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Dosage PlGF, sVEGFR-1, sEng in maternal serum e preeclampsia: preliminary study

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SUMMARY: **Dosage PlGF, sVEGFR-1, sEng in maternal serum e preeclampsia: preliminary study.**

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Preeclampsia is responsible for 10-15% of maternal deaths during pregnancy. According to recent hypotheses on the pathogenesis of the disease, two factors play a key role in the process of remodeling of the maternal arteries: Vascular endothelial growth factor (VEGF) and Placental Growth Factor (PlGF). The VEGF may be particularly active during pregnancy. The receptor of this growth factor, called sVEGFR-1, just because of placental ischemia, appears to be over-expressed in preeclamptic placentas of women and, consequently, this may antagonize the effects of VEGF and PlGF. Other studies have demonstrated the involvement in the pathogenesis of preeclampsia of another antiangiogenic soluble factor produced by the placenta, the soluble endoglin (sEng). Our study evaluates the values of PlGF, sVEGFR-1 and sEng in maternal serum in order to assess their predictive value for the onset of preeclampsia. Between 2011 and 2012, at the Institute of Pathology Obstetrics and Gynaecology of the University Hospital "Policlinico-Vittorio Emanuele" of Catania were enrolled 20 women in the first trimester of pregnancy divided into two groups, at-risk group (12) and control group (8). For 3 of the 12 patients in the risk group, was given the diagnosis of pre-eclampsia. The median concentration of sEng and sVEGFR-1 was significantly higher in women compared to diseased women at risk and to the controls while the median concentration of PlGF was much lower in women sick than the other two groups, with highly significant differences (P <0.01).

RIASSUNTO: **Dosaggio di PlGF, sVEGFR-1, sEng nel siero materno e preeclampsia: studio preliminare.**

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La preeclampsia è responsabile del 10-15% delle morti materne in gravidanza. Secondo recenti ipotesi sulla patogenesi della malattia, due fattori giocano un ruolo chiave nel processo di rimodellamento delle arterie materne: Vascular Endothelial Growth Factor *(VEGF) e* Placental Growth Factor *(PlGF). Il VEGF può esser particolarmente attivo durante la gravidanza. Il recettore di questo fattore di crescita, denominato sVEGFR-1, proprio a causa dell'ischemia placentare, risulta essere sovraespresso nelle placente delle donne preeclamptiche e, di conseguenza, questo può antagonizzare gli effetti del VEGF e del PlGF. Altri studi hanno dimostrato il coinvolgimento nella patogenesi della preeclampsia di un altro fattore solubile antiangiogenetico prodotto dalla placenta, l'endoglina solubile (sEng). Il nostro studio valuta i valori di PlGF, sVEGFR-1 ed sEng nel siero materno al fine di valutare la loro predittività per l'insorgenza di preeclampsia. Tra il 2011 e il 2012, presso l'Istituto di Patologia Ostetrica e Ginecologica dell'Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele" di Catania sono state arruolate 20 donne al primo trimestre di gravidanza divise in due gruppi, gruppo a rischio (12) e gruppo controllo (8). Per 3 delle 12 pazienti del gruppo a rischio, è stata posta la diagnosi di preeclampsia. La concentrazione mediana di sEng e sVEGFR-1 è risultata decisamente più elevata nelle donne malate rispetto alle donne a rischio e ai controlli, mentre la concentrazione mediana di PlGF è risultata molto più bassa nelle donne malate rispetto agli altri due gruppi, con differenze altamente significative (P<0,01).*

KEY WORDS: Preeclampsia - PlGF - sVEGFR-1 - sEng.
Preeclampsia - PlGF - sVEGFR-1 - sEng.

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Introduction

The Society for the Study of Hypertension in Pregnancy (SSHP) describes the pre-eclampsia as a state where the pregnant, normotensive before pregnancy, has systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg in 2 determinations spaced at least 6 hours, arising after 20 weeks of gestation and associated to proteinuria (≥ 300 mg in 24 h).

Pre-eclampsia affects 2-5% of pregnancies in western countries and complicates up to 10% of pregnancies in developing countries. The severity of the disease is responsible for 10-15% of maternal deaths during pregnancy (1).

