

## Original Research

# Urban Rural Comparison Of Vitamin D Status In Mothers And Their Neonates

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## ABSTRACT

Vitamin D deficiency is widely prevalent in our population in spite of changes on guidelines about the regular supplementation of vitamin D during pregnancy and infancy. The maternal vitamin D levels has impact on pregnancy outcome and neonate health. There is dire need of data from various parts of India to plan the appropriate management plan. **Aims and objectives:** a) To study the vitamin D status in rural & urban mothers and their neonates. b) To study the correlation of vitamin D status among above groups. **Results:** The Urban group, 47.50% of newborns were male and 52.50% were female, while in the Rural group 71.25% were male and 28.75% were female. The Mean±SD of birth weight of newborn in the Urban and in the Rural groups was 3188.75±176.93 gms and 3182.13±243.52 gms respectively with a p value (0.450). The time duration (Mean±SD) of sun exposure / day in the last trimester of pregnancy in the Urban and Rural groups was 0.9938±0.59 hrs and 1.2375±0.74 hrs respectively, with a p value (0.019). The mean maternal vitamin D levels was 13.79±6.57 ng/ml in the Urban group and 16.93±7.24 ng/ml in the Rural group with a p value of 0.003. The mean of cord blood vitamin D levels was 13.66±7.61 ng/ml in the Urban group and 16.67±7.94 ng/ml in the Rural group with p value of 0.000. Maternal vitamin D levels showed a strong positive correlation with cord blood vitamin D levels in the Urban group (r = 0.808, p<0.001). Similarly, there was a strong positive correlation in the Rural group also (r = 0.740, p<0.001). **Conclusion:** Our study demonstrated that the prevalence of vitamin D deficiency in pregnant mothers and their neonates is high in both the Urban and Rural groups.

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## INTRODUCTION

Vitamin D deficiency is pandemic, yet it is the most under-diagnosed and under-treated as well overtreated nutritional deficiency in the world.<sup>[1-3]</sup> Recent research has endorsed that vitamin D has an enormous role in providing innate immunity as well as prevention of diseases in children and adults involving cardiovascular, endocrinal and respiratory systems in addition to its role in calcium metabolism.<sup>[7-10]</sup> The Indian Council of Medical Research (ICMR) recommends a daily dietary intake of 800 mg of calcium and 400 IU of vitamin D in adolescent females and 1200 mg of calcium and 400 IU of vitamin D in pregnant females.<sup>[12]</sup> However the daily dietary intake of calcium of both urban and rural population has been observed to be low as compared to the recommended figures.<sup>[13-15]</sup> Women in the reproductive age group have been observed to be insufficient or deficient in vitamin D to the extent of 76% (South India)<sup>[16]</sup>, 84.3% urban and 83.6% rural (Central India)<sup>[5]</sup> and 58.5% (North India).<sup>[17]</sup> Various factors that are likely to cause this deficiency are poverty

leading to poor dietary intake, skin pigmentation of Indian population (more sun exposure is required to produce vitamin D due to excess melanin) and decreased sun exposure due to cultural practices.<sup>[18]</sup> Other factors may be high fiber and phytate intake that depletes vitamin D levels, pollution and increasing urbanization that results in poor outdoor activity. The problem is further aggravated by the many prevalent social and cultural practices in India that preclude the adequate exposure of adolescent girls and young women to sunshine.<sup>[5]</sup> Fractional calcium absorption increases from 35% in the non-pregnant state to 60% during the third trimester of pregnancy. Serum concentrations of 1,25(OH)<sub>2</sub>D increase 50–100% over the non-pregnant state during the third trimester but as the receptors do not increase to that extent there is increase in free 1,25(OH)<sub>2</sub>D levels.<sup>[19]</sup> During pregnancy, maternal serum concentrations of 1,25(OH)<sub>2</sub>D, the circulating form of vitamin D do not correlate with dietary vitamin D intake. The mechanism underlying the increased serum 1,25(OH)<sub>2</sub>D concentrations during pregnancy

