



Association of Antiphospholipid Antibody and Vitamin D in Women Suffering from Recurrent Pregnancy Loss

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ABSTRACT:

BACKGROUND:

Recurrent pregnancy loss is a heterogenous reproductive problem with multiple etiologies. Antiphospholipid antibody has been associated with recurrent miscarriage. Vitamin D₃ helps to support successful implantation through immunological action. Vitamin D₃ deficiency highly associated with recurrent miscarriage.

OBJECTIVE:

To find association between antiphospholipid antibody and vitamin D₃ (1, 25 dihydrocholecalcifrol) in women with recurrent pregnancy loss.

PATIENTS AND METHODS:

A comparative cross sectional study done at Al-Elwiya Maternity Teaching Hospital in Baghdad including A 58 participants suffering from recurrent miscarriage and another 58 control group. Vitamin D₃ level and antiphospholipid antibody were analyzed and data have arranged for analysis.

RESULTS:

Vitamin D₃ was found to be low in 96.6% of women with recurrent miscarriage the mean was (11ng/ml) which is significantly lower than mean vitamin D₃ of the control group (37.6 ng/ml (p<0.001))

Vitamin D₃ deficiency and Antiphospholipid antibodies were present in 29.3% of women with recurrent miscarriage. There were a highly significant association between vitamin D₃ deficiency and antiphospholipid antibodies in women with recurrent miscarriage (p<0.001).

CONCLUSION:

The antiphospholipid antibodies and vitamin D₃ deficiency were found to be highly associated together with recurrent miscarriage.

KEYWORDS: Vit D, Recurrent Pregnancy Loss.

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INTRODUCTION:

Recurrent pregnancy loss (RPL) is defined as two or more failed pregnancies, which have been documented by either ultrasound or histopathological examination⁽¹⁾. The definition of recurrent pregnancy loss (RPL) has long been debated and differs among international societies. According to the American Society for Reproductive Medicine, it is defined as two or more clinical pregnancy losses (documented by ultrasonography or histopathologic examination), but not necessarily consecutive^(2,3). Royal College of Obstetricians and Gynecologists, and the European Society for Human Reproduction

and Embryology, the RPL refers to three consecutive pregnancy losses, including not visualized ones⁽³⁾.

The incidence of RPL varies widely between reports because of the differences in the definitions and criteria used, it affects about 0.4-1% of couples. Accepted etiologies for RPL include parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, certain uterine anatomic abnormalities, and the antiphospholipid antibody syndrome (APS). Other possible etiologies include additional endocrine disorders, heritable and/or acquired

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thrombophilias, immunologic abnormalities, and environmental causes. After evaluation for these causes, more than 33% of all cases remain unexplained^(2,3).

Vitamin D deficiency is endemic for both men and women and across different age groups. Low vitamin D level has been linked to major common health problems in populations, particularly inflammatory conditions such as cardiovascular disease, cancers, diabetes, autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and others⁽⁴⁾. In addition, vitamin D deficiency is a predisposing factor for various female diseases such as osteoporosis, uterine fibroids, endometriosis, and breast cancer and a risk factor for obstetrical complications such as gestational diabetes and preeclampsia⁽⁵⁾. Recently, reported that 47.4% of women with recurrent pregnancy losses (RPL) have vitamin D deficiency. In women with RPL, decreased vitamin D level was associated with the increased prevalence of antiphospholipid antibody, antinuclear antigen antibody, anti-ssDNA, and anti-thyroperoxidase antibody. In addition, peripheral blood CD19+ B and CD56+ NK-cell levels and NK cytotoxicity were significantly higher in women with low vitamin D level when compared with those of normal vitamin D level⁽⁶⁾. It has been reported that vitamin D could prevent the occurrence of antiphospholipid syndrome (APS) by reducing the expression levels of anti-β2 glycoprotein and tissue factor in RPL cases with APS⁽⁷⁾.

