Serum Vascular Endothelial Growth Factor in Pregnant Women with Different Types of Assisted Reproductive Techniques

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Abstract

Background: Vascular endothelial growth factor (VEGF) is crucial for the implantation of a fertilized egg and for the development of placental blood vessels. This study was conducted to detect the serum VEGF level of pregnant with different types of assisted reproductive techniques in relation to the age group, body mass index (BMI), and type of infertility. **Objectives:** The aim of this study was to determine the levels of serum VEGF in different types of assisted pregnancy. **Materials and Methods:** Enzyme-linked immunosorbent assay was used for the detection of VEGF levels in the serum of pregnant women in the first trimester of pregnancy with a spontaneous pregnancy (SP), pregnant women by intrauterine insemination (IUI) or *in vitro* fertilization-embryo transfer (IVF), in comparison with nonpregnant women (NP). **Results:** A highly significant increase was seen in the serum VEGF within the different studied pregnant groups compared to that of NP and no difference between IUI and IVF ($P < 0.01$). Statistical analysis showed a nonsignificant relation between the VEGF serum levels and the increase in age or BMI ($P > 0.05$). No significant differences were seen in VEGF serum levels between pregnant women with primary or secondary types of infertility ($P > 0.05$). **Conclusion:** Statistical analysis showed a significant increase in the serum of VEGF in IUI and IVF comparison to SP groups, whereas there is no difference between IUI and IVF. Age, BMI, and type of infertility showed no significant effect on serum VEGF levels.

Keywords: *In vitro* fertilization, intrauterine insemination, vascular endothelial growth factor

Introduction

The family of VEGF is a growth protein involving the VEGF-A to VEGF-F, endocrine gland-derived VEGF, and placental GF.[1] One of the most effective and particular angiogenic cytokines is VEGF. It is a 23–45 kDa glycoprotein that binds heparin.[2] Vascular endothelial growth factor (VEGF) is a necessary component of angiogenesis and is crucial for the growth of the placenta,[3] development of blood and lymphatic vessels,[4] cell proliferation and differentiation regulation, endothelial cells survival, and permeability of vasculature.[5] It activates and binds to the VEGF receptor (VEGFR), mediated by tyrosine kinase and then it influences the different physiological changes.[6,7] In postnatal development, several researches showed the important role of VEGF in organ development and body growth[8,9] and activation of monocytes and macrophages.[10] In tumors, it can assist in its growth and metastasis.[11] Early in the first trimester of healthy pregnancies, maternal serum VEGF levels were found to rise. The corpus luteum, endometrium, and placenta were known as a source of VEGF.[12] It also controls the growth and differentiation of cytotrophoblasts through VEGF receptors which are found on cytoplasmic membranes of trophoblasts, so it orientates the implantation and is considered one of the fundamental roles in placental and early embryonic development.[13] The normal level of serum VEGF can help in homeostasis and

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then maintenance of a healthy pregnancy. In case of disturbances in VEGF concentration, pregnancy complications may occur.[14] Intrauterine insemination (IUI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and frozen embryo transfer (FET) are several forms of assisted reproductive methods. The IUI and the IVF are the most commonly used.[15] Since VEGF has an important role in the fertilized ovum implantation process and in the vascular formation during the process of placental development in the pregnancy period, the aim of the this subject was to clarify the serum level of VEGF in women with SP, and in women that pregnant by artificial fertility treatments (IUI or IVF) with primary or secondary types of infertilities, in comparison with NP of different age groups and body mass indices.

Due to the needs of the developing fetus, a time of higher metabolic demands and a change in the woman's physiology occurs during pregnancy. At this point, inadequate intake of micronutrients or inadequate stores can have adverse effects on the mother and the fetus.[16]

Materials and Methods

Samples collection

This prospective study was carried out in Taiba Center for Infertility Treatment, IVF, and ICSI Babylon/Iraq from November 2022 to February 2023. This study was approved by the University of Babylon/College of Medicine Ethical Committee. Informed consent was obtained from all pregnant and non-pregnant participants before the data collection. From 486 pregnant women, only 150 randomly selected healthy pregnant women with uncomplicated pregnancy and within the inclusion, criteria were selected. Fifty women were with SP, 50 had undergone IUI, and 50 women had undergone IVF. They had singleton pregnancy with alive baby in the first trimester of pregnancy.

