The Role of Homocysteine and CA125 in the Differentiation between Ectopic Pregnancy and Spontaneous Abortion in First Trimester

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ABSTRACT:

BACKGROUND:
Ectopic pregnancy and spontaneous abortion are the two common complications seen in early pregnancy. While spontaneous abortion is the most common complication of pregnancy, ectopic pregnancy is the most life threatening emergency in early pregnancy.

OBJECTIVE:
To investigate the diagnostic value of serum homocysteine and CA-125 levels for early diagnosis and differentiation for early pregnancy complications.

PATIENTS AND METHODS:
The study included 90 pregnant women, aged from 21 to 40 years old presented with a gestational age ranging between 5-13 weeks. The patients were divided into three groups, group A included 30 cases of ectopic pregnancies, group B Included 30 cases who presented with spontaneous abortions and group C included 30 cases who presented as normal uncomplicated pregnancies and were followed up till their normal term deliveries and was considered as the control group. All women in the studied groups underwent CA125 and serum homocysteine measurements by radioimmune assay and Enzyme-linked immunosorbent assay (ELISA) respectively.

RESULTS:
Regarding group A, the majority of cases had low levels of serum homocysteine on admission (60%) and only one case changed from low to high level after one week from surgical termination of pregnancy. The CA125 readings were subdivided into two subdivisions: the first subdivision was the ruptured ectopic pregnancy cases on admission and diagnosis and they were 20 out of the 30 cases of ectopic pregnancy, half of these cases (50%) had high CA125 levels on admission and 3 patients had returned to normal after one week from surgical termination of pregnancy, in the second subdivision (unruptured ectopic pregnancies which were10 cases), the vast majority of patients (90%) had normal CA125 levels on admission and all of them had returned to normal after one week from the surgical termination of pregnancy. Regarding group B, (56.7%) of patients had high levels of serum homocysteine on admission and none of them had returned to normal value after one week from spontaneous or interventional completeness of the abortion, while the results of CA125 showed that (63.3%) of patients had high levels of this tumor marker on admission and 6 cases had returned to normal levels after one week from spontaneous or interventional completeness of the abortion.
In group C, the majority of the cases were within normal ranges of both CA125 and serum homocysteine both at admission and one week follow up. (90% and 93.3% regarding CA125) and (76.6% and 70% regarding serum homocysteine).

CONCLUSION:
The measurement of serum homocysteine and CA125 was found to be an effective method for early diagnosis and differentiation between spontaneous abortion and ectopic pregnancy.

KEYWORDS: pregnancy, spontaneous abortion, ectopic pregnancy, homocysteine, CA 125

INTRODUCTION:

Abortion is defined as the spontaneous loss of a pregnancy prior to viability, taken legally as a gestation date of 23 weeks +6 days [(1)]and it is the most common complication in the first half of pregnancy. Its incidence varies roughly between 10-20% [(2)].There are two main types of abortion, spontaneous and induced.

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There are various stages and types of spontaneous abortion including (threatened, inevitable, incomplete, complete, missed abortion, and fetal/embryonic demise) [3]. On other hand, ectopic pregnancy (EP) occurs when a fertilized egg implants somewhere other than the main cavity of the uterus[4]. The incidence of ectopic pregnancy is 11.1/1000 pregnancies. The IVF and related treatment increase the likelihood of ectopic pregnancy, reaching up to 1-3%[5].There are different types of ectopic pregnancy, tubal and non tubal. Both ectopic pregnancy and abortion are the two common complications seen in early pregnancy [6],however, their management modalities are different and ectopic pregnancy is regarded as the most common life threatening emergency in early pregnancy [7].CA-125(cancer antigen 125) tumor marker is a cell-surface antigen derived from the surface coelomic epithelium, including the mucosa of the entire female genital tract and maternal decidua. Therefore, extensive tissue destruction in both tubal and uterine abortions has been shown to result in a significant increase of CA-125 levels [8]. CA125 level increases in early pregnancy. Levels peak during the first trimester of pregnancy, between 6-7 weeks and drop to non-pregnant values in second and third trimesters[9]. CA125 is also elevated immediately after birth, implicating the disintegration of the maternal decidua (i.e. blastocyst implantation and placental seperation) as a possible cause of the tumor marker elevation [10]. Homocysteine (Hcy) is a non-protein α-amino acid. It is a homologue of the amino acid cysteine and can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins [11]. Hcy was identified as an important intermediate [12]. The discovery of patients with inherited or "inborn" errors of hcy metabolism gave a valuable insight into the roles of enzymes in this process. It was found that many of these enzymes needed B vitamins, including vitamins B12, B6 and folate, to act as "cofactors" to help them work properly [12]. In pregnancy, hcy levels tend to decrease. Elevated hcy levels may be associated with some fetal abnormalities and with potential blood vessel problems in the placenta, causing abruption. There may also be an association with pre-eclampsia[13].

