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What is in common between preeclampsia, HPS70 and medieval headwear? Part II. Serum HSP70 in superimposed preeclampsia: original study

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The objective: **to investigate serum heat shock protein 70 (HSP70) concentration in pregnant women with chronic hypertension and superimposed preeclampsia. To assess the prospects of using HSP70 as a superimposed preeclampsia predictor.**
Materials and methods**. The original prospective cohort single-center observational study included 105 pregnant women with chronic hypertension and 34 healthy pregnant women as a control group. Serum HSP70 was measured via enzyme-linked immunosorbent assay.**
The first measurement point of serum HSP70 was at 28 weeks of gestation, the second measurement point was at 36 weeks in case of absence of preeclampsia or at 29-35 weeks in case of preeclampsia. If signs of preeclampsia appeared later than 36 weeks, additional HSP70 measurement was not performed.
Statistical analysis was conducted using EZR 1.55 software.
Results**. In the study group (105 pregnant women with chronic hypertension), after delivery 30 patients had signs of superimposed preeclampsia (superimposed preeclampsia subgroup) and 75 persons had no signs of superimposed preeclampsia (chronic hypertension subgroup). In the control group (34 healthy pregnant women) 3 patients after delivery had preeclampsia signs (preeclampsia subgroup) and another 31 had no signs of preeclampsia (healthy subgroup).**
Serum HSP70 levels, obtained at the first measurement point (28 weeks) was statistically significantly different between three subgroups: patients with chronic hypertension vs. patients with superimposed preeclampsia ($p<0.01$), healthy pregnant women vs. chronic hypertension women ($p<0.0001$), healthy persons vs. superimposed preeclampsia persons ($p<0.0001$).
Serum HSP70 concentrations at the second measurement point (36 weeks, or 29-35 weeks in case of preeclampsia) also had a statistically significant difference for each pair of subgroups ($p<0.001$). Given the small number of persons in the preeclampsia subgroup (3 women), it was not included in the calculations.
No statistically significant difference between serum HSP70 levels in the first and second measurement points was found in healthy pregnant women subgroup. In the subgroup of pregnant women with chronic hypertension without superimposed preeclampsia complications, a statistically significant increased serum HSP70 concentration was found at 36 weeks compared to 28 weeks ($p<0.0001$). Even bigger growth of HSP70 levels compared to 28 weeks occurred in case of joining preeclampsia to chronic hypertension ($p<0.0001$).
Conclusions**. The data suggest an increased concentration of HSP70 in pregnant women with superimposed preeclampsia compared to pregnant women with chronic hypertension of the corresponding term. Thus, it is possible to assume that HSP70 plays a role in superimposed preeclampsia pathogenesis.**
The increased serum HSP70 levels in pregnant women with chronic hypertension, compared to healthy pregnant women of the corresponding gestational age was determined. Therefore, it can be argued that HSP70 has an influence on the course of chronic hypertension during pregnancy.
No statistically significant dependance of serum HSP70 level in healthy pregnant women on gestational age was found. The increase in the level of HSP70 in pregnant women with chronic hypertension with increasing gestational age is most likely due to the progression of hypertensive disorders and/or related conditions. The highest HSP70 increase was observed in pregnant women with chronic hypertension with the addition of preeclampsia.
The use of HSP70 as the only predictor of superimposed preeclampsia is not effective, given its non-specificity, but the use of this indicator in combination with other markers is promising and requires further study.
Keywords: *HSP70, heat-shock protein 70, preeclampsia, superimposed preeclampsia, chronic hypertension, pregnancy.*

Що спільного між прееклампсією, HSP70 та середньовічним капелюхом? Частина II. Сироватковий HSP70 при накладеній прееклампсії: оригінальне дослідження

О. К. Попель, Д. О. Говсєєв

Мета дослідження: вивчення концентрації heat shock protein 70 (HSP70) у сироватці крові вагітних із хронічною гіпертензією та накладеною прееклампсією. Оцінювання перспективи використання HSP70 в якості предиктора накладеної прееклампсії.

