



## The Yolk Sac Abnormalities, Maternal Serum Level of Cancer Antigen 125 (CA-125) and Beta Human Chorionic Gonadotropin (B-HCG) as an Early Predictors of First Trimester Pregnancy Loss in Patients with Threatened Miscarriage

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### Abstract

**Background:** Pregnancy loss before 20 weeks is considered a miscarriage, as is the loss of a fetus weighing less than 500 grams before viability. A medical emergency, threatened miscarriage affects 15–25% of pregnancies.

**Aim and objectives:** The goal of this study was to assess the predictive value of maternal blood levels of Cancer Antigen 125 (CA-125) and beta-human chorionic gonadotropin (B- HCG) in individuals at risk of miscarriage during the first trimester.

**Subjects and methods:** This study was a prospective cohort study. This study included 120 pregnant women with threatened abortion between (6-11 weeks) and followed up till end of 14th week. Results: 36(30%) of pregnant women aborted, while 84(70%) of women continued till 14th weeks of pregnancy. At a cut-off value of 45 U/ml, the CA125 test was shown to have a sensitivity of 88.9% and a specificity of 77.5%, respectively, while also having a positive predictive value of 79.8% and a negative predictive value of 87.5%. At a cut-off value of 18.501 mIU/ml, the B-HCG test's sensitivity and specificity were determined to be 96.3 and 88.9, respectively, with a positive predictive value of 89.7% and a negative predictive value of 96%.

**Conclusion:** Even before fetal morphology can be investigated sonographically, abnormalities in the size of the yolk sac can be utilized as a good prognostic sign of early pregnancy loss. Pregnancy viability can be estimated from first trimester serum CA 125 and Beta HCG measurements.

**Key words:** Yolk Sac, CA-125, B-HCG, Threatened Miscarriage.

## 1. Introduction

Loss of a pregnancy before 20 weeks is considered a miscarriage, as is the loss of a fetus weighing less than 500 grams before viability. (1).

Threatened miscarriage is a common medical problem that represents 15% to 25% of all pregnancies (2).

One of the most common pregnancy problems is a spontaneous abortion. There are currently nine hormonal methods (serum human chorionic gonadotropin [HCG], estradiol [E2], estrone, estriol, progesterone, human placental lactogen, cortisol [Cort], urine HCG, and urine estrogen) and several sonographic parameters that have been reported as possible predictors of miscarriage that go into the diagnosis of spontaneous abortion. Among them are the presence and characteristics of the yolk sac (3),

the size of the gestational sac (4), the rate of heartbeat in the fetus, and the rate of embryonic development.

HCG, or human chorionic gonadotropin, is a glycoprotein composed of alpha and beta units that is released by placental syncytiotrophoblasts. The production of HCG peaks at around 10 weeks of pregnancy and then continues throughout the duration of the pregnancy. Possible miscarriage, blighted ovum, or ectopic pregnancy are all possible outcomes associated with a low B-HCG level. If your B-HCG level is high, it might mean you're carrying more than one baby, you have molar pregnancy, or your due date was off .(5)

The study's overall objective was to assess the predictive value of maternal blood levels of Cancer Antigen 125 (CA-125) and (B- HCG) in cases of impending miscarriage.

### **Patients and methods**

This study was a prospective cohort study, conducted at any pregnant females with threatened abortion from 6 weeks to 11 weeks of gestation attending Obstetrics & Gynecology Emergency Department or the Antenatal Clinic in Suez Canal University Hospital in Ismailia.

### **Inclusion criteria**

Singleton pregnancy from 6 to 11wks of gestation, Maternal age less than 40 years old,

Proved diagnosis of threatened abortion by having vaginal bleeding, potentially mild lower abdominal pain, a closed cervical os and  $\pm$  appearance of cardiac activity.

