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Impact of Maternal and Neonatal Vitamin D Status on the Development of Congenital Anomalies in Egyptian Model

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Abstract

BACKGROUND: Vitamin D (VD) deficiency is a significant issue during pregnancy. During embryogenesis, VD is essential for cellular growth and differentiation. VD deficiency during pregnancy is linked to a number of maternal and neonatal complications.

AIM: The purpose of this study was to look into the possible link between maternal and neonatal VD status and the development of congenital anomalies (CAs).

PATIENTS AND METHODS: A casecontrol study with 30 mothers and their neonates born with gross structural CAs as cases and 30 mothers and their healthy neonates as controls from Ain Shams University Children Hospital. Serum 25-hydroxyvitamin D (25[OH]VD) levels were determined using maternal and neonatal blood samples. The 25(OH) VD concentration was divided into four categories: severe deficiency (<10 ng/ml), deficiency (<20 ng/ml), insufficient (20–29 ng/ml), and sufficient >30 ng/ml. Above 150ng/ml, there is a high risk of toxicity.

RESULTS: Within 29 days postpartum, there were statistically significant differences between the two groups in maternal and neonatal VD serum levels. The mean maternal VD level was 23.8 ng/ml in cases versus 42.13 ng/ml in controls (p = 0.000). The mean neonatal VD level in cases was 15.97 ng/ml, compared to 28.9 ng/ml in controls (p = 0.000). Both maternal and neonatal VD levels were found to have a significant positive correlation with birth weight.

CONCLUSION: A low level of VD in the mother is linked to an increased risk of CAs in the offspring. As a result, improving the periconceptional maternal VD status is advised.

Introduction

Congenital anomalies (CAs), also known as congenital malformations or birth defects, are functional or structural defects that occur during the development of the foetus. Its cause is unknown in half of the cases, genetic in 30–40%, and environmental in 5–10%. Teratogens have been found in pharmaceuticals, infectious agents, and environmental toxins [1].

Vitamin D (VD) is a steroid hormone that can be synthesised endogenously as well as a lipid-soluble vitamin. It is essential for calcium (Ca)-phosphorus (P) homeostasis [2]. VD deficiency during pregnancy has been linked to the development of a variety of CAs in offspring, including congenital heart disease, congenital neural tube defect, congenital diaphragmatic hernia (CDH), and others [3].

Molecular and genetic studies confirm that VD plays a role in epigenetic modification and influences the risk of a variety of other human diseases, including autoimmune disorders [4]. Through its nuclear receptor (VD receptor [VDR]), VD regulates the essential pathways of cellular metabolism and differentiation (VDR). Furthermore, VD has a significant impact on the regulation of cell replication [5].

The purpose of this research was to look into the potential role of maternal VD levels as an etiological factor in congenital defects in offspring and to look for the possible link between maternal and neonatal VD status and the development of CAs. Furthermore, the effect of various factors on maternal VD level, such as maternal age, parity, antenatal care, and multivitamin intake during pregnancy, was investigated. Our study included two groups: one of neonates diagnosed with a congenital anomaly (Group 1a and their mothers Group 1) and another of age-matched normal neonates (Group 2a and their mothers Group 2) who were free of any CAs.

As a result, it is recommended that VD deficiency in mothers and their offspring be reviewed so that strategies to prevent the impact of VD deficiency on the fetus can be implemented.

Subjects and Methods

In this observational Case–Control study, 60 neonates and their mothers were divided into two groups: The mothers of the 30 neonates born with gross structural CAs were included in Group 1 (case mothers). Thirty neonates with gross structural CAs were included in Group 1a (Cases). Group 2 (control mothers) consisted of the mothers of the 30 healthy neonates. Thirty healthy neonates with no CAs were included in Group 2a (Controls).

Exclusion criteria

Neonates born to consanguineous parents, The presence of more than one anomaly or congenital syndrome, Maternal exposure to teratogenic drugs or STORCH infection during pregnancy, Maternal smoking or alcohol consumption during pregnancy, Maternal disease that has been identified (gestational DM, hypothyroidism, others).