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Матеріали та методи. Проведено оригінальне проспективне когортне одноцентрове обсерваційне дослідження, що включало 105 вагітних із хронічною гіпертензією та 34 здорові вагітні у якості контрольної групи. Вміст HSP70 у сироватці крові визначали за допомогою імуноферментного аналізу.
Перша точка дослідження сироваткового HSP70 була обрана у 28 тиж, друга точка – у 36 тиж у разі відсутності прееклампсії або у 29–35 тиж у разі приєднання прееклампсії. Якщо ознаки прееклампсії з'являлись пізніше 36 тиж гестації, додаткове визначення вмісту HSP70 не проводили.
Для статистичного аналізу застосовували програмне забезпечення EZR 1.55.
Результати. З групи дослідження (105 вагітних із хронічною гіпертензією) після пологів 30 породіль мали ознаки накладеної прееклампсії (підгрупа накладеної прееклампсії), 75 породіль не мали ознак накладеної прееклампсії (підгрупа хронічної гіпертензії). З контрольної групи (34 здорові вагітні) після пологів 3 породіллі мали ознаки прееклампсії (підгрупа прееклампсії), 31 породілля не мала ознак прееклампсії (підгрупа здорових).
У першій точці дослідження (28 тиж) сироваткові рівні HSP70 продемонстрували статистично значущу різницю між трьома підгрупами: при порівнянні вагітних із хронічною гіпертензією та накладеною прееклампсією – на рівні $p<0,01$, здорових вагітних із вагітними з хронічною гіпертензією – на рівні $p<0,0001$, здорових вагітних із вагітними з накладеною прееклампсією – на рівні $p<0,0001$.
Сироваткові концентрації HSP70 у другій точці дослідження (36 тиж, або у 29–35 тиж у разі приєднання прееклампсії) також мали статистично значущу різницю для кожної пари підгруп на рівні $p<0,001$. Підгрупу прееклампсії, ураховуючи малу чисельність (3 породіллі), не включали у розрахунки.
У підгрупі здорових вагітних не виявлено статистично значущих змін сироваткових рівнів HSP70 у першій та другій точках дослідження. У підгрупі вагітних з існуючою раніше гіпертензією, яка не ускладнилась накладеною прееклампсією, було виявлено статистично значуще зростання сироваткового рівня HSP70 у терміні 36 тиж порівняно з 28 тиж ($p<0,0001$). Ще більше підвищення рівня HSP70 порівняно з 28 тиж фіксували у разі приєднання прееклампсії до хронічної гіпертензії ($p<0,0001$).
Висновки. Отримані результати свідчать про підвищену концентрацію HSP70 у вагітних із накладеною прееклампсією порівняно з вагітними у відповідному терміні з хронічною гіпертензією. Отже, можна припустити участь HSP70 у патогенезі накладеної прееклампсії.
Установлено зростання у сироватці крові рівня HSP70 у вагітних із хронічною гіпертензією порівняно із сироватковими рівнями у здорових вагітних у відповідний гестаційний термін. Отже, можна стверджувати, що HSP70 має значення у перебігу хронічної гіпертензії під час вагітності.
Не було виявлено статистично значущої залежності рівня HSP70 у здорових жінок від гестаційного терміну. Зростання рівня HSP70 у вагітних із хронічною гіпертензією при збільшенні терміну гестації зумовлено, скоріш за все, прогресуванням гіпертензивних розладів та/або станів, з цим пов'язаних. Найбільш виражене підвищення вмісту HSP70 спостерігалося у вагітних із хронічною гіпертензією при приєднанні прееклампсії.
Використання HSP70 як єдиного предиктора накладеної прееклампсії не є ефективним, ураховуючи його неспецифічність, однак застосування даного показника у комплексі з іншими маркерами перспективне та потребує подальшого вивчення.
Ключові слова: *HSP70, heat shock protein 70, прееклампсія, накладена прееклампсія, хронічна гіпертензія, вагітність.*

Chronic hypertension during pregnancy has become more common in recent decades. For example, in the United States of America (USA) in 1996, only 0.97% of pregnant women had chronic hypertension, in 2008 – 1.76% [1], in 2017 – 2.0%, in 2019 – 2.3% [2]. Today chronic hypertension prevalence among pregnant women varies from 1% to 5% depending on the region [3–6], it is higher in low- and middle-income countries [6, 7].

Pregnant women with chronic hypertension have an increased risk of preeclampsia development. 17-50% of pregnant women with chronic hypertension are diagnosed with superimposed preeclampsia, this number varies significantly in different populations [1, 6, 8, 9, 10]. According to the results of a meta-analysis, which combined 795 221 pregnant women from 55 studies in the USA up to 2013 year, 25.9% developed superimposed preeclampsia (95%CI 21.0% - 31.5%) [5]. According to retrospective study of the World Health Organization (WHO) database, conducted in 2014 year, chronic hypertension was significantly increasing preeclampsia risk, the adjusted odds ratio was 8.32 (95%CI 7.13–9.72) [11]. Women in whom hypertension progresses and secondary organ damage occurs, have an increased frequency of preeclampsia development, with secondary hypertension reaching 75% [1].

Superimposed preeclampsia is associated with a greater number of complications for the mother, fetus, and newborn [1, 3, 5, 9–12]. A further study of superimposed preeclampsia pathogenesis and search for its predictors is required [13, 14].

Currently, the following processes are considered to be part of preeclampsia pathogenesis: placental ischemia due to disruption of cytotrophoblast invasion and spiral artery remodeling leads to an imbalance of pro- and anti-angiogenic factors, generalized endothelial dysfunction, excessive inflammatory response and oxidant stress [14]. These conditions, as well as hemodynamic stress, are triggers of heat shock protein 70 (HSP70) overexpression [15].

Our meta-analysis, which included 16 studies regarding serum HSP70 levels, having a total of 751 patients with preeclampsia and 719 healthy patients in the control group (part I of the article), allowed us to conclude that the HSP70 concentration in women with preeclampsia is higher than in healthy pregnant women of corresponding gestational age [15]. Studies of serum HSP70 in pregnant women with chronic hypertension and superimposed preeclampsia are scarce.

A. Molvarec et al. (2006) studied serum HSP70 levels in pregnant women with hypertensive disorders during pregnancy, including 20 patients with superimposed preeclampsia [16]. A statistically significant difference was obtained between serum HSP70 concentrations in pregnant women with superimposed preeclampsia compared to corresponding values in the group of healthy pregnant women. Serum HSP70 levels in pregnant women with superimposed

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preeclampsia had no statistically significant difference compared to corresponding levels in the preeclampsia and gestational hypertension groups. The study had limitations due to its power and case-control design.

However, a large number of scientists have paid much attention to study of HSP70 role in hypertension [15, 17–23] and related cardiovascular diseases [24], including atherosclerosis [25, 26], heart failure and ischemic heart disease [27–30], vascular disease [31].

