating levels of the anti-angiogenic factors sFlt-1 and sEndoglin are elevated in preeclampsia (PE) and fetal growth restriction (FGR), mainly secreted from placental trophoblast. This study aims to identify the contributory role of monocyte Flt-1 and endoglin expression in PE and FGR.
Study design: A prospective cross-sectional study was conducted and patients recruited from four clinical groups including normal pregnancy, PE, FGR and PE + FGR. Peripheral blood samples and cord blood were collected from 54 pregnant women between 24–40 weeks of gestation. Monocyte subset distribution was assessed using CD14 and CD16 expression and the surface expression of Flt-1, endoglin, CD86 and CD163 assessed by flow cytometry. We compared these factors between (1) clinical groups. (2) monocyte subset (3) monocyte polarization and (4) gestational age.
Results: Across all clinical groups, Flt-1 was mainly expressed by classical and intermediate monocytes, but no differences between clinical groups were observed. Surface expression of endoglin was higher on intermediate and non-classical monocytes and decreased in PE + FGR total monocytes. Flt-1 and endoglin expression correlated with increasing gestational age as well as higher CD86/CD163 ratio favouring M1 polarisation. The fetal monocyte endoglin expression was increased in FGR.
Conclusion: We conclude that monocyte Flt-1 and endoglin expression increase with gestational age and with M1 polarization suggesting their upregulation with inflammatory changes in monocytes. Endoglin expression by M1 monocytes may play a part in increased cardiovascular risk associated with preeclampsia. Endoglin expression on fetal monocytes is increased in FGR as a likely response to placental injury.

Introduction

Circulating levels of the anti-angiogenic factors fms-like tyrosine kinase (sFlt-1) and soluble endoglin are elevated in preeclampsia and fetal growth restriction [1–4]. Studies to date have focused on placental trophoblast expression of Flt-1 and endoglin [5–7]. A contribution to the pathogenesis of preeclampsia and fetal growth restriction by Flt-1 and endoglin production by other cell types such as endothelial cells, monocytes and macrophages in the placenta and maternal peripheral circulation, has not been well explored. Monocytes are a functionally heterogeneous cell type with plasticity in subset types and polarization. The contribution of monocyte subsets to the angiogenic milieu in the pathogenesis of preeclampsia and fetal growth restriction is not established.

Circulating monocytes [8,9] express Flt-1 as a cell surface molecule, through which they bind VEGF and PlGF [10]. Both VEGF and PlGF stimulate tissue factor production, chemotaxis and trigger of further release of VEGF [11]. Flt-1 is also a mediator of pro-coagulant activity [10]. Both PlGF and VEGF activate the Flt-1–dependent signaling pathways of PI-3 K, p38 kinase, Akt, and ERK1/2 in primary human monocytes, leading to the activation of several intracellular signaling pathways that are critically involved in primary monocyte chemotaxis [12]. These data suggest that Flt-1 is a cell surface marker as well as a biologically functional molecule for monocyte-macrophage lineages. Any expression of sFlt-1 from these cells could be an additional, extra-placental, source of sFlt-1 that contributes to the pathogenesis of preeclampsia while surface expression of Flt-1 may play a pro-angiogenic role. In addition, the strong placental expression of PlGF could contribute to the marked angiogenesis seen in the growing placenta in part by its chemo-attraction of monocytes [13,14]. While there is some data available on monocytes in

* Corresponding author.
E-mail address: indika.alahakoon@health.nsw.gov.au (T.I. Alahakoon).

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preeclampsia, there are no published studies exploring peripheral monocyte-derived angiogenic factors in fetal growth restriction or in the fetal / placental circulation.

Endoglin (CD105) is a component of the TGF-β receptor system and acts as a co-receptor for TGF- β1 and TGF- β3 with high affinity [15–17], playing a critical role in maintaining cardiovascular homeostasis [18]. While endoglin is primarily expressed on endothelial cells [19] and induces pro-angiogenic proliferation and migration of these cells, it has also been demonstrated on macrophages [20], erythroid precursors [21], syncytiotrophoblast [22], monocytes [20], and stromal cells [23]. Soluble endoglin (sEng) inhibits TGFβ1 binding to its receptor, disordering signaling and preventing stimulation of endothelial nitric oxide synthase and vessel dilatation and is known to be elevated in preeclampsia, fetal growth restriction [7], and severe placental disease [24,25]. While monocytes can express endoglin, whether they do so in preeclampsia and fetal growth restriction has not been demonstrated.

