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# Comparation Level of Nitric Oxide (NO), Thromboxane A2(TXA2), Prostaglandin E2(PGE2) and Prostacyclin (PGI2) in the Plasma among Normal and Preeclampsia Pregnantwomen

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## Abstract

Introduction: Among all hypertensive disorders, preeclampsia is as a multisystem disorderandit is the major and prevalent factor for defection or death in the fetus and mothers. There are many evidence regarding damage of endothelial cells and change in their function that might have important role in pathogenesis of preeclampsia. Most of the researches has been focused on the role of Vasoactive Eicosanoids .In addition, Nitric Oxide as a vasodilator componenthas important role during pregnancy. Therefor, the present study was designed to comparationlevel of NO, TXA2, PGE2 and PGI2 in plasma among normal and preeclampsia pregnant women. Material and Methods: In this study, blood samples were collected from 40 pregant women and 40 pregnant with preeclampsia. The Nitrite and Nitrate (NO2-/NO3-) the stable and product NO in plasma were measured by colorimetric method (Griees reaction). The stable metabolits of thromboxane A2 (thromboxane TXB2), PGI2 (*6*-keto-prostaglandin Fl*a*) and PGE2 in plasma sample were measured by reverse- phase HPLC in ng/ml level. Statistical analysis was performed using T-test and SPSS software. Results: the level of NO in the plasma of preeclampsia group was 36.22 ±5.4 µmol/l as compared to normal group (56.72±11.3) µ mole/l and PGI2, PGE2 and TXA2 in the palsma of preeclampsic women were 22.35±14.40, 92.57±46.18 , 2082.25±694 ng/ml respectively. meanwhile the level of them for control (normal) group were 118.07 ± 39.81(P<0.001), 240.92 ± 122.87(P<0.001) and 450.22 + 330.93(P<0.001), respectively, The ratio of TXA2/PGI2, used to express relative vasoconstriction vs. vasodilation effect, was 83% higher in preeclampsia women to normal pregnant women (P<0.001). Conclusion: The result of this study showed the significant reduction of NO, PGI2 *a* ,PGE2 and an increase in TXB2 in preeclampsia as compared to normal pregnant women, which- suggests the possible role of above compounds in the pathogenesis of preeclampsia. Thus the measurements of these compounds study the levels of nitric (NO) thromboxane A2 (TXA2) prostaglandin E2 (PGE2) and prostacyclin (PGI2) in plasma of normal. Could be useful in diagnosis of early-preeclampsia and possible control of these factors or prevention of side effects of preeclampsia.

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## Introduction

Pre-eclampcia is detected as blood pressure syndrome accompanied with proteinuria in the pregnancy. Blood pressuremore than 140/90 mmHg after twentieth week of pregnancy also proteinuriamore than 300 mg during 24 hours or 1 plus in urine strip test. Approximately 5 percent of all pregnancies get complicated with pre-eclampcia.chance of occure this problem is depend on economical condition so thatit had an increase to 10% amonglow income women whereas it haddecline to 2% among rich women (Leeman et al., 2016) pre-eclampcia is a multisystem disorder and it is the most and prevalent factor for mortality and defectionin the fetus and mothers (Lecarpentier et al., 1983). Pre-eclampcia can be classified as slight or severe, generally based on level of blood pressure and involvement or lack of involvement body systems. The unpredictable process of this syndrome that varies from slow advancement to rapi. One of the syndromes which is derivated ofPre-eclampcia is HELLP that is characterise with hemolysis, high level of liver enzymes and low platelets count (Leeman et al., 2016; Gedik et al., 2017). Etiology of this disease still isunknown but there are evidences about damage of endothelial cells and change their performance which these facts indicated they have an important role in pre-eclampcia pathogenesis (Adekola et al., 2015). Increase of blood vessels reaction and damage ofendothelial in pre-eclampcia state leads to change sensitivity of blood vessels thanVasopressin hormons and Eicosanoid and lead to general contraction in the arterioles so that Increase of vascular contraction because of increase resistance than blood flow and arterial hypertension (Leeman et al., 2016). Most of researches have had focus on the role of Vasoactive Eicosanoids, as thelevel of Thromboxane A2 had increase, Prostaglandin and prostacycline E2 had decline in the normal pregnancy rather than women with pre-eclampcia that these conditions results to vascular contraction and sensitivity to angiotensin II (Broegger etal., 2016). The studies showed that imbalance between Prostaglandin PGI2 (blood vessels dilator) and thromboxane A2 (tightener of blood vessels) are the most important reasons responsible in pre-eclampcia (Lee et al., 2015;