HSP70 performs both chaperone functions and has cytokine activity, leading to either immunogenic tolerance or immunogenic reactivity depending on the context [18, 19]. Therefore, HSP70 either enables the body to adapt to hemodynamic stress or becomes involved in the progression of hypertensive disorders.

Some works consider the possibility of immune therapy during hypertension [23, 29–33]. This is interesting because up to 15–19% of arterial hypertension cases are cases of resistant hypertension – patients do not reach arterial pressure target values despite medication composed of three drugs of certain groups in maximum or near-maximum doses [34–36]. Given the high frequency of preeclampsia in pregnant women with chronic hypertension and lack of treatment options for superimposed preeclampsia other than delivery, even the appearance of works in such direction looks perspective.

A prospective cohort single-center observational study of pregnant women with chronic hypertension and superimposed preeclampsia was designed and conducted in Kyiv City Maternity Hospital No. 5 (KCMH No. 5) in Kyiv (Ukraine). The initial hypothesis was that HSP70 levels in blood serum increase both in superimposed preeclampsia and in pregnant women with chronic hypertension.

Purpose of the study: investigation of serum heat shock protein 70 (HSP70) concentration in pregnant women with chronic hypertension and superimposed preeclampsia. Assessment of the prospects of using HSP70 as a superimposed preeclampsia predictor.

MATERIALS AND METHODS

The study was conducted and presented based on CONsolidated Standards Of Reporting Trials (CONSORT-Outcomes 2022) guidelines – part of Enhancing the QUAlity and Transparency Of health Research (EQUATOR) system [37].

The study included pregnant women who were observed and gave birth in KCMH No. 5 from March 1, 2021 to March 15, 2023.

Sample size estimation was based on the data of previous authors [16, 38–40], assuming a statistical power of 80% and a significance level of $p = 0.05$, as well as considering data on the prevalence of superimposed preeclampsia and preeclampsia in the population [1–9]. In addition to HSP70, the study investigated the ratio of fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF), so the sample size was calculated for each variable, the maximum of them was chosen as the required size samples for research. Another 10% was added to the required sample size to compensate for possible losses during the study.

Therefore, the research group (chronic hypertension) included 119 patients with essential chronic hypertension, singleton pregnancies, without fetal malformations, at 24–28 weeks. Exclusion criteria were type 1 and type 2 diabetes, autoimmune diseases with potential vascular complications, cancer, human immunodeficiency viruses (HIV) or syphilis infection. The control group (healthy) consisted of 38 pregnant women, enrolled in the study at 24–28 weeks, with a singleton pregnancy without extragenital diseases and obstetric complications at the beginning of the study.

The main outcome in the chronic hypertension group was the occurrence of superimposed preeclampsia. The patient was diagnosed with chronic hypertension if arterial hypertension existed before pregnancy or was recorded at least twice before 20 weeks of gestation. The criteria for arterial hypertension during pregnancy were systolic blood pressure (SBP) of 140 mmHg and/or diastolic blood pressure (DBP) of 90 mmHg. The same values of SBP and DBP were chosen as arterial hypertension criteria before pregnancy for the following reasons.

In 2017, the American College of Cardiology (ACC) and the American Heart Association (AHA) began implementing lower blood pressure targets in order to reduce cardiovascular events and deaths [41]. The blood pressure threshold for arterial hypertension diagnosis was lowered: stage 1 arterial hypertension (stage 1 AH) in case of 130–139 mmHg SBP, 80-89 mmHg DBP, whereas the traditional definition of arterial hypertension (SBP ≥ 140 mm Hg, DBP ≥ 90 mmHg) became stage 2 arterial hypertension (stage 2 AH) [41]. But currently, the American College of Obstetricians and Gynecologists (ACOG) guidelines for hypertension during pregnancy do not use the ACC/AHA criteria [1, 42]. There is still no evidence that arterial hypertension treatment and aspirin prophylaxis reduces perinatal and maternal risk in pregnant women with chronic hypertension who meet the current ACC/ANA criteria for stage 1 AH, although studies are being conducted [43–50]. Current guidelines in Ukraine also still use the traditional classification of arterial hypertension, and women were diagnosed according to it before pregnancy [51, 52]. Therefore, in our study, the term «chronic hypertension» was used for pregnant women who meet the current ACC/ANA criteria for stage 2 AH.

Pregnant women with chronic hypertension were diagnosed with superimposed preeclampsia according to ACOG recommendations either in the event of sudden increase of initial blood pressure, which was previously well controlled, need for antihypertensive therapy rapid increase; the sudden proteinuria onset or an increase in proteinuria present before pregnancy and/or in the first trimester, or the occurrence of organ disorders [42].

The first measurement point of serum HSP70 was chosen at 28 weeks. The second measurement point – at 36 weeks in the absence of preeclampsia or at 29- 35 weeks in case of preeclampsia signs. If preeclampsia signs appeared at 37 weeks or later, additional blood sampling was not performed. Participants were excluded from the study if 2 blood samples of serum HSP70 were not obtained according to the study protocol, or if before obtaining both samples,

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the pregnant woman had spontaneous labor, premature rupture of the amniotic membranes, or premature delivery for reasons unrelated to preeclampsia onset.

