

## Low Level of Alpha-1 Antitrypsin in Prediction of Spontaneous Recurrent Abortion

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**Abstract: Objective:** The aim of this study is to evaluate the relation between the level of alpha-1 antitrypsin (AAT) and spontaneous recurrent abortion. **Background:** Abortion is the most common complication of pregnancy; spontaneous abortion is a termination of pregnancy before the fetal viability. Although 15% of clinically recognized pregnancies result in abortion, total reproductive losses are reportedly closer to 50%. **Patient and method:** This prospective case-control study consisted of patients with recurrent spontaneous abortion (n=25) and healthy pregnancies in the first trimester (n=25). Blood samples were assayed for AAT concentration and activity. **Results:** There was statistically significant increase in AAT during normal pregnancy and decline in study group of recurrent abortion ( $2.12 \pm 0.35$ ,  $1.65 \pm 0.10$  mg/ml,  $p < 0.001$  HS). No statistical significant differences were noted regarding maternal age, BMI between the study and control groups ( $26.7 \pm 2.52$ ,  $27.8 \pm 3.05$ ,  $p = 0.15$  NS,  $26.7 \pm 3.26$ ,  $27.3 \pm 3.77$ ,  $p = 0.59$  NS). And also no statistical significant differences were noted between the groups regarding gestational age ( $10.0 \pm 1.50$ ,  $10.2 \pm 1.24$ ,  $p = 0.68$  NS). Parity has no statistical significance as the control group was ( $2.28 \pm 0.73$ ) but study group has no parity. **Conclusion:** This study demonstrate a significant decline in circulating AAT in patients with recurrent pregnancy loss in first trimesters compared with normal pregnant women in the same trimesters. So determination of  $\alpha$ 1AT may be useful for the prediction of pregnancy outcome in first trimester.

[Mohamed Farag EL-sherbiny, Ashraf Nassif El- Mantwe, Nasra Said Mohamed. **Low Level of Alpha-1 Antitrypsin in Prediction of Spontaneous Recurrent Abortion.** *Nat Sci* 2018;16(2):58-61]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). <http://www.sciencepub.net/nature>. 9. doi:[10.7537/marsnsj160218.09](https://doi.org/10.7537/marsnsj160218.09).

**Keywords:** Alpha-1 antitrypsin and abortion.

### Introduction:

Abortion is the most common complication of pregnancy, spontaneous abortion is a termination of pregnancy before the fetal viability. Although 15% of clinically recognized pregnancies result in abortion, total reproductive losses are reportedly closer to 50% (1), (2). Although etiology of recurrent miscarriage can be broadly categorized into genetic, infective, structural, endocrinal, immunological background, there is disagreement among clinicians as to the numerical contribution of each of these factors. However, to date, the cause is not established in more than 50% of couples, and several alleged causes of recurrent pregnancy loss are controversial. Recent studies suggest that inflammatory and thrombotic pathways might be involved in disease pathogenesis. For example, the development of an intact placenta involves perfusion-dependent events that might be impaired by local microthrombi (3). Previous studies showed that AAT increases by about 100% in the third trimester, increase in AAT was hypothesized to be a result of the increased estrogen concentrations inducing AAT synthesis by the liver. Alpha-1 antitrypsin is an acute phase protein; antitrypsin functions to protect tissues from released proteolytic enzymes and also AAT is an anti-inflammatory circulating serine-protease inhibitor that controls tissue degradation and inflammation (4).

Also AAT rises in response to infection, trauma and estrogen; thus, in appropriately low levels of AAT may have a negative influence during pregnancy in all its vital roles due to its relative insufficiency. In the light of other studies about consideration of AAT that have been shown that it increase during normal pregnancy, it is responsible to suggest that failure to elevate AAT during pregnancy may represent relative functional deficiency as Alpha-1-Antitrypsin (AAT) promotes angiogenesis and vascularization of the endometrium (5), (6).

### Patients and methods:

#### Study design:

This prospective case-control study will be conducted on 25 pregnant woman coming in current spontaneous abortion and having a history of unexplained spontaneous recurrent abortion (study group) and 25 pregnant woman with healthy pregnancy in the first trimester (control group) with good obstetric history and having at least one living child.

The levels and activity of AAT in maternal serum will be evaluated and compared in both groups.

#### Sample size estimation will be according to:

$$N = \frac{2 \text{standard deviation} \times K}{E^2}$$

Standard deviation: Population of previous literature (0.4).

K: constant (7.8) from statistical table.

E<sup>2</sup>: Minimal change in mean that would be clinically useful.

#### Setting:

This study will be done at Benha university hospital and will start in 2014.

#### Inclusion criteria for the study group:

1. History of first trimester recurrent ( $\geq 2$ ) unexplained miscarriage (cases).

2. **Parity:** Pregnant patient in the first trimester diagnosed as having current spontaneous abortion and having no living children.

3. Maternal age ranges between 21 and 43years.

4. **Gestational age:** First trimester.

5. **Body mass index:** ranges between 18-35.

