

First trimester spontaneous abortion: a clinicopathological view

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Abstract

A study of 24 cases of first trimester spontaneous abortions and ten normal ongoing first trimester pregnancies are included. Clinical information (maternal age, gestational age, gravidity, parity, history of prior live or dead babies, residency, history of other diseases, infectious and non infectious.) and laboratory assessment of the cases (complete blood count, biochemical assay of serum malondialdehyde level and pathological confirmation of abortion with measurement of trophoblastic villous diameter). Most cases were found to be within the age group (30-39 years) with higher gravidity and history of previous abortion. Hematological profile (except ESR) and serum malondialdehyde show no significant difference between abortion cases and those with normal first trimester pregnancy, but the malondialdehyde level have a strong statistical relation with trophoblastic villous diameter indicating the possibility of underlying relationship between oxidant injury and villous diameter leading to hydropic or even molar changes.

Key words: Abortion, malondialdehyde, trophoblastic villi

Introduction

Spontaneous abortion is the most common complication of pregnancy and is defined as the passing of a pregnancy prior to completion of the 20th gestational week. It implies delivery of all or any part of the products of conception, with or without a fetus weighing less than 500 g. (Rebecca, 2005). The true incidence of spontaneous abortion is unknown, about 15% of clinically evident pregnancies and 60% of chemically evident pregnancies end in spontaneous

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abortion. Eighty percent of spontaneous abortions occur prior to 12 weeks' gestation. (Zinaman *et al*, 1996).

The incidence of abortion is influenced by the age of the mother and by a number of pregnancy-related factors, including a history of a previous full-term normal pregnancy, the number of previous spontaneous abortions, a previous stillbirth, and a previous infant born with malformations or known genetic defects. Additionally, parental influences, including balanced translocation carriers and medical complications, may influence the rate of spontaneous abortion (Cunningham *et al*, 2010).

Other factors known to be associated with miscarriage include advanced maternal age and a maternal history of recurrent losses (i.e., two or more such outcomes in previous pregnancies), as well as maternal infections such as *Listeria monocytogenes*, *Toxoplasma gondii*, and rubella (Rosai 2004). Poorly controlled maternal insulin-dependent diabetes and thyroid autoimmunity are also linked to spontaneous abortion (Kleinhaus, *et al* 2006) as are antiphospholipid antibodies and factor V Leiden. (ACOG, 1995) Environmental toxins related to this outcome include benzene, gasoline, hydrogen sulfide, lead, and mercury as well as tobacco, alcohol, and caffeine. (Sharara 1998). Lifestyle factors, diet, deficiencies of folic acid or vitamin B12, and maternal obesity may also play a role. (Reznikoff, 2002).

An elevation in serum malondialdehyde (MDA) level is parallel to an increase in lipid peroxidation rate (Esterbauer *et al.*, 1991). At the same time, elevation in lipid peroxide level indicates the presence of oxidative tissue damage as a result of impaired antioxidant defense mechanism (Şehirli *et al.*, 2007) and this oxidative stress may cause the death of cells. (Ramaekers *et al.*, 1997).

Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA). The quantization of MDA is widely used as an indicator of lipid peroxidation (Simsek *et al* 2006).

The etiology of recurrent pregnancy loss remains unclear and is a scientific challenge. Oxidative stress may have a role in the etiology of recurrent pregnancy loss with no known etiology.

This study was a clinicopathological view for the abortion cases trying to clarify some associating or causative factors.

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Material and method

Over a period of 4 months from January to April 2011, twenty four cases of spontaneous abortions in Koya general hospital were sent for histopathological evaluation at our private clinical laboratory. The following information obtained from each patient including full obstetrical history (maternal age, gestational age as confirmed by sonography, gravidity, history of prior live or dead babies, residency, and history of other diseases infectious and non infectious).

Blood samples were obtained from each patient for (Complete Blood Picture, biochemical assay of serum malondialdehyde level and the abortuses tissue were submitted for routine Hematoxylin and Eosin histopathological evaluation).

Microscopic sections were evaluated for villous diameter, and examination of five largest non hydropic villi per section were randomly taken for systematic microscopic examination.

Another ten cases of normal ongoing first trimester pregnancies (as confirmed by ultrasound) were included as a control group. Cases included in the study showed no evidence or past history of genetic diseases, infectious diseases, hypertension, or diabetes.

For the assessment of correlation between various parameters, a statistical Correlation, Analysis Of Variance (ANOVA) were calculated and tested for significance.