### **Exclusion criteria**

Patients with high-risk pregnancy (hypertension, diabetic, renal, hepatic, cardiac), Patients with blighted ovum, Patients with molar pregnancy, Patients with ectopic pregnancy, Patients with ovarian masses with pregnancy, cases with history of pelvic inflammatory disease. Patients with multiple pregnancy, missed abortion or habitual abortion, Refusal of the participants.

### **Ethical committee**

The Suez Canal University Faculty of Medicine ethical committee and hospital administration board approved this study, and patients were informed of their participation in the study in a simple language. Discussion included aim of the study, study methodology, source of funding, and the institutional affiliation of the researcher. Confidentiality of patient personal information was maintained at all stages of the study. Administrative approval was taken (from the department, the hospital and the faculty of medicine). Privacy of the patient was respected. All data was used in the present research only.

### **Methodology**

The following procedures were performed on every patient: Complete medical, family, and pregnancy background (Appendix II): Background information (such as age, smoking status, and education level). The onset, duration, and frequency of lower abdominal pain; vaginal bleeding; urinary symptoms (dysuria, frequency, urgency); and the color and itchiness of the vaginal discharge; as well as the patient's parity, number of abortions, and method of delivery in previous pregnancies; and the patient's gestational age. medical history, examination (abdominal, vaginal), Lab assessment. All enrolled patients underwent the following four visits at different mean gestational ages with one week interval between each visit.

### **Statistical analysis**

Spss (Statistical Package for the Social Sciences) (version 22.0. IBM Corp. Released 2013) was used to evaluate and interpret the acquired data. IBM Corp., Armonk, New York. Measures of central tendency and dispersion for the attributes under study. Student's t-test was used to compare the two groups on the continuous variables. The correlation between these categories was analyzed using the Chi-square test. Predictive thresholds for maternal serum quantitative B-HCG, CA125 level, and anomalies of the yolk sac were established using receiver-operating characteristic (ROC) analysis. We determined the likelihood ratios, as well as the sensitivity, specificity, positive predictive values, and negative predictive values. In order to characterize the connection between the variables, a Pearson correlation analysis was performed. The threshold for statistical significance is set at a P-value of less than 0.05.

