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The relationship between complete hydatidiform mole and serum Vitamin D level: a prospective case-control study

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Abstract. – OBJECTIVE: This study aimed to determine whether or not there was a relationship between complete hydatidiform mole (CHM) and serum Vitamin D level by comparing CHM patients with two control groups and to determine whether or not Vitamin D deficiency is a risk factor for CHM.

PATIENTS AND METHODS: This prospective study included 30 patients diagnosed with CHM (case group), 30 patients in the first trimester of a healthy pregnancy (control group), and 30 healthy non-pregnant subjects (control group). A record was made of serum 25-hydroxyvitamin D (25-OH D vitamin) levels, age, body mass index (BMI), gravida, parity, and the number of abortus. The serum 25-OH D vitamin levels were examined in each group and compared between groups.

RESULTS: The 25-OH D vitamin level of all the patients in the study was determined as 11.16±8.64 ng/mL. No significant difference was determined between the groups in respect of 25 OH-D vitamin levels. When comparisons were made between the four subgroups according to the 25-OH-D level, no significant difference was determined between the CHM and control groups. When the patients were separated as obese and non-obese groups, no significant difference was determined between the groups.

CONCLUSIONS: Severe deficiency, deficiency, or insufficient levels of serum Vitamin D are not thought to be risk factors for CHM patients.

Key Words:
Complete hydatidiform mole, Hydatidiform mole, Serum 25-OH D vitamin, Vitamin D deficiency, Vitamin D insufficiency.

Introduction

Hydatidiform mole (HM) is part of a disease group classified as gestational trophoblastic disease (GTD), which originates from the placenta and has the potential to locally invade the uterus and metastasis. However, the pathogenesis is not similar to that of GTD as the maternal tumor originates from gestational tissue, not from maternal tissue[1]. HM comprises two separate types: complete hydatidiform mole (CHM) and partial HM. Although molar pregnancies are benign, they are accepted as pre-malignant as there is the potential to become malignant. The risk of gestational trophoblastic neoplasia (GTN), which is a malignant disease, is higher in CHM than in partial HM[1,2]. The reported incidence of HM shows great variations across different regions of the world. High rates have been reported in Latin America, Asia, and the Middle East, in a wide range (23-1,299 per 100,000 pregnancies)[3]. The etiology of HM has not yet been fully understood[4]. Risk factors for HM include an extreme maternal age (≤15->35 years), ethnicity, genetic factors, miscarriage, and restricted diet[5].

Vitamin D is a vitamin produced in the skin with the effect of ultraviolet light, which is soluble in fat and steroid hormones. It can also be obtained exogenously from the diet or food supplements[6]. 25-hydroxy vitamin D (25-OH-D) is the vitamin D form in circulation and generally the measured form[7]. Vitamin D deficiency is common in pregnancy, and a recent study reported the prevalence of vitamin D deficiency in pregnancies in Turkey as 81.4-86%[8]. Vitamin D receptors are located in many bodily systems such as the immune system, cardiovascular system, and genitourinary system[9]. In the female reproductive system, there are vitamin D receptors in the endometrium, ovaries, and placenta[10]. Vitamin D has an important role in processes such as implantation in pregnancy, immune functions, angiogenesis, inflammation,

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and glucose metabolism[11]. Studies in literature have determined that low vitamin D levels are associated with adverse pregnancy outcomes, such as pre-eclampsia, pre-term birth, small for gestational age (SGA), and neonatal hypocalcemia[12].

This study aimed at determining whether or not there is any relationship between vitamin D level and CHM, for which the etiology has not yet been determined and has the potential to become malignant. It was also aimed at determining whether or not vitamin D deficiency is a risk factor for CHM, and thereby contributing to the literature.

Patients and Methods

This prospective, case-control study included 30 patients diagnosed with CHM (case group), 30 patients in the first trimester of a healthy pregnancy (control group A), and 30 healthy non-pregnant subjects (control group B) who presented at the Obstetrics and Gynaecology Clinic of Dicle University Medical Faculty Hospital between January 2021 and January 2022. Approval for the study was granted by the Ethics Committee of Dicle University Medical Faculty (decision No. 96, dated: 07.01.2021). All study procedures complied with the Helsinki Declaration. Before inclusion in the study, all the patients and control subjects provided written informed consent.