The control (non-pregnant) group consists of 50 apparently healthy women with a negative pregnancy test. They were randomly selected, not taking any form of oral contraceptive pill, had regular menstrual cycles, and had access to service at the same centers at the same period. In this study, any women with chronic diseases, gestational diabetes mellitus, heart failure, and renal diseases were excluded.

Serum assay

Venous blood was aspirated using disposable syringes, left for[10-20] minutes at room temperature for clotting, and after that centrifuged at 3000 RPM at 4°C for 20 min. The supernatant was collected carefully in labeled Eppendorf tubes and then frozen at –20 C0 until the time of its use. The assay for the human VEGF was done using an enzyme-linked immune sorbent assay (ELISA) kit (Biont, Shanghai/ China, Catalog No: YLA1208HU). The Biotin double antibody sandwich technology was used. Kit sensitivity was 10.42ng/L. The samples were tested in a duplicate manner according to the manufacturer's instructions. The inter- and intra-assay coefficients of variation were <6% and <5.5%, respectively.

The parameters like patients' age, body mass index (BMI), and type of infertility[17] were all analyzed. From the first day of the last menstrual cycle, the gestational age was calculated. According to the formula weight/height2, the BMI was calculated.[18]

Statistical analysis

By using SPSS statistical software version 23, analysis was carried out. Descriptive statistics were drawn for all studied parameters. Assessment of normality was performed using the Kolmogorov–Smirnov test. Data were described as percentages and mean with standard deviation (SD). Level of significance between pregnant and nonpregnant women was analyzed using an independent t test and Mann–Whitney U test for parametric and non-parametric variables, respectively. The one-way analysis of variance (ANOVA) was used to examine the age and BMI. A value of P = 0.05 was considered statistically significant.

Ethical approval

According to the declaration of Helsinki, the ethical principles of this study were conducted. The study design and the subject information and agreement form were reviewed and approved by a local ethics committee according to document number 11-1 on 27 Apr 2023.

Results

Sample distribution

A total of 150 pregnant women in the first trimester of pregnancy with a mean age of 28.90 ± 5.97 years, and 50 aged matched (P = 0.069) apparently healthy non-pregnant women with a mean age of 27.26 ± 6.84 years were participated in the study. The highest number of pregnant and non-pregnant participants were seen within the age group,[21-28] as shown in Table 1.

Regarding the BMI, the highest percentage of NP, SP, and IUI were seen as weight, and the highest percentage of women with IVF were seen as obese. In all the studied groups, no one seen as underweight [Table 2].

The highest percentage of women with primary infertility have undergone IVF, whereas highest percentage of women with secondary infertility were undergone IUI, as shown in Table 3.

Statistical analysis also showed non- significant differences between the pregnant groups of Age.

Statistical analysis also showed non- significant differences between the pregnant groups of body mass index.

Table 1: Distribution of pregnant women according to the age groups

Age (Years)	(SP) (No.50)	(IUI) (No.50)	(IVF) (No.50)	*P* Value
Mean ± SD	28.18 ± 6.30	30.0 ± 5.80	30.08 ± 5.59	0.222
Age groups (years)		Pregnant (No.& %)		Total pregnant (No.& %)
18–25	15(30%)	12(24%)	12(24%)	39 (26.0%)
26–33	21(42%)	21(42%)	22(44%)	64(42.7%)
Above 34	14(28%)	17(34%)	16(32%)	47(31.3%)
Total (No.& %)	50(100%)	50(100%)	50(100%)	150(100%)

*Significant at $P < 0.05$

Table 2: Distribution of pregnant women according to the body mass index (BMI)

BMI kg/m2	(SP) (No.50)	(IUI) (No.50)	(IVF) (No.50)	*P* Value
Mean ± SD	28.03 ± 4.14	26.96 ± 3.80	29.014 ± 5.39	0.079
BMI/ kg/m2 Groups		Pregnant (No.& %)		Total pregnant (No.& %)
Underweight (≤ 19.9)	0(0%)	0(0%)	0(0%)	0 (0%)
Normal weight (20–24.9)	14(28%)	18(36%)	13(26%)	45(30%)
Overweight (25–29.9)	24(48%)	20(40%)	17(34%)	61(40.7%)
Obese (30 or more)	12(24%)	12(24%)	20(40%)	44(29.3%)
Total (No.& %)	50(100%)	50(100%)	50(100%)	150(100%)

