

# Th1/Th2 Cytokine Balance and SOCS3 Levels of Female Offspring Born from Rats with Gestational Diabetes Mellitus <sup>[1]</sup>

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## Abstract

Diabetes mellitus during pregnancy is a metabolic disorder that is very important in regard to both the health of the mother and the baby. We determine the effect of diabetes mellitus generated by streptozotocin with different dosages in pregnant rats on serum Th1/Th2 cytokine balance and SOCS3 levels. Twenty-one pregnant rats in their late pregnancy were used for this purpose. The rats were divided into three groups randomly and the rats in the first group were used as the control group. Streptozotocin was administered intraperitoneally at a dosage of 40 mg/kg to the rats in the second group and at a dosage of 60 mg/kg to the rats in the third group. One female offspring of each rat was decapitated and the blood of the decapitated rats was collected and Th1 [Interferon gamma (IFN $\gamma$ ), Interleukin 2 (IL-2), Tumor necrosis factor alpha (TNF $\alpha$ )], Th2 [Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 10 (IL-10)] cytokine levels were measured using a multiplex immunoassay based on xMAP<sup>®</sup> detection technology and the suppressor of cytokine signaling 3 (SOCS3) levels were measured using a commercial ELISA kit. The IFN- $\gamma$  (1.59 $\pm$ 0.28 pg/mL) levels in group 1 were lower than those in the other groups. The TNF- $\alpha$  (2.24 $\pm$ 0.20, 2.21 $\pm$ 0.19 pg/mL) levels in groups 2 and 3 were higher than in group 1, and the IL-4, IL-6, IL-10 and SOCS3 concentrations were not significantly different among groups. The SOCS3 levels of the offspring were not different among the groups, and the IFN- $\gamma$  and TNF- $\alpha$  blood concentrations were increased; the Th1/Th2 cytokine balance was shifted toward Th1. We have observed that the SOCS3 levels of offspring born from mothers generated to be diabetic by administration of streptozotocin in late pregnancy were not different among various groups, and the IFN- $\gamma$  and TNF- $\alpha$  blood concentration were increased. As a result, the Th1/Th2 cytokine balance was shifted toward Th1. This suggests that more prominent cellular immunity.

**Keywords:** Cytokine, Diabetes Mellitus, Offspring, Pregnancy, Rat, SOCS3

## Gebelikte Diabetes Mellitus Şekillenmiş Ratlardan Doğan Dişi Yavrularda Th1/Th2 Sitokin Dengesi Ve SOCS3 Düzeyleri

### Özet

Gebelikte şekillenen diabetes mellitus, hem anne sağlığı hem de yavru sağlığı için son derece önemli ve giderek görülme sıklığı artan bir metabolik bozukluktur. Bu hastalığın gelişimi ve tedavisine yönelik yeni araştırmalara ihtiyaç vardır. Bu çalışmada da ileri gebe ratlarda farklı dozlarda streptozotosinle oluşturulan diabetes mellitusun yavruların, kan serumlarındaki Th1/Th2 sitokin dengesi ve SOCS3 düzeyleri üzerine etkisinin belirlenmesi amaçlanmıştır. Bu amaçla, 21 adet ileri gebe rat kullanıldı. Ratlar rastgele üç gruba ayrılarak 1. gruptaki hayvanlar kontrol grubu olarak ayrıldı. Streptozotosin 2. gruptaki hayvanlara 40 mg/kg, 3. gruptaki hayvanlara 60 mg/kg dozda intraperitoneal olarak uygulandı. Daha sonra hayvanların doğumları takip edildi. Yavrular 1 aylık olduktan sonra her hayvanın 1 dişi yavrusu dekapite edildi. Dekapite edilen hayvanların kanları alındı ve kan serumlarında Th1 [Interferon gamma (IFN $\gamma$ ), Interleukin 2 (IL-2), Tumor necrosis factor alpha (TNF $\alpha$ )], Th2 [Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 10 (IL-10)] sitokin düzeyleri xMAP<sup>®</sup> tespit teknolojisini esas alan multiplex immünoassay ve Suppressor of cytokine signalling 3 (SOCS3) düzeyleri ise ticari ELİSA kiti ölçüldü. Sonuç olarak, grup 1'deki IFN- $\gamma$  (1,59 $\pm$ 0,28 pg/ml) değerinin diğer gruplara nazaran daha düşük olduğu, grup 2 ve 3'teki TNF- $\alpha$  (2,24 $\pm$ 0,20, 2,21 $\pm$ 0,19 pg/ml) değerlerinin de grup 1'den fazla olduğu, tüm gruplardaki IL-4, IL-6, IL-10 ve SOCS3 konsantrasyonlarının karşılaştırılması sonucunda ise gruplar arasında istatistiki anlamda bir fark olmadığı belirlendi. Sonuç olarak; Th1/Th2 sitokin dengesinin Th1'e doğru kaydığı gözlemlendi. Bu da hücresel immünitenin daha ön planda olduğunu göstermektedir.

