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Markers of implantation in ectopic and high-risk early eutopic pregnancies

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Abstract: Introduction: This study focused on the assessment of HSP-10, HSP-27 and PSG-11 which are one of the first detectable serum pregnancy proteins. Contrary to ultrasound imaging, biochemical methods allow to clarify the pathogenesis and pathomechanism of high-risk pregnancies, fetal anomalies, and abnormal fetal implantation. Early serum concentration estimation of HSP-10, HSP-27 and PSG-11 may be very useful not only in prognosis of pregnancies of unknown localization (PUL), but also as markers of ectopic pregnancies.
Objectives: The aim of the study was to evaluate the expression of HSP-10, HSP-27, PSG-11 implantation proteins in ectopic and eutopic pregnancies, and their mutual correlations.
Patients and Methods: The study involved 42 healthy women who were hospitalized, due to symptoms of imminent miscarriage, risk of spontaneous abortion, or the diagnosis of an ectopic pregnancy. The subjects were subdivided into two equal groups of 21 women who consented to participate in this clinical trial. Biochemical assays were performed involving PSG-11, HSP-27, and HSP-10 serum concentration.
Results: Serum concentration levels of HSP-10, HSP-27, and PSG-11 were significantly higher in pregnancies at risk of spontaneous abortion as compared to ectopic pregnancies.
Conclusions: The results of the study indicate high value of PSG-11, HSP-27 and HSP-10 serum concentrations as predictors of correct implantation site. This may be very useful in prognosis of pregnancies of unknown localization (PUL) and early conservative/surgical ectopic pregnancies treatment if necessary to preserve maximum fertility.

Key words: early pregnancy, pregnancy of unknown localization, PSG-11, HSP-27, HSP-10.

Introduction

Biochemical studies, especially hormonal assays, constitute a powerful, albeit secondary, diagnostic tool for the detection of ectopic pregnancies and pregnancies at a high risk of spontaneous abortion/miscarriage. In contemporary medicine, the rapidly evolving ultrasound has become a vital instrument of great diagnostic sensitivity and specificity. Serial determination of proteins and fetal steroids, along with ultrasonographic specificity and sensitivity, allows not only to diagnose an ectopic pregnancy with a 95–99% accuracy, but also to establish the prognosis of high-risk early eutopic pregnancies. The most commonly monitored protein of placental origin is β-subunit of human chorionic gonadotropin (β-hCG) [1, 2], while progesterone remains the main monitored steroid hormone [3]. Serum profiles of these substances are most often used to confirm pregnancy, monitor and forecast further embryonic development, as well as to evaluate placental efficiency and the wellbeing of the developing fetus [4]. A widespread application of biophysical methods such as fetal ultrasound monitoring is the reason why biochemical monitoring has become largely underestimated. Contrary to ultrasound imaging, biochemical methods allow to clarify the pathogenesis and pathomechanism of high-risk pregnancies, fetal anomalies, and abnormal fetal implantation. Detection of protein markers present in the serum of pregnant women constitutes the foundation of a non-invasive biochemical monitoring of proper blastocyst implantation and fetal health [5]. Serum concentrations of certain proteins in pregnancy i.e. PSG9 (pregnancy-specific beta-1-glycoprotein 9), PSG11 (pregnancy-specific beta-1-glycoprotein 11), PAPP-A (pregnancy-associated plasma protein A), PSG3 (pregnancy-specific beta-1-glycoprotein 3), HSP-10 (heat-shock protein 10), HSP-27 (heat-shock protein 27), Ca 125, as well as inhibins A and B, allow to assess the site of implantation and ongoing pregnancy [6–9]. Our study focused on the assessment of HSP-10, which is encoded on chromosome 2 and is an oligomer of 6 to 8 identical subunits [10]. Furthermore, HSP-10 in combination with HSP-60 impedes its ATPase activity. HSP-10 is present in the maternal serum after fertilization of the ovum. The media used in the IVF-ET demonstrate the presence of HSP-10 protein 48 hours after fertilization [11]. Its presence may also be demonstrated in the cervical mucus and the amniotic fluid [12, 13]. The current generation of pregnancy tests focuses on the presence of β-subunit of chorionic gonadotropin (β-hCG), which appears in the serum no earlier than 6 days after fertilization [14]. It is accepted that HSP-10 is the earliest detectable protein in maternal serum early in pregnancy [15, 16]. Apart from implantation processes, HSP-10 is involved in carcinogenesis of colorectal cancer [17, 18], and may be used as a marker of remission in ovarian cancer [19]. In addition, HSP-10 further exhibits anti-inflammatory activity, most likely via interactions with extracellular HSP-60 protein fraction [20].