Miscarriage is a relatively common event, occurring in 15%–25% of pregnancies, and increasing in prevalence with maternal age⁽²⁾. Indeed, the risk is between 9% and 12% in women aged ≤35 years but increases to 50% in women aged >40. Miscarriage can be further classified as embryonic loss when it occurs before 10 gestational weeks and fetal loss when it occurs after 10 gestational weeks, because factors associated with each may differ⁽¹⁾.

Aim of study is to find association between antiphospholipid antibody and Vitamin D 1,25 dihydrocholecalciferol in women with recurrent pregnancy loss.

PATIENTS AND METHODS:

Design, settings & sampling

The current study is a comparative cross sectional in Al-Elwiya Maternity Teaching Hospital in Baghdad. The study was approved by *Gynecology & Obstetrics Scientific Council of Arab Board for Health Specialties* and oral informed consent was

taken from the participants before being enrolled in the study.

They were collected while they are in the hospital with sign and symptom of miscarriage and having two or more previous miscarriages, a 58 participant were collected during period of study. A similar number of pregnant women with no such history were selected while attending outpatient antenatal care clinic and considered as control group.

Inclusion criteria

1. Pregnant women with recent miscarriage.
2. Previous 2 or more miscarriage.
3. Gestational age ≤ 22 weeks.
4. Normal uterine cavity.

Exclusion criteria

1. Single miscarriage.
2. Poorly controlled types 1 and 2 diabetes mellitus.
3. Hypertension.
4. Thyroid disorders.
5. Adrenal disorders.
6. Inflammatory and infectious diseases.
7. Hereditary diseases.
8. Polycystic ovarian syndrome.

Data collection

Data of study participants were collected directly by the researcher through a prepared questionnaire. The design of the questionnaire was accomplished by the supervisor. It included the followings:

- Sociodemographic characteristics of study participants: Age and occupation.
- Gestational history of study participants: History of gravidity, parity and abortion, in addition to gestational age.
- Past medical and surgical history of study participants: Medical and surgical problems.
- Family history of study participants.
- Socioeconomic history of study participants.
- Investigations findings of study participants: Vitamin D and antiphospholipid antigens (M&G).

After taking history and examination, an ultrasound examination was done to confirm their pregnancy condition; the participants were found to be either suffering from threatened or missed miscarriage. A sample of 5 ml blood was taken from each study participant and sent to Laboratory of the hospital.

Laboratory testing: The Antiphospholipid IgG and IgM were measured by using ELISA kits with normal value up to 10U/ml. High-performance liquid chromatography methods quantitate 25-hydroxy vitamin D. HPLC methods are available in kit form (Hitachi High-Technologies

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Corporation Tokyo, Japan, and Thermo Fisher Scientific, Sunnyvale, CA) in an effort to standardize test quality and to make the assays more cost effective and less labor intensive.

Vitamin D deficiency diagnosis was based on levels <20 ng/mL; while vitamin D insufficiency was defined as levels ranging from 20 to 29.9 ng/mL, while normal level was 30 ng/mL and more.

Statistical analysis:

The data were analyzed using Microsoft excel program and Statistical Package for Social Sciences (SPSS) version 23. Outcomes of analysis were arranged in scales variables (means & standard deviation) and in categorical variables. Chi square test was used for comparison between categorical data (Fishers exact test applied when expected variable was less than 20% of total). Independent sample t-test was used to compare between two means. The level of significance (p value) was set as ≤ 0.05 .

RESULTS:

The study included 58 pregnant women with recurrent pregnancy loss with mean age of

28.7 \pm 7.1 years; 5.2% of them were in age group of less than 20 years, 48.2% of them were in age group 20-29 years and 46.6% of them were in age group 30-39 years. Most of pregnant women with recurrent pregnancy loss were housewives (94.8%).

Regarding family history of recurrent abortion was positive only in 8.6% of them. The socioeconomic history was low in 24.2%, moderate in 67.2% and high in 8.6% of them.

