**Association of Maternal Vitamin D Status and Risk of Preeclampsia**

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**Abstract**

To examine serum vitamin D concentrations from early pregnancy until delivery in women who did and did not develop preeclampsia. This longitudinal study was carried out in Pune, India. A total of 1154 women with singleton pregnancies were recruited in early pregnancy from 2 hospitals. Blood samples were collected and stored at four time points across gestation: V1=11-14 weeks, V2=18-22 weeks, V3=26-28 weeks and V4=at delivery. 108 women developed preeclampsia (PE), and 216 did not develop PE (Non-PE) were randomly selected from the remainder. Serum 25-hydroxy vitamin D concentrations (25(OH)D) were estimated in their samples using commercially available ELISA kits. Independent t-tests were used to compare 25(OH)D between PE and non-PE groups. Logistic and linear regression was used to examine associations of 25(OH)D with the risk of preeclampsia and birth outcomes respectively, after adjusting for confounders. The mean (SD) 25OHD at V1 was 21.95 (19.64) in the Non-PE group and 17.76 (13.21) in the PE group. A decrease in the concentrations of vitamin D (ng/ml) in mid pregnancy (V2) and at delivery was associated with increased risk of preeclampsia (0.31 [95% CI 0.11, 0.86] p=0.024 and 0.24 [95% CI 0.08, 0.77] p=0.016) respectively.Our finding of lower vitamin D concentrations in mid-pregnancy, before women developed clinical preeclampsia, suggests that vitamin D may have a role in its pathophysiology.

**Keywords:** Longitudinal, Preeclampsia, Pregnancy, Vitamin D, 25 hydroxy vitamin D

**Introduction**

Preeclampsia is a hypertensive disorder of pregnancy associated with abnormal placentation. It is a major cause of maternal morbidity and mortality.1 It affects 2-10% pregnancies worldwide2 and 8-10% of pregnancies in India (National Health Portal, 2016).3 The etiology of preeclampsia remains unclear.4 Vitamin D is critical for normal placental development; it has an important role to play in endometrial decidualization and cytotrophoblast invasion. A recent review article suggests that vitamin D deficiency may increase the risk of disorders such as preeclampsia where the placenta plays a central role in its pathophysiology.5

Maternal vitamin D deficiency is common among pregnant women in developing countries; in India, 60-96% of pregnant women are reported to be vitamin D deficient.6 Studies by us and others have reported low maternal vitamin D concentrations in women with preeclampsia at the time of delivery.7-13 However, limited studies have examined serial concentrations across gestation in preeclampsia and are inconsistent. Agudelo-Zapata et al., 2018 report no association between vitamin D and the risk of preeclampsia14 in contrast Raia-Barjat et al., 2021 found a strong, inverse association between serum 25(OH)D concentrations and the risk of placenta-mediated complications.15

A better understanding of vitamin D status and metabolism across gestation is needed, in regions of the world where maternal vitamin D status is suboptimal. This study explores the role of vitamin D (a potentially modifiable causal factor) in the etiology of preeclampsia. The aim of the present study is to serially examine maternal vitamin D status from early pregnancy until delivery and to examine the association of vitamin D status in the mother and the risk of preeclampsia.

**Materials and methods**

**Subjects**

This longitudinal study was carried out at the Interactive Research School for Health Affairs (IRSHA), Pune, India and was approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee. The Institutional Ethics Committee Number is IEC/ 2015/37, dated 03.10.2015, Indian Council of Medical Research (ICMR) Grant no.5/7/1069/13-RCH,dated 31.03.2017. Samples collected under the ICMR REVAMP: Research Exploring Various Aspects and Mechanisms in Preeclampsia study were used for this study. Women were recruited in early pregnancy and followed up until delivery at two hospitals in Pune; the Department of Obstetrics and Gynecology, Bharati Hospital, and Gupte Hospital, Pune. The detailed study protocol has been published.16

In brief, pregnant women visiting both hospitals were invited to join the study at their first antenatal visit, and were recruited after giving written consent. Fig 1 shows the details of the number of women recruited in the study. Maternal blood samples (10 ml) were collected at four time points across gestation that is 11-14 weeks of gestation (V1), 18-22 weeks (V2), 26-28 weeks (V3) and at the time of delivery (V4). Maternal blood samples collected in the non EDTA coated tubes were centrifuged at 3000 rpm for 20 min to separate serum. Serum samples were aliquoted and stored at -800C until further analysis.

