CLINICAL VALUE OF PLACENTAL GROWTH FACTOR AS A DIAGNOSTIC MARKER IN TUBAL PREGNANCY

A thesis
submitted *for partial fulfillment of Master Degree in Obstetrics & Gynecology*

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بسم الله الرحمن الرحيم

صدق الله العظيم

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Ahmed Samir Ahmed Aly

Contents

List of tables

List of figures

PROTOCOL

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Introduction

Ectopic pregnancy presents a major health problem for women of child bearing age , it is a result of flaw in the human reproductive physiology that allows the conceptus to implant and mature outside the endometrial cavity , which ultimately ends in death of the fetus (**Sameena et al 2009**). Tubal ectopic pregnancy is an important cause of maternal morbidity that can be fatal if left undiagnosed due to the risk of potential tubal rupture and haemorrhage (**Lewis ., 2007**).

The incidence of tubal ectopic pregnancy is increasing worldwide most likely due to arising incidence of pelvic inflammatory diseases caused by Chlamydia-Trachomatis infection and the increased use of assisted reproductive techniques(**Farquhar., 2005 & Walker., 2007**). During the past 25 years,the incidence of ectopic pregnancy has progressively increased while the morbidity and mortality associated with it has decreased , and the treatment options available have progressed from salpingectomy by laparotomy to conservative surgery by laparoscopy and medical therapy. This transition from surgical to medical management has been attributed to early diagnosis by the use of sensitive assays for beta subunit HCG and high definition trans abdominal and trans vaginal ultrasound(**Sameena et al ., 2009**).

Implantation in the Fallopian tube occurs in 1-2% of pregnancies in the developed world and ectopic pregnancy remains a leading cause of pregnancy-related first trimester deaths. It is associated with severe pelvic pain,acute hypovolemic shock and the need for blood transfusion as well as emergency surgical treatment with prolonged recovery. In the long term,complications of treatment include ongoing pelvic pain , de novo adhesion formation , impairment of future fertility prospects and an increased chance of further ectopic pregnancy(**Horne et al ., 2011**).

Increased serum concentrations of vascular endothelial growth factor (VEGF) and decreased serum levels of leukaemia-inhibiting factor (LIF),progesterone(P),pregnancy associated plasma protein A(PAPP-A),human placental lactogen (HPL) and glycodelin (GLY) have been previously associated with failed pregnancy. In an emergency set-up, it would be extremely valuable if a single combined measurement of these new markers could differentiate not only between an ectopic pregnancy and a healthy intrauterine pregnancy , but also between ectopic pregnancy and an abnormal intrauterine pregnancy. Such a correlation would decrease the time to diagnosis and reduce the possibility of tubal rupture and its sequelae (**Daponte et al ., 2005**).

During implantation the conceptus interacts with the local environment to facilitate its growth and development. One of the key features of successful implantation is the establishment of a supportive vascular network. Neo-vascularization depends on the induction of secreted pro-angiogenic growth factors (**Horne et al., 2011**).

Intrauterine implantation has been associated with the activity of placental growth factor(PLGF)which is a secreted pro-angiogenic protein with similarities to vascular endothelial growth factor (VEGF). It has been identified at the implantation site and acts on neighboring cells, notably endothelial cells, through the receptors flt-1(VEGF receptor 1) and fkl-1/KDR (VEGF receptor 2) to facilitate the development of a local blood supply . The normal response to implantation is an augmented secretion of PLGF , and this increase is reflected systemically , such that it can be measured in serum **(Horne et al ., 2011**).

Serum PLGF concentrations were shown to be significantly lower in women with tubal ectopic pregnancies (too low to be determined) and the non-viable intrauterine pregnancies compared with viable intrauterine pregnancies . Despite similar HCG concentrations, the PLGF assay was sufficiently sensitive to differentiate a tubal ectopic pregnancy from a non-viable intrauterine pregnancy (**Horne et al ., 2011**).

Aim of the work

The aim of this study is to evaluate the possible value of placental growth factor as a promising diagnostic biomarker for ectopic pregnancy.

Patients and methods

Data sources

This is a prospective case control study that will be carried up on 40 cases recruited from reception room or out patient clinics of Ain Shams University Maternity Hospital.