Little is known about the pro and anti-angiogenic factor expression by different subsets of monocytes. Monocytes are subdivided into 3 subsets based on the expression of CD14 and the CD16 receptors, classical monocytes that lack CD16 ($CD14^{+}{}^{+}CD16^{-}$), and two subsets that express CD16 intermediate ($CD14^{++}CD16^{+}$), and the non-classical ($CD14^{+}CD16^{++}$) [26]. The intermediate and non-classical subsets are considered inflammatory monocytes [26]. The surface markers KDR and endoglin are suggested to be expressed on intermediate monocytes [27], at a low threshold. Monocyte chemotaxis towards the angiogenic ligands VEGF and PlGF was reduced in CD16+ intermediate and non-classical monocytes compared to CD16- classical monocytes [28]. Currently there is no data available on the relative levels of endoglin expression by different monocyte subsets.

Monocytes and macrophages can be phenotypically polarized by the environment to mount specific functional programs. Polarized activation of cells of the monocyte-macrophage lineage into classically activated inflammatory (M1) and alternatively activated healing (M2), cells is an operationally useful, simplified descriptor of the functional plasticity of these cells [29]. A transition from pro-inflammatory M1 to anti-inflammatory M2 phenotype is characterized by changes in cell surface marker expression including increases in CD163, CD206, and CD11b, which are distinctive of M2 monocytes and macrophages [30]. CD86 is a cell surface protein strongly expressed by M1-type macrophages / monocytes [31–33]. The monocyte expression of anti-inflammatory molecule, CD163 and the CD86/CD163 ratio can be used as a marker of M1 and M2 phenotypes [34]. M2 polarized macrophages play a role in resolution of inflammation, accompanied by reduced pro-inflammatory cytokine secretion [35]. Differentiation of monocytes into M1 and M2 macrophages play a central role in wound healing [36]. The association of monocyte polarization into M1/M2 phenotypes and correlation with angiogenic factor expression has not been described in the literature.

The current study explores the associations of peripheral monocyte expression of angiogenic factors (Flt-1 and endoglin), with clinical groups (normal, preeclampsia, fetal growth restriction and preeclampsia + fetal growth restriction), monocyte subsets (classical, non-classical and intermediate), polarization (by ratio of CD86/CD163), as well as by gestational age. We examined both maternal and fetal monocytes.

Materials and methods

A prospective cross-sectional study was conducted. Pregnant women between 24–40 weeks of gestation dated by first trimester ultrasound, who satisfied the selection criteria, confining at an Australian tertiary hospital during 2013–2014 were recruited from ultrasound service, antenatal ward, and antenatal clinics into four clinical groups of normal pregnancy, preeclampsia, fetal growth restriction and preeclampsia + fetal growth restriction. The normal pregnancy samples were collected at the routine antenatal clinics for gestations less than 37 weeks and prior to elective repeat caesarean section at over 37 weeks. The study was conducted with the approval of the institutional Human Research Ethics committee. Written consent was obtained from all participants in the study.

All patients classified as preeclampsia in this study satisfied the ISSHP 2014 criteria for preeclampsia [37]. Fetal growth restriction was defined as birth weight less than 10^{th} centile with elevated umbilical artery Doppler systolic / diastolic ratio or Resistance Index > 95th centile for gestation. All patients with preeclampsia, preeclampsia + fetal growth restriction or fetal growth restriction underwent antenatal ultrasound examination after 24 weeks of gestation and within 7 days of delivery. Patients with pre-existing hypertension, renal disease, pre-existing diabetes, gestational diabetes and multiple pregnancies were excluded from the study.

Flow cytometry

Maternal venous blood samples were collected prior to delivery in EDTA tubes. Blood counts were performed. Cells were stained at room temperature using antibodies against CD14 (BD 560349, Biosciences, USA), CD16 (Ab 140477, Abcam, USA), Flt-1 (FAB321 A, R&D), endoglin (BD561443), CD86 (BD 555658 Biosciences, USA) and CD163 (BD 556018, Biosciences, USA). Incubation with cell surface antibodies was followed by Optilyse C for lysing red blood cells and fixation. Monocyte phenotype assessment and cell marker profile was assessed by flow cytometry using a BD Canto II flow cytometer (BD Biosciences, USA) and Flow Jo software version 10.6 (Tree star, Inc., Ashland, OR, USA). A fluorescence minus one control and a three-color panel were used for data acquisition. The median fluorescence intensity (MFI) was calculated for each marker in each clinical group, Flt-1 and endoglin expression were examined and correlated with clinical group, monocyte subset and CD86/CD163 as an indicator of the degree of monocyte M1/M2 polarization.

