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ASSESSMENT OF ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVITY AND OXIDATIVE STRESS MARKERS IN PREGNANT WOMEN AT HIGH RISK FOR HYPERTENSIVE DISORDERS

Abstract. *The study evaluated the effects of combined prophylactic therapy on nitric oxide system and oxidative stress markers in pregnant women at high risk for hypertensive disorders. Sixty-four participants were randomized to receive standard prophylaxis or additional L-arginine and antioxidant-micronutrient complex (folic acid, vitamin E, docosahexaenoic acid, vitamin D, potassium iodide). After treatment, the combined therapy group showed higher endothelial nitric oxide synthase activity, nitric oxide metabolites, and glutathione reductase activity, indicating improved endothelial function and antioxidant defense.*

Introduction

Hypertensive disorders of pregnancy (HDP) remain a leading cause of maternal and perinatal morbidity and mortality worldwide, despite ongoing improvements in obstetric care and increasing awareness of long-term risks for both mother and child [1-3]. Preeclampsia (PE), the most severe form of HDP, is characterized by impaired vascular adaptation, endothelial dysfunction, and systemic inflammation, resulting in adverse perinatal outcomes [1,4]. Oxidative stress (OS) and inadequate placental vascular remodeling are recognized as central mechanisms contributing to the disease process [5-7].

Normal pregnancy requires substantial cardiovascular and metabolic adaptation supported by finely balanced redox regulation and adequate nitric oxide (NO) bioavailability [8,9]. Disruption of this equilibrium leads to excessive formation of reactive oxygen and nitrogen species, oxidative damage to macromolecules, and impaired endothelial function [9-11]. The placenta is particularly vulnerable to oxidative imbalance; insufficient trophoblast invasion and incomplete spiral artery remodeling contribute to fetal growth restriction and the

later clinical manifestations of PE [12-14]. Intermittent perfusion and hypoxia-reoxygenation cycles further amplify placental injury through redox-dependent and reperfusion mechanisms [15,16].

Importantly, disturbances in redox homeostasis and NO pathways may appear early in gestation, well before the clinical onset of hypertensive complications, highlighting opportunities for preventive intervention in women at high risk for HDP [5,8,17]. Preventive strategies increasingly aim to counteract early endothelial dysfunction. L-arginine, the principal substrate for NO synthesis, may promote vasodilation and improve uteroplacental perfusion. Recent meta-analyses of randomized trials suggest a reduced risk of PE with L-arginine prophylaxis in at-risk pregnancies, although the certainty of evidence remains low to moderate [18-20].

In parallel, antioxidant interventions have been investigated to enhance placental tolerance to oxidative stress by modulating the balance between reactive oxygen and nitrogen species. Systematic and network meta-analyses have reported potential benefits of antioxidant regimens in reducing the risk of PE and/or gestational hypertension, although high heterogeneity and limited evidence quality warrant cautious interpretation [7,21,22]. A combined approach that simultaneously enhances NO pathways and antioxidant capacity may therefore offer stronger protection against early vascular dysregulation than monotherapy.

However, despite encouraging clinical observations, biochemical evidence supporting the effectiveness of combined prophylactic therapy remains limited. Studies evaluating longitudinal changes in NO-related and oxidative stress biomarkers are particularly scarce. Monitoring such dynamics may be critical for assessing treatment effectiveness, improving risk stratification, and identifying personalized preventive strategies.

Objective

To evaluate nitric oxide system parameters in pregnant women at high risk for hypertensive disorders by assessing longitudinal changes in endothelial nitric oxide synthase activity, stable nitric oxide metabolites, and glutathione reductase activity during combined prophylactic therapy.

Materials and Methods

This prospective randomized controlled study included 64 pregnant women at high risk for HDP, enrolled at 12-13 weeks of gestation. Risk stratification followed national clinical guidelines based on the presence of one major or at least two moderate risk factors [23]. All participants received standard prophylaxis recommended by the Ministry of Health of Ukraine: acetylsalicylic acid (150 mg/day) from 12 to 36 weeks of gestation and calcium supplementation (1-

2 g/day) from 16 weeks onward [24]. Random allocation was performed using a computer-generated randomization list (simple randomization, 1:1 ratio) into two study groups: C1 and C2.