HSP-27 is yet another protein which is encoded by the HSPB1 gene, located on chromosome 3. It is found in many types of tissues, especially muscle tissue, where

it is found in high concentrations in muscle tissue cytosol, but also appears in the perinuclear vicinity, reticulum, and nucleus. The main function of this protein is to ensure thermotolerance and cytoprotection of cells exposed to stress. HSP-27 is also involved in inhibition of apoptosis [21] and its decreased expression in systemic lupus erythematosus pregnant patients is associated with higher miscarriage rate [22, 23]. Another function of this protein is cell purification by the activation of proteasomes, which degrade irreversibly denatured proteins and stimulate the pathway of NF-kappa-B, which is important in the process of implantation, development of immune tolerance, and carcinogenesis [24, 25]. The protein is present in the placenta and there is a visible decrease in its concentration as the labor progresses [26].

PSG-11 (pregnancy specific glycoprotein b-11) was the last investigated marker. In order to achieve adequate pregnancy development in mammalian species, a correct sequence of vascular processes between the embryo and the decidua ought to take place. This increased blood flow is associated with proper angiogenesis, vasodilation, and vascular remodeling [27, 28]. Proteins from the PSG family take an active part in that process by interacting with VEGF (vasoendothelial growth factor) and TGF-b pathways [29]. Previous studies reported lower concentrations of proteins from the PSG family to be associated with reduced fetal growth [30, 31]. Furthermore, a relationship between abnormally low concentrations of PSG proteins and the development of preeclampsia was also reported [32]. So far, the literature reports have failed to identify the exact steps of the correlation cascade when these proteins arrive at their aberrant expression, resulting in abnormal migration and implantation of the embryo.

Patient and methods

The aim of the study was to evaluate the expression of HSP-10, HSP-27, PSG-11 implantation proteins in ectopic and eutopic pregnancies, and their mutual correlations.

The study involved 42 healthy women who were hospitalized at the Clinic of Gynecological Endocrinology, Jagiellonian University Medical College, due to symptoms of imminent miscarriage, risk of spontaneous abortion, or the diagnosis of an ectopic pregnancy. The subjects were subdivided into two equal groups of 21 women who consented to participate in this clinical trial. Biochemical assays were performed at the Department of Biochemistry, UJCM Children's Hospital.

The following were performed in all participants:

— detailed OB-GYN interview
— physical examination as well as a complete OB-GYN exam using ultrasound
— blood pressure measurements
— anthropometric measurements, including height and weight
— biochemical tests involving PSG-11, HSP-27, and HSP-10.

Inclusion criteria: Healthy women, regardless of age or ethnicity, diagnosed with pregnancy of incorrect location (e.g. ectopic tubal pregnancy, ovarian, abdominal, or cervical) or intrauterine pregnancy at risk of miscarriage/spontaneous abortion with symptoms including vaginal bleeding, pain in the mid-lower abdomen, as well as 'silent' dilatation of the cervix, were included in the study.

Exclusion criteria: The study excluded women currently taking medication due to the following coexisting diseases: hypertension, coronary heart disease, diabetes, active and chronic inflammation, autoimmune diseases, liver disease, kidney disease, cancer, thrombophlebitis or history of venous thromboembolic disease, cholelithiasis without history of cholecystectomy, illnesses involving seizures including epilepsy, and migraine. Smoking and alcohol consumption were included in the interview.

Biochemical assays were performed at the Department of Laboratory Diagnostics, Children's Clinical Hospital, Jagiellonian University. Testing and analyses of the implantation markers were carried out with enzyme-linked immunosorbent assay (ELISA) using reagent kits from Stressgen Biotechnologies. Each assay was performed in duplicate. The standard curve was generated for each set of assays from standards of known concentrations for each protein.

In order to determine statistical significance of differences between the groups, as well as the possible correlations between them, the following tests were used to assess the variables: Student's t test for independent variables, Pearson's correlation coefficient, and chi-square test. Written informed consent was obtained from all participants. Local Ethics Committee approved of the study.

Results

The study included 42 women: 21 diagnosed with ectopic pregnancy and 21 with pregnancies at risk of miscarriage. Mean β-HCG concentration in both groups was 7300 IU/mL. Mean maternal age was 28 years. No differences between BMI and duration of pregnancy calculated from the date of the last menstrual cycle were found (Table 1).