The case group included patients diagnosed with CHM. Control group A included pregnant patients in the first trimester with a healthy, single, intrauterine, live fetus. Control group B included healthy females of reproductive age who were not pregnant. Patients or control subjects were excluded from the study if they were taking vitamin D or calcium supplements, had a history of molar pregnancy or had any systemic disease or other diseases that would prevent 25-OH-D vitamin absorption.

For each study participant, age, body mass index (BMI), gravida, parity, number of abortus, and serum 25-OH D vitamin levels were recorded. The serum 25-OH-D vitamin levels were measured before uterine evacuation in patients with suspected CHM on transvaginal ultrasonography. The definitive diagnosis of CHM was made from the histopathological examination. In the control groups, serum 25-OH-D levels were measured on the first presentation. As 25-OH-D vitamin values show seasonal changes, for each patient diagnosed with CHM, one patient with 25-OH-D levels examined on the same day was assigned to each of the control groups to provide homogeneity between the groups. BMI was calculated according to pre-pregnancy values as kg/m^2.

A 5 ml sample of peripheral blood was collected from each patient. The samples were stored in anticoagulant tubes and prepared by centrifugation at 4°C for 15 mins. The supernatants obtained were stored at -20°C until assay. Vitamin D levels were determined using high-performance liquid chromatography (HPLC). The vitamin D kit was measured in two copies, according to the manufacturer's instructions (Shimadzu LC-20A, Immuchrom GmbH). According to the Endocrine Society Guidelines, a 25-OH D vitamin level <20 ng/mL (50 nmol/L) was defined as vitamin D deficiency, <10 ng/mL was defined as severe vitamin D deficiency, and 25-OH D vitamin level of 20-29 ng/mL (525- 725 nmol/L) was defined as vitamin D insufficiency[13]. The 25-OH-D levels were examined within each group and were compared between groups.

Statistical Analysis

Data obtained in the study were statistically analyzed using SPSS for Windows version 24.0 software (IBM Corp., Armonk, NY, USA). The normality of the distribution of continuous variables was tested with the Shapiro-Wilk test. One-way ANOVA (for data with normal distribution) or Kruskal-Wallis' tests (for non-normal data) were used in the comparisons of numerical data between groups. The Chi-square test was applied to investigate relationships between categorical variables. Descriptive statistics were stated as mean ± standard deviation (SD) values. A value of $p < 0.05$ was accepted as statistically significant.

Results

The mean age of the whole study sample was calculated as 30.52±8.63 years, BMI as 24.63±4.43 kg/m^2, and 25-OH D vitamin level as 11.16±8.64 ng/mL. Of these patients, severe 25 OH-D vitamin deficiency was determined in 48 (53.3%), deficiency in 31 (34.4%), and insufficiency in 7 (7.8%). According to the BMI values, 76 (84.4%) patients were not obese.

No difference was determined between the CHM and control groups in respect of age, parity, and BMI (p>0.05). The number of gravida and abortus in the healthy first-trimester pregnancy control group was determined to be significantly higher than in the other two groups (p<0.05). No significant difference was determined between

Table I. Evaluation of demographic and clinical values.

	Complete hydatidiform mole case group (n=30) mean±SD	First trimester of a healthy pregnancy control group A (n=30) mean±SD	Non-pregnant subjects control group B (n=30) mean±SD	p
Age	29.57 ± 9.39	30.33 ± 6.78	31.67 ± 9.58	0.639
	Median [25%-75%]	Median [25%-75%]	Median [25%-75%]	
Gravida	4 [2-5]	5 [3-6]	0.5 [0-3]	0.001*
Parity	2 [0-4]	2 [1-3]	0 [0-3]	0.254
Abortus	0 [0-1]	1 [0-2]	0 [0-0]	0.001*
BMI (kg/m^2)	23 [20-27]	24 [21-27]	24 [22-28]	0.493
25-OH-D vitamin levels (ng/ml)	6.82 [4.8-11.5]	10.38 [6.29-14]	10.61 [5.92-15.22]	0.111

*p<0.05 statistically significant; One-way ANOVA or Kruskal-Wallis' test for numerical. BMI: Body mass index; SD: Standard Deviation.

the groups in respect of 25 OH-D vitamin levels (p=0.111) (Table I).

When the 25-OH-D vitamin levels were compared between the four subgroups (severe deficiency, deficiency, insufficiency, normal), no significant difference was determined between the CHM and control groups (p=0.442). In the CHM group, the level of 25-OH-D was determined to be severely deficient in 20 (66.7%) patients and deficient in 8 (26.7%). The 25-OH-D level was not determined as normal in any patient in the CHM group (Table II).