Inclusion criteria

***Cases*

Patients who were initially either admitted to emergency room or seen at antenatal clinics with symptoms as (amenorrhea,unilateral lower abdominal pain,vaginal bleeding or fainting attack) or ultrasound findings suggestive of ectopic pregnancy as identification of adnexal gestational sac with an embryo and cardiac activity with empty uterus.

***Controls*

- Normal pregnant females with definite viable intrauterine pregnancy in the first trimester as proven by LMP or ultrasound finding of intrauterine gestational sac with positive cardiac activity.
- Patients undergoing surgical management of embryonic missed abortion (ultrasound confirmed non viable intrauterine pregnancy).

Patients will be divided into three groups;

1) The first group : will include 15 pregnant women with suspected ectopic pregnancy.
2) The second group : will include 10 pregnant women undergoing surgical management of an embryonic missed abortion .
3) The third group : will include 15 women with normal uncomplicated first trimester intrauterine pregnancy.

Exclusion criteria

1- Women who refuse to participate in the study.
2- Women with medical disorders as hypertension,diabetes mellitus,SLE,…….etc.
3- Patients under treatment with hormonal therapy.
4- Multiple fetal gestations.

All patients will be subjected to:

1. **Verbal Consent to participate in the study**

2. **History taking**

Detailed history is taken including personal history as regard name,age,duration of marriage and special habits , menstrual history as regard rhythm,length,duration and amount of flow of menstrual cycle and first day of last menstrual period , obstetric history regarding gravidity and parity and number,mode and outcome of previous pregnancies and deliveries , past history as diseases,drug intake or surgical operations , contraceptive history regarding last contraceptive method used,its duration and cause of discontinuation and present history as regard duration of marriage,symptoms suggestive of early pregnancy,symptoms confirming pregnancy and symptoms suggestive of abnormal pregnancy as loin pain and vaginal bleeding.

3. **General,abdominal and pelvic examination**

General examination: including **general appearance** as constitution and gait.,**vital signs** as pulse.blood pressure and temperature ,**complexions** as pallor,jaundice and cyanosis and **signs of shock** as pallor,sweating,cold clammy skin,oliguria and fainting.

Abdominal examination:-

Inspection as abdominal contour , respiratory movement and abdominal skin.

Palpation :** Superficial for any abdominal swelling ,tenderness and rigidity.

** Deep palpation for abdominal organs and abdominal masses.

Pelvic (local) examination:-

Inspection of external genitalia and vagina for lesions , signs of trauma or infection and amount of blood.

Gentle bimanual examination for detection of:-

- Size and consistency of the uterus.
- Open or closed cervix.
- Cervical motion tenderness.
- Adnexal mass and / or tenderness.

4. Ultrasonography scanning (abdominal and vaginal)

Vaginal u/s is more accurate than abdominal u/s to diagnose ectopic pregnancy , and it is used to confirm the presence of a gestational sac outside the uterus or empty uterus with positive pregnancy test.

5. Laboratory investigations

** Estimation of B.subunit HCG for suspected cases

By using ablood sample that is collected aseptically into a clean tube without anticoagulants then serum is separated and used for analysis by HCG kits produced by ACON company (ACON laboratories,Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121 , USA).

** Estimation of placental growth factor (PLGF)

Serum placental growth factor will be measured using:

Quantitative human PLGF immunoassay manufactured and distributed by: R&D systems , Inc. 614 McKinley place NE – Minneapolis , MN 55413 United States of America , for the quantitative determination of human placental growth factor concentrations in serum.

Statistical analysis

Collected data will be spread on Microsoft Excel Worksheet, version 2007. statistical analysis will be performed using statistical package for social sciences (SPSS) for Windows. Variables will be presented as range,mean and standard deviation (for numeric parametric data). range,median and interquartile range (for numeric non parametric data). And number and proportion (for categorical data).

Variables of the three groups will be compared using

**student t test (for numeric parametric data)

**Mann-Whitney's U test (for numeric non-parametric data)

**Chi-square test (for categorical data).

Association between variables will be assessed using Pearson's correlation coefficient (for metric variables) and Spearman's correlation coefficient (for rank variables) .Receiver operator characteristics (ROC) curve will be constructed for finding the best cutoff level for diagnosis.

Diagnostic validity will be assessed using the terms of sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios. Significance level will be set at 0.05.