Changes in Serum Levels of Vascular Endothelial Growth Factor in Males and Females Throughout Life

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OBJECTIVES: *To study serum levels of vascular endothelial growth factor (VEGF), a potent angiogenic factor, during distinct periods of the female life span and compare them with corresponding levels of age-matched males. It is hypothesized that VEGF might be increased at periods of enhanced angiogenesis.*

METHODS: *Venous blood was drawn from healthy females (n = 59) and males (n = 53) divided into six groups: fetuses (cord blood), neonates, children, adults (same females in the proliferative and secretory phases of their menstrual cycle), pregnant, and elderly (postmenopausal). Serum VEGF levels were measured by an enzyme immunoassay.*

RESULTS: *Females showed 49% higher serum VEGF levels than males (t = 2.74, P = .01). Cord and neonatal blood levels were significantly increased compared with those of adults (t = 2.41, P = .02, and t = 5.81, P = .0001, respectively). All female age groups presented higher serum VEGF levels than the group of women in the proliferative phase of the cycle; nevertheless, VEGF levels in the secretory phase did not differ (t = 1.85, P = .07).*

CONCLUSIONS: *Serum VEGF levels are higher in females than in males and during life periods characterized by enhanced growth and development, implying increased rates of angiogenesis. (J Soc Gynecol Investig 2000;7:309–12)*

KEY WORDS: Angiogenesis, vascular endothelial growth factor, menstruation, neonate, postmenopause.

Angiogenesis, the formation of new blood vessels from an existing vasculature, is a complex multistep process initiated by the secretion and action of soluble angiogenic factors. Thus, degradation of extracellular matrix proteins and activation, proliferation, migration, and organization into tubular structures of vascular endothelial cells and pericytes take place.[1,2] Angiogenesis plays a key role in physiologic processes involving neovascularization, such as ovulation, placentation, and embryogenesis, and is also central to several pathologic conditions such as tumor growth and metastasis.[3]

Among the growth factors with angiogenic activity, one of the most potent is vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF).[4,5] The active form of VEGF is a homodimeric cytokine of molecular weight 34–46 kD. The variation in size is due to alternative exon splicing, which produces four different isoforms of 121, 165, 189, and 206 amino acids, the last three of which have heparin binding activity.[6,7] Many cell types produce VEGF,[8] with $VEGF_{165}$ being the predominant soluble isoform.[9]

In two previous studies[10,11] we showed increased circulating levels of angiogenic factors, among them VEGF, soon after birth, attributed to the enhanced angiogenesis encountered perinatally. We also documented undetectable serum VEGF levels in pregnancy, probably due to local and systemic binding of VEGF to the soluble form of the VEGF receptor flt-1 produced by the placenta.[12,13]

In this study, we hypothesized that other life periods with presumed high rates of angiogenesis (eg, periods of growth and development or menstruation) also could be characterized by increased circulating VEGF levels. Therefore, we investigated serum levels of VEGF in females of all age groups and compared them with corresponding values in age-matched males.

MATERIALS AND METHODS

This study, which was approved by the ethics committee of our teaching hospital, was composed of healthy subjects (59 females and 53 males) who gave written informed consent for themselves or for their infants. They were allocated in six groups as follows: Group 1 consisted of 11 fetuses (six females and five males). They were all born by normal vaginal delivery after an uncomplicated term single pregnancy. Gestational age (mean ± standard deviation [SD] [range]) was 38.4 ± 1.2 (37–41) weeks. Blood was drawn from the umbilical cord at delivery, thus reflecting fetal state. Group 2 included 26 full-

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Table 1. Geometric Mean Values and 95% Confidence Intervals of VEGF Levels by Gender in Six Groups Investigated

Group	*n*	Males $\bar{x}$ (95% CI)	*n*	Females $\bar{x}$ (95% CI)	*t**	*P*
1. Cord blood	5	220 (86–564)	6	303 (199–461)	−0.85	.44
2. Neonates	11	423 (318–564)	15	498 (361–688)	−0.83	.42
3. Children	12	107 (53–215)	11	240 (165–350)	−2.25	.05
4. Adults	10	158 (104–242)	10‡	113 (77–166)	1.13	.22
			10§	63 (29–138)		
5. Pregnant women			11	†		
6. Elderly	11	100 (58–174)	10	265 (149–469)	−2.75	.02

Data show comparison between males and females for each group (*t*-test and *P* value)
* Comparisons were performed using log values.
† All measurements were below the lower limit of detection.
‡ Women in the proliferative phase of the menstrual cycle.
§ Women in the secretory phase of the menstrual cycle.