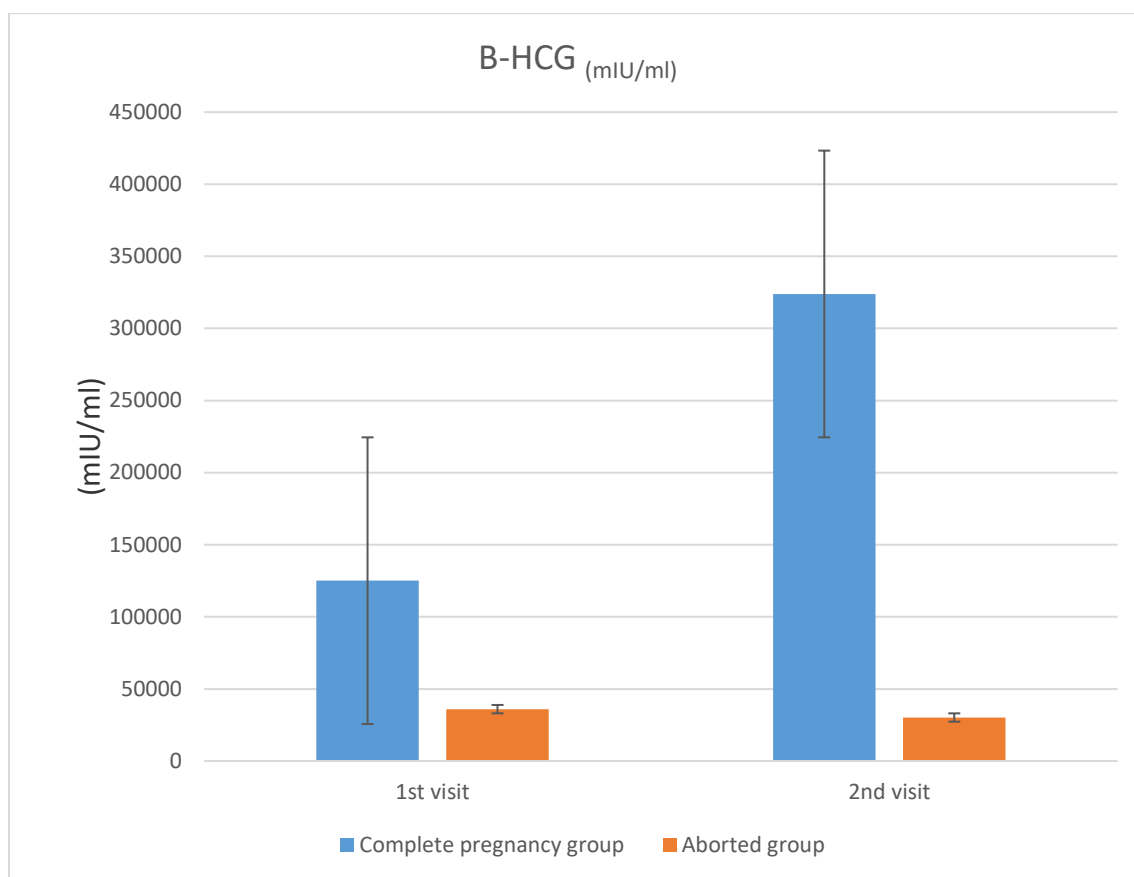
## Results

**Table 1:** Distribution of the two studied groups according to demographic data.

|                               | <b>Complete pregnancy group (n = 84)</b> | <b>Aborted group (n = 36)</b> | <b>P-value</b> |
|-------------------------------|--|-------------------------------|----------------|
| <b>Age (years)</b>            |  |                               |                |
| <b>Mean ± SD</b>              | 26.6 ± 4.40                              | 28.06 ± 4.35                  | 0.0901         |
| <b>Education(no, %)</b>       |  |                               |                |
| <b>No education</b>           | 30(35.7%)                                | 9(25%)                        | 0.9322         |
| <b>Middle /secondary</b>      | 31(36.9%)                                | 4(11.1%)                      |                |
| <b>High education</b>         | 23(27.3%)                                | 23(63.9%)                     |                |
| <b>Working status (no, %)</b> |  |                               |                |
| <b>Employee</b>               | 13(15.5%)                                | 7(19.4%)                      | 0.323          |
| <b>Manual worker</b>          | 9(10.7%)                                 | 7(19.4%)                      |                |
| <b>House wives</b>            | 62(73.8%)                                | 22(61.1%)                     |                |
| <b>Smoking(no, %)</b>         |  |                               |                |
| <b>Yes</b>                    | 2(2.4%)                                  | 6(16.7%)                      | 0.000*2        |
| <b>No</b>                     | 82(97.6%)                                | 30(83.3%)                     |                |
| <b>Weight (kg)</b>            |  |                               |                |
| <b>Mean ± SD.</b>             | 80.6 ± 9.0                               | 82.7± 7.4                     | 0.2221         |
| <b>Height (cm)</b>            |  |                               |                |
| <b>Mean ± SD.</b>             | 167.8 ± 8.0                              | 168.5 ±7.8                    | 0.6651         |
| <b>BMI (kg\cm2)</b>           |  |                               |                |
| <b>Mean ± SD.</b>             | 28.8 ± 4.3                               | 29.3 ± 4.07                   | 0.5641         |
| <b>BMI (kg\cm2)</b>           |  |                               |                |
| <b>Normal</b>                 | 14(16.7%)                                | 5(13.9%)                      | 0.6192         |
| <b>Overweight</b>             | 37(44%)                                  | 18(50%)                       |                |
| <b>Mild obesity</b>           | 26(31%)                                  | 8(22.2%)                      |                |
| <b>Moderate obesity</b>       | 7(8.3%)                                  | 5(13.9%)                      |                |

Student t test, 2. Fisher exact test. 3. Chi square test. Abbreviations: SD: Standard deviation, BMI: Body Mass Index.

