

Changes in the concentration of sHLA-I and selected cytokines in pregnancy complicated by antiphospholipid syndrome

Zmiany stężenia sHLA-I i niektórych cytokin w przebiegu ciąży powikłanej zespołem antyfosfolipidowym

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Summary

Objectives: To compare the serum concentrations of the selected cytokines from subsequent trimesters of antiphospholipid syndrome complicated pregnancies.

Material and Methods: This study included 43 pregnant women (23 patients diagnosed with antiphospholipid syndrome and 20 controls). IL-2R, IL-4, IL-10, INF- γ , TNF- α , and class I sHLA concentrations were analyzed by ELISA in the 1st, 2nd and 3rd trimester.

Results: INF- γ levels were below the method detection threshold and therefore were not included in the final analysis. No significant changes in IL-2R, IL-4, IL-10, TNF- α , and class I sHLA concentrations were observed in subsequent trimesters in the group of women with antiphospholipid syndrome. Significant decrease in IL-10 in the 2nd trimester along with significant increase in TNF- α in the 3rd trimester were noted amongst the control subjects. The only significant inter-group difference was observed in the 1st trimester when mean TNF- α level was significantly higher among antiphospholipid syndrome women if compared to controls.

Conclusions: The observed lack of significant differences between antiphospholipid syndrome affected and control group pregnancies in their sHLA class I and cytokine concentrations may be related to the fact that the treatment of antiphospholipid syndrome was implemented early and continued throughout all trimesters of the pregnancy.

Key words: **antiphospholipid syndrome / class I sHLA / cytokines / pregnancy /**

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Streszczenie

Cel pracy: Porównanie stężeń surowiczych wybranych cytokin w kolejnych trymestrach ciąży powikłanej zespołem antyfosfolipidowym.

Materiał i Metoda: Badaniem objęto 43 ciężarne (23 pacjentki z zespołem antyfosfolipidowym i 20 zdrowych kobiet z grupy kontrolnej). Stężenia IL-2R, IL-4, IL-10, INF- γ , TNF- α oraz sHLA klasy I oznaczano metodą ELISA w I, II i III trymestrze ciąży.

Wyniki: Wartości INF- γ w badanej grupie znajdowały się poniżej progu detekcji i nie zostały uwzględnione w dalszej analizie. W grupie ciężarnych z zespołem antyfosfolipidowym nie stwierdzono znamiennych różnic pomiędzy stężeniami IL-2R, IL-4, IL-10, TNF- α i sHLA klasy I oznaczanymi w kolejnych trymestrach ciąży. Natomiast w grupie kontrolnej w II trymestrze odnotowano istotny statystycznie spadek stężenia IL-10, a w III trymestrze – znamienny wzrost poziomu TNF- α .

Jedyna istotna różnica pomiędzy grupą badaną i kontrolną dotyczyła stężenia TNF- α w I trymestrze, które było znamienne wyższe wśród ciężarnych z zespołem antyfosfolipidowym.

Wnioski: Brak istotnych różnic pomiędzy pacjentkami z zespołem antyfosfolipidowym a ciężarnymi z grupy kontrolnej w zakresie stężenia sHLA klasy I i niektórych cytokin może wynikać z faktu wczesnego rozpoczęcia terapii zespołu antyfosfolipidowego i jej kontynuacji w kolejnych trymestrach ciąży.

Słowa kluczowe: **zespół antyfosfolipidowy / sHLA klasy I / cytokiny / ciąża /**

Introduction

The etiology of antiphospholipid syndrome (APS) remains to be fully elucidated though previously it has been associated with a complex and abnormal immunological response. The syndrome is clinically associated with incidents of venous and arterial thrombosis, pregnancy failures and circulation of antiphospholipid antibodies [1, 2]. As the pathogenesis of spontaneous abortions found to co-occur in this syndrome is not exactly known, novel diagnostic and therapeutic options for pregnancy loss prevention are being investigated [1].

Cytokines play a vital primary role in the development of an array of autoimmune diseases, including APS [2, 3]. Cytokines tend to promote hyperactive coagulation, however, this effect is associated with infection as well as autoimmunity [4]. This effect is most pronounced for TNF- α which stimulates thrombin production, and IL-6 which promotes the release of tissue factors and initiation of the extrinsic coagulation cascade [5].