New hypotheses have recently been formulated on the pathogenesis of pre-eclampsia: several angiogenic factors are believed to be an important part of the pathophysiology of pre-eclampsia and involved in the early stages of the disease (2, 3).

In normal placental development, two factors play a key role in the process of remodeling of the maternal arteries: vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), that performs a function similar to VEGF. These factors are released into the bloodstream from the trophoblast migration (4). While during normal pregnancy uterine spiral arteries are invaded by cytotrophoblast and remodeled to ensure an adequate placental blood flow for the nutritional needs of the fetus, in the placenta of a woman with pre-eclampsia this trophoblast invasion does not occur normally and the flow blood is reduced leading to placental ischemia.

The VEGF may be particularly active during pregnancy. The receptor of this growth factor, called Flt1 (fms-like tyrosine kinase 1, also known as soluble VEGF receptor 1, sVEGFR-1), appears to be over-expressed in the placentas of pre-eclamptic women and may antagonize the effects of VEGF and PlGF (5). In addition, the circulating sFlt1 in patients with pre-eclampsia was associated with a reduction in the levels of VEGF and PlGF free.

The reduction of these vasodilators factors can contribute to endothelial dysfunction and multi-organ disease that involves all the districts of the organism.

More recent studies have demonstrated in the pathogenesis of pre-eclampsia the involvement of another antiangiogenic soluble factor produced by the placenta, the soluble endoglin (sEng). This protein is the isoform of soluble CD105 (belonging to the family of receptors for the TGF beta) and in pre-eclamptic women is specially expressed on the cytoplasmic membranes of endothelial cells and on the syncytium trophoblast (6-9). The sEng has its antiangiogenic effects inducing eNOS and antagonizing vasodilation mediated by TGF beta.

Since the pathogenesis of pre-eclampsia is not yet clear, prophylaxis is essential and knowledge of the involvement of these angiogenic factors in the onset of disease could be very helpful. The early detection of alteration of their values in maternal serum up to 5 weeks before the development of the signs and symptoms of the disease may be an important result to limit the damage of the maternal-fetal disease. To pregnant women with abnormal values of PlGF, sVEGFR-1 and sEng is recommended a calorie intake of 1800-2000 kcal, 1 g/kg/day of protein and low-salt regime, blood pressure monitoring and possible anti-hypertensive therapy, aspirin therapy and sulfate magnesium in the prophylaxis of eclamptic crisis, monitoring of fetal growth and possible corticosteroid therapy for fetal lung maturation (10, 11).

Numerous studies have shown elevated serum levels of sVEGF-1 in women with pre-eclampsia compared with normal pregnancies (6, 12, 13).

Levine et al., in a 2004 study, reported an average of the sVEGFR-1 value of 4382 pg/mL in women with pre-eclampsia and an average value of 1643 pg/mL in the control group. Similar values have been reported by other studies, and most of them agree in demonstrating that an higher level of sVEGFR-1 is proportionally predictive for the development of pre-eclampsia. It is important to note that this increase of the sVEGFR-1 levels in serum is detectable up to 5 weeks before the onset of symptoms (6, 14, 15).

In the serum of pre-eclamptic women low levels of PlGF have also been demonstrated (13, 16, 17) and this is probably due to the binding with high levels of sVEGFR-1 rather than to a low production of the placenta. In pre-eclampsia, the PlGF decrease in serum occurs 9-11 weeks before the development of hypertension and proteinuria, with a further significant decrease in 5 weeks before manifesting the disease (18-20).

In the same study by Levine et al., in the serum of women with preeclampsia we can observe an average concentration of PlGF of 137 pg/mL compared to 669 pg/mL in controls (50) and similar results have been reported by other groups (18, 21).

Recently, in a 2010 study, Sunderji et al. showed high plasma levels of sFlt-1 (91,514 pg/mL) and reduced levels of PlGF (12.1 pg/mL) in 38 patients with pre-eclampsia compared to 388 normotensive controls with mean values of sVEGFR-1 equal to 2416 pg/mL and PlGF equal to 477 pg/mL (22).

In an attempt to improve the predictive value of sVEGFR-1 and PlGF, these markers have been studied in combination with other biomarkers.

