

## PREDICTIVE VALUE OF THE TRIPLE MARKERS: SERUM PROGESTERONE, CA 125 AND BETA – HCG FOR THE POSSIBLE OCCURRENCE OF MISCARRIAGE

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### Abstract

**Background and aim of the study:** Spontaneous abortion represents a common pregnancy adverse outcome and is a serious emotional burden for women. Loss of pregnancy is a distressing problem for both the patient and physician. The clinical diagnosis of threatened abortion is presumed when any bloody vaginal discharge or bleeding appears during the first trimester of pregnancy. In the year 2000, Egypt recorded high incidence of spontaneous miscarriage amounting to 10.5 %.

It was thought of interest, therefore, to evaluate the value of measurement of serum progesterone,  $\beta$ - hCG and CA- 125 as possible parameters to predict the occurrence of miscarriage. To achieve this goal, it was thought fruitful to compare data from control pregnant women with that obtained from women with a previous history of miscarriage. The work aimed to discover factors responsible for spontaneous miscarriage early enough to allow possible intervention.

**Methods:** A prospective study was conducted on 80 pregnant women with normal menstrual cycles before pregnancy between the sixth and eighth gestational weeks in the Department of Obstetrics, Misr University for Science and Technology Teaching Hospital. Eighty women with natural singleton intrauterine pregnancies, 41 were primigravid (control), and 39 with previous history of miscarriage (cases).

At the time of initial recruitment, the purpose of the study was explained to the participants and they were informed of the need for follow-up contact. Maternal age ranged

from 18 to 35 years (mean 27.5 years). Subjects were divided into three groups: Group I (control), Group II (cases of abortion), and Group III (ongoing pregnancy cases).

The markers studied included: CA- 125, beta human chorionic gonadotropin ( $\beta$ -HCG), and progesterone, as well as the ultrasonographic markers as Embryonic/Fetal heart rate (E/FHR), Gestational sac diameter (GSD), Crown-rump length (CRL) and the Growth rate of the gestational sac.

**Results:** Ultrasound carried out by the obstetrician was performed to the selected patients on their first visit at the obstetrics and gynaecology outpatient clinic at Misr University for Science and Technology Teaching Hospital. The following parameters were recorded: fetal pulsation and viability, matching of the gestational sac measurements with the calculated gestational age, presence of retro-placental hematoma, presence of inadequate chorio-decidual reaction, and screening of any congenital anomalies as Down's syndrome. It was then repeated at 20 weeks gestation for follow-up, where 7 negative fetal pulsations were discovered for pregnant women with previous history of miscarriage, 3 of them showed oligohydramnios with amniotic fluid index (AFI- 2) (normal: 10 – 20) and another 3 with normal appearance, while only one case showed congenital renal agenesis. In the present study, pregnant women with previous history of single miscarriage showed significantly lower levels of  $\beta$ -hCG when compared with control primigravid women. A significantly lower level in  $\beta$ - hCG was seen in aborted cases in pregnant women with previous history of miscarriage. The level of  $\beta$ - hCG, was  $105 \times 10^3 \pm 15.26 \times 10^3$  IU/ml in women who continued their pregnancy. On the other hand, the level in the aborted cases was  $42 \times 10^3 \pm 21.32 \times 10^3$  IU/ml. The pregnant women with previous history of single miscarriage showed significantly lower levels of progesterone when compared with control primigravid women. The level of progesterone, was  $35.29 \pm 2.16$  ng /ml in women who continued their pregnancy. On the other hand, the level in the aborted cases was  $19.51 \pm 2.66$  ng /ml ( $P < 0.01$ ). By contrast, pregnant women with previous history of single miscarriage showed significantly higher levels of CA- 125 when compared with control primigravid women. The level of CA-125, was  $19.37 \pm 1.64$  U/ml in women who continued their pregnancy, whereas, the level in the aborted cases was  $41.04 \pm 7.55$  U/ml ( $P < 0.01$ ).

