



Inducible nitric oxide synthase in the placenta of pregnant women with preeclampsia.

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ABSTRACT.

Introduction. During pregnancy, changes in blood pressure is a major cause of maternal and fetal death worldwide, 12% of all pregnancies develop preeclampsia (PEC). The oxide nitric synthase endothelial enzyme (eNOS) generates nitric oxide continuously, producing vasodilation. The inducible isoform (iNOS) is found in macrophages, smooth muscle cells and endothelial cells, and other locations as platelets, hepatocytes, tumor cells and lymphocytes. The main objective of this work was to detect iNOS by fluorescein in placental tissue.

Methodology. Childbirth after were performed 1X1X0.5cm cuts on the surface of the cotyledons, were placed in 10% formalin were embedded in paraffin for microtome cuts and fix them on lamella. Were subsequently washed with buffer solution, oxygenated water with methanol, using also pig serum 10% (Rockland®, D305, lot # 421), solution-RabbitAb anti-iNOS (Calbiochem®482728), streptavidin-fluorescein (Calbiochem®, Cat. No.189734). Fluorescence microscope Carl Zeiss brand was used, and the Image-Pro Plus, 7.0 software.

Results. Nineteen placentas of pregnant women with PEC were collected and 19 without the pathology. It find increased uptake of fluorescein in erythrocytes in the maternal placentas no PEC, the patients who developed PEC was remarkable decreased catchment of fluorescein by state of gravity.

Conclusions. There is an obvious decrease in activity of iNOS during preeclampsia, which is a possible explanatory factor on the process of vasoconstriction during pregnancy, manifested by elevated blood pressure.

INTRODUCTION.

During pregnancy, changes in blood pressure is a major cause of maternal and fetal death worldwide, 12% of all pregnancies develop preeclampsia (PEC)^{1,2,3,4,5,6}. The enzyme endothelial nitric oxide synthase (eNOS) generates nitric oxide continuously, producing vasodilation^{7,8}. The inducible isoform (iNOS) is it found in macrophages, smooth muscle cells and endothelial cells, and other locations as platelets, hepatocytes, tumor cells and lymphocytes^{9,10}. The main objective of this work was to detect iNOS with fluorescein in placental tissue.

METODOLOGY

The samples were washed with PBS solution, 7.2 pH, three times for 2 minutes, subsequently treated with hydrogen peroxide at 3% with methanol, for 15 minutes at room temperature, to prevent endogenous peroxidase activity, after this phase was again washed three times for 2 minutes.

The fabric was covered with pig serum (Rockland®, D305, Lot # 421) 10% solution in sterile PBS for 30 minutes at room temperature, as a preventive measure for nonspecific binding, washing again, as mentioned in the previous step. The was tissue immediately covered with a solution containing antibodies anti-inducible nitric oxide synthase (Calbiochem®, Cat No. 482728, Rabbit anti-iNOS pAb.) diluted 1: 500 with sterile PBS solution for one hour at room temperature. After washing again with PBS, for 30 minutes, the tissue was covered with streptavidin-fluorescein (Calbiochem®, Cat No. 189,734) diluted 1:100 with sterile PBS solution, washing during the same time, at room temperature, protected from light.

Finally, washed again, and the presence of iNOS was visualized by fluorescence microscope, Carl Zeiss mark and the Image-Pro Plus 7.0 software.

RESULTS

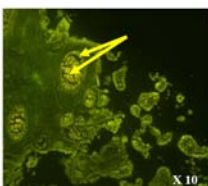
The presence of iNOS in placental tissue from pregnant healthy, demonstrated by fluorescein uptake was higher in erythrocytes, opposite to that described by various authors, who point out that there is a greater presence of the enzyme in macrophages and in endothelial cells, endothelial tissue there is little apparent halo, as shown in figure 1, picture A.

For samples of placental tissue, from pregnant women with mild PEC, similar uptake of fluorescein compared to pregnant without pathology, that is, there is growing evidence of the presence of the enzyme in the cytosol of the erythrocytes, with notable absence in macrophage (picture B).

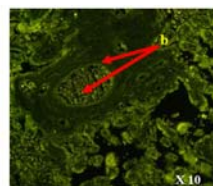
In placental tissue samples belonging to pregnant with PEC moderated is evident the fewer captured of fluorescein in the erythrocyte (picture C).

Placental samples from pregnant women with PEC severe is further reduced level uptake of fluorescein erythrocytes and on endothelial tissue (picture D and E).

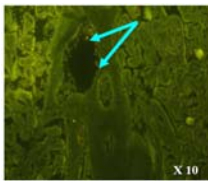
Only one case of PEC severe showed increased accumulation of fluorescein in the endothelial tissue (picture E, F and G), erythrocytes show decreased uptake of the compound, compared with cuts of pregnant without PEC.



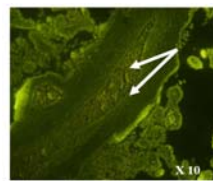
Picture A



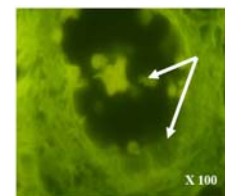
Picture B, PEC mild



Picture C, PEC moderate



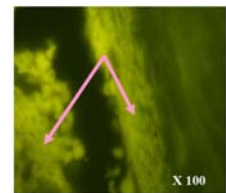
Picture D, PEC severe



Picture E, PEC severe



Picture F, PEC severe



Picture G, PEC severe

Figure 1. Histological sections of placental tissue, fluorescein-labeled with different levels of uptake of the compound, to erythrocyte level and endothelial tissue, picture A) pregnant healthy, erythrocytes and endothelial halo fluorescein (yellow arrows); B) PEC mild, erythrocytes with compound lesser degree than pregnant without PEC (red arrows); C) PEC moderate, fluorescein decreased uptake by erythrocytes (blue arrows); D, E, F and G) PEC severe, uptake reduced fluorescein by erythrocytes (white arrows and purple).

CONCLUSIONS.

There is an obvious decrease in activity of iNOS during preeclampsia, which is a possible explanatory factor on the process of vasoconstriction during pregnancy, manifested by elevated blood pressure.

The technique described allowed achieve the goal, making a notable difference between the normal and the pathological (PEC), highlighting differences by the progress and severity of preeclampsia.

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Acknowledgments.

To the executives of the Hospital of the Women Zacatecana, and women pregnant of the Medical Unit.