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Original Research Article

Prevalence of vitamin D3 deficiency and its correlation with high-risk pregnancy pre-eclampsia and eclampsia study from central India

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ABSTRACT

Background: To study the relationship between vitamin D3 deficiency and pre- eclampsia and eclampsia.
Methods: It is case control study, The study was conducted in the department of medicine Pt. J. N. M. and BRAM hospital, Raipur (CG) among the patients diagnosed as pre-eclampsia and eclampsia admitted in Obs. and Gynae wards. Study group (Group A) comprised of 80 patients diagnosed as pre-eclampsia and eclampsia presented at term Control group (Group B) comprised of 50 individuals from normal healthy population (ANC patient at term which was normotensive)
Results: It is case control study. In this study 130 patients were included of which study group comprised of 80 patients and control group comprised of 50 patients. it has been observed that vitamin D deficiency was prevalent among the study group and control group however statistically significant higher number of cases in study group (toxemia of pregnancy) were vitamin D deficient as compared to control group. In our study 95% cases had lower level of vitamin D (35% insufficient and 60% deficient) as compared to 72% (46% insufficient and 28% deficient) in control. Out of these 60% of cases in study group vs. 28% cases in control group were seriously vitamin D deficient (vitamin D level <30 nmol/l).
Conclusions: Vitamin D deficiency and insufficiency was found to be associated with normal pregnancy (control group), preeclampsia and eclampsia. In present study direct correlation was observed in preeclampsia and eclampsia with low vitamin D levels.

Keywords: Preeclampsia pregnancy induced hypertension, PIH, Vitamin vascular endothelial growth factor

INTRODUCTION

A specific importance for vitamin D during pregnancy is suggested by the presence of vitamin D receptors (mediating hormonal effects) and vitamin D hormonal activator enzyme (CYP27B1) in pregnancy specific tissues such as the placenta and in the deciduae. Poor vitamin D status, based on low circulating 25-hydroxyvitamin D (25OHD) concentration, has been described in pregnant women in several countries, Kimball et al.[1] Attaining adequate vitamin D status during pregnancy is important for maternal and infant health. A lack of vitamin D during pregnancy results in poor fetal and infant bone mineralization that may persist into later life (Weiler Het et al and Javaid et al.[2] Also low maternal vitamin D has been associated with an increased risk of lower birth weight (Brooke OG et al), type 1 diabetes, Hypponen et al and asthma Camargo et al in the offspring.[3,4] Low maternal vitamin D has also been associated with an increased risk of pre-eclampsia Bodnar et al.[5] In a prospective nested case–control study, Bodnar et al reported that mean serum 25OHD measured early in pregnancy (<22 weeks of gestation) was lower in 55 women who subsequently developed pre-eclampsia than in 219 control women (45.4 versus 53.1 nmol/l; p<0.01).[5] Further, there was a dose-response relationship between 25OHD concentration and risk of pre-eclampsia such that each 50 nmol/l decrease in

25OHD concentration doubled the risk of pre-eclampsia. Also, in a recent large Norwegian cohort study done by Haugen et al women taking supplements containing vitamin D pre-pregnancy, in the first trimester and in late pregnancy had a lower risk of developing pre-eclampsia than those who did not (odds ratio 0.81, 95% CI 0.68-0.97).[6]

Vitamin D deficiency among women of reproductive age is of particular concern because it can have negative consequences for mother, fetus, infant, and child, Dawodu and Wagner.[7] Recent research has suggested that vitamin D deficiency may put women of reproductive age at greater risk for preeclampsia during pregnancy, breast cancer, and premenstrual syndrome, Hyppınen, Bodnar, Evans et al, John et al and Bertone-Johnso.[3,4,8-10] The maternal mid gestation vitamin D deficiency was associated with increased risk of severe preeclampsia Baker et al.[11] Keeping this in mind I intend to measure the levels of vitamin D and to find out any association between serum vitamin D levels in patients of preeclampsia and eclampsia and its effect on pregnancy outcome.

METHODS

It is a case control study the study was conducted in the department of medicine Pt. J. N. M. and BRAM hospital, Raipur (CG) among the patients diagnosed as pre-eclampsia and Eclampsia admitted in obs. and gynae wards from year 2013 to 2015. Study group (Group A) comprised of 80 patients diagnosed as pre-eclampsia and eclampsia presented at term control group (Group B) comprised of 50 individuals from normal healthy population (ANC patient at term which was normotensive), ethical approval was obtained.

