Mini Review

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Could molecular assessment of calcium metabolism be a useful tool to early screen patients at risk for pre-eclampsia complicated pregnancy? Proposal and rationale

Abstract: One of the most frequent causes of maternal and perinatal morbidity is represented by hypertensive disorders during pregnancy. Women at high risk must be subjected to a more intensive antenatal surveillance and prophylactic treatments. Many genetic risk factors, clinical features and biomarkers have been proposed but none of these seems able to prevent pre-eclampsia onset. English literature review of manuscripts focused on calcium intake and hypertensive disorders during pregnancy was performed. We performed a critical analysis of evidences about maternal calcium metabolism pattern in pregnancy analyzing all possible bias affecting studies. Calcium supplementation seems to give beneficial effects on women with low calcium intake. Some evidence reported that calcium supplementation may drastically reduce the percentage of pre-eclampsia onset consequently improving the neonatal outcome. Starting from this evidence, it is intuitive that investigations on maternal calcium metabolism pattern in first trimester of pregnancy could represent a low cost, large scale tool to screen pregnant women and to identify those at increased risk of pre-eclampsia onset. We propose a biochemical screening of maternal calcium metabolism pattern in first trimester of pregnancy to discriminate patients who potentially may benefit from calcium supplementation. In a second step we propose to randomly allocate the sub-cohort of patients with calcium metabolism disorders in a treatment group (calcium supplementation) or in a control group (placebo) to define if calcium supplementation may represent a dietary mean to reduce pre-eclampsia onset and to improve pregnancy outcome.

Keywords: bone metabolism; calcium supplementation; pre-eclampsia; pregnancy outcome; serum biochemical profile.

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Background

High blood pressure (BP) in pregnancy, with or without proteinuria, is one of the most frequent causes of maternal and perinatal morbidity [1]. About 5% of all pregnancies are complicated by hypertension, associated in half of the cases with pre-eclampsia and annually responsible for up to 60,000 maternal deaths [2, 3]. It is already known that hypertensive disorders during pregnancy may be responsible of both acute (abruptio placentae, cerebrovascular accident, organ failure, and disseminated intravascular coagulation) and chronic complications (fetal intrauterine growth retardation, preterm birth and neonatal intensive care) [4–6]. Despite nowadays diagnostic criteria of pregnancy-induced hypertension and pre-eclampsia being well defined [4], neither specific test nor daily blood pressure monitoring appear able to prevent or identify women at risk of hypertension/pre-eclampsia before clinical effect manifestation [3, 4]. Unfortunately, after

diagnosis some cases are not responsive to the conservative treatment and, despite high intensive maternal-fetal surveillance and multiple antihypertensive treatments, sometimes delivery represents the only option to definitively resolve this obstetrical complication independently from the gestational age.

It is intuitive that both strategies useful in early detection of women at potential increased risk as well as effective prophylactic treatment options are urgently needed [1].

Literature review and analysis of evidence

The first limitation in the assessment of screening and prophylactic strategies is due to the incomplete and not well-defined pathological mechanism involved in the disease onset.

