NOVEL SCREENING METHODS AND INTERVENTIONS IN PREECLAMPSIA

Ph.D. Thesis Booklet

Balázs Mészáros M.D.

Semmelweis University Doctoral School

Division of Molecular Sciences

SEMMELWEIS UNIVERSITY

Supervisor:	Sándor Valent M.D., Ph.D.
Official reviewers:	Bence Kozma M.D., Ph.D.
	Márton Keszthelyi M.D., Ph.D.
Head of the Complex Examination Committee:	Attila Bokor M.D., Ph.D.
Members of the Complex Examination Committee:	Ábel Altorjay M.D., Ph.D.
	Réka Brubel M.D., Ph.D.

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1. INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific disorder marked by new-onset hypertension after 20 weeks of gestation, often accompanied by maternal organ dysfunction or uteroplacental abnormalities, though proteinuria is no longer essential for diagnosis. It affects 2–8% of pregnancies globally and is a major cause of maternal and neonatal morbidity, particularly in low-income countries. The pathogenesis involves abnormal placental development and the release of antiangiogenic factors, which lead to systemic endothelial dysfunction. Currently, delivery of the fetus and placenta remains the only definitive treatment, underscoring the need for improved screening and treatment options. Early identification of high-risk patients is crucial, with known risk factors including multifetal gestation, preexisting hypertension, diabetes, obesity, and advanced maternal age.

Inflammation plays a role in PE development, and the neutrophil-to-lymphocyte ratio (NLR), an affordable and accessible inflammatory marker, has emerged as a promising predictor of the condition. Studies and meta-analyses have consistently reported elevated NLR in patients with preeclampsia, supporting its potential role in early detection. Pravastatin, a hydrophilic statin, has shown potential in reducing antiangiogenic markers like sFlt-1 and increasing PlGF, thereby countering endothelial dysfunction. Its favorable safety profile, especially during early unintentional pregnancy exposure, makes it a focus of growing research interest. A 2021 meta-analysis found no significant risks of stillbirth or elective abortion linked to statins, though spontaneous abortion risk may increase. While simvastatin may be more potent in reducing sFlt-1 secretion, pravastatin shows similar benefits without the toxic effects seen in other statins. Its unique metabolism and demonstrated efficacy in animal and cell models position pravastatin as a promising therapeutic agent in preeclampsia prevention.

2. OBJECTIVES

The aim of my PhD research was to explore novel screening methods and treatments for preeclampsia by identifying predictive markers and evaluating preventive therapies.

First, I conducted a meta-analysis focusing on the neutrophil-to-lymphocyte ratio (NLR) in the first trimester to determine its value as an early predictor of preeclampsia.

Second, I performed a systematic review and meta analysis on pravastatin therapy, examining its effectiveness and safety in reducing the risks of preeclampsia, intrauterine growth restriction (IUGR), NICU admissions, and preterm births when administered before 20 weeks of gestation. Overall, my work aimed to both identify high-risk patients early and evaluate a potential treatment to improve maternal and fetal outcomes.

3. METHODS

To ensure methodological rigor, both meta-analyses were planned using a PRISMA checklist and followed the MOOSE methods.

3.1 Methods and materials for the first-trimester preeclampsia screening meta-analysis

Two independent researchers collected data for the meta-analysis from PubMed, Scopus, Web of Science, Cochrane Library, and Embase databases. Discrepancies were resolved through consensus or by consulting a third reviewer when it was necessary.

During the time of screening, the research group aimed to identify studies reporting NLR values during the first trimester of pregnancy among women who subsequently developed preeclampsia. These values were then compared to those women in the control groups who maintained normotensive pregnancies without obstetrical complications.

From the studies selected for further review, the following data were extracted: the study objectives, the number of patients with mild preeclampsia, the number of patients with severe preeclampsia, the total number of preeclamptic patients, the number of control (healthy, normotensive) pregnant patients, the timing of data collection (trimester, weeks), NLR values of patients with mild preeclampsia and their corresponding standard deviations, NLR values of patients with severe preeclampsia and their corresponding standard deviations, NLR values of all preeclamptic patients and their corresponding standard deviations, NLR values of healthy, normotensive patients (control group) and their corresponding standard deviations, and p-values.

Additionally, both researchers recorded the titles, authors, publication years, publishers, and DOIs (digital object identifier) of the articles.

The Newcastle–Ottawa scale (NOS) was utilized to assess the quality of the included studies. According to the authors, all included articles received 6 or more stars on the NOS.

The mean difference (MD) with a 95% confidence interval (CI) was employed to represent the effect size. This involved extracting the

number of patients, mean, and standard deviation (SD) of the variable of interest for both the "preeclampsia" and "without preeclampsia" (i.e., control) groups from the studies. The MD is calculated as the mean of the "preeclampsia" group minus that of the "without preeclampsia" group. In cases where means and SDs were provided for moderate and severe preeclampsia subgroups separately, we combined them using established formulae.

Forest plots were used to graphically summarize the results. The confidence interval of each individual study was calculated based on the t-distribution. Additionally, where applicable, we reported prediction intervals (i.e., the expected range of effects of future studies) of results.

