Original Article
The relationship between serum 25-hydroxyvitamin D, free estriol levels and complications during pregnancy, adverse pregnancy outcomes, and fetal growth in pregnant women from Chongqing

Honglu Wei[1,2], Zhiwei He[3], Jin Li[3], Min Zhang[4], Weixian Chen[1]

[1]Department of Laboratory Medicine of The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China; [2]Department of Laboratory Medicine of The Fifth People's Hospital of Chongqing, Chongqing, China; [3]Ultrasonic Department of The Fifth People's Hospital of Chongqing, Chongqing, China; [4]Department of Obstetrics of The Fifth People's Hospital of Chongqing, Chongqing, China

Abstract: Objective: To explore the relationship between these factors and pregnancy complications, adverse pregnancy outcomes, and fetal growth, and to provide a reference for the reasonable supplementation of vitamin D in pregnant women in Chongqing, China. Methods: 267 pregnant women participated in our study from 2016 to 2018. The patients were divided into two groups according to their complications and adverse outcomes. They were divided into the healthy group (group A) and the group with pregnancy complications and adverse outcomes (group B). The clinical information and B-ultrasound data of the pregnant women were collected. 25(OH)D was done and the µE3 levels were tested in the second trimester to calculate the µE3 multiple of the medians of the MOM values. Results: The Serum 25(OH)D level was negatively correlated with adverse pregnancy outcomes (r=-0.121, P=0.048). There was a negative correlation between 25(OH)D level and 38w-39w6d fetal abdominal circumference (P=0.012), and there was a positive correlation with fetal birth length. The µE3 level in the placental adhesion group was different from the level in the healthy group (P=0.044). µE3 and µE3 MOM were independent influencing factors of placental adhesion (P=0.003, OR=0.02), (P=0.004, OR=31.244). The µE3 MOM value in the gestational diabetes group was different from the value in the healthy group (P=0.022). There was a significant difference in the µE3 MOM value between the amniotic fluid index ≥8 cm group and the < 8 cm group (P=0.030). A statistical difference existed in the level of µE3 between grade III and grade II in placental maturity (P=0.011). The µE3 level was positively correlated with the biparietal diameter and with the femur length of the 15w-17w fetus (P=0.001), and negatively correlated with the biparietal diameter and femur length of the 33w-35w6d (P=0.022). The µE3 MOM value was negatively correlated with the 33w-35w6d fetal biparietal diameter (P=0.020) and head circumference (P=0.042). Conclusion: The levels of 25(OH)D, µE3, and µE3 MOM are related to fetal growth. 25(OH)D, µE3, and µE3 MOM values have positive effects on preventing pregnancy complications and adverse outcomes.

Keywords: 25 hydroxy vitamin D, free estriol, gestational complications, delivery outcomes, fetal growth

Introduction

Vitamin D deficiency is a global problem and is common in the general population. According to published studies, pregnant women in the United Kingdom, India, Greece, Australia, New Zealand [1-4], and other countries generally lack vitamin D. The proportion of the pregnant women with serum vitamin D sufficiency in Beijing, Shanghai, Guizhou, Anhui and other places is also less than 10% [5-8]. The classic function of vitamin D is to regulate calcium and phos-

phorus metabolism [9]. Recent studies have shown that vitamin D plays an important role in other physiological processes and is associated with various diseases such as immune regulation, infection, cardiovascular disease, and cancer [10, 11]. The level of vitamin D during pregnancy may play an important role in maternal health and fetal development [12, 13]. Free estriol (µE3) is a metabolite of estradiol that can be used to determine placental function and predict fetal status. Some studies have found that a low maternal uE3 level in the sec-

ond trimester is associated with fetal growth restriction, preterm birth, and decreased birth weight [14].

By investigating the status of vitamin D and μE3 in pregnant women living in Chongqing, this study aimed to explore the effects of serum vitamin D, μE3, and μE3 MOM levels on pregnancy complications, delivery outcomes, and fetal growth.

