INTRODUCTION

Ectopic pregnancy means implantation of fertilized ovum in a location other than the endometrium. Ectopic pregnancy represents 1-2% of all pregnancy ***(Walker, 2007).*** Ninety - eight percent occurs in the oviducts, of these tubal pregnancy: 80% are ampullary, 12%are isthmic, 6%occurs with the fimbriae, 2% are interstitial, 1.4%abdominal, 0.15% cervical and 0.25% ovarian ***(Heinberg, 2010).***

Ectopic pregnancy remains a leading cause of pregnancy related first trimester morbidity and mortality. The classical symptoms of ectopic pregnancy are pelvic pain and vaginal bleeding after a period of amenorrhea ***(Kaplan and Moskos, 1996).*** These are nonspecific symptoms that may also present in threatened or spontaneous abortion ***(Della-Giustuna, 2003).***

The symptoms may be severe including severe pain and hypovolemic shock that may need blood transfusion ***(Varma and Gupta, 2009).*** The most common signs of ectopic pregnancy include normal to slightly enlarged uterus, pelvic pain with cervical manipulation, palpable adnexal mass. However, no combination of physical examination findings can reliably exclude ectopic pregnancy. Complications include emergency surgical treatment, long term complications as de novo adhesion formation, impairment of future fertility and increased chance of further ectopic pregnancy ***(Della-Giustuna, 2003).***

The most common gestational age at time of diagnosis is 6 to 10 weeks but ectopic pregnancy can be discovered until time of delivery ***(Xiao and Sun, 2005)***. Current diagnosis of tubal ectopic pregnancy involves a combination of transvaginal ultrasound and measurement of serum human chorionic gonadotropin (HCG) concentration. However, accurate and early diagnosis remains problematic and only 50%of ectopic pregnancy is diagnosed at initial time of presentation ***(Robson and Shea, 1996),*** and this rate has not improved in the last decade despite advances in ultrasound imaging technology ***(Wedderburn and Warner, 2010)***.

So there is an urgent unmet need for a biomarker for tubal ectopic pregnancy to allow quicker, accurate diagnosis and facilitate earlier and less invasive treatment ***(Elson and Jurkovic, 2004; Cartwright and Duncan, 2009).***

During implantation, the conceptus interacts with the local environment of the endometrium to facilitate its growth and development. One of the key features of successful implantation is the establishment of a supportive vascular network ***(Tony and Leavenworth, 2007).***

Neo-vascularization depends on the induction of secreted proangiogenic growth factors. These are regulated by a combination of paracrine signaling molecules and hypoxia ***(Smith, 2000).***

Intrauterine implantation has been associated with the activity of placental growth factor (PLGF) ***(Tony and Leavenworth, 2007).*** PLGF is a secreted proangiogenic protein with similarities to vascular endothelial growth factor, it has been identified at the implantation site and acts on neighboring cells, notably endothelial cells, through the receptors flt-1 (VEGF) recptor1 and FK1-1/KDR VEGF reptors2) to facilitate the development of local blood supply ***(Torry and Leavenworth, 2007).***

The role of PLGF in the development of an intrauterine vascular network is highlighted by its relationship to preeclampsia, which is associated with reduced placental vascularization ***(Red-Hores, 2004).***

Lower maternal serum levels of PLGF in early pregnancy correlate with a greater risk of developing preeclampsia in the third trimester ***(Baumann and Surbek, 2007).***

To grow, and cause harm ectopic pregnancy needs to develop a supportive blood supply, and angiogenesis also occurs at tubal implantation sites. It is not known whether PLGF is involved in increasing the vascularization of the fallopian tube in ectopic implantation. However, it is known that another proangiogenic growth factor; VEGF is involved. VEGF and its receptors are up regulated at the tubal

implantation site in ectopic pregnancy compared with elsewhere in the fallopian tube ***(Lam and Briton Jones, 2004)***. In addition, serum VEGF is increased in women with ectopic compared with intrauterine pregnancies ***(Felemban and Sammour, 2002).***

The normal response to implantation is an augmented secretion of PLGF, and this increase is reflected systemically, so that it can be measured in serum ***(Mu and Chen, 2001; Liang, 2003)***.

