Original Article

Hypovitaminosis-D: A Predictive Risk Marker for Preeclampsia in 3rd Trimester of Pregnancy

Rida Fatima, Muhammad Shafiq, Muhammad Ammar Yaser, Muhammad Ali, Samia Jawed, Rana Khurrum Aftab and Farhat Ijaz

Objective: To compare the Vitamin-D in serum of normotensive pregnancy and preeclamptic pregnancy.
Methods: This case-control study was done at Lady Aitchison Hospital, Lahore. 47 pregnant females were assigned to preeclamptic group (cases) and 47 pregnant females assigned to normotensive group (control). The enzyme linked immunosorbent assay (ELISA) method was utilized to determine serum vitamin-D levels.
Results: The mean Vitamin-D levels were 15.74 ± 3.50 in cases and 33.26 ± 11.94 in control group; the levels were significantly lower in cases as compared to controls with p-value < 0.001.
Conclusions: The findings of this study concluded that Vitamin-D levels are less in preeclamptic than in Normotensive Pregnancy and could be marked as a risk factor for preeclampsia.
Keywords: pregnancy, preeclampsia, vitamin-D.

Introduction

Preeclampsia (PE) is one of the commonest pathology of pregnancy defined as hypertensive disorder of pregnancy, clinically characterized by persistently raised blood pressure (≥140mmHg/≥90mmHg), proteinuria and edema, after 20 weeks of gestation.[1,2] It is a common cause of both maternal and fetal morbidity and mortality with a global incidence of 2%-10%.[3] In Pakistan its prevalence is 19%.[4] In year 2015 World Health Organization (WHO) reported maternal mortality rate (MMR) of 178 in Pakistan.[5] Hypertensive disorder of pregnancy as PE, is responsible for about 15% of maternal deaths.[6] Etiology is unknown but researchers have implicated many factors such as obesity, diabetes mellitus, micronutrient deficiencies (Vitamins and Minerals), genetics and advance maternal age[7] and found an association between micronutrient deficiency and PE prevalence.[1-3,8] The Institute of medicine United States recommends that vitamin D levels should be >20ng/ml for women of reproductive age.[9] The increases of vitamin-D in early pregnancy is attributed to its increase synthesis and decrease catabolism, especially in decidual and placental tissue.[10] Vitamin D required locally for induction of immune tolerance to implantation and successful maintenance of pregnancy, via dampening of T helper cells-1 (Th1) immune function, PE has been associated with an increased release of Th1 cytokines e.g. tumor necrosis factor-a (TNF-α). In normal cultured trophoblasts, TNF-α expression is inhibited by active form of vitamin D, i.e. TNF-α and vitamin-D are mutually inhibitory and PE placentas may have decreased ability to convert vitamin-D to active form as compared with normal placental tissue.[11] Researchers have found few causes for PE like immune irregulation and dysfunctional trophoblastic invasion and also discovered a link between these possible causes and vitamin-D deficiency.[12] Overall the epidemiological evidence is conflicting[9] such as a study done at Netherlands in 2015 and at Paris by Benachi A, showed that there is no significant association between the PE cases and decreased vitamin-D serum levels in early pregnancy but they also found that women with sufficient vitamin D levels in their serum showed no sign and symptoms of PE in their third trimester of pregnancy.[13,14] Another study done at India by Dhillon shows a strong positive relationship between vitamin-D deficiency and PE development.[15] Increasing trend of vitamin D supplementation awareness for the prevention and treatment of PE by WHO has increased the popularity of vitamin D trials.[16] In light of all these epidemiologic conflicts, increasing MMR and WHO emphases, our study is designed with the object to measure vitamin-D status in our PE female population and to understand it's possible role in PE etiology.

Methods

This case-control study was done at Lady Atichison hospital, Lahore with a sample size of 94 pregnant females selected by purposive nonprobability sampling technique. Written informed consent was

taken from subjects. Informative brochure was given to all the subjects.