Material and methods

General information

The women who were routinely examined and delivered in the obstetrics department of Fifth People's Hospital of Chongqing from 2016 to 2018 were selected for this retrospective study.

Inclusion criteria: Pregnant women 12 to 55 years of age who were routinely examined and who delivered in the obstetrics department of Fifth People's Hospital of Chongqing. The exclusion criteria were: Patients diagnosed with liver or kidney dysfunction, rickets, patients who were malnourished, etc. Other examination data were incomplete. The patient's basic information included age, gestational complications, and adverse outcomes, B-ultrasound data of the fetal growth index for each pregnancy, the last B-ultrasound data of the amniotic fluid index and placental maturity before delivery, and birth weight and length. The information was obtained from the patients' medical records. The gestational complications or adverse outcomes evaluated in this study included gestational diabetes, gestational hypertension, hypothyroidism during pregnancy, thrombocytopenia during pregnancy, postpartum hemorrhage, premature rupture of membranes, intrauterine distress, placental adhesions, and so on. The patients were divided into two groups according to their complications and adverse outcomes. In the healthy group (group A), the patients had a good pregnancy outcome. In the pregnancy complications and adverse outcomes group (group B), the pregnant women were healthy before their pregnancy and had relevant diseases or adverse pregnancy outcomes. This study was approved by the Ethics Committee of Fifth People's Hospital of Chongqing. All study participants provided written informed consent before participating in the study.

Methods

All pregnant women received health education and guidance for clinical nutrition during the perinatal period, and 4 ml fasting blood was taken in the morning for a laboratory analysis. All pregnant women's μE3 levels were measured in the second trimester, and the μE3 MOM value was calculated, which is the ratio of the serum μE3 level to the median serum μE3 level in normal pregnant women at the same gestational age. 25(OH)D level was also routinely tested.

Instruments and reagents

25(OH)D was tested using Siemens' ADVIA Centaur XP automatic chemiluminometer. In brief, an antibody to 25(OH)D is precoated onto a microtiterplate, and serum samples and calibrators are added. Free 25(OH)D is captured during this first incubation step, and after washing, a second incubation with a biotin-labeled 25(OH)D analog reacts with non-occupied antibody binding sites (a competitive immunoassay). Finally, after washing and incubating with a streptavidin-peroxidase conjugate, the absorbance (at 450 nm) is measured by using a plate spectrophotometer, where the concentration of free 25(OH)D in the sample is inversely proportional to the absorbance in each sample well. The assay calibration was done against a symmetric dialysis method. The original kit from Siemens was used. The μE3 was tested using a Beckman ACCESS2 automatic chemiluminometer. The original kit of Beckman Coulter was used. The reagents were used within the validity period, and the quality control products of Bole Company of the United States were used for the daily quality control monitoring.

Reference range

Vitamin D deficiency was defined as serum 25(OH)D concentration < 20 ng/ml. Vitamin D insufficiency was defined as serum 25(OH)D concentration > 20 ng/ml and < 29 ng/ml. The sufficiency was defined when the serum 25(OH)D level was greater than 30 ng/ml. Vitamin D was considered excessive when the serum 25(OH)D level > 100 ng/ml.

Statistical analysis

The statistical analysis was performed using SPSS 23.0 software. A t-test was used for the

Table 1. The relationship of 25(OH)D, μE3, and μE3 MOM levels between the adverse outcome and healthy groups