#### Inclusion criteria for the control group:

The same as the study group except that they are currently pregnant in first trimester and have at least one living child and have good obstetric history with no history of abortion.

#### Exclusion criteria:

1- Absence of inclusion criteria.

2- Multiple gestation pregnancy.

3- Any factor which might contribute as a cause to recurrent miscarriage as uterine structural anomalies, autoimmune diseases,....etc.

#### Method:

- Before inclusion in the study, all patients will sign an informed consent, all included patients will undergo the following;

- History taking include: Personal history will be taken with special attention to age. Present history will include: weeks of current pregnancy (first trimester). Past history will be mentioned regarding medical history (such as: antiphospholipid syndrome, diabetes, hypothyroidism and anemia) and/or surgical history (such as previous caesarean sections, laparoscopy for infertility and appendectomy).

Full obstetric history will be mentioned in details: full term, preterm, miscarriages and all living pregnancies.

After inclusion in either group and undergoing of general, abdominal and local examination. The following will be done:

- **Vaginal ultrasound:** Will be done once included in the study to ascertain the diagnosis of abortion for study group and the exact weeks of gestation and normal fetal cardiac activity for control group.

-If ultrasound findings were not confirmed to either of our group, the patient will be excluded (10 patients were excluded) and replaced by another one.

-Estimation of the level of alpha-lantitrypsin (AAT) in serum in both groups. on the same day of u/s

and at conformal of inclusion in either control or study groups, a 5ml of venous blood will be withdrawn. the blood samples of both groups will be collected and allowed to clot and then centrifuge for 10 minutes. Two milliliters of serum will then transfer into sterile tubes and frozen until assay. AAT levels will be determined by human ELISA.

All patients of the study group will be admitted to the hospital while pregnant women of the control group will have their ultrasound and AAT evaluation during their antenatal visit.

#### Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (Spss Inc, Chicago, ILL Company). Data were expressed as mean  $\pm$  standard deviation, median, IQR and range. Data were tested for normality using Shapiro-Wilks test, assuming normality at  $P > 0.05$ , using Student "t", if normally distributed, or Man Whitney U test and Spearman's correlation coefficient (  $\rho$  ) if not normally distributed. ROC curve was used to determine cutoff value of AAT with optimum sensitivity and specificity in prediction of spontaneous abortion. The accepted level of significance in this work was stated at 0.05 (  $P < 0.05$  was considered significant),

-  $P$  value  $> 0.05$  is non significant (NS)

-  $P < 0.05$  is significant (S)

-  $P \leq 0.001$  is highly significant (HS)

Mean =

Is the sum of the values in a set of data divided by the number of the values in the set. It is denoted by the sign  $\bar{X}$  (called X bar).

$$\bar{X} = \frac{\sum X}{n}$$

Where: X: Denotes any value of observation.

$\sum$ : The Greek capital letter sigma, means the sum of.

N: The number of observations.

#### 3. Results:

Socio-demographic characters of the studied groups were summarized in Table 1. There were no significant differences in age, BMI.

( $26.7 \pm 2.52$ ,  $27.8 \pm 3.05$ ,  $p = 0.15$  and  $26.7 \pm 3.26$ ,  $27.3 \pm 3.77$ ,  $p = 0.59$ ) respectively as  $P > 0.05$ .

Also in the study there is no statistically significant difference between both groups regarding gestational age and Gravidity ( $10.0 \pm 1.50$ ,  $10.2 \pm 1.24$ ,  $p = 0.68$ ,  $2.8 \pm 0.76$ ,  $3.28 \pm 0.73$ ,  $p = 0.028$ ) respectively. But Parity has no statistical significance as the control group was 2.28 but study group has no parity according to inclusion criteria of the study Table 2.

Table 1: Socio-demographic characters of the studied groups

Variable	Patients (N=25)			Controls (N=25)			St."t"	P
	Mean	±SD	Range	Mean	±SD	Range		
Age (ys)	26.7	2.52	21-30	27.8	3.05	23-35	1.46	0.15 (NS)
BMI (kg/m <sup>2</sup> )	26.7	3.26	21.48-32.44	27.3	3.77	19.53-35.16	0.54	0.59 (NS)

Table 2: Obstetric history among the studied groups

Variable	Patients (N=25)			Controls (N=25)			St."t"	P
	Mean	±SD	Range	Mean	±SD	Range		
Gestational age (w)	10.0	1.50	7-13	10.2	1.24	8-12	0.41	0.68 (NS)
Gravidity	2.8	0.76	2-4	3.28	0.73	2-5	2.26	0.028 (S)
Parity	---	---	-----	2.28	0.73	1-4	-----	-----

The decline in mean AAT values was highly significant between the study group and the control group (  $1.65 \pm 0.10, 2.12 \pm 0.35, p < 0.001$  ) respectively Table 3.