Statistics

The statistical software packages SPSS for windows Version 21 and SPLUS version 8 were used. The patient numbers used in this study were considered adequate from previous work on similar exploratory studies. Similar work has been published on sample numbers ranging from 5 to 10 in each group. ANOVA for multiple comparisons was used to assess the association between tested variables. Kruskal Wallis non-parametric analysis of variance was used to test for homogeneity across the four clinical groups for each of the variables; percentage of monocytes, percentage of monocyte subsets, median fluorescence intensity of CD86, CD163, Flt-1 and endoglin. Where heterogeneity was identified, post hoc Mann-Whitney tests were used for pairwise comparisons between normal pregnancy and each of the clinical groups as well as between each of the pathological groups. The Spearman's rank correlation was used to quantify the extent of the association between monocyte surface Flt-1, endoglin expression and gestational age in normal pregnancies. Data were also analyzed for any association between Flt-1, endoglin expression and monocyte subtype as well as monocyte polarization and pro-inflammatory status as defined by CD86/CD163 ratio.

Results

Maternal and neonatal demographic data, clinical characteristics and results are presented for 54 maternal and 27 fetal

Table 1
Maternal and fetal demographic data and clinical characteristics of the study population. Results are presented as mean ± SD for each continuous variable unless otherwise specified. * Significantly different from normal pregnancy. † Significantly different to PE. # Significantly different to FGR. $p < 0.05$. LSCS Lower segment caesarean section.

Experimental Groups	Normal	PE	FGR	PE + FGR
Number of maternal samples	24	9	12	9
Number of cord blood / fetal samples	8	4	9	6
Maternal age (years)	29.4(3.6)	27.9(6.1)	28.3(4.9)	33.0(7.1)* †
BMI	27.3(5.3)	29.6(8.1)	24.2(5.3) †	28.2(6.3)
Gestation at sample collection (weeks)	34.8(4.2)	35.7(3.3)	34.8(4.2)	32.3(2.3)
Gestation at delivery (weeks)	39.2(0.8)	36.2(3.5)*	35.5(3.6)*	32.68(2.5)* † #
Birth weight (g)	3316(505)	2749(821)*	1879(670)* †	1509(540)* †
Primiparous (%)	37.5	77.8*	58.3	88.9*
Antihypertensive treatment (%)	0%	88%	0%	44%
Smoking	8.3%	0.0%	8.3%	7.4%
Mode of delivery (Rate of LSCS)	71%	78%	100%	100%

samples for pregnancies from 24 to 40 weeks of gestation. There were no statistically significant differences noted between the pathological (preeclampsia, preeclampsia + fetal growth restriction and fetal growth restriction) groups in gestational age at sample collection. Gestational age and weight at delivery were lower in study groups compared to controls (Table 1). There was a 71% caesarean section rate in the normal pregnancy controls due to the inclusion of elective caesarean deliveries at term.

Maternal monocyte Flt-1 and endoglin expression

Circulating maternal monocytes expressed Flt-1 and endoglin as surface markers (Table 2). Although the median fluorescence intensity of Flt-1 expression of total monocytes was not different across the clinical groups, differences were observed in the monocyte subsets with Flt-1 mainly expressed by classical and intermediate monocytes (Fig. 1A).

The expression of endoglin MFI appears to be significantly decreased in preeclampsia + fetal growth restriction for total monocytes and classical monocytes, as compared to the normal pregnancies (Table 2). In all clinical groups, the expression of endoglin was significantly higher in the intermediate monocytes compared to classical monocyte subtype (Fig. 1B).

Fetal monocyte Flt-1 and endoglin expression

The results for fetal monocyte expression of Flt-1 and endoglin are presented in Fig. 2 and Table 3. The percentage and MFI of fetal monocytes Flt-1 expression did not show a statistically significant differences between the clinical groups. The results did not vary for the different subsets. The endoglin expression however was increased in the fetal growth restriction group for total monocytes as well as all the subtypes.

Correlation of Flt-1 and endoglin expression with gestation

Differential distribution of maternal monocyte Flt-1 and endoglin expression with increasing gestation in third trimester of pregnancy was assessed in 24 pregnancies from 26 weeks to 40 weeks of gestation. Spearman's rank correlation between gestational age in normal third trimester pregnancy and the markers of interest Flt-1 and endoglin showed that the percentage of monocytes expressing Flt-1 and endoglin did not change with gestation but the monocyte Flt-1 MFI (correlation coefficient 0.402, $p = 0.049$) and endoglin MFI (correlation coefficient 0.457, $p = 0.025$) showed a statistically significant increase with gestation (Fig. 3).

