BENHA MEDICAL JOURNAL bmj
Print ISSN 1110-208X
Online ISSN 2357-0016

Original article

Vitamin D Supplementation on Unexplained Early Recurrent Pregnancy Loss

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Received: 29 March 2022

Accepted: 25 July 2022

Abstract:

Background: A low level of vitamin D is strongly correlated with a decreased calcium level, which in turn leads to inadequate mineralization of bones with subsequent development of rickets in children or osteoporosis in adults. It results not only in bone deformation, but also in high susceptibility of falls and bone fractures. Thus, proper vitamin D supplementation according to recent standards is essential for maintenance of the body homeostasis. The aim of the present study was to clinically assess the effect of empirical vitamin D supplementation on unexplained recurrent early pregnancy loss. **Methods:** This study was a prospective study (Clinical trial study) which included 80 women suffered from unexplained RPL. They were selected carefully from outpatient clinic in Banha University Hospitals. **Results:** Parity showed a significant difference between both groups; it ranged from 0-2 in group A and from 0-3 in group B. No significant differences were noted between both groups regarding age. No CMV, toxoplasmosis, or rubella infections were reported in both groups. HCT was significantly higher in group A (37.3 %) compared to group B (36.3%). No positive APA was detected in both groups. Abortion showed non-significant difference between both groups. **Conclusion:** Vitamin D minimize the incidence of abortion among women with unexplained recurrent early pregnancy loss, but this decrease was not significant. The effects of vitamin D on the immune regulation of RPL indicate that vitamin D might be used as an alternative therapy in the future.

Keywords: Vitamin D Supplementation; Unexplained; Recurrent; Pregnancy Loss.

Introduction:

Recurrent pregnancy loss (RPL) is defined as two or more failed clinical pregnancies as documented by ultrasonography or histopathologic examination, also known as three consecutive pregnancy losses ***(1)***.

Pregnancy loss is an adverse outcome of pregnancy in which conception does not result in a live-born child. Of clinically recognized pregnancies, 10–15% terminate with spontaneous loss. Early pregnancy loss (spontaneous miscarriage) is defined as the spontaneous termination of pregnancy before 12 gestational weeks. The great majority of pregnancy losses occur early, before 12 weeks' gestation; hence, the pathophysiology is enough to be of major concern. Several mechanisms have previously been described for the pathogenesis of recurrent PL, including chromosomal anomalies, hormonal problems, uterine abnormalities, infections, autoimmune disorders and thrombophilias, and up to 50% of cases of recurrent PL will not have a clearly defined etiology ***(2).***

Vitamin D, a steroid hormone, is well known to be involved in calcium-phosphate homeostasis and bone metabolism ***(3).***

In the past decade, non-skeletal effects of vitamin D via non-genomic responses have been reported in many organs .The target organs for the non-classical actions of the vitamin D include the adaptive and innate immune systems, pancreatic β-cells, the heart and cardiovascular system, the brain and reproductive tissues ***(4).***

Tissue responses to vitamin D include regulation of hormone secretion, modulation of immune responses, and a control of cellular proliferation and differentiation ***(5).***

Vitamin D was also reported to inhibit proliferation of T helper 1 (Th1) cells and limit their production of cytokines, such as interferon gamma (IFN-γ), interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α). Conversely, vitamin D induces T helper 2 (Th2) cytokines, such as IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 ***(6).***

A dominant Th2 immune response is important to maintain maternal–fetal relationship for successful pregnancy. In contrast autoimmunity and dysregulated cellular immune reactions may be responsible for immunological alterations leading to RPL ***(7).***

Furthermore, in many studies vitamin D has been presented as a modifiable environmental factor for Th1-mediated autoimmune disease and appears to be important for susceptibility to and severity of the disease ***(8).***

Vitamin D also regulates B cell immunity ***(9).***It down-regulates the proliferation and differentiation of B lymphocytes and inhibits IgG production.