**Fig. 1: Number of women recruited in the study**

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V1=11–14weeks, V2=18–22 weeks, V3= 26–28 weeks, and V4= at delivery; PE: Preeclampsia; n: Number of subjects

Women were categorized as having preeclampsia (PE). If there was presence of proteinuria (300mg/Dipstick reading of 1+) and high blood pressure (defined as systolic BP >140 mmHg and/or diastolic BP >90 mmHg) on at least two measurements taken at least 4 hours apart) after 20 weeks of gestation. Additionally, in the absence of proteinuria, presence of severe features like thrombocytopenia, epigastric pain, pulmonary edema, renal insufficiency, impaired liver function or new-onset headache or visual disturbances along with the presence of high blood pressure was diagnosed as PE (Magee et al., 2022). Non-preeclampsia (Non-PE) women include pregnant women without preeclampsia. ‘Select Cases’ in SPSS was used to choose 2 normotensive controls for every case of preeclampsia delivering in the same month. The present study includes a total of 324 pregnant women (216 Non-PE and 108 PE women).

Maternal characteristics such as age (at V1) and body mass index (BMI), standard of living index (SLI) and clinical information and gestation were recorded at all the time points across gestation. In this study cohort, none of the pregnant women smoked. Infant birth weight and length were recorded using a digital weighing scale and an infantometer.

Serum 25(OH)D concentrations in ng/ml for all the time points were estimated using AC-57SF1, 25- Hydroxy Vitamin Ds Enzyme Immunoassay (EIA) kit (AC-57SF1, IDS, Boldon, UK). 25(OH)D concentrations were considered sufficient if the concentrations were more than 30 ng/ml, insufficient if in the range of 20–30 ng/ml and deficient if it was less than 20 ng/ml.17 To ascertain analytic quality all standards were analyzed in duplicate and the coefficient of variations (CV) for the intra and interassay variations were less than 5%. Each kit was provided with two quality controls to assess the validity of the results obtained with each plate. The control samples provided by the manufacturer were within the recommended range that is for Control 1 (AC-5805A) is 12.3-22.8 ng/ml and Control 2 (AC-5805B) is 36.8-60.0 ng/ml. The lower limit of detection of the assay is 2.7 ng/ml.

**Dietary assessment of intake of vitamin D rich foods**

The dietary intake of the women enrolled in the study was recorded using a Food Frequency Questionnaire (FFQ) at V1, V2 and V3. The vitamin D rich foods were identified with the help of Indian Food composition table 2017 by ICMR National Institute of Nutrition (NIN). Based on this, vitamin D rich foods include amaranth seed, sorghum, maize, ragi, whole wheat, black gram, lentil, peas, rajmah, soya bean, amaranth leaves, beans, lady finger, mango, papaya, pomegranate, gingelly seeds, walnut, ground nut, apricot, cashew nut, egg and marine fish. The FFQ taken was based on the recall of food consumed during the last 1 month.

**Statistical Analysis**

Values are expressed as mean ± SD for normally distributed continuous variables or as median and interquartile range for non-normal variables and as percent (%) for categorical variables. SPSS/PC+ statistical package (Version 28, Chicago, IL, USA) was used to analyze the data. The mean values of the estimates of various parameters for the PE group were compared with those of Non-PE group at conventional levels of significance (p < 0.05) using t-tests. Univariate associations between vitamin D concentrations and potential confounders: maternal age, body mass index (BMI), standard of living index (SLI) score, hospital and gestational diabetes mellitus (GDM) were examined. Chi-square tests were used for catagorical variables. Multiple logistic regression was used to investigate the association between vitamin D status at each time point and the risk of preeclampsia, unadjusted and then adjusted for confounders, and is expressed as an odds ratio (95% confidence intervals). Associations of vitamin D concentrations with birth outcome (birth weight and length) were analyzed using multiple linear regression after adjusting for maternal age, maternal BMI, gestational age and baby gender. The variable sample number (n) in vitamin D concentrations was due to either samples below detection limit of the assay or serum samples were not available for that timepoint.