Patients included in our study were pts of preeclampsia having blood pressure ≥140/90 mmHg after 20 weeks, urine albumin ≥300 mg /24 hour or 1+ by dipstick method. And in eclampsia preeclampsia patients complicated with generalized tonic convulsion and/or coma. We excluded pregestational diabetes mellitus, chronic hypertension, chronic renal disease, known case of seizure disorder, urinary tract infection any liver disease.

Statistical methods

The calculation of mean and standard deviation was done by method described by Park.

$\chi^2 = \sum(O_i\text{-}E_i)^2/E_i$

Mean

Individual observation is added together and divided by the number of observations, process of adding together is known as summation and is denoted by Σ or S. individual observation is denoted by sign x at arithmetic mean is denoted by sign Σ. Total number of observations is denoted by 'n'.

Test of significance

Chi-square test

This was done to find out significance of difference observed between two variables in following steps-First expected proportion of cases calculated from the observed and then X_2 applied were

$X^2=\sum(0\text{-}E)^2/ E$

If the p value <0.05 the difference of the two sets of observation was considered significant otherwise insignificant.

RESULTS

Analysis of prevalence of vit D deficiency in pre-eclampsia and eclampsia with the control population

In present study it has been observed that vitamin D deficiency was prevalent among the study group and control group however statistically significant higher number of cases in study group (toxemia of pregnancy) were vitamin D deficient as compared to control group.

In our study 95% cases had lower level of vitamin D (35% insufficient and 60% deficient) as compared to 72% (46% insufficient and 28% deficient) in control. Out of these 60% of cases in study group vs. 28% cases in control group were seriously vitamin D deficient (vitamin D level <30 nmol/l) which was statistically significant (p=0.0003) and 35% of cases in study group vs. 46% cases in control group were having insufficient vitamin D level (vitamin D level ≥ 30-≤75 nmol/l) which has border line significance (p=0.059).

Analysis of prevalence of vit D deficiency in pre-eclampsia and eclampsia

In our study, we found that among the preeclampsia patient 95.24% had low vitamin D level (36.50% insufficient and 58.74% seriously deficient) vs. 94.12% cases among the eclampsia patient (29.41% insufficient and 64.71% seriously deficient). This difference was statistically insignificant (p1=0.43; p2= 0.15). Thus, it seems that vitamin D level does not affect the progression of the disease from preeclampsia to eclampsia.

Analysis of prevalence of vitamin D deficiency in study and control group based on rural and urban area

In our study, vitamin D deficiency found in 15 (44.12%) patients from rural area and 33 (70.74%) patients from urban area in study group whereas 6 (30%) patients from rural area and 8 (26.66%) patients from urban area in the control group. Vitamin D insufficiency was found in 18 (52.94%) patients from rural area and 10 (21.74%) patients from urban area in study group whereas 6 (30%) patients from rural area and 17 (56.66%) patients from urban area

in control group. Normal vitamin D level was found in 1 (2.94%) patient from rural area and 3 (6.52%) patients from urban area in study group whereas 8 (40%) patients from rural area and 5 (16.66%) patients from urban area in control group.

Table 1: Prevalence of normal, insufficient and deficient vitamin D level in cases and controls.

Vitamin D level (nmol/l)	Cases, n=80		Controls, n=50		Z test	P value
	N	%	N	%		
>75	4	5	13	26	39.18	0.0001
≥30-≤75	28	35	23	46	3.56	0.059
<30	48	60	14	28	8.78	0.003
Total	80	100	50	100		

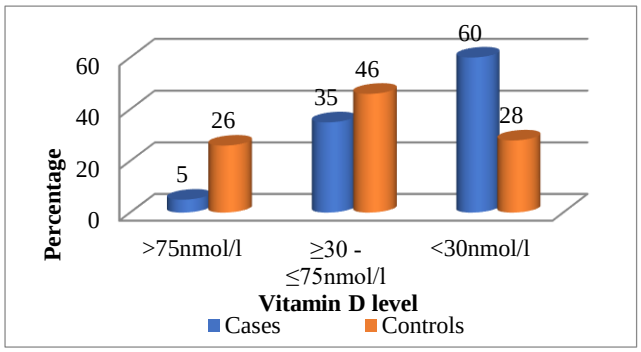

Figure 1: Prevalence of normal, insufficient and deficient vitamin D level in cases and controls.