Recent studies [6, 7] have indicated that the balance of the Th1/Th2 response among women with recurrent spontaneous abortions is disturbed. The excess of Th1 cytokines (IL-2, INF- γ , TNF- α) has an unfavorable influence on the course of the pregnancy – immediate embryotoxic activity and destruction of the trophoblast tissue. In normal pregnancies, decreased expression of a number of cytokines, namely IL-2 and INF- γ , as well as an increase in levels of IL-4 and IL-10 were observed [8]. IL-10 inhibits the production of certain cytokines such as IL-4, IL-5 and INF- γ , inhibiting secretion of growth factor and chemokines involved in autoimmunologic processes [9]. IL-6 is a strong promoter of the humoral autoimmune response by activation of an IL-4 stimulated increase in antiphospholipid autoantibody production [10].

TNF- α also plays a key role in the development of APS. This cytokine, by adhering to the phospholipid anions of the cell membrane, forms an antiphospholipid antibody binding complex which induces thrombus formation and apoptosis. It has also been suggested that selective induction of the non-classical HLA I class antigens [11] and their soluble forms occurs under the

influence of cytokines [12]. Soluble HLA class I antigens (sHLA I) are a normal component of human serum and other body fluids [13-15]. They play an important role in the induction of maternal immunological tolerance for the fetus as an allograft.

Objectives

The aim of this work was to compare serum concentrations of the selected cytokines (IL-2R, IL-4, IL-10, INF- γ , TNF- α and class I sHLA) from the 1st, 2nd and 3rd trimesters of antiphospholipid syndrome complicated and uncomplicated pregnancies.

Material and methods

For this study, 43 pregnant women were enrolled and followed-up at the Department of Fetal-Maternal Medicine, Pomeranian Medical University, Szczecin (Poland). Study participants were assigned to one of the two groups. The study group (APS) consisted of 23 pregnant women (mean age 29.5 \pm 1.08 years) with a history of 5 pregnancy failures defined by either of the following: 1) intrauterine fetal death after 10 weeks of gestation, 2) one eclampsia-associated preterm birth (prior to the 34th week of gestation) of a morphologically normal fetus, 3) severe pre-eclampsia and/or placental failure, or 4) three spontaneous consecutive abortions prior to the 10th week of pregnancy. Diagnostic laboratory criteria included the detection of anti-phospholipid antibodies (anti-cardiolipin antibodies, and lupus anti-coagulant), anti-beta antibodies, anti-nDNA antibodies, anti-nucleosome antibodies, anti-histone antibodies, anti-neutrophilic cytoplasmic antibodies, and anti-endothelial cell antibodies. Diagnosis of antiphospholipid syndrome was based thusly on laboratory tests, clinical presentation and the Sapporo criteria [16].

All subjects from the APS group underwent treatment throughout the entire period of pregnancy and during labor. All pregnant women underwent psychological counseling. Control group was comprised of 71 pregnant multiparous women (mean age of 28.7 \pm 0.72 years) with a history of at least one successful pregnancy with no failures.

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The authors were granted the permission of the Pomeranian Medical University Commission for Bioethics to conduct the study, and received the informed consent of all the patients participating in the program.

Blood was collected from the 1st, 2nd and 3rd trimesters of pregnancy. IFN- γ levels were analyzed by ELISA using validated (Amersham, UK) kits (Bender MedSystems, Denmark). Assay sensitivity was 1 pg/ml. TNF- α , IL-4, IL-10, and IL-2R were measured by ELISA method with monoclonal specific antibodies and the Quantikine HS kit (R&D Systems Europe Ltd, UK). Assay sensitivities of 0.5pg/ml for IL-10, 0.22 pg/ml for IL-4, 10 pg/ml for IL-2R, and 0.32pg/ml for TNF- α were used.