**Results**

This study was initiated from June 2017 to September 2020. A total of 1154 women delivered in this study, of which 1096 were singleton pregnancies and 108 women developed preeclampsia. The mean (SD, range) gestational age of clinical diagnosis of PE was 34.3 (4.94, 24.0 – 40.6) weeks. In our study, out of the total number of preeclampsia 86 women delivered at term and 22 women delivered preterm. A total of 23 women had severe preeclampsia.

**Maternal and Neonatal Characteristics**

In comparison to the women without preeclampsia, women with preeclampsia had a higher weight (p < 0.01), BMI (p < 0.01) and systolic and diastolic blood pressure (p < 0.01 for both) at all four time points across gestation. Gestational age at birth (p < 0.01) and the baby’s birth weight were lower (p < 0.05) in the PE compared to the Non-PE group (Supplementary Table. 1).

**Vitamin D supplements during pregnancy**

The percentages of women taking vitamin D supplements were similar in the PE and Non-PE groups. Women taking vitamin D supplements in the Non-PE group at V1, V2 and V3 was 48.7%, 88.7%, 94.7% respectively while in the PE group it was 49.0%, 91.7%, 97.5% respectively. The mean (SD) vitamin D concentrations for women taking vitamin D supplements in the Non-PE group at V1, V2, V3 and V4 was 24.60 (19.57), 23.84 (21.25), 23.06 (17.16) and 17.20 (9.24) respectively and in the PE group it was 18.35 (14.50), 17.45 (9.14), 16.85 (8.80) and 20.97 (7.66) respectively. The mean vitamin D concentrations for women taking vitamin D supplements were significantly lower in PE group compared to the Non-PE group at V1 (p < 0.01), V2 (p < 0.01) and V3 (p < 0.05) respectively.

The mean (SD) vitamin D concentrations in ng/ml in the women taking vitamin D supplements at V1, V2, V3 and V4 was 22.56 (18.39), 21.81 (18.51), 21.28 (15.48) and 19.35 (8.26) respectively. The mean (SD) vitamin D concentrations in the women not taking vitamin D supplements at V1, V2, V3 and V4 was 18.70 (17.25), 16.09 (15.49) , 18.08 (11.45) and 20.06 (16.59) respectively. At V2, the vitamin D concentrations were significantly lower in the women not taking supplements as compared to the women taking vitamin D supplements (p < 0.01).

**Dietary intake of vitamin D rich foods**

Table. 1 shows the frequency of intake of vitamin D rich foods in Non-PE and PE groups. In this study, the dietary intake of vitamin D rich foods was found to be similar in both the groups.

**Table. 1 Frequency of intake of vitamin D rich foods in Non-PE and PE groups**

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamin D (n%)** | **PE** | **Non-PE** | **p** |
| **V1 (11-14 wks)** | | | |
| **Upto 2 times in a day** | 27 (27.3%) | 46 (22.9%) | 0.657 |
| **2-3 times a day** | 41 (41.4%) | 84 (41.8%) |
| **> 3 times a day** | 31 (31.3%) | 71 (35.3%) |
| **V2 (18-22 wks)** | | | |
| **Upto 2 times in a day** | 15 (17.4%) | 26 (13.8%) | 0.171 |
| **2-3 times a day** | 36 (41.9%) | 63 (33.3%) |
| **> 3 times a day** | 35 (40.7%) | 100 (52.9%) |
| **V3 (26-28 wks)** | | | |
| **Upto 2 times in a day** | 11 (14.3%) | 19 (9.8%) | 0.563 |
| **2-3 times a day** | 24 (31.2%) | 62 (32.0%) |
| **> 3 times a day** | 42 (54.5%) | 113 (58.2%) |

**Maternal serum vitamin D concentrations at different time points of gestation**

Serum vitamin D concentrations at V1 were lower in women who subsequently developed PE compared to the Non-PE women (p = 0.068) (Table. 2). At V2, vitamin D concentrations were found to be significantly lower in women who subsequently developed preeclampsia as compared to the Non-PE women (p < 0.05). Serum vitamin D concentrations at V3 were comparable between the two groups. Serum vitamin D concentrations at V4 were significantly lower in women with preeclampsia compared to the women without preeclampsia (p < 0.01).