**Conclusion:** The results of this present study indicated that serum progesterone combined with quantitative serum  $\beta$ - hCG and CA-125 measurements are not only diagnostic but also have good predictive value for pregnancy outcome when used as combined triple markers.

**Keywords:** Spontaneous abortion – pregnancy – progesterone –  $\beta$ - hCG – CA-125 – threatened miscarriage – triple markers

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## Introduction

Miscarriage, or the threat of miscarriage, is a traumatic event that can have psychological consequences for the potential parents (Lok and Neugebauer, 2007). Miscarriage is the spontaneous loss of a fetus before it is capable of surviving outside the uterus; this is generally defined as being before 24 completed weeks of gestation (Cunningham, 2005).

Threatened miscarriage is manifested by vaginal bleeding, with or without abdominal pain, while the cervix is closed and the fetus is viable and inside the uterine cavity. Once the cervix begins to dilate, miscarriage and pregnancy loss are inevitable. When the fetus is non-viable and the cervix is closed, this is known as missed miscarriage or missed abortion (Cunningham, 2001).

Threatened miscarriage is a common complication, occurring in about 20% of all clinically recognized pregnancies. If bleeding occurs during pregnancy, in the case of a viable fetus, the incidence of miscarriage can be around 20% or even up to 30%, depending on the severity and risk factors (Weiss *et al.*, 2004). Threatened miscarriage is associated with chromosomal abnormality of the conceptus in over 50% of cases (Szabo and Szilagyi, 1996). Other risk factors for miscarriage include maternal age over 34 years (Falco *et al.*, 1996), maternal infection such as genital herpes simplex, human immunodeficiency virus-1 and vaginal colonization of group B streptococci (Temmerman *et al.*, 1992).

Other risk factors for miscarriage include maternal endocrine abnormalities such as uncontrolled diabetes mellitus and insufficient production of progesterone by the corpus luteum, polycystic ovary syndrome, maternal autoimmune factors such as phospholipids antibodies, and a previous history of two or more miscarriages (Cunningham, 2001).

The risk of miscarriage is dependent also on maternal serum biochemistry as progesterone, hCG and CA-125. Several studies demonstrated an increasing risk of miscarriage with declining serum progesterone levels. Levels of <5 ng /ml were associated with a spontaneous miscarriage in 86% of cases compared with only 8% at levels of 20–25 ng /ml (McCord *et al.*, 1996). As seen with progesterone, low levels of hCG also predict a higher risk of miscarriage (Camejo *et al.*, 2003). In failed pregnancies, a decline in serum hCG is usually apparent based upon two measurements at least two days apart. A drop of baseline serum hCG level greater than 21 % is strongly predictive of miscarriage (Barnhart, 2009). Elevated levels of cancer antigen (CA-125), which can be associated with damage to the deciduous membrane, have also been proposed as an indicator of miscarriage risk. Sequential determinations of CA-125 in women with bleeding during gestational weeks 6–12 were able to distinguish between women who subsequently miscarried and those who did not (Schmidt *et al.*, 2001). Other studies have pointed to the prognostic value of single measurements of CA-125. One study showed that a cut-off of 125 IU/ml had positive and negative predictive values of 93% and 92%, respectively (Leylek *et al.*, 1997).

The introduction of ultrasound scans in the management of bleeding in early pregnancy has improved the diagnosis by rapid confirmation of viability and has improved the management by introducing prognostic factors such as fetal bradycardia and discrepancy between gestational age and crown-to-rump length (Makrydimas *et al.*, 2003). A number of options are available to try and preserve pregnancy in cases of threatened miscarriage. These include bed rest, abstention from coitus and a simple wait and watch policy, as well as treatment with progesterone or human chorionic gonadotropin (Sotiriadis *et al.*, 2004).

