Vascular Endothelial Growth Factor$_{165b}$ Protein Expression in the Placenta of Women with Uncomplicated Pregnancy

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Abstract

Introduction: Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis. VEGF-A gene produces 6 proteins of varying length from 121-206 amino acids, with VEGF$_{165}$ as the dominant form in human placenta. Recently a VEGF$_{165b}$ has been identified that competitively inhibits VEGF$_{165}$ action by binding to VEGF receptor KDR, and inhibit receptor phosphorylation and downstream intracellular signaling.

Objective: In this study, we have examined the placental expression of VEGF$_{165b}$ in normal women throughout gestation.

Methods: In an IRB approved study, placentas were obtained from normal pregnant women who underwent elective abortion or term delivery. Tissues were collected and dissected in saline to identify chorionic villi without associated decidua. Cytotrophoblast VEGF$_{165b}$ expression was assayed using ELISA kit DY3045 (R&D Systems, Minneapolis, MN). Non-parametric tests considered p<0.05 as significant.

Results: VEGF$_{165b}$ protein was detected throughout gestation. VEGF$_{165b}$ protein expression differed significantly among the trimester groups (p<0.0001), with median VEGF$_{165b}$ protein expression peaking in the second trimester. A significant positive correlation between VEGF$_{165b}$ protein expression and gestational age (GA) in days was noted in the first trimester (rho=0.327, p<0.001) and a negative but insignificant correlation thereafter.

Conclusions: Our findings of VEGF$_{165b}$ in all 231 placentas analyzed suggest that VEGF$_{165b}$ may have a regulatory role in human pregnancy. VEGF$_{165}$ is overexpressed in many cancers while VEGF$_{165b}$ is down-regulated in renal cell carcinoma. We speculate that VEGF$_{165b}$ protein expression in placental tissues could be a physiological phenomenon during placental development to restrain overexpression of VEGF that could lead to pregnancy complications.

Keywords: Chorionic villi; Throughout gestation; Enzyme immune assay

Introduction

Endothelial cells are the cellular organizational unit of vascular structures. They proliferate and migrate with elaborate mechanisms that constrain the growth of the vascular network to the needs of the organ and organisms. Under normal physiological conditions when endothelial cells proliferate, neovascularization is short-lived and not sustained [1]. Stimulators and inhibitors of the angiogenic process work synergistically to maintain a steady state. The factors involved in the angiogenic process include but are not limited to fibroblast growth factor, placental growth factor, vascular endothelial growth factor and its receptors, fibronectin, collagen, integrin, angiogenin and the soluble form of endoglin [2,3]. Among the many angiogenic molecules, VEGF and its receptors Flt-1 and KDR are considered to be of prime importance in embryonic and placental vascular development [4,5]. Targeted homozygous null mutations of VEGFRs showed that hematopoiesis and formation of blood islands and later blood vessels are dependent on VEGFR-2, while endothelial organization during vascular development is more dependent on VEGFR-1 [5]. VEGF mRNA is expressed within first few days following implantation in the trophoblasts [6,7]. In the human fetus, VEGF mRNA is detected in all tissues by 16-22 weeks of gestation [1]. That VEGF plays an irreplaceable role in fetoplacental angiogenesis was elegantly demonstrated by Carmeliet et al. [8] in 1996 when the group showed that mice embryos lacking a single VEGF allele could not survive and died mid-gestation, and that other factors administered to these mice were unable to compensate for the loss of VEGF [8]. Moreover, that placenta is a significant source of VEGF was suggested when post-delivery VEGF concentration was found to decrease in the sera of both pre-eclamptic

and control women [9].