Correlation of anti-angiogenic factor Flt-1 and endoglin expression with polarisation of monocytes into M1/M2 inflammatory phenotypes

Spearman's rank correlations were performed to investigate the relationship between total monocyte Flt-1 and endoglin expression and M1/M2 polarization of monocytes (Table 4). A strongly positive correlation was noted between Flt-1 and CD86 MFI (correlation coefficient 0.643, $p = 0.001$). The correlation between Flt-1 and CD163 MFI (correlation coefficient 0.274, $p = 0.045$) was low but statistically significant. Positive correlations were noted between Flt-1 and endoglin (correlation coefficient 0.547, $p = 0.001$), Flt-1 and CD86/CD163 expression (correlation coefficient 0.412, $p = 0.002$), and between endoglin

Table 2
Maternal monocyte expression of Flt-1 and Endoglin presented for total monocytes and different monocyte subsets. Results are presented as median ± interquartile range for each continuous variable. * Statistically significantly different from normal pregnancy $p < 0.05$. † Statistically significantly different from PE $p < 0.05$.

	Clinical Group			
	Normal Median (Percentile 25, 75)	PE only Median (Percentile 25, 75)	FGR only Median (Percentile 25, 75)	PE + FGR Median (Percentile 25, 75)
Percentage of total monocytes expressing Flt-1	42.0 (33.4, 51.0)	37.6 (30.2, 54.9)	48.2 (25.1, 70.6)	42.4 (37.7, 44.5)
Total monocytes Flt-1 MFI	80.0 (57.0, 126.0)	49.0 (19.0, 71.0)	104.0 (33.5, 194.5)	73.0 (51.0, 102.0)
Classical monocytes Flt-1 MFI	140.0 (81.0, 181.0)	100.0 (65.0, 167.0)	185.5 (96.0, 278.0)	147.0 (102.0, 178.0)
Intermediate monocytes Flt-1 MFI	167.0 (99.0, 238.5)	128.0 (90.0, 204.0)	191.5 (99.5, 337.5)	140.0 (119.0, 212.0)
Non Classical Flt-1 MFI	52.5 (30.5, 98.0)	88.0 (48.0, 93.0)	84.0 (54.5, 103.0)	97.0 (73.0, 160.0)
Percentage of monocytes expressing Endoglin	62.7 (57.1, 76.2)	63.3 (58.8, 73.3)	74.5 (63.0, 82.1)	54.4*† (43.4, 63.6)
Total monocytes Endoglin MFI	330.0 (291.0, 446.5)	300.0 (267.0, 403.0)	362.5 (299.5, 575.5)	264.0* (75.0, 296.0)
Classical monocytes Endoglin MFI	342.0 (302.5, 403.5)	320.0 (308.0, 330.0)	454.5 (314.0, 644.0)	236.0* (216.0, 351.0)
Intermediate monocytes Endoglin MFI	653.0 (523.0, 1034.5)	483.0 (444.0, 896.0)	692.0 (468.0, 937.0)	499.0 (395.0, 595.0)
Non Classical monocytes Endoglin MFI	582.0 (526.0, 811.0)	458.0 (332.0, 678.0)	658.0 (293.0, 725.5)	464.0 (203.0, 641.0)