is not clear. PTH, which is usually considered the stimulus for increased renal hydroxylation of 25(OH)D to 1,25(OH)<sub>2</sub>D, has not been shown to be increased during pregnancy<sup>[20-22]</sup> and it has been speculated that the 1,25(OH)<sub>2</sub>D present in the maternal circulation may be of placental origin.<sup>[23]</sup> The fetus is entirely dependent on the mother for an adequate supply of calcium and 1,25(OH)<sub>2</sub>D, which are believed to cross the placenta. Maternal circulating 1,25(OH)<sub>2</sub>D is the most significant regulator of neonatal circulating 1,25(OH)<sub>2</sub>D concentrations, with underlying genetic factors playing a limited role.<sup>[24]</sup> Transplacental transfer is the main source of vitamin D for the newborns.<sup>[14,26]</sup> Hence, vitamin D deficiency during pregnancy may result in immediate newborn illnesses such as hypocalcemic seizures and poor vitamin D stores.<sup>[5,27]</sup> The less amount of vitamin D in breast milk, little sunlight exposure and low stores obtained from a deficient mother make them prone to low birth weight, small for gestational age<sup>[28]</sup>, neonatal and infantile rickets, kidney disease<sup>[29]</sup>, allergic bronchitis and eczema.<sup>[30]</sup> Recent studies have demonstrated multiple adverse maternal outcomes during pregnancy associated with hypovitaminosis D like preeclampsia, gestational diabetes, growth retardation, multiple sclerosis, schizophrenia, asthma, bacterial vaginosis and postpartum depression.<sup>[32]</sup>

There are variations in the levels of vitamin D in the urban and rural population. There was paucity of data regarding the comparison of vitamin D levels in rural and urban mothers and their correlation with the cord blood vitamin D levels in northern India. This study was planned to see if there is a difference in vitamin D levels in rural mothers as compared to urban mothers in South Western Punjab presenting to this tertiary care hospital.

#### AIMS AND OBJECTIVES

a) To study the vitamin D status in rural & urban mothers and their neonates. b) To study the correlation of vitamin D status among above groups.

#### MATERIAL AND METHODS

The present study was conducted in term neonates (37-41 weeks) born at Guru Gobind Singh Medical College & Hospital, Faridkot over a period of one year from march 2017 to march 2018.

**Study design:** This facility based cross sectional study was done at Guru Gobind Singh Medical College & Hospital, Faridkot, Punjab, India.

**Sample Size:** There were 80 mother-neonate pairs in each (rural and urban) population group to achieve 80% power of study and level of significance 0.05. The sample size has been calculated using the formula:  $n = \left[ \frac{(z_{\alpha} + z_{\beta})}{c} \right]^2 + 3$  where  $z_{\alpha}$  is the standard normal deviate for  $\alpha$  (1.96),  $z_{\beta}$  is the standard normal deviate for  $\beta$  (0.84) and  $c = 1/2 \times \log_e \left[ \frac{(1+r)}{(1-r)} \right]$ . Based upon

the previous literature and expert consensus, sample sizes were explored with correlation coefficient ranging from 0.35 to 0.85. Using a two sided hypothesis test with a significance level ( $\alpha$ ) of 0.05 and 80% power to detect a difference from the null hypothesis correlation of 0.00, the sample sizes ranged from 8 to 61. Assuming a non response rate of 15% the maximum required sample size was 71, so a total of 80 mother-neonate pairs were included for the purpose of this study.

#### Subjects

All consecutive mother – neonate pairs belonging to either rural or urban areas and delivering at G.G.S. Medical College & Hospital, Faridkot who met the inclusion criteria were enrolled for the study. The study subjects were divided into following two groups: Group 1 80 mother-neonate pairs residing in the rural areas.

Group 2 80 mother-neonate pairs residing in the urban areas.