Nandiet al., 2017). Nitric oxide, as a powerful dilator of blood vessels, can have an important role in development of blood vessels during pregnancy period. Change in the production value of NO by endothelium cells can be effective in pre-eclampcia pathogenesis (Khalil & Hardman, 2015). On the other hand, nitric oxide can be initial mediator of lack of blood vessels responsing to vasopressors in pregnancy. Change in production of this material or smooth muscle tissue reaction to nitric oxide can have an appropriate role in pre-eclampcia (Leiva et al., 2016). Recently, different studies have showed various results for the level of NO metabolites (Nitrite and Nitrate) in the blood samples. The reports indicated that the amount of NO metabolites in pre-eclampcia patients is accompanied with reduction, increase and without change rather than normal pregnant women (Khalil & Hardman, 2015; Ashraf et al., 2017; Zaheret al., 2017).Correct management of patients, clinical judgment and perfect knowledge about severity and advancement of disaese and preventing from acute levels of the disease can cause toimprove health in the mothers and fetus. Finally, initial recognition and appropriate treatment cause pregnancy induction and improvement their conditions (Leeman et al., 2016).

In Hence, the aim of this study was measurement the level of nitric oxide level (NO), thromboxane A2 (TXA2), prostaglandin E2 (PGE2) and prostacyclin (PGI2) in pre-eclampcia pregnant insead of normal pregnant women.

## Method

*Subject characteristics*

Thisstudy was case-cntrol,it has doneamong women suffering from pre-eclampcia with blood pressure equal to or more than 140/90 mmHg and proteinuria eqaul to or more than 300 mg during 24 hours or equal to or more than +1 in urine strip test with confirmation of gynecologist and noraml women pregnantwhich referred to Shahid Abadi hospital in the Tehran.Creteria conditions for enterecing were included health water, first pregnancy, cephalic fetus, singleton, and the weeks of 37-40 of pregnancy. Individuals with the history of smoking, chronic systematic diseases (high blood pressure, diabetes, cardiovascular, renal, hepatic and metabolic disorders), chronic infections, and drug consumption were excluded. 10 ml venous blood samplebefore childbearing in sleep position after 15 minutes were collectedin in theplastic pipes contain anticoagulant EDTA-K3 .Also patients restricted from consumption of food materials with Nitrite and Nitrate (Red meat and Bean). After centrifuge (rpm=1000 and 10 min), plasma was separated and kept in -20 degrees centigrade until experiment. Then biochemical parameters were analyzed by Hitachi 912.Then ,0/5 ml of plasma sample are added to 0/5 ml methanol 100% with 0/1% Acetic acid and after 5 minutes vertex, homogenized samplewas centrifuged in 1500 rpmat $4^{C}$for 15 minutes after transfer surface liquid to other pipe andadded 1 ml acetic acid 0/1%, finally the sample with 25% methanol was extracted.