During the observation before the second serum HSP70 sample was obtained, 14 pregnant women were excluded from the study group due to premature rupture of membranes (PROM), premature spontaneous delivery, or delivery not associated with preeclampsia (8 patients), or residence change as a result of the war (6 patients). Losses were less than 5% of the calculated sample, which is statistically insignificant. From control group 4 pregnant women were excluded before the second blood sampling (preterm delivery, premature birth not related to preeclampsia, change of residence). The schematic representation of the study is presented in Figure 1.

Women refrained from eating for 8 hours before blood sampling. Maternal venous blood from the medial cubital vein was collected in standard tubes, centrifuged at room temperature (10 minutes at 3000 rpm to separate serum), serum was collected and stored in Eppendorf-type microtubes at -40°C until analysis.

Serum HSP70 analysis was performed the day after delivery of all women who participated in the study, the obtained results had no influence on the choice of management plan for pregnancy and delivery.

Serum HSP70 levels were measured by an enzyme-linked immunosorbent assay ELISA Kit (Wuhan Fine Biotech Co., Ltd., China, catalog number EH3242) according to the manufacturer's instructions. The standard curve was 31.25 - 2000 pg/ml, sensitivity was 18.75 pg/ml. The optical density was measured at λ=450 nm.

The research protocol was approved by the bioethics committee of the Bogomolets National Medical University (18.10.2021, № 150). Each pregnant woman, who participated in the study, signed an informed consent.

Statistical analysis

The normality of continuous variables was checked via Shapiro-Wilk's W test. The normal distribution hypothesis of HSP70 was rejected ($p<0.01$ at both measurement points for the superimposed preeclampsia subgroup; $p=0.016$ and $p<0.01$ at 1 and 2 measurement points, respectively, for the chronic hypertension subgroup; $p<0.01$ and $p<0.001$ at 1 and 2 points, respectively, for the healthy subgroup), so that, non-parametric statistical tests were used. The Kruskal–Wallis test (non-parametric ANOVA) was used to compare medians in several groups and Mann–Whitney test was used for multiple pairwise comparisons. For categorial variables comparison Chi-square test was applied. Multivariate logistic regression model was built with adjustment for the following variables: maternal age, body mass index (BMI), first birth, smoking status, newborn weight. Statistical analysis was carried out in the EZR 1.55 programs [53].

Normally distributed data was presented as mean and standard deviation, non-normally distributed data was presented as median and interquartile range (Q1–Q3). If the distribution of parameter differed from normal for at least one subgroup, data for this parameter was considered as non-normal. Categorical variables were presented as numbers and percentages.