Results

This study included patients with spontaneous first trimester abortion in whom pregnancy was confirmed by ultrasound and they are arranged in three groups according to maternal age.

Group I (age 20-29 years):

This group included seven patients (29.1% of cases) with a mean age (22.14 ± 1.95) years.

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The mean gestational age for this group was (7.14 ± 2.35 weeks) which was less than the mean for group 2 and 3. Moreover, the mean gravidity for the patients was (2.42 ± 1.49) which was lower than patients in older age groups that was significantly and positively correlated with history of prior live birth (Para), *P value* 0.0001 a result also noticed in patients older than 40 years. All patients had history of previous abortion (mean 1 ± 0.0) which was less than older age group but still higher than control group (mean 0.4 ± 0.54). On the other hand, the mean Malondialdehyde level among this group was (0.94 ± 0.28 $\mu\text{mol/L}$) which was within the normal range value recorded in all study groups, but its level showed appositive correlation with maternal and gestational age. Additionally, the mean trophoblastic villous diameter recorded in abortuses tissue sections was (327.81 ± 73.81 micrometer) that was less than the mean diameter of both group 2 and 3 and it showed a negative correlation with all studied clinical parameters. All hematological parameters assessed were within the normal reference values for pregnant women in the same age, gestational age and control group. Table 1.

Group II (age 30-39 years):

This group included 12 patients (50% of cases) with a mean maternal age (33.75 ± 3.00 years) and showed significant positive relationship with prior history of live births (Para) *P value* 0.0035. The mean gestational age for this group was (7.5 ± 1.9 weeks) which was comparable to those in older age group. The mean gravidity for the patients was (4.75 ± 1.53) which was higher than control group and group 1 but lower than patients in older age groups (≥ 40 years) which was found to be significantly and positively correlated with history previous abortion (*P value* 0.0026). All patients had history of previous abortion (mean 1.83 ± 1.90) which was the highest record among study groups. The mean Malondialdehyde level among this group was (0.869 ± 0.44 $\mu\text{mol/L}$) which was within the normal range value recorded in all study groups, but its level showed a negative correlation with maternal and previous history of live births. The mean trophoblastic villous diameter recorded in abortuses tissue sections was (355.49 ± 117.38 micrometer) that was higher than the mean diameter of group 1 and found to be negatively correlated with maternal age and gravidity. Moreover, the hematological parameters

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assessed were within the normal reference values for pregnant women in the same age, gestational age and control group. Table2.

Group III (≥ 40 years):

This group included 5 patients (20.82% of cases) with a mean maternal age (40.75 ± 0.95 years) and showed a positive relationship with only serum MDA level but was statistically non significant and negatively correlated with other clinical parameters and the mean gestational age for this group was (7.5 ± 1 weeks) which was comparable to those in group 2, but the mean gravidity for the patients was (5.75 ± 2.06) which was the highest record among study groups and significantly correlated with prior history of live babies and history of abortion, *P values* were 0.05 and 0.019 respectively and all patients had history of previous abortion (mean 1.5 ± 0.5) which was lower than group 2 and was significantly and positively associated with prior history of live births (Para), and *P value* 0.0377. On the other hand, the mean Malondialdehyde level among this group was ($1.18 \pm 0.33 \mu\text{mol/L}$) which was within the normal range value recorded in all study groups. Additionally, the mean trophoblastic villous diameter recorded in abortuses tissue sections was (355.75 ± 57.25 micrometer) that was higher than the mean diameter of all study groups and significantly correlated with past obstetrical history (gravida, Para, abortion) and *P value* were 0.05., 0.0082, and 0.05 respectively. Also, all hematological parameters assessed were within the normal reference values for pregnant women in the same age, gestational age and control group except the total leukocyte count that was higher than normal values (mean $11,425 \pm 2.27$ cells/ mm^3) and the serum malondialdehyde level showed no significant difference between patient and control groups (*P value* 0.5), but in patients serum MDA showed strong statistical association with trophoblastic villous diameter (*P value* 0.00). Tables 4 and 5.

ANOVA test showed positive and significant statistical correlation between study groups (*P value* 0.000).