AIM OF THE STUDY:

To investigate the diagnostic value of serum hcy and CA-125 levels for early diagnosis and differentiation between spontaneous abortion and ectopic pregnancy in first trimester period.

PATIENTS AND METHODS:

This is a prospective cohort study conducted at the department of Obstetrics and Gynecology of AL-Yarmouk Teaching Hospital through a period from February 2016 to October 2016.

The study protocol was approved by The Scientific Council of Obstetrics and Gynecology Specialization of the Arab Board of Health Specializations.

The study included 90 pregnant women, aged from 21 to 40 year old within first trimester attending the outpatient clinic with a gestational age range between 5th – 13th weeks. The participants were informed the nature of the study and written consents were obtained from them. The patients were divided into three groups as follows:

-**Group A:** Includes 30 cases presented as ectopic pregnancies in 1st trimester (subdivided into ruptured and unruptured ectopic pregnancy when measuring CA125)

-**Group B:** Includes 30 cases presented as spontaneous abortions in 1sttrimester.

-**Group C:** Includes 30 cases presented as normal uncomplicated pregnancies on their booking visit and were followed up until their normal term deliveries, this cohort was considered the control group.

Exclusion Criteria:

- History of cervical pathology or any surgical operations.
- History of medical ovarian stimulation used for conception or IVF.
- History of chronic medical diseases blood dyscrasias, or gynecological diseases that elevate serum CA125 level.
- Previous/current history of malignancies or history of intake of chemotherapy or immunotherapy for any cause.
- Fetal malformation, abnormal placentation, or Chorioamnionitis.

Detailed history, general, and obstetrical examination were done for all patients. Maternal blood samples were collected at the time of admission (before any intervention and before

receiving any medication) for the 3 groups (A, B and C) and were sent to the laboratories for the blood group and cross match -if needed-, full blood count, and random blood sugar. Ultrasonography also performed to confirm the diagnosis. Other blood samples were sent for serum Hcy and serum CA125 levels for the studied groups after one week of intervention (medical or surgical)

Radioimmune Assay method was used for the measurement of CA125. It is a one-step (sandwich) radioimmune assay. Wallace brand Wizard 1470 Automatic Gamma Counter Laboratory testing machine was used for this purpose.

Enzyme-linked immunosorbent assay (ELISA) kit was used for Hcy measurment. This method is based on biotin double antibody sandwich technology to assay human homocysteine. BioTech brand ELx800 Laboratory testing machine was used for this purpose.

Statistical Analysis:

The SPSS software program, version 20, was used for all computerized statistical analyses. The results were expressed as mean ± SD (standard deviation), or frequency & percentage. Continuous normally distributed variables were compared by analysis of variance test (ANOVA). Categorical variables were compared using Chi-square X^2 tests. p - value equal or less than 0.05 was considered to be statistically significant.

RESULTS:

This study involved 90 pregnant women in their first trimester divided into three groups: ectopic pregnancy (Group A), spontaneous abortion (Group B), and normal term delivery pregnancies (Group C). Their ages were ranged between 21 to 40 years old.

