Italian Journal of
Gynæcology & Obstetrics
September 2019 - Vol. 31 - N. 3 - Quarterly - ISSN 2385 - 0868

D-Chiro inositol phosphoglycan (IPG-P) as a potential urinary marker to predict preeclampsia

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ABSTRACT
Preeclampsia is a pathological condition that can complicate human pregnancy with a frequency between 2 and 7% of healthy nulliparous women worldwide. Inositol phosphoglycans P type (IPG-P) are phospholipids that exert an insulin mimetic activity and increase during pregnancy in fetal and maternal compartments with a clear higher concentration in pregnancies complicated by preeclampsia. A comprehensive review of the literature was performed to identify the most relevant studies about this topic. Many authors in the last decades investigated IPG-P modifications in different compartments of preeclamptic pregnancies, finding an increased concentration of this phosphoglycans in villous stroma, cord blood, amniotic fluid and in maternal urines even weeks before the clinical onset of the disease. According to these findings, urinary IPG-P can be a reliable test to identify women at risk of preeclampsia weeks before the clinical presentation of the maternal syndrome. This test showed a good sensitivity and specificity and it is based on a low-cost and direct assay that makes this method of great interest especially in low-and middle-income countries. Further longitudinal studies in different ethnic groups are warranted to demonstrate a transverse efficacy of the test.

Keywords:: Preeclampsia; IPG-P; Inositol phosphoglycan; D-Chiro inositol phosphoglycans; urinary inositol phosphoglycans; urinary test; screening test; diagnostic test; urinary marker; insulin resistance.

SOMMARIO
La preeclampsia è una condizione patologica che può complicare la gravidanza con una frequenza tra il 2 ed il 7% in una popolazione di nullipare sane. Il
fosfatidilinositolo tipo P (IPG-P) è un fosfolipide che possiede attività insulino-mimetica e che aumenta durante la gravidanza sia nei compartimenti materni che in quelli fetali, con una concentrazione chiaramente aumentata nelle gravidanze complicate da preeclampsia. E' stata eseguita una dettagliata revisione della letteratura per identificare gli studi più rilevanti riguardanti questo argomento. Molti autori negli ultimi decenni hanno analizzato le modificazioni del IPG-P nei diversi tessuti e fluidi in gravidanze con preeclampsia, riscontrando un aumento di concentrazione di questo fosfoglicano nello stroma villoso, nel sangue cordonale, nel liquido amniotico e nelle urine materne, anche settimane prima dell'esordio clinico. Secondo questi risultati la ricerca dei IPG-P urinari può essere un test affidabile per identificare le donne a rischio di preeclampsia con un anticipo di anche alcune settimane rispetto alla presentazione clinica della patologia. Questo test ha dimostrato una buona sensibilità e specificità ed è basato su un saggio diretto ed a basso costo, fatto che lo rende un test di grande interesse soprattutto nei paesi in via di sviluppo. Sono tuttavia necessari studi condotti su differenti gruppi etnici al fine di dimostrare un'efficacia più trasversale di questa metodica.

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DOI: 10.14660/2385-0868-130

INTRODUCTION

Preeclampsia is a pathological condition that can complicate human pregnancy with a frequency between 2 and 7% of healthy nulliparous woman worldwide[1], increasing its incidence in presence of some risk factors like obesity, insulin resistance, previous history of hypertensive disorders of pregnancy, chronic hypertension, and medical assisted pregnancies. It is the second leading cause of maternal death behind postpartum hemorrhage[2].

We can divide preeclampsia risk factors into two groups: one linked to immunology and placenta (medical assisted pregnancies, primipaternity, and sperm exposure)[3,4] and the other linked to maternal metabolic syndrome (obesity, diabetes mellitus, insulin resistance, chronic hypertension, renal disease, and low birth weight)[5]. According to present knowledge, this may lead to two types of preeclampsia: "early onset", mainly associated with immune adaptation disorders, and "late onset", associated with metabolic disorders[6].

It is thought that the aberrant maternal reaction to the placental factors, leads to the clinical syndrome[7] characterized by new hypertension (arterial blood pressure ≥ 140/90) and organ damage (mainly kidney and liver) that develops later than 20 weeks of gestation often with spontaneous remission after delivery[8]. This can be matched also to a fetal syndrome, which can occur when the disease arises in the late second or early third trimester causing IUGR, oligohydramnios and abnormal blood flow in fetal and maternal compartments[9].

