

Lena Nowak-Los¹, Grazyna Odrowaz-Sypniewska¹, Jacek Michalkiewicz², Izabela Kubiszewska², Jolanta Zegarska³, Marta Zalewska-Zacharek³

¹Department of Laboratory Medicine, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland

²Department of Immunology, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland

³Department of Obstetrics, Gynaecology and Gynaecological Oncology, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland

Evaluation of serum heat shock protein 70 concentration in women with recurrent miscarriages

Corresponding author:

Lena Nowak-Los, MD PhD
 Department of Laboratory Medicine,
 Nicolaus Copernicus University
 in Torun, Collegium Medicum in
 Bydgoszcz
 Skłodowskiej-Curie Street No 9
 85-094 Bydgoszcz, Poland
 E-mail: l.nowak-los@cm.umk.pl

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ABSTRACT

Background. Heat shock proteins (Hsp) were discovered over 50 years ago and are commonly called 'stress proteins'. Hsp proteins play an important role in a cell, in that they provide protection against cell stress factors and environmentally negative factors. The most conservative, and the best known, heat shock proteins are Hsp 70 subfamily proteins. It has been suggested that an increase of Hsp 70 in the blood during pregnancy has a negative impact. The aetiology of recurrent miscarriages in more than 60% of women remains unexplained. Therefore the aim of this study was to evaluate the usefulness of Hsp 70 assessment in the diagnosis of recurrent miscarriages.

Material and methods. The study group consisted of 100 women (aged 36.0 ± 4.9 years) who had experienced repeated miscarriages. The reference group consisted of 60 women (aged 36.1 ± 3.6 years), who had been pregnant at least twice and who had given birth by a spontaneous labour without complications. Hsp 70 was determined in the serum.

Results. We found no significant differences in the Hsp 70 concentration between the women with recurrent miscarriages and the reference group. While median serum Hsp 70 was the most elevated in the women with the highest number of miscarriages, this difference was not significant.

Conclusion. Based on the obtained results, it is difficult to determine whether Hsp 70 plays a causative role in recurrent miscarriages. However, taking into account the fact that the role of Hsp 70 in the course of normal and pathological pregnancy is not yet completely understood, it may be worth expanding the study to include a larger group of women with recurrent miscarriages.

Key words: recurrent pregnancy loss, heat shock protein (Hsp 70)

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Introduction

Heat shock proteins (Hsp) were discovered over 50 years ago and are commonly called 'stress proteins'. They occur both in the cells of prokaryotes and eukaryotes organisms, and they have a dual activity which results from their distribution in the cell. Extracellular proteins are involved in the immune response, while intracellular proteins play a protective role [1, 2].

These proteins belong to a family of polypeptides with a highly conservative primary structure. Depending on the molecular weight within the heat shock proteins family, several subfamilies can be distinguished, including macromolecular proteins (Hsp 100,

Hsp 90), medium-molecular proteins (Hsp 70, Hsp 60, Hsp 40), and low-molecular weight proteins (Hsp 27, Hsp 10).

It is believed that Hsp 70 subfamily proteins are the most conservative and most thoroughly-established group of heat shock proteins. In the literature there are several reports on the involvement of the Hsp 70 protein in various disease entities, such as cardiovascular, cancer, acute infections, asthma, and pregnancy pathology [3–5]. Particularly noteworthy is the role of protein Hsp 70 in the pathology of pregnancy. It has been suggested that heat shock protein Hsp 70 may play a role in embryogenesis and reproduction. As is well-known, extracellular protein Hsp 70 has the ability to induce

a proinflammatory (Th1) immune response. In a normal pregnancy, the concentration of this protein in the blood decreases. It is believed that an elevated level of this protein in the blood of a pregnant woman has a negative impact on the pregnancy [6–8]. The aim of this study was to evaluate the concentration of Hsp 70 protein in the serum of women with recurrent miscarriages.

Material and methods

Studies were conducted in the Department of Laboratory Medicine, Collegium Medicum in Bydgoszcz, University of Nicolaus Copernicus in Torun. The study group consisted of 160 women aged 24–44 years (mean 36.1 ± 4.4 years) of whom 100 had experienced recurrent pregnancy loss (mean age 36.0 ± 4.9 years), who reported to the Genetic Clinic at the University Hospital. Women with recurrent miscarriages were divided in terms of firstly the number of miscarriages and secondly

the incidence of early pregnancy. The coexistence of chronic diseases (e.g. diabetes, hypertension), metabolic diseases, hormonal disorders, thrombophilic, anatomical defects, infections, antiphospholipid syndrome, and systemic lupus erythematosus was excluded. To the reference group 60 non pregnant women (aged 36.1 ± 3.6 years) were qualified, aged from 30 to 41, who had been pregnant at least twice and who had given birth by a spontaneous labour without complications. Based on the taken history data, the coexistence of chronic diseases prior to the study enrollment was excluded in these subjects (Tab.1).

All the women were informed about the study purpose and gave informed, written consent for it. Blood was collected from the women with recurrent miscarriages in an outpatient setting at least six weeks after the miscarriage. The obtained sera were stored at -70°C until measurement. To determine the serum concentration of human heat shock protein 70 HSP-70, an ELISA test kit from EIAab (China) was used. The minimal detectable concentration of Hsp 70 was 0.039 ng/mL.