Recently some theories have been postulated (abnormal maternal immune response to the allogenic fetus, dysregulation of placental oxygen supplies, defective angiogenesis, dysregulation of several key angiogenic factors expression, defective trophoblastic invasion) but the exact patho-physiological mechanism is not still completely understood [2, 7, 8]. In fact, many proposed diagnostic tools failed to demonstrate advantages as well as a large part of prophylactic treatment sometimes administered. Ultrasound investigation of uterine arteries blood flow impedance in the second trimester of pregnancy has been proposed to detect women at high risk of pre-eclampsia (which is associated to a defective trophoblast differentiation and an impaired invasion of uterine spiral arteries) but large scale studies demonstrated that this technique is burdened by low positive predictive value [9]. Anyway, several studies aimed to anticipate the timing of patients screening in first trimester, proposed the association between some serum biomarkers assay (such as ADAM12 and PAPP-A) and maternal ultrasound velocimetry, but this combination did not appear to increase the sensitivity and positive predictive value of ultrasound alone [10–13]. Regarding the prophylactic treatment of mild/moderate pregnancy-related hypertension by antihypertensive drugs, clear advantages of pharmacological treatments in preventing proteinuria/pre-eclampsia were not reported when compared to placebo or no treatment [4, 14]. Also nutritional and dietary interventions have been proposed in pre-eclampsia prevention. A normal diet without salt restriction is advised, particularly close to delivery, consequently reducing intravascular volume [4]. A role of fish oil supplementation as well as vitamins and nutrient supplements in the prevention of hypertensive disorders and pre-eclampsia was not demonstrated [4, 15, 16] while antioxidants, calcium and magnesium dietetic supplementation seem to be useful [2]. In fact, in women at high risk of pre-eclampsia development with a low calcium intake (<600 mg or 900 mg/die depending on the author), a daily 1000 mg calcium supplementation during pregnancy seems to halve the risk of pre-eclampsia [4, 17, 18]. A theoretical increased risk of kidney stone formation has not been demonstrated and no other adverse effects of calcium intake have been documented. Intuitively, calcium supplementation would seem to be the only safe and useful prophylactic treatment for pregnancy-related hypertensive disorders but the available evidence, also affected by patients' selection bias, are still conflicting [17–20]. An inverse relationship between calcium intake and hypertensive disorders during pregnancy was firstly described in 1980 [1]. Evidence was subsequently confirmed by epidemiological and clinical studies reporting both that calcium supplementation might reduce the incidence of high BP and pre-eclampsia in women with low calcium intake and that pre-eclampsia is associated with hypocalciuria or high calciuria/creatinine ratio, hypocalcemia and low dietary calcium intake [1, 14, 21]. Calcium homeostasis is physiologically guaranteed by a perfect balance between intestinal absorption, renal excretion and skeletal release. During pregnancy and lactation calcium request is increased since the fetus depends completely on maternal mineral resources. Maternal hypocalcemia may stimulate either parathyroid hormone (PTH) and renin release, increase intracellular calcium levels in vascular smooth muscle or consequently induce vasoconstriction [20]. The correlation between low calcium intake and vasoconstriction has been demonstrated also in the utero-placental system: patients receiving calcium supplementation during third trimester of pregnancy showed an improvement of both umbilical and uterine artery hemodynamics features [22]. Recently, studies on trophoblastic cells of women affected by pre-eclampsia demonstrated that Ca^{2+} transport proteins were overexpressed probably as a mechanism to adequately balance intra/extra cellular Ca^{2+} levels and guarantee the necessary exchange between maternal and fetal compartments [23, 24]. Ca^{2+} overexpression seems to be directly induced by maternal hypocalcemia (responsible for vasoconstriction and reduced fetal blood flow) and indirectly by hypovascularization and hypoxic status that further worsen calcium exchange causing trans-membrane Ca^{2+} transport proteins overexpression. A maternal physiological hypocalcemia usually occurs during pregnancy because of at

least three conditions: lower gastro-enteric adsorption, physiological hemodilution and increased glomerular filtration (particularly in the third trimester of pregnancy when pre-eclampsia incidence reaches its peak) and progressive increase of fetal calcium request. The compensatory mechanism to restore an adequate calcemia in the absence of dietary supplementation is based on both PTH release (to mobilize calcium from bone reservoir) and renin-angiotensin cascade (to reduce glomerular calcium loss) [2, 14, 25]. Kumar et al. demonstrated that in a large part of cases this maternal compensation appears to be able to restore an adequate calcium serum level despite bone metabolism being strongly shifted with osteoclastic activity [26]. Previous reports investigating calcium metabolism pathway in pre-eclamptic women demonstrated that Ca^{2+} serum levels is reduced if compared to healthy ones only when calcium intake is very low and pre-eclampsia is overt [27, 28]. Probably when the physiological compensatory mechanism fails to restore and maintain adequate calcium serum levels, clinical and biochemical pre-eclampsia manifests.

Proposal: hypothesis and rationale

Our proposal is to fully investigate the pathway of maternal calcium metabolism in the first trimester of pregnancy in order to screen and identify all patients at increased risk of pre-eclampsia.

Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) super-family marker and a key regulatory factor of bone metabolism. It is secreted by osteoclasts and involved in absorption, regulation and inhibition of osteoclast maturation through the RANK-L pathway. It is also a potential pro-angiogenic factor, involved in vascular endothelial cells protection [2]. Cross-linked C-telopeptides of type I collagen (CTX) and type I procollagen N propeptide (P1NP) are two bone metabolism markers, involved respectively in bone resorption and formation [29]. A slight decrease of all these markers in the initial phases of pregnancy (due to physiologic hemodilution) and a progressive increase (probably related to physiological bone resorption during pregnancy) has been demonstrated. However, the increasing trend of these markers has been shown to be higher in pre-eclamptic women compared to normal pregnancies [26, 30].