Complete history and general physical examination was done. Cases (PE) and controls (normotensive) were selected for a gestational age (28-40 weeks of gestation) and maternal age (18 to 35 years). Cases were recruited on the basis of Systolic BP ≥140mmHg and Diastolic BP ≥90mmHg with previously normal BP, dipstick shows ≥1+ proteinuria and edema and controls were recruited on the basis of Systolic BP 110-120mmHg and Diastolic BP 70-80mmHg, no proteinuria and no edema. Any subject who is Obese (BMI ≥35 kg/m^2) or diagnosed case of chronic hypertension, diabetes mellitus, gestational diabetes, multiple pregnancies has been excluded from the study. 5ml venous blood was taken under aseptic measures and Serum vitamin D was assessed by enzyme linked immunosorbent assay (ELISA) method. Statistical **Analysis:** Data was transferred to Statistical Package for Social Sciences (SPSS-21). Quantitative variables like gestational age, maternal age, weight and heights, blood pressure, BMI, serum vitamin D were presented as mean±S.D. Comparison of normotensive pregnancy and PE pregnancy in two groups apply independent sample t- test. Correlation coefficient was used to see the linear trend between vitamin-D and quantative variables. Shapiro wilk's test & Mann and Whitney U test was applied to analyze the difference between two groups for all quantitative variables. P-value ≤0.05 was taken as significant.

Results

The study was conducted with 47 PE women and a comparative group of 47 other pregnant women who were normotensive throughout their pregnancies. The difference for Quantative variables was highly significant when compared between two groups and all these had higher values in PE group vs normotensive group i.e mean age (27.9 years vs 24.9 years, p-value <0.001), mean heart rate (94 beats per minute was 75 beats per minute, p-value <0.001). Respiratory rate and BMI both were significantly higher in PE group with p-value< 0.001. The mean systolic blood pressure for PE group was 140 (140-150) and that for the normotensive was 110 (100-120) mmHg. Similarly the diastolic blood pressure was 100 (90-110) and 70 (70-80) for two groups respectively.

The difference for both variables was highly significant with p-value <0.001. **(Table-1)** Vitamin D level in normotensive group was 33.3±11.9 ng/ml and in PE group was 15.7±3.5 ng/ml. Among PE group 75.0% of the cases had vitamin D levels below 19.0ng/ml, and among normotensive group 75.0% had above 27.0ng/ml and 50% had above 30.0 ng/ml, with p-value <0.001. **(Fig-1**) Among normotensive group vitamin D had no significant correlation with quantative variables. Among PE group vitamin D had just significant and negative correlation of -0.292 with p-value 0.046. **(Table-2)**

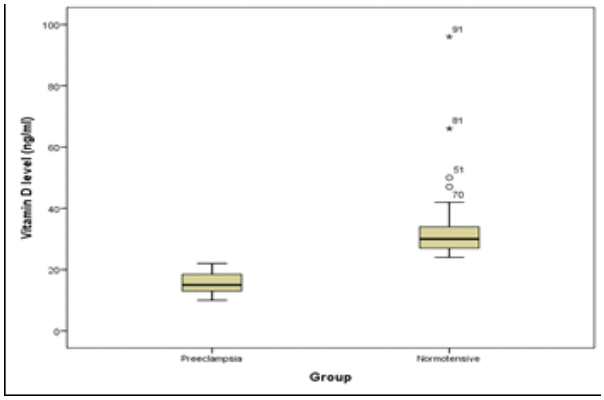

Fig-1: Box plot presenting distribution of serum vitamin-D levels in two groups.

Table-1: Distribution of heart rate, respiratory rate, BMI, Systolic and diastolic blood pressure comparison between women with and without preeclampsia.

Variables	Heart Rate		Respiratory Rate		BMI (kg/m2)		Systolic blood pressure		Diastolic blood pressure	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Mean	95	77	17	16	31.3	28.3	147	110	101	74
Standard deviation	06	04	01	01	2.8	4.1	09	09	09	05
Percentile 25 =Q1	94	78	17	16	31.5	28.4	140	110	100	70
Percentile 75 = Q3	90	75	16	16	29.7	24.6	140	100	90	70
Comparison	99	80	17	16	33.3	32.0	150	1201	110	80
Comparison	Mann Whitney u=0.0	p-value 0.001	Mann Whitney u=453	P-value 0.001	Mann Whitney u=625.5	p-value 0.001	Mann Whitney u=0.0	p-value 0.001	Mann Whitney u=0.0	p-value 0.001

**P-value ≤0.05 was taken as significant.*

Table-2: Correlation of vitamin D with other parameters in women with preeclampsia

		Age	Gest. Age	Heart Rate	Systolic blood pressue	Diastolic blood pressure	Resp. Rate	BMI
Vitamin-D	Pearson correlation	0.156	-0.125	-0.246	-0.208	-0.292	0.027	-0.016
	Sig (2-tailed)	0.295	0.404	0.095	0.161	0.046	0.857	0.913
	n	47	47	47	47	47	47	47