With these immune modulatory effects of vitamin D, it has been speculated that vitamin D could act as an immune regulator during implantation and play an important role in reproductive capacity ***(10).***

In early pregnancy, trophoblasts produce and respond to vitamin D, and some investigators have demonstrated that vitamin D influences local anti-inflammatory responses and induces decidualization for successful pregnancy ***(11).***

Others find that vitamin D sufficiency is associated with increased IVF pregnancy rates ***(12).*** The role of vitamin D in human reproduction has been increasingly recognized as important. However, the effect of vitamin D in recurrent pregnancy losses (RPL) has not been confirmed yet.

The aim of this study is to clinically assess the effect of empirical vitamin D supplementation on unexplained recurrent early pregnancy loss.

Patients and Methods:

Study setting :

The study had been conducted at the obstetrics and gynecology department, Benha University Hospital, during the period from January 2021 to december 2021.

This prospective clinically controlled study include 80 women suffered from unexplained RPL. They were selected carefully from outpatient clinic in Banha University Hospitals. They were divided into two groups:

I. **Study group:** (40 patients) women were given empirical vit D supplementation.
II. **Control group**: (40 patients) women with no addition of vit D supplementation.

The study was approved by the ethical committee of Benha Faculty of Medicine. A written informed consent was taken from all participants, before their enrollment in the study.

Inclusion Criteria for groups :

1. Age group : 20y - 35y
2. Pregnant females from 5 weeks (positive pregnancy test).
3. With a history of early unexplained RPL (defined as three or more consecutive spontaneous abortions prior) <12 weeks.

Exclusion Criteria for groups:

1. Any woman on vit D supplementation for the last 3 months.
2. Women with known causes of RPL:

- Chromosomal disorders generally detected on karyotyping.
- Women with antiphospholipid syndrome.
- Women with chronic exposure to toxins and alcohol.
- Women with uterine anomalies.
- Women with infections as toxoplasmosis, cytomegalo virus, rubella.
- Women with luteal phase defect.
- Women with autoimmune disease.

3. Medical disorders with pregnancy:

- Women with severe renal dysfunction.
- Women with severe liver disease.
- Women with thyroid, parathyroid or adrenal disorders.
- Women with type 1 diabetes.

Methods

Patients were subjected to:

Complete history taking:

1. Personal history including: Name, Age, marital state, address.
2. Menstrual history: including age of Menarche, menstrual disturbance, dysmenorrhea, related symptoms.
3. Obstetric history.
4. Present history: of chronic diseases and medication.
5. Past history of HTN, DM.
6. Family history of similar condition or diabetes.

A general questionnaire were completed through a face-to-face interview conducted by the researchers including:

The participants' socio-demographic characteristics and information about prior abortion, smoking, alcohol consumption, sunscreen usage, diseases, medications, and use of food supplements.

Examination:

A. General examination:

- **Vital signs** (Blood pressure, Temperature, Heart rate, Respiratory rate),
- **Signs of** (Pallor, Cyanosis, Jaundice, and Lymph node enlargement).
- **Body Mass Index(BMI):**

$$= \frac{\textbf{Weight in Kg}}{(\textbf{Heigh} \square \textbf{ in meters})^2}$$

-It was obtained from their weight and height recorded by midwifes during the first prenatal consultation at the beginning of the first trimester of pregnancy

-An individual would be considered to be underweight if his/her BMI was in the range of 15 to 19.9, normal weight if the BMI was 20 to 24.9, overweight if the BMI was 25 to 29.9, and obese if it was 30 to 35 or greater ***(13).***

- **Laboratory investigation:**
  - **Complete blood picture (CBC):** hemoglobin concentration (Hb %), red blood cells (RBCs), white blood cells (WBCs), platelet count.
  - **Random blood sugar**
  - **TSH**
  - **Anticardoilipin antibody Ig G, Ig M.**

Statistical Analysis:

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) **(**Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage , quantitative continues group represent by mean ± SD , the following tests were used to test differences for significance;., correlation by Pearson's correlation or Spearman's . P value was set at <0.05 for significant results & <0.001 for high significant result.