When the patients in the CHM and control groups were separated as obese and non-obese groups, no significant difference was determined between the groups in respect of the 25-OH-D levels (p>0.05) (Table III).

Discussion

Uncertainty remains around the relationship between vitamin D levels and the risk of pregnancy-related complications. Vitamin D deficiency is a common diagnosis in pregnant women worldwide[14]. Dietary restriction is known to be among the risk factors for HM, but within these dietary limitations, there is no clear information about whether vitamin D deficiency is a risk factor for CHM[5]. Therefore, this study was conducted to determine whether or not vitamin D deficiency is a risk factor for CHM. The results of the study demonstrated that vitamin D deficiency and insufficiency were common in all the patients included in the study, and there was no significant difference in the rates of vitamin D deficiency and insufficiency in the CHM group compared with the control groups.

Studies in the literature related to vitamin D deficiency were examined[14-20]. In a study by Umar et al[15] of 30 pre-eclamptic and 30 normotensive pregnancies, vitamin D deficiency was determined in 95% of all the patients. Sahhaf et al[16] examined 25 OH-D levels between a group of ectopic pregnancies and a control group, and reported vi-

Table II. Comparison of 25 OH vitamin D levels between groups.

	Complete hydatidiform mole n (%)	First trimester of a healthy pregnancy (control group A) n (%)	Non-pregnant subjects (control group B) n (%)	p
25-OH-D vitamin levels (ng/ml)				0.442
Severe deficiency (<10)	20 (66.7)	14 (46.7)	14 (46.7)	
Deficiency (10-19)	8 (26.7)	12 (40)	11 (36.7)	
Insufficiency (20-29)	2 (6.7)	2 (6.7)	3 (10)	
Normal (>30)	0 (0)	2 (6.7)	2 (6.7)	

p<0.05 statistically significant; Chi-square test for categorical data.

Table III. Comparison of 25 OH vitamin D levels between obese and non-obese patients in the groups.

	Complete hydatidiform mole n (%)	First trimester of a healthy pregnancy (control group) n (%)	Non-pregnant subjects (control group) n (%)	*p*
	n (%)	n (%)	n (%)	
Non-obese (BMI<30 kg/m^2)				0.588
25-OH-D vitamin levels (ng/ml)				
Severe deficiency (<10)	18 (66.7)	11 (45.8)	13 (52.0)	
Deficiency (10-19)	7 (25.9)	11 (45.8)	8 (32.0)	
Insufficiency (20-29)	2 (7.4)	1 (4.2)	3 (12.0)	
Normal (>30)	0 (0)	1 (4.2)	1 (4.0)	
Obese ((BMI≥30 kg/m^2)				0.620
25-OH-D vitamin levels (ng/ml)				
Severe deficiency (<10)	2 (66.7)	3 (50)	1 (20)	
Deficiency (10-19)	1 (33.3)	1 (16.7)	3 (60)	
Insufficiency (20-29)	0 (0)	1 (16.7)	0 (0)	
Normal (>30)	0 (0)	1 (16.7)	1 (20)	

p<0.05 statistically significant; Chi-square test for categorical data. BMI: Body mass index.

tamin D deficiency in 60.66% of all the patients, and vitamin D insufficiency in 21.33%. Consistent with the findings in the literature, severe vitamin D deficiency was determined in 53.3% of the current study patients, vitamin D deficiency in 34.4%, and vitamin D insufficiency in 7.8%. Vitamin D deficiency must not be overlooked, especially in the geographic region where this study was conducted. As exposure to sunlight is low and few people can afford vitamin D supplements because of socioeconomic conditions, the high rate of vitamin D deficiency is not surprising in this population. Efforts must be made to prevent this by determining the negative effects of vitamin D deficiency, in particular on pregnant women.