In earlier studies VEGF A was considered as VEGF. The other isoforms of VEGF include VEGF B, VEGF C, VEGF D and VEGF E [10]. By the 1990s more than 20,000 papers were published that recognized VEGF as a pro-angiogenic tumor-enhancing endothelial-specific growth factor [11]. The VEGF A gene generates several proteins by alternative splicing [12] a process by which particular exons of a gene may be included within or excluded from the final processed mRNA. The process allows multiple proteins to be formed from the same gene that differ in their molecular masses, their expression patterns and in their biochemical and biological properties. The proteins produced by alternative splicing of VEGF A vary in length from 121-207 amino acids and include $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$ [11-13]. $VEGF_{121}$ and $VEGF_{165}$ are efficiently secreted by cytotrophoblasts, while $VEGF_{189}$ and $VEGF_{206}$ have a strong affinity for cell-surface proteoglycans and largely remain cell bound [12,13]. Conventionally, all the VEGF isoforms are pro-angiogenic. In placental tissues, even though $VEGF_{121}$ and $VEGF_{189}$ can be present, $VEGF_{165}$ is considered the most dominant isoform [14]. In 2002, Bates et al. [15] identified a VEGF isoform which was identical in length to $VEGF_{165}$ but the C-terminal exon had an open reading frame of six amino acids that were strikingly different from that of $VEGF_{165}$. Instead of the usual six amino acids CDKPRR, the six amino acids in the newly identified protein were SLTRKD. The characteristics of this newly found protein are that it antagonizes effects of $VEGF_{165}$ and is down-regulated in a renal cell carcinoma [15] and metastatic melanoma [16]. Bates et al. [15] named this new protein $VEGF_{165b}$. $VEGF_{165b}$ binds to VEGF receptor KDR and inhibits its downstream signaling pathway [17]. In the present study we investigated the placental expression of $VEGF_{165b}$ throughout gestation in women with uncomplicated pregnancy.

Materials and Methods

The investigative protocol for the study was approved by the Human Subject Ethics Committee of the Bronx Lebanon Hospital Center, New York, protocol #10101304. Discarded placental tissue samples were collected within approximately 30 min of the procedures from normal pregnant women who underwent elective termination of pregnancy at 6 weeks to 23 weeks and 6 days, and from normal women who delivered uncomplicated singleton pregnancies at term. Placentas from missed abortion, or from pregnancies complicated with diabetes, hypertension, chronic renal disease, and chronic peripheral vascular disease or with major fetal anomalies were excluded. The placental tissues collected from term deliveries were taken from one of the peripheral cotyledons. The approved protocol allowed collection of some clinical information regarding the women which included: maternal age, parity, race, gestational age as determined by ultrasound or by initial date of the last menstrual period, reason for pregnancy termination (whether elective, for maternal medical reasons or for fetal indications), method of delivery (caesarean versus vaginal delivery), and medicine(s) administered to induce termination of pregnancy. Tissues and clinical information were de-identified before exiting the delivery suites. Placental samples from pregnancies 7-23 weeks and 6 days were collected from the elective termination group, and placental samples from 37 to 42 weeks of gestation were collected to constitute the term delivery group.

Placental tissues were processed to obtain chorionic villi samples by a method described in detail earlier [18]. Briefly, placental tissues were collected within 30 min of elective first or second trimester pregnancy terminations and term deliveries from women with uncomplicated pregnancy. The tissues collected from term deliveries were obtained from one of the peripheral cotyledons; in case of elective pregnancy termination, the location of the tissue sampled within the placenta could not be determined. Placental tissues were thoroughly washed in cold saline to remove maternal blood and were then dissected in saline to collect only free floating chorionic villi, not anchored to the basal plate nor emerging from the chorionic plate surface vessels. Sections of each chorionic villi sample were then placed in separate tubes bearing identical study number and were transported to the laboratory on ice and stored at -80°C until assay.

Chorionic villi $VEGF_{165b}$ protein expression was determined by using Human $VEGF_{165b}$ Duoset ELISA kit from R&D Systems (item #DY3045), Minneapolis, MN) as per the manufacturer's protocol. A Tecan infinite 200 Pro microplate reader (Tecan Systems Inc., San Jose, CA) set at 450 nm with wavelength correction set at 540 nm measured the absorbance. The sensitivity of the ELISA assay was 62.5 pg/ml. The manufacturer claims no cross reactivity of the captured monoclonal antibody used in the assay kit with recombinant human $VEGF_{121}$, $VEGF_{162}$, $VEGF_{165}$ and recombinant VEGFR3/Fc, VEGF R1/Fc or VEGF R-2/Fc Chimeras. The intra-assay and inter-assay variations were 3% to 5%.