Group	25(OH)D		μE3		μE3 MOM	
	$\overline{X} \pm S$	P	$\overline{X} \pm S$	P	$\overline{X} \pm S$	P
Healthy group (group A)	12.88±7.02		1.71±0.76		1.48±0.53	
Adverse outcome group (group B)	12.38±7.61*	0.048*	1.65±0.58	0.958	1.55±0.51	0.18
Gestational diabetes	11.65±7.66*	0.037*	1.45±0.55	0.08	1.43±0.46*	0.022*
Hypothyroidism	12.77±6.47	0.944	1.83±0.72	0.501	1.68±0.49	0.815
Thrombocytopenia	9.02±3.48	0.073	1.54±0.35	0.643	1.40±0.26	0.181
Postpartum hemorrhage	10.68±6.12	0.084	1.81±0.66	0.486	1.53±0.44	0.37
Premature rupture of membranes	12.88±6.54	0.788	1.72±0.59	0.44	1.61±0.59	0.713
Fetal intrauterine distress	9.53±4.69*	0.031*	1.56±0.61	0.744	1.56±0.65	0.534
Placental adhesion	17.03±11.15	0.93	1.28±0.45*	0.044*	1.5±0.58	0.403

Note: *P < 0.05 indicates statistically significant compared with the healthy group.

normal distribution of the measurement data. A Wilcoxon signed-rand test was used for the data that did not satisfy the normal distribution. The relationship between the factors and adverse pregnancy outcomes was analyzed using a multivariate logistic regression analysis. The Spearman rank correlation analysis method was used. An F test was used for the multiple sets of measurement data, and the posttest LSD method was used for the comparisons between groups.

Results

The basic results between the adverse outcome group and healthy group

A total of 267 pregnant women were enrolled in the study, including 131 (49.1%) in the healthy group (A) and 136 (50.9%) in the adverse outcome group (group B). The patients ranged from 17 to 36 years old (27.04±3.25). Four of them were younger than 21 years old, 262 were 22-34 years old, and one was over 35 years old.

The overall levels of 25(OH)D, μE3, and μE3 MOM in pregnant women

Among the 267 pregnant women, the serum 25(OH)D ranged from 4.2 to 51.6 ng/ml. 86.5% (231/267) of the patients had a vitamin D deficiency, and 11.2% (32/267) patients had a vitamin D insufficiency. 2.2% (6/267) cases were adequate in vitamin D. There was no vitamin D excess in the pregnant women. The results of this study showed that 97.8% of pregnant women had 25(OH)D deficiency. In Group

A, 88.55% (116/131) patients had 25(OH)D deficiency, 9.92% (13/131) patients had 25(OH)D insufficiency. 1.53% (2/131) cases were adequate in 25(OH)D, of which the 25(OH)D concentrations were greater than 40 ng/ml. In group B, 113 cases (83.1%) were deficient in 25(OH)D, 19 cases (14%) were insufficient in 25(OH)D, and 4 cases (2.9%) were sufficient in 25(OH)D. No case in group B had 25(OH)D concentration greater than 40 ng/ml. Overall, 48.9% of pregnant women with 25(OH)D deficiency and 59.4% with 25(OH)D insufficiency had pregnancy complications or adverse outcomes.

The serum μE3 concentrations of the pregnant women ranged from 0.41 to 4.8 mmol/L, and the μE3 MOM value ranged from 0.41 to 3.6. The μE3 MOM value of less than 0.5 was found in 2 cases (0.7%), with one case in group A and one in group B.

Comparison of 25(OH)D, μE3, and μE3 MOM levels between the adverse outcome and healthy groups

*Wilcoxon signed-rand test (**Table 1**):* The results showed that there was a difference of 25 (OH)D concentration between group B, the gestational diabetes group and the healthy group (group A) (P < 0.05). The differences between the hypothyroidism group, the placental adhesion group, the thrombocytopenia group, and the postpartum hemorrhage group were not statistically significant.

The differences in μE3 concentration and μE3 MOM between group A and group B were not

Table 2. The correlation between 25(OH) D, μE3, and μE3 MOM levels and adverse outcomes

Factor	r	P
25(OH)D	-0.121	0.048
μE3	-0.003	0.958
MOM	0.078	0.201

Adverse mean: Gestational diabetes, Hypothyroidism, Thrombocytopenia, Postpartum hemorrhage, Premature rupture of membranes, Fetal intrauterine distress, Placental adhesion.