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Table 1: Study parameters in group 1: (Age 20-29) years (7 cases)

Parameter		Age	Gestation	Gravida	Para	abortion	MDA	Villous diameter
Age	correlation		+	-	-	-	+	-
	<i>P value</i>		0.1551	0.4567	0.5655	0.3487	0.2945	0.466
Gestation	Correlation	+		-	+	-	+	-
	<i>P value</i>	0.1551		0.4567	0.7831	0.1008	0.6974	0.6953
Gravida	Correlation	-	+		+	+	-	-
	<i>P value</i>	0.4567	0.7572		0.0001*	0.3881	0.8393	0.4838
Para	correlation	-	+	+		+	-	-
	<i>P value</i>	0.5655	0.7831	0.0001*		0.3599	0.7705	0.4425
Abortion	Correlation	-	-	+	+		-	+
	<i>P value</i>	0.348	0.1008	0.3881	0.3599		0.8365	0.7184
MDA	Correlation	+	+	-	-	-		-
	<i>P value</i>	0.2945	0.6974	0.8393	0.7705	0.8365		0.1782
Villous Diameter	Correlation	-	-	-	-	-	-	
	<i>P value</i>	0.466	0.6953	0.4838	0.4425	0.7184	0.1782	

+: Positive correlation

-: Negative correlation

*: Significant ($P \text{ value} \leq 0.05$)

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Table 2: Study parameters in group 2 (Age 30-39) (12 cases)

parameter		Age	Gestation	Gravida	Para	abortion	MDA	Villous diameter
Age	correlation		-	+	+	-	-	+
	<i>P value</i>		0.4318	0.9889	0.0035*	0.1976	0.8574	0.0859
Gestation	Correlation	-		+	-	+	+	-
	<i>P value</i>	0.4318		0.0839	0.0819	0.0080*	0.7611	0.4292
Gravida	Correlation	+	+		+	+	+	-
	<i>P value</i>	0.9889	0.0839		1.0000	0.0026*	0.6789	0.4059
Para	correlation	+	-	+		+	-	+
	<i>P value</i>	0.0035*	0.0819	1.0000		0.0370	0.8519	0.1829
Abortion	Correlation	-	+	+	-	+	+	-
	<i>P value</i>	0.1976	0.0080*	0.0026*	0.0370*		0.5284	0.2252
MDA	Correlation	-	+	+	-	+		+
	<i>P value</i>	0.8574	0.7611	0.6789	0.8519	0.5284		0.0815
Villous diameter	Correlation	+	-	-	+	+	+	
	<i>P value</i>	0.0859	0.4292	0.4059	0.1829	0.2252	0.815	

+: Positive correlation

-: Negative correlation

*: Significant ($P \text{ value} \leq 0.05$)

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Table 3: Study parameters in group 3: (age ≥ 40 years) (4 cases)

Parameter		Age	Gestation	Gravida	Para	abortion	MDA	Villous diameter
Age	correlation		-	-	-	-	+	-
	<i>P value</i>		0.8259	0.1134	0.245	0.3487	0.1539	0.2768
Gestation	Correlation	-		+	+	+	+	+
	<i>P value</i>	0.8259		0.4341		0.4226	0.8649	0.2046
Gravida	Correlation	-	-		+	+	-	+
	<i>P value</i>	0.1134	0.8259		0.05*	0.019*	0.4587	0.05*
Para	correlation	-	+	+		+	-	+
	<i>P value</i>	0.2457	0.2222	0.05*		0.0377*	0.55	0.0082
Abortion	Correlation	-	+	+	+		-	+
	<i>P value</i>	0.0955	0.422	0.0198	0.0377*		0.357	0.05*
MDA	Correlation	+	+	-	-	-		-
	<i>P value</i>	0.1539	0.864	0.458	0.55	0.357		0.6377
Villous Diameter	Correlation	-	+	+	+	+	-	
	<i>P value</i>	0.27	0.2040	0.05*	0.0082*	0.05*	0.6377	

+: Positive correlation

-: Negative correlation

*: Significant ($P \text{ value} \leq 0.05$)

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Table 4: Comparison of study parameters between patient and control groups