AIM OF THE WORK

To estimate placental growth factor level in ectopic pregnancy in comparison to missed abortion and normal intrauterine pregnancy to demonstrate if there is a statistically significant difference in between which may enable it to be used as a biomarker in diagnosis of ectopic pregnancy.

Chapter (1)

ECTOPIC PREGNANCY

An ectopic pregnancy is a complication of pregnancy in which the embryo implants outside the uterine cavity. With rare exceptions, ectopic pregnancies are not viable. Furthermore, they are dangerous for the mother, since internal heamorrhage is a life-threatening complication. Most ectopic pregnancies occur in the Fallopian tube (so-called tubal pregnancies), but implantation can also occur in the cervix, ovaries, and abdomen. An ectopic pregnancy is a potential medical emergency, and, if not treated properly, can lead to death ***(WHO, 2010)***.

In a normal pregnancy, the fertilized egg enters the uterus and settles into the uterine lining where it has plenty of room to divide and grow. About 1% of pregnancies are in an ectopic location with implantation not occurring inside of the womb, and of these 98% occur in the Fallopian tubes ***(Barnhart et al., 2006)***.

Detection of ectopic pregnancy in early gestation has been achieved mainly due to enhanced diagnostic capability. Despite all these notable successes in diagnostics and detection techniques ectopic pregnancy remains a source of serious

maternal morbidity and mortality worldwide, especially in countries with poor prenatal care ***(WHO, 2010)***.

Classification

Tubal pregnancy

The vast majority of ectopic pregnancies implant in the Fallopian tube. Pregnancies can grow in the fimbrial end (5% of all ectopic pregnancies), the ampullary section (80%), the isthmus (12%), and the cornual and interstitial part of the tube (3%) ***(Aneeshkumar & Venkateswaran, 2012)***.

The incidence of tubal ectopic pregnancy is increasing worldwide most likely due to a rising incidence of pelvic inflammatory disease caused by Chlamydia trachomatis infection and the increased use of assisted reproductive techniques ***(Shaw et al., 2010)***.

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. Every year in the UK there are 11 000 cases of tubal ectopic pregnancy (11.5 per 1000 pregnancies) and four maternal deaths due to ruptured tubal ectopic pregnancies (0.4 per 1000 tubal ectopic pregnancies) ***(Lewis 2007)***.

Mortality of a tubal pregnancy at the isthmus or within the uterus (interstitial pregnancy) is higher as there is increased vascularity that may result more likely in sudden major internal hemorrhage. A review published in 2010 supports the hypothesis that tubal ectopic pregnancy is caused by a combination of retention of the embryo within the fallopian tube due to impaired embryo-tubal transport and alterations in the tubal environment allowing early implantation to occur ***(Shaw et al., 2010)***.

Non-tubal ectopic pregnancy

Two percent of ectopic pregnancies occur in the ovary, cervix, or are intra-abdominal. Trans-vaginal ultra-sound examination is usually able to detect a cervical pregnancy. An ovarian pregnancy is differentiated from a tubal pregnancy by the Spiegelberg criteria ***(Zhang et al., 2008)***.

While a fetus of ectopic pregnancy is typically not viable, very rarely, a live baby has been delivered from an abdominal pregnancy. In such a situation the placenta sits on the intra-abdominal organs or the peritoneum and has found sufficient blood supply. This is generally bowel or mesentery, but other sites, such as the renal (kidney), liver or hepatic (liver) artery or even aorta have been described. Support to near viability has occasionally been described, but even in third world countries, the diagnosis is most commonly made at 16 to 20 weeks

gestation. Such a fetus would have to be delivered by laparotomy ***(Zhang et al., 2008)***.

