Vitamin D and Preeclampsia

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Preeclampsia (PE) is a set of clinical symptoms that appears after the 20th week of pregnancy. It is a multi-organ disease characterized by hypertension and proteinuria, and in the absence of proteinuria—an impairment of the functions of the internal organs.
With regard to the multiple mechanisms of action of Vit D, its deficiency seems to be one of the possible factors conducive to PE development. It has been suggested that the consequence of low Vit D levels may be the appearance of an early, severe form of PE, and its supplementation may be a protective factor against its recurrence in subsequent pregnancies [232].
The relationship between Vit D and PE development may explain its impact on implantation, angiogenesis, and endothelial status, regulation of the immune response, effect on RAAS, and calcium metabolism.
preeclampsia vitamin D prophylaxis

1. Pathogenesis of Preeclampsia

Despite significant advances in research on PE pathophysiology, its cause has not been definitively settled. It has been demonstrated that its development is associated with the presence of the placenta, and the processes that initiate it begin at the time of abnormal trophoblast invasion in early pregnancy. As a result, they lead to the development of trophoblast/placental hypoxia and consequently to the development of oxidative stress and endothelial dysfunction in the later phases of the disease, which are manifested by clinical symptoms. The only effective way to treat PE is delivery which indicates its relationship with the presence of the placenta.

A two-stage model for PE development has been proposed. The first stage involves incomplete remodeling of spirals arteries in the uterus, which leads to hypoxia of the placenta. In the second stage, anti-angiogenic factors responsible for endothelial damage are released from the hypoxic placenta into the maternal circulation.

The trophoblast implantation involves its invasion into the uteroplacental arteries and then their transformation into dilated, inelastic tubes, which provides increased blood flow without maternal vasomotor control. The purpose of this process is to provide increased perfusion of the intervillous space. In the case of inadequate trophoblast invasion and lack of transformation of spiral arteries, relative hypoxia of the placenta with the development of oxidative stress occurs [1]. Trophoblast hypoxia could explain the death of cells, mainly in the mechanism of apoptosis [2][3]. These processes occur early in pregnancy; trophoblast implantation is completed by the 16–17th week. The critical issue remains the cause of abnormal trophoblast implantation. Many researchers suggest an

impaired response of the maternal immune system or abnormal development of maternal immune tolerance to the development of the allogenic fetus [3][4].

Several studies have been conducted on immune changes within the preeclamptic decidua. They have shown excessive activation of neutrophils and monocytes, which synthesize large amounts of pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8 [5][6].

In addition, CD4+ and CD8+ T cells together with natural killer cells (NKc) and dendritic cells (DCs) show a different response in women with PE compared to healthy pregnant women [7][8]. An animal model has shown that decidual natural killer cells (dNKc) knockout mice did not develop spiral arteries [9]. It has been revealed that dNKc, by releasing pro-apoptotic factors during normal pregnancy, can lead to apoptosis in vascular smooth muscle cells (VSMC) and endothelial cells, which are essential in the process of spiral arteries remodeling [10].

Abnormal remodeling of spiral arteries entails a disorder of placental function, which is the source of many factors entering the maternal circulation responsible for increased inflammatory response, oxidative stress, apoptosis, and generalized endothelial dysfunction, which is an essential pathophysiological change in PE, explaining the development of clinical symptoms [11]. These include generalized vasoconstriction and restricted organ perfusion. Factors that adversely affect endothelial function such as obesity, diabetes, malnutrition intensify the maternal response to signals from the hypoxic placenta and thus promote PE development [12]. It has been suggested that endothelial dysfunction could be more pronounced in PE than in GH, which explains less severe clinical symptoms and a better prognosis [13][14].

The endothelium has autocrine, paracrine, and endocrine properties. It is responsible for the synthesis of numerous vasodilators (nitric oxide (NO), prostacyclin I2 (PGI2), endothelium-derived hyperpolarizing factor (EDHF), bradykinin, histamine, serotonin, substance P), and vasoconstrictors (endothelin-1 (ET-1), angiotensin II (ANG-II), thromboxane A2 (TX2), prostacyclin H2 and reactive oxygen species (ROS)). The imbalance between them and the predominance of the synthesis of vasoconstrictive factors are responsible for developing many pathological processes, including preeclampsia. Endothelial dysfunction is connected with the presence of at least one of the following changes: the decrease in the NO synthesis and bioavailability, higher adhesion molecules and inflammatory genes expression, intensified ROS synthesis, impaired endothelium-dependent vasorelaxation, decreased fibrinolysis and enhanced endothelial permeability [15]. Hypoxia and oxidative stress have been thought to disrupt the placental synthesis of pro-angiogenic and anti-angiogenic factors, which play a key role in the pathogenesis of PE [16]. It is characterized by a reduced concentration of pro-angiogenic factors and a predominance of anti-angiogenic factors [17]. The characteristic shift in balance favoring anti-angiogenic factors is present from the beginning of pregnancy and impairs the trophoblast implantation [18][19].