*Significant at $P < 0.05$

Table 3: Distribution of pregnant women according to the types of infertility

Types of infertility	IUI (No.& %)	IVF (No.& %)
Primary infertility	24(48%)	29(58%)
Secondary infertility	26(52%)	21(42%)
Total (No.& %)	50(100%)	50(100%)

Table 4: Relation of serum levels of VEGF between the different groups

Group	VEGF Mean ± SD (ng/L)	*P* Value
NP	347.410 ± 44.993	0.00001
SP	405.284 ± 59.611	
NP	347.410 ± 44.993	0.00001
IUI	434.021 ± 57.790	
NP	347.410 ± 44.993	0.00001
IVF	444.670 ± 54.494	
SP	405.284 ± 59.611	0.0166
IUI	434.021 ± 57.790	
SP	405.284 ± 59.611	0.0001
IVF	444.670 ± 54.494	
IUI	434.021 ± 57.790	0.3503
IVF	444.670 ± 54.494	

$P > 0.05$: Non-significant; * $P < 0.05$ significant; **$P < 0.01$: Highly Significant

Serological analysis results

In comparison with the NP group, statistical analysis showed a highly significant increase in serum VEGF in the SP, IUI, and IVF pregnant groups ($P < 0.01$). A significant increase was also seen in the serum of the IUI and IVF groups ($P < 0.05$) in comparison with the SP group. A nonsignificant difference was seen between the IUI and IVF groups ($P > 0.05$), as shown in Table 4.

This study showed that the serum VEGF level showed a non-significant relation with the increase in the maternal age ($P > 0.05$) or increase in the BMI ($P > 0.05$), as shown in Table 5.

In women with IUI, the serum levels of VEGF in primary and secondary types of infertility were 410.720 ± 22.760 and 422.146 ± 25.788 ng/L, respectively. Statistical analysis showed a non-significant difference present between them ($P = 0.644$), as shown in Table 6.

In women with IVF, the serum levels of VEGF in primary and secondary types of infertility were 421.677 ± 23.786 ng/L and 423.884 ± 20.782 ng/L, respectively. Statistical analysis showed a nonsignificant difference present between them ($P = 0.765$).

Discussion

A total of 150 pregnant women in the first trimester of pregnancy and 50 aged matched apparently healthy NP women were considered for the study. Statistical analysis showed a highly significant increase in VEGF serum level in the different studied pregnant groups in comparison with the NP group ($P < 0.001$).

Previous studies by Ren *et al.*,[19] and Tandon *et al.*[20] found a highly significant increase in the total immunoreactive VEGF in pregnant compared to NP. Evans *et al.*[29] else that the VEGF serum levels are increased significantly during the first trimester of SP. Fasouliotis *et al.*[30] found that women who underwent IVF therapy showed high serum VEGF. But Rahmatullah *et al.*[31] found a significantly

Table 5: Serum levels of VEGF in the different pregnant studied groups in relation to the age group and BMI

Age group (year)	Mean ± SD VEGF (ng/L)		Mean ± SD VEGF (ng/L		Mean ± SD VEGF (ng/L)	
	SP	*P* Value	IUI pregnant	*P* Value	IVF pregnant	*P* Value
18–25	400.779±53.240	0 .877	405.572±54.516	0 .052	426.419±45.581	0.160
26–33	401.997±68.771		441.998±50.897		445.879±66.388	
Above 34	410.303±59.118		450.759±59.857		461.018±48.008	
Normal weight	417.304±62.094	0 .223	437.983±50.622	0.750	467.089±63.680	0.290
Overweight	394.707±47.672		432.788±48.147		441.794±53.735	
Obese	381.713±64.322		423.101±76.103		435.955±49.855	

P >0.05: Non-significant

Table 6: Relation of serum levels of VEGF with the type of infertility in IUI and IVF pregnant group

Types of infertility	IUI Mean ± SD VEGF (ng/L)	*P* Value	IVF mean ± SD VEGF (ng/L)	*P* Value
Primary infertility	410.720 ± 22.760	0.644	421.677 ± 23.786	0.765
Secondary infertility	422.146 ± 25.788		423.884±20.782	

P > 0.05: Non-significant

higher VEGF level in infertile women undergoing ICSI protocol, compared to NP.