Group C1 (n = 32) received standard prophylaxis plus a combined regimen with endothelial-protective and antioxidant properties: Ogestan capsules (containing folic acid 400 μg, cholecalciferol 5 μg, docosahexaenoic acid 200 mg, vitamin E 12 mg, and potassium iodide 150 μg; administered once daily) and L-arginine aspartate oral solution (200 mg/mL; 5 mL three times daily). Group C2 (n = 32) received only standard prophylaxis.

Biochemical evaluation of NO-related markers was performed twice: at enrollment (baseline) and after completion of prophylactic therapy (≥36 weeks of gestation). For dynamic comparison, two post-treatment subgroups were defined: CC1 and CC2, corresponding to Groups C1 and C2 after therapy. Subgroup CC2 included 31 participants (one woman delivered prematurely at 35 weeks before the second sampling), while CC2 comprised all 32 participants who completed both assessments.

Measured parameters included endothelial nitric oxide synthase (eNOS) activity, stable nitric oxide metabolites (NOx), and glutathione reductase (GR) activity. eNOS activity was quantified spectrophotometrically by monitoring the decrease in NADPH absorbance at 340 nm over four minutes, with 1 mM N-nitro-L-arginine used to confirm reaction specificity; results were expressed as nmol/mg protein/min. GR activity was measured spectrophotometrically by assessing the oxidation of NADPH at 340 nm in the presence of oxidized glutathione over five minutes and expressed as μmol/g protein/min. NOx concentration was determined using the Griess reaction after enzymatic reduction of nitrates to nitrites, with absorbance recorded at 540 nm and expressed as μmol/L [25]. All laboratory analyses were conducted at the Educational and Scientific Medical Laboratory Centre of Zaporizhzhia State Medical and Pharmaceutical University using certified equipment and validated protocols. The study complied with ICH/GCP standards, the Declaration of Helsinki (1964, as amended), the Council of Europe Convention on Human Rights and Biomedicine, and relevant Ukrainian legislation. Written informed consent was obtained from all participants.

Statistical analysis was performed using STATISTICA 13 and Microsoft Excel. Data were tested for normality using the Shapiro-Wilk test. Between-group comparisons were performed using the Mann-Whitney U test, and within-group comparisons using the Wilcoxon signed-rank test. Continuous variables are presented as median [Q1-Q3]. A p-value < 0.05 was considered statistically significant.

Results and Discussion

At baseline, no statistically significant differences were observed between Groups C1 and C2 for any of the studied markers ($p > 0.05$) (Table 1).

Table 1

Comparative analysis of oxidative stress markers in Groups C1 and C2

Marker	Group C1	Group C2	p-value
eNOS (nmol/mg protein/min)	22.05 [20.50-24.25]	22.25 [21.18-24.25]	$p > 0.05$
NOx (µmol/L)	21.30 [19.77-22.85]	21.65 [19.35-23.25]	$p > 0.05$
Glutathione reductase (µmol/g protein/min)	7.05 [6.67-7.83]	7.05 [6.62-7.62]	$p > 0.05$

Note: Data are expressed as median [Q1-Q3], where Q1 and Q3 represent the 25th and 75th percentiles, respectively.

Following the prophylactic course, all three parameters: eNOS activity, NOx concentration, and GR activity were significantly higher in Group CC1 compared to CC2 ($p < 0.05$) (Table 2).

Table 2

Comparative analysis of oxidative stress markers in Groups CC1 and CC2

Marker	Group CC1	Group CC2	p-value
eNOS (nmol/mg protein/min)	45.35 [40.20-54.73]	20.90 [19.40-21.95]	$p < 0.05$
NOx (µmol/L)	24.20 [21.70-29.73]	19.00 [17.15-23.15]	$p < 0.05$
Glutathione reductase (µmol/g protein/min)	12.55 [12.10-13.12]	6.70 [6.50-7.50]	$p < 0.05$

Note: Data are expressed as median [Q1-Q3], where Q1 and Q3 represent the 25th and 75th percentiles, respectively

Longitudinal analysis revealed significant post-treatment increases in Group CC1: median eNOS activity rose to 45.35 nmol/mg protein/min, NOx to 24.20 µmol/L, and GR activity to 12.55 µmol/g protein/min (all $p < 0.05$). (Fig. 1).