The essential pro-angiogenic factors in pregnancy are vascular endothelial growth (VEGF) and placental growth (PlGF). VEGF plays an important role by attaching and activating the two-cell surface receptor tyrosine kinases, vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1). Furthermore, vascular endothelial growth factor receptor 2/kinase insert domain receptor (VEGFR-2/KDR), which is present on endothelial cells, stimulates their

proliferation and the release of the plasminogen activators [20]. Its pro-angiogenic activity is expressed through these mechanisms [21]. VEGF has been postulated to play an important role in maintaining endothelial integrity. A link between VEGF and placental oxidative stress has been suggested. In patients with severe preeclampsia, changes in VEGF concentration resulting from hypoxia may cause an increase in the activity of 5' adenosine monophosphate-activated protein kinase (AMPK) [22]. AMPK plays an important role in many of the cellular energy and metabolic processes. It affects angiogenesis within the placenta, and its activity increases under hypoxia conditions observed in preeclampsia [23].

Another pro-angiogenic factor important for the proper development of pregnancy is PlGF which regulates endothelial cell adhesion and chemotaxis. PlGF is thought to enhance the pro-angiogenic effect of VEGF [24][25]. The transforming growth factor-β (TGF-β) family has been shown to play an important role in endothelial cell growth and angiogenesis, modulates the immune response and thus regulates many placental functions [26]. It has been found that TGF-β enhances the expression of VEGF, and its concentration is significantly reduced in PE [27]. The main anti-angiogenic agents whose role in the pathogenesis of preeclampsia has been described are VEGF receptors (VEGFR1 and VEGFR2) and soluble endoglin (sEng). VEGFR1 is also known as fms-like tyrosine kinase-1 (sFlt-1) [28]. It has been shown that sFlt-1 by binding VEGF and PlGF reduces the formation of vessels within the trophoblast [29][30]. It has been observed that an increase in its levels accompanied by a decrease in PlGF concentration correlates with the PE severity [31].

With the limited perfusion and hypoxia that characterize PE, the placenta produces large amounts of sFlt-1 and sEng, one of the potent anti-angiogenic factors, which both are thought to be responsible for endothelial damage and PE symptoms [32][33]. It has been shown that sEng by disturbing TGF-β1 signaling in endothelium cells reduces vasodilation and limits the pro-angiogenic effect [34]. On the pregnant rodents model, Venkatesha et al. have shown that the administration of sEng significantly increases blood pressure and develops mild proteinuria. In contrast, the administration of sFlt-1 results in the development of severe hypertension and severe proteinuria and the appearance of HELLP (hemolysis, elevated liver enzymes, low platelets count) syndrome symptoms. sENG together with sFlt-1 can inhibit the action of both TGF-β1 and VEGF [35].

These observations confirm the results of studies by other authors recognizing sFlt-1 as the main anti-angiogenic factor involved in the PE development [29].

It has been reported that the activation of eNOS (endothelial nitric oxide synthase) and the NO release, the potent vasodilator, is inhibited by sEng, which significantly limits the proper growth and invasion of the trophoblast [36]. On the other hand, VEGF and PlGF positively affect the synthesis and bioavailability of NO [37][38].

sFlt-1 by inhibiting PlGF and VEGF leads to a decrease in NO synthesis, which is additionally disturbed by oxidative stress and ROS. These observations confirm that the synthesis and release of NO are dependent on the balance between pro-angiogenic and anti-angiogenic factors. Disturbance of this balance in favor of anti-angiogenic factors adversely affects the release of NO [39].

Increased inflammation observed in PE, which is expressed for example by elevated TNF-α (tumor necrosis factor α) concentrations, is associated with an increase in the expression of adhesive molecules ICAM1 (intercellular adhesion molecule 1), VICAM1 (vascular cell adhesion molecule 1), and endothelin 1 (ET-1), the potent vasoconstrictor, which are all markers of endothelial damage [40][41][42].

The mechanism of action of anti-angiogenic factors and the imbalance between pro- and anti-angiogenic factors partly explain the stages of the pathogenetic pathway in the development of PE. In addition, the assessment of the sFlt/PlGF ratio is of prognostic importance to predict the severity of PE complications: the increased sFlt/PlGF ratio anticipates the appearance of adverse outcomes within two weeks [43][44].

Hypoxia-inducible factor α (HIF1α) is a molecular factor that combines placental hypoxia with downstream mediators of PE. The synthesis of HIF1α has been shown to be intensified in placental hypoxia. It has also been observed that HIF1α is a factor inducing the synthesis and release of sFLT-1 in placental explants [45].