The research was carried out at Ain Shams University Children's Hospital in Cairo, Egypt. Cases were sourced from our neonatal intensive care unit (NICU). The patient was recruited from January 2020 to July 2021. The caregivers gave informed written consent.

Inclusion criteria

Neonates born with gross structural CAs.

All subjects will be tested. The mothers' complete history will be taken, with a special emphasis on obstetric history: Age, parity, time of last delivery, route of delivery, antenatal follow up, maternal VD supplementation (Dose, Duration, and Form), maternal dietetic history, and family history. A comprehensive clinical examination of the neonate, including (anthropometry, neurological, cardiac, chest, and abdominal examination). A thorough surgical evaluation by general surgeon was performed to rule out any surgical interference.

Sample size justification

Power and sample size calculator program was used for calculations of sample size, we were needed to study 30 infants or more per group to be able to reject the null hypothesis that the population means of the two groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

Laboratory

Routine investigations including (complete blood picture and metabolic profile). Maternal and Neonatal serum 25-hydroxyvitamin D (25[OH]VD) concentrations using Immunodiagnostic Systems enzyme-linked immunosorbent assay (ELISA) using the Stat Fax 2100, USA. The kit is a solid phase ELISA, based on the principle of competitive binding. Anti-VD antibody-coated wells are incubated with VD standards, controls, samples, and VD-Biotin conjugate at room temperature for 45 min. During the incubation, a fixed amount of biotin-labeled VD competes with the endogenous VD in the sample, standard, or quality control serum for a fixed number of binding sites on the anti VD antibody. Following a wash step, bound VD -Biotin is detected with Streptavidin-Horseradish Peroxidase (SA-HRP). SA-HRP conjugate immunologically bound to the well progressively decreases as the concentration of VD in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 15 min, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional to the amount of 25-OHVD in the sample. The assay measures both the 25-OHVD 2 and D3. The total assay procedure run time is 75 min.

Test interpretation

The 25(OH)VD concentration was stratified into a severe deficiency <10 ng/ml, deficiency <20 ng/ml, insufficiency 20–29 ng/ml, and sufficiency >30 ng/ml. Status above 150 ng/ml presents high risk of toxicity.

Imaging

Echocardiography to assess any structural cardiac anomalies. Abdominal and cranial Ultrasound to detect any abdominal CAs. Definition of CAs according to the classification of the WHO, 2016 [6]. X-Ray to confirm any skeletal anomalies if present. Others (According to the recorded anomaly).

Statistical analysis

Statistical presentation and analysis of the present study were conducted, using the mean, standard deviation and Student t-test (Unpaired), Linear Correlation Coefficient (r) Chi-square, analysis of variance and receiver operating characteristic

Table 1: Patients group and control group laboratory and demographic data

Parameters	Patients group	Control group	Test value	p-value
	n = 30 (%)	n = 30 (%)		
Calcium				
No	14 (46.7)	10 (33.3)	1.111	0.292
Yes	16 (53.3)	20 (66.7)		
Vitamin D				
No	12 (40.0)	9 (30.0)	0.659	0.417
Yes	18 (60.0)	21 (70.0)		
Continuous	5 (27.8)	8 (38.1)	0.464	0.496
Interrupted	13 (72.2)	13 (61.9)		
Duration of Vit. D intake (months)				
Mean ± SD	3.50 ± 1.50	3.57 ± 1.43	–0.152	0.880
Range	1–6	1–6		
Maternal age				
Mean ± SD	28.23 ± 3.81	27.00 ± 3.82	1.251•	0.216
Range	23–39	20–35		
Parity of mother				
Multi para	25 (83.3)	22 (73.3)	0.884*	0.347
Primi gravid	5 (16.7)	8 (26.7)		
Number of Births				
Mean ± SD	2.77 ± 1.38	2.07 ± 0.87	2.349•	0.022
Range	1–6	1–4		
Antenatal care*				
No	9 (30.0)	7 (23.3)	0.341*	0.559
Yes	21 (70.0)	23 (76.7)		
Neonate Gender				
Male	21 (70.0)	14 (46.7)	3.360*	0.067
Female	9 (30.0)	16 (53.3)		
Birth weight				
Mean ± SD	2645.00 ± 655.68	2908.33 ± 153.16	–1.983•	0.052
Range	1100–3900	2300–3600		
Gestation				
Full term	20 (66.7)	27 (90.0)	4.812*	0.028
Preterm	10 (33.3)	3 (10.0)		
Order of birth				
Mean ± SD	3.10 ± 1.24	2.33 ± 0.92	2.715•	0.009
Range	1–6	1–4		
Mode of delivery				
CS	27 (90.0)	18 (60.0)	7.200*	0.007
NV	3 (10.0)	12 (40.0)		