Statistical Analyses

The statistical software package SPSS, version 24 (IBM Corporation, Armonk, NY) was used for statistical analyses. It needs to be emphasized that the statistical software package was first used to test for normality of the data which revealed that (1) the skewness and kurtosis data for the variable was not within ± 1.96, (2) the Shapiro-Wilk test significance value was p<0.05, (3) visual inspection of the histogram showed that the data was skewed to the right, (4) Normal Q-Q plots showed that the dots were not linear, and (5) the Box Plots too confirmed that the data was not normally distributed. Hence, standard t-test or ANOVA could not be used for statistical analyses; andnon-parametric statistics had to be applied. The data was grouped by trimesters and the following statistical analyses were performed: 1) Kruskal Wallis test, which is an alternative test to one way ANOVA was used to explore the difference in $VEGF_{165b}$ protein expression among the trimester groups. This test does not provide post–hoc test in the same way as the one way ANOVA does, just informs whether the groups are statistically different from one another. For this we applied Mann Whitney U test to compare two groups at a time against each other. Since our data was not normally distributed we also could not use the Pearson correlation which evaluates the linear relationship between two continuous variables. Instead the non-parametric Spearman Rank correlation coefficient test was applied to summarize the strength and direction of a relationship between the two variables. A p<0.05 was considered statistically significant.

Results and Discussion

In this study we have focused our attention solely in delineating the $VEGF_{165b}$ protein expression profile in chorionic villi tissue in normal human pregnancy. The demographic characteristics of women from whom placental samples were obtained showed that there was no significant differences in maternal age among the first, second and third trimester groups (26.5 ± 6.4, 26.5 ± 6.4 and 28.56 ± 6.6, respectively). Race/ethnicity was self-reported and the distribution pattern showed that women in the study could be

Table 1: Chorionic villi VEGF$_{165b}$ protein expression throughout gestation in normal pregnancy.

Groups	N	VEGF$_{165b}$ (pg/100 mg tissue) Median	VEGF$_{165b}$ (pg/100 mg tissue) 25th Percentile	VEGF$_{165b}$ (pg/100 mg tissue) 75th Percentile
1st Trimester	101	158.11	89.24	231.78
2nd Trimester	56	437.09	294.62	631.08
3rd Trimester	74	239.68	155.35	342.76

Note: VEGF$_{165b}$: Vascular Endothelial Growth Factor$_{165b}$, GA: gestational age in days. The first trimester placental chorionic villi samples were from $7^{0/7}$-$12^{0/7}$ weeks gestation, the median GA was 8 weeks; second trimester were from $12^{1/7}$ to $23^{6/7}$ weeks, the median GA was 15 weeks 3 days; and the third trimester term were from $37^{0/7}$ to $41^{4/7}$ weeks of gestation, the median GA 39 weeks 1 day. Homogenized human placental chorionic villi samples were analyzed using assay kit from R&D Systems (Item #DY3045, Minneapolis, MN) to determine VEGF$_{165b}$ protein expression.

Table 2: Spearman's correlation between chorionic villi VEGF$_{165b}$ protein expression and gestational age in days throughout normal human pregnancy.

	Control Variables	VEGF$_{165b}$
First Trimester	GA Correlation Coefficient	0.314
	Significance (2-tailed) N=101	0.001
Second Trimester	GA Correlation Coefficient	-0.102
	Significance (2-tailed) N=56	0.454
Third Trimester	GA Correlation	-0.063
	Significance (2-tailed) N=74	0.593

Note: VEGF$_{165b}$: Vascular Endothelial Growth Factor$_{165b}$; GA: Gestational age in days. Homogenized human placental chorionic villi samples were assayed for VEGF$_{165b}$ protein expressions using Duoset assay kit from R&D System (Item #DY3045, Minneapolis, MN). Results show significant positive correlation between the protein and gestational age in days only in the first trimester (rho=+0.314, p=0.001). In the second and third trimester, the correlations were negative but insignificant.

grouped as 30% Black, 60% Hispanic, 1% Caucasian and 9% of other ethnic origin. A total of 101 placental tissues were collected from the first trimester and the median gestational age of the group was 8 weeks. In the second trimester 56 placental tissues were collected, and the median gestational age of the group was 15 weeks and 3 days. In the term delivery group 74 placental samples were collected and the median gestational age of the group was 39 weeks and 1 day.