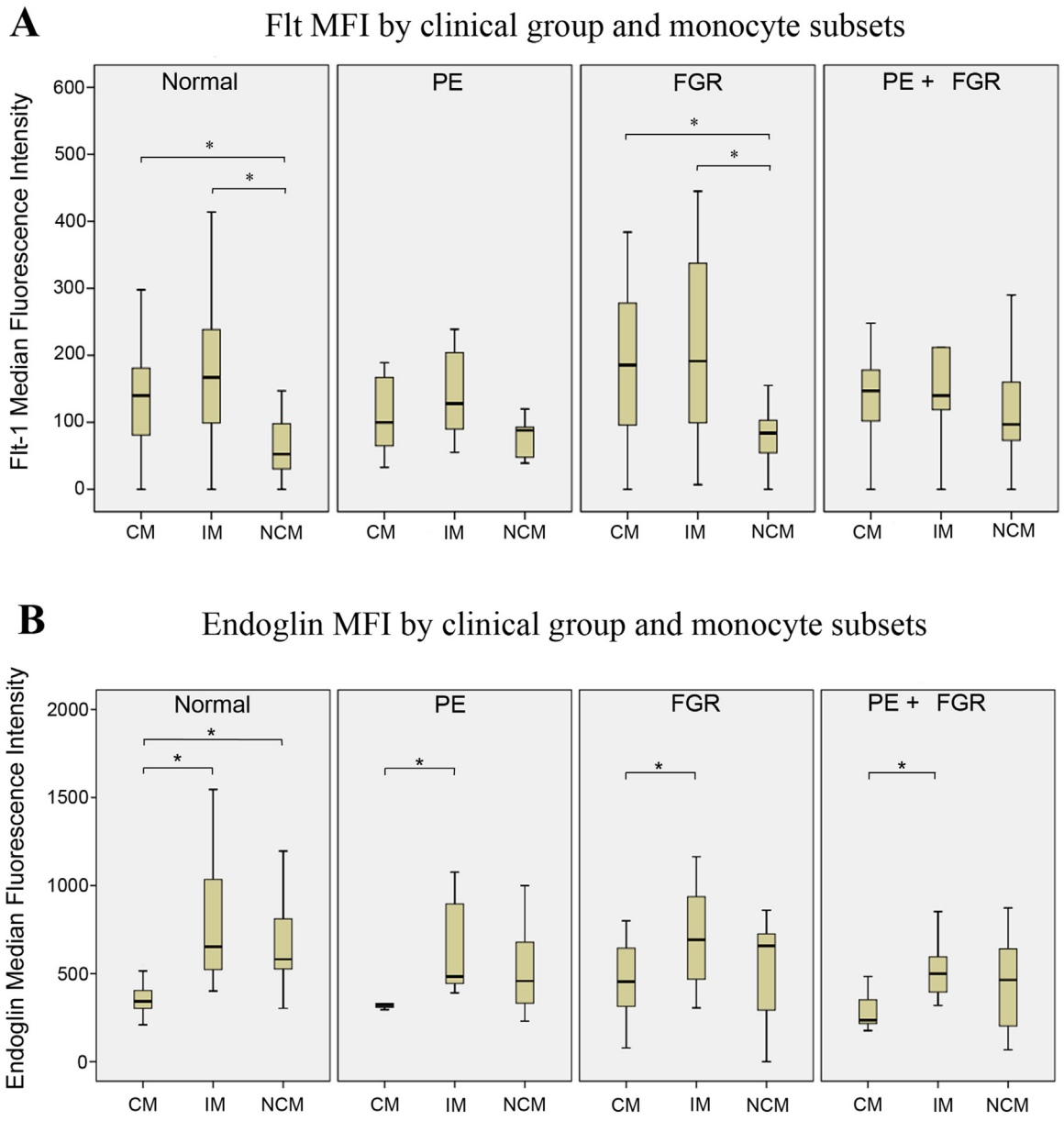

Fig. 1. Analysis of maternal monocyte surface (A) Flt-1 and (B) endoglin expression (MFI) according to the subtype and clinical group. Results are presented as median and interquartile range. * Statistically significant difference between groups $p < 0.05$.
CM = Classical monocytes, IM = Intermediate monocytes, NCM = Non classical monocytes.

and CD86/CD163 expression (correlation coefficient 0.45, $p = 0.001$) (Fig. 4A and B).

Discussion

The current study has contributed to the literature by documentation of Flt-1 and endoglin expression by maternal and fetal monocytes in normal and complicated pregnancies as well as exploration of these markers by monocyte subtype and polarization.

While it is established that soluble Flt-1 and soluble endoglin play a significant role in the pathogenesis of preeclampsia [7,24,38], this is the first study to document maternal and fetal monocyte expression of Flt-1 and endoglin in preeclampsia and fetal growth restriction. This study has clearly shown that circulating monocytes from maternal circulation express Flt-1 and endoglin as surface markers and that gestational variations in the monocyte Flt-1 and endoglin expression are evident in normal third trimester of pregnancy. While the percentage of monocytes expressing Flt-1 did not show any difference with gestation, the demonstrated gestational increase in intensity of Flt-1 and endoglin expression (MFI) may reflect the increased inflammatory nature of late gestation [39].

Lack of variation in maternal monocyte surface expression of Flt-1 between clinical groups suggests that Flt-1 is unlikely to be a significant contributor to the circulatory anti-angiogenic factors in preeclampsia and fetal growth restriction. The monocyte Flt-1 has been described to have a significant role in the chemotaxis of peripheral monocytes and tissue macrophages [10]. It is possible that surface expression of Flt-1 on monocytes in preeclampsia, particularly by non-classical monocytes, may have a functional role in chemotaxis and autocrine expression.

This study further explored the circulating monocyte Flt-1 and endoglin expression by subset and clinical groups. Variable expression of Flt-1 and endoglin was noted with the different monocyte subset populations, suggesting different functional roles for the different monocyte subsets in the angiogenic contribution.