**Table. 2 Serum concentrations of vitamin D across gestation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time Points** | **Vitamin D (ng/ml)**  **(Median (IQR))** | | |
| **PE** | **Non-PE** | **p** |
| V1 | 13.88  (8.63-21.96) | 14.63  (08.65-32.09) | 0.068 |
| V2 | 15.41  (10.76-22.68) | 16.86  (10.99-26.94) | 0.016 |
| V3 | 15.72  (9.85-22.30) | 19.06  (11.37-27.57) | 0.093 |
| V4 | 16.01  (8.85-23.88) | 17.10  (10.82-26.46) | 0.009 |

All values are gestational age adjusted values, Values are expressed as median and interquartile range; PE: Preeclampsia; p: Level of significance, V1=11–14weeks, V2=18–22 weeks, V3=26–28 weeks, and V4=at delivery; The number of samples analyzed for vitamin D at each time point is as depicted in Figure 1.

**Vitamin D status in Non-PE and PE groups across gestation**

Percentages of women with vitamin D deficiency in the PE group were 68.8%, 65.6%, 65.4% and 61.4% at V1, V2, V3 and V4 respectively. The percentages in the Non-PE group were 62.8%, 58.2%, 53.1% and 59.2% at V1, V2, V3 and V4 respectively. At V2, we found that the percentage of women with sufficient vitamin D concentrations was significantly lower in the PE group compared to the Non-PE group (p = 0.035). We also found that percentage of women in the deficient and insufficient group was significantly higher in the PE group compared to the Non-PE group at V4 (p = 0.027 and p = 0.033 respectively). In the current study, there were no women in the preeclampsia group who had sufficient vitamin D levels throughout pregnancy.

**Association of maternal vitamin D concentrations with risk of preeclampsia**

To assess the effect of maternal serum 25(OH)D on the odds of having a diagnosis of preeclampsia, a multiple logistic regression analysis was performed (Table. 3). The unadjusted models showed low vitamin D concentrations were associated with an increased risk of preeclampsia at V2 (OR 0.35 [95% CI 0.15, 0.83] p=0.017) and V4 (OR 0.29 [95% CI 0.11, 0.74] p=0.010). These associations remained significant after adjusting for the confounding factors.

**Table. 3 Unadjusted and adjusted odds ratios for preeclampsia according to vitamin D status across gestation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **25(OH)D, ng/ml** | **n** | **Unadjusted**  **OR of preeclampsia**  **(95% CI)** | **p** | **Adjusted**  **OR of preeclampsia**  **(95% CI)** | **p** |
| V1 | 294 | 0.46 (0.20,1.07) | 0.070 | 0.57 (0.22,1.47) | 0.237 |
| V2 | 308 | 0.35 (0.15,0.83) | 0.017 | 0.31 (0.11,0.86) | 0.024 |
| V3 | 289 | 0.42 (0.17,1.06) | 0.065 | 0.48 (0.16,1.45) | 0.190 |
| V4 | 293 | 0.29 (0.11,0.74) | 0.010 | 0.24 (0.08,0.77) | 0.016 |

CI=confidence interval, OR=odds ratio, n=number, Adjusted for maternal age, BMI, SLI score, hospital and GDM.

**Associations of maternal vitamin D concentrations with birth outcome**

Multiple linear regression showed no association between vitamin D concentrations at any time points with the baby’s weight and length (Table. 4).

**Table. 4 Multiple linear regression analysis of maternal vitamin D concentrations with birth weight and length**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Time**  **Points** | **Newborn Weight** | | | | **Newborn Length** | | |
| **n** | **B(CI)** | **p** | **n** | | **B(CI)** | **P** |
| V1 | 294 | 26.64  (-175.07,228.35) | 0.795 | 270 | | -1.34  (-2.92,0.26) | 0.099 |
| V2 | 308 | 64  (-140.06,268.05) | 0.538 | 283 | | -1.18  (-2.69,0.34) | 0.126 |
| V3 | 288 | 97.74  (-100.88,296.34) | 0.334 | 270 | | 0.34  (-1.36,2.02) | 0.700 |
| V4 | 293 | 177.36  (-029.23,383.93) | 0.092 | 272 | | 0.26  (-1.40,1.91) | 0.762 |