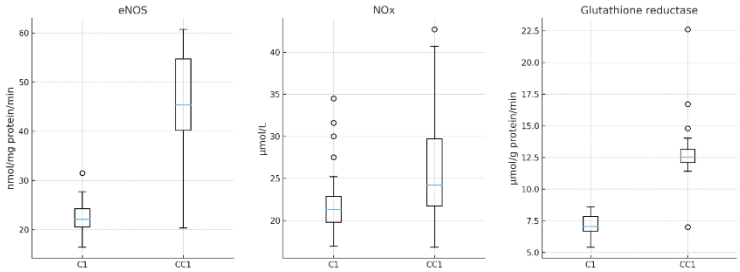

Fig. 1. **Comparison of oxidative stress markers, group C1 and group CC1**

In contrast, Group CC2 showed no statistically significant changes compared with baseline ($p > 0.05$). (Fig. 2).

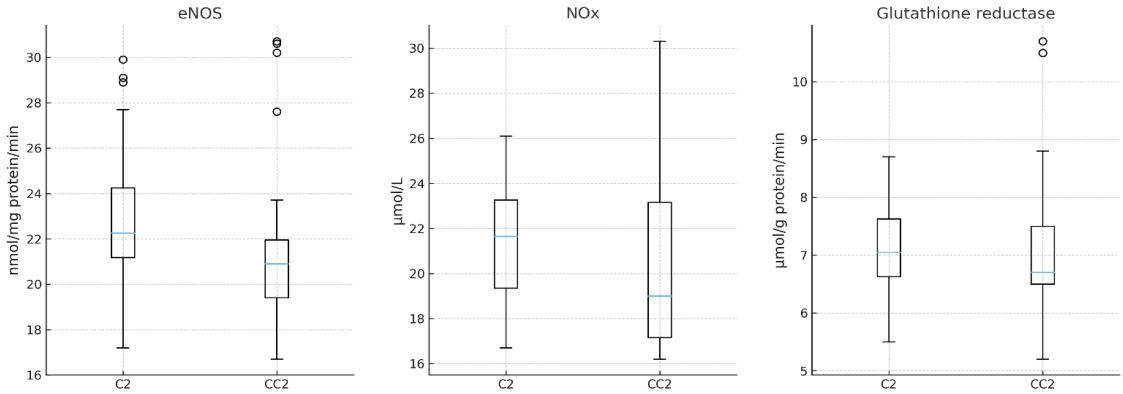

Fig. 2. **Comparison of oxidative stress markers, group C2 and group CC2**

In summary, these findings align with the established understanding of oxidative stress as a central mechanism in PE pathogenesis [1,3,5]. Excessive generation of reactive species, an imbalance between pro-oxidants and antioxidants, and diminished radical-scavenging capacity contribute to endothelial injury underlying the systemic manifestations of PE [2,5,11]. A decline in GR activity indicates activation of nitrosative stress and a reduction in systemic antioxidant capacity [8]. Similarly, reduced eNOS activity and NOx levels reflect impaired endothelium-dependent vasodilation, promoting vasospasm and ischemia [3,5].

The combined preventive regimen – particularly L-arginine and docosahexaenoic acid – demonstrated favorable effects on redox balance and endothelial function, suggesting its potential as an individualized prophylactic approach for women at high risk of PE. These results support the feasibility of modulating oxidative stress pathways as a therapeutic strategy for preventing HDP.

Conclusions

After prophylactic treatment, the group receiving additional endothelial-protective and antioxidant therapy (Group CC1) demonstrated significant improvements in all biochemical markers:

– Endothelial nitric oxide synthase activity increased to 45.35 [40.20-54.73] nmol/mg protein/min.

– Glutathione reductase activity increased to 12.55 [12.10-13.12] µmol/g protein/min.

– Stable nitric oxide metabolite concentration increased to 24.20 [21.70-29.73] µmol/L (all $p < 0.05$).

In contrast, the group that received standard prophylaxis alone (Group CC2)

showed no statistically significant changes in these markers ($p > 0.05$).

The combined prophylactic regimen comprising L-arginine and and a multicomponent supplement providing folic acid, vitamin E, docosahexaenoic acid, vitamin D, and potassium iodide appeared to improve endothelial nitric oxide production, strengthen antioxidant defense mechanisms, and reduce nitrosative stress. These findings support the clinical relevance of the combined prophylactic regimens used for the prevention of hypertensive disorders in high-risk pregnancies.

Moreover, endothelial nitric oxide synthase, stable nitric oxide metabolites, and glutathione reductase may be considered promising biochemical indicators for monitoring the effectiveness of preventive interventions and for early detection of oxidative-stress-related disturbances during pregnancy. Their use could contribute to improved risk stratification and targeted prevention of preeclampsia.

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