### **Subjects and Methods**

This prospective study was conducted on 80 pregnant women with normal menstrual cycles before pregnancy between the sixth and eighth gestational weeks in the Department of Obstetrics, Misr University for Science and Technology Teaching Hospital. Approval of the ethical committee was obtained, and all women provided written informed consent. Eighty

women with natural singleton intrauterine pregnancies, 41 were primigravid (control), and 39 with previous history of miscarriage (cases) were included in this study.

Pregnant women with thyroid dysfunction, *diabetes mellitus*, and pre-existing hypertension were excluded from this study.

At the time of initial recruitment the purpose of the study was explained to the participants and they were informed of the need for follow-up contact. Maternal age ranged from 18-35 years (mean 27.5 years). Subjects were divided into three groups: Group I (control), Group II (cases of abortion), and Group III (continuous cases).

Venous blood samples (5 ml) were collected at admission from all subjects. Three ml blood was put into plain tubes. The samples were allowed to clot and the sera were separated after centrifugation at 3,000 rpm for 10 min. Analysis of samples was carried out in the Chemical Pathology department of Misr University for Science and Technology Teaching Hospital. Free  $\beta$ -HCG, progesterone, and CA-125 were measured using Vitek Immuno Diagnostic Assay System (VIDAS) Serum Progesterone which is an automated quantitative test for use on the VIDAS family instruments for the quantitative measurement of progesterone,  $\beta$ -hCG, and CA-125 in the human serum (EDTA), using the ELFA technique (Enzyme Linked Fluorescent Assay).

### Statistical analysis

Analysis of data was carried out using SPSS 10 (SPSS, Chicago, IL, USA). In addition sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and odds ratios were calculated. Values of  $P < 0.05$  were considered statistically significant.

### Results

Mean age, parity, systolic blood pressure (SBP), and diastolic blood pressure (DBP) of the subjects were comparable in both groups. Table 1 shows the demographic and hemodynamic findings in both groups.

As shown in Tab.1, systolic blood pressure, diastolic blood pressure, body weight, and temperature did not differ between the two groups of pregnant women and were in the normal range for pregnant women without cardiovascular complications. Also the age of women in both groups did not significantly differ ( $P > 0.05$ ).

**Table (1):** Demographic features and hemodynamic findings in the two studied groups

	<b>Control (n= 41)</b> <b>(Primigravid women)</b>	<b>Cases (n= 39)</b> <b>(Pregnant women with history of miscarriage)</b>	<b>P</b>
<b>Age (yrs.)</b>			
Minimum - maximum	19-34	18-35	
Mean $\pm$ SE	26.05 $\pm$ 0.55	26.21 $\pm$ 0.71	0.861
<b>SBP (mmHg)</b>	116.34 $\pm$ 1.30	116.92 $\pm$ 1.48	0.908
<b>DBP (mmHg)</b>	69.39 $\pm$ 1.08	70.90 $\pm$ 1.37	0.556
<b>Weight (Kg)</b>	82.12 $\pm$ 1.54	81.19 $\pm$ 1.69	0.685
<b>Temperature (<math>^{\circ}</math>C)</b>	36.99 $\pm$ 0.04	37.02 $\pm$ 0.04	0.771

Data are expressed as mean  $\pm$  standard error.