term, appropriate for gestational age, 10-day-old, healthy, breast-feeding neonates (15 females, and 11 males). Group 3 consisted of 23 healthy children (11 females and 12 males), presenting for blood group typing. Age for males was 7.9 ± 2.7 (4–11.5) years and for females 8.6 ± 2.4 (5.5–11) years. Eight of the 12 boys were in Tanner stage 1, whereas three were in Tanner stage 2 and one was in Tanner stage 3. Concerning girls, two were in Tanner stage 1, three were in Tanner stage 2, three were in Tanner stage 3, and three were in Tanner stage 4. Group 4 was 20 adults (ten females and ten males) all part of the medical personnel. Females had regular menstrual cycles and were not receiving hormonal contraception. Blood was drawn both from the proliferative (midfollicular) and secretory (midluteal) phases of the cycle. Their age was 29.0 ± 5.9 (23–38) for males and 30.6 ± 5.1 (21–40) years for females. Group 5 included 11 term pregnant women before initiation of labor, aged 27.3 ± 5.5 (20–39) years. Finally, group 6, referred to as the elderly group, was composed of 21 adults (ten postmenopausal women and 11 men over 55 years of age). The age for females was 62.8 ± 8.6 (49–78) years, and for males was 64.1 ± 5.7 (55–79) years. All postmenopausal women presented with second-degree uterine prolapse, had a negative histology report for neoplasia after diagnostic curettage, and were not receiving any type of hormone replacement therapy. These women were 2–26 years postmenopausal (mean ± SD; 10.9 ± 6.2). Cigarette smoking was limited to <10 cigarettes/day in six women of reproductive age, three pregnant women, and four postmenopausal women, as well as in seven adult and eight elderly men.

We drew 1 mL of blood from all subjects and collected it in pyrogen-free tubes. It was immediately separated by centrifugation after clotting and was kept frozen at −20C until assay. The analysis was performed by an enzyme immunoassay (human VEGF Quantikine; R&D Systems, Minneapolis, MN). The minimum detectable concentration was 9 pg/mL. Intra- and interassay coefficients of variation were 4.8% and 6.6%, respectively.

Statistical Analysis

The data were not normally distributed; thus, values were log-transformed and the geometric means with 95% confidence limits were calculated. Multiple linear regression procedures were then applied (log values), calculating the percent difference of the point estimate with 95% confidence intervals (CIs). In the first step of the analysis, serum levels of VEGF were the dependent variable and gender (males as reference) and the five investigated age groups (adults as reference) the independent ones. In the second step of the analysis, only females were included. The dependent variable was again serum levels of VEGF and the independent variables were the same as for the above age groups (women in the proliferative phase of the menstrual cycle were taken as the reference). For statistical analysis we used the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Serum levels of VEGF for males and females of the various groups are shown in Table 1. Multiple linear regression analysis of serum VEGF levels by gender in the investigated groups showed a statistically significant difference between males and females (49% higher levels in females with 95% CI 12–97, $P = .01$) (Table 2). Serum levels of VEGF differed significantly between the reference group (adults, group 4), and (1) cord blood (group 1) (92% higher levels in cord blood, 95% CI 13–228, $P = .02$) and (2) neonates (250% higher levels in neonates, 95% CI 129–434, $P = .0001$). Restricting the multiple linear regression analysis to only females of the six groups (Table 3), serum levels of VEGF in cord blood (group 1), neonates (group 2), children (group 3), and postmenopausal

Table 2. Multiple Linear Regression of VEGF Levels (Log Values as Dependent Variable) and Gender and Five Groups Investigated (as Independent Variables)

Variable	Category	% difference of point estimate	95% CI	*t*	*P*
Gender	Male	Reference			
	Female	49	12–97	2.74	.01
Groups	4. Adults	Reference			
	1. Cord blood	92	13–228	2.41	.02
	2. Neonates	250	129–434	5.81	.0001
	3. Children	19	−23–83	0.78	.44
	5. Elderly	20	−23–87	0.82	.42

Data are derived percent difference of point estimate with 95% CI and corresponding *t* and *P* values for both genders.

women (group 6) were significantly higher than levels in women in the proliferative phase of the menstrual cycle (reference group) by a difference of 169% with 95% CI 32–449 ($P = .01$), 342% with 95% CI 142–709 ($P = .0001$), 113% with 95% CI 16–289 ($P = .02$), and 135% with 95% CI 27–336 ($P = .01$), respectively. However, serum levels of VEGF in the same cohort of adult women in the secretory phase of the menstrual cycle (group 4b) were lower than in the reference group by 44%, with 95% CI −70–4, a difference which was not statistically significant ($P = .07$). The group of pregnant women was not included in the analyses because serum levels of VEGF in this group were below the lower limit of detection of the enzyme immunoassay.