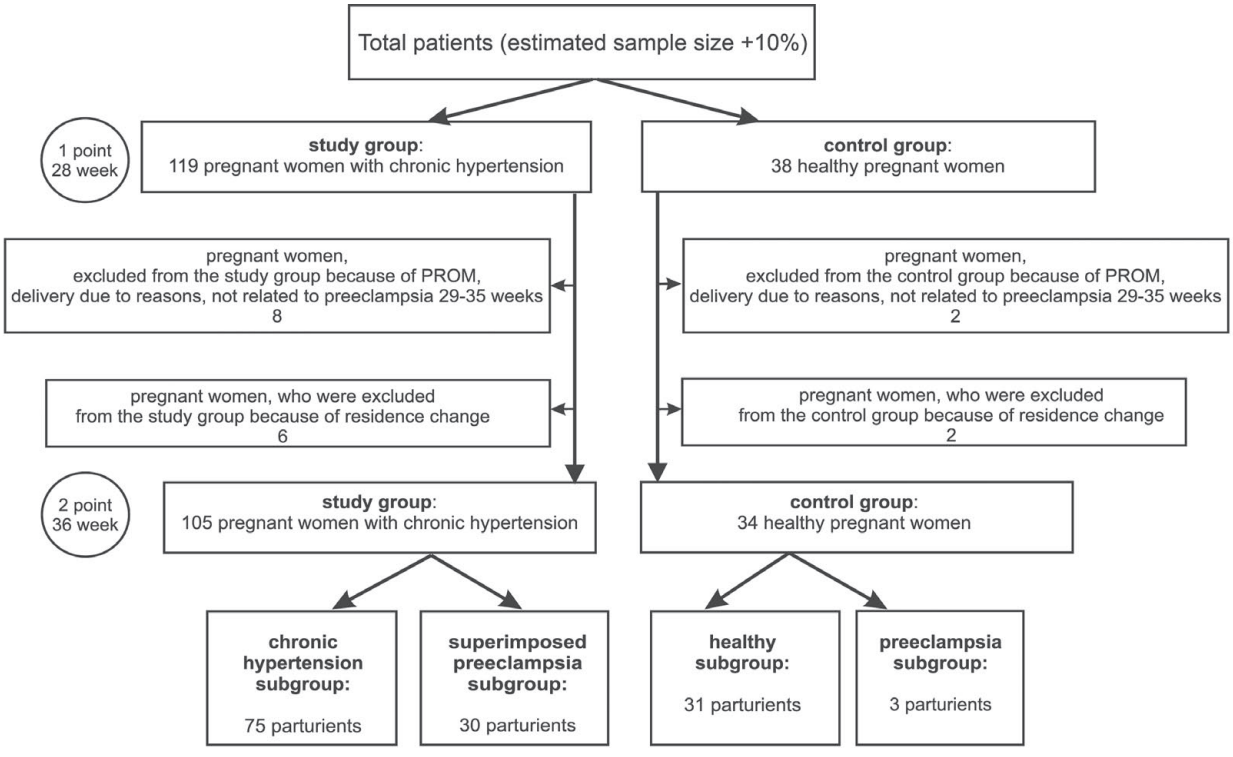

Fig. 1. Schematic representation of the study

RESULTS AND DISCUSSION

After observation, the study group consisted of 105 pregnant women with chronic hypertension and the control group of 34 healthy pregnant women. The study group (105 pregnant women with chronic hypertension) at the time of delivery, had 30 parturients (28.6%) with superimposed preeclampsia signs (superimposed preeclampsia subgroup), 75 parturients (71.4%) without superimposed preeclampsia signs (chronic hypertension subgroup). As for control group (34 pregnant women), at the time of delivery, 3 patients (8.8%) developed preeclampsia (preeclampsia subgroup), 31 patients (91.2%) had no signs of preeclampsia (healthy subgroup). Characteristics of patients in the study and control groups are presented in Table.

A statistically significant difference in means of maternal age at delivery was found between certain subgroups: healthy vs. chronic hypertension ($p<0.01$), healthy vs. superimposed preeclampsia ($p=0.03$). No statistically significant difference in maternal age at delivery between chronic hypertension subgroup and superimposed preeclampsia subgroup was found.

The medians of BMI, recorded at the first visit during pregnancy, in subgroups with chronic hypertension and superimposed preeclampsia were higher than in the healthy subgroup ($p<0.0001$). No statistically significant difference in BMI median between chronic hypertension and superimposed preeclampsia subgroups was found.

There was almost no difference between the relative number of primiparas in the subgroups of chronic hypertension and superimposed preeclampsia (48% vs. 50% respectively); the healthy control subgroup had 74.2% of primiparous women. Comparison of three subgroups together gave a statistically significant difference ($p=0.04$). Nevertheless, subsequent multiple comparisons did not find any statistically significant difference. This suggests that sample of bigger size must be used for investigation of primiparas proportions.

Childbirth in the superimposed preeclampsia subgroup occurred earlier than in both chronic hypertension ($p<0.01$) and healthy subgroups ($p<0.0001$), and chronic hypertension subgroup had early labor than healthy subgroup ($p<0.01$).

During the evaluation of newborns weight, a statistically significant difference was found in the average weight between superimposed preeclampsia and healthy subgroup ($p=0.046$).

Pregnant smokers were more common in the superimposed preeclampsia subgroup (16.7%) than in chronic hypertension (4%) and healthy subgroups (3%). Three subgroups comparison found a statistically significant difference ($p=0.04$), however, multiple comparisons afterwards gave no statistically significant difference.

The subgroup of preeclampsia was not analyzed due to a small size (3 pregnant women).

For all pregnant women the assessed HSP70 concentration was above the sensitivity limit of the laboratory test. Median levels of HSP70 at 1 measurement point differed in three subgroups: chronic hypertension 0.347 (0.293–0.382) ng/ml, superimposed preeclampsia 0.390 (0.355–0.455) ng/ml, healthy 0.213 (0.194–0.295) ng/ml (chronic hypertension vs. superimposed preeclampsia $p<0.01$, chronic hypertension vs. healthy $p<0.0001$, superimposed preeclampsia vs. healthy $p<0.0001$).

Median levels of HSP70 at the second measurement point also had a statistically significant difference between all pairs of subgroups at the $p<0.001$ level: chronic hypertension 0.367 (0.316–0.410) ng/ml, superimposed preeclampsia 0.429 (0.392–0.518) ng/ml, healthy 0.236 (0.192–0.313) ng/ml.

Clinical characteristics and serum HSP70 concentrations, study group (chronic hypertension subgroup and superimposed preeclampsia subgroup) and control group (healthy subgroup and preeclampsia subgroup)

Subgroups	Study group – 105 parturients		Control group - 34 parturients		Upper value of p		
	Chronic hypertension subgroup n=75	Superimposed preeclampsia subgroup n=30	Healthy subgroup n=31	Preeclampsia subgroup n=3	a	b	c
Relative number of parturients	71.4%	28.6%	91.2%	8.8%			
Age (years)*	35.0 (31.0–38.0)	33.0 (31.0–38.8)	30.0 (26.0–34.0)	35.0 (34.0–36.5)	<0.01	=0.03	NS
BMI (kg/m2)*	33.0 (29.3–35.9)	33.7 (30.5–37.0)	25.5 (23.4–27.1)	26.6 (25.7–26.9)	<0.0001	<0.0001	NS
Primiparas	36 (48%)	15 (50%)	23 (74.2%)	3 (100%)	NS	NS	NS
Smokers	3 (4.0%)	5 (16.7%)	1 (3.3%)	0 (0%)	NS	NS	NS
Gestational age at delivery (weeks)*	39.0 (38.0–39.0)	37.5 (36.0–38.8)	40.0 (39.0–40.0)	39.0 (35.0–39.5)	<0.01	<0.0001	<0.01
Fetal birth weight (grams)*	3400 (3025–3650)	3170 (2340–3645)	3540 (3195–3705)	2960 (2220–3205)	NS	=0.046	NS
HSP70, 1 point (ng/ml)*	0.347 (0.293–0.382)	0.390 (0.355–0.455)	0.213 (0.194–0.295)	0.366 (0.327–0.394)	<0.0001	<0.0001	<0.01
HSP70, 2 point (ng/ml)*	0.367 (0.316–0.410)	0.429 (0.392–0.518)	0.236 (0.192–0.313)	0.467 (0.407–0.496)	<0.0001	<0.0001	<0.001

Notes: *median, Q1 – Q3; NS - not significant.

a – Statistically significant difference between subgroups: healthy vs. chronic hypertension.

b – Statistically significant difference between subgroups: healthy vs. superimposed preeclampsia.

c – Statistically significant difference between subgroups: chronic hypertension vs. superimposed preeclampsia.