ELISA was used for analysis of the class I sHLA concentrations. Maxisorb plates (NUNC, Denmark) were coated overnight at 4°C, with 100 μ l 1:500 dilution of rabbit anti-mouse IgG. The plates were washed 3 times with 300 μ l of 0.02% PBS/Tween 20 (PBST) solution and incubated for one hour in room temperature with 100 μ l 1:500 dilution of anti-HLA I W6/32 (DAKO, Denmark) serum. Plates were washed again (3 times, 300 μ l of 0.02% PBS/Tween 20), blocked for one hour in room temperature with 300 μ l 3% BSA (Amersham, UK) and then washed. Standard serum (100 μ l, kindly provided by prof. dr. hab. med. H. Grosse-Wilde, Immunology Institute Essen, Germany) or 100 μ l of the sample serum was added onto the plate and incubated for 1 hour in room temperature. Subsequent steps involved 5-fold wash in PBST, 1 hour of room temperature incubation with 100 μ l of the serum containing peroxidase-stained anti- β 2 microglobulin (DAKO, Denmark), followed by 10-fold PBST washing. For the color reaction, orthophenylenodiamin (DAKO, Denmark) was used with the reaction inhibited after 15 minutes with 0.5 M H₂SO₄. Absorbance was measured at wave lengths of $\lambda=490$ nm and $\lambda_{ref}=630$ nm using Inc analyzer BIO-TEK Instruments, Germany). sHLA-I concentrations were calculated based on the standard curve method with standards of 80ng/ml.

Cytokine concentrations were presented as arithmetic means, their standard deviations (SD) and 95% confidence intervals. Their normal distribution was tested by Shapiro-Wilk test. Arithmetic means between APS and control groups were compared with the Student t test for independent variables or with the non-parametric Mann-Whitney U test for independent variables whenever feasible. Friedman's non-parametric ANOVA test with Kendall's agreement coefficient were used to compare differences between mean cytokine concentrations observed in subsequent trimesters. Calculations were performed using the Statistica 7 (StatSoft®, Poland) package, with statistical significance defined as $p \leq 0.05$.

Results

INF- γ levels were below the method detection threshold and therefore were not included in the final analysis. The levels of other cytokine studied are presented in the Table I.

No significant changes in IL-2R, IL-4 and IL-10 concentrations were observed in subsequent trimesters in the group of women with APS, whereas significant decrease in IL-10 was noted in the 2nd trimester amongst the control subjects. Nonetheless, IL-2R, IL-4 and IL-10 concentrations in APS patients were not significantly different if compared to controls at any time point studied.

In the APS group, TNF- α levels did not change significantly in subsequent trimesters, whereas significant increase in TNF- α

was noted in the 3rd trimester amongst the control subjects. The only significant inter-group difference was observed in the 1st trimester when mean TNF- α level was significantly higher among APS women if compared to controls.

No significant changes in sHLA I concentrations were recorded in all trimesters of pregnancy, either in the group of women with APS or amongst the control subjects. Inter-group differences in sHLA I levels were statistically insignificant at any time point studied.

Discussion

APS was previously linked to pregnancy loss, thrombotic events, thrombocytopenia, and multi-organ damage including the heart and central nervous system [17]. In this syndrome, inflammation alters the immunologic response in pregnancy by the release of proinflammatory chemokines and cytokines [18]. sHLA is mainly produced in the liver by peripheral lymphocytes [15], however numerous clinical studies indicate that changes in sHLA concentration are related to immunological stimulation, especially in the setting of increased cellular metabolic rates [12, 13, 37]. Indeed, higher serum sHLA concentrations were observed among patients with autoimmune disease [19].

Soluble class I HLA particles (HLA-A, HLA -B, HLA -C and HLA -G) modulate peripheral immune tolerance and act in concert with CD8+ lymphocytes by inducing Fas ligand (FasL) expression and secretion of soluble FasL, ultimately leading to apoptosis of activated cells resulting from Fas/FasL interactions [20]. This may be one of the possible reasons as to why increased serum concentrations of sHLA class I particles exert such a strong immunosuppressive effect and potentially lead to the disruption of immunological homeostasis in pregnant women.