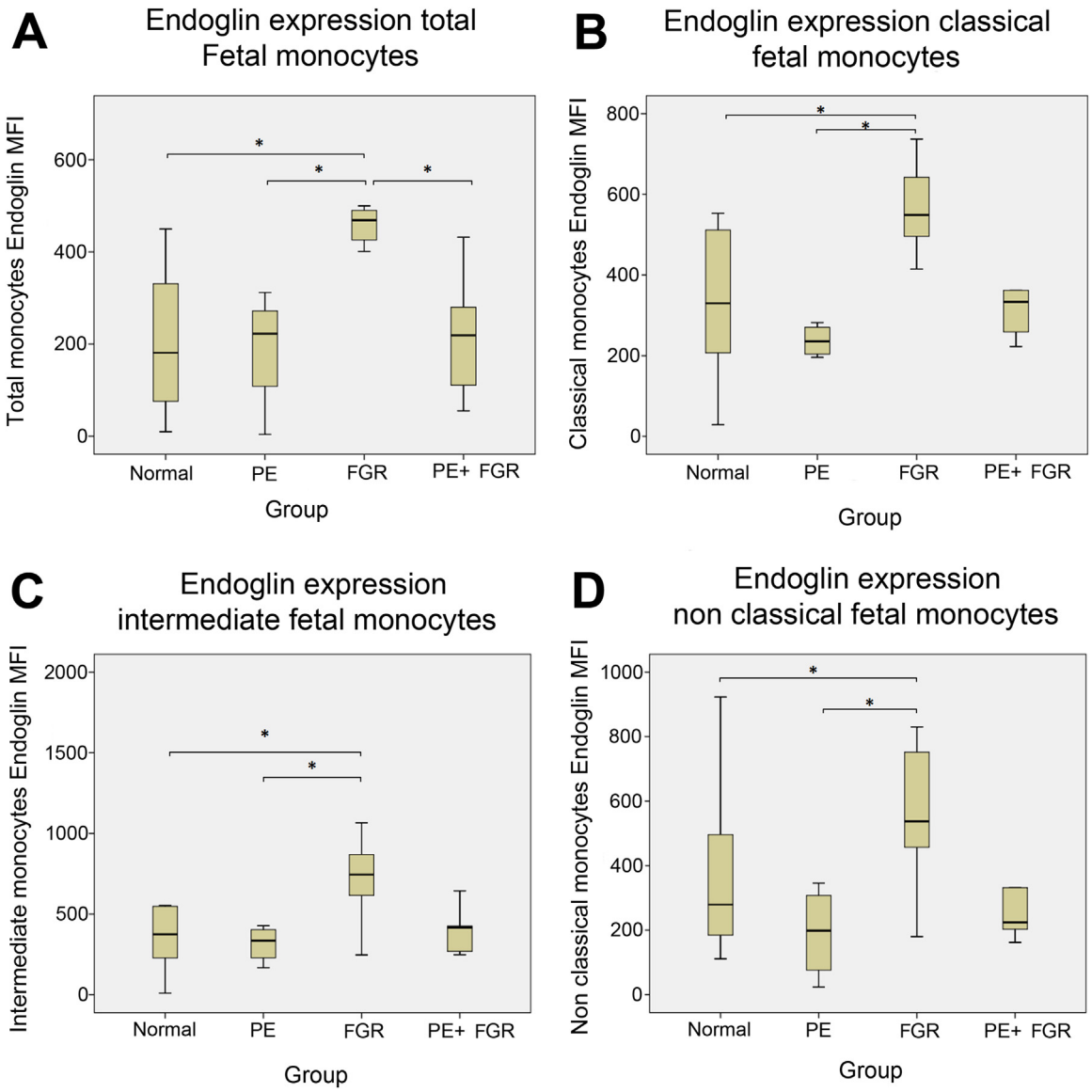

Fig. 2. Fetal monocytes expression of Endoglin. A: Total monocytes B: Classical monocytes. C: Intermediate monocytes. D: Non-classical monocytes. The results are presented as median ± interquartile range. * Statistically significant differences between clinical groups $p < 0.05$.

Table 3
Fetal monocyte expression of Flt-1 and Endoglin as markers of anti-angiogenic activity. *Significantly different from normal pregnancy. # Significantly different from PE. †Significantly different from FGR. Results are presented as median ± interquartile range for each continuous variable. For PE, 3 out of 4 MFI values for Flt-1 were not higher than the isotype control, resulting in a median value of 0.