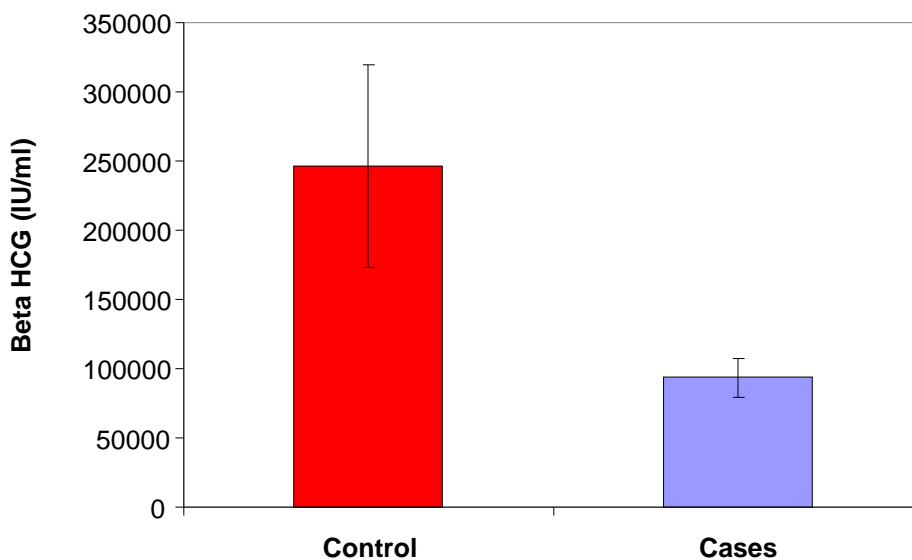
Pregnant women with history of miscarriage showed a significant lower levels of human chorionic gonadotropin. Control primigravid women had a mean value of  $246 \times 10^3 \pm 73.4 \times 10^3$  as compared with  $93.4 \times 10^3 \pm 13.56 \times 10^3$  in the pregnant women with history of miscarriage ( $P < 0.01$ ) (Tab. 2, Fig. 1).

**Table (2): Human Chorionic gonadotropin in control pregnant women and pregnant women with previous history of miscarriage**

Biochemical parameters	Control (n= 41) (Primigravid women)	Cases (n= 39) (Pregnant women with history of miscarriage)	P
$\beta$ -hCG (IU/ml)	$246 \times 10^3 \pm 73.4 \times 10^3$	$93.4 \times 10^3 \pm 13.56 \times 10^3$	0.012*

Data are expressed as mean  $\pm$  standard error

p < 0.05 = significant



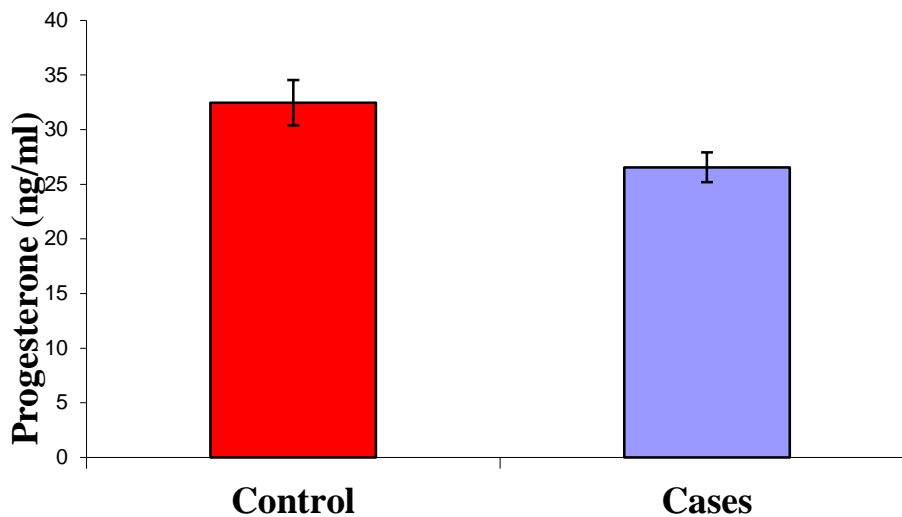
**Fig. (1): Comparison between mean values of  $\beta$ - hCG in control primigravid women and pregnant women with history of miscarriage.**

\*= P < 0.05

Pregnant women with history of miscarriage showed significant lower levels of progesterone. Control primigravid women had a mean value of  $32.46 \pm 2.07$  as compared with  $26.55 \pm 1.36$  in the pregnant women with history of miscarriage (P < 0.01) (Tab.3 Fig. 2).

