

## Uloga IL-2 i reumatoidnog faktora u ponovljenom spontanom pobačaju

### The Role of IL-2 and rheumatoid factor in recurrent spontaneous abortion

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#### Sažetak

**Cilj:** Ponovljeni spontani pobačaj (PSP), tj. ponavljanje triju ili više uzastopnih pobačaja, pojavljuje se u približno 1-2% žena u reproduktivnoj dobi. Pretpostavlja se da bi određeni broj ponovljenih gubitaka trudnoće mogao biti uzročno povezan s imunošću. Svrha ove studije bila je odrediti ulogu interleukina-2 (IL-2) i reumatoidnog faktora (RF) u PSP.

**Materijali i metode:** Ovo je istraživanje parova provedeno na dvije različite skupine. Prva se skupina sastojala od 56 žena koje su imale tri ili više PSP, a druga od 63 zdrave žene bez pobačaja u anamnezi i uz barem jednu uspješnu trudnoću. U uzorcima seruma ispitana je prisutnost IL-2 pomoću metode ELISA i RF testom lateks-aglutinacije.

**Rezultati:** Dvije od 56 žena u prvoj skupini imale su IL-2 u serumu. U drugoj skupini nijedna žena nije imala IL-2 u serumu. Također, 53,5% žena u prvoj skupini i 6% žena u drugoj skupini bilo je pozitivno na RF pri različitim titrovima. Postojale su statistički značajne razlike između skupina I i II s obzirom na RF ( $P = 0,002$ ).

**Zaključci:** Koncentracija IL-2 ne utječe na ishod trudnoće, dok je prevalencija RF u žena s PSP bila viša nego u normalnih ispitanica. Stoga se preporučuje ispitivanje uloge RF u vezi s ishodom trudnoće.

**Ključne riječi:** IL-2, reumatoidni faktor, ponovljeni spontani pobačaj

#### Abstract

**Objective:** Recurrent spontaneous abortion (RSA), the repetition of three or more consecutive abortions, occurs in approximately 1–2% of reproductive-aged women. It has been postulated that a number of repeated pregnancy losses may be related to immune causes. The purpose of this study was to determine the role of Interleukin (IL)-2 and Rheumatoid Factor (RF) in RSA.

**Materials and methods:** This case control study was carried out on two different groups. Group I consisted of fifty-six women with a history of three or more RSA and group II consisted of sixty-three healthy women who have had no abortion history, with at least one successful pregnancy. The sera were examined for the presence of IL-2 by ELISA method, and RF by latex agglutination test.

**Results:** Two out of 56 women in group I had IL-2 in serum. None of the women in group II had IL-2 in serum. Also, 53.5% of women in group I and 6% of women in group II were positive for RF in different titers. There were significant differences between groups I and II for RF ( $P = 0.002$ ).

**Conclusions:** Serum IL-2 concentration does not affect pregnancy outcome, and the prevalence of RF in RSA women was higher than in normal controls. It is therefore recommended that the role of RF in pregnancy outcome should be investigated.

**Key words:** IL-2, rheumatoid factor, recurrent spontaneous abortion

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#### Uvod

Ponovljeni spontani pobačaji (PSP) još uvijek predstavljaju učestali reproduktivni problem širom svijeta; tri ili više pobačaja zahvaća 1-2% žena reproduktivne dobi (1). Unatoč nekoliko utvrđenih etioloških čimbenika, uzrok PSP se

#### Introduction

Recurrent spontaneous abortions (RSA) are still a frequent reproductive problem worldwide, with three or more affecting 1-2% of women of reproductive age (1). Despite several well established etiologic factors, the cause