Maternal morbidity and mortality from extra-uterine pregnancy are high as attempts to remove the placenta from the organs to which it is attached usually lead to uncontrollable bleeding from the attachment site. If the organ to which the placenta is attached is removable, such as a section of bowel, then the placenta should be removed together with that organ. This is such a rare occurrence that true data are unavailable and reliance must be made on an ecdotal reports. However, the vast majority of abdominal pregnancies require intervention well before fetal viability because of the risk of hemorrhage ***(Zhang et al., 2008)***.

Heterotopic pregnancy

In rare cases of ectopic pregnancy, there may be two fertilized ova, one outside the uterus and the other inside. This is called a heterotopic pregnancy. Often the intrauterine pregnancy is discovered later than the ectopic, mainly because of the painful emergency nature of ectopic pregnancies. Since ectopic pregnancies are normally discovered and removed very early in the pregnancy, an ultrasound may not find the additional pregnancy inside the uterus. When hCG levels continue to rise after the removal of the ectopic pregnancy, there is the chance that a pregnancy inside the uterus is still

viable. This is normally discovered through an ultrasound ***(Ludwig, 2008)***.

Although rare, heterotopic pregnancies are becoming more common, likely due to increased use of IVF. The survival rate of the uterine fetus of an ectopic pregnancy is around 70%. Successful pregnancies have been reported from ruptured tubal pregnancy continuing by the placenta implanting on abdominal organs or on the outside of the uterus ***(Ludwig, 2008)***.

Persistent ectopic pregnancy

A persistent ectopic pregnancy refers to the continuation of trophoplastic growth after a surgical intervention to remove an ectopic pregnancy. After a conservative procedure that attempts to preserve the affected fallopian tube such as a salpingotomy, in about 15-20% the major portion of the ectopic growth may have been removed, but some trophoblastic tissue, perhaps deeply embedded, has escaped removal and continues to grow, generating a new rise in β-hCG levels. After weeks this may lead to new clinical symptoms including bleeding. For this reason β-hCG levels may have to be monitored after removal of an ectopic pregnancy to assure their decline, also methotrexate can be given at the time of surgery prophylactically ***(Bangsgaard et al., 2003)***.

Epidemiology

An ectopic pregnancy is defined as implantation of the fertilized ovum outside the uterine cavity. In the UK during the period 2006-2008 more than 35000 ectopic pregnancies were estimated to have occurred and there were 6 maternal deaths resulting from ectopic pregnancy. This rate has declined since the last triennial report and is the lowest since figures were first estimated in 1988. Whether this is a trend or an anomaly remains to be seen ***(CMACE, 2011)***.

During the 1980s, and 1990s, the incidence of ectopic pregnancy in developed countries increase by factor of three to four to reach 100 to 170 per 100000 women aged 15- 44 years ***(Barnhart et al., 2006)***.

The majority of ectopic pregnancies (98%) are tubal in origin while a small proportion may be interstitial, primary abdominal, ovarian or cervical. 80% of tubal ectopics are located in the ampullary segment of the tube ***(Cunningham et al., 2001)***.

Although hospital-based African studies indicate EP incidence has probably increased in Africa in recent decades, major methodological limitations in the published literature make it impossible to draw formal conclusions concerning the incidence of ectopic pregnancy in Africa in recent years. As in

industrialized countries, pelvic inflammatory disease (PID) associated with sexually transmitted diseases (STDs) must be considered as the most important risk factor for EP in developing countries. In African developing countries, a majority of hospital-based studies have reported EP case fatality rates of around 1-3%, 10 times higher than that reported in industrialized countries ***(Goyaux et al., 2003)***.

