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MaternalSeruma Disintegrin andMetalloprotease Protein-12 (ADAM 12) In First Trimester as a Marker of EarlyAdverse Pregnancy Outcomes

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Abstract:
Background:*ADAM 12, a multidomain polypeptide, is a placental protein, that is involved in growth and differentiation of placenta and fetus secreted from the invasive extra villous trophoblasts andtrophoblasts in distal anchoring columns in early gestation placental villi,ADAM12, existing as two distinct splice variants (ADAM12L and ADAM12S), promoting cell migration and invasion of the placenta, poor invasion of the placenta leads to adverse first trimester outcomes.*
Objectives:*To determine whether the maternal serum concentration of A disintegrin and metalloprotease protein12 (ADAM12) in first trimester can be considered as an indicatorof first-trimester spontaneous abortions, missed abortions, ectopic pregnancies and hydatidiform moles.*
Patients and methods*:Thiscase control study was carried out during period from the 1st of March to the 30th of November 2016 at Baghdad Teaching Hospital inObstetrics and Gynecologydepartment. Two hundred pregnant women were enrolled in this study, one hundred of them were control group and the remainder (n=100) are divided to complete abortion (n=25), missed abortion (n=25), ectopic pregnancy (n=25), molar pregnancy (n=25). The maternal serum concentration of ADAMs were measured between (5-9+6wks) of gestation using enzyme-linked immunosorbent assay (ELISA).*
Results*:The level of ADAM12 increased with gestational age in the control group. The meanlevel of ADAM12 in spontaneous abortion 10.3 ng/ml , in ectopic pregnancy 4.9ng/ml and in hydatidiform mole 6.4 ng/mlgroups which were higher than that in the control , while the mean level of ADAM12 in the missed abortion group 1.6 ng/ml which was not significant from the control (2.8 ng/ml) . Logistic regression analysis demonstrated that ADAM level was significantly predicting ectopic pregnancy, molar pregnancy and spontaneous abortion with OR (0.3), OR (0.4) , and OR (1.8) respectively .*
Conclusions:*The level of the maternal serum ADAM-12 in early pregnancy can be considered as an indicator of early adverse pregnancy outcome.*
Keywords:*ADAM12, ectopicpregnancy, molar pregnancy, spontaneousabortion.*

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I. Introduction

Pregnancyis the term used to describe the period in which a woman carries a fetus usually inside of the uterus[1,2] Fertilization describes the process by which a ("zygote") is formed , if fertilization does occur, the zygote divides and differentiates into a "*pre-embryo*" while being carried down the fallopian tube toward the uterus **[3].** Cell division of the zygote , resultsinblastocyst[4] The period from 3 – 8 weeks in human is called the *Embryonic Stage*: it is the period in which cell division and cellular differentiation of the zygote occur**[5,6]** The period from the beginning of the 9th week to birth is known as the *fetal period*. It is characterized by maturation of tissues and organs and at this stage the risk of miscarriage decreases sharply[7,8] Any pregnancy in the first trimester may end either in:Healthy viable fetus,Miscarriage , Ectopic pregnancy(the implantation is in the wrong place) and Molar pregnancy**(**a premalignant tumor that develops in the uterus). [9, 10].

A Disintegrin and A Metalloprotease protein 12 (ADAMS12) is a cellular Trans membrane polypeptide chain composed of unique multiple domains; the signal peptide, the prod main, the metalloprotease, the disintegrin, the cysteine-rich, and the EGF (epidermal growth factor-like domains), as well as the trans membrane segment and the cytoplasmic tail **[11.12]**. they play pivotal roles in the proteolytic degradation of the extracellular matrix for cell invasion **[13]**,cell adhesion, cell fusion signaling **[14,15]**, fertilization and

ectodomainshedding [16]**.** The multidomain structure allows the ADAM family of proteins to perform such various physiological tasks [17]. Human **ADAM12** exists in 2 variants by alternative splitting of ADAM 12 gene which locates in human chromosome 10q26.2. [18,19]. ADAM 12 level increase in pregnancy , where as in non pregnant women , its level is undetected only in some diseases or cancers , gestational age is likely to be a key factor in ADAM 12 levels , it rises from 5 weeks of gestation [20,21], this increase in serum levels of ADAM12 with gestation presumably reflects its increased production, as well as its improved transport due to the establishment of the uteroplacental circulation**.** While in non-pregnant women its level about zero [22,23]. The source of ADAM 12S is suggested to be the placenta [24,25].