Table 3. Relationship between 25(OH)D3, μE3, μE3 MOM levels and amniotic fluid index

Group	$25(OH)D_3$	μE3	μE3 MOM
≥8 cm	12.77±7.42	1.65±0.66	1.51±0.54
< 8 cm	10.12±4.10	1.82±0.61	1.77±0.47
t	1.681	-1.136	-2.188
P	0.094	0.257	0.030

statistically significant. However, the μE3 concentrations in the placenta adhesion group and the μE3 MOM levels in the gestational diabetes group were different from those in group A (P < 0.05).

Spearman correlation test: A Spearman correlation analysis was used to determine the relationship between the maternal 25(OH)D, μE3, and μE3 MOM levels and gestational complications or adverse outcomes (**Table 2**). The analysis showed that only 25(OH)D had a negative correlation with adverse pregnancy outcomes (r=-0.121, P < 0.05).

Multi-factor logistic regression analysis: The healthy group was the control group, and the gestational diabetes group was the disease group. A multivariate logistic regression analysis was performed with 25(OH)D, μE3, and μE3 MOM as independent variables. The results showed that 25(OH)D, μE3, and μE3 MOM were not the independent influencing factors (P > 0.05).

The healthy group was the control group and the placenta adhesion group was the disease group. A multivariate logistic regression analysis was performed with VD, μE3, and μE3 MOM as independent variables. The results showed that VD was not an independent influencing factor (P > 0.05). μE3 and μE3 MOM were the independent influencing factors (P=0.003,

OR=0.02), (P=0.004, OR=31.244), indicating that the lower the they are, the more likely the placental adhesion will occur.

The relationship between 25(OH)D, μE3, μE3 MOM and the amniotic fluid index

According to the amniotic fluid index of the last B-ultrasound before delivery, all the patients were divided into two groups, the index < 8 cm and the ≥8 cm groups. The data accorded with a normal distribution. A t test was used to analyze the differences in 25(OH)D, μE3, and MOM between the two groups. The results showed that the expression level of μE3 MOM in the amniotic fluid index ≥8 cm group was significantly lower than it was in the < 8 cm group (P < 0.05) (**Table 3**).

The relationship between 25(OH)D, μE3, μE3 MOM and placental maturity

According to the placental maturity of the last B-ultrasound before delivery, the pregnant women were divided into three groups (Grade I, Grade II, and Grade III). An F-test was used for the multi-group measurement data that conformed to the normal distribution, and the post-test LSD method was used for the comparisons between groups. It was found that only μE3 was significantly different in terms of placental maturity between grades III and II (**Table 4**).

The relationship between 25(OH)D, μE3, μE3 MOM and the fetal growth index

A Spearman rank correlation analysis was used to find the association between double top diameter and head-hip diameter at 10-14 weeks of pregnancy, double top diameter and femur length at 15-17 weeks, biparietal diameter, head circumference, abdominal circumference, tibia length, femur length, and body length at 28-30 weeks, 33-35+6 weeks, 38+39+6 weeks, and body weight at birth with 25(OH)D, μE3, and μE3 MOM.

The results showed that 25(OH)D was negatively correlated with the fetal circumference of 38-39+6 weeks, and positively correlated with the birth length of the fetus. The level of μE3 was positively correlated with the biparietal diameter and femur length of the fetus at 15-17 weeks of pregnancy. There was a negative correlation between the bimodal diameter of the fetus and the length of the femur at -35+6 wee-

Table 4. The relationship between 25(OH)D, μE3, μE3 MOM and placental maturity

Placental maturity	25(OH)D level	μE3	MOM
Grade I	9.72±3.61	1.61±0.36	1.21±0.43
Grade II	12.04±6.02	1.84±0.7	1.58±0.53
Grade III	12.42±7.06	1.56±0.56*	1.49±0.5
F	0.508	4.628	1.896
P	0.603	0.011	0.153

Note: *P < 0.05 means statistically significant compared with grade II.

ks. The level of μE3 MOM was negatively correlated with the biparietal diameter and head circumference of 33-35+6 weeks. The differences were statistically significant (P < 0.05) (**Table 5**).