#### Inclusion Criteria

1. Babies delivered at Guru Gobind Singh Medical College, Faridkot by any mode of delivery
2. Babies having birth weight of  $\geq 2500$  grams
3. Gestational age of 37-41 completed weeks
4. Babies of either sex

#### Exclusion criteria

##### A. For baby

1. Gestational age  $< 37$  weeks and  $> 41$  weeks
2. Small for date babies
3. Large for date babies
4. Requiring resuscitation more than initial steps
5. Multiple births
6. Babies of retrovirus positive mothers
7. Born with any major congenital anomaly
8. Parents not consenting for the study
9. Failure to obtain sample from umbilical cord due to any reason

##### B. For mother

1. History of malabsorption syndrome Parenteral intake of Vitamin D in past
2. History of Chronic liver disease
3. History of Renal disease
4. History of treatment with antitubercular or antiepileptic drugs in the previous three months
5. History of Metabolic Bone Disease
6. History of Thyroid and Parathyroid disorders
7. Clinical features suggestive of osteomalacia
8. Delivery of the mother within one hour of reporting to the hospital

#### METHOD

The detailed history and examination was done for each mother & neonate dyad. The average amount of sun exposure in the mothers was calculated by asking them how much time they remained outdoors daily in

the first week of each month during last trimester. The percentage of body surface area (BSA) exposed was calculated by using Rule of Nine.<sup>[58]</sup>To assess the amount of sun exposure in the mothers according to weekly duration of exposure and percent body surface area exposed, the Sun Index was calculated as per following formula:<sup>[59]</sup> Sun Index = (minutes of sun exposure per week) × (fraction of body surface area (BSA) exposed to sunlight) Mothers were also asked about the intake of vitamin D rich foods like liver, egg yolk, butter, cheese, fish etc. It was also noted whether these mothers were receiving supplemental calcium, vitamin D or both. Apgar score at 1 min, 5 min and requirement of resuscitation

was recorded. Birth weight, crown-heel length, anterior fontanelle, posterior fontanelle, and head circumference of newborns were noted. The levels of vitamin D in both mother and baby were recorded. Five millilitre of blood was taken from umbilical vein under aseptic condition in a plain vial. Similarly mother's sample was also taken from a peripheral vein immediately after delivery. Both samples were sent to the laboratory immediately. 25(OH)D was measured by chemiluminescent immunoassay method. The serum was separated by centrifugation for 10 minutes at 2500 rpm and stored at -20°C until the analysis for estimation of 25(OH)D.

The subjects were classified as per below according to recent consensus:<sup>[2]</sup>

Classification	25(OH)D levels in ng/ml
Deficient	<20
Insufficient	21 to 29
Sufficient	≥30

Data was collected, compiled and analyzed. Definition of urban area was used as per the census of India:<sup>[61]</sup>

- All places with a Municipality, Corporation or Cantonment or Notified Town Area
- All other places which satisfied the following criteria:
  - A minimum population of 5,000
  - At least 75% of the male working population was non-agricultural
  - A density of population of at least 400 per sq. km.

The following definition of rural area was used as per the census.

- A population of less than 5,000
- Density of population less than 400 per sq. km.
- More than 25 per cent of the male working population is engaged in agricultural pursuits

**Ethical clearance:** The protocol was submitted for ethical clearance from Guru Gobind Singh Medical College and Hospital and its approval was taken. It was also submitted to the thesis committee of the institute for permission.

**Statistical analysis :** The Data was entered in the form of a data matrix in Microsoft® Excel 2007 and analyzed statistically using IBM® SPSS® version 20.0.0. Descriptive statistics were calculated as frequencies for categorical variables and means and standard deviation for continuous variables. The association between the categorical variables was explored by using Pearson's chi-square test or Fisher's exact test, where applicable. The difference of continuous variables, among two groups was explored by using independent t-test in case of normally distributed data and Mann-Whitney U test in case the data was not normally distributed. p value of <0.05 was considered statistically significant.