Risk factors

There are a number of risk factors for ectopic pregnancies. However, in as many as one third to one half no risk factors can be identified. Risk factors include: pelvic inflammatory disease, infertility, use of an intrauterine device (IUD), previous exposure to DES, tubal surgery, intrauterine surgery (e.g. D&C), smoking, previous ectopic pregnancy, and tubal ligation ***(Bogdanskiene et al., 2006)***.

In theory an abnormal conceptus could be predisposed to ectopic implantation due to delayed migration but studies have not confirmed an important role for chromosomal abnormalities in the etiology of ectopic pregnancies ***(Bogdanskiene et al., 2006)***.

Although older texts suggest an association between endometriosis and ectopic pregnancy this is not evidence based suggests no such association ***(Goddijn et al., 1996)***.

Risk factors for ectopic pregnancy can be divided into high, moderate, or low risk factors ***(Barnhart et al., 2006)***.

High risk factors:

- ***Previous ectopic pregnancy***

Women who have had conservative treatment for ectopic pregnancy are at high risk (15 percent overall) for recurrence. This risk is related to both the underlying tubal disorder that led to the initial ectopic pregnancy and to the choice of treatment procedure ***(Barnhart et al., 2006)***.

- ***Tubal pathology and surgery-***

The major cause of ectopic pregnancy is disruption of normal tubal anatomy from factors such as infection, surgery, congenital anomalies or tumors ***(Barnhart et al., 2006)***.

Reconstructive surgery

The association between tubal reconstructive surgery and subsequent ectopic pregnancy depends upon the condition of the tube, the type of surgery, and the surgeon's expertise, the tubal surgery itself is not the main cause of ectopic pregnancy, rather, the underlying tubal damage resulting from prior pelvic inflammatory disease or a prior ectopic pregnancy is the major cause ***(Bouyer et al., 2003).***

- ***Sterilization***

The estimated failure rate during the first year after tubal sterilization ranges from 0.1 to0.8 percent, approximately one-third of these pregnancies are ectopic .the risk of ectopic pregnancy was higher in women sterilized before the age of 30 ***(Barnhart et al., 2006)***.

- ***In-utero DES exposure***

Women exposed to diethylstilbestrol (DES) in utero (also known as "DES daughters") also have an elevated risk of ectopic pregnancy, up to 3 times the risk of unexposed women. It has also been suggested that pathologic generation of nitric oxide may decrease tubal ciliary beats and smooth muscle contractions and thus affect embryo transport, which may consequently result in ectopic pregnancy ***(Al-Azemi et al., 2009)***.

- ***Intrauterine contraceptive devices:***

IUDs, like all contraceptive methods decrease the risk of both intrauterine and extra uterine pregnancy .These women using an IUD have a lower risk of ectopic pregnancy than those not using contraception. However, the IUD does not prevent ovulation, and it is more effective at preventing intrauterine than extra uterine pregnancy. This account for most of higher risk of ectopic pregnancy for IUD users than nonusers: IUDs

prevent 99% of intrauterine pregnancies and only 95% of extra uterine pregnancies. However, the reasons for implantation outside the uterus in women using IUDs are unknown, but may be attributed to the mild inflammation caused by the IUD, resulting in declination of the endosalpinx and therefore delayed ovum transport, leading to ectopic implantation. Women with IUDs account for 25-30 of all ectopic pregnancy cases according to the data of the Auvergne register ***(Goksedef et al., 2011).***

Moderate risk factors:

- ***Previous genital infections***

Pelvic infection (eg, nonspecific salpingitis, Chlamydia, gonorrhea), especially recurrent infection, is a major cause of tubal pathology and therefore the increasing incidence of ectopic pregnancy ***(Patil, 2012).***

- ***Infertility***

The incidence of ectopic pregnancy is higher in the infertility population, although this could reflect the increased incidence of tubal abnormality in this group of women. Several reports have also suggested an association between fertility drugs and ectopic pregnancy, which may be related to altered tubal function secondary to hormonal fluctuation. One large multicenter study found that women taking clomiphen citrate