ADAM 12 have many roles in pregnancy such as possesses gelatinase activity which cleaves extracellular matrix proteins, such as gelatin, and help extra villous trophoblasts to invade endometrium and myometrium during pregnancy. also participates in cell signaling and increases insulin growth factor by the action of its metalloprotease domain on the insulin growth factor binding protein 3 and 5 (IGFBP 3 & 5). [15,16, 17,18 ,22]

Aim Of This Study: To determine whether the concentration of A disintegrin & metalloprotease protein 12S (ADAM 12) measure in serum during first trimester could be considered as an indicator of early adverse pregnancy outcome .

II. Patients And Methods

This study is a case-control study, carried out during the period from the 1st of March 2016 to the 30th of November 2016, at Baghdad teaching hospital/medical city complex in Obstetrics and Gynecology department**.**

Two hundred pregnant women with gestational age 5- 9 +6weeks were enrolled in this study and they were divided into the following groups:

Group A: 100 pregnant women with viable intrauterine pregnancy between (5-9^{+6} weeks) as a control group **.**

Group B: 100 pregnant women presented at the outpatient department with either abdominal pain &/or vaginal bleeding and were admitted as inpatientsthen grouped into groupsaccording to their diagnosis and to the specific ultrasound criteria of each of first trimester complicationsas follows :

1. 25 women with complete spontaneous abortion. The report of ultrasound must be: Empty uterus with endometrial thickness at least 16 mm or small retained pieces of conception with previous ultrasound report of healthy viable intrauterine pregnancy.
2. 25 women with ectopic pregnancy.
3. 25 women with missed abortion
4. 25 women with molar pregnancy.

Full history taken from all pregnant women (control & cases) including demographic details (name, age and occupation), history regarding her recent pregnancy (gestational age was calculated depending on her last menstrual period and by ultra-sonographic measurement of the crown-rump length**.**

Exclusion criteria:

pregnant womenabove 40 years old ,Patient with twin pregnancies or higher order pregnancies **,** Patient with medical diseases (rheumatoid arthritis, asthma, cardiac hypertrophy) **,** Patient with a known or suspicious cancers **,** Any patient with history of previous pre-eclampsia, or strong history of Down syndrome or Intrauterine growth restriction**.**For confirmation of the diagnosis, in patient with incomplete abortion, missed abortion, suspected molar pregnancies, placentaltissue wassent to histopathology, patient with ectopic pregnanciesspecimens of salpingectomy wassent for histopathology. After confirmation by histopathology the patients were included in the study.Five milliliters of maternal blood aspirated peripherally from the ante cubital vein from the all subjects included in the study, and left to clot for 30 minutes at room temperature before centrifugation for 15 minute, then the serum is collected and stored at less or equal to -20 centigrade Celseus until assayed.Sera were assayed using the ADAM12 Quantikine ELISA kit (R&D systems)

- Sensitivity of the test: the minimum detectable dose of human ADAM 12 ranged from **0.016-0.076 ng/ml**. The mean MDD was **0.030 ng/ml.**
- Normal range of ADAM 12 from sera of apparently healthy non pregnant volunteers = **zero to 0.473 ng/L**.

III. StatisticalAnalysis

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 21 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Kolmogorov Smirnov analysis verified the normality of the data set**.** Multiple contingency tables conducted and appropriate statistical tests performed, fishers exact test used for categorical variables and One way ANOVA analysis was used to compare between more than two means. ROC curve was

used to clarify validity tests. In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the result presented as tables.

