NITRIC OXIDE AS A MARKER OF ENDOTHELIAL CELL DYSFUNCTION IN PRE-ECLAMPSIA

Thesis

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List of abbreviation

ACOG	America Collage of Obstetricians and Gynaecologists
ACTH	Adrenocorticotrophic Hormone
AFP	Alfa Feto Protein
ALT	Alanine tansaminase
ANP	Atrial Natruritic Peptide
AST	Aspartate transaminase
AT III	Antithrombin III
BMI	Body mass index
cGMP	Cyclic guanosine monophosphate
cNOS	Constitutive Nitric oxide synthase
CSF	Cerebrospinal fluid
DIC	Disseminated intravascular coagulopathy
DNA	Deoxyribonucleic acid
EDRF	Endothelium derived relaxing factor
eNOS	Endothelial nitric oxide synthase
FGR	Fetal growth retardation
HCG	Human chorionic gonadotrophin
HELLP	Hypertension,Elevated Liver Enzymes,Low Platelets Syndrome
HLA	Human leukocytic antigen
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
iNOS	Inducible Nitric oxide synthase
ISSHP	International society for the study of hypertension in pregnancy
IUGR	Intrauterine growth restriction
IUK/Cr	Inactive urinary kallikarein to creatinine ratio
LDH	Lactate dehydrogenase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NO	Nitric oxide

NO_2	Nitrogen dioxide
NOS	Nitric oxide synthase
nNOS	Neuronal Nitric oxide synthase
PAF	Platelet activation factor
PAI-1	Plasminogen activator inhibitor-1
PGF	Placental growth factor
PGI_2	Prostacyclins
Rh	Rhesus factor
ROC	Receiver operator characteristics
SD	Standard deviation
SPSS	Statistical programme for social sicnece
TAT	Thrombin antithrombin III complex
TNF-α	Tumar necrosis factor-alpha
TRAP	Total radical antioxidant potential
TXA_2	Thromboxane A_2
VEGF	Vascular endothelial growth factor
vWF	Von wilbrand factor

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INTRODUCTION

Pre-eclampsia is one of the most common health problems experienced by pregnant women and it appears in approximately 5-8% of pregnancies later than 20 weeks. This disease which is characterized by hypertension, edema and proteinuria has a variety of serious pregnancy complication which leads to both fetal and maternal morbidity and mortality. ***(American College of Obstetricians and Gynecologists, 2002).***

Pre-eclampsia is defined as a pregnancy-specific occlusive vascular disorder characterized by endothelial cell dysfunction and increased platelet aggregation ***(laivuori et. al., 1999).***

Even though etiology and pathogenesis of pre-eclampsia could not be clarified definitely until now, it is considered that the basic problem is decreased placental bleeding due to abnormal cytotrophoblast invasion and extensive endothelial damage ***(Granger et. al., 2001).***

Endothelial dysfunction has been proposed as a central feature of the pathophysiology of pre-eclampsia resulting in altered vascular reactivity, activation of the coagulation cascade, and loss of vascular integrity ***(Roberts and Redman, 1993).***

Nitric oxide (NO) is recently hypothesized to have a key role in the pathology of PET. It is synthesized from L-arginine, which is an amino acid, by the enzyme "nitrite oxide synthase (NOS)", (NO) is an effective free radical which inhibits thrombocyte aggression and leads to vasodilatation in vessels ***(Weiner and Thompson, 1997).***

NO is a gaseous molecule, its half-life is so short (nearly 4 seconds) and rapidly converts into its metabolites nitrite (NO_2) and nitrate (NO_3) ***(Buhimschi et. al., 1998).***

It is known that both NO production and response to NO increase in normal pregnancy, and it is thought that this increase plays a role in many physiological mechanisms which provide continuation of the pregnancy ***(Rosselli et. al., 1998).***

Pathak et. al., 2000 has found serum nitrite/nitrate levels in pre-eclamptic pregnant women to be significantly higher when compared to normotensive pregnant women. They also declared a direct proportion between the severity of pre-eclampsia and nitrite/nitrate levels. ***Shaamash et. al., 2000*** have also indicated a positive correlation between the severity of pre-eclampsia and the amount in maternal and fetal circulation of (NO) metabolites.