Taking 25(OH)D as the dependent variable, the relevant index was selected as the independent variable for the multiple linear correlation analysis. It was found that only the 38w-39w6d abdominal circumference had a negative relationship with 25(OH)D. Taking μE3 as the dependent variable and selecting the relevant index as the independent variable to do a multiple linear correlation analysis, it was found that the 15w-17w double top diameter had a certain positive relationship with the μE3. 33w-35w6d double top diameter and had a certain negative relationship with μE3. Taking μE3 MOM as the dependent variable and selecting the relevant index as the independent variable for the multiple linear correlation analysis, it was found that no index was associated with μE3 MOM.

Discussion

As a special population, pregnant women have an uneven nutritional intake during pregnancy. More protein-rich, high-calorie food is consumed, and the amount of exercise during pregnancy is greatly reduced, leading to weight gain in pregnant women. During pregnancy, both the mother and child experience tremendous growth and physiological changes. The fetus and accessory tissues are foreign bodies for the mother. In order not to exclude these useful foreign bodies, the mother's immune surveillance system must be adjusted to ensure tolerance to these foreign proteins from the paternal line, so that it protects them from immune damage. An immune imbalance

caused by various factors in the process will induce diverse complications such as gestational diabetes, pregnancy-induced hypertension, and can eventually lead to fetal distress, the premature rupture of membranes, postpartum hemorrhage and other adverse outcomes. Among the 267 pregnant women included in the study, more than half (136 cases) of the patients had gestational complications or adverse pregnancy outcomes. It was too late to intervene when these gestational complications or adverse outcomes occurred. The health of the mothers and children should be given a high priority. Therefore, using simple serum markers for prediction before complications or adverse outcomes and carrying on early intervention are important steps to take in avoiding or reducing the occurrence of pregnancy complications or adverse outcomes.

In China, pregnant women generally exercise less, have an increased awareness of sun protection, and have a lack of awareness of additional vitamin D supplementation. It leads to a prevalence of vitamin D deficiency in pregnant women. According to relevant statistical data, the proportion of vitamin D deficiency in Beijing, Shanghai, and Guizhou is above 90%. The results in this study showed that the proportion of vitamin D deficiency in pregnant women in Chongqing reaches 97.8%. It is especially important for pregnant women to supplement vitamin D in Chongqing. We should not only pay attention to nutritional supplements from pre-pregnancy to pregnancy, but we should also pay attention to the supplements throughout women's gestational age. Studies have shown that 20 ng/ml circulating 25(OH)D can meet human physiological needs [15]. However, The Endocrine Society believes that the concentration should be at least 30 ng/ml [16], while the circulating 25(OH)D level is required to reach 40 ng/ml during pregnancy [17]. At present, there is still controversy about the amount of vitamin D supplementation. Some experts recommend that pregnant women and lactating women supplement with 600 IU per day, while some suggested that 1000-2000 IU should be added every day [18], and some other people suggested 4000 IU per day. In addition to the recommended daily supplement, the vitamin D content in the blood should also be measured to determine the actual deficiency of vitamin D

Table 5. The Relationship between 25(OH)D, μE3, μE3 MOM and the fetal growth index