p > 0.05: Non significant (NS), p < 0.05: Significant (S), p < 0.01: Highly significant (HS), *: Chi-square test, •: Independent t-test.

curve by SPSSV17. Unpaired Student t-test: used to compare between two groups in quantitative data. Chi-square test: used to study the association between two variables or comparison between two independent groups as regards the categorized data.

Table 2: Comparison between patients group and control group regarding maternal and neonatal vitamin D level and degree of deficiency

Parameters	Patients group	Control group	Test value	p-value
	n = 30 (%)	n = 30 (%)		
Maternal vitamin D level in ng/ml within 28 days postpartum				
Mean±SD	23.87 ± 11.55	42.13 ± 22.69	–3.930•	0.000
Range	7–50	10–90		
Maternal vitamin D category*				
Severe deficiency	3 (10.0)	0 (0.0)	9.615*	0.022
Deficiency	7 (23.3)	4 (13.3)		
Insufficiency	12 (40.0)	7 (23.3)		
Sufficiency	8 (26.7)	19 (63.3)		
Neonatal vitamin D level in ng/ml within 28 days of life				
Mean ± SD	15.97 ± 7.05	28.9 ± 13.00	–4.790•	0.000
Range	5–40	12–70		
Neonatal vitamin D category*				
Severe deficiency	4 (13.3)	0 (0.0)	21.621*	0.000
Deficiency	18 (60.0)	5 (16.7)		
Insufficiency	7 (23.3)	15 (50.0)		
Sufficiency	1 (3.3)	10 (33.3)		

p > 0.05: Non significant (NS), p < 0.05: Significant (S), p < 0.01: Highly significant (HS), *: Chi-square test, •: Independent t-test.

Linear Correlation Coefficient (r): used for detection of correlation between two quantitative variables in one group. Probability (p-value): $p < 0.05$ was considered significant., $p < 0.001$ was considered as highly significant., $p > 0.05$ was considered insignificant.

Table 3: Correlation of maternal Vitamin D level with maternal age, total number of births, and birth weight

Parameters	Neonatal Vitamin D level in ng/ml within 28 days of life		Maternal Vitamin D level in ng/ml within 28 days postpartum	
	R	p-value	R	p-value
Maternal age	–0.258	0.169	0.251	0.182
Number of Births	0.376*	0.040	0.243	0.196
Birth Weight	–0.372*	0.047	0.375*	0.041

Results

In group(1a); there was nine neonates (30%) had congenital gastrointestinal tract (GIT) anomalies, including 3 neonates (10%) with tracheoesophageal fistula, two neonates (6.7%) with congenital hypertrophic pulmonary stenosis, two neonates (6.7%) with Exomphalos, one neonate (3.3%) with ileal atresia, and one neonate (3.3%) with Gastroschisis.