Table 1. Study group characteristics.

Characteristics	Patients with ectopic pregnancy	Patients with risk of miscarrige
Number of cases — n	21	21
Mean age (min-max)	28 (23–25)	28 (22–23)
Mean BMI (min-max)	24.4 (17.9–34.0)	22.8 (17.8–30.1)
Percentage of obese patients (BMI >30)	9.6% (n = 2)	4.8% (n = 1)
Percentage of underweight patients (BMI <18.5)	4.8% (n = 1)	9.6% (n = 2)
Weeks of pregnancy	6–8	6–9

However, lower concentrations of all proteins were observed in the ectopic pregnancy group.

Mean serum HSP-10 concentrations were 65.29 ug/ml (SD 59.32) and 120.12 ug/ml (SD 39.82), P = 0.001 in the ectopic and eutopic pregnancy groups, respectively (Fig. 1). The difference in mean serum concentrations was 54.95 ug/ml (95% CI for the difference of 42.85–66.82).

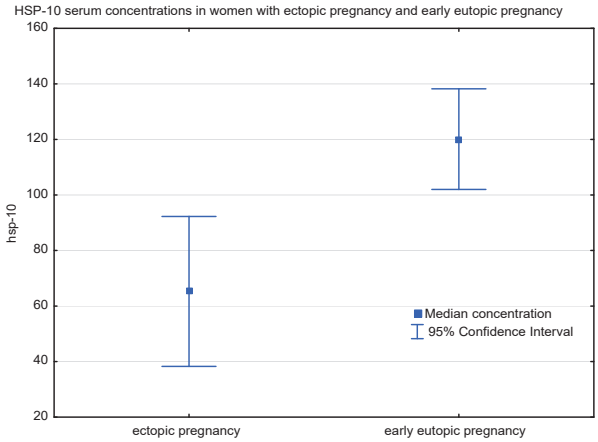

Fig. 1. HSP-10 serum concentrations in women with ectopic pregnancy and early eutopic pregnancy.

Mean serum HSP-27 concentrations were 33.24 ug/ml (SD 31.35) and 66.55 ug/ml (SD 13.97), *P* <0.001 in the ectopic and eutopic pregnancy groups, respectively (Fig. 2). The difference in mean serum concentrations was 33.31 ug/ml (95% CI for the difference of 25.84–40.73).

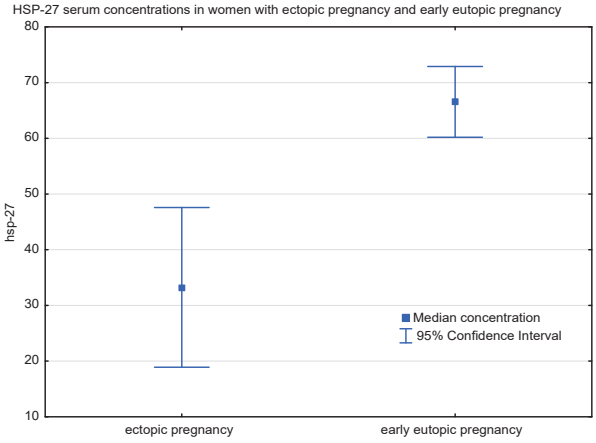

Fig. 2. HSP-27 serum concentrations in women with ectopic pregnancy and early eutopic pregnancy.

Mean serum PSG-11 concentrations were 13.34 ug/ml (SD 14.73) and 30.44 ug/ml (SD 8.8), *P* <0.001 in the ectopic and eutopic pregnancy groups, respectively (Fig. 3). The difference in mean serum concentrations was 17.10 ug/ml (95% CI for the difference of 12.90–21.25).

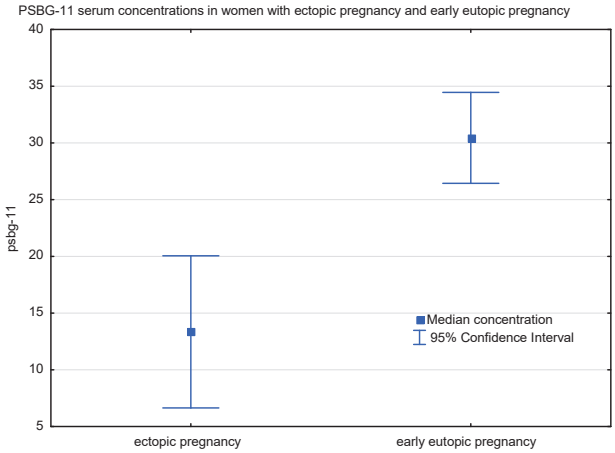

Fig. 3. PSB-11 serum concentrations in women with ectopic pregnancy and early eutopic pregnancy.