Mean gravidity of pregnant women with recurrent pregnancy loss was 5.6 \pm 2.1; 41.4% of them had gravida of 3-4, 34.5% of them had gravida of 4-6 and 24.1% of them had gravida of more than 6.

Mean parity was 2 \pm 2; 29.3% of them were nulliparous, 60.4% of them had parity of 1-3 and 10.3% of them had parity of more than 3. Mean miscarriage history was 3 \pm 1; 86.2% of them had miscarriages of 2-3 and 13.8% of them had miscarriages of more than 3. Mean gestational age was 14.2 \pm 4.5 weeks; 25.9% of them had GA of 6-12 weeks, 44.8% of them had GA of 13-18 weeks and 29.3% of them had GA of 19-22 weeks as shown in table 1.

Table 1: Gestational history of pregnant women with recurrent pregnancy loss.

Variable	No.	%
Gravidity mean \pm SD (5.6 \pm 2.1)		
3-4	24	41.4
4-6	20	34.5
>6	14	24.1
Total	58	100.0
Parity mean \pm SD (2 \pm 2)		
Nulliparous	17	29.3
1-3	35	60.4
>3	6	10.3
Total	58	100.0
Miscarriage history mean \pm SD (3 \pm 1)		
2-3	50	86.2
>3	8	13.8
Total	58	100.0
Gestational age mean \pm SD (14.2 \pm 4.5 weeks)		
6-12 weeks	15	25.9
13-18 weeks	26	44.8
19-22 weeks	17	29.3
Total	58	100.0

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Mean vitamin D of pregnant women with recurrent pregnancy loss was 11 ± 9.5 ng/ml; 96.6% of them had low vitamin D level. Mean APL IgM of pregnant women with recurrent pregnancy loss was 5.4 ± 4.2 MPL-U/ml; 19% of them had positive APL-IgM. Mean APL IgG of pregnant women

with recurrent pregnancy loss was 5.8 ± 4.6 GPL-U/ml; 15.5% of them had positive APL-IgG. Vitamin D deficiency and APL was present among 29.3% of pregnant women with recurrent pregnancy loss, as shown in table 2.

Table 2: Investigations findings of pregnant women with recurrent pregnancy loss.

Variable	No.	%
Vitamin D mean±SD (11 ± 9.5 ng/ml)		
Normal	2	3.4
Low	56	96.6
Total	58	100.0
APL IgM mean±SD (5.4 ± 4.2 MPL-U/ml)		
Positive	11	19.0
Negative	47	81.0
Total	58	100.0
APL IgG mean±SD (5.8 ± 4.6 GPL-U/ml)		
Positive	9	15.5
Negative	49	84.5
Total	58	100.0
Vitamin D deficiency and APL		
Positive	17	29.3
Negative	41	70.7
Total	58	100.0

A highly significant association was observed between low vitamin D level and pregnant women with recurrent pregnancy loss ($p < 0.001$), mean vitamin D of pregnant women with recurrent pregnancy loss was 11 ng/ml which was significantly lower than mean vitamin D of 37.6 ng/ml ($p < 0.001$). There was a significant association between positive APL IgM and recurrent pregnancy loss ($p = 0.05$), mean APL IgM

was significantly higher among pregnant women with recurrent pregnancy loss ($p < 0.001$). A highly significant association was observed between positive APL IgG and pregnant women with no pregnancy loss ($p < 0.001$). There was a highly significant association between positive vitamin D deficiency and APL with recurrent pregnancy loss ($p < 0.001$) as shown in table 3.

Table 3: Distribution of investigations findings according to study groups.

Variable	Recurrent PL		No PL		P
	No.	%	No.	%	
Vitamin D					
Normal	2	3.4	54	93.1	<0.001 *S
Low	56	96.6	4	6.9	
Mean±SD (ng/ml)	11 ± 9.5		37.6 ± 6.5		<0.001 **S
APL IgM					
Positive	11	19.0	4	6.9	0.05 *S
Negative	47	81.0	54	93.1	
Mean±SD (U/ml)	5.4 ± 4.2		2.4 ± 2.1		<0.001 **S

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APL Ig G					0.02**S
Positive	9	15.5	2	3.4	
Negative	49	84.5	56	96.6	
Mean±SD (U/ml)	5.8±4.6		2.9±2.5		<0.001**S
Vitamin D deficiency and APL					<0.001**S
Positive	17	29.3	0	-	
Negative	41	70.7	58	100.0	

*Chi-square test, **Independent sample t-test, NS=Not significant, S=Significant.