Table 4: Correlation between maternal Vitamin D category with antenatal history, Vitamin D, and calcium supplementation during pregnancy

Parameters	Maternal Vitamin D category		Test value	p-value
	Deficiency	Sufficiency		
	n = 22 (%)	n = 8 (%)		
Antenatal care				
No	7 (31.8)	2 (25.0)	0.130*	0.719
Yes	15 (68.2)	6 (75.0)		
Birth weight				
Mean ± SD	2600.00 ± 625.86	2768.75 ± 762.95	–0.617•	0.542
Range	1100–3800	1800–3900		
Gestation				
Full term	15 (68.2)	5 (62.5)	0.085*	0.770
Preterm	7 (31.8)	3 (37.5)		
Number of births				
Mean ± SD	3.05 ± 1.40	3.25 ± 0.71	–0.393•	0.697
Range	1–6	2–4		
Maternal age				
Mean ± SD	28.14 ± 3.75	28.50 ± 4.24	–0.227•	0.822
Range	23–39	23–35		
Parity of mother				
Multi para	17 (77.3)	8 (100.0)	2.182*	0.140
Primi gravida	5 (22.7)	0 (0.0)		
Number of births				
Mean ± SD	2.68 ± 1.52	3.00 ± 0.93	–0.551•	0.586
Range	1–6	2–4		
Mode of delivery				
CS	19 (86.4)	8 (100.0)	1.212*	0.271
NV	3 (13.6)	0 (0.0)		
Calcium				
No	10 (45.5)	4 (50.0)	0.049*	0.825
Yes	12 (54.5)	4 (50.0)		
Vitamin D				
No	7 (31.8)	5 (62.5)	2.301*	0.129
Yes	15 (68.2)	3 (37.5)		
Continuous	4 (26.7)	1 (33.3)	0.055*	0.814
Interrupted	11 (73.3)	2 (66.7)		
Duration of Vitamim D intake				
Mean ± SD	3.47 ± 1.60	3.67 ± 1.15	–0.204•	0.841
Range	1–6	3–5		

p > 0.05: Non significant (NS), p < 0.05: Significant (S), p < 0.01: Highly significant (HS), *: Chi-square test; •: Independent t-test.

Seven neonates (23.3%) had congenital heart anomalies, two (6.7%) had Fallot tetralogy, two (6.7%) had transposition of the great arteries, one (3.3%) had Coarctation of the aorta, one (3.3%) had Pulmonary stenosis, and one (3.3%) had Dextrocardia with large ventricular septal defect. Seven neonates (23.3%) had congenital central nervous system (CNS) anomalies, with three neonates having congenital CNS anomalies (10 %) Four neonates representing (13.3%) had congenital Urogenital anomalies inform of horseshoe

Table 5: Relation between maternal and neonatal with demographic and laboratory parameters

Parameters	Maternal Vitamin D level in ng/ml within 28 days postpartum		Neonatal Vitamin D level in ng/ml within 28 days of life	
	Mean ± SD	p-value	Mean ± SD	p-value
Gestational Age				
Full term	25.3 ± 11.19	0.345 (ns)	17.65 ± 7.54	0.063 (ns)
Preterm	21 ± 12.3		12.6 ± 4.6	
Parity of Mother				
Multi Para	25.16 ± 11.91	0.174 (ns)	14.84 ± 5.36	0.048 (s)
Primi Gravida	17.4 ± 7.23		21.6 ± 11.84	
Mode of Delivery				
CS	24.33 ± 11.92	0.516 (ns)	16.3 ± 6.92	0.452 (ns)
NV	19.67 ± 7.57		13 ± 9.17	
Calcium intake during pregnancy				
No	24.5 ± 11.3	0.784 (ns)	15.93 ± 8.37	0.978 (ns)
Yes	23.31 ± 12.1		16 ± 5.94	
Vitamin D intake				
No	27.83 ± 12.16	0.126 (ns)	14.33 ± 4.38	0.308 (ns)
Yes	21.22 ± 10.64		17.06 ± 8.32	
Vitamin D regimen				
Continuous	24.4 ± 14.62	0.449 (ns)	16.6 ± 13.69	0.891 (ns)
Interrupted	20 ± 9.13		17.23 ± 5.96	
Antenatal Care				
No	20.67 ± 10.78	0.329 (ns)	19 ± 9.07	0.125 (ns)
Yes	25.24 ± 11.84		14.67 ± 5.76	
Neonate Gender				
Male	22.57 ± 12.21	0.357 (ns)	15.86 ± 5.99	0.899 (ns)
Female	26.89 ± 9.78		16.22 ± 9.51	

kidney, congenital solitary kidney, polycystic kidney, and cloacal extrophy, each of them representing (3.3%) and three neonates representing (10%) had congenital respiratory anomalies inform of CDH.