During a healthy pregnancy, there is an increase in metalloproteinases (MMs) activity to ensure proper trophoblast implantation which requires the destruction of the extracellular matrix. The invasive potential of extravillous trophoblast (EVT) cells relates to MMP-2 and MMP-9 expression [46]. Reduced activity of metalloproteinases is associated with PE development [47]. This observation is confirmed by the results of studies indicating the relationship of vasoconstriction typical for PE with reduced expression of MMP-2 and MMP-9. Chen et al. have reported a different effect of pro- and anti-angiogenic factors on MMP-2 activity in placental tissues and vascular wall. sFlt-1 lowered the activity of these molecules, and VEGF reversed this process and improved placentation [48].

During physiological pregnancy, the phenomenon of increased production of PGI2 as a platelet inhibitor and vasodilator and a limitation of the synthesis of TX2 responsible for platelet activation and vasoconstriction is observed. In PE, endothelial dysfunction results in the peroxidation of endothelial lipids and the limitation of antioxidant processes. Lipid peroxidation activates cyclooxygenase (COX—cyclooxygenase), which is responsible for the synthesis of TX2 thromboxane, disturbing the TX2/PGI2 balance in favor of TX2 [49]. Although progesterone is the hormone responsible for the proper development of pregnancy, its excess can lead to a decrease in the synthesis of prostacyclin and an increase in the production of thromboxane [50].

In a healthy pregnancy, activation of the renin-angiotensin-aldosterone system (RAAS) is observed, which leads to an increase in the concentration of renin, angiotensinogen, and angiotensin II [51]. Many authors have so far postulated that RAAS has a significant impact on the development of preeclampsia. In PE, RAAS is inhibited, confirmed by a reduced serum concentration of angiotensin I, angiotensin II, aldosterone, an increase in renin plasma activity, and the concentration of antibodies to the angiotensin II type 1 receptor (ATR1-AA). These antibodies are responsible for stimulating the signaling ATR1 and, as a result, for increasing blood pressure [52][53][54]. However, it seems that the role of this system in the pathogenesis of preeclampsia has not been definitively determined. Many researchers believe that it has a significant impact on the development of PE. However, there is

a growing body of evidence that although RAAS plays an important role in the development of pregnancy, its importance in the pathogenesis of PE is not significant except ATR1-AA [55].

Current reports also emphasize the importance of disorders of the methionine-homocysteine system and cellular mechanisms of oxygen sensing in the process of abnormal trophoblast invasion and placental hypoxia [56][57]. Hyperhomocysteinemia is associated with PE development, and it is thought to be responsible for endothelium dysfunction caused by thrombosis [58]. One of the causes of hyperhomocysteinemia is MTHFR 677TT genotype, whose relationship with the PE development is postulated. Micronutrients such as folic acid and riboflavin have been shown to reduce homocysteine levels significantly [59]. **Figure 1** shows the main stages in PE pathogenesis.