Statistical analysis

Because of nonparametric distribution of Hsp 70 protein concentration, a nonparametric U Mann-Whitney test was used for statistical analysis. Statistical analysis of the results of the study was performed using Excel 2007 of the Microsoft Office® 2007 package and Statistica 9.1 by StatSoft, Inc. (2010).

Results

There was no significant difference in the concentration of serum Hsp 70 in women with recurrent pregnancy loss compared to the reference group (Tab. 2). The impact of the number of miscarriages on Hsp 70 protein concentration was not significant, in spite of the highest median Hsp 70 in women with the highest number of lost pregnancies (Tab. 3). Also there were no significant differences in concentrations of Hsp 70 in women with recurrent pregnancy loss but who had given birth at least once compared to those who had never given birth (Tab. 4). It is however worth noting

Table 1. Characteristics of the study and reference groups

Parameter	Study group	Reference group
Number (N)	100	60
Age (years)	36.0 ± 4.9	36.1 ± 3.6
Karyotype 46, XX N (%)	100 (100%)	60 (100%)
Previous pregnancies N (%)	18 (17%)	60 (100%)
Number of miscarriages		
3 miscarriages N (%)	88 (86%)	0 (0%)
4 miscarriages N (%)	10 (9%)	0 (0%)
5 miscarriages N (%)	2 (6%)	0 (0%)
Smoking N (%)	22 (21%)	No data
Coexistence of other diseases N (%)	0 (0%)	No data

Table 2. The Hsp 70 concentration in women with recurrent pregnancy loss and in the reference group

Parameter	Statistical parameter	Study group	Reference group	p
Hsp 70 protein [ng/mL]	N	100	60	0.97
	Me	1.57	1.73	0.97
	Q1	1.21	1.24	0.97
	Q3	2.25	2.06	0.97

N — number of subjects; Me — median; Q1 — first quartile; Q3 — third quartile; p — p-value

Table 3. Comparison of the Hsp 70 values in patients with recurrent pregnancy loss based on the number of miscarriages

Parameter	Statistical measures	Number of miscarriages			p
		3 miscarriages	4 miscarriages	5 miscarriages	
Hsp 70 protein [ng/mL]	N	88	10	2	0.72
	Me	1.57	1.28	2.47	0.72
	Q1	1.25	1.19	0.85	0.72
	Q3	2.17	1.82	2.66	0.72

N — number of subjects; Me — median; Q1 — first quartile; Q3 — third quartile; p — p-value

Table 4. Concentration of Hsp 70 in women with recurrent pregnancy loss who had at least once given birth and in those who had never given birth

Parameter	Statistical measures	PN ₀ *	PN ₁ **	p
Hsp70 protein [ng/mL]	N	82	18	0.15
	Me	1.70	1.43	
	Q1	1.28	1.07	
	Q3	2.37	1.57	

N — number of subjects; Me — median; Q1 — first quartile; Q3 — third quartile; p — p-value; *PN₀ — women with recurrent pregnancy loss who had not given birth; **PN₁ — women with recurrent pregnancy loss, who had given birth at least once

that median Hsp 70 was higher in women with recurrent miscarriages who had never given birth.

Discussion

It is known that in a normal pregnancy the concentration of the Hsp 70 protein is reduced, and that it shows a positive correlation with gestational age and an inverse correlation with maternal age. This protein has the ability to produce extracellular pro-inflammatory state Th1 response-dependent, and consequently may lead to rejection of the foetus by the mother as semiallograft. It is believed that a reduction in the circulating levels of the Hsp 70 protein can affect the immune system to maintain tolerance to the foetus in a woman's body [9]. Only a few papers in the literature have described Hsp 70 protein levels among patients at risk of preterm birth and intraamniotic infections [9, 10].

To the best of our knowledge, this is the first study on the Hsp 70 concentration in the serum of women with recurrent miscarriages. We did not observe significant differences in the Hsp 70 concentration between the group of women with recurrent miscarriages and the reference group. The only important finding was that Hsp 70 concentration in women with fewer than five miscarriages was significantly lower than in women with five or more miscarriages (Me = 1.57 ng/mL v. 2.47 ng/mL), although because of the small sample size the difference was not statistically significant.

There is no earlier data available to compare our results with those of others. However, it is worth men-

tioning that Hsp 70 measurements were performed at least six weeks after the miscarriage, which means after the puerperium period. If the blood sample had been taken immediately after a miscarriage, then the obtained results might have been different. This represents a certain limitation of our study. However, taking blood samples for diagnostic purposes from women with recurrent miscarriages six weeks after an incident is a routine medical procedure.

The obtained results provide some information. But in order to broaden this, it would be good to measure the Hsp 70 twice, immediately after a miscarriage and again after six weeks. Because the role of the Hsp 70 protein in the course of normal and pathological pregnancy is not yet completely understood, it seems to us necessary to continue such studies with a larger group of women. To determine whether an elevated concentration of Hsp 70 is preceded by failures related to pregnancy, prospective studies should be carried out.

Conclusion

Currently, it is difficult to determine whether the Hsp 70 protein plays a role in the pathogenesis of recurrent miscarriages.

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Conflict of interest statement: The authors declare no conflict of interests.

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