*sample Extraction*

After preparation of the cartridge, the sample with 25% methanol passed from column quickly and then, 5 ml acetic acid 0/1 and 5 ml methanol 25% with 0/1% acetic acid passed, respectively. And output liquid wasdelebrated and in the final phase, to extract Eicosanoids, 2/4 ml methanol 90% with 0/1% Acetic Acid passed and collect output liquid in clean pipe and evaporate extracted liquid with Nitrogen gas and supply achieved sediment with adding 200 Micro Liter of methanol 50% to volume and after mild vertex and passing from syringe filter 0/22 Micron, achieved sample was ready for injection to HPLC column (Aghazadeh et al., 2015).

*NO measurement*

NO measurement is performed with assistance of Nitric Oxide (NO2-/NO3-) assay kit based on instruction of manufacturer company of assay (design, Canada) with colorimetric method and sensitivity of 0/222 Micromole/Liter and cv-2/8% for Nitrite and sensitivity of 0/622 Micromole .Liter and cv-2/9% for Nitrate. After measuring Nitrite and Nitrate (after exact calculation based on formula), these two metabolites are reported as the amount of Nitric Oxide (NO).

In this method, Nitrite is combined withgrease reagent and achieved colorful complex with light attraction in wavelength of 550 nanometer is measured based on standard curve in density of Micromole/Liter. To level ofNitrate, with enzymatic conversion of Nitrate to Nitrite, rest of phases are performed like Nitrite measurement levels and real density of Nitrate is determined with deduction from Nitrite amount and total of these two metabolites (Nitrite and Nitrate) are reported as the amount of Nitric Oxide (Gedik et al., 2017; Zaher et al., 2017).

*Simultaneous measurement, PGE2, 6-keto-PGFIa and TXB2 by HPLC*

Plasma level of stable metabolites of Thromboxane A2 (thromboxane B2), Prostacyclin PGI2-6-keto), Prostaglandin (F1a and prostaglandin E2 after extraction steps with liquid chromatography with high efficiency- reverse phase (RP-HPLC) is measured in the Nano Gram/ml level.

*Equipmentand Reagents*

Used HPLC device includes pump, vaccum degasing, UV injector, and detector of Perkin Elmer Series Company (200 HPLC system, USA and C18 chromatography column of Knauer, Berlin company, solvent outgassing device, nitrogen gas capsule, syringe filter 0/22 micron from cartridge and polypropylene kind (C18, 100 mg, 3ml) of Kinesis Ltd and Cambridge shire company) iceman centrifuge and PH meter device.

The standards of PGE2, 6-keto-PGF1a (PGI2 stable metabolite) and TXB2 (TXA2 stable metabolite) of Caymman, USA company and acetonitrile, methanol, ethanol, phosphoric acid, acetic acid glacial 100% and distilled water with HPLC grade of Darmstadt, Germany, Merck provided.

*HPLC method for TXB2, 6-keto-PGFla and PGE2*

Mobile phase includes volume 32/8 of acetonitrile and 67/2 distilled water that after solvent outgassing, supply PH to 3/3-3/5 by acid phosphoric. Moreover, injection of sample in the volume of 20 microliter is performed in wavelength of 195 Nano meter and passing mobile phase solvent with current speed of 1/5 ml/ min (Aghazadeh et al., 2015; Regnier & Gooding, 2018).

After injection of standards, recovery is about 80-100% and retention time reported (table1).In addition, all peaks are determined obviously and separately based on chromatogram (density of 5000 Nano Gram/ml) and standard curve for PGE2, 6-keto-PGFla and TXB2 is depicted with r=0/9912, r=0/9991 and r=0/9986, respectively(Figure 1).