Table 6: Category and subtypes of congenital anomalies included

Congenital Anomaly	Patients group	
	No.	%
GIT	9	30.0
Tracheo esophageal fistula	3	10.0
CHPS	2	6.7
Ileal atresia	1	3.3
Gastrosciezies	1	3.3
Exomphalos Major	1	3.3
Omphalocele	1	3.3
Cardiology	7	23.3
Fallot tetrology	2	6.7
TGA	2	6.7
Coarcatation of aorta	1	3.3
Critical Pulmonary Stenosis	1	3.3
VSD, Dextrocardia	1	3.3
Urology	4	13.3
Horse shoe kidney	1	3.3
Solitary Kidney	1	3.3
Polycystic kidney	1	3.3
Cloacal extrophy	1	3.3
Neurology	7	23.3
Arnold chiari	3	10.0
Enchephalocele	2	6.7
Hydrochephalus	1	3.3
Meningeomyelocele	1	3.3
Respiratory	3	10.0
Diaphragmatic hernia	3	10.0

TGA: Transposition of the Great Arteries, GIT: Gastrointestinal tract, VSD: Ventricular septal defect.

In our study, 10.0% of mothers in Group 1 had severe VD deficiency, 23.3% had deficient VD levels, 40% had insufficient VD levels, and 26.7% had adequate VD levels.

In our study, 13.3% of neonates had a severe VD deficiency, 60% had a deficient VD level, 23.3% had an insufficient level of VD, and 3.3% had an adequate level of VD. In Group 2, however, 16.7% of neonates had a deficient VD level, % had an insufficient level of VD, 33.3% had an adequate level of VD, and no neonates had a severe deficiency of VD level.

We assumed that strict antenatal care with an obstetrician would reduce the risk of maternal VD deficiency, but our findings revealed that there was no statistically significant relationship between antenatal care and maternal VD deficiency. This finding could be the result of a late pregnancy diagnosis, for example, many women are diagnosed to be pregnant in the 3rd or 4th month of pregnancy when almost all fetal organs have already formed. Other possible explanations include the fact that most obstetricians only prescribe iron and folic acid, ignoring VD and other multivitamins.

There is a lack of studies on the effect of antenatal care on maternal periconceptional VD level, which is important data to assess the effect of antenatal care on improving maternal VD status, and no studies on the relationship between maternal and neonatal VD level in CAs.

Table 7: Relation between degree of maternal Vitamin D deficiency with neonatal type of congenital anomaly

Category of congenital anomaly	Maternal Vitamin D category		Test value	p-value
	Deficiency	Sufficiency		
	n = 22 (%)	n = 8 (%)		
Gastero-Enterology	6 (27.3)	3 (37.5)	2.788	0.594
Cardiology	4 (18.2)	3 (37.5)		
Urology	3 (13.6)	1 (12.5)		
Neurology	6 (27.3)	1 (12.5)		
Respiratory	3 (13.6)	0 (0.0)		
Needed Surgical intervention	6 (27.3)	0 (0.0)		

p > 0.05: Non significant (NS), p < 0.05: Significant (S), p < 0.01: Highly significant (HS), *: Chi-square test, •: One- way ANOVA test.

Although we divided our cases into groups based on whether the neonate had congenital heart defects, GIT anomalies, CNS anomalies, Respiratory anomalies, or Genitourinary anomalies, as well as the subtype of congenital defect, we were unable to link an increased risk of certain congenital defects to low maternal or neonatal VD status. Only in cases of maternal and neonatal VD deficiency did our study find an increased overall risk of CAs (Tables 1-8).

