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Biochemical marker for prediction of pregnancy outcome in cases of recurrent pregnancy loss

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Abstract

This study had been designed to predict pregnancy outcome by studying the relation between serum βHCG, progesterone and CA125 and the occurrence of miscarriage in the first trimester, in cases with history of recurrent pregnancy loss. The study was carried out on 150 pregnant women in Immamin Al-Kadumin Medical City. They were divided into three groups. The control group comprising 50 normal pregnant women with no history of miscarriage(Group A), the recurrent aborters group comprising 50 pregnant females with history of two or more 1st trimester pregnancy losses (Group B) and the last group of 50 patients who failed to complete the 1st trimester of pregnancy (aborting for the first time)during the study(Group C). All were singleton pregnancies of 6-11 weeks of gestation. All groups were subjected to history taking and thorough physical examination. Serial maternal serum levels of βHCG, progesterone and CA125 were determined .further subdivision according to the gestational age within each age group was done. Group 1 with 6-7 weeks,group11with 8-9 weeks and group111 with10-11weeks.This subdivision was done in an effort to limit the variability and increase the comparability of the results of the studied cases. Results revealed that serum β-HCG showed a sensitivity of 100%,a specificity of 50%, a ppv of 50%and a npv of 100%.serum progesterone showed a sensitivity of 24%,a specificity of 73%, a ppv of 55.07% and a npv of 85.18%,while serum CA125 showed a sensitivity of 15.6%,a specificity of 58.59%,a ppv of 16.32% and a npv of 57.42%. In conclusion the value of CA125 in recurrent abortions is still unclear and cannot be recommended on routine basis. On other hand β-HCG is highly sensitive as a single serum measurement for the prediction of pregnancy outcome. The effectiveness of progesterone estimation obviously wouldn't depend only on absolute levels or even rising levels during pregnancy.

Keywords: Recurrent miscarriage, **β**-hcg, CA125, Progesterone.

Introduction

The lack of well- defined criteria to differentiate between pregnancy that will spontaneously resolve and those that will not is an ongoing problem for expectant management and is certainly off-putting for patients and clinicians. Expectant management often takes weeks to complete and success rates vary widely. Failure of expectant management after prolonged follow–up is particularly disappointing for women and reduces overall benefits of the management strategy. A number of novel biochemical markers of the luteal-trophoblastic axis have emerged in the last decade (Carmona *et al.,* 2003).

Normal early pregnancy: Fertilization, implantation and early development; Following ovulation the ovum is taken up by the fimbrial end of the fallopian tube and is taken medially by the rhythmical action of the cilia. Fertilization of the ovum by spermatozoa occurs either in the peritoneal cavity or within the fallopian tube. Placentation ,along with other early gestational processes such as implantation ,is one of the most important determinants of pregnancy outcome (Jauniaux and Burton, 2005).

Miscarriage: Epidemiology and etiology, miscarriage is an intrauterine pregnancy that ends spontaneously before the fetus has reached viability. This is currently defined by the World Health Organization as the spontaneous expulsion from its mother of a fetus weighing less than 500 g or before 24 weeks of gestation. A missed miscarriage is defined as an embryonic pregnancy or where there is early fetal demise but the gestational sac remains in utero. An incomplete miscarriage is one where part but not all of the products of conception have been passed from the uterus. A complete miscarriage is one where all of

the products of conception have been passed and the uterus is now empty (Wang *et al.,* 2003).

Miscarriage is the most common complication of early pregnancy. It has been estimated that the overall miscarriage rate is around 40%. The majority of these losses occur before the missed menstrual period but bleeding complicates 21% of clinically detected pregnancies and 12-15% are lost. Miscarriage accounts for 50000 inpatient admissions to hospitals in the UK annually.

Types of abortion: Threatened miscarriage presentation with vaginal bleeding, pelvic pain and cervical os closed. Inevitable miscarriage presentation with vaginal bleeding, pelvic pain and cervical os open. Incomplete miscarriage presentation with vaginal bleeding, pelvic pain and cervical os open and some products of conception may be identified vaginally. Complete miscarriage presentation with cervix open and all products of conception expelled .Missed miscarriage presentation with vaginal bleeding, loss of pregnancy symptoms, usually no pelvic pain and cervical os closed. Septic presentation with intrauterine infection. Recurrent miscarriage presentation with three or more miscarriage (Everett *et al.,* 1987; Geyman *et al.,* 1999; Graziosis *et al.,* 2004; Trinder *et al.,* 2006).