ne može odrediti u gotovo 50% slučajeva. Pretpostavlja se da određeni broj tih ponovljenih gubitaka trudnoće može biti povezan s imunskim uzrocima (2). Odgovor T-pomoćničkih stanica (Th) nakon aktivacije je funkcionalno obilježen citokinima koje te stanice proizvode. Stanice Th1 uglavnom luče interleukin (IL)-2, interferon (IFN)- $\gamma$  i čimbenik nekroze tumora (TNF)- $\beta$  (3). IL-2 se izrazito ističe među citokinima koji su osobito povezani s preživljavanjem zametka. Chaouat i sur. (1990.) su pokazali da primjena IL-2 kod gravidnih miševa uzrokuje pobačaj (4). Reumatoidni faktori (RF) su antitijela koja prepoznaju udio Fc molekula IgG kao svoje vlastite antigene. RF mogu biti bilo kojeg izotipa imunoglobulina (IgM, IgG, IgE). Većina klinički izmjerenih RF su razreda IgM (5). Među zdravom populacijom učestalost pojedinaca pozitivnih na RF iznosi od 1,3 do 4% u bijelaca pa do 30% u nekim plemenima sjevernoameričkih indijanaca (6). Čini se da mala podskupina B-stanica koje izražavaju CD5, a poznate su kao B-1a-stanice, stvaraju RF (7). Mnoge su studije razjasnile temeljnu ulogu T-stanica u promjeni razreda u kojoj stvaranje citokina ima važan učinak na vrstu imunoglobulina koju stvaraju B-stanice (8).

Cilj ove studije bio je usporediti koncentracije IL-2 (citokina Th1) i RF u serumu žena s anamnezom PSP s koncentracijama u kontrolnoj skupini.

## Materijali i metode

### Ispitanice

Ovo je istraživanje parova provedeno u dvije različite skupine upućene u Istraživački i klinički centar za neplodnost Yazd. Prva se skupina sastojala od 56 žena s anamnezom tri ili više PSP, a druga je obuhvaćala 63 zdrave žene bez pobačaja u anamnezi te s barem jednom uspješnom trudnoćom. Uzorci pobačenog zametka imali su normalan kariotip, negativan nalaz za TORCH, bili su bez anatomskih ili endokrinih problema uz odsutnost antifosfolipidnih antitijela razreda IgM i IgG, antikardiolipinskih antitijela razreda IgM, IgG i IgA te antinuklearnih antitijela razreda IgM i IgG. Sve su slučajeve činili primarni PSP. Dobiveni su demografski podatci, o dobi, broju pobačaja i gestacijskoj dobi posljednjeg pobačaja za žene iz skupine s PSP, te o broju trudnoća za žene u kontrolnoj skupini. Lokalno institucionalno prosudbeno tijelo je odobrilo eksperimentalne postupke, a od svake je sudionice dobiven potpisani informirani pristanak.

### Metode

Prikupljeni su uzorci krvi (5 mL) i serumi su ispitani na prisutnost IL-2 prema uputama proizvođača (Bender Medsystem GmbH, Beč, Austrija). Ukratko, postupak se sastojao od sljedećih koraka: 1) 100  $\mu$ L svakog uzorka u duplikatu je dodano u jažicu; također, ista je količina razrjeđivača uzorka stavljena u jažice za slijepu probu; 2) 50  $\mu$ L razri-

of RSA cannot be determined in almost 50% of cases. It has been postulated that a number of these repeated pregnancy losses may be related to immune causes (2). The response of T helper (Th) cells following activation is characterized functionally according to the cytokines they produce. Th1 cells secrete mainly interleukin (IL)-2, interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\beta$  (3). IL-2 figures prominently among cytokines that are particularly related to the survival of the conceptus. Chaouat et al. (1990) showed that administration of IL-2 to pregnant mice causes abortion (4).

Rheumatoid factors (RF) are antibodies that recognize the Fc portion of IgG molecules as their own antigens. RFs can be of any immunoglobulin isotype (IgM, IgG, IgE). Most of the RFs measured clinically are of IgM class (5). Among healthy population, the frequency of RF positive individuals ranges from 1.3-4% in Caucasians to 30% in some tribes of North American Indians (6). A small subset of B cells that express CD5, known as B-1a cells, appear to produce RF (7). Many studies have illustrated the essential role of T cells in class switching where cytokine production has an important impact on the type of immunoglobulin produced by B cells (8).

The aim of this study was to compare the levels of IL-2 (Th1 cytokine) and RF in the sera of women having history of RSA with those of the control group.