All 231 placental chorionic villi samples were analyzed by enzyme-linked immunoassay method to determine VEGF$_{165b}$ protein expression. The median, 25th percentile and 75th percentile VEGF$_{165b}$ protein concentrations of the three trimester groups are presented in Table 1. Median VEGF$_{165b}$ concentration was the lowest in the first trimester; the protein expression peaked in the second trimester, and declined in the third. Differences in VEGF$_{165b}$ protein expression among the three trimester groups analyzed using Kruskal Wallis test showed significant differences (p<0.0001). Pairwise comparison of the trimester groups using Mann Whitney U test further revealed that each trimester group was also significantly different from the other at p<0.0001 level. Figure 1 depicts the chorionic villi VEGF$_{165b}$ protein expression pattern by trimester group in women with uncomplicated pregnancy. Spearman's correlation was used to exam the correlation between chorionic villi VEGF$_{165b}$ expression and GA, and the results revealed a significant positive correlation only in the first trimester (rho=+0.314, p<0.001, Table 2). In the second and third trimesters, the correlation between VEGF$_{165b}$ and GA was negative even though it was not statistically significant (Table 2). Ethnic variations or parity did not affect placental VEGF$_{165b}$ levels. In the study, the distribution pattern of women who were either given misoprostol or dinoprostone at the time of pregnancy termination or prior to delivery was as follows: In the first trimester, 6 out of 101 women had received misoprostol; in the second trimester, 23 out of 56 women had received misopoistol; and in the third trimester, only 1 woman was administered with dinoprostone. The chorionic villi VEGF$_{165b}$
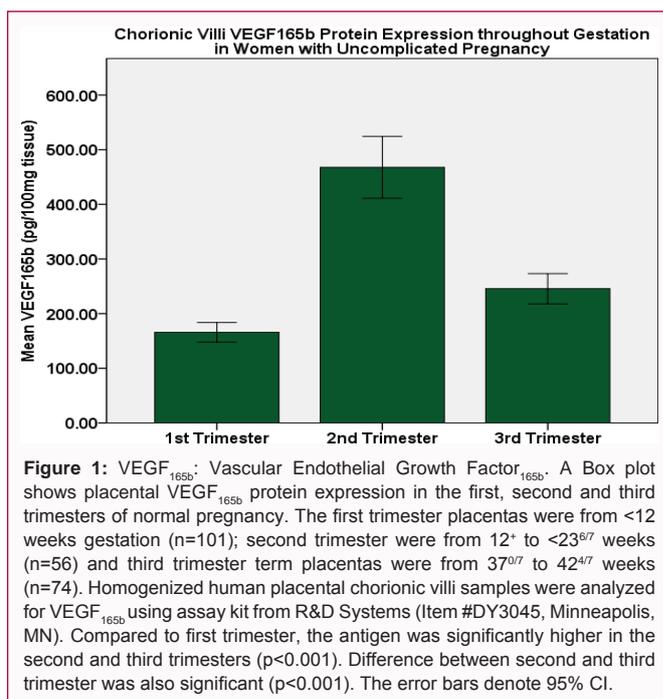

Figure 1: VEGF$_{165b}$: Vascular Endothelial Growth Factor$_{165b}$. A Box plot shows placental VEGF$_{165b}$ protein expression in the first, second and third trimesters of normal pregnancy. The first trimester placentas were from <12 weeks gestation (n=101); second trimester were from 12^+ to $<23^{6/7}$ weeks (n=56) and third trimester term placentas were from $37^{0/7}$ to $42^{4/7}$ weeks (n=74). Homogenized human placental chorionic villi samples were analyzed for VEGF$_{165b}$ using assay kit from R&D Systems (Item #DY3045, Minneapolis, MN). Compared to first trimester, the antigen was significantly higher in the second and third trimesters (p<0.001). Difference between second and third trimester was also significant (p<0.001). The error bars denote 95% CI.

protein expression pattern between prostaglandins administered (n=30) and un-administered (n=201) groups showed a significantly higher expression of the protein in the prostaglandins administered group (p<0.0001).