**OBSERVATIONS AND RESULTS**

**Table 1 : Demographic Profile Of Subjects**

Sex	Study Group			
	Urban		Rural	
	(n)	(%)	(n)	(%)
Male	38	47.50	57	71.25
Female	42	52.50	23	28.75
NVD	44	55.00	36	45.00
LSCS	36	45.00	44	55.00
Upper middle	22	27.50	5	6.25
Lower middle	58	72.50	69	86.25
Upper lower	-	-	6	7.50
Calcium and vitamin D was taken	70		68	
Calcium and vitamin D was not taken	10		12	

Table 1 that in the Urban group, 47.50% of newborns were male and 52.50% were female, while in the Rural group 71.25% were male and 28.75% were female. On comparison by Chi-square Test, the male: female ratio in the two groups was not similar.

**Table 2: Anthropometric Parameters Of Newborns In The Two Study Groups**

Anthropometric parameters	Study Group		p value
	Urban	Rural	
Birth weight(gms) (Mean±SD)	3188.75±176.93	3182.13±243.52	0.450
Length(cms) (Mean±SD)	48.68±0.46	48.80±0.59	0.236
Head circumference(cms) (Mean±SD)	34.60±0.40	34.62±0.42	0.727

Table 2 show that the Mean±SD of birth weight of newborn in the Urban and in the Rural groups was 3188.75±176.93 gms and 3182.13±243.52 gms respectively with a p value (0.450) . The Mean±SD of length of newborns in the Urban and in the Rural groups was 48.68±0.46 cms and 48.80±0.59 cms respectively with a p value (0.236).The Mean±SD of head circumference of newborns in the Urban and in the Rural groups was 34.60±0.40 cms and 34.62±0.42 cms respectively with a p value (0.727) . Thus, the two groups were comparable with respect to all the three anthropometric parameters studied.

**Table 3 : Duration Of Sun Exposure And Sun Index In Mothers In The Study Groups**

Study Group	Sun exposure in hours / day (Mean±SD)	p value
Urban	0.9938±0.59	0.019
Rural	1.2375±0.74	
Urban	68.27±50.98	0.241
Rural	80.79±66.42	

Table 3 shows that the time duration (Mean±SD) of sun exposure / day in the last trimester of pregnancy in the Urban and Rural groups was 0.9938±0.59 hrs and 1.2375±0.74 hrs respectively, with a p value (0.019). Thus, the sun exposure in the Rural group was significantly more. To assess the amount of sun exposure in the mothers according to weekly duration of exposure and percent body surface area exposed, the Sun Index was calculated as per following formula:<sup>[59]</sup>Sun Index = (minutes of sun exposure per week) × (fraction of body surface area (BSA) exposed to sunlight)The sun index (Mean±SD) in the Urban and Rural groups was 68.27±50.98 units and 80.79±66.42 units respectively, with a p value (0.241).

**Table 4 : Maternal Vitamin D Levels In The Two Study Groups**

Classification	STUDY GROUP				p value
	URBAN		RURAL		
	(n)	(%)	(n)	(%)	
Deficient (≤ 20ng/ml)	64	80.00	56	70.00	0.465
Insufficient (21-29ng/ml)	16	20.00	23	28.75	0.262
Sufficient (≥30ng/ml)	-	-	1	1.25	-
Total	80	100.00	80	100.00	

Table 4 show that only one mother (Rural group) had sufficient vitamin D levels, no mother in the Urban group was sufficient in vitamin D levels. 80.00% of mothers in the Urban group and 70.00% of mothers in the Rural group had deficient vitamin D levels (p=0.465), while 20.00% of mothers in the Urban group and 28.75% of mothers in the Rural group had insufficient vitamin D levels (p=0.262). So there was no significant difference in percentage of mothers having low vitamin D levels in the two study groups.