IV. Results

A total of two hundred pregnant women in first trimester were included in this study , one hundred of them considered as (control group) and remaining one hundred divided to four groups (ectopic , missed , molar and spontaneous) . all these pregnant women divided to three groups according to the age .

Table 1 :showedno significant difference in maternal age between control and study groups (p=0.1), but There was a significant association between women with molar pregnancy and increased gestational age (p=0.001).

Table1: Distribution Of Age And Gestational Age According To Study Groups.

Variable	Control		Ectopic		Molar		Missed		Spontaneous		P
	No.	%	No.	%	No.	%	No.	%	No.	%	
Age											**0.1***
<20 years	**26**	**6.0**	**0**	**-**	**4**	**16.0**	**2**	**8.0**	**0**	**-**	
20-29 years	**46**	**66.0**	**20**	**80.0**	**15**	**60.0**	**17**	**68.0**	**13**	**52.0**	
30-40 years	**28**	**28.0**	**5**	**20.0**	**6**	**24.0**	**6**	**24.0**	**12**	**48.0**	
Mean age	**27.3±3.2**		**27.4±2.6**		**28.1±1.7**		**27.1±3.3**		**26.8±1.1**		
Gestational age											
5th week	**7**	**7.0**	**2**	**8.0**	**2**	**8.0**	**0**	**-**	**0**	**-**	
6th week	**20**	**20.0**	**5**	**20.0**	**0**	**-**	**4**	**16.0**	**5**	**20.0**	
7th week	**43**	**43.0**	**9**	**36.0**	**2**	**8.0**	**8**	**32.0**	**2**	**8.0**	
8th week	**16**	**16.0**	**6**	**24.0**	**11**	**44.0**	**11**	**44.0**	**11**	**44.0**	**0.001***
9th week	**11**	**11.0**	**2**	**8.0**	**0**	**-**	**1**	**4.0**	**7**	**28.0**	
10th week	**3**	**3.0**	**1**	**4.0**	**10**	**40.0**	**1**	**4.0**	**0**	**-**	
Mean gestational age	**6**		**6**		**7**		**7**		**7**		

There was a highly significant association between higher ADAM mean levels and increased gestational age of the control pregnant women. Table2.

Table 2: Distribution of ADAM mean according to gestational age of control pregnant women.

GA groups	ADAM
	Mean±SD
5th week	**2.3±1**
6th week	**2.3±2.2**
7th week	**2.6±2.1**
8th week	**5±3.7**
9th week	**7.8±4.1**
10th week	**8.2±4.5**
P value*	**<0.001**

Most of healthy control pregnant women had normal obstetrical history and only 7% of them had abnormal obstetrical history as shown inTable 3.

Table 3: Distribution of pregnant control women characteristics.

Variable	No.	%
Obstetrical history of controls		
Normal	**93**	**93.0**
Abnormal	**7**	**7.0**
Total	**100**	**100.0**

Higher ADAM mean level was significantly associated with pregnant women with spontaneous abortion (p<0.001). Post Hoc test showed a significant higher mean of ADAM of women with ectopic pregnancy in comparison to controls (p<0.001), in same direction ADAM mean was significantly higher among women with molar pregnancy and spontaneous abortion in comparison to controls (p<0.001). No significant difference in ADAM mean between women with missed abortion and controls (p=0.9). table 4**.**

Table 4: Distribution of ADAM meanin the study groups.