Our study showed that age, educational level, working status, weight, height and BMI showed statistical insignificant differences between both groups as  $p > 0.05$ , While smokers percentage was significantly higher among aborted group (16.7%) than complete pregnancy group (2.4%) ( $p = 0.009$ ). (table1)



**Figure1:** Serial measurements of B-HCG among the study groups.

This figure showed that B-HCG measurements showed significant increase among the complete pregnancy group, while it showed significant decrease among the aborted group ( $p < 0.001$ ).

**Table2:** Presenting symptoms of threatened abortion in both groups.

|                             | Complete pregnancy group (n = 84) | Aborted group (n = 36) | P-value  |
|-----------------------------|-----------------------------------|------------------------|----------|
| <b>Vaginal bleeding</b>     | 84(100%)                          | 36(100%)               | 1.001    |
| <b>Duration (days)</b>      | 2.32±1.16                         | 1.92±0.8               | 0.0532   |
| <b>Lower abdominal pain</b> | 47(56%)                           | 33(91.7%)              | <0.001*1 |
| <b>Duration (days)</b>      | 1.48±1.5                          | 2.06±1.07              | 0.039*2  |
| <b>UTI Symptoms</b>         | 48(57.1%)                         | 20(55.6%)              | 0.8721   |
| <b>Vaginal discharge</b>    | 1(1.2%)                           | 8(22.2%)               | <0.001*1 |
| <b>White</b>                | 1(1.2%)                           | 5(13.9%)               |          |
| <b>Yellow</b>               | 0(0%)                             | 3(8.3%)                | <0.001*1 |
| <b>Itching</b>              | 1(1.2%)                           | 5(13.9%)               | 0.009*1  |

Chi square test, 2. Student t test.

Abbreviations: UTI: Urinary Tract Infection.

Our study showed that aborted group had significantly higher prevalence of lower abdominal pain, vaginal discharge and itching than complete pregnancy group ( $p < 0.05$ ). (table 2)

**Table3:** Correlation between ultrasound findings throughout visits among complete pregnancy group.

|                                 | Complete pregnancy group (n = 84) |           |           |           | P-value |
|---------------------------------|-----------------------------------|-----------|-----------|-----------|---------|
|                                 | 1st visit                         | 2nd visit | 3rd visit | 4th visit |         |
| <b>GA (weeks)</b>               | 6.5±1.56                          | 9.21±1.5  | 10.21±1.5 | 11.21±1.5 |         |
| <b>Yolk sac characteristics</b> |                                   |           |           |           |         |
| <b>Presence</b>                 | 84(100%)                          | 84(100%)  | 81(96.4%) | 61(72.6%) | 0.7231  |
| <b>Number</b>                   |                                   |           |           |           |         |

|                           |          |           |           |           |        |
|---------------------------|----------|-----------|-----------|-----------|--------|
| <b>Disappear</b>          | 0(0%)    | 0(0%)     | 3(3.6%)   | 23(27.4%) | 0.1421 |
| <b>One</b>                | 84(100%) | 84(100%)  | 81(96.4%) | 61(72.6%) |        |
| <b>Two</b>                | 0(0%)    | 0(0%)     | 0(0%)     | 0(0%)     |        |
| <b>Size</b>               | 5.0±0.66 | 5.29±0.74 | 5.17±1.19 | 4.09±2.46 | 0.0862 |
| <b>Shape</b>              |          |           |           |           |        |
| <b>Rounded</b>            | 84(100%) | 84(100%)  | 81(96.4%) | 61(72.6%) | 0.7231 |
| <b>Oblong</b>             | 0(0%)    | 0(0%)     | 0(0%)     | 0(0%)     |        |
| <b>Regularity</b>         |          |           |           |           |        |
| <b>Irregular</b>          | 0(0%)    | 1(1.2%)   | 0(0%)     | 0(0%)     | 0.6791 |
| <b>Regular</b>            | 84(100%) | 83(98.8%) | 81(96.4%) | 61(72.6%) |        |
| <b>Echogenicity</b>       |          |           |           |           |        |
| <b>Not calcified</b>      | 84(100%) | 84(100%)  | 81(96.4%) | 61(72.6%) | 0.1161 |
| <b>Calcified</b>          | 0(0%)    | 0(0%)     | 0(0%)     | 0(0%)     |        |
| <b>Amount of bleeding</b> |          |           |           |           |        |
| <b>Minimal</b>            | 0(0%)    | 24(28.6%) | 84(100%)  | 61(72.6%) | 0.0791 |
| <b>Moderate</b>           | 0(0%)    | 0(0%)     | 0(0%)     | 0(0%)     |        |
| <b>Severe</b>             | 0(0%)    | 0(0%)     | 0(0%)     | 0(0%)     |        |
| Doppler examination       |          |           |           |           |        |
| <b>Cardiac pulsation</b>  | 84(100%) | 84(100%)  | 84(100%)  | 84(100%)  | 1.001  |