Table 1: chromatographic behavior of retention time and efficiency (%) PGE2, TXB2, PGI2 (6-keto-PGF1a)

| | Retention time (min) | Recovery (%) |
|---|---|---|
| -keto-PGF1α6PGI2() | 6:30 | 4±92 |
| TXB2 | 11:40 | 4±87 |
| PGE2 | 20:20 | 4±93 |

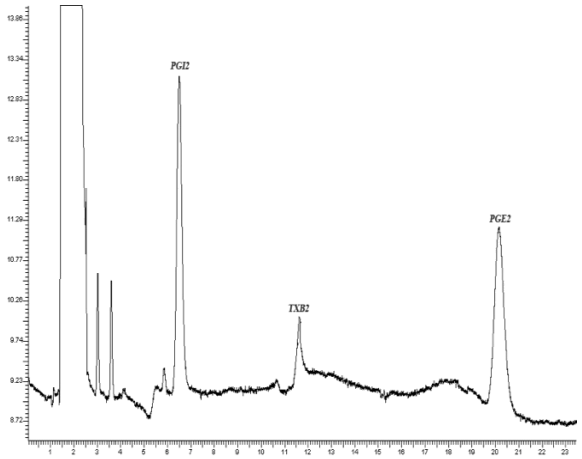


**Figure 1.** RP-HPLC chromatogram for standards of PGE2, TXB2, PGI2 (6-keto-PGF1a) in density of 5000 Nano Gram/ml

## Results:

The results of this study have showed which average age in the control group and pre-eclampcia patients were 23/7±4/7 and 28/5±5/5 respectively. Also, pregnancy weekswere evaluated as 37/9±1/1 in the pre-eclampcia patients and 39/2+-2/1 in the control group. The amount of systolic and diastolic blood pressure was evaluated as 148±14/3 and 96/3±10/5 in pre-eclampcia and 73/7±8/1 mmHg and 113/5±6/9 mmHg in control group, respectively (table 2). NO plasma level was 36/22±5/69 Mmole/l in the pre-eclampcia group and 56/72±11/13 Mmole/l in control group. Moreover, level of PGI2, PGE2 and TXA2 in the serumamong pre-eclampcia group and control group representived in the Table 3.

In addition, relation of level TXA2 rather than PGI2 which is as retractor of blood vessels against blood vessels dilator, among women suffering from pre-eclampcia have showedconsiderable increase in comparison with normal pregnant women. (Table 3)

Table 2: clinical indexes in pre-eclampcia and normal pregnant groups

| variable | Normal | Pre-eclampsia |
|---|---|---|
| Age | 23/7±4/7 | 28/5±5/5 |
| Pregnancy week | 39/2±2/1 | 37/9±1/1 |
| Systolic pressure | 96/3±10/5 | 148±14/3 |
| Diastolic pressure | 73/7±8/1 | 113/5±6/9 |
| Body Mass Index | 28/1±4/3 | 32±5/5 |
| (BMI) | | |

Table 3: level of PGI2, PGE2, TXA2 and NO in pre-eclampcia and normal pregnant groups

| | Normal | pre-eclampcia | P-value |
|---|---|---|---|
| NO (µmol/L) | 56/72±11/13 | 36/29±5/6 | < 0/001 |
| PGI2 (ng/mL) | 118/07±39/81 | 22/35±14/40 | < 0/001 |
| PGE2 (ng/mL) | 240/92±122/87 | 92/57±46/18 | < 0/001 |
| TXA2 (ng/mL) | 450/22±330/293 | 208/25±699/44 | < 0/001 |
| TXA2/PGI2 | 3/81 | 93/16 | < 0/001 |

Results of this study indicated level of PGI2, PGE2 and NO had decline. In spite level of TXA2 has had increase in the pre-eclampcia group and these results were in stark contrast among normal pregnancy in the period of their pregnancy. These results have showed the relevanceamong affects of these factors in pathogenesis of pre-eclampcia and relation between their demographic characteristics were complication. Measuring these combinations may be helpful as auxiliary tests in recognition of pre-eclampcia or even its evaluation and determination of its intensity. On the other hand, by measuring these components earlier than second third months of pregnancy, maybe we be able to recognize or increase of probability of pre-eclampcia for preventive operations and treatment. After all , maybe we will be able to help to decline the danger of pre-eclampcia with control the level of TXA2, NO, PGI2 and PGE2 parameters by controling food regimes, herbs, and consumption of effective drugs in the perod of their pregnancy.

