

Original Article

Correlation between Serum Vitamin D and Calcium Levels in Missed Miscarriage

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Received 1 January 2022; Accepted 18 January 2022
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Abstract

Pregnancy is considered physiological stress, during which a woman's normal static metabolism becomes dynamic anabolism and significant changes are observed in biochemical factors. This study aimed to assess the relationship of serum vitamin D and calcium levels in a pregnant woman with a missed miscarriage. A comparison was performed among 160 women, including 80 females with missed miscarriage (as the study group) and 80 pregnant women (as the control group) in the first and second trimester of pregnancy (before the end of the 24th week of the pregnancy). The results of the comparison showed that there was an insignificant change in serum calcium, while there was a significant reduction in serum vitamin D ($P \leq 0.05$). It was also revealed that, in comparison to normal controls, there was a significant increase in the ratio of serum calcium/vitamin D ratio in cases of missed miscarriage ($P \leq 0.05$). Based on the results of the study, it can be concluded that the estimations of serum vitamin D and calcium/vitamin D ratio in certain pregnancies can be considered valuable parameters in predicting missed miscarriage.

Keywords: Missed miscarriage, Serum calcium, Serum vitamin D

1. Introduction

One of the most complications of early pregnancy is a miscarriage, which is also referred to as spontaneous abortion. At the time of termination of pregnancy, it occurs spontaneously before reaching the survival period by the pregnant woman (24 weeks). Miscarriage has been defined by the World Health Organization as the extraction or expulsion of a fetus or an embryo weighing ≤ 500 g from its mother when it cannot survive independently. Usually, the fetus attains the weight of 500 g at about 22 weeks, which is 154 days of gestation, when this happens, the removed fetus is known as abortion and the recommended term for spontaneous abortion is miscarriage (1). Missed abortion, which is also referred to as missed (silent) miscarriage, is described as the dead products of

conception that have been retained for weeks or days within the uterus with a closed cervix. About 15% of medically confirmed pregnancies are affected by missed abortion. There is no complete understanding of the pathogenesis of missed miscarriage, and it is often believed that there are several factors contributing to the occurrence of this complication (2). Nutritional deficiencies are one of the risk factors reported for this complication since a woman's nutritional status affects her health and the consequences of pregnancy, as well as the health of the newborn fetus (3). Before fertilization and during pregnancy, women are advised to consume prenatal multivitamins daily (4). It has been recommended by the American College of Obstetricians and Gynecologists that 1,000 mg of calcium should be consumed daily by pregnant and

breastfeeding mothers that are aged 19 and above, while a little more is required by teenage mothers. They require a large amount of calcium in their bodies to maintain healthy bones, the rest of which is stored in their bodies to support the baby's growth. In this respect, it is suggested by experts that a minimum of 1,300 mg of calcium be taken daily by pregnant teens that are 18 years and below (5). Vitamin D, a class of fat-soluble hormones, is a steroid vitamin. Vitamin D and active metabolite 1, 25-dihydroxyvitamin D (1,25(OH)₂ D) have classical actions on calcium balance and bone metabolism. Based on the recommendations given by the UK Chief Medical Officers and the guidance of the National Institute for Health and Care Excellence 2012, the importance of vitamin D should be communicated to all pregnant and breastfeeding women, and 10 mg (400 units) of the supplement should be taken daily (6). This study was conducted to correlate the relationship of both vitamin D and calcium with a missed miscarriage.

2. Materials and Methods

2.1. Study Design and Sampling

This prospective case-control study that conducted in the Department of Obstetrics and Gynecology, Al Imamain Al Kadhmain Medical City, over 12 months (April 1st 2018-April 1st 2019). The study group consisted of 80 cases who had missed miscarriage in the 1st or 2nd trimester in less than 24 weeks of pregnancy and were hospitalized in the ward to terminate the pregnancy. Informed consent was obtained from women to participate in the study. The control group consisted of 80 pregnant women with viable embryos, who were in the 1st and 2nd trimesters less than 24 weeks of pregnancy. The pregnant women were regularly visited for outpatient care at the clinic. Inclusion criteria were being in the age range of 18-35 years, having all pregnancies as a singleton before the end of the 24th week of pregnancy, and lacking more than 3 abortions and not less than 1. On the other hand, pregnant women with related maternal medical conditions that could result in fetal compromise, on

medications for chronic diseases (such as thyroid disease, diabetes mellitus, and parathyroid disease), with multiple pregnancies, and receiving calcium and/or magnesium within this pregnancy were excluded from the study.