Table 2: Comparison of vitamin D in preeclampsia and eclampsia patients.

Vitamin D level (nmol/l)	Pre-eclampsia, n=63		Eclampsia, n=17		Z test	P
	N	%	N	%		
>75	3	4.76	1	5.88	0.47	0.49
≥30-≤75	23	36.50	5	29.41	0.62	0.43
<30	37	58.74	11	64.71	0.15	0.69
Total	63	100	17	100		

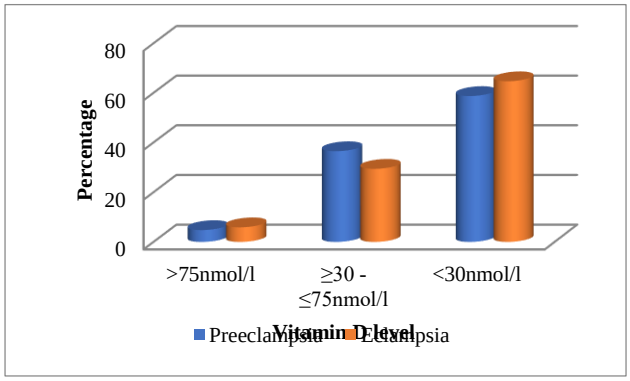

Figure 2: Comparison of vitamin D in preeclampsia and eclampsia patients.

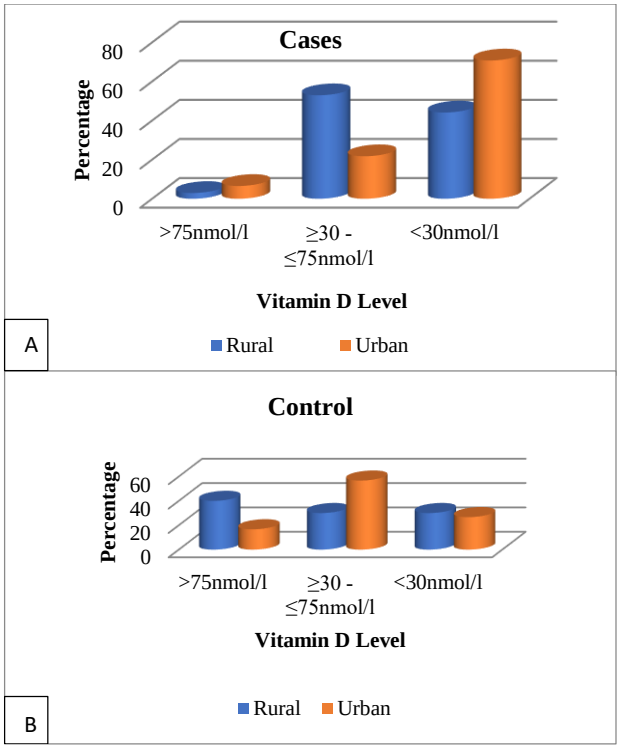

Figure 3 (A and B): Prevalence of normal, insufficient and deficient vitamin D level in cases and control residing in rural and urban area.