Concentration levels of HSP-10, HSP-27, and PSG-11 were significantly higher in pregnancies at risk of spontaneous abortion as compared to ectopic pregnancies.

A linear regression model was created in order to assess the impact of maternal age, BMI, and gestational week on serum protein concentration (Table 2).

HSP-10: The linear regression model displayed significance only in the diagnosis of ectopic pregnancy or pregnancies at risk of spontaneous abortion. Maternal age, BMI, and gestational age were not included in the final model. The resulting final regression equation explains only 22% of the variation, with a standard error of 50.5 (representing 54% of the average protein levels in the group of respondents), signifying a need to look for other factors affecting the level of HSP-10.

HSP-27: The linear regression model displayed significance only in the diagnosis of ectopic pregnancy or pregnancies at risk of spontaneous abortion. Maternal age, BMI, and gestational age were not included in the final model. The resulting final regression equation explains only 31% of the variation, with standard error of 24.37 (representing 49% of the average protein levels in the group of respondents), signifying a need to look for other factors affecting the level of HSP-27.

PSG-11: The linear regression model displayed significance only in the diagnosis of ectopic pregnancy or pregnancies at risk of spontaneous abortion. Maternal age,

Table 2. Impact of maternal age, BMI, and gestational week on serum protein concentration.

Parameter	Regression for HSP-10	Regression for HSP-27	Regression for PSBG-11
Age — *P*	0.227	0.702	0.109
BMI — *P*	0.373	0.776	0.876
Pregnancy week — *P*	0.815	0.499	0.856
Diagnosis	<0.001	<0.001	<0.001
P value	0.017	0.003	0.001
Coefficient of determination (R2) of the output model	19%	27%	33%
P value of the final model	0.001	<0.001	<0.001
Coefficient of determination (R2) of the final model	22%	31%	33%
Standard deviation (Mean %)	50.5 (54%)	24.37 (49%)	12.13 (55%)

regression equation explains only 33% of the variation, with standard error of 12.13 (representing 55% of the average protein levels in the group of respondents), signifying a need to look for other factors affecting the level of PSG-11.

Discussion

Biochemical diagnosis is the key to a better understanding of the processes of implantation in both, ectopic and eutopic pregnancies. In this study, significantly higher levels of HSP-10, HSP-27, and PSG-11 implantation proteins were observed in pregnancies with normal site of implantation. Furthermore, by improving detection of these proteins, the effect of treatment of ectopic pregnancies may be monitored, especially in pregnancies of unknown localization (PUL). Early diagnosis of PUL constitutes the greatest challenge. The earlier the ectopic pregnancy is diagnosed, the sooner the conservative treatment with methotrexate can be applied. If introduced early, the conservative treatment can be exceptionally effective. Additionally, by minimizing the dosage of methotrexate, the side effects are also minimized, resulting in unimpaired fertility [33]. Low level of variation coefficient in determining protein levels and high standard error suggest the existence of an additional, yet to be discovered, variable which affects the level of protein in ectopic pregnancies or gestations at risk of miscarriage/spontaneous abortion.

Further studies of PUL cases are required to confirm the results obtained in this study.

At present, lack of rapidly available laboratory diagnostics, which might produce the results of serum protein concentrations of HSP-10, HSP-27, and PSG-11 in <24 hours, constitutes the most serious limitation.

The results of the study indicate high value of PSG-11, HSP-27 and HSP-10 serum concentrations as predictors of correct implantation site. This may be very useful in prognosis of pregnancies of unknown localization (PUL) and early conservative/surgical ectopic pregnancies treatment if necessary to preserve maximum fertility.

Contribution statement

Jakub Wyroba and Agnieszka Rajtar-Ciosek conceived the idea for the study. Jakub Wyroba and Olga Kacalska-Jansen contributed to the design of the research. All authors were involved in data collection. Dorota Babczyk analyzed the data. Andrzej Zmaczyński coordinated funding for the project. All authors edited and approved the final version of the manuscript.

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Conflict of interest

None declared.

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