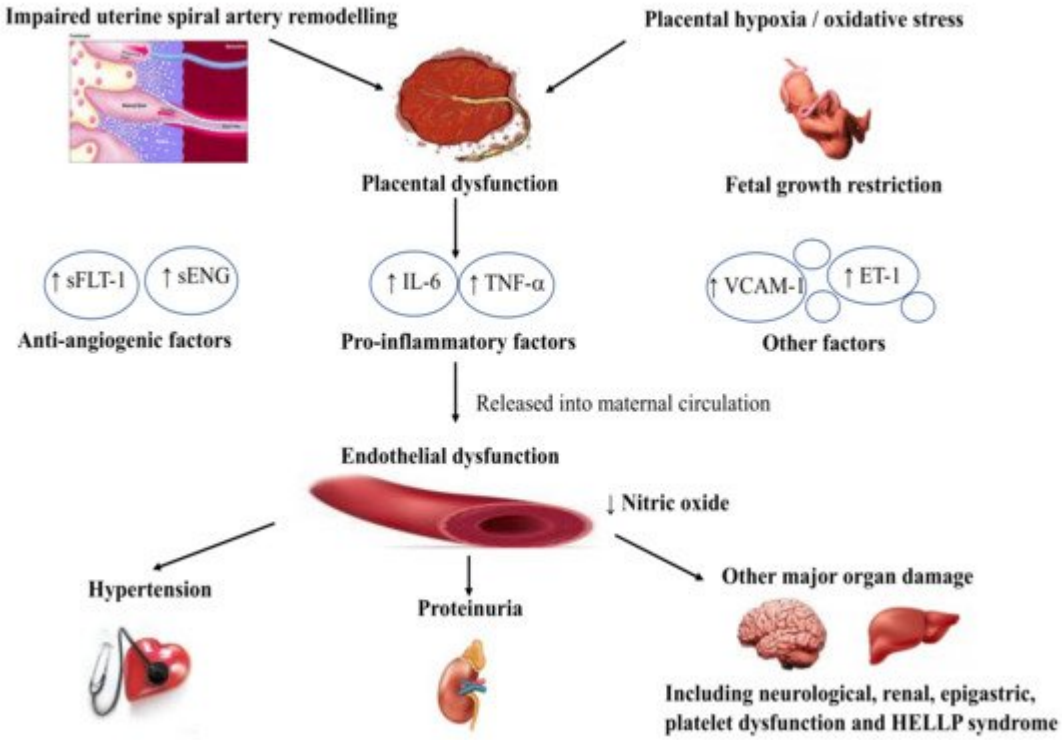

Figure 1. Main stages in PE pathogenesis. sEnd—soluble endoglin, sFlt-1—fms-like tyrosine kinase-1, VICAM1—vascular cell adhesion molecule 1, IL-6—interleukin 6, TNFα—tumor necrosis factor α, ET-1—endothelin-1, HELLP—hemolysis, elevated liver enzymes, low platelets count.

2. Vitamin D and Preeclampsia—Experimental Research

With regard to the multiple mechanisms of action of Vit D, its deficiency seems to be one of the possible factors conducive to PE development, which is confirmed by many reports [60]. Studies conducted by Baca et al. have shown associations between allelic variation in Vit D metabolism genes and PE [61]. It has been suggested that the consequence of low Vit D levels may be the appearance of an early, severe form of PE, and its supplementation may be a protective factor against its recurrence in subsequent pregnancies [62].

The relationship between Vit D and PE development may explain its impact on implantation, angiogenesis, and endothelial status, regulation of the immune response, effect on RAAS, and calcium metabolism.

The main theoretical basis for the use of Vit D in the prevention of preeclampsia is presented in **Figure 2**.

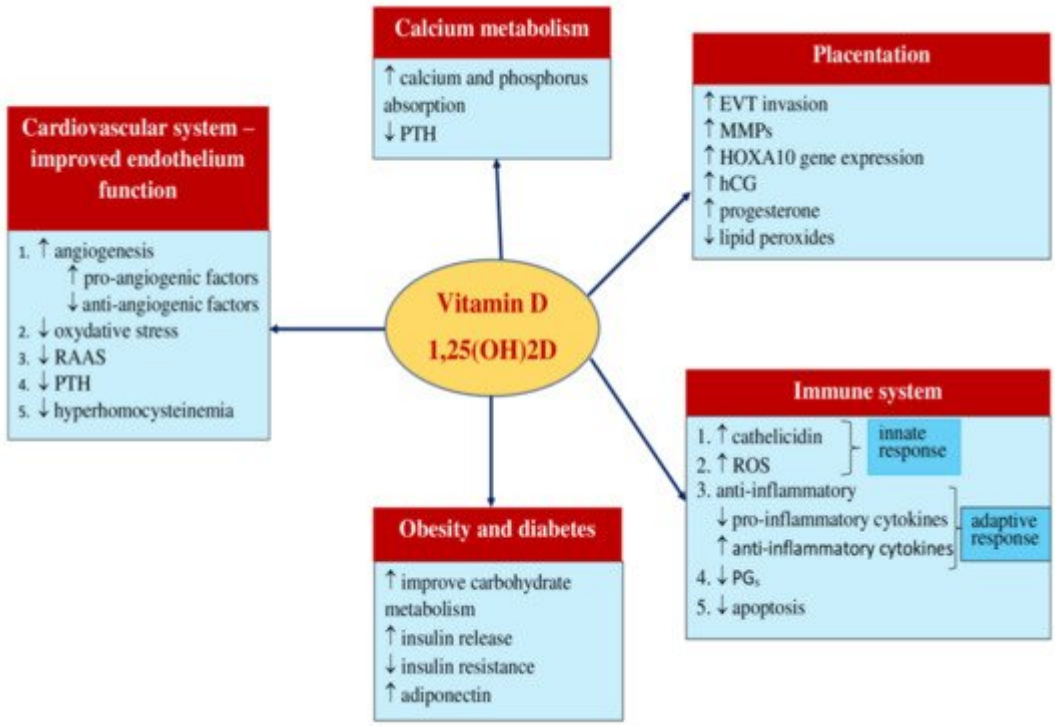

Figure 2. Theoretical basis for the use of Vit D in the prevention of preeclampsia. 1,25(OH)2D - 1,25-dihydroyxvitamin D, PTH – parathyroid hormone, RAAS - renin-angiotensin-aldosterone system, EVT – extravillous trophoblast, MMPs - metalloproteinases, hCG – human chorionic gonadotropin, ROS - reactive oxygen species, PGs - prostaglandins.