The American Society for Reproductive Medicine defines recurrent pregnancy loss as two or more failed pregnancies (documented by ultrasound or histopathological examination) and suggests some assessment after each loss with a thorough evaluation after three or more losses (PCASRM, 2008).

Biochemistry in early pregnancy:

Human chorionic gonadotropin: The glycoprotein hormone human chorionic gonadotropins (hCG) has a molecular weight of 36,700d and was first identified in 1927. It consists of two dissimilar subunits, α and β, which are glycosylated non-covalently bound. Synthesis of hcg occurs predominantly within trophoblast cells of the blastocyst, the α and β subunits being coded for separately on chromosomes 6 and 19 respectively. It has been shown that its level in maternal serum doubles over 1.4-1.6 days from the time of first detection to the thirty-fifth day of pregnancy ,and then doubles over 2.0-2.7 days from thirty-fifth to the forty–second day (Urbancsek *et al.,* 2002; Yang *et al.,* 2003). The half-life of hCG is 32 to 37hrs and the levels of hCG are approximately 1000 IU/L at around 4 weeks of pregnancy, the time of initial visualization of a gestational sac on transvaginal ultrasound scan (Trinder *et al.,* 2006).

Biochemical markers, Serial measurements of serum hCG are often used in the assessment of pregnancies of unknown location and a large number of women are subjected to invasive diagnostic procedures. The use of hCG ratios (hCG 48hrs/hCG 0hrs) In the prediction of recently been assessed in a prospective cohort study, also found to perform only slightly better than single serum progesterone (Area under curves 0.756 and 0.678 respectively) (Bignardi *et al.,* 2010).

Biochemical markers of miscarriage: Biochemical markers have been used to predict early pregnancy outcome. Studies have demonstrated the variable expression of a single glycoisoform of hCG in early successful and failed pregnancies (Lessey, 2003). These studies use specific antibodies to C5 hCG(monoclonal antibody B152) which is a hyperglycoyslated structure related to choriocarcinoma hCG(H-hCG) have been described in the first 5-6 weeks gestation, with a subsequent decline in expression as pregnancy progresses but which persists in failing pregnancy. Whether over expression of H-hCG is an isolated phenomenon or is associated with variable expression of other isoforms is not known (Ayaty *et al.,* 2007).

Steroids:

Progesterone: Progesterone is a C-21 steroid hormone derived from cholesterol. It is one of the primary products of the corpus luteum and plays a pivotal role in the establishment and maintenance of pregnancy (Spencer and Bazer, 2004). As progesterone has shorter half –life than hCG, the progesterone level will reflect any change in the dynamics of the pregnancy earlier. In their prospective cohort studies found that of the single biomarkers, progesterone had the diagnostic accuracy in predicting pregnancy viability (Schindler, 2004; Plante *et al.,* 2008).

17**α**-hydroxyprogesterone: 17α-hydroxyprogesterone (17-OHP) is a steroid secreted in parallel to progesterone from the corpus luteum. The plasma concentration of 17-OHP rises steeply following conception to levels of 2.6ng/ml in the third week of pregnancy to 5.8ng/ml at the fifth week and then declines to reach a nadir in the 13th week.17-OHP values reflect corpus luteum function, since the placenta does not have 17α-hydroxlase to participate in the production of this metabolite (Schindler, 2004). Little is known of the functional role of 17-OHP but levels have been shown to be lower in nonviable intrauterine pregnancies and ectopic pregnancies in a small number of studies (Check *et al.,* 1990).

Serum CA-125: Levels are increased in early pregnancy and immediately after birth (Ayaty *et al.,* 2007; Bellon *et al.,* 2009), implicating the disintegrations of the maternal decidua (i.e, blastocyst implantation and placental separation)

as a possible source of the tumor marker elevation (Predanic, 2000; Schmidt and Rein, 2001). The serum CA-125 level is higher in normal pregnancy compared to ectopic pregnancy 2-4 weeks after a missed menses due to impaired interaction between the fetal trophoblast and tubal mucosa (Schmidt and Rein, 2001). Sequential determination of maternal CA-125 measurement appear to be a highly sensitive prognostic marker in the patient with viable pregnancy at an abortion risk (Predanic, 2000). Transient elevation of the CA-125 level occurs in maternal serum during early pregnancy and just after delivery because of the destruction of decidual tissues may cause this transient elevation of CA-125. Therefore the elevated serum CA-125 level in women with normal intrauterine pregnancies may be clinically useful in early pregnancy monitoring. This test is rather sensitive to differentiate the normal pregnancy and threatened abortion. It had been found that women with symptoms of imminent abortion ,who have a CA-125 level of ≥ 43.IU/ml, should be considered at a greater risk of miscarriage (Chetty *et al.,* 2011).