In this study, it was found that sHLA-I serum concentrations of pregnant women with the diagnosed antiphospholipid syndrome were not significantly different in all three pregnancy trimesters. It is difficult to compare these results with any reference data, as, to the best of our knowledge, no papers on sHLA class I and cytokine concentration changes throughout consecutive trimesters of antiphospholipid syndrome complicated pregnancy have been published so far. However, other authors have investigated the concentrations of these molecules among both pregnant and non-pregnant women with APS. Ronin-Walknowska et al. [21], when analyzing sHLA-I levels among patients with high risk pregnancies (intra-uterine growth restriction, pre-eclampsia) and APS, found that increased sHLA levels correlated positively with IFN- γ concentration. Comparisons to the results obtained in our study are difficult due to differences in the measurement methods.

Inostroza et al. [14], found significant increases of serum sHLA class I in the 1st and 2nd trimester of uncomplicated pregnancies, with a notable drop observed in the 3rd trimester and during labor. Moreover, during delivery sHLA-I concentrations proved to be lower in the umbilical than maternal blood. Similar results were obtained by Russwurm et al. [10], with observed significant decrease of sHLA concentrations in the 3rd trimester of pregnancy. The same research team found that sHLA-II concentrations were the highest in the 2nd trimester of pregnancy, with a tendency for subsequent decrease during the 3rd trimester until labor. However, no significant changes of INF- γ and IL-6 serum concentrations during various periods of pregnancy and

Table 1. Arithmetic means, standard deviations (SD) and 95% confidence intervals (95%CI) of selected serum cytokine concentrations among pregnant women with antiphospholipid syndrome (APS) and controls.

Trimester	APS group (n=23)		Control group (n=20)		p value
	mean±SD (95%CI)	range	mean±SD (95%CI)	range	
IL-2R (pg/ml)					
I	1078.95±629.67 (799.78-1358.13)	324-3068	1019.38±280.02 (870.16-1168.59)	551-1707	0.745 ¹
II	887.86±456.20 (710.96-1064.75)	270-2171	977.50±240.81 (857.75-1097.25)	580-1436	0.156 ¹
III	802.82±360.61 (642.93-962.71)	357-1765	926.89±239.97 (811.23-1042.56)	610-1269	0.210 ²
p value ³	0.300	-	0.292	-	-
IL-4 (pg/ml)					
I	0.39±0.10 (0.34-0.43)	0.25-0.69	0.35±0.06 (0.32-0.38)	0.24-0.46	0.406 ¹
II	0.42±0.17 (0.35-0.48)	0.26-1.02	0.39±0.08 (0.35-0.43)	0.27-0.57	0.719 ¹
III	0.39±0.14 (0.32-0.45)	0.24-0.87	0.45±0.13 (0.39-0.52)	0.31-0.84	0.134 ²
p value ³	0.367	-	0.092	-	-
IL-10 (pg/ml)					
I	5.06±5.25 (2.73-7.38)	0.60-24.60	6.15±5.99 (2.96-9.35)	0.85-26.40	0.553 ²
II	5.10±5.74 (2.87-7.32)	0.34-24.60	4.62±3.90 (2.68-6.56)	0.29-17.60	0.758 ²
III	8.48±14.70 (1.96-14.99)	0.42-69.90	6.82±7.14 (3.38-10.26)	0.42-34.40	0.657 ²
p value ³	0.131	-	0.002	-	-
TNF-α (pg/ml)					
I	11.37±9.01 (7.37-15.36)	2.50-32.90	6.06±4.20 (3.82-8.29)	1.40-17.90	0.035 ²
II	21.44±35.29 (7.48-35.40)	1.60-178.50	6.95±4.51 (4.71-9.19)	1.00-19.10	0.100 ¹
III	24.48±42.33 (5.71-43.25)	2.90-161.00	53.62±71.79 (19.02-88.23)	1.80-268.20	0.116 ²
p value ³	0.273	-	0.005	-	-
sHLA-I (ng/ml)					
I	672.82±590.84 (410.85-934.78)	265-2999	583.50±245.54 (452.66-714.34)	297-1043	0.790 ¹
II	840.85±1350.70 (306.53-1375.17)	204-7403	466.50±157.82 (388.02-544.98)	216-759	0.348 ¹
III	874.00±1536.80 (192.62-1555.38)	227-7617	496.16±190.06 (404.55-587.76)	200-900	0.814 ¹
p value ³	0.905	-	0.794	-	-

¹Mann-Whitney U test for independent variables, ²Student t test for independent variables, ³Friedman's non-parametric ANOVA

labor have been observed. Rebmann et al. [13], found that both in umbilical blood and amniotic fluid, sHLA-I levels tend to be lower than in maternal blood.