Results

Parity showed a significant difference between both groups; it ranged from 0-2 in group A and from 0-3 in group B (P-value = 0.025).

All cases are rural working outdoor.

No significant differences were noted between both groups regarding age (P-value = 0.869), BMI (P-value = 0.128), gravidity (P-value = 0.84), number of abortions (P-value = 0.364), education level (P-value = 0.245), and sunscreen use (P-value = 0.502) ***(Table 1)***.

HCT was significantly higher in group A (37.3 %) compared to group B (36.3%); P-value was 0.003. No significant differences were reported between both groups regarding Hb (P-value = 0.144), platelets (P-value = 0.45), RBS (P-value = 0.376), and TSH (P-value = 0.211) ***(Table 2).***

The outcome of therapy (continued pregnancy, and abortion) showed non-

significant difference between both groups (P-value = 0.650) ***(Table 3& figure 1).***

Table (1) General characteristics in both groups

		Group A (n = 40)	Group B (n = 40)	P-value
Age (years)	Mean ±SD	27 ±4	27 ±4	0.869
BMI	Mean ±SD	28 ±5	26 ±6	0.128
Gravidity	Median (range)	5 (3 - 8)	5 (3 - 8)	0.74
Parity	Median (range)	1 (0 - 2)	1 (0 - 3)	0.025
Number of abortions	Median (range)	3 (1 - 5)	3 (2 - 6)	0.364
Smoking	n (%)	0 (0.0)	0 (0.0)	-
Alcohol	n (%)	0 (0.0)	0 (0.0)	-
Education level	Low n (%)	23 (57.5)	28 (70.0)	0.245
	High n (%)	17 (42.5)	12 (30.0)	
Sunscreen usage	n (%)	18 (45.0)	21 (52.5)	0.502

Independent t-test or Mann Whitney U test was used for numerical data. Chi-square test was used for categorical data
BMI: Body mass index

Table (2) Laboratory findings in both groups

		Group A (n = 40)	Group B (n = 40)	P-value
Hb (g/dl)	Mean ±SD	10.8 ±0.7	11.1 ±0.7	0.144
HCT (%)	Mean ±SD	37.3 ±1	36.3 ±1.7	0.003
Platelets	Mean ±SD	227 ±31	221 ±34	0.45
RBS (mg/dl)	Mean ±SD	101 ±15	104 ±14	0.376

TSH	Mean ±SD	2.677 ±1.089	2.378 ±1.03	0.211

Independent t-test was used

Table (3) Outcome of pregnancy in both groups

		Group A (n = 40)	Group B (n = 40)	P-value
Outcome	Continued n (%)	18 (45.0)	15 (37.5)	0.650
	Abortion n (%)	22 (55.0)	25 (62.5)	