2.1. Trophoblast

The potential Vit D contribution in placentation has been suggested [63]. However, the exact role of vitamin D in this process has still not been settled.

It has been shown that 1,25(OH)2D affects the expression of the HOXA10 gene which is responsible for the implantation and trophoblast invasion into the decidua [64]. A beneficial effect of Vit D on pregnancy development could be observed only if supplementation is initiated during placental implantation [65]. Studies by Barrer D et al. have revealed that Vit D indirectly by intensifying the synthesis of progesterone and human chorionic gonadotrophin (hCG) may improve trophoblast implantation [66]. Although human decidual cells at the fetal-maternal interface synthesize 1,25(OH)2D via CYP72B1 [67], however it has been observed that cultured syncytiotrophoblast cells from preeclamptic placentas have only one-tenth activity of this enzyme compared to the normal cells [68].

The molecular mechanisms explaining the Vit D effect on EVT cells' migratory and invasive properties are not fully understood. Vitamin D has been shown to regulate the actin cytoskeleton in trophoblast cells. Results of in vitro studies conducted by Chan et al. have suggested that under the influence of 1,25(OH)2D or 25(OH) there is a significant improvement in the invasion of human EVT. They have confirmed the role of Vit D and indicated that its appropriate level could improve this process, and thus, it may constitute one of the protective elements against the PE development [69]. CYP27B1, VDR, VDBP, 25-hydroxylase, and 24-hydroxylase expression has been found in syncytial trophoblasts responsible for invasion [68]. The balance between these enzymes is significantly disturbed in the placental tissue from patients with PE. In preeclamptic placentas, increased expression of CYP27B1, CYP24A1 and reduced CYP2R1 and VDR 25-hydroxylase have been demonstrated compared to healthy placentas, indicating impaired Vit D metabolism in preeclampsia. In addition, the presence of a hypoxic-inducing agent responsible for the development of oxidative stress was found in preeclamptic placental tissue. It has been shown that in placentas derived from healthy women under its influence, changes similar to those observed in preeclamptic placentas occur [70].

Zabul et al. have pointed to the potential significance of an adequate placental concentration of 1,25(OH)2D in PE prevention. They believe that calcitriol by competitive inhibition of placental cytochrome P450scc restrains the excessive synthesis of lipid peroxides and progesterone promoting PE development [71].

The process of trophoblast implantation requires the destruction of the extracellular matrix, for which metalloproteinases are responsible. It has been shown that the reduced levels of vascular MMP-2 and MMP-9 are responsible for vasoconstriction and, as a result, lead to the development of GH and PE [46]. Results of research conducted by Ganguly et al. have indicated that Vit D by enhancing the expression of MMP-2 and MMP-9 promotes the migration and invasion of human EVT in the 1st trimester of pregnancy [64].

2.2. Angiogenic Factors and Endothelium

Vitamin D significantly affects blood vessels and angiogenesis. It is postulated that it may play a beneficial role in preventing endothelial damage and controlling blood pressure in pregnant women with preeclampsia [72]. Under the Vit D influence, the activation of endothelium cells caused by cytokines is limited as well as TNF-

-induced expression of adhesive molecules [73][74]. The results of the Shulz et al. study have shown that gene expression for anti-angiogenic factor (sFlt-1) and surprisingly, pro-angiogenic factor (VEGF) was significantly inhibited at a 25(OH)D concentration ≥ 100 ng/mL compared to the lower 25(OH)D levels. These authors believe that adequate Vit D supplementation ensuring this 25(OH)D level may reduce the risk of PE development [75]. However, most studies have indicated that vitamin D upregulates VEGF gene expressions [76][77][78]. Grundmann et al. have observed that by increasing VEGF expression and pro-matrix metalloproteinase (pro-MMP-2) activity, Vit D induces angiogenesis in endothelial progenitor cells [79]. It has been found that by restoring functional properties of endothelial colony-forming cells (ECFC), which are endothelial progenitor cells, and participate in vasculogenesis and endothelial repair, Vit D may reduce the severity of PE symptoms resulting from endothelial damage [80].

Brodowski et al. have also confirmed the beneficial 1,25(OH)2D influence on endothelial progenitor cells, which allows reversing endothelial damage characteristic of PE [81].

2.3. Immune System

The immunomodulatory properties of Vit D may explain its favorable effect on reducing the risk of PE development [82]. Vitamin D limits the overexpression of Th1, which is characteristic of placentas in preeclampsia [83]. Expression of pro-inflammatory cytokines such as TNF-α and IL-6 was inhibited in placental tissues collected from patients with PE and treated with 1,25(OH)2D compared to trophoblast cell cultures without 1,25(OH)2D [84].