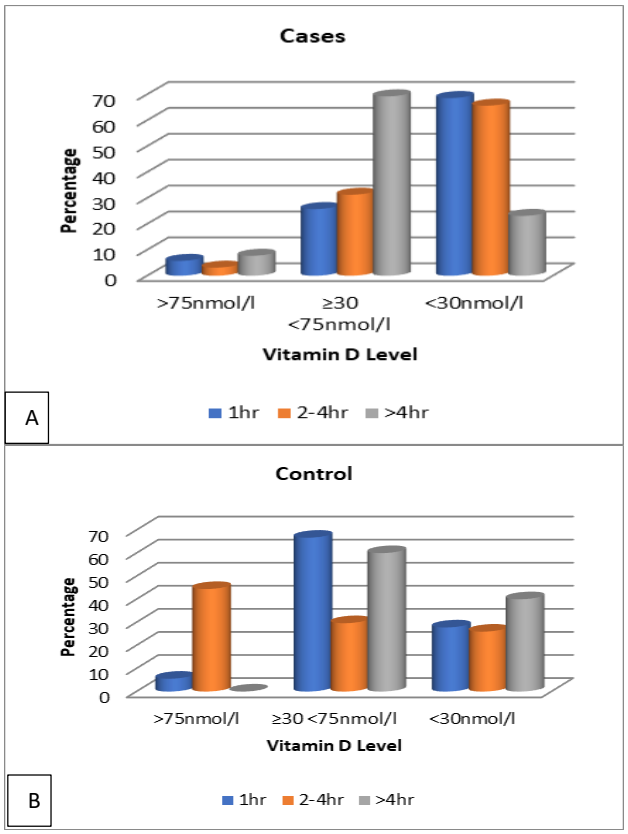

Figure 4 (A and B): Prevalence normal, insufficient and deficient vitamin D level in cases and control depending upon sun exposure (Hours/day).

The mean vitamin D level in rural population was 37.88±20.11 nmol/l in cases vs. 58.30±23.42 nmol/l in control (p=0.001) where in urban population was 26.84±18.52 nmol/l in cases vs. 46.93±20.99 nmol/l in control, this was found to be statistically significant (p=0.0001).

Table 3: Prevalence of normal, insufficient and deficient vitamin D level in cases and controls residing in rural and urban areas.

Vitamin D level (nmol/l)	Cases, n=80				Controls, n=50			
	Rural		Urban		Rural		Urban	
	N	%	N	%	N	%	N	%
>75	1	2.94	3	6.52	8	40	5	16.66
≥30-≤75	18	52.94	10	21.74	6	30	17	56.66
<30	15	44.12	33	70.74	6	30	8	26.66
Total	34	100	46	100	20	100	30	100

Table 4: Prevalence of normal, insufficient and deficient vitamin D level in cases and controls depending upon sun exposure (Hours/day).

Category	Cases, n=80							Controls, n=50						
Sun exposure (Hours /day)	Normal (Vit D level >75 nmol/l)		Insufficient (Vit D level ≥30 <75 nmol/l)		Deficient (Vit D level <30 nmol/l)		Total	Normal (Vit D level >75 nmol/l)		Insufficient (Vit D level (≥3-<75 nmol/l)		Deficient (Vit D level <30 nmol/l)		Total
	No	%	No	%	No	%		No	%	No	%	No	%	
1	2	5.71	9	25.71	24	68.57	35	1	5.56	12	66.67	5	27.78	18
2-4	1	3.12	10	31.25	21	65.62	32	12	44.44	8	29.63	7	25.96	27
>4	1	7.69	9	69.23	3	23.08	13	0	0	3	60	2	40	5
Total							80							50

Analysis of prevalence of vitamin D deficiency in study and control group based on sun exposure

In our study we found that cases having daily sun exposure less than 1 hour**,** the mean vitamin D level was 26.02±18.51 nmol/l in study group while 42.75±16.55 nmol/l in control group which was statistically significant (p<0.0001). Those patients having daily sun exposure 2-4 hour**,** the mean vitamin D level was 32.26±8.46 nmol/l in study group while 58.06±24.43 nmol/l in control group, this was also found to be statistically significant (p<0.0001) and the patients who had daily sun exposure greater than 4 hours the mean vitamin D level was 44.53±21.76 nmol/l in study group while 47.32±22.05 nmol/l in control group (p=0.94). From above observation it was found that the mean vitamin D level in study group was significantly lower than the control group irrespective of the duration of sun exposure. Also, there is significantly low vitamin D level in the patients who had less sun exposure.

Analysis of prevalence of vitamin D deficiency in study and control group based on religion

In our study, among the study group of 80 patients, 74 patients were Hindu and 6 patients were Muslim while in control group out of 48 patients were Hindu and 2 were Muslim.