## Materials and methods

### Patients

This case control study was carried out on two different groups referred to Yazd Research and Clinical Centre for Infertility. Group I consisted of 56 women with a history of three or more RSA and group II consisted of 63 healthy women who had no abortion history, with at least one successful pregnancy. Abortion samples were normal in karyotype, TORCH negative and had no anatomical or endocrine problems, absence of anti-phospholipid antibodies of both IgM and IgG classes, anti-cardiolipin antibodies of IgM, IgG and IgA classes, and antinuclear antibodies of IgM and IgG classes. All cases were primary RSA. Demographic information such as age, number of abortions, gestational age at the last abortion for RSA women, and the number of pregnancies in control group were provided. The local Institutional Review Board approved experimental procedures, and signed informed consent was obtained from each participant.

### Methods

Blood samples (5 mL) were collected and sera were examined for the presence of IL-2 according to manufacturer's instructions (Bender Medsystem GmbH, Vienna, Austria). The procedure is explained briefly as follows: 1) 100  $\mu$ L of each sample in duplicate was added to the well; also,

jeđenog Biotin-konjugata je dodano u sve jažice i inkubirano na sobnoj temperaturi tijekom 2 sata; 3) mikrotrake s jažicama isprane su četiri puta i 100  $\mu$ L razrijeđenog Streptavidin-HRP je dodano svim jažicama; 4) uzorci su najprije inkubirani na sobnoj temperaturi tijekom 1 sata i zatim isprani 4 puta; 5) konačno je otopina TMB-supstrata dodana svim jažicama i inkubirana na sobnoj temperaturi 15 minuta; 6) enzimska je reakcija zaustavljena pipetiranjem 100  $\mu$ L otopine za zaustavljanje reakcije u jažice i apsorbanacija svake mikrojažice je očitana na 450 i 620 nm, kao referentnim valnim dužinama.

Osjetljivost analize IL-2 iznosila je 3,5 pg/mL. Ukupan koeficijent varijacije iz dana u dan iznosio je 5,2%, a u seriji 8%. Prisutnost RF (antihumanog imunoglobulina) određena je testom lateks-aglutinacije (Bionic, Iran) prema uputama proizvođača, a prisutnost aglutinacije je smatrana pozitivnom. Pozitivni su uzorci ispitani u različitim razrjeđenjima do titra 1/64.

### Statistička analiza

Statistička je analiza provedena pomoću programskog paketa SPSS V 12.0 (SPSS Inc, Chicago, IL). Hi-kvadrat test je primijenjen za usporedbu pojavnosti RF među skupinama.

### Rezultati

Prosječna dob žena u prvoj skupini bila je  $28 \pm 4$  godine (raspon 21-42), a u drugoj skupini  $27 \pm 5$  godina (raspon 18-37). Među ženama iz dviju skupina nije bilo značajne razlike u dobi. Broj gubitaka trudnoće je varirao od 3 do 10 ( $4 \pm 1$ ). Prosječna gestacijska dob kod posljednjeg pobačaja bila je u prvoj skupini  $11,45 \pm 2,85$  tjedana. Dvije od 56 žena u skupini I imale su IL-2 u serumu (koncentracije 35 pg/mL i 10 pg/mL). IL-2 nije nađen ni kod jedne žene iz skupine II. Također, 53,5% žena u skupini I i 6% žena u skupini II bilo je pozitivno na RF u različitim titrovima (Slika 1.). Postojale su statistički značajne razlike između skupina I i II za RF ( $P = 0,002$ ).

### Rasprava

Prošlih je godina postignut određen napredak u boljem razumijevanju ponovljenog pobačaja te ponudi dijagnostičkih pomagala i obradi. PSP je još uvijek najčešća komplikacija tijekom trudnoće (9). Stvaranje citokina i razdoba imunih stanica tijekom trudnoće može pružiti važne informacije za predviđanje ishoda trudnoće, npr. termina ili gubitka trudnoće (10). U ovoj smo studiji određivali citokin IL-2 u žena s anamnezom PSP te u žena reproduktivno podudarne dobi bez pobačaja. Iako nismo mogli otkriti IL-2 u serumima kontrolne skupine, o povišenim koncentracijama IL-2 u normalnoj trudnoći već je izvještavano (11). Takva razlika u rezultatima može biti povezana s do-