In a recent study by Kusanovic et al. (23), the plasma concentrations of PlGF, sVEGFR-1 and sEng have been measured in two time intervals, between weeks 6-15 and 20-25, because multiple measurements may be more explanatory than a single measurement and the ratio of PlGF and soluble endoglin (sEng) is strongly predictive for the early onset of pre-eclampsia, having a sensitivity of 100% and a specificity of 98.3%.

The results of these new combinations of angiogenic factors are encouraging, but the limited data available on those combinations do not still support their routine use (24, 25). Further studies are needed to define the sensitivity and specificity of these new combinations as well as the predictability of their positive values in the diagnosis of pre-eclampsia (26).

Our study, in agreement with the literature collection, proposes to consider the values of PlGF, sVEGFR-1 and sEng in order to assess their predictive power in developing of pre-eclampsia, since the therapeutic difficulties in the treatment of pathology are often great.

The identification of a biophysical or a biochemical test that can indicate early onset of pre-eclampsia is therefore a particularly important objective for many clinical implications.

Materials and methods

In 2011 e 2012 the Institute of Gynecologic and Obstetric Pathology of "Policlinico-Vittorio Emanuele" University Hospital in Catania enrolled 20 women at 3 months of gestation.

Based on most relevant clinical characteristics, 12 of them were included in a risk of pre-eclampsia group (maternal age > 35, nulliparity, BMI, previous pre-eclampsia, smoking, thrombophilia, kidney disease, twin pregnancy), while 8 were included in a control group (no risk factors). Women with hypertension developed before 20 weeks of gestation, chromosomal and congenital anomalies were excluded. All patients provided written informed consent before test.

Blood pressure, BMI increase and kidney function were valued every month, paying attention on any appearance of proteinuria, while fetal growth and morphology, placental and amniotic fluid were valued every 3 months by ultrasound examination.

Blood samples were collected at 16, 24 and 30 weeks of gestation, centrifuged at 5000 rpm for 5' and stored at -80 °C until assayed.

Concentrations of endoglin (sEng), PlGF and sVEGFR1 were determined using ELISA assays (ABCAM AB100507, AB119613 e AB100629). Immunoassays were performed according to the manufacturer's recommendations. The test sensitivity was respectively 10 pg/ml, 2 pg/ml e 4 pg/ml for each test.

The Shapiro-Wilk test was used to analyze data distribution (d.n.s.). For comparisons among control, risk of pre-eclampsia and pre-eclampsia groups the two-way ANOVA test was used for normally distributed data. The same test was used for analyze the correlation between data, gestational age and the other clinical characteristics.

Results

3 of the 12 patients in the risk of pre-eclampsia group developed pre-eclampsia, defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy (SSHP): arterial hypertension (systolic blood pressure >140 mm Hg and diastolic blood pressure >90 mm Hg) in 2 measurements spaced at least 6 hours and proteinuria >300 mg in a 24-h collection appeared after 20 weeks of gestation.

The values of sEng and sVEGFR-1 showed a slight increase at 30 weeks, compared to those at 16 and 24 weeks in all patients of which we have at least two samples, although not statistically significantly (dns). The values of PlGF showed an irregular distribution in the samples at 16, 24 and 30 weeks, with no detectable trend.

Concentrations of sEng

In at-risk women who developed pre-eclampsia the median concentration (124.1 pg/ml) was higher than in at-risk women who have not developed the disease (13 pg/ml), and than in controls (9.65 pg/ml) as shown in Figure 1, with highly statistically significant difference ($P < 0.01$) for both comparisons. There were no statistically significant differences between the median concentrations of at-risk group and the control group ($P > 0.05$) (Fig. 1).

Similar results were also seen in the comparison between the mean values of the three groups (Fig. 2).

Concentrations of PlGF

In pre-eclamptic pregnant women the median concentration (0.07 pg/ml) was much lower than in

at-risk women without pre-eclampsia (1.58 pg/ml) and than in controls (1.75 pg/ml) as we can see from Figure 3, with P<0.05 (significant difference) and P <0.01 (highly significant difference), respectively. There were no statistically significant differences between the median concentrations of at-risk group and the control group (P>0.05) (Fig. 3).

Similar results are also observed when comparing the mean values of the three groups (Fig. 4).