The results of studies among women with PE have shown that compared to healthy ones, they were characterized by significantly lower Vit D levels and elevated levels of IL-6, although no correlation was observed between their concentrations [85].

It has been suggested that it also regulates the proper response of the maternal immune system to the placenta, which prevents the release of anti-angiogenic factors [86].

2.4. RAAS

Although the ultimate role of RAAS in the development of PE has not been clearly defined, it has been shown that ATR1-AA are responsible for the development of hypertension [55]. In an animal model, it has been demonstrated that the Vit D administration significantly reduces the blood pressure induced by ATR1-AA [54].

3. Vitamin D and Preeclampsia Risk

Due to the multitude of functions of vitamin D, especially its immunomodulatory properties and its beneficial effect on angiogenesis and vascular endothelium, its use in preventing preeclampsia seems attractive. The results of several experimental, clinical, observational, and randomized studies and meta-analyses on this issue have been published. Searching the PubMed database using the keywords “vitamin D” and “preeclampsia” only from the last ten years gives the result of 360 articles. However, only a tiny percentage of them attempted to answer whether Vit D can effectively prevent PE.

This chapter presents the results of randomized controlled trials and meta-analyses that have been published over the past ten years. Electronic databases PubMed has been searched using keywords such as “Vitamin D” and “preeclampsia”. Only articles available in English were considered. Only 5 out of 14 published RCTs and 16 out of 30 meta-analyses provided information on the effect of vitamin D on preeclampsia.

The results of the selected RCTs, which have been released within the last ten years and present the information on the Vit D influence on PE risk, are presented in **Table 1**.

Table 1. Selected randomized placebo-controlled trials on vitamin D influence on PE risk.

Author	Aim of the Study	Size of Groups	Vit D Dose (IU) and Duration of Treatment	GA at the Entry to the Study	Main Outcome
Mirzakhani et al. 2016 [87]	PE risk	Vit D (SG) 408 CG 408	4400 daily 400 daily	10–18th week	PE incidence SG 8.08% CG 8.33%, NS RR 0.97 95% CI: 0.61–1.53
Rostami et al. 2018 [88]	Vit D status screening	Screened Vit D 800 Without Vit D 200 Non screened 900	50,000–300,000 weekly or monthly; 6–12 weeks	<14th week	Screening reduces PE risk by 60% RR 0.40 95% CI: 0.30–0.60
Karamali et al. 2015 [89]	PE risk	Vit D (SG) 30 CG 30 patients with high PE risk	50,000 every 2 weeks	20–32nd week	PE incidence SG 3.3% CG 10% *p* = 0.3
Sablok et al. 2015 [90]	Pregnancy complication risk	Vit D (SG) 120 CG 60	60,000–120,000 every 4 weeks	20–32nd week	PE incidence SG 11.1% CG 21.1% *p* = 0.08
Ali et al. 2019 [91]	PE risk	Vit D (SG) 83 CG 81	4000 daily	at 13th week up to 12th week after delivery	PE incidence SG 1.2% CG 7.4% *p* = 0.049

PE—preeclampsia; Vit D—vitamin D; SG—study group; CG—control group; GA—gestational age; P—statistical significance; RR—relative risk; CI—confidence interval.

The presented papers assessed not only the effectiveness of Vit D administration in PE prevention, but their authors also analyzed the relationship of Vit D concentration with the risk of PE [87][90][88][89][91], and the legitimacy of Vit D deficiency screening to prevent PE [88]. Additionally, studies by Mirzakhani et al. determined the expression of 348 Vit D-dependent genes in preeclamptic patients [87].

According to the results of the presented RCTs, it might be concluded that Vit D supplementation seems ineffective in the prevention of PE. Mirzakhani et al., Sablok et al. and Karamali et al. have not demonstrated a beneficial effect of Vit D supplementation in reducing the risk of PE, even despite the inclusion of this treatment in the second trimester of pregnancy (Mirzakhani et al.) [87][90][89]. In contrast, in Sablok et al. and Karamali et al. studies, Vit D was offered late, between the 20th–32nd week [90][89]. For these cases, the late start of Vit D administration and the lack of adequate concentration during placental implantation seem to have influenced the results. Although the

difference in doses used in these studies is significant 4400 vs. 50,000 IU, and different administration regimens have been used, it does not affect the results. In contrast, the results of the study conducted by Ali et al. have indicated the effectiveness of the 4000 IU dose in preventing PE. In this research, all patients were started with Vit D supplementation at week 13, which may have significantly affected the outcome [91]. The group studied by Karamali et al., although consisting of patients at high risk of developing PE, was tiny. Hence the results of this study may not be representative [89]. It is also noteworthy that the very wide range of doses from 4400 IU per day [87] through 50,000 [88][89] to even 300,000 [88] was provided at various time intervals in the studied groups. So far, no organization recommends such high doses of vitamin D (50,000 and 300,000 IU) during pregnancy. Low doses of vitamin D (400 IU) [92] or no treatment was offered to pregnant women in control groups [8][90][88][89].