	Clinical Group			
	Normal Median (Percentile 25, 75)	PE only Median (Percentile 25, 75)	FGR only Median (Percentile 25, 75)	PE + FGR Median (Percentile 25, 75)
Percentage of monocytes expressing Flt-1	10.0 (6.6, 28.9)	32.3 (12.6, 49.0)	17.6 (12.7, 43.0)	30.4 (12.3, 37.7)
Total monocytes Flt-1 MFI	6.0 (0.0, 49.0)	43.5 (8.5, 87.5)	30.0 (15.0, 64.0)	34.0 (16.0, 87.0)
Classical monocytes Flt-1 MFI	58.0 (43.5, 98.0)	0.0 (0.0, 55.5)	42.0 (26.0, 137.0)	68.0 (34.0, 142.0)
Intermediate monocytes Flt-1 MFI	107.0 (68.0, 182.5)	41.5 (13.5, 128.0)	50.0 (43.0, 218.0)	157.5 (77.0, 223.0)
Non Classical Flt-1 MFI	72.0 (67.5, 140.5)	61.5 (0.0, 168.5)	48.0 (4.0, 80.0)	107.0 (71.0, 152.0)
Percentage of monocytes expressing Endoglin	49.7 (36.3, 59.3)	52.5 (39.9, 58.7)	77.9 *# (74.5, 81.9)	59.5 † (46.8, 73.5)
Total monocytes Endoglin MFI	181.0 (76.0, 331.0)	222.5 (108.5, 272.0)	469.0 *# (426.0, 490.0)	219.0 † (111.0, 280.0)
Classical monocytes Endoglin MFI	330.0 (207.0, 511.5)	235.5 (204.0, 270.5)	549.0*# (496.0, 642.0)	333.5 (259.0, 362.0)
Intermediate monocytes Endoglin MFI	375.0 (228.5, 548.0)	335.0 (229.0, 403.5)	745.0 *# (616.0, 869.0)	417.0 (269.0, 426.0)
Non Classical monocytes Endoglin MFI	279.0 (184.5, 496.0)	198.5 (76.0, 307.5)	537.0*# (457.0, 752.0)	224.0 (203.0, 332.0)

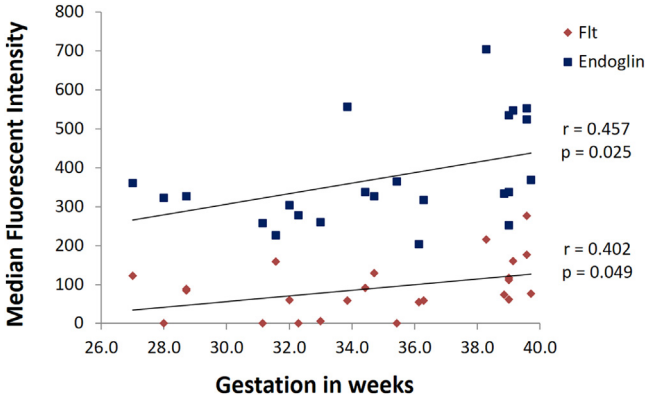

Fig. 3. Mean fluorescent intensity of Flt-1 and Endoglin with increased gestation in normal pregnancies. There was a moderate and statistically significant correlation seen between gestational age and MFI of both Flt-1 and Endoglin. r = Correlation coefficient, Spearman rank correlation. Significance $p < 0.05$.

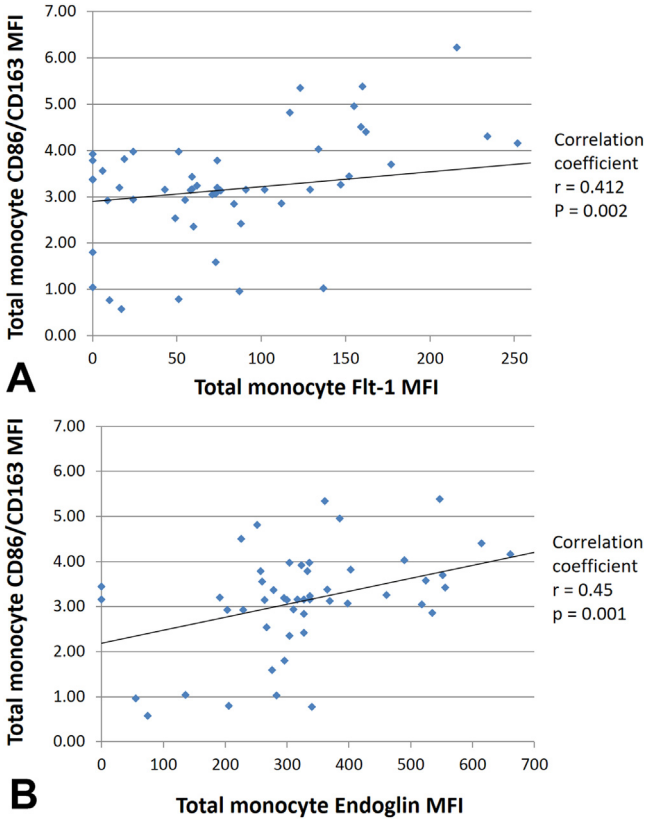

Fig. 4. Spearman's rank correlations for relationships between total monocyte Flt-1 MFI (A), Endoglin MFI (B) and markers of monocyte polarisation, CD86/CD163 MFI. r = Spearman's rank correlation. Correlation is significant at $p < 0.05$ level (2-tailed).