Ultrasound in early pregnancy: Recent ROCG guidelines recommend that all early pregnancy assessment units (EPAUs) should have access to TVS with staff appropriately trained in its use. The gestational sac is the first pregnancy structure that can be detected by ultrasound .

Aim of this study: To predict pregnancy outcome by studying the relation between serum βHCG, progesterone and CA-125 and the occurrence of miscarriage in the first trimester, in cases with history of recurrent pregnancy loss.

Materials and Methods

Patients: The study was carried out on 150 pregnant women of comparable age (range from 17-39) and gestational age in Al-Immamen Al-Kadhimin Teaching Hospital, from January 2013 till October 2013. They were divided into 3 groups . the control group comprising 50 normal pregnant women with no history of miscarriage (Group A), the recurrent aborts group comprising 50 pregnant females with history of two or more 1st trimester pregnancy losses (Group B) and the last group of 50 patients who failed to complete the 1st trimester of pregnancy (Aborting for the first time) during the study (Group C). All were singleton pregnancies of 6-11 weeks of gestation. all groups were subjected to history taking and thorough physical examination. maternal serum levels of βHCG, progesterone and Ca125 were determined.

Biochemical studies: The level of βhcg was estimated by a single sample at the time of ultrasound scan. Blood was collected by vein puncture, allowed to clot and serum separated by centrifugation at room temperature . The sera were collected and stored in deep freeze (-70^{o}C) to be assessed quantitatively when suitable. The technique used in the present work is the diagnostic kit prepared by euro genetics; Belgium (Headquarters tessendrlo–belgium) using Bhcg elisa coated microtiterstrips. Estimation of progesterone in serum was carried out using the kit manufactured by spectria progesterone (i) 125 coated tube radio-immunoassay. The technique is principally based on the widely used radioimmunoassay method. Determination of cancer antigen CA125 was obtained by using a special kit for the detection of CA125 based on a new antibody (Monoclonal) that binds to CA125 specifically .Expected values for the best by the manufactures in normal conditions are less than 37 IU/ml in serum.

Statistical analysis: Data were statistically described in terms of range, mean, standard deviation (±SD), median, mode and frequencies (number of cases)and relative frequencies (Percentages). Comparison between different groups in the present study was done using Student t test for comparing continuous data when normally distributed and Mann Whitney U test when not normally distributed. For comparing categorical data, Chi square (x^2) test was performed. A probability value (p value) less than 0.05 was considered significant. All statistical calculation were done using computer statistical programs SPSS ver.20 (Statistical package for the social science; SPSS Inc. Chicago, IL, USA).

Results and Discussion

One hundred and fifty cases were included in this study. Their mean ages ±SD were 26.44±5.43 years (range from 17-39 years). There is no statistical difference between groups regarding the age (p value = 0.468).

Biochemical markers are increasingly being used as an adjunct to ultrasonography and this thesis explored the use of novel biochemical markers in the diagnosis and management of early pregnancy problems (Chetty *et al.,* 2011). The symptoms of bleeding and pain in early pregnancy are very common and can be very common and can be very worrying for women; this can lead to suitable cases being missed (Elson *et al.,* 2003; Chetty *et al.,* 2011). It is clinically important to predict the outcome of patient with history of recurrent abortion at an early stage of gestation (Schindler, 2004).

Table (1): Evaluation parameters of different markers in different gestational age groups

Markers	sensitivity	specificity	PPV	NPV
Serum B-hcg	100%	50%	50%	100%
progesterone	24%	73%	55.07%	85.18%
Ca 125	15.6%	58.59%	16.32%	57.42%

Table (2): Evaluation parameters of serum β-hCG in different gestational age groups.

Serum B-hcg	sensitivity	specificity	PPV	NPV
6-7 weeks	100%	50%	55.55%	100%
8-9 weeks	100%	58.82%	51.72%	100%
10-11 weeks	100%	36.36%	43.24%	100%

Table (3): Evaluation parameters of serum progesterone in different gestational age groups.