Concentrations of sVEGFR-1

In pre-eclamptic patients the median concentration (33.11 pg/ml) was significantly higher than in at-risk women (1.84 pg/ml) and than in control (2.25 pg/ml) as shown in Figure 5, with P <0.01 (highly significant difference) for both comparisons. There were no statistically significant differences between the median concentrations of at-risk group and the control group (P>0.05) (Fig. 5).

Similar results were seen in the comparison between the mean values of the three groups (Fig. 6).

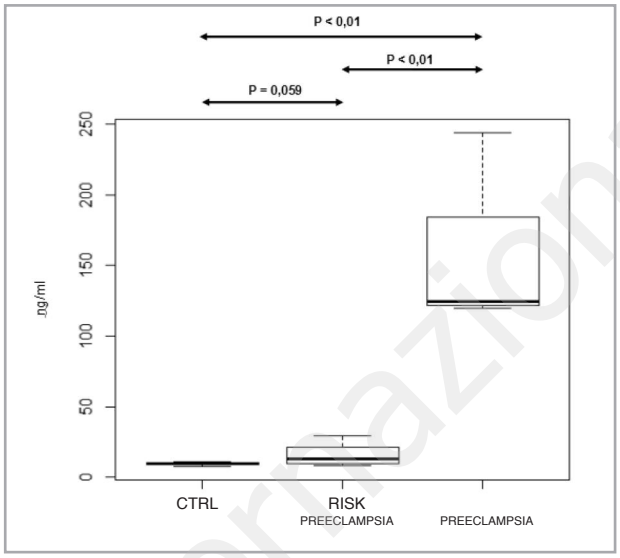

Fig. 1 - Comparison values sEng between control group (CTRL), risk group and group with overt preeclampsia.

Discussion

In the study of incidence of risk factors for pre-eclampsia, we found that 3 patients with pre-eclampsia have two characteristics in common: BMI> 30 and nulliparity. While a high BMI is also found in other at-risk patients, nulliparity appears to be only present in pre-eclamptic pregnants and therefore seems to be correlated to the development of pre-eclampsia. This hypothesis requires further confirmation on a much wider sample of at-risk patients.

No clinical feature of patients at risk of developing pre-eclampsia was also significantly related to an increase of the values of sEng and sVEGFR-1 and to a decrease of the values of PlGF, as found in pre-eclamptic women.

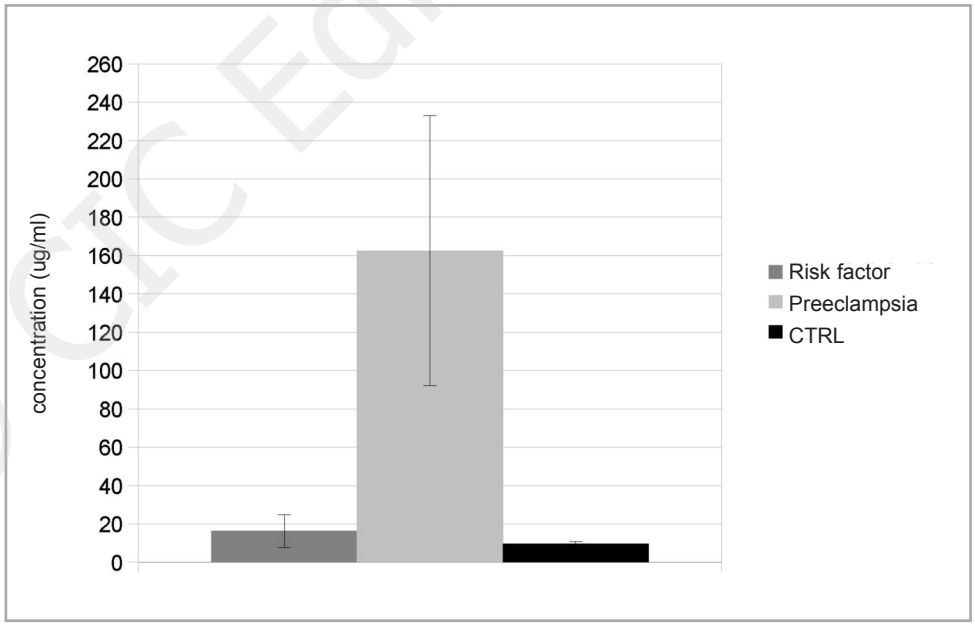

Fig. 2 - sEng: comparison means between control group, risk group and group with overt preeclampsia.