Table 3: Comparing the studied groups regarding serum  $\alpha 1$  antitrypsin level

Variable	Patients (N=25)			Controls (N=25)			MWU test	P
	Mean	±SD	Range	Mean	±SD	Range		
AAT	1.65	0.10	1.39-1.82	2.12	0.35	1.68-3.34	5.81	<0.001 (HS)

#### 4. Discussion:

Our prospective study compared the levels and activity of circulating AAT in patients with recurrent pregnancy loss, and normal pregnancies. The study demonstrates a significant decline in circulating AAT concentrations among pregnant women with recurrent pregnancy loss, as compared with normal pregnancies. These results are in agreement with the study performed at 2013, which found decline in the levels of AAT with recurrent abortion and this study is the first to examine the correlation between AAT and pregnancy loss. Previous studies have shown differences in plasma AAT concentrations in hypertensive pregnancies with and without preeclampsia, compared with normal pregnancies. No correlation was found between premature rupture of membranes and the levels or activity of plasma AAT (7). The mechanism behind recurrent abortions has been studied extensively, yet markers that might help in detecting predisposition are still absent. Possible known causes include uterine structural anomalies, abnormal genetics, endocrine factors, immune disorders, embryo-fetal infections and thrombophilias (2), (8).

This study was conducted on 50 patient (25 of normal pregnancy and 25 of recurrent abortion) after general examination and routine investigations. AAT was measured in the first trimester. There was statistically significant increase in AAT during normal

pregnancy and decline in study group of recurrent abortion.

However, the cause is frequently either indefinable or multifactorial AAT is the main serum inhibitor of serine proteases and thus controls tissue degradation and inflammation. In addition, it is an acute phase reactant that protects tissues against the release of proteolytic enzymes. AAT rises in response to infection, trauma and estrogen; thus, inappropriately low levels of AAT may have a negative influence during pregnancy in all its vital roles due to its relative insufficiency. Proteases were shown to exert both negative and positive effects on pregnancy outcome. For example, matrix metalloproteinases (MMPs), the various cathepsins and urokinase plasminogen activators, were shown to regulate trophoblast invasion and implantation by facilitating degradation of extracellular matrix proteins and cell migration. In contrast, the decidua was shown to express protease inhibitors that restricted trophoblast invasiveness (9). MMPs were shown to facilitate embryo implantation by promoting angiogenesis and endometrium vascularization (5), (6). On the other hand, excessive activity of proteases is associated with increased tissue injury, chemokine release and proinflammatory cytokine secretion, which activates decidual and endometrial leukocytes and results in trophoblast apoptosis and pregnancy loss (10).

Although AAT was shown to inhibit the activity of cathepsins, tissue plasminogen activator and kallikrein, which are implicated in trophoblast invasion and implantation, AAT has also been shown to enhance VEGF production and facilitation of smooth muscle myocyte migration. Thus, AAT also promotes angiogenesis and vascularization of the endometrium. Taken together, these studies implicate the necessity of strict regulation of protease activity for successful pregnancy outcomes. Furthermore, these studies suggest that the reduced AAT protease inhibitory capacity observed in both sporadic and recurrent spontaneous abortion groups may contribute, at least in part, to fetal loss (11).

In our study as there is reduced level of AAT observed in the results of the study group (abortion) in relation to the control (normal pregnancy) so it suggests that adequate levels and activity of AAT may serve to create a sustaining environment that can facilitate successful pregnancy outcomes. Future studies should examine greater cohort numbers, earlier time-points, the influence of antithrombotics and the possibility of safe AAT augmentation therapy for individuals within sufficient circulating AAT levels and activity in order to improve pregnancy outcome. The result of our study agrees with the result of study that was done before in 2013 and demonstrated that low level of circulating alpha-1 antitrypsin are associated with spontaneous abortions. And also the results of our study agree with the study that done at 2014 and its result were that  $\alpha 1$  antitrypsin were elevated significantly ( $p \leq 0.05$ ) in all women with pregnancy complications compared with control and may play a role in monitoring the pregnancy complications depending on the fact that  $\alpha 1$ -AT is acute phase protein that protect tissues by its protease inhibitory mechanism and possesses anti-inflammatory activity.

Our findings suggest that further studies should investigate a possible causative relationship between AAT deficiency and the occurrence of pregnancy loss. If such an association were to be found, it could lead to future prevention of pregnancy loss in selected patients by screening for AAT levels, and by subsequent AAT augmentation therapy as readily available for genetic AAT deficiency (12).

### Conclusion:

Our findings revealed that level of alpha one antitrypsin in the maternal serum could play a significant role in pregnancy outcome.

This study demonstrates a significant decline in circulating AAT in patients with recurrent pregnancy loss in first trimesters compared with normal pregnant women in the same trimesters as  $p < 0.001$  which is highly significant. So determination of  $\alpha 1$ AT may be useful for the prediction of pregnancy outcome in first trimester.

The results of our study showed that reduced level of AAT observed in the study group in relation to control one and this agrees with the fact that AAT is an acute active protein and has anti-protease activity that protects tissues and affects outcome of pregnancy.

So our recommendation is to involve alpha one antitrypsin as one of routine investigations for cases of recurrent abortion as such association between AAT and pregnancy loss might lead to future prevention of pregnancy loss in selected patients by administration of AAT accordingly.

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