Introduction. Pregnancy in women with type 1 diabetes mellitus (T1DM) is associated with 2- to 4-fold increased risk of gestational hypertension, preeclampsia (PE), preterm delivery, and perinatal mortality as compared to background population. For many years pregnancy in women with diabetic nephropathy (DN) has been regarded to be accompanied with an even higher risk of perinatal complications and the risk of decline in maternal kidney function leading to ESRD. In an unselected population of pregnant women with T1DM DN is present in 5% to 10% of patients. DN is probably the most commonly CKD seen in pregnancy.

In some studies devoted to long-term effect of gestation on renal function in women with DN, pregnancy was not associated with a greater decline in kidney function or impaired long-term maternal survival (especially in cases with initially normal serum creatinine). Other studies reported the increased risk of more intensive deterioration of kidney function during and after pregnancy with earlier ESRD development and requirement for renal replacement therapy than it would have been expected without pregnancy.

DN may seriously impact the course of pregnancy and mediates its adverse effect through 3 main mechanisms: development of severe hypertension with subsequent deterioration of kidney function in a mother; preterm delivery due to high maternal blood pressure and pre-eclampsia; fetal intrauterine growth restriction and fetal distress caused by placental dysfunction. High creatinine concentrations (>176 µmol/L), proteinuria within the nephrotic range (>3 g/24 h), pre-existing cardiovascular disease and severe hypertension is associated with a high risk for poor maternal and fetal outcomes. Thus, hypertensive disorders (either pre-existing, or gestational and, in particular, PE) may play an essential role in determining maternal and fetal outcomes in patients with pre-existing T1DM and DN.

PE is reported in up to 64% of women with pre-existing DN. The prevalence is higher in the presence of reduced kidney function, microalbuminuria (or nephrotic proteinuria) and hypertension at the start of pregnancy. Preterm delivery before 34 gestational weeks due to PE has been reported in up to 45% mothers with pre-existing DN. Prediction of hypertension will allow to apply corresponding preventive measures, closer surveillance and to improve maternal/perinatal outcomes in these patients.

The objective of the study was to detect factors which may contribute to and predict preeclampsia development in T1DM patients with concomitant DN on the basis of evaluation of clinical and laboratory parameters, profiles of polymorphic variants of genes, as well as gene-gene and gene-environment interactions.
Materials and methods: The study was accomplished according to “a case-control study” design with retrospective and prospective data involved. The inclusion criteria were T1DM preceding pregnancy (Classes B to T according to the White classification); development of PE during the current pregnancy; singleton pregnancy; completion of the informed consent to participate in the study. Patients were excluded from the study due to the following conditions: history of chronic hypertension; history of molar fetal hydrops (confirmed by US and/or pathomorphology); multiple pregnancy; smoking; patient’s desire to withdraw from the study in each stage. PE was reported according to the pre-defined diagnostic criteria based on Ukrainian national guidelines, as well as diagnostic criteria established by ACOG, RCOG, SOGC, NICE and National Working Group on Hypertension in Pregnancy. DN and its stages were verified in compliance with E. C. Mogensen classification. Pregnant women who met eligibility criteria underwent standard examination and were divided into two groups: group I included 30 T1DM patients with superimposed PE; group II – 30 T1DM pregnant women without PE. Molecular genetic testing was used for detection of 7 polymorphic variants within 5 genes: A1166C_AT2R1; C108T_PON1; Thr83Ala_ and T138C_MGP; 4b/4a_ and G894T_eNOS; I/D_ACE. Genetic investigation included allele-specific PCR with subsequent restriction analysis. The collected data were analyzed using the Student’s t-test, Pearson’s chi-square test with subsequent odds ratio (OR) and 95% confidence interval (95%CI) calculation (SPSS 17.0 software package). The relative quality of statistical models for a given set of data was measured with Akaike information criterion. Binary logistic regression was used in order to confirm the validity of the generated prediction models. Gene-gene and gene-environment interactions were studied using entropy-based MDR method (software version 2.0) and MDR permutation testing. The differences were considered to be statistically significant with p-value <0.05.