**. Correlation is significant at the 0.05 level (2-tailed).*

Discussion

Molecular basis of this condition is unresolved in literature. Researchers have postulated that fluctuations in maternal nutrients level may be the precipitating cause of elevated blood pressures in PE and have harmful effect on the pregnant mother and growing fetus and possibly complicate PE. In our study the mean age for cases (27.89±4.13 years) and for controls (24.87±3.79 years) shows p-value <0.001 and BMI in cases and controls (31.28±2.82 and 28.25±4.05) respectively shows p-value <0.001. These findings are in consistent with the finding shown by Kanagal et al study that the mean age of women with PE was higher than normotensive controls (27.45±4.33 yrs vs 25.87± 3.11 yrs p-value 0.023) and mean BMI was significantly higher in PE than normotensives (27.07±3.07 kg/m2 vs 24.9 ±2.32 kg/m2 p<0.001)[17] also supported by the observation shows by Bodnar LM et al.[18] Significant reduction in vitamin-D seen by many researcher but In 2016 Umar N did a research and on comparison of vitamin D levels between two groups, it was observed that the difference between groups was insignificant with p-value of 0.21.[19] A nested case control study by Powe et al. found no association between first trimester vitamin D and subsequent PE but importantly even Powe et al. agreed that at very low levels of vitamin D (<15ng/dl), there was a significant association between vitamin D and occurrence of PE.[20] In India, Dhillon Mk and his fellows research study found a statistically significant association of low serum vitamin-D levels (p-value< 0.04).[15] Díaz L findings shows that in PE increase Vitamin D catabolism by TNF-α, leading to its low circulating level in turn contribute to the lower calcium levels seen in patients with PE.[21] Bodnar LM results showed that vitamin D deficiency at 22 weeks of pregnancy was a strong independent risk factor for PE & risk more than doubled for each 50 nmol/L decrease in maternal vitamin-D levels.[18] The limitation of our study is the absence of detailed dietry history of the subjects. So, the impact of the inadequate intake of this hormone on PE could not be ascertained in our study. Moreover sample size must be increased; the population size should also be expanded to other cities. This will cause further confirmation of the results of our present study.

Conclusion

This study concludes a significant reduction in serum vitamin D levels in PE women when compared to normal pregnant women in their 3rd trimester of pregnancy. Maternal deficiency of micro nutrient like vitamin-D shows a relationship with underlying pathology of PE.

Department of Physiology
SIMS/Services Hospital, Lahore
www.esculapio.pk

References

1. Ugwuja E, Famurewa A, Ikaraoha C. Comparison of serum calcium and magnesium between preeclamptic and normotensive pregnant Nigerian women in Abakaliki, Nigeria. Ann Med Health Sci Res. 2016;6(1):33-7.
2. Pairu J, Triveni G, Manohar A. The study of serum calcium and serum magnesium in pregnancy induced hypertension and normal pregnancy. Int J Reprod Contracept Obstetr Gynecol. 2017;4(1):30-4.
3. Ramachandra C, Rekha R, Roopa R, Shankaregowda S, Nirupama Y. Comparative study of the serum calcium level in normal pregnancy and pre-eclamptic women in a tertiary centre in India. Int J Med Res Rev. 2016;4(05):834-8.
4. Tariq M, Rehman H, Tayyab M, Kamal F, Yasmeen N, Sultan F. Clinipathological study of pre-eclampsia. Biomedica. 2000;16(1):60-5.
5. WHO U. UNFPA, World Bank Group and the United Nations Population Division. Trends in Maternal Mortality: 1990 to 2015. Organization. 2015;32(5):1-55.
6. Shah N, Hossain N, Shoaib R, Hussain A, Gillani R, Khan NH. Socio-demographic characteristics and the three delays of maternal mortality. J Coll Physicians Surg Pak. 2009;19(2):95-8.
7. Ephraim RKD, Osakunor DNM, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: a casecontrol study in the Cape Coast metropolis, Ghana. BMC Pregnancy Childbirth. 2014;14(1):1-8.
8. Gupta T, Wahi S, Gupta N, Arora S, Gupta S, Bhatia P. Correlation of vitamin D levels in term normotensive & pre-eclamptic patients in labor. J Obstetr Gynecol India. 2016;66(3):154-9.
9. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. editors. Institute of Medicine (US) Committeeto Review Dietary reference intakes for adequacy: calcium and vitamin D. 2011. National Academies Press (US).
10. Achkar M, Dodds L, Giguere Y, Forest JC, Armson BA, Woolcott C, et al. Vitamin D status in early pregnancy and risk of preeclampsia. Am J Obstet and gynecol. 2015;212(4):1-14.
11. Washington K, Ghosh S, Reeves IV. A Review: Molecular Concepts and Common Pathways Involving Vitamin D in the Pathophysiology of