Many of the earlier studies have confirmed that VEGF$_{165}$ is the dominant form present in human placenta [10-15]. A number of studies have previously reported that placental VEGF$_{165}$ increase with GA only in the first trimester of human pregnancy [19-21], thereafter, both VEGF and its receptor VEGFR-2 decline with advancing GA [22]. Immunohistochemical studies have localized VEGF in villous cytotrophoblast in these first trimester placental samples [21]. In the present study, results of Spearman correlation mirrors a similar scenario for placental VEGF$_{165b}$ in the first trimester of pregnancy, showing a significant positive correlation between VEGF$_{165b}$ and GA (rho=+0.314, p=0.001, Table 2). To our knowledge no other report on VEGF$_{165b}$ protein expression in the first trimester of human pregnancy is currently available in the literature. However, that both forms of VEGF, VEGF$_{165}$ and VEGF$_{165b}$, can simultaneously increase in placental tissues has been previously reported by Bates et al. [14] using third trimester placental tissue samples.

The results of the present study underscores that VEGF$_{165b}$ protein is not only expressed in the first trimester of human placenta but the antigen was also detected in appreciable amounts in all 231 placental samples; collected throughout gestation from first, second and third trimesters to term. We suggest that VEGF$_{165b}$ protein may

be involved in the regulation of placental angiogenesis, particularly in early pregnancy and perhaps throughout gestation as well. The GA-specific variations in $VEGF_{165b}$ protein expression in chorionic villi as depicted in Figure 1 show that the expression of the antigen peaked in the second trimester. In the third trimester the protein concentration was lower than that of the second trimester but remained significantly higher than that of the first trimester (Table 1, Figure 1).

An interesting feature of trophoblasts is its tendency to closely resemble the morphologic appearance of the neighboring maternal tissue while still retaining certain inherent structural and biochemical features. This unique feature of cytotrophoblasts has elegantly been described by Yeh and Kurman [23]. The authors described that in the decidua, intermediate trophoblasts tend to be polygonal, in myometrium between smooth muscle bundles they are spindle-shaped, and when they replace the endothelial cells in the spiral arteries they are flattened. The trophoblasts not only mimic the morphologic appearance of these cells but mimic the functions of these cells as well. Hence it is not surprising that VEGF an endothelial cell specific growth factor is released by trophoblasts during pregnancy.

Vasculogenesis and angiogenesis are physiological processes involved in normal human pregnancy. During pregnancy, endothelial cells originate from primitive progenitor cells, expand in number and become organized and assemble into ordered vascular structures [24]. In humans, placental vasculogenesis is evident by approximately 21-22 days post conception [25]. At this stage, cords of hemangiogenetic cells are present and some demonstrate primitive lumen formation. These cords further develop such that by approximately 28 day post conception most villi show the presence of capillary structures [25]. Histomorphometric [26] and immunohistochemical [27] analyses of normal pregnancies terminated between 6-15 weeks of gestation demonstrate an increase in vascularity of the villi, with a steady increase in vascular volume. Branching angiogenesis is recognized to be the only mode of angiogenesis during this time [24].