Parameter	Patient (24 cases)	Control (10 cases)	<i>P value</i>
PCV	36.38 + 0.89	36.4 + 1.17	0.64
ESR	12.38 + 1.69	21.8 + 4.08	0.01
Platelets	253.84+23.45	282.2+25.31	0.88
Reticulocyte	0.15+0.14	0.25+1.16	0.17
Total WBC	0.99+10.08	8.09 + 0.65	0.1
Neutrophils	63.23+2.65	61.8+1.94	0.19
Lymphocytes	34.54+2.83	35.4 + 1.73	0.07
Monocytes	1.54+0.31	1.2 + 0.36	0.09
Eosinophils	1.46+0.37	1.5 + 0.34	0.54
MDA	1.01±0.1	1.11±0.11	0.5
Maternal age	31.43+7.2	27 + 4.39	0.26
Gestation age	1.8+7.5	6.6 + 1.67	0.20
Gravida	2.09+4.22	2.8+1.3	0.15
Para	1.5+2.6	1.4+0.89	0.054
Abortion	1.52+1.5	0.4 + 0.5	0.1

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Table 5: General statistical correlation between different clinical parameters in study groups

	Age	Gestation	Gravida	Para	Abortion	MDA	Diameter
Age		0.7		0.0006*	0.80	0.0006*	0.5
Gestation	0.7		0.11	0.7	0.0092*	0.60	0.6
Gravida	0.0073*	0.11		0.0003*	0.0028*	0.91	0.91
Para	0.0006*	0.7	0.0003*		0.51	0.79	0.7
Abortion	0.80	0.53	0.0028	0.51		0.65	0.65
MDA	0.5	0.6	0.91	0.7	0.65		0.000*
Diameter	0.5	0.6	0.9	0.79	0.65	0.000*	

Discussion

Spontaneous abortion still represents a major ,poorly understood clinical problem with a variety of causes and most cases occur during the first trimester of pregnancy(less than or equal to 13 gestational weeks (Margareta 2002).

Early abortion specimens are one of the most common specimens submitted to pathology. They are quite varied in their composition; they may consist of blood clot admixed with minimal tissue, fragmented villous tissue and fetal parts, a completely intact gestational sac, or anything in between. (Rebecca 2005).

In this study, the incidence of first trimester abortions was found to be 18.22 %(74 cases out of 406) and this is comparable to the world wide record of clinical abortion cases of 15 % (Zinaman *et al.*, 1996,Rosai 2004)despite the fact that the real incidence is much higher due to very early missed cases and those not receiving proper clinical evaluation.

The mean maternal age for the study groups are (31.43 ± 7.22 years) higher than the control group (27.6 ± 4.39 years) and other study with mean age (24.78 years)(Clarisa *et al.*,2005) as compared to the current study when age as a risk factor for abortion was detected in group 2(30-39 years)

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Maternal age has showed a an increase in trophoblastic villous diameter as the older the patient develop larger villi that may contribute to subsequent hydropic or molar changes despite the fact that period of fetal retention in uterus has been associated with villous diameter (Carien *et al.*, 1998), although some authors consider size of villous diameter an inaccurate and insensitive parameter. (Carien *et al.*, 1998).

It is reported that the increase in maternal age reduces the risk of abortion (Sundeil *et al.*, 1990) this in part may be attributed to the age related higher birth ranks.

Women with abortion were presented at higher gestational age in the study group (7.54 ± 1.89 weeks) which was more than the control group (6.6 ± 1.6 weeks) as it showed a strong statistical relationship with history of prior abortion (*P value* 0.008) in group two only that may includes another influencing factor beside genetic anomalies that are known to play a critical role in many early miscarriages (Kazerouni *et al.*, 2009). Other factors known to be associated with miscarriage include advanced maternal age and a maternal history of recurrent losses (i.e., two or more such outcomes in previous pregnancies), as well as maternal infections (Kleinhaus, *et al.*, 2006).

As gestational age increases, the increased blood volume, increased uterine size, and increased fetal and placental tissue could increase the risk of complications such as abortions.(Mona *et al.*, 1999) and most pregnant women seek for prenatal care late toward the end of first trimester. The variability of gestation age at early pregnancy is non dependant by sonography(Kazerouni, *et al.*, 2009)Although there is good data showing that hCG is the most valuable diagnostic test before 6 weeks gestation,(Clarisa *et al.*, 2005).

Because gestational age is strongly associated with pregnancy loss rates, (Aaron *et al* 2006) some maternal infection associated with congenital infection occurring at around 7-8 weeks of gestation. (Margareta *et al.*, 2002).

Normal mesenchymal villi found throughout gestation and when maximum (0-8weeks) their size 120-250 micron which is less than the mean record in the current study (372.7 micron) in group 2. But in early abortion specimens, pathological changes often do not provide information on the cause of the pregnancy loss. The few exceptions noted above include abnormalities of the implantation site vessels and excessive inflammation and necrosis.