Serum Progesterone	Sensitivity	Specificity	PPV	NPV
6-7 weeks	55%	53.12%	39.5%	60.71%
8-9 weeks	33.33%	79.41%	58.82%	84.37%
10-11 weeks	0%	78.78%	0%	60%

Table (4): Evaluation parameters of serum CA125 in different gestational age groups.

Serum CA125	Sensitivity	Specificity	PPV	NPV
6-7 weeks	10%	50%	11.11%	47.05%
8-9 weeks	0%	44.11%	0%	50%
10-11 weeks	37.5%	62.79%	50%	72.97%

The prognostic predictive value of maternal serum CA125 measurement was investigated in different studies with conflicting results. In one study, the mean serum CA125 level of the patient with unfavorable pregnancy outcome was significantly higher than that of the patient a favorable outcome. When the cut-off level of maternal serum CA125 was taken as >65IU/ml in the first and>60IU/ml in the second measurement of the study group ,the risk of termination of the pregnancy by spontanouse abortion was 83.3% in the patients with elevated serum CA125 levels, also this study found that the mean serum CA125 level of the patient with an unfavorable pregnancy outcome was significantly higher than that of the patient with favorable outcome (Ocer *et al.,* 1992). Sherif *et al.* (2000) also found that the serum CA125 may be developed as a cheap, sensitive and specific predictor of outcome in cases of threatened abortion. Although CA125 levels seem to be predictive of clinical pregnancy ,they are not predictive of its outcome. Higher CA125 concentration may reflect higher endometrial receptivity but do not predict the number or viability of implanted embryos (Carranza-Lira *et al.,* 2000; Urbancsek *et al.,* 2005). Serum β-hcg showed a sensitivity of 100%, a specificity of 50%, a PPV of 50% and a NPV of 100% with relatively equal values in different age groups. This matches well with other investigators who found that the best predictor of ongoing pregnancy was βhcg concentration (Anin *et al.,* 2004).

In the present study, in the control group mean progesterone values were within the normal ranges described. Moreover, serum progesterone showed no significant difference in serum levels of women of different gestational ages in the control group. The effectiveness of progesterone estimation obviously wouldn't depend only on absolute levels or even rising levels during pregnancy. At least the effectiveness would also depend on endometrial and vascular bed receptivity to progesterone and probably also on the original basic structure of the tissue in question (Sherif *et al.,* 2000; Carranza-Lira *et al.,* 2000). We investigated three serum markers to predict pregnancy outcomes at the earliest stage. Like other studies, we found that the serum hcg level measurement is the best and most reliable biomarker for predicting pregnancy outcome. Serum progesterone and serum CA125 measurement are of some, but limited use in the assessment of early pregnancy complication and that a cutoff value on which we can evaluate 1st trimester pregnancies cannot be devised. Therefore, routine assessment of serum progesterone and CA125 levels to predict pregnancy outcomes seems to be unwarranted (Elson *et al.,* 2003). It should always be kept in mind that it is not possible to identify the cause of recurrent early pregnancy loss in approximately half of the cases and this could be the limiting

factor for any biochemical marker (Urbancsek *et al.,* 2005).

Conclusions

The value of CA125 in recurrent abortions is still unclear and cannot be recommended on routine basis. On the other hand β-HCG is highly sensitive as a single serum measurement for the prediction of pregnancy outcome. The effectiveness of progesterone estimation obviously wouldn't depend only on absolute levels or even rising levels during pregnancy.

Recommendation

We recommend the use of β-HCG as a marker for the estimation of recurrent miscarriage in the future.

References

Anin, S.A.; Vince, G. and Quenby, S. 2004. Trophoblast invasion. Hum. Fertil., 7: 169-174.

Ayaty, S.; Roudsari, F.V. and Tavassoly, F. 2007. CA125 in normal pregnancy and threatened abortion. Iranian J. Reprod. Med., 5: 57-60.

Bellon, S,.; Anfossi, S.O. and Brien, T.J. 2009. Generation of CA125 specific cytotoxic T lymphocytes in human leukocytes antigen–A2,1 positive healthy donors and patient with advanced ovarian cancer. American J. Obstet. Gynecol., 200: 75e1-e10.

Bignardi, T.; Condous, G. and Kirk, E. 2010. Viability of intrauterine pregnancy in women with pregnancy of unknown location :prediction using human chorionic gonadotropin ratio vs. progesterone .Ultrasou. Obstet. Gynecol., 35: 656-661.