Table 8: Relation between maternal and neonatal Vitamin D level with type of congenital anomaly

Parameters	Maternal Vitamin D level in ng/ml within 28 days postpartum		Neonatal Vitamin D level in ng/ml within 28 days of life	
	Mean ± SD	p-value	Mean ± SD	p-value
Type of congenital anomaly				
Gastroenterology	21.33 ± 11.25	0.567	12.67 ± 2.83	0.019
Cardiology	29.14 ± 17.74		15.14 ± 5.27	
Urology	25 ± 3.56		11.25 ± 4.5	
Neurology	24.29 ± 8.75		22.71 ± 9.64	
Respiratory	16.67 ± 5.13		18.33 ± 5.69	

Discussion

VD is crucial for fetal development because of its important role during cell proliferation, differentiation, and maturation processes. Suboptimal VD concentrations may affect early organogenesis and subsequently affect later health and disease. Based on previous studies suggesting that low VD concentrations

may lead to suboptimal placentation, organogenesis, and fetal skeletal growth, we hypothesized that low VD concentrations may lead to increased risks of CAs [7].

In our study, there was a statistically significant relationship between maternal VD level and gestational age, but no relationship was found between maternal VD level and mother's age, parity, number of births, multivitamin intake during pregnancy, or neonatal VD level. Of note; the current studied mothers were <30 years, this comes in agreement of one study that revealed that VD deficiency was more in younger women below 30 years old (43.2%, p = 0.032), housewives (65.3%, p = 0.008) and those on low incomes (49.2%, p = 0.03) [8].

On the contrary to our results; VD supplement intake (89.7%, p < 0.001) were significantly lower in deficient pregnant women [9]. Furthermore, no statistically significant relationship was found between neonatal VD and mother's age, parity, gestational age, multivitamin intake during pregnancy, or maternal VD level. The current study found a statistically significant difference between groups 1 and 2 in terms of maternal and neonatal VD levels in ng/ml within 29 days of delivery. The mean maternal VD level in group 1 was 23.8 ng/ml, whereas the mean maternal VD level in group 2 was 42.13 ng/ml (p = 0.001). While Group 1 had a mean neonatal VD level of 15.97 ng/ml, Group 2 had a mean neonatal VD level of 28.9 ng/ml (p = 0.000). In concordance to our results; the study done by Koster *et al*. They analyzed the association between both maternal VD level and development and CHD in offspring: mean VD level in cases mothers compared to controls was (20.43 ng/ml vs. 32.45 ng/ml, p = 0.020) [10].

Furthermore, the study of Dave A *et al*. revealed that nearly half of the studied pregnant women had VD deficiency (48.2%) [11]. Furthermore, other study designed in South Carolina found 41% of pregnant women with deficiency of VD (25[OH]D <50 nmol/L) and another 41% were insufficient with a level of (25[OH]D 50–80 nmol/L). 4 Furrthermore, the study by Merewood *et al*. found 62% of Caucasian pregnant women and 96% of African American pregnant women were deficient or insufficient (25[OH]D <80 nmol/L) in VD during early pregnancy. Another study examined the prevalence of VD deficiency and insufficiency in 80 pregnant African American adolescents and revealed that 52% and 36% of African American adolescents were low (25[OH]D <50 nmol/L) in VD [12], [13], [14].

In the study done in India by Ghosh and Bhardwa that was planned to evaluate the level of 25-(OH)VD in cord blood in cases of CAs and its association with visible CAs: mean VD level in cases neonates compared to controls was (26.86 ng/ml vs. 39.91 ng/ml, p = 0.000) [15].

In our study, there was statistically significant positive correlation between maternal VD level and birth weight while no statistically significant correlation found between maternal VD level and age or total number of births. Many studies revealed that maternal VD levels were positively correlated with birthweight centile [16], and that VD deficiency had up to 2.4-fold risk of having a small birth weight baby [17]. Furthermore, the study by Dave *et al*. found that VD deficient mothers had more growth-restricted babies (80%) [11].

Our results showed also that there was statistically significant positive correlation between neonatal VD level and birth weight and there was negative correlation between neonatal VD level and order of birth while no statistically significant correlation found between neonatal VD and age of mother.