The Age distribution and the percentage for each studied groups are demonstrated by table 1 which reveals that the majority of Group C are within the younger age groups (83.3% of them within 21-30 years old), while the older age groups (36-40y) are grouped towards the cases of spontaneous abortion and ectopic pregnancy cases.

Table 1: Age distribution of the studied groups.

Studied groups	Age range and percentage							
	21-25		26-30		31-35		36-40	
A	5	16.6%	6	20%	11	36.6%	8	26.6%
B	4	13.3%	2	6.6%	15	50%	9	30%
C	15	50%	10	33.3%	3	10%	2	6.6%

The distributions of the CA125 levels among the studied groups on admission are shown in table 2. On admission group A was subdivided into 2 subgroups (ruptured and unruptured). Half (50%) of the ruptured ectopic pregnancy cases (which were 20 cases), had high CA125 level and the other half (50%) had normal levels. Nine cases (90%) of the unruptured ectopic pregnancy group (which included 10 patients) had normal CA25 level and only one case (10%) had high CA125 level on admission.

In group B (30 cases), 19 cases(63.3%)had high level of CA125 and 11 cases (36.7%)had normal levels of CA125 on admission.

Regarding group C, 27 out of 30 cases had normal CA125 level on admission and only 3 cases (10%) had high levels.

Table 2 reveals that the majority of normal pregnancy (90%) and unruptured ectopic pregnancy (90%) had normal levels of CA125, while the majority of spontaneous abortion cases (63%) and half the cases (50%) of ruptured ectopic pregnancy had high levels of CA125. The P value of this statistical association was < 0 .001 which is highly significant.

Table 2:Relationship between type of pregnancy outcome and CA125 level on admission.

Studied groups		CA125 level on admission		
		Normal	Elevated	Total
Group A (ruptured)	count	10	10	20
	%	50.0%	50.0%	100%
Group A (unruptured)	count	9	1	10
	%	90%	10%	100%
Group B	count	11	19	30
	%	36.7%	63.3%	100%
Group C	count	27	3	30
	%	90.0%	10.0%	100%
Total		57	33	90
		63.3%	36.7%	
X2= 22.967		d.f.= 3	*P < 0 .001*	

The relationship of CA125 level among the three studied groups after one week from the first investigation is shown in table 3 which reveals that CA125 levels of 6 cases (20%) of spontaneous abortion and 3of 10 cases (30%) of ruptured ectopic pregnancy had returned to normal after one week from therapeutic termination of pregnancy of these cases, while only one case of unruptured ectopic pregnancy (10%) and one case of the control group (3.3%) had returned to normal after one week.

The P value of this statistical association was 0.002 which is highly significant.

Table 3: Relationship between the studied groups and CA125 level after one week.

Studied groups		CA125 Level after one week		Total
		Normal	Elevated	
Group A (ruptured)	Count	13	7	20
	%	65.0%	35.0%	100%
Group A (unruptured)	Count	10	0	10
	%	100%	0%	100%
Group B	Count	17	13	30
	%	56.7%	43.3%	100%
Group C	Count	28	2	30
	%	93.3%	6.7%	100%
Total	Count	68	22	90
	%	75.6%	24.4%	100%
X2= 15.371		d.f.= 3	*P = 0 .002*	

The distribution of the hcy levels on admission among the three studied groups is illustrated table 4.In group A(ectopic pregnancy group) was as follow, 9 cases had normal hcy levels, 3 cases had high levels and 18 cases had low levels. Of the 30 cases of group B (spontaneous abortion), 12 cases had normal hcy level and 17 cases had high levels and only one case had low level on admission. In group C (normal pregnancy), 23 cases had normal hcy levels, 5 cases had high levels and 2 cases had low levels on admission.

The table shows that the majority of cases of normal pregnancy (76.7%) had normal levels of hcy, and the majority of cases of spontaneous abortion (56.7%) had high hcy levels, while the majority of cases of ectopic pregnancy (60.0%) had low hcy levels.

Table 4: Relationship between type of pregnancy outcome and Homocysteine level on admission.