Chi-square test was used

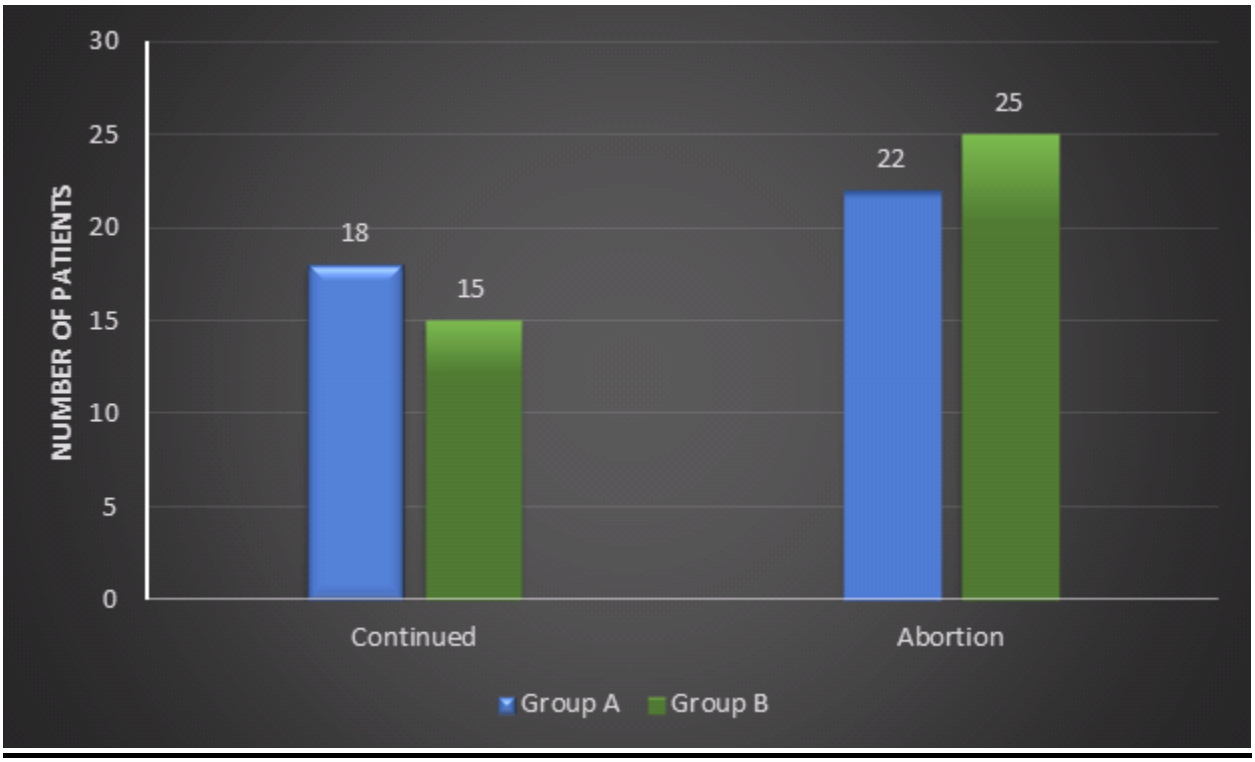

Fig. (1) Outcome of pregnancy in both groups

Discussion

In our study, as regard General characteristics in both groups; Parity showed a significant difference between both groups; it ranged from 0-2 in group A and from 0-3 in group B (P-value = 0.025). No significant differences were noted between both groups regarding age (P-value = 0.869), BMI (P-value = 0.128), gravidity (P-value = 0.84), number of abortions (P-value = 0.364), education level (P-value = 0.245), and sunscreen use (P-value = 0.502).

Our results were supported by study of other researchers ***(14)*** as they reported that there were no significant differences were noted between their studied groups as regard age, BMI, educational level and gravidity. Three out of eighty patients (one in the intervention group and two in the control group) were excluded due to unwillingness to participate and 77 patients were finally evaluated in the final analysis. The average age of the patients was equal to 26.1±4.28 and 26.29±4.43 years in the intervention and control groups, respectively (p=0.85).

Also, other researchers ***(15)*** revealed that there were no significant differences in age and BMI between the two groups. The mean age and BMI of the patients with unexplained recurrent fetal loss were 23.3 ± 6.00 and 24.3 ± 5.00 years, and those of the control group were 25.6 ± 6.00 (p = 0.71) and 23.5 ± 6.03 (p = 0.51), respectively.