In the first trimester human placenta, maternal oxygenized blood is prevented from entering the intravillous space by a trophoblastic plug which creates a low oxygen environment [28,29]. Such low oxygen state is indispensable for optimal fetal and placental growth [30]. It has been suggested that VEGF is involved in the formation and maintenance of the trophoblastic plug throughout first trimester of normal pregnancy [31], to protect the fetoplacental unit against oxidative insult, at a time when the first trimester placenta has not yet developed an adequate defense mechanism [28,29,32]. In the first 10 weeks of pregnancy when the oxygen tension of trophoblastic villi is particularly low, up-regulation of both VEGF mRNA [33] and VEGF antigen occur [19-21]. Immunohistochemical studies have localized VEGF in villous cytotrophoblasts in the first trimester and in syncytiotrophoblasts thereafter; and diffused VEGF staining in the villous stroma at term [6,21]. Villous trophoblasts cultured at 1% O_2 express higher levels of VEGF A, Flt-1, and sFlt-1 mRNA compared to cells cultured under normoxic condition [34]; emphasizing that low oxygen status induces placental angiogenesis. In the present study, we report that placental $VEGF_{165b}$ increase with GA in the first trimester of pregnancy. Since placental oxygen status during this time is reported by other investigators to be low [28,29], hence, our finding suggests, that $VEGF_{165b}$ can affect placental angiogenesis in low oxygen conditions as well.

A characteristic feature of human pregnancy is that the trophoblast

plugs that previously block the spiral arteries are removed at around 10-12 weeks of gestation, and the placental milieu at this time switches from a low oxygen to a normoxic environment [28,32]. During this switch, the trophoblasts also switch from a proliferative to an invasive phenotype [6,28,30]. Matrix degrading enzyme, specifically matrix metalloproteinase-9 (MMP-9) is reported to stimulate placental cytotrophoblasts along this invasive pathway [6]. As the infiltration of the decidua by the trophoblasts proceeds it resembles tumor cell invasion. However, the cytotrophoblast invasion is finely controlled and the depth of the invasion is only allowed to progress as far as the first third of the myometrium [35]. In our previous studies, we have shown that both placental tumor necrosis factor-α (TNF-α) and MMP-9 significantly increase in the second trimester of normal pregnancy in comparison to the first trimester values, and at this time the two proteins bear a positive correlation between them [18,36]. We have suggested that TNF-α, by inducing the synthesis of MMP-9 in the second trimester, may have allowed significant degradation of the extracellular matrix; whereby larger number of cytotrophoblasts could reach the deeper layers of the endometrium [36], and the coordinated involvement of the two proteins in the second trimester of pregnancy may be necessary for a successful pregnancy outcome. Optimal growth of the fetus and the placenta is not only dependent on the development of fetoplacental vasculature but how well the maternal vasculature is remodeled to meet the growing demands of the fetus. Differentiation of cytotrophoblasts to invasive phenotype and degradation of extracellular matrix are two processes that are essential for appropriate remodeling of the spiral arteries. The remodeling process begins at the end of the first trimester and continues into the second trimester of pregnancy. Since $VEGF_{165}$ inhibits both the differentiation of cytotrophoblast to invasive phenotype as well as the activity of MMP, particularly that of MMP-9; hence, the decrease in placental $VEGF_{165}$ protein expression beyond the first trimester of pregnancy as reported by other investigators [22], seems not only logical but necessary as well.

In the second trimester of human pregnancy, the placenta experiences dramatic growth. Mayhew [37] has reported that by term, the villous undergo substantial remodeling characterized by a 56-fold increase in capillary volume in the peripheral villi. He has further demonstrated a biphasic growth pattern during fetoplacental angiogenesis. Before mid-gestation, the growth is slow with branching angiogenesis being the dominant pattern. After that, the growth pattern is rapid, with a shift towards non-branching angiogenesis, when the capillaries increase in their mean length rather than in their cross-sectional area due to endothelial cell proliferation [37]. Our finding of a significant increase in placental $VEGF_{165b}$ protein expression in the second and third trimester of pregnancy compared to first trimester (Table1, Figure 1) therefore seems to be a physiological phenomenon whereby $VEGF_{165b}$ fulfills the angiogenic function of $VEGF_{165}$ in its absence. We hypothesize that the switch between $VEGF_{165}$ and $VEGF_{165b}$ occurs when the partial pressure of oxygen in the placenta switches at the end of the first trimester from a low to a normoxic state; favoring placental release of $VEGF_{165}$ when oxygen pressure is low and placental $VEGF_{165b}$ in normoxic condition. That switch in the partial pressure of oxygen at the end of first trimester of normal pregnancy can modify the expression pattern of two proteins has been reported earlier, between placental derived growth factor and VEGF [38].

