

A STUDY OF SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN ECTOPIC PREGNANCY: COULD THIS BE A RELIABLE DIAGNOSTIC SERUM BIOMARKER OF ECTOPIC PREGNANCY?

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Received : 08/02/2023
Received in revised form : 11/03/2023
Accepted : 03/04/2023

Keywords:
VEGF, β -hCG, Ectopic pregnancy,
Normal intrauterine pregnancy

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DOI: 10.47009/jamp.2023.5.3.53

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 245-249



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Abstract

Background: Ectopic pregnancy is a common life threatening complications of early pregnancy refers to a gestation in which the fertilized ovum implants itself outside the uterus, usually in one of the fallopian tubes. Vascular endothelial growth factor (VEGF) an angiogenic factor and plays a key role in establishment of viable pregnancy. It participates in the process of implantation and placentation, and it is significantly increased in early pregnancy complications. **Objectives.** The study was to evaluate and compare serum vascular endothelial growth factor and beta-human chorionic gonadotropin in women with ectopic pregnancy and normal intrauterine pregnancy and to correlate the levels of Serum Vascular endothelial growth factor (VEGF) and β -hCG in women with ectopic pregnancy and normal intrauterine pregnancy. **Methodology:** It is a prospective, case controlled, hospital based study in which Serum levels of vascular endothelial growth factor and beta-human chorionic gonadotropin were measured by enzyme linked immuno sorbent assay (ELISA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy in the wards of Rajkiya mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer after taking approval from ethical committee. These values were compared by t-test. By determining cut-off levels of these parameters the sensitivity and specificity of them in prediction of ectopic pregnancy was estimated. **Results:** The mean serum level of VEGF was significantly higher and serum β -hCG level was significantly lower in patients with EP than in women with normal intrauterine pregnancy ($p < 0.001$). **Conclusions:** The increased serum VEGF levels and low levels of β -hCG in women with Ectopic Pregnancy in comparison to Normal Intrauterine Pregnancy can serve as an excellent diagnostic tool for the prediction of ectopic pregnancy, so that 40-50% of initially misdiagnosed cases which resulted earlier in significant morbidity and mortality, can be overcome.

INTRODUCTION

Ectopic pregnancy (EP) is a potentially life threatening condition, as it is still a major cause of maternal morbidity and mortality, resulting for 9-13% of all pregnancy-related deaths.^[1] Despite the introduction of highly sensitive assays for the estimation of serum human chorionic gonadotropin (hCG) and an increase in the sensitivity of transvaginal sonography (TVS), it is believed 40-50% of cases initially are misdiagnosed.^[2] Ectopic pregnancy is the implantation of an embryo outside of the uterine cavity most commonly in the fallopian tube. Smooth muscle contraction and ciliary beat within the fallopian tubes to assist in the transport of an oocyte or embryo. Damage to the fallopian

tubes, usually secondary to inflammation, induces tubal dysfunction which can result in retention of an oocyte or embryo. There are several local factors, such as toxic, infectious, immunologic and hormonal that can induce inflammation. There is upregulation of pro-inflammatory cytokines following tubal damage, this subsequently promotes embryo implantation, invasion and angiogenesis within the fallopian tube.^[3]

Vascular endothelial growth factor (VEGF) is a most potent angiogenic factor^[4,5] which has an important role in establishment of a viable pregnancy, participating in the process of implantation and placentation. This acts as a major modulator of vascular growth, remodelling, and permeability in endometrium, decidua,

trophoblast^[6,7,8,9]. VEGF is also indispensable for trophoblast development during the vascular development of the embryo.^[10] The secretion and expression of the VEGF is dependent on local conditions, such as hypoxia, and it has been observed that the cellular VEGF is increased in hypoxic conditions.^[11,12,13] The implantation environment in oviduct is very different from that of well-vascularised endometrium, and the production and secretion of VEGF may be affected in EP.^[14,15]

Human chorionic gonadotropin (hCG) composed of two dissimilar subunits alpha (α) and beta (β), linked together with hydrogen and disulphide bonds. It is a peptide hormone produced in pregnancy that is made by the embryo soon after conception and later by the syncytiotrophoblast (part of the placenta). Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for a pregnancy in humans. Early pregnancy testing in general is based on the detection or measurement of β -hCG. Because an early and accurate laboratory diagnosis of tubal EP could assist clinical management, so various laboratories have directed their research toward biochemical markers of tubal EP.^[16,17,18,19]

The study was aimed to evaluate serum vascular endothelial growth factor and beta-human chorionic gonadotropin in women with ectopic pregnancy and normal intrauterine pregnancy, so that the importance and the usefulness of measuring VEGF

levels in order to diagnose EP can be evaluated and it could serve as reliable serum biomarker of EP. The levels of serum VEGF and β -hCG were also correlated in EP and normal IUP cases.