	25(OH)D		μE3		MOM	
	r	P	r	P	r	P
10w-14w Double top diameter	0.069	0.481	0.074	0.450	0.030	0.756
10w-14w Head-hip diameter	0.05	0.619	0.099	0.330	-0.080	0.429
15w-17w Femur top diameter	0.098	0.158	0.429*	0.001	0.044	0.523
15w-17w Femur length	0.063	0.369	0.372*	0.001	0.024	0.730
28w-30w Double top diameter	0.177	0.052	0.026	0.779	-0.062	0.499
28w-30w Head circumference	0.148	0.105	0.100	0.273	0.060	0.513
28w-30w Abdominal circumference	0.077	0.399	0.019	0.834	-0.007	0.941
28w-30w Humerus	0.113	0.217	-0.035	0.702	-0.007	0.941
28w-30w Femur	0.146	0.107	-0.019	0.834	-0.024	0.796
33w-35w6d Double top diameter	0.089	0.481	-0.285*	0.022	-0.288*	0.020
33w-35w6d Head circumference	0.186	0.138	-0.229	0.067	-0.253*	0.042
33w-35w6d Abdominal circumference	0.056	0.656	-0.188	0.134	-0.097	0.442
33w-35w6d Humerus	0.122	0.333	-0.137	0.277	-0.113	0.369
33w-35w6d Femur	0.140	0.266	-0.276*	0.026	-0.167	0.183
36w-37w6d Double top diameter	0.038	0.661	-0.043	0.617	-0.023	0.790
36w-37w6d Head circumference	-0.028	0.746	-0.130	0.128	-0.040	0.644
36w-37w6d Abdominal circumference	-0.111	0.195	-0.005	0.952	0.088	0.306
36w-37w6d Humerus	-0.087	0.310	0.011	0.898	0.044	0.610
36w-37w6d Femur	0.072	0.399	0.028	0.744	0.069	0.424
38w-39w6d Double top diameter	-0.044	0.620	-0.006	0.947	-0.026	0.773
38w-39w6d Head circumference	-0.068	0.443	-0.010	0.908	0.020	0.819
38w-39w6d Abdominal circumference	-0.221*	0.012	-0.066	0.460	-0.042	0.638
38w-39w6d Humerus	-0.059	0.504	-0.087	0.329	-0.021	0.811
38w-39w6d Femur	0.031	0.731	-0.099	0.262	0.014	0.873
Gestational age	-0.011	0.862	-0.092	0.133	-0.040	0.514
Birth length	0.125*	0.041	-0.077	0.211	-0.025	0.685
Birth weight	0.012	0.851	-0.109	0.076	-0.083	0.180

Note: *P < 0.05 indicates that the correlation coefficient is statistically significant.

and to assess the sufficiency of vitamin D supplementation.

Vitamin D plays a role in low-intensity chronic inflammation and type 2 diabetes (TD2M) insulin resistance. Insulin resistance and low-intensity chronic inflammation are risk factors for TD2M, so the status of vitamin D is related to the occurrence of TD2M. Estriol is a weak estrogen agonist which increases weight and resists insulin in pregnant women. These changes promote gestational diabetes. In this study, the 25(OH)D, μE3, and μE3 MOM values of patients with gestational diabetes were lower than those of the healthy controls. μE3 MOM was more meaningful than μE3. Therefore, people with a slightly lower MOM value of 25(OH)D and μE3 should pay special attention to the risk of developing gestational diabetes, which can be used

as a monitoring indicator for high-risk pregnant women. Intrauterine distress is an important indicator of caesarean section. This study found that the level of 25(OH)D in pregnant women with intrauterine distress was significantly lower than it is in healthy pregnant women. Therefore, those with low 25(OH)D levels should pay attention to the risk of intrauterine distress. Some researchers believe that the level of μE3 in the second trimester is not related to adverse pregnancy outcomes [19]. This study also confirmed this view, but μE3's low level increases the risk of gestational diabetes. There was also a significant decrease in μE3 in pregnant women with placental adhesions, and μE3 is an independent risk factor for placental adhesions. There are also reports of an increased risk of the premature rupture of membranes in pregnant women with abnormal levels of μE3

[20], but no similar results were found in this study. It is possible that the level of μE3 MOM in pregnant women involved in this study was no less than 0.4, resulting in a difference in the final results.