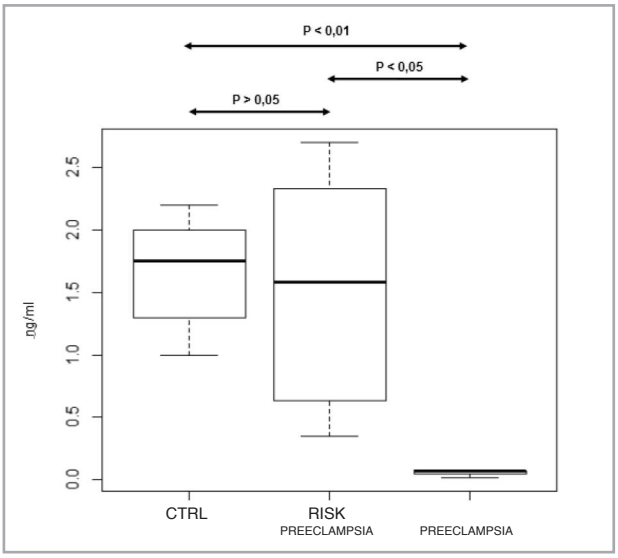

Fig. 3 - Comparison values PlGF between control group (CTRL), risk group and group with overt preeclampsia.

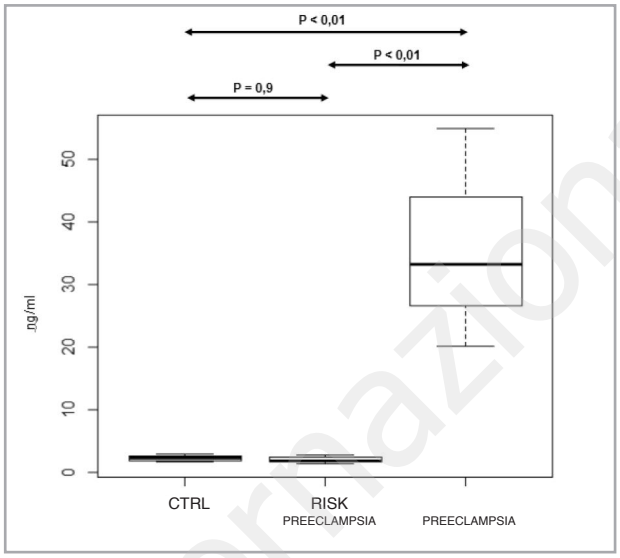

Fig. 5 - Comparison values sVEGFR-1 between control group (CTRL), risk group and group with overt preeclampsia.

For all three factors measured, the patient DS (no. 5: at-risk, not pre-eclamptic) has exhibited a trend similar to pre-eclamptic women, although the values in her samples were much lower. In the history of this patient, unlike the others in the same group, there is a past history of pre-eclampsia. It's possible, therefore, that a history of previous pre-eclampsia represents another important risk factor in the development of the disease, as nulliparity, although this condition should be investigated through the study of other similar cases.

Conclusions

In our study, the difference of the values of sEng, PlGF and sVEGFR-1 between the pre-eclamptic women, at-risk women and the control group was highly significant. The median concentration of sEng and sVEGFR-1 was significantly higher in pre-eclamptic women than in at-risk women and controls while the median concentration of PlGF was much lower in pre-eclamptic women than in the other two groups.