**TABLE 5: CORD BLOOD VITAMIN D LEVELS IN THE TWO STUDY GROUPS**

Classification	STUDY GROUP				p value
	URBAN		RURAL		
	(n)	(%)	(n)	(%)	
Deficient (≤ 20ng/ml)	65	81.25	58	72.50	0.528
Insufficient (21-29ng/ml)	11	13.75	20	25.00	0.106
Sufficient (≥30ng/ml)	4	5.00	2	2.50	0.414
Total	80	100.00	80	100.00	

Table 5 show that only 5.00% of the newborns in the Urban group and 2.50% of the newborns in the Rural group had sufficient vitamin D levels ( $p=0.414$ ). 81.25% of the newborns in the Urban group and 72.50% of the newborns in the Rural group had deficient vitamin D levels ( $p=0.528$ ) and 13.75% of the newborns in the Urban group and 25.00% of the newborns in the Rural group had insufficient vitamin D levels ( $p=0.106$ ). So, there was no significant difference in percentage of newborns having low vitamin D levels in the two study groups. The mean maternal vitamin D levels was  $13.79\pm 6.57$  ng/ml in the Urban group and  $16.93\pm 7.24$  ng/ml in the Rural group

with a p value of 0.003 (significant). So, vitamin D levels in the mothers of the Rural group was significantly higher than the vitamin D levels in the mothers of Urban group. However, the means did not reach sufficiency in both the study groups. The mean of cord blood vitamin D levels was  $13.66\pm 7.61$  ng/ml in the Urban group and  $16.67\pm 7.94$  ng/ml in the Rural group with p value of 0.000 (highly significant). So, vitamin D levels in cord blood of the Rural group were significantly higher than the vitamin D levels in cord blood of the Urban group. However, the means did not reach sufficiency in both the study groups.

**Table 6: Correlation Of Vitamin D Levels In Cord Blood & Maternal Blood In The Two Study Groups**

Study Group	Spearman's correlation coefficient	p value
Urban	0.808	0.000
Rural	0.740	0.000

Table 6 shows the correlation between cord blood and maternal blood vitamin D levels in the two study groups, which was explored by using the nonparametric spearman's coefficient. Maternal vitamin D levels showed a strong positive correlation with cord blood vitamin D levels in the Urban group ( $r = 0.808$ ,  $p < 0.001$ ) (Figure 13 a). Similarly, there was a strong positive correlation in the Rural group also ( $r = 0.740$ ,  $p < 0.001$ ) (Figure 13 b).

## DISCUSSION

The present study was a facility based, cross sectional study to compare the vitamin D status in mother-neonate pairs residing in Urban and Rural areas. 80 term mother-neonate pairs were studied in each area. 80% of mothers and 81.25% of neonates in the Urban area were found to be deficient vitamin D levels ( $\leq 20$  ng/ml). In the Rural area 70% of mothers and 72.50% of neonates were found to be deficient. In our study, we found that in the Urban group, 47.50% of newborns were male and 52.50% were female, while in the Rural group 71.25% were male and 28.75% were female. The male : female ratio in the two groups was not similar. (Table 1) In a similar study, conducted at Appalachia (Eastern United States), Cottrell et al<sup>[54]</sup> found that in the Urban area 55.22% of newborns were male and 44.78% were female, whereas in the Rural area 49.25% newborns were male and 50.75% were female. In another study conducted by Naik et al<sup>[52]</sup> on cord blood vitamin D levels in term neonates in Kozhikode, Kerala, 44% of newborns were male and 56% were female. In our study Mean $\pm$ SD of birth weight of newborns in the Urban and in the Rural groups was  $3188.75\pm 176.93$  gms and  $3182.13\pm 243.52$  gms respectively with a p value of 0.450 (not significant). The Mean $\pm$ SD of length of newborns in the Urban and in the Rural groups was  $48.68\pm 0.46$  cms and  $48.80\pm 0.59$  cms respectively with a p value of 0.236 (not significant). Similarly, the Mean $\pm$ SD of head circumference of newborns in the Urban and in the Rural groups was  $34.60\pm 0.40$  cms and  $34.62\pm 0.42$