In fact Shen et al. demonstrated in patients affected by pre-eclampsia the OPG protein and mRNA level in placentas were found abnormal compared with normal pregnancy. So, since these markers appeared closely related

Table 1 Detailed information about the considered serum markers, their measuring units, methods of measurement, analyzer, manufacturer and reference range for 'healthy' and 'estimated at risk' women.

Biomarker	Measuring unit	Healthy subjects	Reference range	Estimated subject at risk	Method principle	Analyzer	Manufacturer
P (phosphorus)	mmol/L	More than 10 years	0.87–1.45	+1 SD	Phosphomolybdate method (colorimetric)	Cobas c 702	Roche Diagnostics, GmbH
CA (calcium)	mmol/L	0–18 years	2.2–2.7	−1 SD	Ortocresolftaleina (colorimetric)	Cobas c 702	Roche Diagnostics, GmbH
PTH (parathormone whole molecule 1-84 aa)	ng/L	More than 18 years, females All ages	2.1–2.55 4.6–26.8	+1 SD	Chemiluminescent immunoassay	LIAISON XL	DiaSorin Inc.
P1NP (type I procollagen N propeptide)	mg/L	More than 10 years	28–128	−1 SD	Chemiluminescent immunoassay	IDS-iSYS	PANTEC S.r.l.
CTX (β-crosslaps)	pg/mL	Females pre-menopause	<573	+1 SD	Electrochemiluminescent immunoassay (ECLIA)	Cobas e 601	Roche Diagnostics, GmbH
OPG (osteoprotegerin)	pmol/L	19–96 years	1.8	+1 SD	Sandwich enzyme linked immunosorbent assay (ELISA)	Manual method (microplate)	Biomedica Gruppe

with pregnancy outcome, authors suggested them as possible markers useful for pre-eclampsia screening [31]. In addition to OPG, Dorota et al. demonstrated that all markers of bone turnover are increased in patients with pre-eclampsia when compared to healthy normotensive pregnant women [32].

On this basis, a screening test investigating bone markers turnover at the end of first trimester of pregnancy may represent a low cost, large scale applicability and feasibility tool able to early predict women at risk of pre-eclampsia onset with a good sensitivity and cost-effectiveness.

So, we propose a biochemical screening (OPG, CTX, P1NP, PTH, Ca^{2+} and PO_4^3 serum levels assay) of maternal calcium metabolism pattern during first trimester of pregnancy (12th gestational week) to discriminate patients who will present an early osteoclastic shift of bone metabolism and potentially may benefit from calcium supplementation (Table 1). In a second step we propose to randomly allocate the sub-cohort of patients with calcium metabolism disorders in a treatment group (calcium supplementation) or in a control group (placebo) to define if calcium supplementation may represent a dietary mean to reduce pre-eclampsia onset and to improve pregnancy outcome.

We choose 12 weeks of gestational age for markers assay because at this time patients usually routinely have a blood assay. So, delaying the screening at second trimester of pregnancy cannot really allow us to estimate benefits of prophylactic calcium supplementation (in treatments group) because some cases of pre-eclampsia may develop during the second trimester of pregnancy.

Conclusions

If our data will confirm our hypothesis and rationale, the obstetric dilemma about cause-effect relationship between pre-eclampsia and calcium intake may be solved. Although a relationship between calcium and pre-eclampsia seems demonstrated, it is unclear if there is a primary unknown pathophysiologic mechanism that alters calcium homeostasis leading to pre-eclampsia or if calcium deficiency is implied in a pathophysiologic pathway leading to pre-eclampsia. Certainly no evidences exist regarding pregnancy phase in which metabolic alterations onset occurs and remains unclear when these markers have to be investigated. When a pathophysiologic mechanism, an early diagnostic algorithm and proper markers assays of pre-eclampsia will be defined, calcium supplementation may probably represent a low cost and useful treatment to prevent its related clinical diseases and organ damages with a substantial improvement in health care policies and a reduction in social-economic costs.

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