A finding of the present study is that a statistically significant difference in $VEGF_{165b}$ concentrations was noted between women

who had received misoprostol (n=30) for pregnancy termination versus those who did not (n=201) (p<0.0001). In our outpatient clinic, misoprostol was administered orally at a dose of 200 ug to 400 ug, approximately two hours prior to termination of pregnancy. The functional significance of the effect of misoprostol on chorionic villi VEGF$_{165b}$ protein expression remains to be established.

Our study has limitations. First, we have analyzed VEGF$_{165b}$ protein expression by the enzyme immunoassay method even though we realize that the data would have been strengthened if we could have applied immunohistochemistry, western blot or polymerase chain reaction (PCR) methods to confirm our results. Second, availability of a cell culture facility would have allowed us to show the effect of different levels of VEGF$_{165b}$ on receptor phosphorylation and any potential downstream intracellular signaling. Third, in the present study we were particularly interested in determining VEGF protein expression throughout normal gestation in human pregnancy. Since VEGF$_{165}$ is reported by other investigators to decline after the first trimester of pregnancy [6,22], we in this study have therefore focused our attention primarily on the protein expression of VEGF$_{165b}$. Fourth, inclusion of other VEGF isoforms involved in placental angiogenesis would have been desirable. However, addition of all these factors would have made the study more complex and any conclusions drawn from the study would have been extremely difficult to interpret. Fifth, it would have been useful if we could have collected more placental tissues samples from women administered with either PGE1 or PGE2 in the first and third trimester of pregnancy. To our knowledge, the effect of misoprostol on VEGF$_{165b}$ has not been reported earlier. This was an unexpected finding of the present study hence, collection of a greater number of placentas from women administered with PGE1 or PGE2 in the first and third trimester of pregnancy would not have been contemplated. The strength of our study is our relatively larger sample size in each of the trimester group. Additionally, this is the first study reporting on VEGF$_{165b}$ protein expression in chorionic villi throughout gestation in normal human pregnancy which has not been reported earlier. Furthermore, the EIA method used for the study specifically detects free form of VEGF$_{165b}$, and based on the manufacturer's claim the monoclonal capture antibody used in the kit does not cross react with recombinant human VEGF$_{121}$, VEGF$_{162}$, VEGF$_{165}$, or with recombinant human VEGF R1/Fc, VEGF R2/Fc or VEGF R3/Fc chimeras. The detection method used was additionally sensitive that allowed the detection of VEGF$_{165b}$ at a dose as low as 62.5 pg/ml.

Future research directions in this area include measuring both VEGF$_{165}$ and VGEF$_{165b}$ protein expression simultaneously, using other techniques such as western blot, immunohistochemistry and PCR methods. It is also essential to compare placental VEGF$_{165b}$ status between normal and complicated pregnancies. Future studies should also include assessing the effect of different levels of VEGF$_{165b}$ on receptor phosphorylation and on any potential downstream intracellular signaling, and correlating placental VEGF$_{165b}$ expressions under hypoxic versus normoxic conditions. Such studies could elucidate the role, if any, of VEGF$_{165b}$ in placental angiogenesis in normal and abnormal gestation.

Conclusion

The presence of VEGF$_{165b}$ in chorionic villi tissues throughout gestation as revealed in the present study suggests that this cytokine may play a role in placental vascular development during normal human pregnancy. Over expression of VEGFA has been reported in most human cancers [5,11,16], while VEGF$_{165b}$ is shown to be down-regulated [11,15-17]. We hypothesize that VEGF$_{165b}$ protein expression in placental tissues could act during placental development to balance the effects of VEGF that could lead to pregnancy complications.

Declaration of Interest

The authors declare no conflicts of interest with respect to the research, authorship and/or publication of this article.

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