The level of amniotic fluid index is an important indicator of B-ultrasound monitoring in the third trimester of pregnancy. Too little amniotic fluid can lead to intrauterine hypoxia and adverse pregnancy outcomes. This study found that the μE3 MOM value of the pregnant women with less than an 8 cm amniotic fluid index was significantly higher than that of the higher than 8 cm amniotic fluid index pregnant women. Therefore, the risk of amniotic fluid reduction can be predicted by the mid-pregnancy μE3 MOM value, so that measures can be taken in advance to reduce the occurrence of fetal hypoxia caused by low amniotic fluid and avoid the forced termination of pregnancy due to less amniotic fluid. The placenta is an important organ for maternal and child substance exchange. Its maturity affects the exchange of nutrients. The ability of the grade III placenta to transport oxygen and nutrients is reduced, which is unfavorable for fetal development. Fetal development in pregnant women with placental maturity grade III before 37 weeks of gestation may be affected specially. This study found that pregnant women with a placental maturity of grade III had lower levels of mid-pregnancy μE3 than those with a maturity of grade II, so the level of placental development can be assessed by the level of μE3 in the second trimester.

In early pregnancy, bones and muscles begin to grow, including the formation of the arms, legs, vertebrae, and neck. In the third trimester, fetuses primarily gain weight through the accumulation of fat mass and bone density [21]. The results of this study showed that the levels of μE3 and μE3 MOM in the second trimester (15-17 weeks) were positively correlated with the biparietal diameter and femur length of the fetus during the same period. Therefore, fetal growth can be assessed by simultaneous μE3 and μE3 MOM levels. At 34 weeks of gestation, the femoral head is ossified. During this period, fetal bone age can be confirmed using a fetal femur to determine whether fetal growth meets gestational age. This study found that fetal femur length was positively correlated with the 25(OH)D level, but the difference was not obvi-

ous. The double top diameter and femur length were significantly negatively correlated with μE3, and the double top diameter and head circumference were significantly negatively correlated with the μE3 MOM level. Therefore, the early prediction of fetal growth can be made by the mid-pregnancy μE3 and μE3 MOM levels. At 38w-39w6d, the fetal abdominal circumference was significantly negatively correlated with 25(OH)D level. Vitamin D probably has some effect on improving maternal blood glucose and insulin homeostasis which inhibit the fetal overgrowth in the third trimester of pregnancy. As a result, the pregnant woman with higher 25(OH)D levels have fetuses with smaller abdominal circumferences. At the time of birth, the length of the fetus is significantly positively correlated with the level of 25(OH)D during pregnancy. The higher the 25(OH)D level, the higher the birth length of the fetus.

In summary, this study shows that vitamin D deficiency or insufficiency in pregnant women in Chongqing is very common. Vitamin D, μE3, and μE3 MOM levels are closely related to pregnancy complications, fetal growth, and adverse pregnancy outcomes. In pregnant women's pregnancy care, in addition to supplementing the effective concentration of vitamin D, vitamin D, μE3, and μE3 MOM can be used as a serological indicator to predict the occurrence of pregnancy and adverse pregnancy outcomes. They can facilitate intervention and improve the quality of maternal and child health in advance.

Disclosure of conflict of interest

None.

Address correspondences to: Weixian Chen, Department of Laboratory Medicine of The Second Affiliated Hospital of Chongqing Medical University, No. 74, Linjiang Road, Yuzhong District, Chongqing 400010, China. Tel: +86-023-62896109; E-mail: rj2msnv@163.com

References

[1] Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM and Cooper C; Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008; 62: 68-77.

[2] Sahu M, Bhatia V, Aggarwal A, Rawat V, Saxena P, Pandey A and Das V. Vitamin D deficiency in

rural girls and pregnant women despite abundant sunshine in northern India. Clin Endocrinol (Oxf) 2010; 70: 680-4.

[3] Nicolaidou P, Hatzistamatiou Z, Papadopoulou A, Kaleyias J, Floropoulou E, Lagona E, Tsagris V, Costalos C and Antsaklis A. Low vitamin D status in mother-newborn pairs in Greece. Calcif Tissue Int 2006; 78: 337-342.