2.2. Blood Sampling and Serum Vitamin D and Calcium Analysis

In order to perform serum vitamin D and serum calcium analysis, 4 ml of venous blood was drawn from each pregnant woman in the study and control groups. The blood was collected by vacuum blood collecting needle without using any squeezing on the vein and was sent to be centrifuged at 3,000 rpm in an RC3 centrifuge for 10 min at room temperature. Subsequently, each sample was divided into two tubes and labeled and stored at -15°C. Quantitation of total serum vitamin D was measured using the commercial enzyme-linked immunosorbent assay kits. Vitamin D levels were categorized into > 30 ng/ml as sufficient, 20-30 ng/ml as insufficient level, and < 20 ng/ml as deficient. In order to perform the calcium analysis, a manual colorimetric method was conducted using Erba Chem 7 Plus V2 Biochemistry Analyzer.

2.3. Statically Analysis

The collected data were analyzed in SPSS software (version 22) using frequency and percentage for categorical data and mean median, and SD for continuous data. A Chi-square test was used to assess the association between variables and Person correlation to show the correlation between continuous data. A T-test was employed for evaluating differences between the mean and median of continuous variables. A p-value of ≤ 0.05 was considered significant.

3. Results

A total number of 160 women participated in this study, out of which, 80 cases had missed miscarriage (as the study group), while the remaining 80 subjects had healthy normal pregnancies (as the control group). The collected data were analyzed using descriptive and inferential statistics. A p-value of ≤ 0.05 was considered statistically significant. A comparison was

carried out between the participants in terms of their age and gestational age (Table 1). No statistical differences were observed in the mean serum calcium level between the subjects in the two groups. However, there was a significantly lower level of vitamin D in women with a missed miscarriage, compared to those with normal pregnancies (Figures 1 and 2) (Table 2). The mean age of the participants in the study group was

obtained at 28.6 years and their mean gestational age was estimated at 11.22 weeks ($P \leq 0.05$).

The results showed that the ratio of calcium/vitamin D was significantly different in both groups. Moreover, it was observed that the ratio of calcium/vitamin D was significantly higher in women with a missed miscarriage than in women with normal pregnancies ($P \leq 0.05$) (Table 3).

Table 1. Age and gestational age of study and control groups

	Study group Mean±SD	Control group Mean±SD	P-value
Frequency	80	80	
Age (year)	28.625±6.350935	27.35±5.703289	0.335
Gestational age (week)	11.225±2.514831	11.125±2.694323	0.774

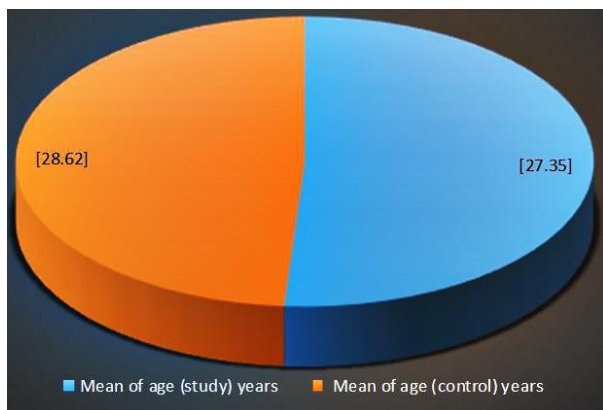


Figure 1. Mean age of the study and control groups

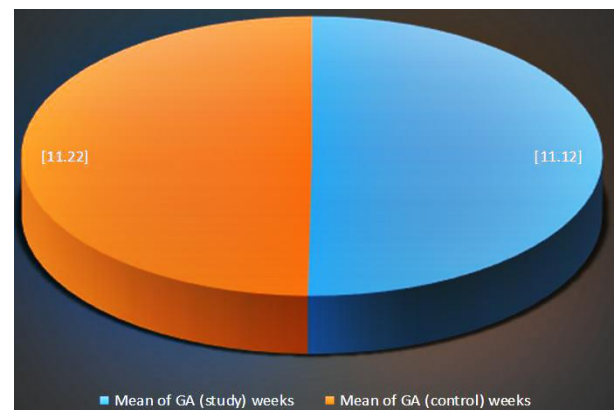


Figure 2. Mean pregnancy age of the study and control groups

Table 2. Serum calcium and vitamin D levels in the study and control groups

Serum level (mg/dl)	Study group Mean±SD	Control group Mean±SD	P-value
Calcium (mg/dl)	8.5425±0.7790	8.74±0.7351	0.253
Vitamin D (ng/ml)	20.3125±4.1669	29.7675±7.978	0.0001