**Table (3): Progesterone in control pregnant women and pregnant women with previous history of miscarriage**

Biochemical parameters	Control (n= 41) (Primigravid women)	Cases (n= 39) (Pregnant women with history of miscarriage)	P
Progesterone (ng/ml)	32.46 ± 2.07	26.55 ± 1.36	0.020*

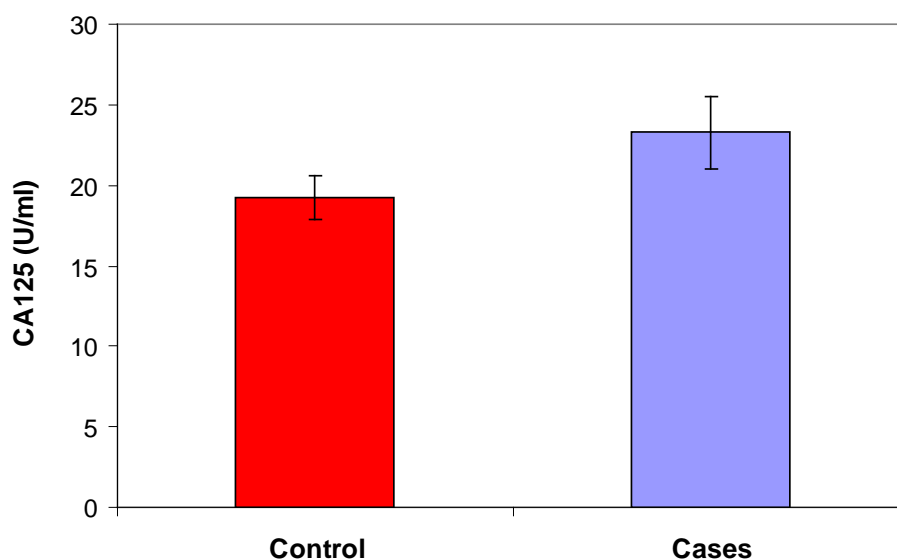
**Fig. (2): Comparison between mean values of progesterone in in control pregnant women and pregnant women with previous history of miscarriage.**\*= $P < 0.05$ 

Pregnant women with history of miscarriage showed a slight non - significant higher levels of cancer antigen- 125. Control primigravid women had a mean value of  $19.21 \pm 1.37$  as compared with  $23.26 \pm 2.29$  in the pregnant women with history of miscarriage ( $P > 0.05$ ) (Tab. 4, Fig. 3).



**Table (4): Cancer antigen- 125 in control pregnant women and pregnant women with previous history of miscarriage**

Biochemical parameters	Control (n= 41) (Primigravid women)	Cases (n= 39) (Pregnant women with history of miscarriage)	P
CA- 125 (U/ml)	19.21 ± 1.37	23.26 ± 2.29	0.129



**Fig. (3): Comparison between mean values of CA- 125 in control pregnant women and pregnant women with previous history of miscarriage.**

When comparing pregnant women with a previous history of abortion who continued their pregnancy with those of pregnant women with a previous history of abortion who had aborted for the second time, different magnitudes of the three markers were obtained. As can