Among the cases 44/74 (59.46%) Hindu and 4/6 (66.66%) Muslim had vitamin D level less than 30 nmol/l while in control 12/48 (25%) Hindu and 2/2 (100%) Muslim had vitamin D level less than 30 nmol/l. Vitamin D level between 30-75 nmol/l there was found in 27/74 (36.49) Hindu and 1/4 (16.67%) Muslim in cases while 23/48 (47.9%) Hindu in control. Vitamin D level greater than 75 nmol/l was found in 3/74 (4.05%) Hindu and only 1/4 (16.67%) in Muslim patients in cases while 13/48 (27.1%) Hindu patients in control. From above observation we could not draw firm conclusion as the number of Muslim patients in our study was small.

DISCUSSION

The study entitled "study of association of vitamin d deficiency in patients of preeclampsia and eclampsia*"* was conducted in the department of medicine Pt. J. N. M. and BRAM hospital, Raipur (CG) and hospital among 80 patients diagnosed as pre-eclampsia and eclampsia admitted in Obs. and Gynae wards. 50 normal pregnant women were used as control.

The mean vitamin D level in study group was 31.82±19.82 nmol/l vs. 52.16±22.18 nmol/l in control group showing statistically significant difference (p=0.0001).

Similar observation was found one of the studies in her study, Author found that adjusted serum 25(OH)D concentrations in early pregnancy were lower in women

who subsequently developed pre-eclampsia compared with controls [geometric mean, 45.4 nmol/liter, and 95% confidence interval (CI), 38.6-53.4 nmol/liter, vs. 53.1 and 47.1-59.9 nmol/liter; p<0.01]. Early-pregnancy maternal 25(OH)D concentration less than 37.5nmol/liter was associated with a 5-fold increase in the odds of preeclampsia (adjusted OR, 5.0; 95% CI, 1.7-14.1), independent of race/ethnicity, season, gestational age, pre pregnancy BMI, and education, Bodnar et al.[5]

Found association between the reduced risk of preeclampsia and vitamin D supplementation in infancy. He concluded that risk of pre-eclampsia was halved (OR 0.49, 95% confidence interval (CI) 0.26-0.92) in participants who had received vitamin D supplementation regularly during the first year of life and this association was not affected by adjustment for own birth order, birth weight, gestational age, social class, Hyppo¨nen et al.[3]

Haugen conducted cohort study and demonstrated a 27% reduction in risk of preeclampsia (OR=0.73 [0.58-0.92]) for women taking 10-15 microg/d as compared with no supplements. No association was found between intake of vitamin D from the diet alone and the occurrence of preeclampsia, Haugen et al.[6]

There was an inverse association between maternal 25(OH) D concentration at mid gestation and risk of severe preeclampsia. There were no cases of severe preeclampsia among women with serum 25(OH)D concentrations greater than 135 nmol/liter, Baker et al.[11]

In our study we also found the significant association of vitamin D deficiency and preeclampsia as observed by other studies throughout the world. In our study the control group also comprised of pregnant women and we found that the vitamin D deficiency in pregnant women was 74%. Similar observation was made by various studies.

It was observed 74% of pregnant women had vitamin D deficiency in India, Sahu et al.[12]

Another study found the prevalence of vitamin D deficiency was 84% among pregnant women in India which correlated significantly with serum 25(OH) D status of their newborn. Sachan et al.[13]

It was found that 67% of pregnant women had vitamin D deficiency [serum 25(OH)D concentration, 50 nmol/L]. Krishnaveni et al.[14]

It was found that prevalence of vitamin D deficiency was 69.9% among pregnant women in their study. Tahir and Naqui et al.[15] It is observed that nearly 42% of the cases had vitamin D deficiency and 14% had vitamin D insufficiency in the first trimester, Dasgupta et al.[16]

in residents of a North Indian village, it was found that the mean 25(OH)D values of all subjects in the rural area was 36.4 ±22.5 nmol/l/L. Males had significantly higher 25(OH)D values than females. When compared to urban subjects, the mean 25(OH)D value of rural males and females was six and three folds higher, respectively, Goswami et al.[17]

It was observed That 25(OH)D levels of rural adult subjects were significantly higher (p<0.001) than that of urban adult subjects in both males and female groups, Harinarayan et al.[18]

The mean vitamin D level in rural population was greater than urban population (urban: 15.5±0.3 ng/ml, rural: 19±0.9 ng/ml), Harinarayan et al.[18]