Flt-1 was mainly expressed on classical and intermediate cells. This finding is consistent with previous literature suggesting that monocyte chemotaxis towards the angiogenic ligands VEGF and PlGF is reduced in CD16+ intermediate and non-classical monocytes compared to CD16- classical monocytes [28]. The differences in chemotactic function may relate to their lower level of Flt-1 protein expression. Such differences may predict different functional roles of monocyte subsets in vascular repair, arteriogenesis and atherogenesis [28]. The membrane bound and circulating forms of Flt-1 and endoglin have different functions, with pro-angiogenic activity of the membrane bound forms and anti-angiogenic activity of the soluble forms. They may be controlled by different regulatory mechanisms and may reflect opposing regulatory mechanisms of the angiogenesis milieu.

The association of monocyte polarization into M1/M2 phenotypes in vivo and correlation with angiogenic factors Flt-1 and endoglin expression as presented has not been previously described in the literature. This study has demonstrated a moderate association between M1 polarization and surface expression of endoglin and Flt-1 on monocytes. While Flt-1 weakly correlated with CD163, the findings are consistent with Flt-1 related M1 upregulation of macrophages demonstrated in preeclamptic decidua [40] and may reflect monocyte activity such as chemotaxis. The inflammatory intermediate monocyte subset has been shown to be increased in preterm preeclampsia and fetal growth restriction as well as term pregnancy [34]. The current study findings of a gestational increase in Flt-1 and endoglin as well as with M1 polarization may represent an increase in the Flt-1 and endoglin expression by more inflammatory monocytes. With Flt-1 associated with monocyte recruitment, then this may lead to an increased influx of inflammatory monocytes into the placenta.

Interestingly, M1 polarization has also been shown to be atherogenic [41]. The long term increased vascular risk associated with preeclampsia is now well established [42]. The role of endoglin in cardiovascular homeostasis has been established. Increased expression of endoglin is associated with angiogenesis and myocardial fibrosis after infarction leading to increased risk of heart failure [18]. Soluble endoglin post myocardial infarction is an indicator of poor prognosis [43]. It is interesting to speculate, and investigate in the future, whether the M1 monocyte /macrophage related plaque formation and endoglin plays a role in adult as well as placental vascular disease. Up-regulation of endoglin in M1

Table 4
Spearman rank correlations for relationships between total monocyte Flt-1 MFI, Endoglin MFI, CD86 MFI, CD163 MFI and CD86/CD163 MFI ratio. *Correlation is significant at $p < 0.05$ level (2-tailed).

Variable 1	Variable 2	Correlation coefficient	Significance (2 tailed)	Interpretation
Total monocyte Flt-1 MFI	Total monocyte Endoglin MFI	0.547	p < 0.001*	Moderate positive correlation Statistically significant
Total monocyte Flt-1 MFI	Total monocyte CD86 MFI	0.643	p < 0.001*	Strong positive correlation Statistically significant
Total monocyte Flt-1 MFI	Total monocyte CD163 MFI	0.274	P = 0.045*	Low positive correlation Statistically significant
Total monocyte Flt-1 MFI	Total monocyte CD86/CD163 MFI ratio	0.412	P = 0.002*	Moderate positive correlation Statistically significant
Total monocyte Endoglin MFI	Total monocyte CD86 MFI	0.571	p < 0.001*	Moderate positive correlation Statistically significant
Total monocyte Endoglin MFI	Total monocyte CD163 MFI	0.145	P = 0.297	No correlation
Total monocyte Endoglin MFI	Total monocyte CD86/CD163 MFI ratio	0.45	P = 0.001*	Moderate positive correlation Statistically significant

monocytes as well as by the trophoblast may play a role in the pathogenesis of increased long-term cardiovascular risk associated with preeclampsia.

The findings of this study are exploratory. The data and the interpretations are limited by small patient numbers and the cross-sectional design of the study. Further study on monocyte expression of Flt-1 and endoglin and correlation with soluble forms may help to shed further light on the monocyte contribution to the pathogenesis of preeclampsia and fetal growth restriction.

We conclude that circulating monocytes from maternal and fetal circulation express Flt-1 and endoglin as surface markers and that their expression increase with gestational age and M1 polarization suggesting their upregulation with inflammatory changes in monocytes. Increased fetal monocyte expression of endoglin expression may be a response to placental injury.

Conflict of interest

The authors declare no competing interests.

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