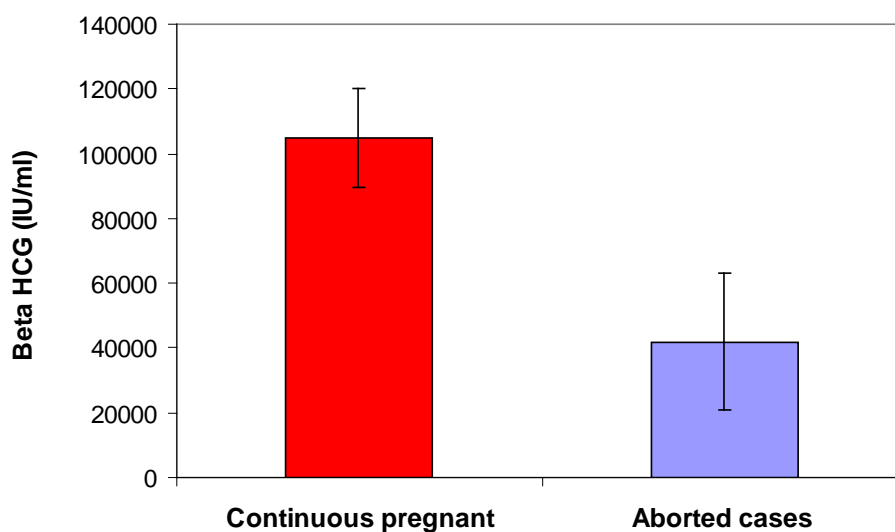
be seen in Tab. 5 and Fig. 4, 5, and 6 a significantly lower levels in  $\beta$ - hCG and highly significant decreased levels in progesterone and highly significant increased levels in cancer antigen – 125 were seen in aborted cases in pregnant women with previous history of miscarriage. The levels of  $\beta$ - hCG, progesterone and cancer antigen- 125 were  $105 \times 10^3 \pm 15.26 \times 10^3$ ,  $35.29 \pm 2.16$ ,  $19.37 \pm 1.64$  respectively in women who continued their pregnancy. The levels in the aborted cases were  $42 \times 10^3 \pm 21.32 \times 10^3$ ,  $19.51 \pm 2.66$ ,  $41.04 \pm 7.55$  respectively.

**Table (5): Beta - Human Chorionic gonadotropin, progesterone and cancer antigen – 125 in pregnant women with previous history of miscarriage: changes in aborted cases**

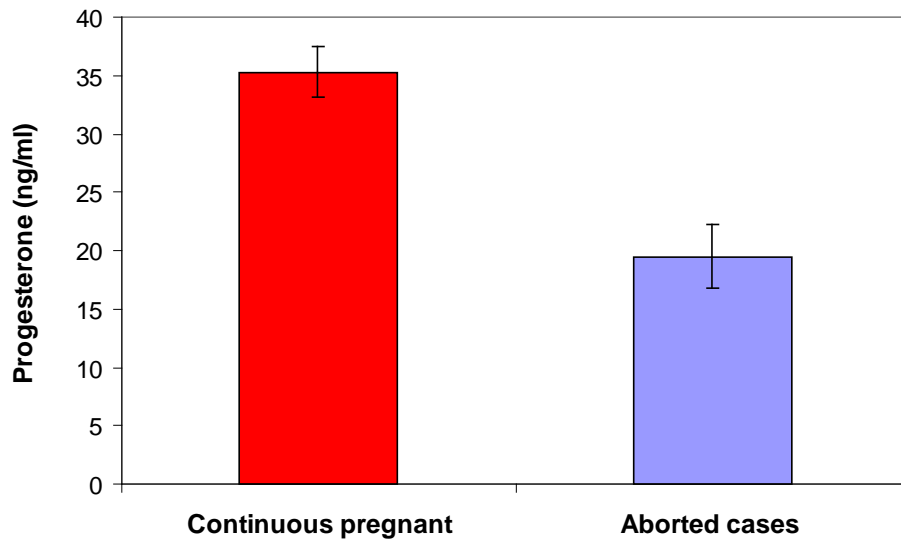
Biochemical parameters	Continuous (n= 32)	@ Aborted cases (n=7)	P
$\beta$ -hCG (IU/ml)	$105 \times 10^3 \pm 15.26 \times 10^3$	$42 \times 10^3 \pm 21.32 \times 10^3$	0.032*
Progesterone (ng/ml)	$35.29 \pm 2.16$	$19.51 \pm 2.66$	0.003**
CA125 (U/ml)	$19.37 \pm 1.64$	$41.04 \pm 7.55$	0.004**

Data are expressed as mean  $\pm$  standard error. \* $p < 0.05$ = significant. \*\* $p < 0.01$ = Highly significant.

@: 7 negative fetal pulsations were discovered for pregnant women with previous history of miscarriage, 3 of them showed oligohydramnios with amniotic fluid index (AFI- 2) (normal: 10 – 20) and another 3 with normal appearance, while only one case showed congenital renal agenesis.