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However, the pathologic changes in abortion specimens are more often related to the timing of embryonic death and the age of the conceptus at the time of death than to the cause of the pregnancy failure.

Obstetrical history show a strong relationship in the current study in which Gravida 4.22 ± 2.09 Para 2.60 ± 1.59 Abortion 1.52 ± 1.50 (100 %has history of abortion.) while the control group had Gravida 2.8 ± 1.30 , Para 1.4 ± 0.89 , and Abortion 0.4 ± 0.54 . This was close to the record f a comparable age group in that parity and history of previous abortion is a risk factor for subsequent abortions.

Hematological parameters showed that the PCV (mean 36.38 ± 0.89 %) was not significantly different from the control group (36.4 ± 1.17 %) indicating that anemia was not a factor and despite the fact that the hemoglobin is decreased with more advanced pregnancy a and indicates that the bleeding at time of patient presentation was insignificant. (Hasan *et al.*, 2009).

Although the total WBC and differential count in patients was insignificantly higher than control group (*P value* 0.1) but this cannot exclude the possibility of infectious etiology for abortion since about 29.1% of patients were from rural areas beside the high rate of consanguinity marriages that increases the rate of chromosomal abnormalities as an etiology for abortion. Microorganisms like Toxoplasmosis, Listeria, Cytomegalovirus and parvovirus are known to cause sporadic pregnancy loss but this cannot be attributed alone to infectious etiology. (Rosai, 2004).

The high ESR (*P value* 0.01) may be a non specific inflammatory response to the dead embryo or may be due to underlying infectious etiology that needs further evaluation. The reticulocyte count was assessed to detect the possibility of hemolytic anemia specially thalassemia which is a quite challenging problem in this region ,but it showed no significant difference as compared with the control group. (*P value* 0.17)

Malondialdehyde (MDA) was assessed in the study groups as a marker lipid peroxidation of possible oxidative injury in the etiology of abortion, but it showed no significant difference as compared with the control group (*P value* 2) but it showed a high statistical association with increasing maternal age (*P value* 0.0006) that may be explained by the gradual cumulative exposure to environmental oxidant injury.

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MDA also showed a significant statistical association with villous diameter (*P* value 0) indicating that despite the current status of serum MDA levels in study groups may be non reflective to the actual population value due to sample inadequacy and oxidant injury to proliferating embryonic cells remain a crucial event in the pathogenesis of abortion and increase in villous diameter. Some clinicians feel that the intake of fresh food like fruits, raw vegetables with high levels of antioxidants is likely to improve the pregnancy outcome when compared with those taking processed food that may put the estimation of serum MDA as a possible useful marker during different stages of pregnancy for detection and categorization of high risk early pregnancies in which the trophoblastic villi may increase in size with subsequent hydropic or even molar changes.

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مرضية سريرية الاجهاض التلقائي في الثلث الاول من الحمل: نظرة

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الخلاصة

شملت الدراسة 24 من حالات الاجهاض التلقائي و 10 حالات حمل طبيعية في الثلث الأول من الحمل.

تم اخذ المعلومات السريرية (عمر الأم، عمر الحمل، عدد مرات الحمل، عدد الأطفال، حالات سابقة لأطفال احياء او متوفين، محل الإقامة، الأصابة بامراض اخرى معدية وغير معدية) والتقييم المختبري للحالات (صورة الدم الكاملة، التقدير الكيموحيوي لمستوى المالوندايديهايد والتثبت النسيجي المرضي للاجهاض وقياس قطر زغابات الأرومة الغازية).

وجد ان معظم الحالات كانت ضمن الفئة العمرية 30-39 سنة واطهرت المجموعة معدلات اعلى لعدد مرات الحمل وحالات الإجهاض السابقة.

تقييم فحص الدم (عدا معدل ترسب كريات الدم الحمراء) ومستوى المالوندايديهايد اظهرت عدم وجود فرق معنوي بين حالات الأجهاض و حالات الحمل الطبيعية في الثلث الأول من الحمل بينما اظهر المالوندايديهايد علاقة احصائية بقطر زغابات الأرومة الغازية مما يدل على احتمالية وجود علاقة بين الضرر التاكسدي و قطر زغابات الأرومة الغازية التي من الممكن ان تؤدي الى تغييرات مائية او حتى عنقودية.

الكلمات المفتاحية: المالوندايديهايد، زغابات الأرومة الغازية.