MATERIAL AND METHODS

This is a case controlled, prospective study in which the subjects included were 100 of ectopic pregnancy (EP) cases and 100 of normal intrauterine pregnancy (IUP) cases as controls of different age groups (20-40 years) attending the out patient clinics or admitted in the wards of Rajkiya Mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer, after taking approval from ethical committee. Serum levels of VEGF and β -hCG were measured by Enzyme Linked Immuno Sorbent Assay (ELISA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy. SPSS 13/win statistical software was used for analyzing the data. Data were presented as mean \pm standard deviation. A parametric independent sample t-test was used to compare differences between two groups. Level of statistical significance was set at $p < 0.05$. By determining cut-off levels of these parameters the specificity and sensitivity of each in prediction of ectopic pregnancy were estimated.

RESULTS

Demographic data of IUP and EP are shown in tables.

Table 1: Mean serum VEGF and β -hCG values in normal IUP and EP.

S. No.	Parameters	Normal IUP	EP	t- value	P value
1.	Serum VEGF (pg/ml)	26.8 \pm 4.23	260.16 \pm 59.05	40.4	<0.001(HS)
2.	Serum β -hCG (mIU/ml)	1903.0 \pm 425.5	511.7 \pm 245.5	28.3	<0.001(HS)

$P < 0.001$ = Highly Significant(HS)

Table 2: Sensitivity, Specificity and Cut off values of parameters.

S. No.	Parameters	Sensitivity	Specificity	Cut off value
1.	Serum VEGF (pg/ml)	88%	100%	< 200 pg/ml
2.	Serum β -hCG (mIU/ml)	91 %	85%	< 1500mIU/ml

Table 3: Correlation of parameters in IUP and EP.

	Normal Intrauterine Pregnancy	Ectopic Pregnancy	Serum β -hCG
Serum VEGF	$r = 0.113$ $p > 0.10$ (NS)	$r = -0.176$ $p > 0.05$ (NS)	r

In this study the mean levels of Serum VEGF ie 260.16 ± 59.05 pg/ml were significantly higher in ectopic pregnancy than Serum VEGF ie 26.8 ± 4.23 pg/ml in normal intrauterine pregnancy ($p < 0.001$). Similarly β -hCG levels in patients with ectopic pregnancy ie 511.7 ± 245.5 mIU/ml were lower than β -hCG in patients with normal intrauterine pregnancy ie 1903.0 ± 425.5 mIU/ml ($p < 0.001$) [Table 1]. By determining the cut-off level of < 200 pg/ml for Serum VEGF, the sensitivity of single measurement of Serum VEGF level was 88% and specificity was 100%. Similarly at the cut off level of < 1500 mIU/ml for β -hCG the sensitivity of serial measurement of β -hCG was 91% and specificity was 85%. Both the parameters ie Serum VEGF and β -hCG

were of great assistance in early diagnosis of ectopic pregnancy [Table 2]. When in normal IUP the β -hCG was correlated with Serum VEGF there was positive correlation with serum VEGF ie $r = 0.113$ $p > 0.10$ (NS) but when in EP, the β -hCG was correlated with Serum VEGF there was non significant inverse correlation with serum VEGF ie $r = -0.176$ $p > 0.05$ (NS) [Table 3].

In about 85% of normal intrauterine pregnancy, the HCG level will double every 48-72 hours. Further along the pregnancy and the HCG levels gets higher, the time it takes to double can increase to about every 96 hours. Intact β -hCG is present in serum and urine. The concentration of intact β -hCG may reach upto 150,000 mIU/ml. β -hCG levels rise exponentially for the first 8 weeks of pregnancy reaching to peak at 10 weeks after LMP. In the following 10 weeks levels slowly decline to approximately one fifth of peak levels and remain around one fifth of peak levels until term.

But in ectopic pregnancy as the trophoblast is implanted abnormally in fallopian tube the entirely intact HCG is present in serum and urine samples with much lesser proportion of HCG degradation products, β -core fragment, free β -subunit. The levels can be low due to its implantation site, disruption of trophoblast by hemorrhage, or due to embryonic death. So, very low levels of β -hCG can be used as diagnostic tool for identifying ectopic pregnancy. But when serum VEGF values were correlated with β -hCG the Pearson's correlation was $r = 0.113$, $p > 0.10$ showing an insignificant positive correlation among them.