[4] Wilson RL, Leviton AJ, Leemaqz SY, Anderson PH, Grieger JA, Grzeskowiak LE, Verburg PE, McCowan L, Dekker GA, Bianco-Miotto T and Roberts CT. Vitamin D levels in an Australian and New Zealand cohort and the association with pregnancy outcome. BMC Pregnancy Childbirth 2018; 18: 251.

[5] Song SJ, Si S, Liu J, Chen X, Zhou L, Jia G, Liu G, Niu Y, Wu J, Zhang W and Zhang J. Vitamin D status in Chinese pregnant women and their newborns in Beijing and their relationships to birth size. Public Health Nutr 2013; 16: 687-692.

[6] Tao M, Shao H, Gu J and Zhen Z. Vitamin D status of pregnant women in Shanghai, China. J Matern Fetal Neonatal Med 2012; 25: 237-239.

[7] Hong-Bi S, Yin X, Xiaowu Y, Ying W, Yang X, Ting C and Na W. High prevalence of vitamin D deficiency in pregnant women and its relationship with adverse pregnancy outcomes in Guizhou, China. J Int Med Res 2018; 46: 4500-4505.

[8] Chen YH, Fu L, Hao JH, Yu Z, Zhu P, Wang H, Xu YY, Zhang C, Tao FB and Xu DX. Maternal vitamin D deficiency during pregnancy elevates the risks of small for gestational age and low birth weight infants in Chinese population. J Clin Endocrinol Metab 2015; 100: 1912-1919.

[9] Gil A, Plaza-Diaz J and Mesa MD. Vitamin D: classic and novel actions. Ann Nutr Metab 2018; 72: 87-95.

[10] Buggio L, Roncella E, Somigliana E and Vercellini P. Vitamin D and benign gynaecological diseases: a critical analysis of the current evidence. Gynecol Endocrinol 2016; 32: 259-263.

[11] Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N and Zheng SG. Vitamin D and chronic diseases. Aging Dis 2017; 8: 346-353.

[12] Wagner CL, Taylor SN, Dawodu A, Johnson DD and Hollis BW. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients 2012; 4: 208-230.

[13] Hollis BW and Wagner CL. Vitamin D supplementation during pregnancy: improvements in birth outcomes and complications through direct genomic alteration. Mol Cell Endocrinol 2017; 453: 113-130.

[14] Huang T, Hoffman B, Meschino W, Kingdom J and Okun N. Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome. Prenat Diagn 2010; 30: 471-477.

[15] Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary reference intakes: applications in dietary planning. Washington (DC): National Academies Press (US) 2003; 17: 269-270.

[16] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH and Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911-1930.

[17] Wagner CL, Baggerly C, McDonnell S, Baggerly KA, French CB, Baggerly L, Hamilton SA and Hollis BW. Post-hoc analysis of vitamin D status and reduced risk of preterm birth in two vitamin D pregnancy cohorts compared with South Carolina March of Dimes 2009-2011 rates. J Steroid Biochem Mol Biol 2016; 155: 245-251.

[18] ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 495: vitamin D: screening and supplementation during pregnancy. Obstet Gynecol 2011; 118: 197-198.

[19] Settiyanan T, Wanapirak C, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, Traisrisilp K and Tongsong T. Association between isolated abnormal levels of maternal serum unconjugated estriol in the second trimester and adverse pregnancy outcomes. J Matern Fetal Neonatal Med 2016; 29: 2093-2097.

[20] Yazdani S, Rouholahnejad R, Asnafi N, Sharbatdaran M, Zakershob M and Bouzari Z. Correlation of pregnancy outcome with quadruple screening test at second trimester. Med J Islam Repub Iran 2015; 29: 281.

[21] Adams Waldorf KM and McAdams RM. Influence of infection during pregnancy on fetal development. Reproduction 2013; 146: R151-R162.