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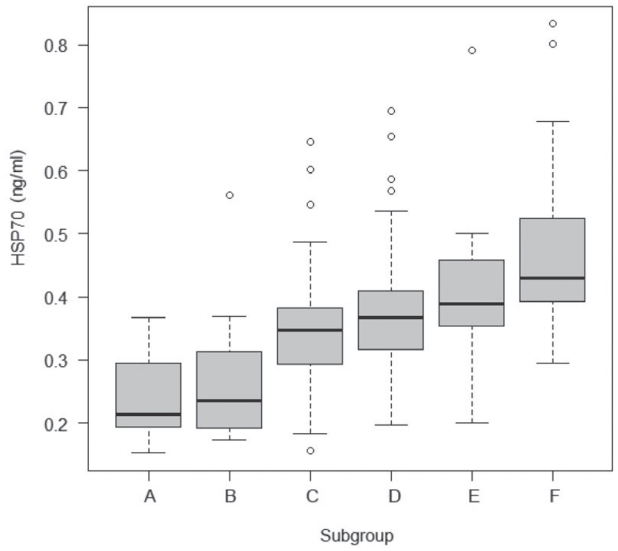

A – healthy subgroup, 28 weeks
B – healthy subgroup, 36 weeks

C – chronic hypertension subgroup, 28 weeks
D – chronic hypertension subgroup, 36 weeks

E – superimposed preeclampsia subgroup, 28 weeks
F – superimposed preeclampsia subgroup, time when preeclampsia signs occurred.

Middle line: median; box: interquartile range; whiskers: 1.5×interquartile range (1.5×IQR); points: outliers.

Fig. 2. HSP70 (ng/ml) in healthy subgroup (A, B), chronic hypertension subgroup (C, D), superimposed preeclampsia subgroup (E, F)

No statistically significant difference in HSP70 levels between two related samples in the healthy subgroup at 28 weeks and 36 weeks was found (p=0.0502). In chronic hypertension subgroup, the corresponding HSP70 levels had a statistically significant increase in the period of 36 weeks compared to 28 weeks (p<0.0001). Superimposed preeclampsia subgroup had even greater growth in HSP70 levels in compared to 28 weeks (p<0.0001).

A graphic representation of HSP70 levels in three subgroups for control points 1 and 2 is presented in Figure 2. Medians, 25th and 75th percentiles (Q1 and Q3) of HSP70 in ng/m, as well as whiskers, which determine non-outliers range, and outliers. For healthy and chronic hypertension subgroups, HSP70 concentrations are shown for 28 and 36 weeks. For the superimposed preeclampsia subgroup, HSP70 levels depicted at 28 weeks and at the time of preeclampsia signs occurrence are given.

Different models for superimposed preeclampsia prediction in study group were built. ROC curve for prediction of superimposed preeclampsia development in pregnant women with chronic hypertension based only on the HSP70 level at 28 weeks (Figure 3) had the area under the curve (AUC) 0.696 (95% CI 0.587–0.806), with optimal cut-off 0.354 ng/ml. This threshold had 76.7% sensitivity and

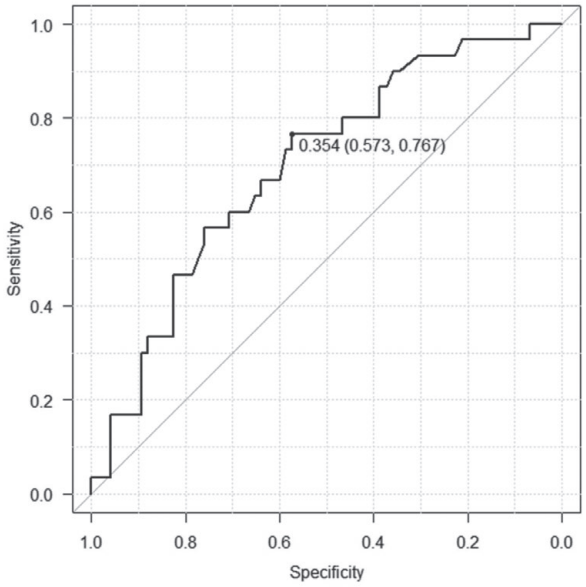

Fig. 3. ROC curve for prediction of superimposed preeclampsia in pregnant women with chronic hypertension via HSP70 levels at 28 weeks

57.3% specificity. This suggests that the use of HSP70 only as a predictor of superimposed preeclampsia at 28 weeks in pregnant women with chronic hypertension is not enough accurate. However, the results improve if used at 36 weeks.