Results: DN was reported in 23 patients of group 1 (76.66%) as compared to 13 patients of group 2 (36.66%) (χ²=8.21; p<0.01; OR=5.68; 95%CI 1.84-17.49). When background clinical characteristics were compared between Group I subjects with and without concomitant DN, patients with nephropathy showed statistically longer duration of diabetes (14.17±0.93 vs. 8.22±2.69 years; p< 0.05); significantly higher prevalence of diabetic retinopathy (82.61% vs.14.28%; χ²=8.41; p=0.004; OR=28.50; 95%CI 2.65-30.64) and no differences in frequencies of other microangiopathies. Further, the profiles of polymorphic variants of genes were investigated in Group 1 patients with and without concomitant DN. Frequencies of MGP -138TC and -138CC genotypes (dominant model) significantly differed in group 1 pregnant women with and without initial DN (χ²=6.41, p=0.03, OR=0.11 95%CI (0.01-0.77) due to their higher prevalence in pregnant women without DN. In order to find out
whether the above-mentioned polymorphic variants of *MGP* and other genes increased the risk of PE development in the absence pre-existing DN, genetic profiles of non-nephropathy patients of both groups were studied. In T1DM patients either with or without nephropathy *ACE* ID and DD genotypes were associated with the increased probability of PE development ($\chi^2=5.78; p=0.03; OR=13; 95\%\ CI\ 1.71-76.08$), while II-genotype significantly attenuated risk of PE ($\chi^2=4.05; p=0.044; OR=0.08; 95\%\ CI\ 0.01-0.79$). Moreover, *MGP* -138TC genotype was found to be more prevalent in non-DN group 1 patients (71.43%) as compared to non-DN group 2 patients (26.32%) ($\chi^2=3.89; p=0.046; OR=7.00; 95\%\ CI\ 2.01-48.31$). The frequencies of *MGP* -138CC genotype were comparable between non-DN patients of both groups. Based on the Akaike information criterion value (AIC=11.12), the supradominant model seemed to be the most reliable in prediction of PE risk within T1DM women. According to this model, the presence of -138TC genotype in patients without pre-existing DN was associated with 7-fold higher risk of PE development as compared to carriers of -138TT and -138CC genotypes (OR=7.00; 95\%CI 1.13-61.57). Taking into consideration the established role of polymorphic variants of *ACE* and *MGP* genes in PE development, we analyzed the occurrence of different combinations of their causative genotypes (MGP_138TC/ACE_ID; MGP_138TC/ACE_DD; MGP_Thr83Ala/ACE_ID; MGP_Thr83Ala/ACE_DD) within patients of both groups. Irrespective of presence or absence of DN, cumulative incidence of such combinations was significantly higher in group 1 in comparison to group 2 ($\chi^2=6.79; p=0.018; OR=4.13; 95\%\ CI\ 1.39-12.27$). This finding supported out suggestion that the presence of *MGP* Thr83Ala and -138TC genotypes in combination with *ACE* DD and ID genotypes enhanced the risk of PE development in T1DM pregnant women regardless of the presence or absence of DN. Taking into consideration the obtained data, we decided to study gene-gene interactions, which might to predispose to PE development in T1DM pregnant women with background DN. Only 2 combinations of polymorphic variants were associated with PE development in this specific population of patients: 1) PON_C108T + ACE (cumulative entropy index elevated by 19.14% in the case of genotypes’ combination); 2) MGP_Thr83Ala + eNOS_G894T (cumulative entropy index increased by 7.51%). We used binary logistic regression to establish whether PON_C108T polymorphism strengthened the impact of DN on PE risk elevation. The generated model with prognostic value of 68.3% showed that in the presence of DN *PON1* -108CT genotype increased the risk of PE by 8-fold ($p=0.004; OR=8.00; 95\%\ CI\ 1.923-33.274$). In contrast, *PON1* 108TT genotype demonstrated limited and insignificant influence on PE development in women with pre-existing DN ($p=0.282; OR=2.462; 95\%\ CI\ 0.476-12.716$). When gene-environment interactions were studied with MDR, systolic blood pressure demonstrated synergistic additive effect with clinical factors.
(age at diagnosis of diabetes and age of a pregnant woman) and diastolic blood pressure – with genetic factors (in particular, with the studied polymorphic variants).

**Conclusions:** In the presence of DN the following factors correlate with preeclampsia development: long diabetes duration (>14 years); diabetic retinopathy; *PON1* genotype 108CT and *ACE* genotypes ID and DD. In the absence of DN *MGP*-138TC genotype plays an important role. II-genotype of *ACE* gene provides protective effect against PE development. The most significant combinations of genes related to PE development are as follows: *ACE* + *PON1*, *MGP* (Thr83Ala) + *eNOS*. Identification of the abovementioned polymorphic variants of genes may be recommended in patients with T1DM and concomitant DN in order to detect women at high risk for PE development.