In our previously published research conducted on women with non-pregnancy related fertility failures, the highest concentrations of sHLA-I were observed in patients with a history of empty gestational sacs (252.3ng/ml), with the lowest concentrations noted amongst women with artificial insemination (IVF) failures (171.8ng/ml). However, these changes were not statistically significant. In women with pregnancy failures, an association was found between high sHLA class I concentrations (513.4±89.5 SEMng/ml) and the presence of the HLA-G 10401 allele, with significant differences in sHLA-I levels observed for all remaining HLA-G alleles [22]. Pfeifer [23] found that in women with IVF failures (early pregnancy loss), sHLA-I and sHLA-G levels in the preovulation period and pregnancy were lower if compared to women with normal pregnancies.

The clinical features and course of APS is linked to a disturbance in the equilibrium between the Th1 and Th2 cytokine expression. In this study, IL-2R, IL-4 and IL-10 did not change significantly in subsequent trimesters in the group of women with APS and were not significantly different compared to the control group.

Mean TNF-α level was higher in the 1st trimester among APS women if compared to controls with an insignificant tendency for increase in subsequent trimesters.

Forastiero et al. [24], in a group of patients with primary antiphospholipid syndrome, identified Th2 related response domination including increased serum IL-6 concentrations and decreased INF-γ and IL-2 levels among non-pregnant women. The same research group observed elevated TNF-α and IL-6 levels with decreased INF-γ levels among patients with APS [25]. Wilson et al. [26], found significantly increased levels of IL-2R in the blood of non-pregnant women with recurrent abortions compared to controls.

McLean et al. [27], found that the IL-2R concentrations were increased in the first trimester of pregnancy among women with recurrent spontaneous abortions if compared to women with no abnormalities in previous gynecological examinations and prior uncomplicated pregnancies. Visvanathan and McNeil et al. [2], when investigating APS affected women with histories of pregnancy loss, recurrent thrombosis and thrombocytopenia, found significantly increased INF-γ levels alongside low IL-4 serum concentrations, probably related to chronic stimulation with β2GP-1 antigen resulting in increased Th1 CD4+ cell production.

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Richaud-Patin et al. [9], observed increased expression of IL-4, IL-6, IL-10 and IFN- γ among non-pregnant systemic lupus erythematosus women compared to healthy individuals.

The exact influence of IL-10 on immunological regulation is to be fully elucidated. It is probable that IL-10 decreases the expression of HLA-DR, HLA-DP and HLA-DQ on the macrophage cell surface, reducing the number of cells involved in antigen presentation and decreasing the number of CD4+ and CD8+lymphocytes involved [28, 29].

TNF- α is involved in regulation of hormone synthesis, placental, and fetal development [30]. It also participates in the pathogenesis of spontaneous abortions [31] and the development of eclampsia [32]. Moreover, it is directly involved in platelet, monocyte and endothelium activation and therefore induction of thrombus formation [25]. Results from independent research carried out on animal models found that increased TNF- α concentrations resulted in the activation of maternal monocytes in the trophoblast and apoptosis induction [33], which might be associated to the pregnancy loss.

Manfredi et al. [34], identified an association between antiphospholipid antibody and TNF- α secretion in macrophages. Bertolaccini et al. [35], among women with APS and a history of thrombosis and pregnancy loss, found TNF- α levels to be increased (2.95 pg/ml) along with the number of TNF A-238*A genotypes (OR 3.95; 95%CI 1.3-11.7; p=0,01) – factors which might influence the development of this clinical syndrome. Berman et al. [36], suggest that monocyte and neutrophil activation associated TNF- α secretion might be a cause of spontaneous abortions by triggering the inflammatory modulator and proteolytic enzyme release on the trophoblast. The same research group observed that antiphospholipid antibodies activate the release of TNF- α into decidual tissue of APS mice, resulting in the rapid increase in the levels of this cytokine both in tissues and blood [36].