The ROC curve for superimposed preeclampsia prediction in pregnant women with chronic hypertension based only on HSP70 levels in 29-35 weeks (Figure 4) had an optimal cut-off 0.389 ng/ml with 80% sensitivity and 66.7% specificity. The AUC for this model is 0.757 (95% CI 0.658–0.856). If the HSP70 concentration exceeded 0.389 ng/ml after 28 weeks in pregnant women

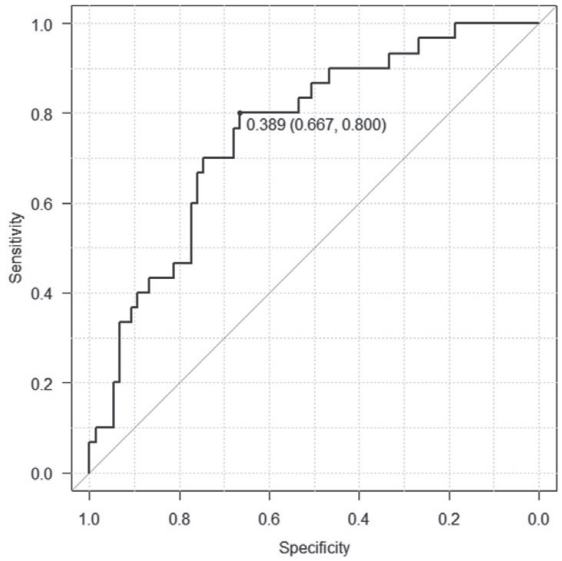

Fig. 4. ROC curve for prediction of superimposed preeclampsia in pregnant women with chronic hypertension via HSP70 levels at 28–35 weeks

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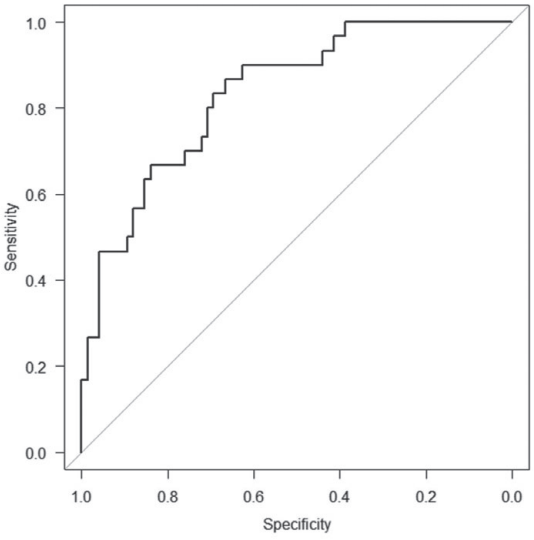

Fig. 5. Multivariate logistic regression model for superimposed preeclampsia prediction based on HSP70 (includes following variables: maternal age, BMI, giving a second or more birth, birth weight and smoking status)

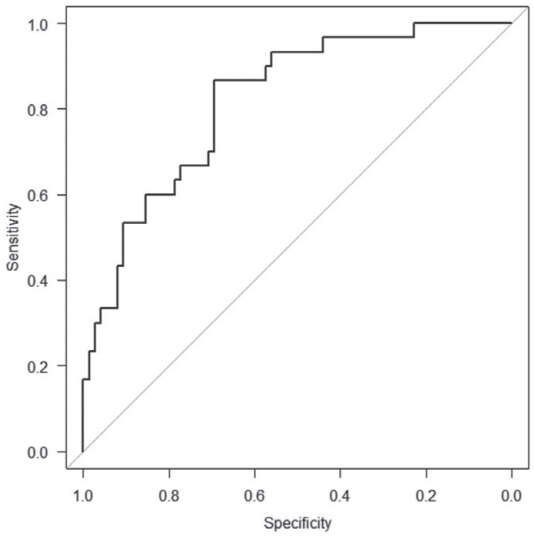

Fig. 6. Multivariate logistic regression model for superimposed preeclampsia prediction based on HSP70 (includes following variables: smoking status, birth weight)

with chronic hypertension, the probability of preeclampsia development increased 4.24 times.

Next, a multivariate logistic regression model for superimposed preeclampsia prediction based on HSP70 was built, taking into account maternal age, BMI, giving a second or more birth, birth weight and smoking status (Figure 5). The AUC is 0.835 (95% CI 0.754 – 0.916), model has 86.4% sensitivity and 67.3% specificity. The following variables were not statistically significant: maternal age (0.053), BMI (0.067), status of giving a second or more birth (-0.59). However, HSP70 level (10.03), smoking status (2.75) and birth weight (-1.39, in kilograms) variables were statistically significant in this model. Therefore, another model was built which includes only statistically significant variables.

The multivariate logistic regression model for prediction of superimposed preeclampsia with adjustment for only statistically significant factors (HSP70, smoking status, birth weight) is shown in Figure 6. The AUC was 0.820 (95% CI 0.735–0.905) with 85.3% sensitivity and 68.9% specificity. Taking into account additional factors allows for increase in prediction accuracy compared to models based only on HSP70.

In our study, serum HSP70 concentrations were higher in patients with superimposed preeclampsia compared to healthy pregnant women and pregnant women with chronic hypertension.

Researchers have repeatedly explored HSP70 level increase in pregnant women with preeclampsia, however, they considered patients without previous hypertensive disorders [15]. Higher HSP70 production in preeclampsia is explained by the response to hemodynamic stress and its participation in the processes of generalized endothelial dysfunction, excessive inflammatory response, and oxidant stress [54, 55].