The infectious diseases are commonly considered to cause recurrent abortion at a rate as low as 4%. Indeed, all microorganisms can produce acute infection and then occasional abortion, but only a few can induce chronic maternal disease capable of causing recurrent abortion. The rate of occasional abortions due to embryo–fetal infections is believed to range from 10 to 15% but this figure is likely to be underestimated, because of subclinical abortions occurring at an early stage of gestation that remain undiagnosed. In fact, the diagnosis of abortion from infection can only be made retrospectively, based on histological examination of fetal and placental tissues and isolation by culture or genomic detection of the suspected infectious agent ***(16).***

The current study showed that as regard Laboratory findings in both groups; HCT was significantly higher in group A (37.3 %) compared to group B (36.3%); P-value was 0.003. No significant differences were reported between both groups regarding Hb (P-value = 0.144), platelets (P-value = 0.45),

RBS (P-value = 0.376), and TSH (P-value = 0.211).

Our results were supported by study of other researchers ***(17)*** as they reported that mean hemoglobin, mean corpuscular volume, red cell distribution width, and white blood cell and platelet levels were similar in both groups (p > 0.05).

However, in the study of other researchers [(18),] there was a statistically significant difference between the groups regarding mean platelets volume (MPV), with P value 0.002.

Autoimmune diseases are known to negatively affect pregnancy outcomes, and are associated with increased rates of recurrent abortions, as well as decreased fertility in women of reproductive age. In the general population, vitamin D deficiency has been associated with an increased susceptibility to autoimmune disease. The mechanism of this association is unclear; however, it has been linked to a negative correlation between vitamin D levels and B-cell activation. Hence, vitamin deficiency would lead to increased auto-antibody formation. The findings of a study of women with RPL, in whom vitamin D was shown to decrease B-cell levels, supports this hypothesis ***(19).***

Antiphospholipid syndrome (APS) is one of the autoimmune disorders which is most linked to negative obstetric outcomes and RPL. The main antiphospholipid antibodies are mainly targeted against cardiolipin, lupus anticoagulant, and b2- glycoprotein I antibodies. The incidence of those antibodies in recurrent miscarriages is considered to be between 15%-20%. other researchers demonstrated a greater vitamin D deficiency in APS patients as compared to healthy controls (49% vs 30%, P < .001), which was also significantly associated with thrombosis (P < .001) ***(20).***

In the study in our hands, as regard Antiphospholipid antibody and abortion in both groups; No positive APA was detected in both groups. Abortion showed non-significant difference between both groups (P-value = 0.496).

Our results were supported by study of other researchers ***(14)*** as they reported that until the end of the study, 5 (12.8%) and 13 (34.2%) spontaneous abortions occurred in total in the intervention and control groups, respectively (p=0.03, OR=3.53, 95% CI= 1.12-11.2). In the logistic regression

analysis, the effect of vitamin D3 and the incidence of abortion is not statistically significant when applying the confounding factors of age, gravidity, number of previous abortions and serum levels of IL-23 (p=0.28, OR=0.37, 95% CI= 0:06 to 2:26).

In the study of other researchers ***(15)***, indicated that the percentage of Tregs in patients with URPL was significantly lower than the control group (2.42 ± 0.27 vs. 3.41 ± 0.29, P= 0.01). The percentage of Th17 cells was significantly greater in URPL (unexplained recurrent pregnancy loss) patients compared to the control group (2.91 ± 0.33 vs. 1.18± 0.15, P=0.001). 1, 25VitD3 treatment significantly increased the percentage of Tregs from the baseline in the URPL group compared to that in the control group (1.23 ± 0.03 vs. 1.00 ± 0.03, P= 0.01). Vitamin D deficiency may be a contributor to recurrent pregnancy loss and suggests supplementation of women with Vit D pre-pregnancy may be protective against URPL.

However, in a prospective cohort study of 1191 women conducted by other researcher, ***(21)*** showed an association between sufficient preconception levels of 25(OH) D3 (≥75 nmol/L) and increased rate of pregnancy and live birth. That study also indicated that vitamin D levels should be increased before pregnancy, not in early pregnancy. An efficacy of vitamin D interventional strategies trial showed that oral supplementation is the best vitamin D supplementation strategy in adults and children.