Model adjusted for maternal age, maternal BMI, gestational age and baby gender; V1=11–14 weeks, V2=18–22 weeks, V3=26–28 weeks, and V4=at delivery; n=number, p: Level of significance; B: regression coefficient; CI: Confidence interval

**Discussion**

The current study reports the vitamin D status across gestation of women who developed preeclampsia and women without preeclampsia. We observed 1) lower concentrations of vitamin D at V2 and at the time of delivery in women with preeclampsia; 2) a decrease in the concentrations of vitamin D in mid pregnancy (V2) and at delivery was associated with increased risk of preeclampsia; 3) and maternal vitamin D concentrations showed no association with the birth outcome.

Various studies have examined the concentrations of vitamin D in women with preeclampsia but these studies have examined vitamin D at the end of pregnancy, generally after the onset of preeclampsia.11-12,18 To the best of our knowledge, very few studies have examined vitamin D concentrations prospectively using serial measurements across gestation from before the clinical onset of the condition. In India, despite the presence of abundant sunlight, there is a high prevalence of vitamin D deficiency in pregnant women which could be due to poor living conditions, economic status and cultural factors.19

In the current study, we found that women with preeclampsia had low concentrations of vitamin D in mid pregnancy compared to the women without preeclampsia and it continued to be low across gestation. A cohort study involving 169 preeclampsia and 1975 control pregnant women reported lower 25(OH)D concentration (25(OH)D <30 nmol/L) at 14 weeks of gestation in women who developed preeclampsia later in pregnancy; the mean (SD) vitamin D concentrations in the control group was 20.92 (6.88) ng/ml while in the PE group it was 18.88 (7.08) ng/ml.20 Bodnar et al., 2007 showed that maternal 25(OH)D during early pregnancy (less than 22 weeks of gestation) was associated with increase in the risk of preeclampsia.13 In contrast, other studies have demonstrated no association between vitamin D levels and risk of preeclampsia.21-24 At delivery, we found significantly low concentrations of vitamin D in women with PE as compared to the Non- PE. These results are in accordance with other published studies.18, 25 A cochrane systematic review examining the effect of vitamin D supplementation during pregnancy on the maternal and neonatal outcomes showed that vitamin D supplementation can reduce the risk of preeclampsia.26 In our study, we found that vitamin D deficiency exists at V2 i.e. before the clinical onset of preeclampsia suggesting that vitamin D deficiency is a likely cause for preeclampsia.

Maternal vitamin D status could alter the risk of preeclampsia through various mechanisms. Preeclampsia is a two-stage disorder where there is reduced placental perfusion which is often secondary to abnormal implantation.27 The poorly perfused placenta secrets toxic factors into the maternal circulation that leads to the maternal clinical features.28 Vitamin D has a key role in decidualization, placental invasion, implantation and immunomodulation.29 It also helps in suppressing pro-inflammatory responses. Therefore, it is plausible that vitamin D deficiency during pregnancy may predispose to the pathophysiology of preeclampsia.

Our results highlight that the percentage of women with sufficient vitamin D concentrations was significantly lower in the PE group than the Non-PE group in mid pregnancy (18-22 weeks). We also found significantly a higher percentage of women with vitamin D deficiency and insufficiency in the PE group as compared to the Non-PE group at the time of delivery. We found that percentages of women taking vitamin D supplements and frequency of intake of vitamin D rich foods were similar in both the groups. However, sun exposure is the major source of vitamin D and we lacked sun exposure data, which is a limitation of this study. Another possibility (reverse causality) is that, although not clinically evident at 18-22 weeks, the process of preeclampsia has already started at this early stage of gestation and is causing a lowering of vitamin D levels. This possibility is supported by the fact that among women taking vitamin D supplements from early pregnancy, vitamin D concentrations were lower in the PE group.