Carmona, F.; Balasch, J. and Creus, M. 2003. Early hormonal markers of pregnancy outcome after *In vitro* fertilization and embryo transfer. J Assist. Reprod. Genet., 20: 521-526.

Carranza-Lira, S.; Blanquet, J. and Tserotas, K. 2000. Endometrial progesterone and estradiol receptors in patients with recurrent early pregnancy loss of unknown etiology-preliminary report. Med. Sci. Monit., 6(4): 759-762.

Check, J.H.; Vaze, M.M. and Epstein, R. 1990. 17-hydroxyprogesterone level as a marker for corpus luteum function in aborters verus nonaborters. Int. J. Fertil., 35: 112-115.

Chetty, M.; Sawyer, E. and Dew, T. 2011. The use of novel biochemical markers in predicting spontaneously resolving 'pregnancies of unknown location. Hum. Repord., 26: 1318-1323.

Elson, J.; Salim, R. and Tailor, A. 2003. Prediction of early pregnancy viability in the absence of an ultrasonically detectable embryo. Ultraso. Obstet. Gynecol., 21: 57-61.

Everett, C.; Ashurst, H. and Chlamers, I. 1987. Reported management of threatened miscarriage by general practitioners in Wessex. BMJ 295: 583-586.

Graziosis, G.; Mol, B. and Ankum, W. 2004. Mangement of early pregnancy loss. Int. J. Gynecol. Obstet., 86: 337-346.

Jauniaux, E. and Burton, G.J. 2005. Pathophysiology of histological changes in pregnancy loss Placenta, 26(2-3): 114-123.

Lessey, B.A. 2003. Two pathways of progesterone action in the human endometrium: Implication for implantation and contraception. Steroid, 68: 809-815.

Ocer, F.; Bese, T. and Saridogan, E. 1992. The prognostic significance of maternal serum CA125 measurement in threatened abortion. European J. Obstet. Gynecol. Reprod. Biol., 46(2-3): 137-142.

Plante, B.J.; Blume, J.D. and Lambert-Messerlian,G. 2008. A multiple marker model to predict pregnancy viability when progesterone is indeterminate. J. Reprod. Med., 53: 243-249.

Practice Committee of the American Society for Reproductive Medicine, (PCASRM). 2008. Definition of infertility and recurrent pregnancy loss. Fertil. Steril., 89: 1603.

Predanic, M. 2000. Differentiating tubal abortion from viable ectopic pregnancy with serum CA125 and beta human chorionic gonadotropin determination. Fertil. Steril., 73: 522-525.

Schindler, A.E. 2004. First trimester endocrinology :consequences for diagnosis and treatment of pregnancy failure. Gynecol. Endocrinol., 18: 51-57.

Schmidt, T. and Rein, D.T. 2001. Forth prognostic value of repeated serum CA125 measurements in first trimester pregnancy. European J. Obstet. Gynecol. Reprod. Biol., 97: 168-173.

Sherif, L.S.; El-Metwaly, A.G.; Shalan, H. and Badawy, A.M. 2000. Can a single serum CA125 assay predict the outcome of threatened abortion. J. Obstet. Gynaecol., 20(1): 65-67.

Spencer, T.E. and Bazer, F.W. 2004. Conceptus signals for establishment and maintenance of pregnancy. Reprod. Biol. Endocrinol., 2: 49.

Trinder, J.; Brocklehurst, P.; Porter, R. 2006. Management of miscarriage; expectant, medical or surgical? Result of randomized controlled trial (miscarriage treatment (MIST)

trial). BMJ 332: 1235-1240.

Urbancsek, J.; Hauzman, E. and Fedorcsak, P. 2002. Serum human chorionic gonadotropin measurements' may predict pregnancy outcome and multiple gestation after *In vitro* fertilization. Fertil. Steril., 78: 540-542.

Urbancsek, J.; Hauzman, E.E. and Lagarde, A.R. 2005. Serum CA125 levels in the second week after embryo transfer predict clinical pregnancy. Fertil. Steril., 83(5): 1414-1421.

Wang, X; Chen, C., Wang, L. 2003. Conception, early pregnancy loss, and time to clinical pregnancy: a population based prospective study. Fertil. Steril., 79: 577-584.

Yang, M.; Lei, Z.M. and Rao, C.V. 2003. The central role of human chorionic gonadotropin in the formation of human placental syncytium. Endocrinol., 144: 1108-1120.