cms respectively with a p value of 0.727 (not significant). Thus, the two groups were comparable with respect to all the three anthropometric parameters studied. (Table 3) In a similar study conducted by Cottrell et al,<sup>[54]</sup> on term & preterm neonates, they found that the birth weight of newborns in the Urban and in the Rural groups was  $3293\pm 62$  gms and  $3141\pm 62$  gms respectively with a p value of 0.16 (not significant). Similarly, the Mean $\pm$ SD of length of newborns in the Urban and in the Rural groups was  $50.11\pm 3.85$  cms and  $49.87\pm 2.64$  cms respectively with a p value of 0.66 (not significant). Similarly, the Mean $\pm$ SD of head circumference of newborns in the Urban and in the Rural groups was  $34.31\pm 2.76$  cms and  $33.93\pm 2.00$  cms respectively with a p value of 0.36 (not significant). In our study, the socio-economic status of the pregnant mothers in the Urban and Rural study groups was lower middle class in 72.50% and 86.25% mothers respectively. Only 27.50% of mothers in the urban group and 6.25% of mothers in the rural group were from upper middle class. There was no mother in either group from upper and lower class. 7.50% of mothers in rural group were from the upper lower class while there was no mother in the urban group from upper lower class. (Table 4) In the present study, we observed that 87.50% of pregnant women in the Urban group and 85.00% of pregnant women in the Rural group were taking calcium and vitamin D supplementation during the antenatal period ( $p = 0.646$ , not significant). (Table 5) All of these mothers received a fixed dose combination of 500 mg elemental calcium and 250 IU of vitamin D as prescribed by gynecologists once a day. The common brands used were Tab Shelcal 500, Tab Ossopan 500, Tab Sandocal 500. This is well below the Recommended Daily Allowance as per ICMR which is 1200 mg calcium and 400 IU of vitamin D.<sup>[12]</sup> An extensive market survey revealed that such a fixed dose combination is not available. In a similar study, by Sachan et al,<sup>[5]</sup> it was found that 72% mothers in the Urban area and 88% mothers in the Rural area

received daily calcium supplements below the RDA during pregnancy. In the present study, we observed that 20.00% of pregnant women in the Urban group and 33.75% of pregnant women in the Rural group were taking vitamin D rich foods like liver, egg yolk, butter, cheese, fish etc. with a *p* value of 0.050 (not significant). (Table 6) Most of the women were consuming 1 or 2 eggs per week and non-vegetarian food like fish or chicken once a week. In a study by Jacquemyn et al,<sup>[50]</sup> on vitamin D levels in maternal serum and umbilical cord blood in a multi-ethnic population in Antwerp, Belgium, it was found that more eggs the mothers ate, lesser was the vitamin D deficiency in maternal blood ( eating 1 to 2 eggs per week, *p* = 0.025; eating 3 to 4 eggs per week, *p* = 0.016 ). In the present study, it was found that the time duration (Mean±SD) of sun exposure / day in the last trimester of pregnancy in the Urban and Rural groups was 0.9938±0.59 hrs and 1.2375±0.74 hrs respectively, with a *p* value of 0.019 (significant). (Table 7) Thus, the sun exposure in the Rural group was significantly more. To assess further, the amount of sun exposure, according to body surface area exposed, the Sun Index was calculated. It was found to be 68.27±50.98 units in the Urban group and 80.79±66.42 units in the Rural group (*p* = 0.241, not significant). (Table 8). In a similar study, by Sachan et al,<sup>[51]</sup> it was found that sun exposure was significantly lower in Urban subjects than in Rural subjects in the last trimester of pregnancy (Urban: 4.1±3.2 h/d × %BSA exposed; Rural: 9.7±8.1 h/d × %BSA exposed; *p* < 0.001). Kumar et al,<sup>[53]</sup> in their study comparing Urban & Rural South Indian mothers, observed that Urban mothers were found to be exposed to sun for lower duration of 0.3 hours (Median) compared to rural mothers 0.5 hours (Median). Similarly the surface area of body exposed to sun was lower in urban mothers (7.5%) compared to rural mothers (15%) with a *p* value of <0.0001. In our study, although the duration of sun exposure was significantly more in the Rural mothers, the sun index was comparable. This may be because, although they remained outdoors for more number of hours yet they kept themselves covered and less body surface area was exposed to the sun. In the present study, only one mother (Rural group) had sufficient vitamin D levels, no mother in the Urban group was sufficient in vitamin D levels. 80.00% of mothers in the Urban group and 70.00% of mothers in the Rural group had deficient vitamin D levels (*p*=0.465), while 20.00% of mothers in the Urban group and 28.75% of mothers in the Rural group had insufficient vitamin D levels (*p*=0.262). So there was no significant difference in percentage of mothers having low vitamin D levels in the two study groups. (Table 9) Kumar et al,<sup>[53]</sup> in a similar comparative study on 91 mothers (Urban=46, Rural=45), observed that only 3 mothers in the Rural group were sufficient in vitamin D, no mother in the Urban group was sufficient (*p* = 0.12, not significant ). 96% of mothers in the Urban group and 84%