HSP70 was detected both in intracellular and extracellular space [54]. HSP70 act inside cells as chaperones and work as part of an integrated network that maintains the stability of proteostasis [56, 57]. Under various factors' influence, cells secrete HSP70 into extracellular space. Living cells, in particular endothelial cells, leukocytes, monocytes, neutrophils, release HSP70 through exosomes [54]. Mechanisms of exocytosis through an increase of intracellular Ca^{2+} have been described [58]. Also, HSP70 is being released from necrotic or apoptotic cells [59, 60]. Some fraction of HSP70 molecules are integrated into cell membrane, which ensures a quick reaction of HSP70 if necessary; also, these molecules can act as targets for autoimmunity [24, 60]. In extracellular space, HSP70 stimulates the innate and adaptive immune system and works as an active mediator of the inflammatory process [60–62].

Preeclampsia and superimposed preeclampsia have similar pathophysiological mechanisms, preeclampsia is associated with serum HSP70 levels increase [15]. Our study provides evidence that serum HSP70 increases in pregnant women with preeclampsia, joined to chronic hypertension, suggesting the involvement of HSP70 both in the pathogenesis of preeclampsia and in the pathogenesis of superimposed preeclampsia.

Also, this study found an increase in HSP70 levels in pregnant women with chronic hypertension compared to healthy pregnant women.

HSP70 role in chronic hypertension is being studied and discussed in scientific community. An increase in HSP70 concentration was found in the serum and kidneys of patients with hypertension [17–20, 63]. In experimental models HSP70 injection induced hypertension and kidney damage [19]. There is data that patients with arterial hypertension who have increased levels of HSP70 face are at higher risk of cardiovascular complications and atherosclerosis [60, 61]. The results of certain works allow to link HSP70 gene polymorphism with essential hypertension [19]. The pathogenesis of these processes is still being studied. Chronic arterial hypertension is accompanied by hemodynamic disturbances, endothelial dysfunction, proatherogenic, proinflammatory and prothrombotic

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reactions, which close the circle and contribute to hypertension increase [63, 64]. Therefore, the presence of mentioned disorders in pregnant women with chronic hypertension can explain an increase of HSP70 level.

Our study found no statistically significant changes in HSP70 levels with the gestational age increases in healthy women, which is consistent with previous authors' results [15].

Our study suggests that HSP70 levels grow in pregnant women with chronic hypertension as gestational age increases from 28 weeks to 36 weeks. This is, most likely, due to peculiarities of pregnancy course on the background of chronic hypertensive disorders and conditions related to them.

During normal pregnancy, changes in the cardiovascular system functioning take place: heart rate increase, volume of circulating blood increase, cardiac output increase and decrease in peripheral resistance, changes in blood pressure [65, 66]. These changes do not occur simultaneously. Heart rate increase has a maximum at 34.1 weeks [67]. Mean arterial pressure decreases at 12-18 weeks, then returns to its initial value at 24-28 weeks with subsequent consistent growth in its level until delivery [67]. The values of cardiac output, heart rate, peripheral resistance and mean arterial pressure showed an association with the presence of chronic arterial hypertension in pregnant women [68, 69].

The endothelium tries to support the barrier function with selective permeability, proper vascular tone, inflammatory response level and maintenance of blood rheological properties state [70, 71]. On the background of several universal disorders in the endothelium in pregnant women with chronic hypertension, there is probably a hemodynamic stress progression as gestational age increases, which intensifies the primary changes. This could explain serum HSP70 growth with gestational age in pregnant women with chronic hypertension.

The similarity of processes in the endothelium in hypertensive disorders determines the response in the form of HSP70 production. The highest HSP70 increase have pregnant women with chronic hypertension in case of joined preeclampsia.

The difference between cells response to chronic and acute stress, the stress of different intensity is still yet to be investigated [55, 72, 73]. There is a need to study magnificent molecules that have a duality of localizations, functions, and actions. Molecules with a wonderful figurative name – chaperon – a medieval headdress, almost a thousand years old hat, which is depicted in ancient paintings, pages of old books and fairy tales. Chaperones are one of the most ancient universal molecules that have not revealed all their secrets to us yet.

Research on HSP70 in chronic hypertension during pregnancy and superimposed preeclampsia is quite rare. The strengths of this study was its planning as prospective cohort type and sufficient number of patients. A limitation of the study was the number of measurement points of serum HSP70 during pregnancy. It would also be interesting to study HSP70 levels at first trimester of pregnancy and at full term in this cohort. We believe that work in this direction will help clarify role of HSP70 in chronic hypertension course during pregnancy and the superimposed preeclampsia development.

CONCLUSIONS

The data suggest that HSP70 is increased in pregnant women with superimposed preeclampsia compared to pregnant women with chronic hypertension of the corresponding term, also demonstrate increased serum HSP70 levels in pregnant women with chronic hypertension compared to healthy pregnant women of the corresponding gestational age. Thus, it is possible to assume that HSP70 plays a role in superimposed preeclampsia pathogenesis, and also that HSP70 has an influence on chronic hypertension course during pregnancy.

HSP70 levels growth as gestational age increases among pregnant women with chronic hypertension is most likely due to the progression of hypertensive disorders and/or conditions associated with them. The highest HSP70 increase was observed in pregnant women with chronic hypertension with the addition of preeclampsia.

The universality of reactions in the body could explain the similarity of pathoanatomical processes in the endothelium in preeclampsia, hypertension, diabetic angiopathy, autoimmune diseases with blood vessel damage, cardiovascular diseases.

The use of HSP70 as a single predictor of superimposed preeclampsia is not efficient given its non-specificity, but its use in combination with other markers is promising and requires further study.

The authors declare no conflict of interest.

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