This was attributable to dress code and occupation, longer duration of exposure to sunlight in rural population but it was found that mean vitamin D level in rural and urban the mean serum 25(OH)D concentration in urban women did not differ significantly from that in rural women (urban: 14.0±9.5 ng/mL; rural: 14.1±8.9 ng/mL), but Sachan et al.[13]

Similar observation was made by they observed that sun exposure contributes in vitamin D level. The overall prevalence of vitamin D deficiency (defined as serum 25(OH)D, <25 nmol=l) was 7% in patients having adequate sun exposure. However, in the subgroup avoiding direct sunshine and abstaining from vitamin D supplementation 32.8% were vitamin D deficient (in winter - spring period), Bro et al.[19]

It observed that there was a significant correlation between daily sun exposure and 25(OH)D levels (r=0.731, p<0.001). They showed that vitamin D deficiency is common in urban north Indian hospital staff. The possible reasons include inadequate sunlight exposure and skin pigmentation in Indians, Arya et al.[20]

It was observed significant correlation between serum 25(OH)D concentration and estimated sun exposure (r=0.185, p<0.001), and between 25(OH)D and percentage of body surface area exposed (r=0.146, p<0.004), Puri et al.[21]

Another study observed A significant difference of baseline serum 25(OH)D levels is found between summer (74.98±19.93) and winter (66.62±20.97) (p<0.001) and concluded that sun exposure affects the serum vitamin D level, Lan-Juan Zhao et al.[13]

It was found that vitamin D deficiency was higher in pregnant women who had less exposure to sun (63.4% vs 59%, p=0.05). We also found that there was significant co-relationship in duration of sun exposure and vitamin D level, Bener et al.[14]

In our study based on Prasad classification of socioeconomic status, vitamin D less than 30 nmol/l was found in 3 (6.25%) cases in study group vs. 1 (7.1%) case in control group among SE class II, 6 (12.5%) cases in

study group vs. 3 (21.4%) case in control group among SE class III, 21 (43.75%) cases in study group vs. 6 (42.85%) case in control group among SE class IV and 18 (37.5%) cases in study group vs. 4 (28.57%) case in control group among SE class V. Vitamin D less between 30-75 nmol/l had found in 1 (4.3%) case in control group vs. no case in study group among SE class II , 3 (10.71%) cases in study group vs. 2 (8.7%) case in control group among SE class III, 10 (35.72%) cases in study group vs. 10 (43.5%) case in control group among SE class IV and 15 (53.57%) cases in study group vs. 10 (43.5%) case in control group among SE class V. Similarly, vitamin D greater than 75nmol/l was found in 6 (46.2%) case in control group vs. no case in study group among SE class II, 2 (50%) cases in study group vs. 4 (30.8%) case in control group among SE class III, 1 (25%) case in study group vs. 2 (15.4%) case in control group among SE class IV and 1 (25%) case in study group vs. 1 (7.6%) case in control among SE class V.

The mean vitamin D level in SE class II was 15±3.6 nmol/l in study group vs. 72.43±22.16 nmol/l in control group (p<0.0001). In SE class III the mean vitamin D level was 36.52±28.2 nmol/l in study group vs. 51.01±27.59 nmol/l in control group (p=0.003). In SE class IV the mean vitamin D level was 30.28±19.8 nmol/l in study group vs. 47.03±19.36 nmol/l in control group (p<0.0001) and in SE class V the mean vitamin D level was 32.55±17.19 nmol/l in study group vs. 45.92±17.96 nmol/l in control group (p<0.0001). However, irrespective of the socioeconomic status there was significantly low level of vitamin D in study group as compared to control group. We also observed that vitamin D deficiency was less in high socioeconomic group as compared to the low socioeconomic group. As the number of cases in different socioeconomic group was too small so no concrete conclusion could be drawn out of it.