Endothelial damage in pre-eclampsia might lead to reduced production of vasodilators such as nitric oxide; however, a recent study has shown that constitutive endothelial nitric oxide synthase could be upregulated by a factor present in plasma from women with pre-eclampsia. Endothelial cells exposed to 2% plasma from pregnancies complicated by pre-eclampsia produce greater amounts of nitric oxide metabolites compared with cells treated with plasma from healthy pregnant women ***(Davidge et. al., 1995).***

AIM OF THE WORK

The Aim of this work is to assess the level of (NO) as biomarker for endothelial cell function in patients with hypertensive disorder in pregnancy.

PATIENTS AND METHODS

This study will be performed in pregnant patients attending ***Ain Shams*** University Maternity Hospital. Patients will be recruited from both the outpatient clinic and inpatient wards.

- **Sample size:** 120 patients.
- **Type of study:** Case control study.
- **Patient groups:** Patients will be divided into four groups:
  1- ***Control:*** Normal healthy pregnant females attending the outpatient clinic for routine Anti natal care.
  2- ***Mild pre-eclampsia:*** 30 patients fulfilling the following criteria.
    - Blood pressure**:**
      - Systolic blood pressure > 140 but less than 160.
      - Diastolic blood pressure > 90 but less than 110.
    - Proteinuria: 300 mg (++) assessed by urine albustix.
    - No symptoms denoting severity (as headache or blurring of vision).
    - Normal investigation for different organ function (as liver and kidney function tests).

3- ***Severe pre-eclampsia:*** 30 patients fulfilling the following criteria.

   - o Blood pressure:
     - Systolic blood pressure > 160.
     - Diastolic blood pressure > 110.
   - o Proteinuria: > 300 (++) assessed by urine albustix.
   - o Symptoms denoting severity (as headache or blurring of vision).
   - o Abnormal different organ function (as liver and kidney function tests) as proven by biochemical investigations.

4- ***Chronic hypertensive:*** 30 patients diagnosed before pregnancy.

- **Exclusion Criteria:**
  - D.M or other endocrinal diseases.
  - Liver diseases.
  - Kidney diseases.
  - Thyroid.
  - Cardiac diseases (ischemic heart diseases).

- **History:** Through history taking from all patients with special emphasizes on:
  - Gestational age (known by LMP or determined by U/S)
  - Any previous disease documented in exclusion criteria.

- Symptoms suggestive of severe PET.
  - Headache.
  - Blurring of Vision.
  - Epigastric Pain.
  - Oliguria or Anuria.

- **Examination:**
  - General: To measure the blood pressure (assessed twice four hours apart in the left lateral position).
  - Abdominal: To assess fundal level and exclude fetal distress.
  - Investigation:
    - Proteinuria (known by albustix in a random urine sample).
    - CBC.
    - Liver and kidney function.
    - Coagulation profile.

- **Methodology:** Sample collection:
  - Consent will be taken from all patients participating in the study.
  - Blood samples will be withdrawn for all patients (5 cc) as a whole venous blood.
  - Also (5 cc) will be taken from umbilical cord immediately after delivery.

- **Timing:**
    - Samples will be taken once PET is diagnosed (whether mild or severe).
    - In the other groups (normal pregnancy and chronically hypertension) samples will be taken at similar matching gestational age.
    - Serum will be collected and Nitric Oxide will be determined. Spectrophotometric quantitation of nitric oxide after conversion into nitrate and nitrite, nitrate is converted into nitrite by NADH dependent enzyme nitrate reductase reaction.
    - The end product is measured at 540nm.
    - Route of delivery, neonatal outcome will be assessed.
    - All data will be statistically analyzed and tabulated.

- **Discussion.**
- **Summary and Conclusion.**

HYPERTENSIVE DISORDERS WITH PREGNANCY

Hypertensive disorders complicating pregnancy are common and contribute greatly to maternal morbidity and mortality. According to the national center for health statistics, gestational hypertension was identified in 3.7% of pregnancies ***(Martin et al, 2002).***The term gestational hypertension is used now to describe any form of new onset pregnancy related hypertension; the term pregnancy induced hypertension was and is still used by some interchangeably with gestational hypertension ***(Cunningham et al, 2005).***

There are five types of hypertensive disease that complicate pregnancy:

1) Gestational hypertension (formerly pregnancy induced hypertension or transient hypertension).
2) Pre-eclampsia.
3) Eclampsia.
4) Pre-eclampsia superimposed on chronic hypertension.
5) Chronic hypertension.

An important consideration in this classification is differentiating hypertensive disorders that precede pregnancy from pre-eclampsia, which is a potentially more ominous disease ***(Cunningham et al, 2005).***