1.McNemar test. 2. Repeated measure ANOVA test. \*Statistically significant as p<0.05.

Abbreviations: HCG: Human Chorionic Gonadotrophin.

Our study showed the results of four visits showed statistical insignificant differences among complete pregnancy group. (Table 3)

**Table4:** Correlation between ultrasound findings throughout visits among aborted group.

|                           | Aborted group (n = 36) |           |           |           | P-value  |
|---------------------------|------------------------|-----------|-----------|-----------|----------|
|                           | 1st visit              | 2nd visit | 3rd visit | 4th visit |          |
| <b>GA (weeks)</b>         | 6.45±1.06              | 9.28±1.5  | 10.28±1.5 | 11.28±1.5 |          |
| Yolk sac characteristics  |                        |           |           |           |          |
| <b>Presence</b>           | 36(100%)               | 36(100%)  | 36(100%)  | 28(77.8%) | 0.7611   |
| <b>Number</b>             |                        |           |           |           |          |
| <b>Disappear</b>          | 0(0%)                  | 0(0%)     | 0(0%)     | 8(22.2%)  | 0.023*1  |
| <b>One</b>                | 34(94.4%)              | 34(94.4%) | 34(94.4%) | 27(75%)   |          |
| <b>Two</b>                | 2(5.6%)                | 2(5.6%)   | 2(5.6%)   | 1(2.8%)   |          |
| <b>Size</b>               | 7.42±0.5               | 7.64±0.49 | 7.61±1.05 | 6.19±3.38 | 0.013*2  |
| <b>Shape</b>              |                        |           |           |           |          |
| <b>Rounded</b>            | 36(100%)               | 36(100%)  | 36(100%)  | 28(77.8%) | <0.001*1 |
| <b>Oblong</b>             | 0(0%)                  | 0(0%)     | 0(0%)     | 0(0%)     |          |
| <b>Regularity</b>         |                        |           |           |           |          |
| <b>Irregular</b>          | 4(11.1%)               | 10(27.8%) | 30(83.3%) | 24(66.7%) | <0.001*1 |
| <b>Regular</b>            | 32(88.9%)              | 26(72.2%) | 6(16.7%)  | 4(11.1%)  |          |
| <b>Echogenicity</b>       |                        |           |           |           |          |
| <b>Not calcified</b>      | 31(86.1%)              | 28(77.8%) | 8(22.2%)  | 6(16.7%)  | <0.001*1 |
| <b>Calcified</b>          | 5(13.9%)               | 8(22.2%)  | 28(77.8%) | 22(61.1%) |          |
| <b>Amount of bleeding</b> |                        |           |           |           |          |
| <b>Minimal</b>            | 0(0%)                  | 19(52.8%) | 18(350%)  | 5(13.9%)  | <0.001*1 |
| <b>Moderate</b>           | 0(0%)                  | 0(0%)     | 15(41.7%) | 22(61.1%) |          |
| <b>Severe</b>             | 0(0%)                  | 0(0%)     | 3(8.3%)   | 9(25%)    |          |
| Doppler examination       |                        |           |           |           |          |
| <b>Cardiac pulsation</b>  | 30(83.3%)              | 20(55.6%) | 10(27.8%) | 6(16.7%)  | <0.001*1 |

1.McNemara test. 2. Repeated measure ANOVA test. \*Statistically significant as p<0.05.