Similar observation was made in one study which found that maternal serum concentrations of 25-(OH) D were significantly greater in the upper socioeconomic group than that in the middle and low socioeconomic groups, Serenius et al.[21] but observed that subjects in Upper socioeconomic strata had higher mean vitamin D level than lower socioeconomic strata (USES-34.4±20.8; LSES-33.4±19.7, p=0.615) but this was statistically non-significant, Puri et al.[22]

Correlation of vitamin D deficiency in cases and control with serum calcium level

In our study low calcium level was found in 59/80 (73.75%) patients' cases vs. 23/50 (46%) in control (p=0.05). Among the cases higher number of patients having serum calcium level less than 8.5 mg/dl had vitamin D level less than 75 nmol/l of them 18/59 (61.01%) patients had vitamin D level less than 30 nmol/l and 22/59 (37.28%) had vitamin D level between 30-75 nmol/l. Only one patient with serum calcium level less than 8.5 mg/dl had to normal vitamin D level i.e., greater than 75 nmol/l.

In control serum calcium level less than 8.5 mg/dl was found in 7/23 (30.44%) patients who had vitamin D level less than 30 nmol/l and 22/59 (37.28%) who had vitamin D level between 30-75 nmol/l. 5/23 (21.74%) patients with serum calcium level less than 8.5mg/dl who had normal vitamin D level i.e., greater than 75 nmol/l.

Thus, significantly higher number of patients having serum calcium level less than 8.5 mg/dl were found in cases as against control at vitamin D level less than 30 nmol/l (61.01% vs. 30.44% in cases and control respectively, p=0.005).

Limitations

Samples, which cannot be evaluated by the one-point calibration due to high 25(OH)- vitamin D concentrations, was diluted maximally 1+1 with ready-prepared 1x wash buffer (e. g. 50µl sample + 50µl 1x wash buffer) and re-assayed.

Whole blood is not suitable as a sample.

In order for the assay results to be considered valid the following criteria were considered.

Optical densities of the calibrator (CAL) can be accepted if they are within range: 0.495-2.167.

The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Deficiency (seriously deficient)-<12 ng/ml, resp. <30 nmol/l, insufficiency (deficient)-12-30 ng/ml, resp. 30-75 nmol/l, sufficiency (adequately supplied)>30 ng/ml, resp. >75 nmol/l.

Conversion factor

1 ng/ml=2.5 nmol/l, 1 nmol/l=0.4 ng/ml

Specificity

The specificity of the antibody was tested by measuring the cross reactivity against a range of compounds with structural similarity to 25(OH)-vitamin D3. The specificity is calculated in per cent, based on the cross-reactivity of these compounds with the anti-25(OH)-vitamin D3 antibody compared to the 25(OH)-vitamin D3 antigen: 25(OH)-vitamin D_3-100.0%, 25(OH)-vitamin D_2- 67.8%, 24, 25(OH)-vitamin D_3-100.0%, Vitamin D_2 (Ergocalciferol)- 0.3%.

CONCLUSION

There was statistically significant higher number of patients in toxemia group as against control, having

vitamin D level less than 30 nmol/l (60% vs.28% in cases and control respectively, p=0.003).

The mean vitamin D level was significantly low in preeclampsia and eclampsia as compared to control (31.82±19.82 nmol/l in cases vs.52.16±22.18 nmol/l in control, this was found to be statistically significant p<0.0001).

There was significantly low vitamin D level in urban as compared to rural population (37.88±20.11 rural vs. 26.84±18.52 urban in cases p<0.0001 and 58.30±23.42 rural vs. 46.93±20.99 urban in cases p<0.0001).

Statistically significant high vitamin D level were found in patients having higher duration of sun exposure in cases as well as in controls.

Hence to conclude, vitamin D deficiency and insufficiency was found to be associated with normal pregnancy (control group), preeclampsia and eclampsia. In present study direct correlation was observed in preeclampsia and eclampsia with low vitamin D levels. Pregnancy outcome like low birth weight, preterm deliveries, lower segment caesarean section and fetal complication (neonatal mortality and requirement of neonatal intensive care unit) were also found to be in direct relation with low vitamin D level (vitamin D level less than 30 nmol/L).

Vitamin D deficiency, which has long been recognized as a contributing factor to poor bone health, has recently been associated with many public health issues that plague many countries world-wide, including cardiovascular disease, hypertension, autoimmune diseases and cancer. The body of evidence that has identified vitamin D as a potentially important factor in preventing many diseases and disorders continues to grow.

Pre-eclampsia is a potentially devastating condition, and it is hoped that future research and resources will help by identifying role of vitamin D in its pathogenesis and effect of vitamin D supplementation to prevent preeclampsia and its adverse pregnancy outcome.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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