All statistical analyses were performed with the usage of R software, with the meta package.

3.2. Methods and materials for evaluating pravastatin's role in preeclampsia prevention

Two independent reviewers gathered data from PubMed, Cochrane, Embase, Web of Science, Scopus, and clinicaltrials.gov databases, covering studies published from January 2003 to July 2022. Language restrictions were not imposed.

Following the selection process, we categorized the studies into the three specified groups.

Two investigators independently assessed the eligibility of retrieved studies based on predetermined criteria. Any disagreements were resolved through consensus and, if needed, a third reviewer was consulted.

Included studies specifically involved the use of statins in human subjects and evaluated their efficacy in preventing and/or treating preeclampsia.

To measure the effect size, we utilized a risk ratio (OR) along with a 95% confidence interval (CI). We employed a random-effects model due to anticipated between-study heterogeneity. Given small sample sizes and instances of zero cell counts, we opted for the exact Mantel-Haenszel method without continuity correction.. Additionally, a Hartung-Knapp

adjustment was applied for conservatism where appropriate. The Paule-Mandel method was used to estimate the heterogeneity variance. Forest plots were employed for graphical representation, with a continuity correction of 0.5 applied for zero cell counts solely for visualization purposes. Due to low study numbers and relatively high heterogeneity, prediction intervals were not presented on plots, and their interpretation was limited. Outlier and influence analyses were less powerful given these factors. All statistical analyses were conducted using R with the meta package.

4. RESULTS

My Ph.D. work explored two key aspects in the context of preeclampsia: the predictive potential of the neutrophil-to-lymphocyte ratio (NLR) and the preventative efficacy of pravastatin.

A meta-analysis of six clinical studies evaluated the use of first-trimester NLR values as early biomarkers for preeclampsia. Initial screening of 324 articles, narrowed to six relevant studies, included 2,469 patients in total. Results revealed an average effect size of 1.082 with a 95% CI of 0.641 to 1.523, indicating NLR's potential predictive value. The between-study heterogeneity was high ($I^2 = 76.5\%$), suggesting variability in true effects. The prediction interval (0.027–2.137) underscores the variability across different populations. These findings support the utility of NLR in early preeclampsia screening, though further validation is needed.

In parallel, a systematic review and meta-analysis were conducted to assess pravastatin's effectiveness in preeclampsia prevention. From 313 initial articles, 5 studies met inclusion criteria for the meta-analysis focusing on pravastatin use before the 20th gestational week.

Pravastatin significantly reduced preeclampsia incidence by 61% (RR = 0.39; 95% CI: 0.186–0.819). Between-study heterogeneity was low ($I^2 = 15\%$), indicating consistent effects across studies. Pravastatin also lowered IUGR risk by 45% (RR = 0.554), although the prediction interval (0.033–9.409) suggested some variability. NICU admissions were reduced by 77% (RR = 0.227), with a strong correlation to higher pravastatin doses. The risk of preterm birth was reduced by 68% (RR = 0.42), with consistent findings across studies ($I^2 = 0\%$).

Further subgroup analyses explored pravastatin use after the 20th week. Though meta-analysis wasn't feasible due to study heterogeneity, results still suggested clinical benefits, particularly when pravastatin was started earlier (20–28 weeks). One study showed reduced preeclampsia incidence and NICU admissions, along with improved birthweight.

In studies addressing pravastatin for treating preexisting preeclampsia, three key investigations highlighted positive outcomes. For example, one trial showed pravastatin prolonged pregnancy by four days and prevented fetal losses, while another reported maternal stabilization with no neonatal adverse effects.

Additional studies examined combination therapies (e.g., pravastatin with aspirin or L-arginine), which demonstrated enhanced benefits, especially in high-risk groups like those with antiphospholipid syndrome (APS) or systemic lupus erythematosus (SLE).

Across all analyses, no serious fetal adverse effects were reported. Mild maternal side effects (e.g., headache, muscle pain) were noted but not clinically concerning.

The cumulative data support the promising role of pravastatin, especially when administered early, in preventing preeclampsia and improving neonatal outcomes.

Meanwhile, NLR appears to be a viable early screening biomarker for preeclampsia, though it may benefit from integration with other markers in predictive models.

Overall, these findings encourage further research into combining NLR and pravastatin in comprehensive screening and prevention strategies for preeclampsia.

5. CONCLUSION

The neutrophil-to-lymphocyte ratio (NLR) appears to be a promising biochemical marker for early detection of preeclampsia. Further research is encouraged to assess its predictive value, especially in combination with other markers, to develop cost-effective screening protocols for the first trimester.

Our meta-analysis indicates that prophylactic pravastatin therapy, when started before the 20th week of gestation, significantly reduces: preeclampsia incidence by 61%, intrauterine growth restriction (IUGR) by 45%, preterm births by 68%, NICU admissions by 77%.

Additionally, no fetal adverse effects were observed, and only mild maternal side effects were reported.

These findings support further investigation into combining predictive markers like NLR with preventive treatments such as pravastatin. This approach may lead to more effective and safer strategies for the early detection and management of preeclampsia.

6. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

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