Considering the probable link between preeclampsia and metabolic dysfunctions many authors in the past two decades investigated the role of inositol phosphoglycans, in particular inositol phosphoglycans P type (IPG-P) in preeclampsia, and its modification in maternal and fetal tissues and fluids, which could be used to foresee the development of this clinical condition.

IPG-P is normally expressed in all insulin-sensitive tissues, exerts insulin mimetic activity on glucose and lipid metabolism, and influences intracellular pathways with short-and long-term effects[10]. This phospholipid molecule is released into caveolae as a result from the cleavage of glycophosphatidylinositol anchors by a specific enzyme (glycosylphosphatidyl inositol phospholipase D, GPI-PLD) which is induced by insulin stimulation[11].

The essential structure of IPG-P is composed by a D-chiro-inositol group and a variable glycan moiety that together mediate the insulin-mimetic properties through the activation of pyruvate dehydrogenase phosphatase, glycan synthase phosphatase, and glycerol-3-phosphate acyltransferase[12].

Placental tissue was shown to be enriched of IPG-P[13] especially in preeclampsia and here these phosphoglycans lead to an unexpected glycogen storage in villous syncytiotrophoblast although IPG-P was not necessarily synthetized in loco[14,15]. Anyway, glucose metabolism is not the only target: IPG-P activates IRS-PI3K-PKB/Akt cascade that promotes cell differentiation, growth and survival and NK cells activation with cytokines production. The complex interaction between immunological alterations, endothelial activation, and insulin resistance is part of a delicate equilibrium in human pregnancy. Vascular development and glucose metabolism are essential for placental and embryo growth, sharing common pathways and intracellular cross-talks. Akt represents the crucial serine/threonine protein kinase activated by insulin and insulin-like growth factors (IGFs)[16,17] and vascular endothelial derived growth factor (VEGF). Their triggering leads to the activation of Ras proteins, receptor kinase cytosolic domains or activation of GPI-PLD with subsequent phosphorylation of PI3-K[18].

Increased IPG-P content in preeclamptic placenta seems to be strictly related to metabolic impairment and insulin resistance. In fact, IPG-P strengthens insulin receptor effects and promotes the activation of PI3-K through IRS-1, while its excessive levels correlate with abnormal phosphorylation of serine residues in IRS-1 and IRS-2 (Insulin Receptor Substrate 1 and 2) and lead to the inactivation of these mediators[19]. This effect can also unbalance vascular constriction/dilatation in vascular cells because of a decreased production of nitric oxide[18]. Loss of PI3-activity contributes to MAP-K unbalanced activation, endothelin-1 increase and vasoconstrictor effects.

IPG-P IN HUMAN PREGNANCY

IPG-P increases during healthy pregnancy in the fetal and in maternal compartments and this is probably due to a fetal production of this second messenger[20]. In physiological conditions, pregnant women release a four-fold higher concentration of IPG-P in urine compared with non-pregnant subjects[20] and this is probably correlated to the physiological insulin resistant state typical of pregnancy[17]; A further increase was reported during active labor[21]. High concentrations of IPG-P can also be found in amniotic fluid[20] and, since amniotic fluid predominantly derives from fetal urine, it is likely that the IPG-P source is the fetus as demonstrated also in fetal urine by Scioscia et al.[20].

In that study, fetal urine drawn directly from fetal bladder or kidney was in equilibrium with amniotic fluid. IPG-P was found in healthy placentas[13] with a significantly higher concentration compared to other parenchymatous organs such as the liver[21]. It was supposed that placenta takes up IPG-P from amniotic fluid and fetal blood, as the placenta was shown to lack the enzyme that is fundamental to release the molecule from the cell surface[22].

IPG-P AND PREECLAMPSIA

A strong correlation between IPG-P in maternal and fetal compartments and preeclampsia was reported[23]. It is also known that women suffering from gestational diabetes have IPG-P urinary concentrations that correlate with blood glucose levels and this supports a role for IPG-P in maternal metabolic control[6]. In fact, in healthy insulin-sensitive tissues, IPG-P plays a role in glucose uptake and lowering glycemia. It was striking that preeclamptic placentas that are rich in the mediator, fail to release IPG-P after insulin stimulation and this was demonstrated to be associated with an impaired activation of insulin signaling pathways[17]. It is linked to an inactivation of IRS1/2 and accumulation of IPG-P in the fetal compartment and may be associated with the local insulin resistance[24].