In another study, ***(22)***, after 2 months of supplementation with 2000 IU vitamin D per day, the levels of vitamin D increased in the RPL patients compared to the control RPL patients who had no supplementation with vitamin D. Compared with normal pregnancy, both the level of vitamin D and the Treg/Th17 ratio were significantly decreased in women with RPL. There was a positive correlation between the level of vitamin D and the Treg/Th17 ratio in the RPL group. Within the RPL group, those who received 2 months of vitamin D supplementation showed a significantly increased Treg/Th17 ratio compared with those without vitamin D supplementation. In vitro analysis showed that adding different concentrations of active vitamin D increased the Treg/Th17 ratio, also the mRNA levels of the vitamin D receptor and the metabolic enzyme CYP24A1 increased significantly.

In another study, ***(23)***; control women had higher levels of 25-hydroxyvitamin D and 1α-hydroxylase than women with PRL (P <

.01); this study also showed an association between vitamin D levels and RPL with odds ratio 1.71, 95 CI 1.2-2.4, P < .001. Other researchers, ***(24)*** reported that decreased expression of 1α-hydroxylase on decidual and villous cells in women with RPL as compared to control women (P = .0001 in villus cells, P-value = .002 in decidual cells).

Other researchers ***(25)*** revealed that women with RPL had lower expression of VDR in the villi and the decidual cells as compared to control women (P < .001). Serum VDR levels were also lower in RPL groups as compared to control women (P = .003). other researchers, ***(26)*** demonstrated that Vitamin D levels did not affect fertility, although lower levels of vitamin D were associated with more cases of late miscarriages P = .03.

Vitamin D employs diverse mechanisms through which it affects the activation and regulation of both the innate and adaptive immunity during normal pregnancy. Firstly, vitamin D plays an important role in modulation of the T-cell subsets. Vitamin D inhibits the release of certain cytokines, including IL-2, IL-12 and IFN-γ, thus impeding Th1 differentiation. Vitamin D has also showed activity against IL-17, therefore diminishing pro-inflammatory innate activation. Moreover, vitamin D increases the production of Treg cells, and IL-4 secretion, promoting the shift to Th2 predominance and enhancing fetomaternal tolerance ***(27).***

Secondly, vitamin D appears to have a direct impact on the activity of innate immunity cells. Vitamin D has an inhibitory effect on dendritic differentiation and maturation and additionally inhibits DC function, leading to decreased activation of T-cell subsets. Vitamin D has also been described as a modulator of the perforin-mediated cytotoxic activity of NK cells, inhibiting the secretion of certain cytokines, such as INF-γ. Treatment with vitamin D has been associated with a significant decrease in NK cells which express CD69 as well as with the increased expression of CD158a and CD158b, molecules which are known to inhibit NK activation. Thus, it is presumable that lower levels of vitamin D may lead to dysregulation of the NK cell's function and increased probability of abortion ***(28).***

Lastly, vitamin D has also been demonstrated as a suppressing factor of various components of innate immunity, such as TLR4, a toll-like receptor which is intrinsically involved in the innate immunity

activation process. Another example is NF-kappa β, a nuclear transcription factor which has an important role as a pro-inflammatory molecule in inflammation and takes a part in the pathogenesis of autoimmune disease ***(29).***

Conclusion:

Vitamin D minimize the incidence of abortion among women with unexplained recurrent early pregnancy loss, but this decrease was not significant. The effects of vitamin D on the immune regulation of RPL indicate that vitamin D might be used as an alternative therapy in the future.

Sources of funding

There was no particular grant for this study from governmental, commercial, or non-profit funding bodies.

Conflicts of interest

No conflicts of interest.

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To cite this article: Seham A. Al-Berry**,** Ahmed S. Saad , Mohamed A. El-Gazzar , Asmaa A. M. Al-Gabali . Vitamin D Supplementation on Unexplained Early Recurrent Pregnancy Loss. BMFJ 2022;39(2): 742-755, DOI: 10.21608/bmfj.2022.130081.1569