Studied group		Homocysteine level on admission			Total
		Normal	Elevated	low	
Group A	Count	9	3	18	30
	%	30.0%	10.0%	60.0%	100%
Group B	Count	12	17	1	30
	%	40.0%	56.7%	3.3%	100%
Group C	Count	23	5	2	30
	%	76.7%	16.7%	6.7%	100%
Total	Count	44	25	21	90
	%	48.9%	27.8%	23.3%	100%
X2 = 47.169		d.f.= 4		P < 0.001	

Table 5 demonstrates the relationship between the levels of hcy and the outcome of pregnancy among the three studied groups after one week from intervention and it reveals that only 2 cases (6.6%) of normal pregnancy outcome cases had increased level of hcy after one week from the first examination, while there was no change in the hcy level results among the cases of spontaneous abortion after one week period. It shows also that only 1 case (3.3%) from the group of ectopic pregnancy group returned to normal level after one week from the termination of pregnancy. The P value of this statistical association was **< 0.001** which is highly significant.

Table 5: Relationship between type of pregnancy outcome and homocysteine level after one week.

Studied group		Homocysteine level on admission			Total
		Normal	Elevated	low	
Group A	Count	10	3	17	30
	%	33.3%	10.0%	56.7%	100%
Group B	Count	12	17	1	30
	%	40.0%	56.7%	3.3%	100%
Group C	Count	21	7	2	30
	%	70.0%	23.3%	6.7%	100%
Total	Count	43	27	20	90
	%	47.8%	30.0%	22.2%	100%
X2 = 40.446		d.f.= 4		P < 0.001	

DISCUSSION:

In spite of various efforts to find an etiological factor for early pregnancy loss, more than 50% of the cases remain unexplained [14]. Although abortion is the most common complication, ectopic pregnancy remains the most common life threatening emergency in early pregnancy [7].

Regarding the demographic distribution of the current study, the results reveal that the older age groups are grouped towards the cases of spontaneous abortion and ectopic pregnancy (63.2% of spontaneous abortion cases and 80% of ectopic pregnancy cases are within 31-40 years old) while the majority of normally ended pregnancies are within the younger age groups (83.3% of them within 21-30 years old).

These findings are comparable with the study of Andersen A-M Nybo et al.[15]which concluded that: (There is an increasing risk of fetal loss with increasing maternal age in women aged more than 30 years. Fetal loss is high in women in their late 30s or older, irrespective of reproductive history. This finding should be taken into consideration in pregnancy planning and counseling) [15].

A French study held by Bouyer J et al. hypothesized that the relationship between increasing maternal age and the incidence of ectopic pregnancy is due to the higher probability of exposure to most other risk factors (i.e. smoking, IUCD …etc). Also the study presumed that with advancing age, the increase in chromosomal abnormalities in trophoblastic tissue and age-related changes in tubal function causes delay in ovum transport, resulting in tubal implantation [16].

Regarding the relationship between the spontaneous abortion and the increase in maternal age, a study held by De La Rochebrochard E et al. [17] postulated that the relationship is multifactorial including a link between increasing age and higher incidence of chromosomal abnormalities.

However, conflicting conclusions have been drawn concerning the possible effect of age on oocyte quality and uterus senescence [17].

In our study, CA125 levels on admission and one week after intervention in spontaneous abortion, found that 19 out of 30 (63.3%) cases had high level of CA125 and only 11 (36.6%) cases had normal levels on admission and 6 cases of the previously high CA125 level cases (31.6%) had returned to normal after one week from therapeutic termination of pregnancy of these cases. The P values of CA125 levels in spontaneous abortion on admission and after one week is < 0.001 and 0.002 respectively which are regarded as highly significant statistical data.