Furthermore, inositol precursors regulate leukocyte interactions and different endothelial cell metabolism routes as well as intracellular signaling pathways[19].

Considering the fetal side, immunostaining for D-chiro inositol in placental tissue demonstrated a higher positivity of villous stroma in early onset preeclampsia, which, considering the inability of placenta to produce the active mediator, suggested a vectorial movement of chiro-inositol from the fetus to the placenta[13]. Moreover, cord blood of fetuses born to preeclamptic mothers showed an increased content of IPG-P[23]. Amniotic fluid also showed higher IPG-P levels in preeclamptic pregnancies than in healthy ones with levels that were a thousand times higher than in maternal urine[20,23].

As previously outlined, IPG-P was found to be heavily increased in both the fetal and the maternal side of preeclamptic pregnancies. The lipidic form can derive from the fetal compartment that leaks out of the placental barrier (due to a reduced presence of glycocalyx proteins on syncytiotrophoblast cells) and cleared by maternal kidneys (21). It was reported that a maternal urine rise of IPG-P could be found about 4 -6 weeks before the clinical onset of this disease[25]. These findings suggested IPG-P as a promising screening test for preeclampsia.

URINE IPG-P AND PREECLAMPSIA

In the last years, many studies assessed the correlation between urinary IPG-P and preeclampsia: it was found that this molecule is increased in pregnant women compared with non-pregnant ones, and it is also considerably increased in women who develop this disease, often well before its clinical onset. Initially, urinary IPG-P levels were calculated using a bioassay (pyruvate-dehydrogenase activity assay) while now they can be easily detected with a polyclonal antibody-based ELISA, which is simpler and cheaper to perform.

In 2006, a prospective study published by Scioscia et al.[20], collecting a mid-stream urine specimen in 109 healthy pregnant women and 66 non-pregnant subjects, demonstrated that the first group had a four-fold higher concentration of IPG-P in urines. In the same year, a prospective case-control study by Paine et al.[23] confirmed an abnormal urine release of IPG-P in preeclamptic women. They enrolled 11 consecutive women undergoing emergency caesarean section for pre-eclampsia or eclampsia and 11 healthy non-laboring controls who underwent elective cesarean section, and collected samples of amniotic fluid, maternal urine, maternal (uterine and antecubital veins) and fetal blood (umbilical artery and vein). IPG-P was found to be higher in amniotic fluid samples than in urine in the same patient, besides IPG-P in the matched urine and amniotic fluid specimens were significantly higher in the preeclampsia group ($p= 0.002$).

Williams et al.[25] published in 2006 a case-control study based on a two-step approach: first they used urine samples from 16 preeclamptic and 16 healthy pregnant women matched for age, parity and weeks of gestation, finding out higher IPG-P readings in the preeclamptic group ($p <0,001$). After that they selected 27 preeclamptic and 47 healthy pregnancies with similar characteristics (age, gestational age, BMI, parity, ethnicity) and pointed out a 30-fold higher IPG-P content in urine samples in the case group ($p <0,001$), without any correlation with gestational age and with blood pressure. In 6 cases they were able to analyze more than one urine sample before the development of the clinical condition, finding out that IPG-P in urines raised up about 6 weeks before the clinical outbreak. In 3 cases they had samples of 6 to 8 weeks before the diagnosis, so they plotted a curve showing a time-related increase in urinary IPG-P, which also kept increasing after diagnosis and then rapidly dropped after delivery[25].

Later, in 2010, it was published a prospective longitudinal study by Paine et al.[26], where 84 high-risk obstetric patients (history of preeclampsia/ eclampsia, IUGR, abruptio placentae, multiple

pregnancy, chronic hypertension or thrombophilia) were scheduled to collect a urine sample every 3-4 weeks (between 11 and 40 weeks of gestation). The study ended with 65 patients who had a normal pregnancy, 10 developed gestational hypertension and 9 preeclampsia. The authors were able to underline a significant difference in urinary IPG-P between preeclamptic group and the other two groups (while there was no difference between healthy and gestational hypertension group). They were also able to define, with the help of ROC curves, a cut-off value of urinary IPG-P of 77.38 U/µmol. Twenty-three healthy women had one or two values beyond the cut-off, but then rapidly went back to normal without developing preeclampsia. Sensitivity and specificity of the test for preeclampsia were 88.9% and 62.7% respectively, with a positive likelihood of 2.38 for a single positive and 4.2 for two positive tests performed at least one week apart.