The processes of implantation and trophoblast invasion which characterize early normal intrauterine pregnancy are accompanied by major changes in the uterine vasculature by vascularisation of corpus luteum and by the development of the villous vasculature connecting embryo and trophoblast. Evans PW *et al.*^[13] reported that concentration of serum VEGF is increased in normal intrauterine pregnancy. It is also suggested that human chorionic gonadotrophin (HCG) influences VEGF production. Exposure of human granulosa

cells to HCG stimulates the expression of VEGF mRNA and administration of HCG in women undergoing in vitro fertilization increases urinary VEGF concentration.

The presence of HCG receptors on trophoblast cells may enable HCG to initiate VEGF transcription in the placenta. HCG has already been shown to have this effect on granulosa cells in the ovary, an action which is likely to promote the vascularisation of the corpus luteum. Serum concentration of both HCG and VEGF increase during the first trimester, so they are inevitably positively correlated.

In ectopic pregnancy subject group, serum VEGF was found to have non significant inverse correlation i.e. $r = -0.176$, $p > 0.05$ with β -hCG. To explain correlation the reason is that immediately after implantation of the blastocyst the developing trophoblast secretes β -hCG into maternal circulation in normal intrauterine pregnancy. Therefore the level β -hCG in blood increases rapidly with maximal level of 50000-100000 mIU/ml attained at about 8-10 weeks of gestation.

So β -hCG rises so rapidly indicating a supramaximal stimulation of the corpus luteum by the trophoblast in early intrauterine pregnancy, but in ectopic pregnancy it is possibly not the corpus luteum which responds poorly to stimulation by β -hCG but insufficient synthesis of β -hCG by the trophoblast. So β -hCG levels are lower. On the other hand due to implantation milieu in fallopian tube is different than endometrium the production and secretion of VEGF is also affected. VEGF is a potent angiogenic factor and its secretion depends on local conditions including hypoxia as stated by Torry, DS *et al.*^[6] In contrast to HCG and progesterone which are trophoblast dependent VEGF is produced both by trophoblast and endometrium as also stated by Evans PW *et al.*^[13] So due to local condition of hypoxia in fallopian tube there is increased production of VEGF. So due to implantation site β -hCG synthesis is low and VEGF synthesis is more. So these are negatively correlated in ectopic pregnancy. These results are similar to Muller MD *et al.*^[33]

DISCUSSION

The word "ectopic" means displaced, and an ectopic pregnancy is when a fertilised egg implants itself outside of the womb (uterus), usually in one of the fallopian fallopian tube, but other possible sites include: cervical, interstitial (also referred to as cornual; a pregnancy located in the proximal segment of the fallopian tube that is embedded within the muscular wall of the uterus), hysterotomy scar, intramural, ovarian, or abdominal. It is a known complication of pregnancy that can carry a high rate of morbidity and mortality when not recognized and treated promptly. It is essential that providers maintain a high index of suspicion for an ectopic in their pregnant patients as they may present with

pain, vaginal bleeding, or more vague complaints such as nausea and vomiting. Approximately 97% of all cases of extrauterine pregnancy the conceptus is implanted within the oviduct, in most cases which is due to the local pathologic conditions that impair its transport to the uterine cavity.^[20] This causes invasion and implantation of the conceptus through the tubal epithelium, which is not primed angiogenically and biochemically for implantation like the well-vascularised and appropriately constructed endometrium.^[21] Ectopic Pregnancies occur because an eggs movement is slowed or obstructed after fertilization, usually because a fallopian tube is scarred, mishappen or possibly infected or inflamed. Disruption of normal tubal anatomy secondary to inflammation induces tubal dysfunction which can result in retention of oocyte or embryo. There can be several local factors, such as toxic, infectious, immunologic, and hormonal, that can induce inflammation. There is up regulation of proinflammatory cytokines following tubal damage; this subsequently promotes embryo implantation, invasion, and angiogenesis within the fallopian tube.^[22]