Katsikis I. et al., concluded that there is a significant elevation of CA125 in spontaneous abortion (80%) and this increase in CA125 level can be used as a predictive factor for spontaneous abortion. The authors speculated that the increase in CA125 level in spontaneous abortion is due to the extensive destruction of decidual and mucous epithelial cells, which have been known to express substantial amount of this marker [18].For the same reason they found that after surgical removal of the necrotic tissue, the CA125 level drops to normal in short time interval (24 hours) which is also comparable with our study regarding the drop in CA125 level in both spontaneous abortion and ruptured ectopic pregnancy after (one week) from termination of the pregnancy. Regarding ectopic pregnancy, Katsikis I. et al., did not found a significant change in CA125 levels both before and after intervention. The authors of the study hypothesized that the unpredicted interaction between ectopic trophoblast and tubal tissue may be responsible for the different findings by different studies regarding CA125 level in ectopic pregnancy[18].

Spitzer M. et al., study had a different conclusion to the question understudy. They concluded that there is a distinct pattern in CA-125 levels during pregnancy and the puerperium. Due to the wide fluctuations in CA-125 levels in very early pregnancy and the immediate postpartum period, CA-125 values during these periods are not useful for clinical correlation with the pathologic conditions known to be associated with elevated levels of CA-125. Also they mentioned that further study is needed to determine whether extreme values in the first trimester or elevated levels after the first trimester are diagnostic or predictive of any conditions related to pregnancy [19].

In the current study, CA125 levels, in cases of ruptured ectopic pregnancies on admission, (50%) had high CA125 level and the other half had normal level. In cases of unruptured ectopic pregnancy on admission, (90%) of them had normal CA125 level and10% had high CA125 level. After one week from therapeutic termination of pregnancy of these cases, (30%) of ruptured ectopic pregnancy and the only 10% of unrupturd ectopic pregnancy which had previously high CA125 level on admission had returned to normal after one week and the P value of CA125 level in ectopic pregnancy on admission and after one week were < 0.001 and 0.002 respectively which are regarded as highly significant.

The above findings are in agreement with a study done by Lilyan WS. et al, which found that CA-125 level was significantly elevated in ruptured tubal ectopic pregnancy than the intact tubal ectopic pregnancy. They presumed that this increase can be explained by the disintegration of maternal deciduas due to blastocyst implantation and concluded that this increase in CA-125 levels can be used as additional test to identify tubal rupture [20].

Schmidt et al., reached a different finding to this study; they concluded that single serum measurements of CA 125 in symptomatic first trimester pregnant patients failed to discriminate spontaneous abortion, ectopic or normal pregnancies[21].

In our study, homocysteine level in cases of first trimester spontaneous abortion on admission revealed that (56.7%) of the cases had high hcy level and only (3.3%) had low level on admission, while there was no change in the hcy level results among the cases of first trimester spontaneous abortion one week after the intervention. These results are in agreement with a study done by Del Biano A et al. which found a high level of hcy in cases of first trimester spontaneous abortion. Also they presumed that the high level of hcy may be associated with folate and vitamin B6 deficiency as a causative factor of first trimester spontaneous abortion [22].

Similar findings of the association between high hcy levels and spontaneous abortion in the first trimester were reached by Mohamed El –Kadi et al., who mentioned that high hcy levels were associated with low folate and vitamin B12 rather than B6 levels [23].

On the other hand, Khong and Hangue in their study did not find a significant association between elevated hcy level and spontaneous abortion risk [24].

When examining the association between ectopic pregnancy and the level of hcy in our study we found that (60%) had low hcy levels on admission and only 10% of cases had high hcy level with a P value < 0.001 which is highly significant. On the other hand, only (3.3%) of ectopic pregnancy cases which had previously low hcy level returned to normal value after one week from the termination of pregnancy. These findings were in agreement with a study done by Kavitha Met al. which concluded that there is a causative association between low circulatory hcy levels and ectopic pregnancy in the first trimester and they presumed that there is an inverse relationship between hcy level and nitric oxide level [25].

CONCLUSION:

The measurement of serum hcy and CA125 was found to be an effective method for early diagnosis and differentiation between spontaneous abortion and ectopic pregnancy. An early measurements of both serum hcy and CA125 as soon as pregnancy is detected can be used to increase the diagnostic accuracy and differentiation between the two conditions.

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