mothers in the Rural group were deficient (*p* = 0.09, not significant ); 4% of mothers in the Urban group and 9% mothers in the Rural group were insufficient (*p* = 0.43, not significant ). In a similar study in Pakistan, Anwar et al,<sup>[56]</sup> on 269 pregnant women (Karachi; Urban=207, Jehlum;Rural=62) found that 99.50% of mothers in the urban area and 89% of mothers in the rural area had vitamin D levels < 50 nmol/l (*p*<0.005) . Thus, our study is similar to the study by Kumar et al<sup>[53]</sup> but Anwar et al<sup>[56]</sup> have found significantly more numbers of mothers deficient in the urban group compared to rural group. In the present study, only 5.00% of the newborns in the Urban group and 2.50% of the newborns in the Rural group had sufficient vitamin D levels (*p*=0.414). 81.25% of the newborns in the Urban group and 72.50% of the newborns in the Rural group had deficient vitamin D levels (*p*=0.528) and 13.75% of the newborns in the Urban group and 25.00% of the newborns in the Rural group had insufficient vitamin D levels (*p*=0.106).. So, there was no significant difference in percentage of newborns having low vitamin D levels in the two study groups. (Table 10) Kumar et al,<sup>[53]</sup> in a similar comparative study on 91 newborns (Urban=46, Rural=45), observed that 4 newborns in the Urban group and 3 in the rural group were sufficient in vitamin D (*p* = 0.36, not significant ), 91% of newborns in the Urban group and 84% newborns in the Rural group were deficient (*p* = 0.35, not significant ); 7% of newborns in the Urban group and 9% newborns in the Rural group were insufficient (*p* = 0.71, not significant ). In a similar study in Pakistan, Anwar et al,<sup>[56]</sup> on 227 neonates ( Karachi; Urban=182, Jehlum;Rural=45 ) found that 97.3% of neonates in the urban area and 82.2% of neonates in the rural area had vitamin D levels < 50 nmol/l (*p*<0.005). Thus, our study is similar to the study by Kumar et al<sup>[53]</sup> but Anwar et al<sup>[56]</sup> have found significantly more numbers of neonates deficient in the urban group compared to rural group. In our study, we found that Mean±SD of maternal vitamin D levels was 13.79±6.57 ng/ml in the Urban group and 16.93±7.24 ng/ml in the Rural group with a *p* value of 0.003 (significant). So, vitamin D levels in the mothers of the Rural group was significantly higher than the vitamin D levels in the mothers of Urban group. (Table 11) A similar study done by Kumar et al<sup>[53]</sup> found vitamin D levels of 29.42±12.2 nmol/l in the Urban group and 35.86 ± 16.3 nmol/l in the Rural group, with a *p* value of 0.037 (significant). In our study also, the maternal vitamin D levels in Rural group were on the higher side. Another similar study done by Anwer et al<sup>[56]</sup> had found maternal vitamin D levels of 13.37±9.1 nmol/L in the Urban group and 28.65±17.95 nmol/L in the Rural group (*p* = <0.0001, highly significant ). Our study is similar to the study done by Kumar et al<sup>[53]</sup> and Anwar et al.<sup>[56]</sup> However, in our study the means did not reach sufficiency in both the groups and in the studies by Kumar et al<sup>[53]</sup> and Anwar et al<sup>[56]</sup> also the means