**Anahtar sözcükler:** Sitokin, Diabetes mellitus, Yavru, Gebelik, Rat, SOCS3



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## INTRODUCTION

Gestational diabetes mellitus (GDM) appearing as a glucose tolerance disorder during pregnancy negatively affects the health of both the mother and the baby. Maternal diabetes creates an unfavorable environment for embryonic and placental development. Both type 1 and type 2 GDM patients are at high risk of developing complications such as abortion, stillbirth, congenital malformations, placental anomalies and intrauterine development impairment <sup>[1]</sup>.

It has been reported that the immune system of the babies born from diabetic mothers may be affected at different levels and even long-term effects such as immunodeficiency have been observed in babies <sup>[2]</sup>.

In particular, the cytokines released from CD4+ (cluster of differentiation 4) T lymphocytes play an important role in the regulation of immunological reactions. Th0 lymphocytes differentiate into two subgroups: Th1 and Th2. IL-2, IL-12, IL-15, IL-18, IFN $\gamma$  and TNF $\beta$  are released from the Th1 group CD4+ T lymphocytes, and IL-4, IL-5, IL-6, IL-10, IL-13 and Granulocyte-macrophage colony-stimulating factor (GM-CSF) are released from the Th2 group CD4+ T lymphocytes. Th1 cells are responsible for cellular immunity whereas Th2 cells are responsible for humoral immunity <sup>[3,4]</sup>.

Etiology, genetics and pathogenesis of type 1 and 2 DM are different. However, there is strong evidence that inflammatory mediators play an important role in the pathogenesis of both diseases <sup>[5,6]</sup>. Loss of beta cells in both type 1 and type 2 DM may be caused by apoptosis and necrosis triggered by inflammatory mediators. In type 1 diabetes is destruction of beta cells due to immune causes. Pro-inflammatory cytokines emerging during the inflammation of pancreatic islets cause apoptosis and necrosis of beta cells <sup>[5,6]</sup>. Both the lack and apoptosis of beta cells and the high levels of pro-inflammatory cytokines during the early periods of the disease are causes of type 2 DM <sup>[7-10]</sup>. Furthermore, it is known that various pro-inflammatory cytokines stimulate insulin resistance <sup>[11-13]</sup>.

Natural inhibitors of some cytokines have recently been defined <sup>[14]</sup>. Suppressor of cytokine signaling (SOCS) proteins are also part of this group of natural inhibitors that regulate IFN- $\gamma$  signals via negative feedback <sup>[14]</sup>. These proteins are inhibitors of the cytokine signal network and are important physiological regulators playing a role in the regulation of innate and adaptive immunity. Signal Transducers and Activators of Transcription (STAT) signal networks play a role in many cellular events, such as the release of cytokines and growth factors. Cytokines lead to cell proliferation or death over gene expression by triggering the JAK/STAT pathway. SOCS3 proteins down-regulate IL-1 signals and restrict the severity and duration

of the cytokine response in a negative direction through STAT3 activation <sup>[15,16]</sup>. These molecules play an essential role in T cell development and differentiation <sup>[17]</sup>.

In this study, we investigated the effect of streptozotocin induced gestational diabetes mellitus on serum Th1/Th2 cytokine balance and SOCS3 levels in pregnant rats.

## MATERIAL and METHODS

### Animals

A total of 21 Wistar rats, aged 3-4 months and weighing 200-250 g, were used in this study. The rats were obtained from Firat University Experimental Research Center. The rats were kept in separate cages in groups of seven, and a 12-h light and 12-h dark regime was applied. The rats were fed as much as they could eat and drink. The Ethical Committee Report was obtained from the Firat University Experiment Animals Ethical Committee (FUHADEK 2014/8).

### Selection of Animals

Twenty-one rats that were detected to be in estrous were selected by vaginal irrigation. The rats were placed in cages in groups of three. One male rat was placed in each case and coitus was followed.

### Vaginal Irrigation

Vaginal irrigations were performed as described by Rişvanlı et al. <sup>[18]</sup>. Irrigations were performed using elastic pipette bulb and pipette tip with distilled water. Fluid obtained by irrigation was placed on the slide and examined under the microscope with 40 $\times$  magnifications. The intensity of the cell types included in the samples were evaluated as +, ++ and +++. Rats with a superficial cell intensity of +++ were accepted as being in estrous. Rats that had spermatozooids in their samples prepared with vaginal irrigation were accepted as being fertilized. The date of examination was accepted as the first day of pregnancy.

### Experimental Groups

The rats were randomly divided into three groups and group 2 and 3 rats were exposed to the following treatment on day 13 of the study:

Group 1: Control group, intraperitoneal normal saline (n=7)

Group 2: Intraperitoneal streptozotocin (SIGMA, USA) 40 mg/kg (n=7) <sup>[19]</sup>,

Group 3: intraperitoneal streptozotocin (SIGMA, USA) 60 mg/kg (n=7) <sup>[19]</sup>.

Streptozotocin was prepared in doses indicated above in solution of 0.1 M citrate phosphate tampon.

Glucose concentrations were measured with a glucometer using blood collected from the tails of the rats 3 days after the injections, on day 16 of pregnancy. Normal blood glucose levels were accepted as 90-110 mg/dl; rats that had a blood glucose level over 250 mg/dl were accepted as diabetic [20].