Conclusions

The observed lack of significant differences between APS affected and control group pregnancies in their sHLA class I and cytokine concentrations may be related to the fact that the treatment of antiphospholipid syndrome was implemented early and continued throughout all trimesters of the pregnancy.

References:

- Berman J, Girardi G, Salmon J. TNF- α is a critical effector and a target for therapy in antiphospholipid antibody-induced pregnancy loss. *J Immunol*. 2005, 174, 485-490.
- Visavanathan S, McNeil H. Cellular immunity to beta 2-glycoprotein-1 in patients with the antiphospholipid syndrome. *J Immunol*. 1999, 162, 6919-6925.
- Fischer K, Collins H, Taniguchi M, [et al.]. IL-4 and T cells are required for the generation of IgG1 isotype antibodies against cardiolipin. *J Immunol*. 2002, 168, 2689-2694.
- Carp H. Cytokines in recurrent miscarriage. *Lupus*. 2004, 13, 630-634.
- Knackstedt M, Zenclussen A, Hertwig K, [et al.]. Th1 cytokines and the prothrombinase [gl2 in stress-triggered and inflammatory abortion. *Am J Reprod Immunol*. 2003, 49, 210-220.
- Wilson R, Jenkins C, Miller H, [et al.]. Abnormal cytokine levels in non-pregnant women with a history of recurrent miscarriage. *Eur J Obstet Gynecol Reprod Biol*. 2004, 115, 51-54.
- Chaouat G, Ledee-Bataille N, Dubanchet S, [et al.]. TH1/TH2 paradigm in pregnancy: Paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. *Int Arch Allergy Immunol*. 2004, 134, 93-119.
- Ng S, Gilman-Sachs A, Thaker P, [et al.]. Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. *Am J Reprod Immunol*. 2002, 48, 77-86.
- Richaud-Patin Y, Alcocer-Varela J, Llorente L. High levels of Th2 cytokine gene expression in systemic lupus erythematosus. *Rev Invest Clin*. 1995, 47, 267-272.
- Russwurm G, Macler A, Fagoaga U, [et al.]. Soluble human leukocyte antigens, interleukin-6 and interferon-gamma during pregnancy. *Am J Reprod Immunol*. 1997, 38, 256-262.
- Hunt J, Orr H. HLA and maternal-fetal recognition. *FASEB J*. 1992, 6, 2344-2348.
- Rebmann V, Passler M, Erhard I, [et al.]. Monitoring of soluble HLA class I size variants after liver transplantation. *Hum Immunol*. 1999, 60, 424-429.
- Rebmann V, Pfeiffer K, Pässler M, [et al.]. Detection of soluble HLA-G molecules in plasma and amniotic fluid. *Tissue Antigens*. 1999, 53, 14-22.
- Inostroza J, Ferrada J, Navarrete C, [et al.]. Soluble histocompatibility class I antigens and beta 2- microglobulin in pregnant females and cord blood samples. *Hum Immunol*. 1997, 54, 63-68.
- Brieva J, Villar L, Leoro G, [et al.]. Soluble HLA class I antigen secretion by normal lymphocytes: relationship with cell activation and effect of interferon-gamma. *Clin Exp Immunol*. 1990, 82, 390-395.
- Wilson W. Classification criteria for antiphospholipid syndrome. *Rheum Dis Clin North Am*. 2001, 27, 499-505.
- Blank M, Shoefeld Y. Antiphosphatidylserine antibodies and reproductive failure. *Lupus*. 2004, 13, 661-665.
- Harel M, Aron-Maor A, Sherer Y, [et al.]. The infectious etiology of the antiphospholipid syndrome: links between infection and autoimmunity. *Immunobiology*. 2005, 210, 743-747.
- Hagihara M, Shimuta T, Yamamoto K, [et al.]. Clinical significance of serum soluble HLA class I antigens in systemic lupus erythematosus. *J Exp Clin Med*. 1993, 18, 61-64.