However, research by Marzakhani et al. has also yielded promising results. It has been shown that the satisfying level of Vit D defined by the authors as >30 ng/mL, observed in both early and late pregnancy, is associated with a significantly lower risk of PE (RR 0.28, 95 CI: 0.10–0.96) [87]. Results of research conducted by Rostami et al. on a large group of patients have shown that screening of Vit D status in pregnant women from the low-risk group allows reducing the risk of PE by 60% (RR 0.40, 95% CI: 0.30–0.60. The NNS (numbers needed to screen) value has been estimated at 11 (95% CI, 8 to 17), which means that screening 11 pregnant women will prevent 1 case of PE. In this study, patients subjected to screening were divided into subgroups depending on the concentration of Vit D—the level of >20 ng/mL was considered sufficient, and the pregnant women did not receive Vit D (control group). The study group had a concentration of Vit D < 20 ng/mL, and a subgroup of moderate (10–20 ng/mL), and severe deficit (<10 ng/mL) has been separated. A dose of 300,000 IU given once to women with moderate and 300,000 IU given twice in women with severe Vit D deficiency and maintenance dose of 50,000 IU 1x per month have been shown to be effective in achieving > 20 ng/mL in the perinatal period (RR 1.7, 95% CI:1.2–24 and RR 2.3, 95% CI: 1.7–3.3, respectively). The authors did not note the significant side effects of such high doses of Vit D in pregnant women, but their effects on offspring were not evaluated. These authors believe that the results of their study raise the question of the effective dose of Vit D in preventing pregnancy complications and suggest that this dose may be significantly higher than the currently proposed [88].

The presented research results have suggested that the critical issue in assessing the role of Vit D in preventing PE may not be its dose but appropriate serum concentration. Vitamin D in the dose according to the serum levels needs to be offered in the 1st trimester and even in the periconception period. However, so far, the optimal concentration of Vit D in pregnancy has not been determined.

The results of the selected meta-analysis on the Vit D influence on PE prevalence published within the last ten years are presented in **Table 2**.

Table 2. Selected meta-analyses on Vitamin D influence on PE risk.

Authors	Studied Group	Number of Participants	Impact on PE	Additional Information
Khaing et al. 2017 [93]	Vit D vs. placebo	357	RR 0.47 95% CI: 0.24–	NNT 17

Authors	Studied Group	Number of Participants	Impact on PE	Additional Information
			0.89	
Palacios et al. 2016 [94]	Vit D vs. no treatment	219	RR 0.52 95% CI: 0.25–1.05	PE occurrence 8.9% vs. 15.5%
Palacios et al. 2019 [95]	Vit D vs. no treatment	499	RR 0.48 95% CI: 0.30–0.79	
Fogacci et al. 2020 [96]	Vit D vs. no treatment Vit D vs. no treatment < 20th week	4777	RR 0.37 95% CI: 0.26–0.52 RR 0.35 95% CI: 0.24–0.50, $p < 0.001$	Increasing dose–decreasing PE risk RR −1.10 95% CI: −1.73–1.46, $p < 0.001$
Gallo et al. 2020 [97]	Vit D vs. no treatment	364	PE RR 0.7 95% CI: 0.4–1.4, NS GH RR 0.8 95% CI: 0.3–2.2, NS	
Pérez-López et al. 2015 [98]	Vit D vs. placebo	877	RR 0.88 95% CI: 0.51–1.52, NS	
Roth et al. 2017 [99]	Vit D vs. no treatment	3398	RR 1.09 95% CI:0.43–2.76, NS	
Aguilar-Cordero et al. 2020 [100]	Random effects meta-analysis 25(OH)D < 75 nmol/L 25(OH)D < 50 nmol/L Fixed effect meta-analysis 25(OH)D < 75 nmol/L	10,979 14,496 10,979 14,469	 RR 1.26 95% CI: 0.87–1.82, NS RR 1.42 95% CI: 0.99–2.04, NS RR 1.44 95% CI: 1.26–1.64	