### Analyses

Afterwards, the rats were placed in separate individual cages and the delivery of each rat was followed. One female offspring of each rat was decapitated after the offspring were 1 month of age. The blood of the decapitated rats was collected and kept at -20°C until the assay after separating the serum. Th1 (IFN- $\gamma$ , IL-2, TNF- $\alpha$ ) and Th2 (IL-4, IL-5, IL-10) cytokine levels were measured with a multiplex immunoassay (Procarta<sup>®</sup> CytokineAssay Service, Diax, Italy) based on xMAP<sup>®</sup> detection technology [21]. SOCS3 levels were measured using a commercial ELISA kit (Catalog No: MBS2019020, MyBiosource Inc., USA) and read by an ELISA reader (BioTek Instruments, USA).

### Statistical Analysis

The Kruskal-Wallis test was used to compare the blood serum levels of the offspring rats among the groups. The significance level was determined by the Mann-Whitney U-test in cases in which significance was present as a result of the Kruskal-Wallis test. Statistical analysis was performed using the SPSS 11.5 program.

## RESULTS

The IFN- $\gamma$  (1.59±0.28 pg/mL) levels in group 1 animals were lower than those of other group rats (P<0.001). In addition, the TNF- $\alpha$  (2.24±0.20 pg/mL and 2.21±0.19 pg/mL) levels in groups 2 and 3 were higher than that of group 1 (P<0.001). The IL-2 concentrations in all groups were determined to be below measurable levels (Table 1).

No significant difference was determined among groups in terms of IL-4, IL-6, IL-10 and SOCS3 concentrations (Table 1).

## DISCUSSION

The cytokine environment around the fetus is important for the continuation of the pregnancy. According to the immunotropic hypothesis described by Wegmann et al. [22], the Th1/Th2 cytokine balance is an important mechanism to maintain the vitality of the fetus in the uterus. Local production of Th2-type cytokines in the fetomaternal relation is required for the constitution of the pregnancy. The Th1/Th2 cell balance is in favor of Th2 in diabetic pregnancies of both animals and humans. However, the Th1/Th2 balance of macrosomic and obese babies born from diabetic mothers is directed toward pro-inflammatory Th1. The direction toward Th1 in obese babies plays a role in the development of diabetogenic status that exhibits hyperglycemia and hyperinsulinemia seen in later years [23]. In this study the IFN- $\gamma$  (Group 2: 2.16±0.24, Group 3: 2.27±0.24 pg/mL) and TNF- $\alpha$  (Group 2: 2.24±0.20, Group 3: 2.21±0.19 pg/mL) levels of female offsprings born from mothers with DM were determined to be higher as well.

TNF- $\alpha$  stimulates insulin resistance by indirectly stimulating the stress hormone or continuous induction of SOCS3 proteins [11,24]. Furthermore, SOCS3 is claimed to inhibit leptin insulin signaling. Growth hormone also causes the development of insulin resistance by causing the release of SOCS3 [25]. Therefore; SOCS3 plays an important role as a candidate gene in the pathogenesis of type 1 diabetes and insulin resistance. In a study by Gylvin et al. [5] no mutation in the SOCS3 gene identification was found in people with type 1 DM. No data were found about the SOCS3 levels of babies born from mothers that had diabetes during pregnancy. In the present study, the serum SOCS3 levels of female offsprings born from mothers with GDM were determined to be different from that of the control group.

The immune status of the babies born from diabetic mothers is important for the postpartum period and also for the rest of the life of the offspring, since any damage to the immune system may cause life-threatening problems also in the future. In conclusion, we demonstrated that

**Table 1.** Comparison of the cytokine and SOCS3 levels among groups

**Tablo 1.** Gruplar arasında sitokin ve SOCS3 düzeylerinin karşılaştırılması

Parameters	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	P
IFN- $\gamma$ (pg/mL)	1.59±0.28 <sup>a</sup>	2.16±0.24 <sup>b</sup>	2.27±0.24 <sup>b</sup>	*
IL-2 (pg/mL)	BD	BD	BD	-
TNF- $\alpha$ (pg/mL)	BD	2.24±0.20 <sup>b</sup>	2.21±0.19 <sup>b</sup>	*
IL-4 (pg/mL)	0.22±0.11	0.22±0.10	0.22±0.08	-
IL-6 (pg/mL)	25.51±1.86	25.43±0.75	24.69±2.05	-
IL-10 (pg/mL)	2.41±0.27	2.44±0.22	2.34±0.24	-
SOCS3 (ng/mL)	0.38±0.08	0.36±0.06	0.33±0.15	-

- The difference between the groups was insignificant (P>0.05); \* The difference between groups was significant (P<0.05); <sup>ab</sup> The difference between the values indicated with different letters in the same line was significant (P<0.001); BD: below detection limits

the IFN- $\gamma$  and TNF- $\alpha$  blood concentrations of offsprings born from experimentally induced GDM female rats were increased where as the SOCS3 levels were not different among groups. As a result, the Th1/Th2 cytokine balance was shifted towards Th1. This suggests that more prominent cellular immunity.

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