Abbreviations: HCG: Human Chorionic Gonadotrophin.

Our study showed that all yolk sac parameters and Doppler examination showed statistical significant differences throughout four visits. (table4)

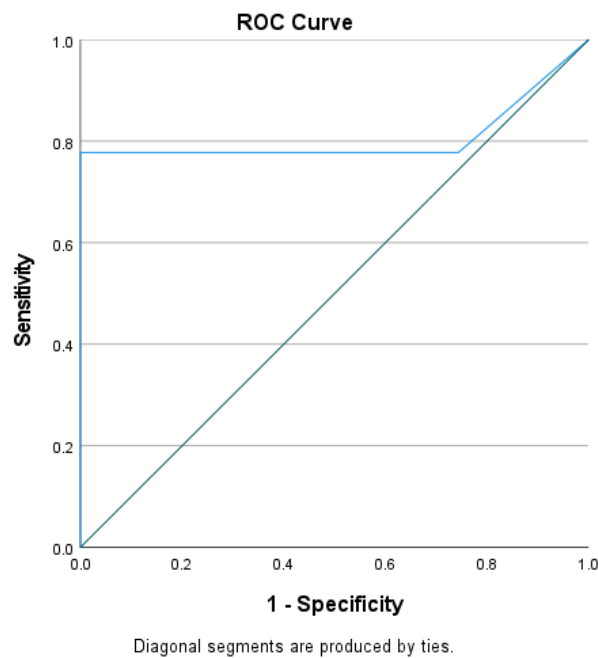
**Table 5:** Correlation between laboratory results in both study groups during visits.

| B-HCG(mIU/ml) | Complete pregnancy group (n = 84) | Aborted group (n = 36) | P-value  |
|---------------|-----------------------------------|------------------------|----------|
| 1st visit     | 125062.8±143211.3                 | 35983.44±99453.1       | <0.001*2 |
| 2nd visit     | 323905.01±373232.9                | 30176.86±19872.36      | <0.001*2 |

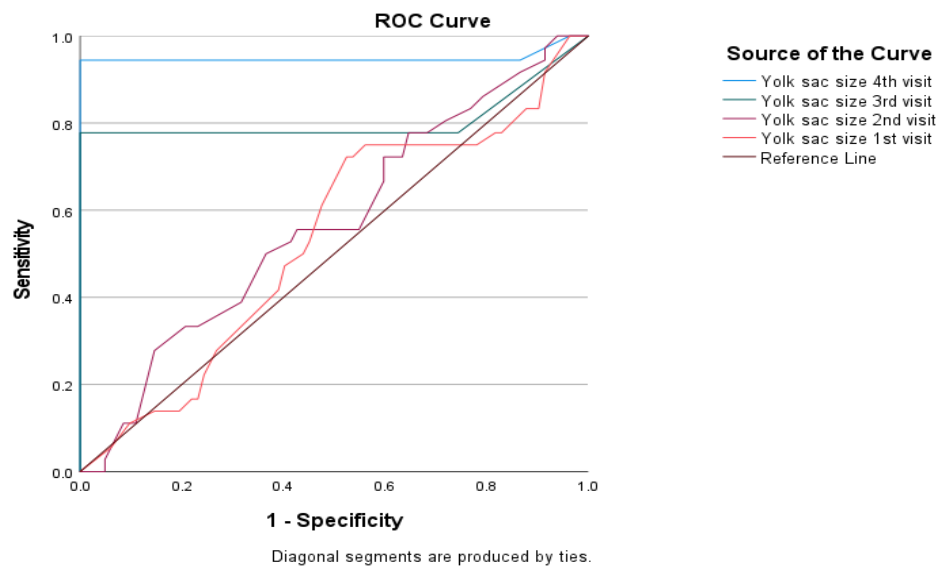
Paired t test. 2. Student t test. \*Statistically significant as p<0.05.