A systematic review and meta-analysis done by Fang *et al*. indicate a consistent association between VD deficiency during pregnancy and an increased risk of low birth weight, In this meta-analysis, the results showed that VD deficiency during pregnancy was likely to have significant positive correlation with LBW (OR ¼ 2.45, 95% CI: 1.91–3.13) [18]. Furthermore, the study by Dave *et al*. the outcome of babies in relation to VD deficiency. Growth restriction was more common in the VD deficient women who delivered more (80%) low birth weight babies (<2.5 kg). The maternal deficiency seems to be affecting the baby growth in utero [11].

Correlation between maternal and neonatal VD level in our study could not be established, there was no statistically significant correlation found. This could be due to several limitations such as low number of population included in our study and also VD levels were assessed only after delivery, which does not represent the levels for the entire duration of pregnancy.

In the study done by Rabbani *et al*. they evaluated the Correlation between maternal and neonatal blood VD level among 213 pregnant women and their neonates and their results showed strong positive association between maternal and newborn 25(OH)D levels (p < 0.001) [19].

Although we classified our cases into groups whether having congenital heart defects, GIT anomalies, CNS anomalies, respiratory anomalies, and genitourinary anomalies and also which subtype of congenital defect, we could not correlate increased risk of certain congenital defects to low maternal or neonatal VD status. Our study showed increased overall risk of CAs due to maternal and neonatal VD deficiency.

In a study conducted in Zakazig University, Egypt by Mokhtar *et al*. they analyzed the association between both maternal VD level and VDR gene Fok1polymorphism and risk of CHD in offspring. Concerning maternal VD level and status among Egyptian healthy control mothers, they concluded that the percentage of mothers having deficient, insufficient, inadequate, and sufficient VD status were 16%, 50%, 22%, and 12%, respectively [20].

In a study done by *Gulenay et al.* aimed to evaluate maternal serum VD levels in neonates with isolated CDH compared to pregnant patients without CDH, The results showed that mean maternal serum VD levels were significantly lower in the study group than in the controls (p: 0.019) [21].

Furthermore, VD concentrations were significantly lower in mothers with fetal anomaly of neural tube origin compared to pregnant with healthy fetus as shown in a study done by Hicran *et al*. [22].

We identified a very high burden of VD deficiency in pregnant mothers and their newborns, exposing them to a higher risk of health problems. Our findings are coherent with the current literature and support the evidence that newborns are dependent on their mothers for the supply of VD during early life.

Hence, we suggest that VD supplementation should be considered for 25(OH)D deficient pregnant mothers and their newborns, and the antenatal visits should include education on the safety and importance of sufficient sunlight exposure during pregnancy. Moreover, pregnancy-specific 25(OH)D cut- offs and dietary recommendations are required for optimal health of the mothers and the newborns.

Conclusion

We have demonstrated that a compromised maternal and neonatal VD status is associated with CAs.

The 1st weeks of pregnancy, when pregnancy is often not yet recognized, are crucial for embryogenesis, which emerges the need of an adequate maternal VD status already in the preconception period. However, evidence concerning adequate and safe intakes of dietary and synthetic VD for this target group is lacking thereby emphasizing the need for research to establish recommendations.

Study Limitation

Unequal number of cases regarding some demographic data

Authors Contributions

All authors equally contributed in the study concept, design, supervision, methodology, data collection, statistical analysis, laboratory workup. All authors reviewed and approved the final manuscript for publication.

SAM: Collected the data, writing manuscript, follow up patients and their supervision; ME: Implementing the study design, supervision of workflow; SAM: Implementing the study design, supervision of workflow; ZI: Performed the surgical evaluation and intervention if needed by the patients, share in writing and final revision of work; HEH: Performed the investigations, interpretation of the results, final revision; OA: Performed the investigations, interpretation of the results, final revision; MK: Collect data, write the thesis; DS: Interpretation of results, follow up of patients, final revision of manuscript; WOA: Follow-up the patient in NICU, write manuscript, interpretation of data

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