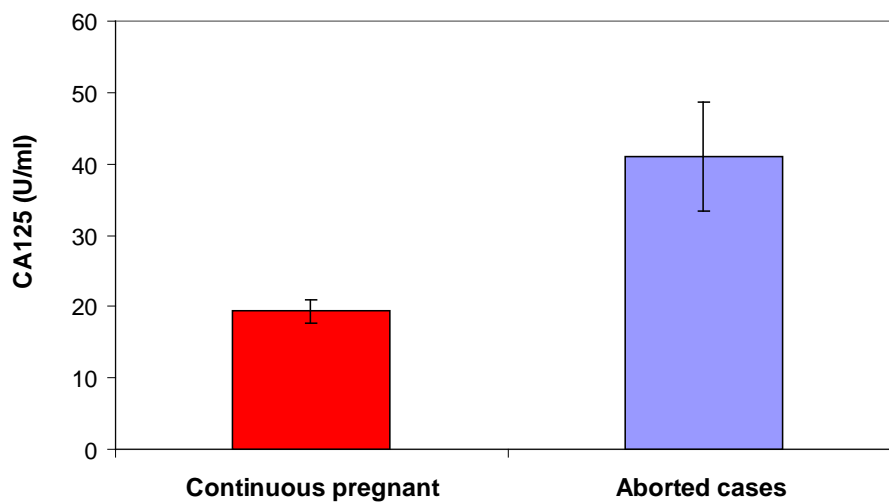


**Fig. (4): Comparison between mean values of beta - hCG in pregnant women with previous history of miscarriage classified according to abortion. \*=  $p < 0.05$**



**Fig. (5): Comparison between mean values of progesterone in pregnant women with previous history of miscarriage classified according to abortion.**

\*= p< 0.01



**Fig. (6): Comparison between mean values of CA -125 in pregnant women with previous history of miscarriage classified according to abortion.**

\*= p< 0.01

**Discussion**

Spontaneous abortion represents a common pregnancy adverse outcome and is a serious emotional burden for women. A prospective study on women with threatened

abortion reported that women older than 34 years had an odds ratio of 2.3 for miscarriage (Falco *et al.*, 1996). Some women bleed in early pregnancy, approximately half of them, will abort (Weiss *et al.*, 2004). Occasionally, bleeding may persist for weeks, and then it becomes essential to decide whether there is any possibility of continuation of the pregnancy or not. The diagnosis of spontaneous abortion currently depends on a combination of ultrasonography and nine hormonal methods including serum human chorionic gonadotropin (hCG), estradiol (E2), estrone, estriol, progesterone, human placental lactogen, cortisol, urine hCG and urine estrogen (Gerhavad and Runnebaum 1984; Zeimet *et al.*, 1998; Osmanagaoglu *et al.*, 2010). Another parameter that could be used as a predictive marker for a spontaneous abortion or subsequent outcome of pregnancy is Cancer Antigen-125 (CA-125).

The ultrasound carried out in the present study played an essential tool, not only to predict pregnancy outcome, but also to confirm the occurrence of miscarriage with the aid of hormonal analysis of the three studied serum markers:  $\beta$ -hCG, progesterone, and CA-125.

In the present study, lower incidence of abortion was shown in pregnant primigravid women as compared with pregnant women with previous history of abortion. In the primigravid group only two out of 41 (4.9 %) pregnant women aborted. By comparison 7 out of 39 (17.9 %) pregnant women with history of miscarriage aborted ( $P < 0.01$ ). The relatively large size of the study sample in the present study may provide an advantage.

Pregnant women with previous history of single miscarriage showed significantly lower levels of  $\beta$ -hCG when compared with control primigravid women. A significantly lower level in  $\beta$ -hCG was seen in aborted cases in pregnant women with previous history of miscarriage. The decreased levels of  $\beta$ -hCG are in agreement with the results reported by Scarpellini *et al.* (1995), Duan *et al.* (2011), Bhattacharya *et al.* (2015), and Darwish *et al.* (2005).