the same amount of sample diluents was placed into blank wells; 2) 50  $\mu$ L of diluted Biotin-Conjugate was added to all wells and was incubated at room temperature for 2 hours; 3) microwell strips were washed 4 times and 100  $\mu$ L diluted Streptavidin-HRP were added to all wells; 4) samples were first incubated at room temperature for 1 hour and then washed 4 times; 5) finally TMB substrate solution was added to all wells and was incubated at room temperature for 15 minutes; 6) the enzyme reaction was stopped by pipetting 100  $\mu$ L of stop solution into the wells and absorbance of each microwell was read at 450 nm and 620 nm as the reference wave length.

The sensitivity of IL-2 assay was 3.5 pg/mL. The overall intra-assay and inter-assay coefficient of variation was calculated to be 5.2% and 8%, respectively. The presence of RF (anti-human immunoglobulin) was determined by a latex agglutination test (Bionic, Iran) according to manufacturer's instructions and the presence of agglutination was considered positive. Positive samples were tested in different dilutions to 1/64 titers.

### Statistical analysis

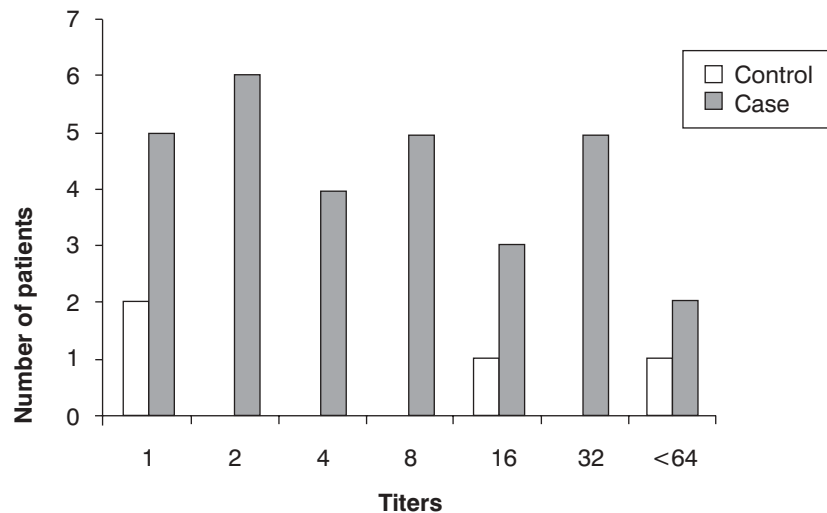
Statistical analysis was performed using statistical software V 12.0 (SPSS Inc, Chicago, IL). Chi-square test was used for comparison of the incidence of RF between groups.

### Results

The mean age of women in group I was  $28 \pm 4$  years (range 21-42) and for those in group II it was  $27 \pm 5$  years (range 18-37). There was no significant difference in the age of women in two groups. Pregnancy loss numbers varied from 3 to 10 ( $4 \pm 1$ ). Mean gestational age in the last abortion was  $11.45 \pm 2.85$  weeks in group I. Two out of 56 women in group I had IL-2 in serum (35 pg/mL and 10 pg/mL). None of the women in group II had IL-2 in serum. Also, 53.5% of women in group I and 6% of women in group II were positive for RF in different titers (Figure 1). There were statistically significant differences between groups I and II for RF ( $P = 0.002$ ).

### Discussion

In recent years, some progress has been made in better understanding of recurrent abortion and in the offer of diagnostic tools and management. RSA is still the most common complication during pregnancy (9). Production of cytokines and distribution of immune cells during pregnancy may provide important information for predicting pregnancy outcome, e.g. the term or loss of pregnancy (10). In this study, we measured IL-2 cytokine in sera of women with history of RSA and in reproductive age-matched women without any abortion. Although we were unable to detect IL-2 in the sera of our control