## Discussion:

Pre-eclampcia is a systemic disease with characteristics of undesirable performance of endothelial, spasm of blood vessels, oxidative stress increase, reduction of antioxidants, hyperlipidemia and activation of coagulation system. During the years, different theories are presented for clarification of pathophysiology of pre-eclampcia including vascular endothelial damage, lack of coordination and adjustment of cardiovascular performances, immunologic phenomenon, and unnatural trophoblastic invasion and inflammation of coagulation system. Despite many studies, etiology of pre-eclampcia has not been determined.

In this study results have shown the plasma level of NO, PGI2 and PGE2 in pre-eclampcia group was lower than control group significantly but plasma level of TXA2 was higher than control group in pre-eclampcia group considerable. Achieved results of study that has done in 2016 have indicated that progress of pre-eclampcia is in increase by connection of TXA2/PGI2 considerably and indicated that vascular retraction begins from second three months and continues until pregnancy finish. Moreover, the results showed that shortage of production of PGI2 is the first factor in beginning of second three month of pregnancy and considered damage of endothelial cells as the reason of reduction of endothelial cells for reduction of synthesis and PGI2 as the main factor of vascular retraction that this results were consensus by results of uor study (Pai et al., 2016).

The study that has done in 2016 indicated that TAX2 levels in pre-eclampsia patients have been shown to reduce the risk of hypertension and reduce the risk of pre-eclampsia in pregnant women, although the increase in TAX2 levels in pregnant women with pre-eclampsia The present study was one of the effective factors in the incidence of pre-eclampsia. However, the result of the present study was not consistent with this study in term of the level of TAX2 in the plasma of these patients (Matsubara et al., 2015) In different studies, the results have shown that NO levels in the pre-eclampsia patients havedecreased which decrease a level of NO leading to an increase in free radicals (ROS) and oxidative stress in these patients . these resultes were consistent with the results of this study in terms of decrease level of NO in the patients with pre-eclampsia. The results of these studies have show the cause of the effect of this factor more clearly in people with pre-eclampsia. (Osol et al., 2017; Snydal et al., 2014). Of course, because of this matter that endothelial cells spatter blood vessels of PGI2 and NO and both of them are effective in hemostasis, permeability, and dilation of blood vesselsand in most of studies of reduction of PGI2, they report NO in the patients with pre-eclampcia, we can reject compensatory response in increase of spatter of NO. (Oyston et al., 2015).

With unknown etiology causes of pre-eclampcia and among different theories, damage of endothelial cells, ischemia in the pair and immunologic factors from fetus are more probable than other theories (akashim et al., 2017). Because normal pregnancy is accompanied with basic changes in cardiovascular system of the mother, she must adjust herself with these changes due to the needs of fetus to growth. Moreover, it seems that initial factor in imbalance of NO, PGE2 and TXA2 is as generalized with advancement of fetus and transfer of antigen factors from fetus to mother (Oyston et al., 2015).

In the recent decades, many studies have done for detecting etiology of pre-eclampcia and different parameters involved in adjustment of blood pressure are considered and different results have achived, the main result is that reduction or increase of most involved parameters in adjustment of blood pressure is created because of one or more factors and they affect the system adjustment of blood pressure and cardiovascular system cascading. On the other hand, since in most of women with pre-eclampcia, with pregnancy finish, pre-eclampcia signs have been removed, we must research for the main reasons in disturbing balance in involved factors of adjustment of blood pressure like NO, PGE2, TXA2, Renin Angiotensin endothelia system and malonedialdehyede. (Oyston et al., 2015; akashima et al., 2017; Snydal & Major, 2014).

## Conclusion:

Different reasons that causes difficulty in judgment and conclusion relates to methods of measurement and contradiction in the results that we can help to etiology of this disease with more researches and introduction of reference and favored methods.

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