Normal tissue function depends on a regular supply of oxygen through the blood vessels. Understanding the formation of blood vessels has become the focus of a major research effort throughout the last decade. Vasculogenesis in the embryo is the process by which new blood vessels are generated de novo from primitive precursor cells. Angiogenesis is a process of a new blood vessel development from pre-existing vasculature. It plays an essential role in embryonic development, normal growth of tissues, wound healing, the female reproductive cycle (i.e. ovulation, menstruation and placental development), as well as a major role in many diseases.^[23] One of the most important growth and survival factors for endothelium is vascular endothelial growth factor (VEGF).^[24] VEGF induces angiogenesis and endothelial cell proliferation and it plays an important role in regulating vasculogenesis. Vascular Endothelial Growth Factor is indispensable for trophoblast development during vascular development of embryo.^[10]

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that plays a key role in vascular growth, permeability and remodelling.^[5] It has a major role in the regulation of angiogenesis in the corpus luteum and the endometrium.^[25] It acts as modulator of permeability in decidua and trophoblast, as well as during vascular development in the embryo, all of which are crucial processes related to normal implantation and placentation.^[11] The secretion and expression of the VEGF is dependent on local conditions, such as hypoxia, and it has been observed that the cellular VEGF is increased in hypoxic conditions.^[11,12,13] The implantation environment in the oviduct is very

different from that of the well vascularised endometrium, and production and secretion of VEGF may be affected in E.P.

β -hCG is a glycoprotein synthesized by the embryo soon after conception and later by the syncytiotrophoblast, and it maintains the corpus luteum during the beginning of pregnancy, causing it to secrete the progesterone. β -HCG production from placenta nourishes the egg after it has been fertilized and becomes attached to the uterine wall. Levels can first be detected by blood test about 11 days after conception and by a urine test about 12-14 days after conception. In general the HCG levels will double every 72 hours. The levels will reach its peak in first 8-11 weeks of pregnancy and then will decline and level off for the remainder of the pregnancy.

But in EP as the trophoblast is implanted abnormally in fallopian tube the entirely intact HCG is present in serum. In urine samples there is much lesser trophoblast proportion of HCG degradation products, β -core fragment and free β -subunit. The level can be low due to its implantation site, disruption due to hemorrhage, or due to embryonic death. So, very low levels of β -hCG can be used as diagnostic tool for identifying EP. The results of the present study were found similar to The results of the present study were found similar to Gharabaghi P *et al.*^[26], Cartwright *et al.*^[27], Barnhart K *et al.*^[28], Jurkovic D *et al.*^[29], Rausch M E *et al.*^[30] and Barash J H *et al.*^[31]

In contrast to HCG and progesterone which are trophoblast dependent VEGF is produced both by trophoblast and endometrium.^[13] So due to local conditions of hypoxia in fallopian tube there is increased production of VEGF. So, due to implantation site β -HCG synthesis is low and VEGF synthesis is more. So these are negatively correlated in ectopic pregnancy. These results are similar to Muller MD *et al.*^[33]. In ectopic pregnancy VEGF levels are highly raised in comparison to normal intrauterine pregnancy. Its reason is considered that the extrauterine implantation environment is very different from well vascularised endometrium. Hypoxia triggers increased VEGF production at the abnormal ectopic implantation i.e. in fallopian tube. Lam PM *et al.*^[34] stated that mRNA expression of VEGF and its receptors (KDR & flt-1) were measured in the implantation and non implantation sites with ectopic pregnancy. The mRNA expression was significantly higher in the implantation site of human oviduct with ectopic gestation compared with non implantation site. Similarly Torry DS *et al.*^[6] and Daniel Y *et al.*^[14] discussed that VEGF is a potent angiogenic factor and its secretion depends on local conditions including hypoxia and so VEGF can be selected as a possible biomarker since serum levels are found significantly higher in women with ectopic pregnancy than in normal intrauterine pregnancy. Similar findings of results has been

noticed in the various studies done by Felemban A *et al.*^[15], Fasouliotis SJ *et al.*^[33], Muller MD *et al.*^[34], Daponte A *et al.*^[35] and Cabar FR *et al.*^[36]

CONCLUSION

In this study levels of Serum Vascular Endothelial Growth Factor were found to be significantly higher in Ectopic Pregnancy in comparison to Normal Intrauterine Pregnancy. Serum beta human chorionic gonadotrophin levels were significantly lower in Ectopic Pregnancy in comparison to Normal Intrauterine Pregnancy. So, Serum Vascular Endothelial Growth Factor can serve as an excellent diagnostic biomarker for the prediction of ectopic pregnancy, so that 40-50% of initially misdiagnosed cases which resulted earlier in significant morbidity and mortality, can be overcome.

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