Pregnant women with previous history of single miscarriage showed significantly lower levels of progesterone when compared with control primigravid women. A highly significant lower level in progesterone was seen in aborted cases in pregnant women with previous history of miscarriage. These results are in agreement with those reported by Katsikis *et al.* (2005), El-Mekkawi *et al.* (2015) and Darwish *et al.* (2005). These authors stated that serum progesterone levels are a good predictive for pregnancy outcome. The results found in the present study indicated that serum progesterone measurement is an

excellent predictor of the risk of miscarriage and can be a good alternative of CA-125 and other markers due its high availability and low cost.

Pregnant women with previous history of single miscarriage showed significantly higher levels of CA- 125 when compared with control primigravid women. A highly significantly higher level in CA-125 was seen in aborted cases in pregnant women with previous history of miscarriage.

Thus, CA-125 is an excellent predictive marker for the risk of spontaneous abortion. This result is in agreement with those found by Scarpellini *et al.* (1995), Katsikis *et al.* (2005), and Bhattacharya *et al.* (2015). The results, however, disagreed with those of Darwish *et al.*, (2005) who stated that the value of CA-125 in abortion is unclear and cannot be recommended on routine basis.

A positive correlation was observed, in the present study, between serum  $\beta$ - hCG and progesterone; a decrease in serum  $\beta$ - hCG was paralleled by a decrease in serum progesterone in aborted cases. An increase in serum CA-125, however, occurred.

It should be remembered that the present work had the advantage of using three different parameters simultaneously, to predict the risk of spontaneous abortion and so pregnancy outcome. Except for Darwish *et al.* (2005), other investigators used either one parameter only (El- Mekkawi *et al.*, 2015, (progesterone)), or two parameters (Scarpellini *et al.*, (1995) and Bhattacharya *et al.*, (2015) ( $\beta$ -hCG + CA-125), Katsikis *et al.*, 2005, (CA-125 + progesterone), and Duan *et al.*, 2011, (progesterone +  $\beta$ -hCG). However, the trend of changes in the parameters studied was similar to that obtained in the present study.

Other factors that might had contributed to miscarriage might include a lower haemoglobin concentrations. Although the number of patients with anaemia was small (5 pregnant women), yet haemoglobin concentration was less in the aborted women by approximately 1 %. This might have contributed to the development of hypoxia which could have shared in miscarriage. It's known that anaemia could cause abortion (Frenkel *et al.*, 2006). However, it should be kept in mind that the magnitude of anaemia found in the aborted cases wasn't major compared to anaemia reported in the cited references. However, it might be an additional factor to the changes in the three major factors described above.

Communications with the pregnant women included in the present study for follow-up was performed and due analysis made. Three patients with previous history of miscarriage exhibited abnormal hormonal levels, while 2 of them suffered threatened abortion. On the other hand, 2 primigravid control women suffered from threatened abortion, while only one woman had abnormal levels of the three markers in study: decrease in serum beta- hCG and progesterone, and an increase in serum CA-125. The susceptible patients had been psychologically reassured that there was a great possibility to continue their pregnancy safely and they were directed to the importance of the four types of rest: psychological, mental, physiological, and sexual. Those who didn't respond appropriately to the non-pharmacological intervention only were ordered oral progesterone (Duphaston) in a starting dose of 40 mg at once then a maintenance dose of 10 mg every 8 hours until symptoms relieved. Eventually, throughout immediate and appropriate intervention, abortion had been prevented and the susceptible patients continued their pregnancy safely and gave birth of healthy well-being babies.

It is concluded that the combined measurement of the three parameters: progesterone,  $\beta$ - hCG and CA-125, can be beneficial in the prediction of first trimester spontaneous miscarriage and gives more accurate predictions and so better results than each alone. However, using serum progesterone and  $\beta$ - hCG alone may be preferred by some clinicians as they have high availability and low cost compared with serum CA-125 which is rare to be used due to its high cost.

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