Preeclampsia. Open J Obstet Gynecol. 2018;8(3):203-6.
12.Wei S, Audibert F, Hidiroglou N, Sarafin K, Julien P, Wu Y, et al. Longitudinal vitamin D status in pregnancy and the risk of pre eclampsia. Int J Obstetr Gynaecol. 2012;119(7):832-9.
13.van Weert B, van den Berg D, Hrudey EJ, Oostvogels AJ, de Miranda E, Vrijkotte TG. Is first trimester vitamin D status in nulliparous women associated with pregnancy related hypertensive disorders? Midwifery. 2016;34:117-22.
14.Benachi A, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, Senat MV, et al. Relationship between vitamin D status in pregnancy and the risk for preeclampsia: A nested case-control study. Clinical nutrition. 2019.
15.Dhillon MK, Bedi GK, Kaur K. Significance of 25 Hydroxy Vitamin D and Calcium in Pregnancy Induced Hypertension. Int J Health Sci Res 2017;7(7):80-5.
16.World Health Organization. WHO recommen-dations for prevention and treatment of preeclampsia and eclampsia. Geneva: World Health Organization; 2011.
17.Kanagal DV, Rajesh A, Rao K, Devi UH, Shetty H, Kumari S, et al. Levels of serum calcium and magnesium in pre-eclamptic and normal pregnancy: a study from coastal India. J Clin Diagnos Res. 2014;8(7):1-4, OC01.
18.Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. Clin J Endocrinol Metab. 2007;92(9):3517-22.
19.Umar N, Tauseef A, Shahzad F, Sabir S, Kanwal S, Akmal A, et al. Serum 25-Hydroxy Vitamin D Level in Preeclamptic and Normotensive Pregnancies. J Coll Physicians Surg Pak. 2016;26(8):673-6.
20.Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA, et al. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension. 2010;56(4):758-63.
21.Díaz L, Noyola-Martínez N, Barrera D, Hernández G, Avila E, Halhali A, et al. Calcitriol inhibits TNF-α-induced inflammatory cytokines in human trophoblasts. J Reprod Immunol. 2009;81(1):17-24.

Answer Picture Quiz

Diagnosis:

1. Median Canaliform Dystrophy Of Heller (MCD).
   It is also known as Dystrophy Unguium Mediana Canaliformis/Solenonychia or Nevus striatus unguis.
   It is a rare acquired condition characterized by midline or paramedian longitudinal split or ridge and canal formation with multiple transverse ridges fanning out in an inverted fir tree pattern.[1] The condition is usually symmetrical and most often effects the thumbs, although other fingers or toes may be involved. It results from temporary defect in the matrix that interfere with nail formation.[2]
   The exact etiology is unknown. The proposed etiopathogenesis is repetitive trauma to the proximal nail fold and cuticle from habitual nail biting or nail picking.[3] Certain drugs like oral isotretinoin and subungual tumors like myxoid tumors, glomus tumors have been described to be causing this deformity. Familal clustering has also been reported.[4]
2. It is very difficult to treat as no consistently successful treatment. Discontinuation of activities causig repetitive trauma to the nail may lead to resolution of lesions within few months. Some cases have shown good results after topical treatment with 0.1% tacrolimus and 0.05% tazarotene ointments.[5] A psychiatrist referral is necessary if history sugesstive of habit tic and/or obsessive compulsive disorder and appropriate psychotropic drug such as fluoxetine, a serotonin reuptake inhibitor (SSRI) should be instituted to prevent irreversible nail damage.[6]

References:

1. Madke B, Gadkari R, Nayak C. Median canaliform dystrophy of Heller. Indian Dermatol Online J 2012;3:224-5.
2. Sweeney SA, Cohen PR, Schulze KE, Nelson BR. Familial median canaliform nail dystrophy. Cutis 2005;75:161-5.
3. Pathania V. Median Canaliform Dystrophy of Heller occurring on thumb and great toe nails. Medical Journal, Armed Forces India. 2016;72(2):178-9.
4. Kota R, Pilani A, Nair PA. Median nail dystrophy involving the thumb nail. Indian J Dermatol 2016;61:120.
5. Kim BY, Jin SP, Won CH, Cho S. Treatment of median canaliform nail dystrophy with topical 0.1% tacrolimus ointment. J Dermatol. 2010;37:573-4.
6. Avhad G, Ghuge P. Median canaliform dystrophy of Heller. Indian Pediatr. 2013;50:1073.