Groups	**ADAM**	***Post Hoc test (P value)***	
	Mean±SD		
Control	**2.8±2.2**		
Ectopic pregnancy	**4.9±3.1**	**Ectopic & control**	**<0.001**
Molar pregnancy	**6.4±3.2**	**Molar & control**	**<0.001**
Missed abortion	**1.6±0.9**	**Missed & control**	**0.9**
Spontaneous abortion	**10.3±2.2**	**Spontaneous & control**	**<0.001**
ANOVA (P value)	**<0.001**		

ADAM12 level of pregnant women was significantly predicting ectopic pregnancy with OR (0.3), ADAM level of pregnant women was significantly predicting molar pregnancy with OR (0.4) and ADAM level of pregnant women was significantly predicting spontaneous abortion with OR (1.8) as shown in table 5 .

Table 5: Multiple logistic regression analysis of ADAM 12 level prediction of pregnancy outcome at first trimester.

Variable	□	**OR**	**P**
Ectopic pregnancy	**-1.1**	**0.3**	**<0.001**
Molar pregnancy	**-0.7**	**0.4**	**<0.001**
Missed abortion	**-0.3**	**0.2**	**0.1**
Spontaneous abortion	**-2.3**	**1.8**	**<0.001**

V. Discussion

ADAM12 is produced by the placenta and is present in the serum of gravid women, but not in the serum of non-pregnant women, but there is no unified level of ADAM 12 S specific to each gestational week[(7)]. The results of the **current study**, that the maternal serum ADAM12 concentration in normal pregnancies increased with gestational age between 5–9 $^{+6}$wks gestation from 2.6 ng/ml to 8.2 ng/ml, these results agree with Sahraravand et al study [(26)] where ADAM 12 increase continuously from week 5 (2.1 microgram/L) to a value of (553.82.1 microgram/L) at 11 week and Yang, Jiexia, et al study [(27)] where ADAM 12 at 5 weeks is 10 microgram/L to 175 microgram/L at 9$^{+6 \text{ days}}$ weeks .

Regarding ectopic pregnancy: thisstudy showed that the mean maternal serum level of ADAM12 in ectopic pregnancy is 4.9±3.1 ng/ml which is significantly higher than the control with P value of < 0.001.This disagree with Yang, Jiexia, et al.[(27)], in which the study involve ***56*** of ectopic pregnancies studied and compared with normal Intrauterine pregnancies, the MOM was 0.460, which is lower than the level in normalintrauterine pregnancies, also disagree with Rauch et al. study [(28)] which found a statistically significant decrease in a disintegrin and metalloprotease protein-12 (ADAM-12) in the sera of patients with Ectopic pregnancy (median 2.5 ng/ml), when compared to women with viable intrauterine pregnancy (median 18.6 ng/ml) , and disagree with Horne et al . study[(29)] which considered ADAM 12 levels had limited value as a diagnostic marker for ectopic pregnancy . the study involved 120 patients with ectopic pregnancy, serum ADAM 12 concentration were increased with histologically – confirmed ectopic pregnancy (median 442 pg/ ml) compared to women with viable intrauterine pregnancies (median 256 pg/ml).This disagreement is due to small sample of study group (ectopic group) .

Regardingspontaneousabortion:In the current study, the mean of ADAM12 in complete spontaneous abortion was 10.3 ng/ml, while in the control group the mean is 2.8 ng/ml, with P value, 0.001, this agree with Yang, Jiexia et al. [(27)] study in which the median of ***9*** patients of complete spontaneous abortions was significantly lower (0.430 MOM), while the control group median was 1.002 MOM. Regarding missed abortion :The current study conclude that the level of ADAM 12 in missed abortion is less than that found in normal pregnancy, this agree with Yang, Jiexia et al.[(27)]studybut in both studies the level of ADAM 12 is not significant from the control.Regarding molar pregnancy: The mean level of ADAM 12 in molar pregnancy in the current study is 6.4 ng/ml which higher than the level in control group with P value <0.001, this agree with Yang, Jiexia et al. [(27)] study MOM 0.037 which studied (***12 patients***) with molar pregnancy.

VI. Conclusion

The level of the maternal serum ADAM- 12 in early pregnancy considered as indicator of early adverse pregnancy outcome.

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