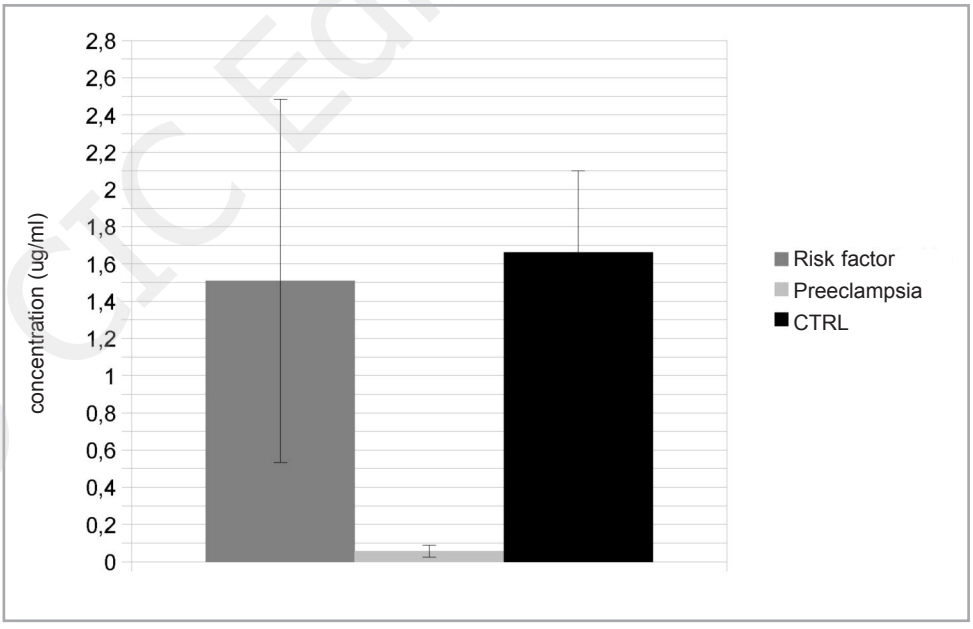

Fig. 4 - PlGF: comparison means between control group, risk group and group with overt preeclampsia.

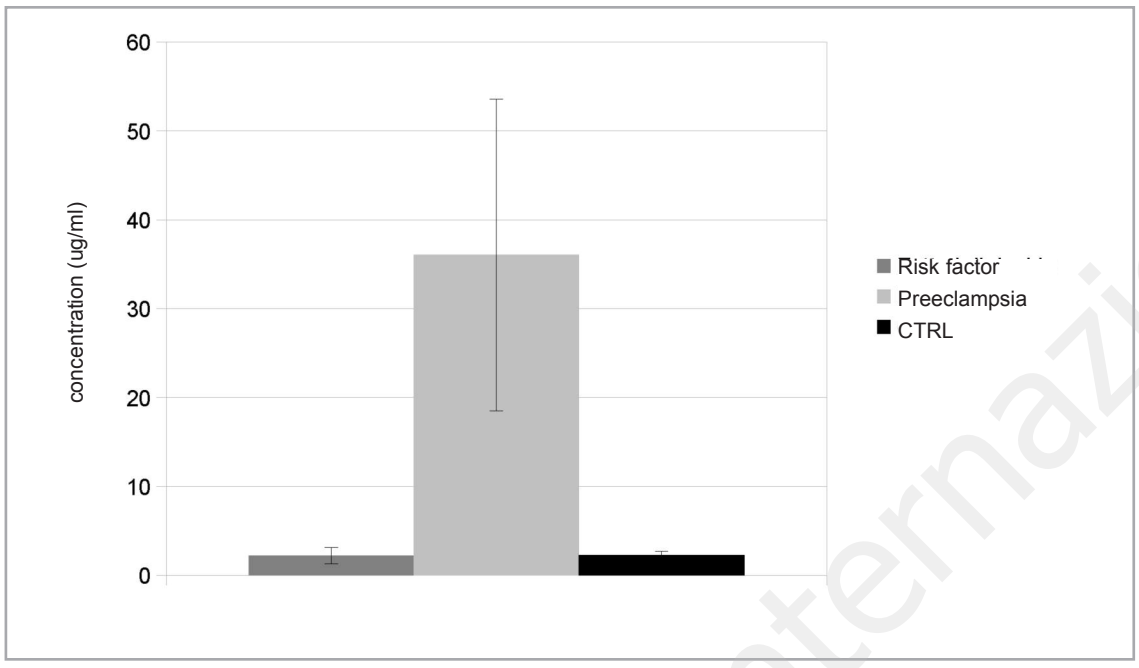

Fig. 6 - sVEGFR-1: comparison means between control group, risk group and group with overt preeclampsia.

In relation to our results, we can conclude, in agreement with the literature reported, that the combined dosage of these factors in maternal serum, in one or more periods gestational, can be considered a predictive marker of the onset of preeclampsia.

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