- Puppo F, Contini P, Ghio M, [et al.]. Soluble human MHC class I molecules induce soluble Fas ligand secretion and trigger apoptosis in activated CD8(+) Fas(CD95)(+) T lymphocytes. *Int Immunol*. 2000, 12, 195-203.
- Ronin-Walknowska E, Sipak-Szmigiel O, Miklaszewicz A, [et al.]. Soluble HLA class I antigens (sHLA-I) and gamma interferon (INF- γ) in high-risk pregnancy. *Archiv Perinat Med*. 2000, 6, 24-28.
- Sipak-Szmigiel O, Ronin-Walknowska E, Cybulski C, [et al.]. Antigens HLA-G, sHLA-G and sHLA-class I in reproductive failure. *Folia Histochem Cytobiol*. 2007, 45, 137-141.
- Pfeiffer K, Rebmann V, Pässler M, [et al.]. Soluble HLA levels in early pregnancy after in vitro fertilization. *Hum Immunol*. 2000, 61, 559-564.
- Forastiero R, Martinuzzo M, Carreras L. Imbalance towards Th2-type response in patients with definite primary antiphospholipid syndrome. *Thromb Haemost*. 2001, 86, 934-935.
- Forastiero R, Martinuzzo M, Larranaga G. Circulating levels of tissue factor and proinflammatory cytokines in patients with primary antiphospholipid syndrome or leprosy related antiphospholipid antibodies. *Lupus*. 2005, 14, 129-136.
- Wilson R, Moore J, Jenkins C, [et al.]. Abnormal II-2 receptor levels in non-pregnant women with a history of recurrent miscarriage. *Hum Reprod*. 2003, 18, 1529-1530.
- MacLean M, Wilson R, Jenkins C, [et al.]. Interleukin-2 receptor concentrations in pregnant women with a history of recurrent miscarriage. *Hum Reprod*. 2002, 17, 219-220.
- Steinborn A, Rebmann V, Scharf A, [et al.]. Soluble HLA-DR levels in the maternal circulation of normal and pathologic pregnancy. *Am J Obstet Gynecol*. 2003, 188, 473-479.
- Heiligenhaus A, Rebmann V, Neubert A, [et al.]. Soluble HLA class I and HLA DR plasma levels in patients with anterior uveitis. *Tissue Antigens*. 2004, 63, 369-375.
- Hunt J, Chen H, Miller L. Tumor necrosis factors: pivotal components of pregnancy? *Biol Reprod*. 1996, 54, 554-562.
- Babbage S, Arkwright P, Vince G, [et al.]. Cytokine promoter gene polymorphisms and idiopathic recurrent pregnancy loss. *J Reprod Immunol*. 2001, 51, 21-27.
- Anim-Nyame N, Gamble J, Sooranna S, [et al.]. Microvascular permeability is related to circulating levels of tumor necrosis factor- α in pre-eclampsia. *Cardiovasc Res*. 2003, 58, 162-169.
- Garcia-Lloret M, Winkler-Lowen I, Guilbert L. Monocytes adhering by LFA-1 to placental syncytiotrophoblasts induce local apoptosis via release of TNF- α . A model for hematogenous initiation of placental inflammations. *J Leukocyte Biol*. 2000, 68, 903-908.
- Manfredi A, Rovere P, Galati G, [et al.]. Apoptotic cell clearance in systemic lupus erythematosus. I. Opsonization by antiphospholipid antibodies. *Arthritis Rheum*. 1998, 41, 205-214.
- Bertolaccini M, Atsumi T, Lanchbury J, [et al.]. Plasma tumor necrosis factor α levels and the -238*A promoter polymorphism in patients with antiphospholipid syndrome. *Thromb Haemost*. 2001, 85, 198-203.
- Berman J, Girardi G, Salmon J. TNF- α is a critical effector and a target for therapy in antiphospholipid antibody-induced pregnancy loss. *J Immunol*. 2005, 174, 485-490.
- Miklaszewicz A, Sipak-Szmigiel O, Ronin-Walknowska E, [et al.]. Analysis of HLA class I and II antigens composition in high-risk pregnancy. *Ginekol Pol*. 2000, 71, 491-499