In early pregnancy, maintenance of a good blood circulation was important for placental growth, in addition, for the fetal growth process, the blood and the oxygen supply are required.[32]

In contrast, Baker *et al.*[33] study revealed an undetectable elevation in serum levels of VEGF in normal pregnant but in late pregnancy in comparison with NP females. However, Lyall *et al.*[21] study noted a decreased serum VEGF level in pregnant compared to the control NP group. These differences in the results may be because the different gestational ages and different methodologies used for the serological analysis.

In this study, a significant increase was also seen in the serum of the IUI and IVF groups (P < 0.001) in comparison with the SP group. This may be due to the drugs, which are used for ovarian hyperstimulation.[22,23]

This study showed that the serum VEGF levels was not correlated significantly to maternal age. This result disagrees with that of Atalay *et al.*[24] study, they found that the serum VEGF levels correlate significantly to maternal age, and the increase in the maternal age is related to elevated serum VEGF levels.

This study showed that the serum VEGF level was not correlated significantly with the BMI. This result disagree with that of Estemberg *et al.*[25] and Zelinka-Khobzey *et al.*,[26] in which they found that the obesity in pregnant is connected significantly with a decreased VEGF synthesis. The maternal obesity might trigger endothelial inflammation and dysfunction.[27] In contrast, another study has documented that the maternal obesity was not related to a reduction in VEGF.[28] These variations in the results could be the result of various gestational ages.

In pregnant women with IUI or IVF, the serological analysis of VEGF in the primary or the secondary types of infertility showed a non-significant difference between them. Significantly a higher level of VEGF has been noted by Hafnerin *et al.*[34] study of women with primary infertility compared with that of secondary type (P = 0.011) on IVF outcome.

Conclusion

In the first trimester of pregnancy, this study found a significantly higher serum level of VEGF in the different studied pregnant groups compared to that of NP. There is no significant difference between IUI and IVF groups. The increased maternal age or BMI is not related significantly to the serum VEGF concentration. There is no appreciable distinction in VEGF serum levels between pregnant women with primary or secondary types of infertility with IUI or IVF fertilization.

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Conflicts of interest

There are no conflicts of interest.

References

1. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 2005;6:1-10.
2. Al-Gazally ME, Al-Saadi AH, Obed AF. Study of the changes of vascular endothelial growth factor (VEGF), antioxidant capacity and oxidative stress in the sera of Iraqi patients with ischemic stroke. J Univ Babylon 2014;22:2372.
3. Al-Moayad HA, Khaleel MF. Association between vascular endothelial growth factor gene polymorphisms and the risk of preeclampsia in Iraqi pregnant women. Iraqi J Med Sci 2022;20:106.