were below 50nmol/l. In the present study, Mean±SD of cord blood vitamin D levels was 13.66±7.61 ng/ml in the Urban group and 16.67±7.94 ng/ml in the Rural group with p value of 0.000 (highly significant). So, vitamin D levels in cord blood of the Rural group were significantly higher than the vitamin D levels in cord blood of the Urban group.(Table 12) A similar study done by Kumar et al<sup>[53]</sup> in South India, found cord blood vitamin D levels of 33.60±15.20 nmol/l in the Urban group and 38.30±18.20 nmol/l in the Rural group with a p value of 0.18 (not significant). Another study done in Pakistan by Anwar et al<sup>[56]</sup> found cord blood vitamin D levels of 19.87±13.55 nmol/l in the Urban group and 29.55±23.12nmol/l in the Rural group (p = 0.0003; highly significant). Cottrell et al,<sup>[54]</sup> in their study in Appalachia (Eastern United States) analysed cord blood samples for vitamin D levels and found mean of 22.81±8.06 ng/ml in rural area and 24.73±10.17 ng/ml in urban area (p = 0.23, not significant).In our study although there was a significant difference in rural and urban groups, the means did not reach sufficiency in both the groups. In two studies by Anwar et al<sup>[56]</sup> and Kumar et al<sup>[53]</sup> also, the means did not reach sufficiency. However, Cottrellet al<sup>[54]</sup> observed mean values of insufficiency. This difference could be because of the difference in geographical location.In the present study, maternal vitamin D levels showed a strong positive correlation with cord blood vitamin D levels in the Urban group (r = 0.808, p<0.001, Figure 13 a). Similarly, there was a strong positive correlation in the Rural group also(r = 0.740, p<0.001, Figure 13 b).(Table 13) Kumar et al,<sup>[53]</sup> in their study, found a moderate correlation of 67.20% mother-neonate pair in the rural population and a low correlation of 48% in the urban area. Anwar et al,<sup>[56]</sup> in this study from Pakistan, observed a strong positive correlation between maternal and neonatal vitamin D levels in the urban area (r = 0.744, p<0.01) and a weak positive correlation in the rural area (r = 0.24, p = 0.016). Both Kumar et al<sup>[53]</sup> and Anwar et al<sup>[56]</sup> have observed a dichotomous phenomenon; as in former study there was a low positive correlation in the urban area, whereas in the later study there was a weak positive correlation in the rural area. In our study, the correlation is strong in both the urban and rural areas. This indicates the possibility of several other yet undetermined factors influencing the foetal levels of vitamin D apart from maternal vitamin D levels.

## CONCLUSIONS

Our study demonstrated that the prevalence of vitamin D deficiency in pregnant mothers and their neonates is high in both the Urban and Rural groups. Despite sufficient number of mother'sreceiving calcium and vitamin D supplementation in the antenatal period, the mean maternal and neonatal vitamin D levels did not reach sufficiency in both the groups. However, the rural group mother-neonate dyad had significantly

more mean values of vitamin D than their urban counterparts. There was a strong positive correlation between the maternal and neonatal vitamin D levels in both the groups. The high prevalence of vitamin D deficiency in our study despite adequate sunshine, our study suggests that there is a need to do further studies to recommend modification in recommended daily allowances of vitamin D for pregnant mothers.

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