**SLIKA 1.** Učestalost titra RF u skupini s PSP (bolesnice) i kontrolnoj skupini.

**FIGURE 1.** The frequency of RF titers in RSA (case) and control groups.

bi ispitanica, kompletima reagencija za mjerenje, rasom i društveno-ekonomskim statusom. Samo su dvije od 56 žena s anamnezom PSP bile pozitivne na IL-2 u serumu. Neke studije ukazuju da limfociti iz periferne krvi žena s PSP, u usporedbi sa ženama s uspješnom trudnoćom, luče povišene koncentracije određenih citokina Th1 kao, primjerice, IL-2 nakon stimulacije mitogenima ili antigenima (12,13). Stvaranje citokina Th1 je, međutim, smanjeno tijekom trudnoće (14). Naši rezultati nisu pokazali sličan obrazac stvaranja IL-2 u ispitivanim uzorcima. Kao dio istraživanja također smo mjerili RF u dvjema skupinama. Rezultati su pokazali da je 53,5% žena s anamnezom PSP bilo pozitivno na RF čija je koncentracija bila značajno viša nego u kontrolnih ispitanica. U nekim je studijama navedeno da određena autoantitijela nepovoljno utječu na tijek trudnoće i fetalnog rasta (15) no jedini članak koji spominje porast RF tijekom trudnoće jest rad Ailusa i sur. (16). U istraživanju autora Ijime i sur. 8,3% žena s anamnezom PSP koje su bile uključene u studiju imalo je RF u serumu (17). RF su autoantitijela povezana s reumatoidnim artritisom koja je moguće otkriti u normalnih pojedinaca, no samo prolazno. Nedavno je dokazano da B-stanice proizvode RF samo kad su aktivirane dvama signalima, tj. jednim zbog uključenosti receptora B-stanica, a drugim zbog prepoznavanja patogeno povezanog molekularnog obrasca preko *Toll-like* receptora. Stoga ta autoantitijela povezuju prirodni i stečeni imuni odgovor (18). Poznate su i uloge nekih infektivnih agenasa u ponovljenim pobačajima. Moguća je, međutim, i određena povezanost između mikrobijalnog pobačaja i stvaranja RF.

group, elevated concentrations of IL-2 in normal pregnancy as compared with non-pregnant controls have been reported (11). This difference in results may be related to the age of patients, detection kits, race and socio-economical status. Only two out of fifty-six women with the history of RSA were positive for IL-2 in serum. There are some studies which indicate that peripheral blood lymphocytes of women with RSA secrete increased concentrations of certain Th1 cytokines, such as IL-2 upon stimulation by mitogens or antigens when compared in women with successful pregnancy (12, 13). However, Th1 cytokine production is down-regulated during pregnancy (14). Our results failed to show similar profile of IL-2 production in the studied sera. As part of this research, also RF was measured in two groups. Our results showed that 53.5% of women with the history of RSA were positive for RF, and its level was significantly higher than in controls. It has been reported that some autoantibodies adversely affect the course of pregnancy and fetal growth (15). For RF, the only report referring to its increase during pregnancy is that of Ailus et al (16). 8.3% of women with RSA history that were studied by Ijima et al. had RF in their sera (17). RFs are autoantibodies associated with rheumatoid arthritis. They can be detected in normal individuals, although transiently. Recently, it has been shown that B cells which produce RF only do so when activated by two signals, one from engagement of the B cell receptor and the other from recognition of a pathogen-associated molecular pattern through a Toll-like receptor. These autoantibodies thus link the innate and acquired immune

## Zaključak

Rezultati koji su postignuti u ovom istraživanju pokazali su da koncentracija IL-2 u serumu nije utjecala na ishod trudnoće. Ipak, prevalencija RF je bila veća među bolesnicama s PSP nego u normalnih žena bez pobačaja. Zbog toga se preporuča istraživanje uloge RF u odnosu na ishod trudnoće.

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response (18). The roles of some infectious agents in recurrent abortions are well known. However, there might be some correlation between microbial abortion and RF production.

## Conclusion

The results of this study showed that serum IL-2 concentration did not affect pregnancy outcome. However, the prevalence of RF was higher among patients with RSA than in normal women with no history of abortion. Therefore, it is recommended that the role of RF in pregnancy outcome should be investigated.

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