Similar results were found in the 2013 in a prospective longitudinal study conducted in a Mauritian population of 416 pregnant women by Dawonauth et al.[27]. They collected urine samples to assess urinary IPG-P levels at each regular visit. 312 women out of this population had a normal pregnancy, 34 developed preeclampsia (8.2%), 56 gestational hypertension (13.5%) and 14 gestational diabetes (3.3%). They found a weak correlation between urinary IPG and maternal age, BMI and weeks of gestation at delivery and no correlation with smoking habit and sex of the fetus, while there was a correlation with gestational hypertension in a previous pregnancy. ROC curves were used to generate a cut-off value that was identified at the moment of the clinical diagnosis as 23.93 AU/nmol of creatinine while the cut-off selected to predict preeclampsia two weeks prior to clinical diagnosis was 9.77 AU/nmol of creatinine. They also found that urinary levels of IPG-P fluctuated with the severity of the symptoms, that they rapidly decreased in postpartum. As in previous studies, some women had a transient elevation of values and, if the subsequent values remained low, they did not develop preeclampsia.

In a more recent prospective longitudinal study, L'Omelette et al.[28] enrolled 1018 pregnant women who collected urinary samples throughout gestation: 80 women developed preeclampsia (7.9%), 161 gestational hypertension (15.8%) and 135 gestational diabetes (13.3%). BMI was found to be correlated to the development of pregnancy complications and Mauritian ethnicity showed a tendency to hypertension. Mean IPG-P levels in urine expressed in unit/nmole of creatinine in normal pregnancies was 7.38 ± 0.49, while in preeclamptic patients was 105.4 ± 5.5 and there was no elevation in urinary IPG-P in gestational hypertension or gestational diabetes.

Table 1 summarizes the main characteristics of the studies previously described.

Table 1
Studies on urinary IPG-P as a diagnostic test for preeclampsia (PE preeclampsia; GH gestational hypertension;)

First autor	Year	Tipe of study	Population	Study population	Cases of PE	Cases of GH	Sensitivity	Specificity	Positive likelihood	Negative likelihood ratio
Williams PJ (25)	2006	Case control study	Europe	74	27	0	N/A	N/A	N/A	N/A
Paine MA (23)	2006	Case control study	South Africa	22	7	1	N/A	N/A	N/A	N/A
Paine MA (26)	2010	Prospective, longitudinal study	Europe	84	9 (10.7%)	10 (11.9%)	88.9%	62.7%	2.38	0.18
Dawonauth L (27)	2013	Prospective, longitudinal study	African Indo-Mauritian ethnic group	416	34 (8.2%)	56 (13.5%)	96,7%	96,7%	29.3	0.03
L'Omelette AD (28)	2018	Prospective, longitudinal study	African Indo-Mauritian ethnic group.	1018	80 (7.9%)	161 (15.8%)	100%	90.3%	10.2	0.01

DISCUSSION

IPG-P concentration in fetal and maternal compartments during pregnancy are known to be correlated to pregnancy insulin resistance. Pregnancies complicated by preeclampsia showed a clear higher IPG-P concentration in both fetal and maternal compartments.

According to the findings presented, urinary IPG-P can be a reliable test to identify women at risk of preeclampsia weeks before the clinical presentation of the disease[23,25,26,28]. This test showed a good sensitivity and specificity and it is based on a low-cost and direct assay that makes this method of great interest especially in low-and middle-income countries where preeclampsia is more frequent[2]. The possibility to detect in advance women at risk of developing preeclampsia can improve secondary prevention and permit the identification of patients to be referred to specific clinical pathways with increased surveillance: this could avoid late presentation of the cases, which leads to less effectiveness of the medical interventions[29]. Further longitudinal studies in different ethnic groups are warranted to demonstrate a transverse efficacy of the test.

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