Abbreviations: HCG: Human Chorionic Gonadotrophin.

Regarding laboratory investigations, B-HCG level was significantly lower among aborted group (30176.86±19872.36) than complete pregnancy group (323905.01±373232.9) (p<0.001) in the first and second visits. (table5)



**Figure2:** ROC curve of the combined CA-125 and B-HCG for prediction of first trimester abortion.



**Figure 3:** ROC curve of yolk sac size for prediction of first trimester abortion.



**Table 6:** Validity of serum level of CA-125, B-HCG and yolk sac size in prediction of abortion in first trimester.

| Test Variable(s) | Result | AUC   | Sensitivity | Specificity | PPV   | NPV   | Accuracy |
|------------------|--------|-------|-------------|-------------|-------|-------|----------|
| 1st visit        |        | 0.903 | 96.4%       | 70%         | 76.2% | 95.1% | 83.2%    |
| 2nd visit        |        | 0.947 | 96.4%       | 91%         | 91.5% | 96.2% | 93.7%    |

Our study showed that the calculated accuracy of lab (CA-125, B-HCG) with US increased in visits from 83.2% in the first visit with 96.4% Sensitivity and 70% Specificity to 93.7% in the second visit with 96.4% Sensitivity and 91% Specificity. (Table 6)

## Discussion

A miscarriage is the loss of a pregnancy before 20 weeks of gestation or the birth of a fetus weighing less than 500 grams before the age of viability (1). One of the most typical problems that might arise during pregnancy is an abortion that occurs by itself. At this time, the diagnosis of an abortion that occurred on its own is made using a combination of nine hormonal tests (serum human chorionic gonadotropin (HCG), estradiol (E2), estrone, estriol, progesterone, human placental lactogen, cortisol, urine HCG and urine estrogen) and physical examinations. There have been several sonographic factors that have been suggested as potential indicators of a miscarriage. These include the presence of the yolk sac as well as its characteristics (3), the size of the gestational sac (4), the heart rate of the fetus, and slowed embryonic development. In the current study, we investigated the anomalies of the yolk sac, the maternal blood level of cancer antigen 125 (CA-125) and the (B-HCG) as early predictors of pregnancy loss in the first trimester in patients whose pregnancies were in danger of being lost. The main results of our study were as following:

Regarding demographic data, our study results demonstrated no statistically significant difference (p value < 0.05) among the 2 groups. Hassan et al, s study results agreed with these findings (6). But our study revealed that there is statistically significant difference among the 2 groups according to smoking habit while smokers percentage was significantly higher among aborted group (16.7%) than complete pregnancy group (2.4%). That is contradicting Hassan et al. study results (6) that found no association between maternal smoking and the risk of spontaneous abortion.

In our study, aborted group had significantly higher prevalence of lower abdominal pain, vaginal discharge and itching than complete pregnancy group. This is in agreement with a recent study which reported that women with recurrent pregnancy loss, had higher pelvic pain and vaginal bleeding (7). While the risk of threatening abortion was highest among those who reported abdominal discomfort (55.9%), those who did not report pain (42.6%) nevertheless had an abortion. (7).

As regards the Yolk sac criteria (size, regularity, echogenicity), our study revealed that first visit was at mean gestational age 6.5wks with no statistically significant differences among the 2 groups , Follow up by TVS revealed yolk sac present in all women in complete pregnancy group , they had rounded , regular shape with mean diameter ranging from 5 mm at first visit to a mean of 5.17 mm at third visit at mean gestational age of 10 wks then declined to a mean of 4 mm at last visit at mean gestational age of 11wks in the complete pregnancy group. This was in contrast to the aborted group where follow up by TVS revealed yolk sac with rounded, irregular, calcified shape with mean diameter of 7.42 mm at first visit at mean gestational age 6.45wks, yolk sac continued to increase slowly till it declined to a mean of 6.19 mm at the last visit at mean gestational age 11.28wks with statistical significant differences throughout the four visits among the 2 groups (p < 0.05). There was a statistically significant difference between the groups beginning around the 9th week of gestation, when the diameter of the yolk sac began to expand more slowly in the aborted group compared to the full pregnancy group, before beginning to decline.