Authors	Studied Group	Number of Participants	Impact on PE	Additional Information
	25(OH)D < 50 nmol/L	1660	*p* < 0.00001 RR 1.47 95% CI: 1.29–1.67	
	Interventional studies Vit D supplementation		*p* < 0.00001 RR 0.68 95% CI: 0.49–0.95	
Akbari et al. 2020 [60]	25(OH)D < 20 ng/ml	21,546	Fixed RR 1.33; *p* < 0.0001; random RR 1.54 *p* = 0.0029	
Fu et al. 2018 [101]	Vit D supplementation	21,127	RR = 0.41 95%CI = 0.22-0.78	
Hyppönen et al. 2014 [102]	Vit D supplementation early in pregnancy	59,789	RR 0.81 95% CI: 0.75–0.87 *p* < 0.000001	
	higher serum 25(OH)D	5058	RR 0.52 95% CI: 0.30–0.89 *p* = 0.02	
	Vit D supplementation	5982	RR 0.66 95% CI: 0.52–0.83 *p* = 0.001	
Aghajafari et al. 2013 [103]	Observational study Insufficient 25(OH)D levels	3190	RR 1.79 95% CI: 1.25–2.58	
	25(OH)D < 75 nmol/L		RR 2.11 95% CI: 1.36–3.27	
	25(OH)D < 50 nmol/L		RR 1.27 95% CI: 0.60–2.42	

Authors	Studied Group	Number of Participants	Impact on PE	Additional Information
Tabesh et al. 2013 [104]	Vit D deficiency 25(OH)D ≤ 50 nmol/L (20 ng/mL),	1775	RR 2.78 95% CI: 1.45–5.33	
	25(OH)D <38 nmol/L (15.2 ng/mL)	931	NS	
Martínez-Domínguez et al. 2018 [105]	First half of pregnancy normal 25(OH)D (≥30.0 ng/mL)	817	RR 0.73 95% CI: 0.35–1.51, NS	
	Insufficient (20.0–29.9 ng/mL)	323	RR 0.79 95% CI: 0.28–2.21, NS	
	Deficient (<20.0 ng/mL)	494	RR 0.67 95% CI: 0.24–1.89, NS	
Kinshella et al. 2021 [106] [102][104][105][107]	Vit D supplementation	1353	RR 0.62 95% CI: 0.43–0.91 NS	Decrease in PE risk by 38%
Yuan et al. 2021 [107]	Low 25(OH)D levels [95][93][94][96][101][102][106]	39,031	RR 1.62 95% CI: 1.36–1.94 $p < 0.001$	

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[99]. Only Gallo et al. assessed the effect of Vit D on PE and GH separately [97]. Other researchers have referred only to preeclampsia.

The inconclusive results of meta-analyses can be explained by the fact that different types of studies were considered in which different doses of vitamin D were analyzed, administered in different time patterns in women with different Vit D concentrations. Similarly, the gestational age when Vit D was offered to pregnant women was heterogeneous and included all trimesters. The authors of the meta-analyses also included in the evaluation studies in which PE was diagnosed based on heterogeneous criteria [60][106][107].

In contrast, the results of meta-analyses assessing the relationship of vitamin D concentrations with the risk of preeclampsia has indicated that its low levels are associated with an increased risk of PE. Higher levels seem to provide protection against PE development [60][101][103][104][107] with the cut-off points used for Vit D concentrations being as follows: <20 ng/mL, <50 ng/mL, and <75 ng/mL. Aghajafari et al. in their meta-analysis, used two discriminators: level 25(OH)D < 50 nmol/L and <75 nmol/L, while the third group was defined as insufficient 25(OH)D levels. They determined the level of <75 nnmol/L for this group and included studies that reported outcomes as proportions of two cut-off categories sufficient and insufficient [103]. A meta-analysis of Hypponen et al. has evaluated the effect of higher serum 25(OH)D levels on the risk of developing PE without specifying a value of

this level. The term was used as defined in each study eligible to their meta-analysis [102]. Only one of the presented analyses by Martínez-Domínguez et al. has indicated that the concentration of Vit D does not affect the risk of PE development [105].

The meta-analysis by Aguilar-Cordero et al. has presented random and fixed effects of meta-analyses of observational studies, which differ significantly. Fixed effects are auspicious and indicate a significant effect of low 25(OH)D concentration on PE risk, while random effects do not confirm such a relationship. The authors decided to make this assessment due to the high heterogeneity of the studies included in the meta-analysis [100].

The authors of the presented meta-analyses have indicated the high heterogeneity of the included studies concerning the dose and type of Vit D supplementation and the duration of its use.
It seems that the fundamental issue, despite the extensive literature, remains the assessment of Vit D concentration in the periconceptional period and/or the early first trimester and defining levels that would allow reducing the risk of PE development. Women with risk factors for Vit D deficiency such as obesity, kidney, liver, thyroid gland diseases, chronic bowel diseases, autoimmune diseases, asthma, diabetes t.2, hypertension, and chronic glucocorticoids, antiepileptic and antiretroviral drug treatment would benefit the most from screening. It appears that patients with risk factors for PE development and Vit D deficiency may require higher doses of vitamin D than commonly recommended for pregnant women.

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