When the studies in the literature investigating the relationship between vitamin D levels and pregnancy complications were examined, it was seen that in a study[17] that investigated whether or not vitamin D level in the first trimester was a risk factor for gestational diabetes mellitus (GDM), there was a significantly greater risk of GDM in pregnant women with a level of 25 OH-D <20 ng/ml. Yue and Ying[18] reported that a sufficient level of 25 OH-D before the 20th week of pregnancy was protective against GDM, levels >20 ng/ml on the first presentation reduced the risk of GDM, and a 25 OH-D level >30 ng/ml made no difference in respect of protection. In a study[18] where it was considered that the pathological changes in GDM occur in early pregnancy, it was emphasized that there is a need for further research on the relationship between pre-pregnancy serum vitamin D levels and the risk of GDM. In a review[19] that examined the relationship between recurrent pregnancy loss (RPL) and vitamin D level, it was reported that the prevalence of vitamin D insufficiency or deficiency was high in women with RPL, and this could be related to immunological irregularity and therefore, RPL. It has also been emphasized that vitamin D could play a role in immune regulation by promoting implantation and a successful pregnancy. In a study[16] that investigated the relationship between ectopic pregnancy and vitamin D levels, the vitamin D levels in patients with ectopic pregnancy were found to be significantly lower than those of the control group with normal pregnancies. Domaracki et al[14] compared the 25 OH-D levels in patients with pre-eclampsia, gestational hypertension, and GDM, with a control group. The results of that study demonstrated that the 25 OH-D level in patients with pre-eclampsia was significantly lower than that of the control group, but no difference was determined between the control group and patients with gestational hypertension and GDM. It was reported that a low level of 25 OH-D could play a role in the etiopathogenesis of pre-eclampsia[14]. As seen in the studies on vitamin D levels and pregnancy-related complications, there remains a lack of certainty. In addition, no study could be found in the literature that has made a detailed investigation of the relationship between vitamin D, for which receptors are found in the placenta, and CHM, which is a disease originating from cytotrophoblasts and syncytiotrophoblasts

with a risk of becoming malignant. In the current study, the serum 25 OH-D levels of CHM patients were compared with those of two control groups, but no significant difference was determined between the groups. As only 4.4% of the patients included in the study had a vitamin D level >30 ng/ml, when the groups were separated into four subgroups according to the vitamin D levels and then compared, no significant difference was determined between the groups. In the previously mentioned study by Yue and Ying[18], obese patients had a higher level of vitamin D binding protein and it was reported that a sufficient 25 OH-D level was very important in protecting obese pregnant patients against GDM. Tanvig et al[20] examined obese pregnant women and determined that making lifestyle changes of diet and exercise during pregnancy significantly increased the serum 25 OH-D levels in late pregnancy and the postpartum period. Vitamin D deficiency was reported to be common in pregnancy and especially in obese pregnant patients[20]. In accordance with these findings, the patients in the current study were separated into obese and non-obese groups, and in the comparison of 25 OH-D levels, no significant difference was determined between the groups.

The etiology of HM has not yet been fully understood. Studies[21-23] related to restricted nutrients in the etiology of HM were examined. In a study by Ferraz et al[21] in respect of limited nutrition, no difference was determined between CHM patients and the other groups included in the study, but it was reported that high exposure to oxidative stress could increase the risk of CHM in healthy pregnancies and even in non-pregnant women. In a study by Kolusari et al[22], catalase, and vitamin A, D, and E levels were determined to be significantly lower in CHM patients than in the control group. In a study[23] that examined folate, vitamin B12, and plasma homocysteine levels in HM patients, the folate level was found to be significantly low and the plasma homocysteine level significantly high in HM pregnancies. It was concluded to be a relationship between plasma folate and homocysteine levels and HM. As previously stated, vitamin D receptors are located in the placenta, and as the vitamin D level is important for the placenta development in early pregnancy, the serum 25 OH-D vitamin levels were compared in this study between CHM patients and control subjects.

Strenghts and Limitations of the Study

The results of the study demonstrated that vitamin D insufficiency or deficiency was not a significant risk factor for CHM. The serum vitamin D level was determined to be low in the CHM group and the reason for a lack of significant difference between the groups could have been due to the low number of patients in the study. Another reason was thought to be that as serum vitamin D deficiency is greater in this region than in other regions, no significant difference emerged from the control groups.

Strong aspects of this study can be said to be that it was prospective and included control groups. Moreover, the patients included in the study were all of the same ethnicity and lived in the same geographical region. To provide homogeneity of the vitamin D levels, for each CHM patient, a patient with serum vitamin D levels examined on the same day was assigned to each of the control groups. A limitation of the study can be considered to be the low number of patients as CHM is not commonly seen.

Conclusions

Severe deficiency, deficiency, or insufficient levels of serum Vitamin D are not thought to be risk factors for CHM patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Approval

Approval for the study was granted by the Ethics Committee of Dicle University Medical Faculty (decision No. 96, dated: 07.01.2021).

Informed Consent

All patients and control subjects provided written informed consent.

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