4. Secker GA, Harvey NL. Regulation of VEGFR signalling in lymphatic vascular development and disease: an update. Int J Mol Sci 2021;22:7760.
5. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and development. Cell 2019;176:1248-64.
6. Bolatai A, He Y, Wu N. Vascular endothelial growth factor and its receptors regulation in gestational diabetes mellitus and eclampsia. J Transl Med 2022;20:400.
7. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.
8. Theis V, Theiss C. VEGF-A stimulus for neuronal development and regeneration in the CNS and PNS. Curr Protein Pept Sci 2018;19:589-97.
9. Smythe G. Role of growth factors in modulation of the microvasculature in adult skeletal muscle. Adv Exp Med Biol 2016;900:161-83.
10. Zittermann SI, Issekutz AC. Endothelial growth factors VEGF and bFGF differentially enhance monocyte and neutrophil recruitment to inflammation. J Leukoc Biol 2006;80:247-57.
11. Trifanescu OG, Gales LN, Tanase BC, Marinescu SA, Trifanescu RA, Gruia IM, *et al*. Prognostic role of vascular endothelial growth factor and correlation with oxidative stress markers in locally advanced and metastatic ovarian cancer patients. Diagnostics 2023;13:166.
12. Evans P, Wheeler T, Anthony F, Osmond C. Maternal serum vascular endothelial growth factor during early pregnancy. Clin Sci 1997;92:567-71.
13. Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol 2016;17:611-25.
14. Zhou LF, Duan T. Research progress of Vascular Endothelial Growth Factor and pregnancy. Prog Mod Obstet Gynecol 2014;2014:146-9.
15. Graham ME, Jelin A, Hoon JAH, Wilms Floet AM, Levey E, Graham EM. Assisted reproductive technology: Short-and long-term outcomes. Develop Med Child Neurol 2023;65:38-49.
16. Black RE. Micronutrients in pregnancy. Br J Nutr 2001;85:S193-7.
17. Benksim A, Elkhoudri N, Addi RA, Baali A, Cherkaoui M. Difference between primary and secondary infertility in Morocco: frequencies and associated factors. Int J Fert Ster 2018;12: 142-6.
18. Yazdani S, Yosofniyapasha Y, Nasab BH, Mojaveri MH, Bouzari Z. Effect of maternal body mass index on pregnancy outcome and newborn weight. BMC Res Not 2012;5:1-4.
19. Ren Y, Wang H, Qin H, Yang J, Wang Y, Jiang S, *et al*. Vascular endothelial growth factor expression in peripheral blood of patients with pregnancy induced hypertension syndrome and its clinical significance. Pakistan J Med Sci 2014;30:634-7.
20. Tandon V, Hiwale S, Amle D, Nagaria T, Patra PK. Assessment of serum vascular endothelial growth factor levels in pregnancy-induced hypertension patients. J Preg 2017;2017:2.
21. Lyall F, Greer IA, Boswell F, Fleming R. Suppression of serum vascular endothelial growth factor immunoreactivity in normal pregnancy and in pre-eclampsia. BJOG 1997;104:223-8.
22. Mattioli M, Barboni B, Turriani M, Galeati G, Zannoni A, Castellani G, *et al*. Follicle activation involves vascular endothelial growth factor production and increased blood vessel extension. Biol Reprod 2001;65:1014-9.
23. Lee A, Christenson LK, Stouffer RL, Burry KA, Patton PE. Vascular endothelial growth factor levels in serum and follicular fluid of patients undergoing in vitro fertilization. Fertil Steril 1997;68:305-11.
24. Atalay MA, Ugurlu N, Zulfikaroglu E, Danisman N. Clinical significance of maternal serum vascular endothelial growth factor [VEGF] level in idiopathic recurrent pregnancy loss. Eur Rev Med Pharmacol Sci 2016;20:14.
25. Karowicz-Bilińska A, Berner-Trąbska M, Kuś E, Brzozowska M, Kowalska-Koprek U. The assessment of leptin concentration and Vascular Endothelial Growth Factor (VEGF) in relation to the body mass index since 20th week of pregnancy. Ginekol Pol 2009;80:5.
26. Zelinka-Khobzey, M, Tarasenko, K, Mamontova, T. Assessment of endothelial dysfunction in pregnant women with obesity and preeclampsia2021 ..
27. Janbakhishov T, Çağlayan E, Acet F, Altunyurt S, Özer E. Effect of vascular endothelial growth factor on fetal vessels among obese pregnant women. Int J Gynaecol Obstet 2020;151:231-6.
28. Ma Y, Zhu MJ, Zhang L, Hein SM, Nathanielsz PW, Ford SP. Maternal obesity and overnutrition alter fetal growth rate and cotyledonary vascularity and angiogenic factor expression in the ewe. Am J Physiol Regul Integr Comp Physiol 2010;299:R249-58.
29. Evans PW, Wheeler T, Anthony FW, Osmond C. A longitudinal study of maternal serum vascular endothelial growth factor in early pregnancy. Hum Reprod 1998;13:1057-62.
30. Fasouliotis SJ, Spandorfer SD, Witkin SS, Liu HC, Roberts JE, Rosenwaks Z. Maternal serum vascular endothelial growth factor levels in early ectopic and intrauterine pregnancies after in vitro fertilization treatment. Fertil Steril 2004;82:309-13.
31. Rahmatullah WS, Al-Obaidi MT, AL-Saadi WI, Selman MO, Faisal GG. Role of Vascular Endothelial Growth Factor (VEGF) and Doppler Sub-endometrial Parameters as Predictors of Successful Implantation in Intracytoplasmic Sperm Injection (ICSI) Patients. Siriraj Med J 2020;72:1.
32. Walter LM, Rogers PA, Girling JE. Differential expression of vascular endothelial growth factor-A isoforms in the mouse uterus during early pregnancy. Reprod Biomed Online 2010;21:803-11.
33. Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. Obstetr Gynecol 1995;86:815-21.
34. Hafner D, Zivkovic SV, Bauman R, Tiljak K, Papić N, Lepej SZ. Follicular fluid vascular endothelial growth factor is associated with type of infertility and interferon alpha correlates with endometrial thickness in natural cycle in vitro fertilization. Reprod Biol 2018;18:289-94.