Yolk sac diameters (YSDs) between 2 and 6 millimeters were associated with a 92% continuation rate of pregnancy, whereas YSDs greater than 6 millimeters were associated with a 20% continuation rate. (4, 8). This agrees with our results showing that the ongoing pregnancy rates for YSD of 5-6 mm and >6mm were 70% and 30% respectively.

Our current study agrees with Hassan et al. study which found that There was a statistically significant split in the outcomes of pregnancy for the two distinct groups (P-Value <0.05). The mean diameter of the yolk sac at the sixth week of gestation was 2.88±1.62 in the group who experienced pregnancy loss,

whereas it was  $4.29 \pm 0.33$  in the group that continued to carry the pregnancy. After that, the yolk sac diameter continued to expand gradually until it began to drop around the 12th week of gestation. (6). This was in agreement with our results.

There was a statistically significant difference between the two groups (P-Value less than 0.05) in terms of the existence of heart pulsation throughout the first trimester of pregnancy. In the complete pregnancy group, cardiac pulsation was preserved in all patients during all visits, while in the aborted group it significantly decreased from 83.3% at the first visit to 16.7% at the last visit. CRL size was higher among complete pregnancy group than aborted group but with statistically insignificant differences ( $p > 0.05$ ).

This study is consistent with the findings of Doubilet et al., who discovered that a heart pulse of less than 90 beats per minute at 6-8 weeks of gestation was associated with an increased risk of the mother passing away in the first trimester of the following pregnancy. (9) .

Balsane et al. (10), Agarwal et al. (11), and Abu Elghar et al. (12) are only a few of the studies that agree with the findings of the current study. Abdulkadhim et al. showed that individuals whose embryonic heart rates were below 100 beats per minute had a poor prognosis. (13).

Regarding laboratory investigations in the present study, B-HCG level was significantly lower among aborted group ( $30176.86 \pm 19872.36$ ) than complete pregnancy group ( $323905.01 \pm 373232.9$ ) ( $p < 0.001$ ). In this study the calculated sensitivity & specificity of B-HCG in the first visit at mean gestational age 6.5 weeks at a cut-off value of 18.501 mIU/ml were 96.3 and 88.9, respectively with 92.6% accuracy. Sensitivity and specificity of B-HCG in the second visit at mean gestational age 9.2 weeks at a cut-off value of 29.706 mIU/ml were decreased to become 89.1 and 72.2, respectively with 80.15% accuracy .

Calculated averages for Serum B-HCG were 10,450.6 in the aborted group and 41,876.1 in the full-term pregnancy group, with a p value  $< 0.001$ . Both the sensitivity and specificity of B-HCG were determined to be 79 and 85.17 at a cutoff value of 20,555 mIU/ml, respectively. (14).

Our study found that, the calculated sensitivity & specificity of combined CA-125 and  $\beta$ -HCG in the first visit at mean gestational age 6.5 weeks were 90.1 and 72.2, respectively with 81.15% accuracy.

In addition, the study conducted by Mansy et al. discovered that the combined sensitivity and specificity of these biomarkers for the prediction of abortion during the first trimester were 99.00 and 82.00, respectively. (14).

## **Conclusion**

Reliable first-trimester abortion prediction might enhance pregnancy outcomes by allowing tighter prenatal surveillance, earlier diagnosis, and faster treatments. Even before sonographically studying fetal morphology, yolk sac size abnormalities can indicate early pregnancy loss. In the first trimester, measuring Beta HCG and CA 125 can indicate pregnancy health.

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