DISCUSSION:

In spite of fact that about one-third of causes of recurrent pregnancy loss were not identified, genetic causes, thyroid dysfunction, uterine anomalies, diabetes mellitus, antiphospholipid antibodies, vitamin D deficiency and others were reported as responsible for recurrent pregnancy loss⁽⁸⁾.

Present study showed that 96.6% of pregnant women with RPL had vitamin D deficiency. This figure is higher than prevalence of (60%) for vitamin D deficiency among pregnant women with recurrent pregnancy loss reported by Mohammed and Al-Qani study in Iraq⁽⁹⁾, and also higher than results of Ghaedi et al⁽¹⁰⁾, study in Iran who reported, prevalence of vitamin D deficiency among pregnant women with RPL was (33.3%) in comparison to (15%) for pregnant women with single pregnancy loss.

Vitamin deficiency in Iraq is reported to be high among females in Northern provinces reaching 82% of all population groups⁽¹¹⁾. High prevalence of vitamin D deficiency in our study might be attributed to many reasons like westernization of dietary habits of our community in last year's and poor antenatal care program of pregnant women.

Current study showed that 19% of pregnant women with RPL had positive IgM for APL and 15.5% of them had positive IgG for APL, while 29.3% of pregnant women with RPL had vitamin D deficiency and positive APL. These findings are higher than results of Upadhyay et al⁽⁸⁾, study in India, which found that 23.6% of pregnant women with RPL had positive APL while 10.9% of pregnant women with RPL had vitamin D deficiency and positive APL and is lower than results of Ota et al⁽⁶⁾, study in USA, which found that 39.7% of pregnant women with RPL had vitamin D deficiency and positive APL.

Al Samarrai et al⁽¹²⁾, study in Iraq reported that antiphospholipid antibodies represented the main cause of recurrent pregnancy loss in mid-trimester of pregnancy.

Several literatures have been found that positive antiphospholipid antibody was accompanied with higher rates of vitamin D deficiency and this link between both vitamin D deficiency and positive APL in pregnancy is related to many bad pregnancy outcomes specifically recurrent spontaneous abortions^(13,14).

A highly significant association is observed between low vitamin D level and pregnant women with recurrent pregnancy loss (p<0.001). This finding is consistent with results of Andersen et al⁽¹⁵⁾, a population based study in Denmark which revealed that vitamin D deficiency in early pregnancy is associated with recurrent pregnancy loss and stated that this vitamin D deficiency is a modifiable risk factor for RPL which could prevented by vitamin D supplementation in early pregnancy. Similarly, Hou et al⁽¹⁶⁾, study in China, stated that deficiency of vitamin D in early pregnancy is associated with pregnancy loss and this risk of pregnancy loss is increased if associated with previous history of pregnancy loss.

An Iraqi study, reported that vitamin D deficiency in early pregnancy with hyperemesis gravidarum and low calcium level of pregnant women were highly related with pregnancy complications like miscarriage and fetal death⁽¹⁷⁾.

Our study showed that means of IgM and IgG for APL of pregnant women with RPL were significantly higher than pregnant women with no pregnancy loss. These findings are in agreement with results of Yassin and Jawad study in Iraq⁽¹⁸⁾, which reported that higher means of IgM and IgG antibodies for antiphospholipid were significantly related to adverse pregnancy outcomes especially recurrent pregnancy loss.

CONCLUSION:

Antiphospholipid antibodies and vitamin D₃ deficiency were found to be highly associated and vitamin D deficiency can play a role in development of recurrent miscarriages.

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