Table 3. Mean of calcium/vitamin D ratio of the study and control groups

Calcium/vitamin D ratio	Study group	Control group	P-value
Frequency	80	80	
Mean±SD	0.4391±0.10	0.3253±0.164	0.00095

4. Discussion

Missed miscarriage is a common complication that affects ~15% of all clinically recognized pregnancies (7). This complication is one of the most popular kinds of early pregnancy loss, and numerous factors have been identified as being responsible for this complication. There has been a significant increase in the incidence of miscarriage in fertile women in the last few years (8). Often, losing a pregnancy causes discomfort to women and their partners, affects them psychologically, and influences social health (9). In addition, this complication is often accompanied by bleeding, which sometimes leads to lethality or disability (10).

According to the findings of our study, there was a statistically significant relationship between the low levels of vitamin D and missed abortion. It was also revealed that the serum 25-hydroxyvitamin D level was lower in mothers with a missed abortion than in those with a normal pregnancy ($P \leq 0.05$). It has been reported that 40-60% of the entire population were vitamin D deficient, including pregnant women (11). The reason for the high rate of this deficiency is attributed to numerous factors, among which, vitamin D is found in a very limited number of foods (e.g., egg yolk, salmon, mushroom, and cod liver oil). Moreover, vitamin D needs sunlight to make the body produce it properly. There are various factors affecting the production and absorption of vitamin D in the body, including season, sunscreen, indoor workplace, clothing type, obesity, pollution, aging, and increased melanin in dark-skinned people.

The findings of various studies have demonstrated the protective role of vitamin D against miscarriage. Vitamin D has been considered a regulator of decasualization; it is mainly regulating the *HOXA10* gene, which is important for the regulation of the development of the endometrium and its receptivity during the period of implantation. Additionally, vitamin D regulates the genes that are involved in trophoblastic angiogenesis and invasion, the main mechanism in the implantation of the embryo and placental function; as a result, fetal growth and development are supported. It

functions as an immune modulator since it has a powerful effect as an anti-inflammatory factor at the maternal-fetal interface that plays a potential role in the maternal-fetal immunologic response. The receptors for vitamin D are expressed on different immune cells that are involved in controlling the pathways for antigen-receptor signaling and the activation of T-cells. Activating $1,25(\text{OH})_2 \text{D}$ has been proposed as an immunotherapies for spontaneous miscarriage, because it down-regulates Th1 cytokines and inhibits the pro-inflammatory secretions of the cytokines, such as interleukin-6, interferon- γ , and tumor necrosis factor- α , in the placenta (6, 12).

Regarding calcium, the results of this study indicated that there was no statistically significant relationship between calcium levels in pregnant women and missed-abortion women as the plasma levels of calcium showed no significant differences between miscarries and controls. This low level of calcium could be due to usual physiologic pregnancy changes that lead to micronutrient deficiency states or because of severe dietary inadequacy. During pregnancy, the total serum concentrations of calcium decrease due to the hem dilution effect. These reductions mainly take place at the albumin-bound fraction for the total calcium as there is a fall in serum albumin, while the ionized calcium levels do not change from that in non-pregnant women (13). In women with adequate calcium intakes, calcium balance is positive early in pregnancy and becomes either neutral or negative in the third trimester (14).

Conclusively, the results showed that the relationship between vitamin D deficiency and missed miscarriage was significant, whereas that between calcium deficiency and missed miscarriage was insignificant ($P \leq 0.05$). Therefore, a total serum $25(\text{OH}) \text{D}$ level is the most important indicator of vitamin D for the body as a source of supply from skin synthesis or nutritional absorption, and calcium levels play an important role in pregnancy.

Authors' Contribution

Study concept and design: S. A. A.

Acquisition of data: S. A. A.
 Analysis and interpretation of data: F. K. A.
 Drafting of the manuscript: E. T. M.
 Critical revision of the manuscript for important intellectual content: E. T. M.
 Statistical analysis: S. A. A.
 Administrative, technical, and material support: S. A. A.

Ethics

The study was approved by the Ethical Committee of the College of Medicine, Al-Nahrain University, Iraq. Informed written consent was obtained from all the participants before starting the study. In addition, the study was reviewed by the Ethical Committee of Al-Nahrain College of Medicine.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to acknowledge Professor Dr Qais Ismaeel Kadhem for his assistance in this study.

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