This study demonstrates that maternal vitamin D status in mid pregnancy is associated with risk for developing preeclampsia. These results are in accordance with the metanalysis and systematic reviews which suggest a relationship between vitamin D deficiency and increased risk of preeclampsia.30-31 This study suggests that maternal vitamin D status may influence risk for preeclampsia. In our study cohort, we found maternal age and body mass index (BMI), standard of living index (SLI) score, hospital and gestational diabetes mellitus (GDM) to be the potential confounding factors. Other studies have also reported similar findings, Bodnar et al., 2007 reported that an increase in BMI from 22 to 34 was associated with 2-fold (95% CI: 1.2, 3.6) increase in the odds of mid-pregnancy vitamin D deficiency (Bodnar et al., 2007). Place of living and lifestyle also influences the vitamin D status (Karras et al., 2018).

In the current study, we found no association between maternal vitamin D status and birth outcome. These findings are in line with a Cochrane review which indicates that maternal supplementation of vitamin D does not affect the birth weight and length.34

A meta-analysis including 7,663 women reports that women diagnosed with vitamin D deficiency (<50 nmol/L) had an increased risk of miscarriage compared with women who were vitamin D replete (>75 nmol/L) (odds ratio, 1.94; 95% confidence interval, 1.25-3.02; 4 studies; n = 3,674; I2 = 18%) (Tamblyn et al., 2022) highlighting the impact of vitamin D on trophoblastic function affecting both miscarriage and preeclampsia risks

We have earlier described that vitamin D may influence the risk of preeclampsia through angiogenesis.36 Findings from our animal studies support a role for vitamin D in promoting angiogenesis in preeclampsia.37 We have also described the possible mechanisms through which vitamin D influences angiogenesis. It may either be Cystathionine β-synthase/Cystathionine γ-lyase genes in the one carbon cycle or by stimulating PI3/AKT pathway through its receptor that is VDR or by regulating renin angiotensin pathway or by regulating the long chain polyunsaturated fatty acids metabolism.36 Our animal studies in preeclampsia have demonstrated that vitamin D status can alter fatty acid concentrations which in turn influence angiogenesis.38 A recent report by Cavoretto and Viganò suggests that there is enough evidence for promoting the measurement of vitamin D levels before conception or in the first trimester of pregnancy (if this was not done earlier) as a prognostic biomarker for miscarriage39. Our findings also support the need to routinely undertake vitamin D measurements in early pregnancy in all patients as Vitamin D levels in early pregnancy were lower in preeclampsia. Nevertheless, there is a need to conduct future RCTs on the role of Vitamin D in pregnancy. A study examining the concentrations of angiogenic growth factors which are critical for normal placental development from early pregnancy will provide a better understanding of the mechanism through which vitamin D influences angiogenesis in preeclampsia.

**Strength and limitations**

The strength of this study is its longitudinal study design. This study is the first to examine the vitamin D concentrations serially across gestation in Indian pregnant women with and without preeclampsia. The current study lacks data on sun exposure and is a limitation of the study. The effect of covariates and risk of bias are likely to have influenced the findings of this study. Furthermore, the absence of significance at V3 need to explored in detail in further studies.

**Conclusion**

This study reports serial measurements of vitamin D across gestation and the risk of developing preeclampsia in the Indian population. Our findings suggest that vitamin D deficiency exists before the clinical diagnosis of preeclampsia and may therefore be an important aetiological factor. However, reverse causality (that vitamin D deficiency is an early manifestation of preeclampsia) cannot be ruled out. Future studies examining the effect of vitamin D supplementation starting in early pregnancy or pre-conceptionally and its influence on risk of developing preeclampsia are warranted.

**Ethical Approval**

The study protocol was performed in accordance with the Guidelines laid down by the Indian Council of Medical Research, Government of India and approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee ( IEC/ 2015/37, dated 03.10.2015, Indian Council of Medical Research (ICMR) Grant no.5/7/1069/13-RCH,dated 31.03.2017).Informed consents were obtained from human participants of this study.

**Author contributions**

Conceptulization : SJ , Investigation : JN , VK , KD, HP, SM, GW, SG , NW Data Curation : KR and CF Writing original draft : JN, Writing – Review and Editing SJ, JN, BK, HPS and CF

**Conflict of Interest statement**

The authors declare that there are no conflicts of interest.

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