Table 3. Multiple Linear Regression of VEGF Levels (Log Values as Dependent Variable) and Six Groups Investigated (as Independent Variables)

Category	% difference of point estimate	95% CI	*t*	*P*
4. Adults*	Reference			
1. Cord blood	169	32–449	2.71	.01
2. Neonates	342	142–709	4.82	.0001
3. Children	113	16–289	2.45	.02
6. Postmenopausal	135	27–336	2.71	.01
4. Adults†	−44	−70–4	−1.85	.07

Data are derived percent difference of point estimate with 95% CI and corresponding *t* and *P* values only for females.
* Women in the proliferative phase of the menstrual cycle.
† Women in the secretory phase of the menstrual cycle.

DISCUSSION

The results of this study indicate that VEGF levels are higher in females than in males ($P = .01$) and in fetuses and neonates after the first week of life ($P = .01$ and $P = .0001$) compared with adults.

Folkman and Klagsbrun stated that physiologic angiogenesis seems to be restricted to the female[14] owing to the cyclic phenomena involving follicular growth and selection as well as growth of the endometrium by dynamic remodeling of its vascular supply.[2] In this respect, estrogens may have a role because existing data suggest that VEGF gene expression is increased by estrogens in the rat and human uterus.[15–18]

The high rates of VEGF in fetuses (cord blood) and 10-day-old neonates are in accordance with the increased rates of growth and development characterizing these age groups and presupposing enhanced rates of angiogenesis. In specific, cord blood levels could primarily be attributed to the vast placental angiogenesis.[2] Although the latter is considered to be diminished at term, fetally derived macrophages within the placental villi continue to express VEGF, the role of which, in this case, is maintenance of the endothelium or regulation of vascular permeability.[19]

For infants beyond the early neonatal period, besides increased growth rates, high VEGF levels might further reflect continuing adaptation demands to extrauterine life of tissues, expressing VEGF mRNA, as epithelial cells of lung alveoli and cardiac myocytes.[20] In those tissues VEGF might regulate normal function by controlling microvascular permeability or maintaining the differentiated state of microvessels.[21]

In children, the significantly lower serum VEGF levels documented in this study in boys compared with girls of a corresponding age may be explained by the fact that puberty, which is characterized by a new growth spurt, starts later in boys than in girls. Thus, in our cohort, girls presented considerably more advanced Tanner stages than boys.

Vascular endothelial growth factor serum levels in adult males and females did not differ significantly in this study. Although women who menstruated normally were included, we assume that the vascular capillary network in men might be more expanded.

Furthermore, no significant difference was found between midfollicular and midluteal serum VEGF values. Previous studies documented that during the late proliferative phase and throughout the secretory phase, a complex subepithelial capillary plexus develops in the endometrium,[2] whereas from days 0 to 25 there is a gradual increase in branching and coiling of spiral arterioles.[2] On the other hand, ovarian angiogenesis takes place during the follicular phase of the menstrual cycle, during ovulation, as well as during the luteal phase for continued support of the corpus luteum.[2,22] Thus, angiogenesis is present in both phases of the menstrual cycle, and secretion of angiogenic factors deriving from the endometrium and/or the ovary is detectable in the periphery.

Regarding the elderly group as a whole, no differences in VEGF serum levels were found compared with younger adults. Nevertheless, it is difficult to explain the higher values of VEGF documented in postmenopausal women compared with proliferative-phase adults. A possibility involves a balancing effort of the female organism to prevent age-associated endothelial dysfunction, which in women occurs abruptly after menopause, but years later than in healthy men of a corresponding age, in whom endothelial responses decline gradually after the fourth decade of life.[23] Nevertheless, Rusnati et al[24] showed that levels of angiogenic basic fibroblast growth factor in human endometrial biopsies increase after menopause, which we were unable to verify in peripheral blood samples.[25]

Finally, our finding that VEGF levels in pregnant women at term were below the limit of detection verifies previous reports.[11,12,13,26,27]

In conclusion, according to this study, VEGF serum levels are higher in females than in males and during life periods characterized by enhanced growth and development, presupposing increased rates of angiogenesis.

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