# TNF-α gene polymorphism and fetal Doppler velocimetry in intrauterine growth restriction

**Agnieszka Seremak-Mrozikiewicz [1], Mariusz Dubiel [1], Krzysztof Drews [1], Saemundur Gudmundsson [2], Przemyslaw M. Mrozikiewicz [3]**

1. Department of Perinatology and Gynecology, University of Medical Sciences, Poznan, Poland
2. Malmo University Hospital, Malmo, Sweden
3. Research Institute of Medicinal Plants, Poznan, Poland

*Correspondence to:* Agnieszka Seremak-Mrozikiewicz, MD
Division of Perinatology and Women's Diseases
Department of Perinatology and Gynecology
University of Medical Sciences, Polna Street 33. 60-535 Poznan, Poland
TEL.: +48 602 250 895; FAX: +48 61 852 74 72
E-MAIL: asm@data.pl

*Submitted: 2008-07-03 Accepted: 2008-07-14 Published online: 2008-08-30*

*Key words:* **intrauterine growth restriction; tumour necrosis factor alpha; genetic polymorphism; Doppler velocimetry**

Neuroendocrinol Lett 2008; **29**(4):493–499 **PMID:** 18766139 NEL290408A29 

## Abstract

**OBJECTIVES:** The intrauterine growth restriction (IUGR) frequently is a cause of fetal morbidity and mortality, and influences perinatal outcome. Several genes have been identified to explain aetiology of IUGR, beside others the gene coding for tumour necrosis factor alpha (TNF-α).

**DESIGN:** To investigate frequency of *Alw*I polymorphism of TNF-α gene and its correlation with TNF-α level in maternal serum, Doppler velocimetry and perinatal outcome in pregnancies suspected for IUGR.

**SETTING:** 42 pregnancies with IUGR and 50 matched healthy pregnant women were included in the study. Maternal venous blood samples were investigated in relationship to blood flow Doppler velocimetry in umbilical (UA) and middle cerebral (MCA) arteries. .*Alw*I polymorphism was analysed using PCR/RFLP assays. TNF-α level was evaluated by immunoelectophoretic method.

**RESULTS:** A higher frequency of mutated *-238A* alleles (13.1% *vs.* 7.0%) and genotypes containing at least one mutated *-238A* allele (23.8% *vs.* 14.0%) were found in the IUGR group. The tendency to the higher TNF-α level in IUGR subgroups with the presence of at least one mutated *A* allele (258.9±231.3 *vs.* 174.1±145.6 pg/ml) was detected. No statistical differences were detected for PI values in UA and MCA arteries considering particular genotypes (*GG vs. GA + AA*) separately in IUGR group.

**CONCLUSION:** Increased UA vascular impedance and signs of brain sparing in MCA are related to IUGR and increased TNF-α level in maternal serum. *Alw*I polymorphism might play a role in IUGR aetiology and influence TNF-α expression in maternal serum, but was not related to Doppler velocimetry or with perinatal outcome.

ORIGINAL ARTICLE

## INTRODUCTION

Fetal growth depends on growth factors levels, oxygen and nutrients balance. Consequently the development of intrauterine growth restriction (IUGR) could be a result of maternal undernutrition coexisting with placental insufficiency [1]. IUGR remains the main cause of placental hypoxemia, fetal morbidity and mortality, however, the pathophysiological processes leading to IUGR are still not clearly understood. Early diagnosis of IUGR could result in lower rates of obstetrics interventions and could improve perinatal outcome [2]. Therefore many authors suggest broad spectrum of investigations in high-risk pregnancies such as IUGR including ultrasound evaluation, blood flow Doppler velocimetry, cytokines activity, and in recent years, the view in genetic basis of this condition that might explain the pathophysiological processes involved [3].

Lymphohematopoietic cytokines play a significant role in many biological mechanisms. Changes in cytokines pattern are also related to IUGR development [4]. An increase cytokine levels has been found in amniotic fluid and in maternal serum in IUGR pregnancies [5, 6]. One of the most investigated cytokine is tumour necrosis factor alpha (TNF-α), which is a potent inducible cytokine with pleiotropic biological effects [1, 4]. In humans TNF-α is a 17 kDa 157 amino acid chain with chemical structure identical to cachectin derived from macrophages. TNF-α circulates in the blood to organs and acts through the specific receptors p75 and p55 [7] to mediate various physiologic and pathophysiologic events. The important function of TNF-α within a complex of cytokine network is activation of multiple signal transduction pathways, induction or suppression of wide variety of genes, including those encoding for other cytokines, adhesion molecules and inducible nitric oxide synthase [8].

Gene encoding for the TNF-α is located on chromosome 6 (6p23-q12 segments). It is about 3 kilo base pairs long and contains 3 introns [9]. Recently, genetic polymorphisms in TNF-α promoter region [10, 11] influencing TNF-α level and activity have been identified. Since the tendency to higher level of TNF-α has been found in the maternal serum and amniotic fluid in the pregnancies complicated by IUGR, it could be suggested the possible role of TNF-α and its polymorphisms in the pathogenesis of fetal hypotrophia [5].

Considering IUGR as a condition commonly connected with increased placental vascular impedance, we hypothesized that genetic polymorphism of TNF-α could also be correlated with disturbances in blood flow in umbilical and middle cerebral arteries in pregnancy affected with IUGR. The aim of this study was to investigate if there was a correlation between increased frequency of *-238G/A Alw*I restriction polymorphism of TNF-α gene and TNF-α level in maternal serum, Doppler velocimetry and perinatal outcome in pregnancies suspected for IUGR.

## MATERIAL AND METHODS

*Patients*

Forty-two pregnancies suspected for IUGR were analysed (mean maternal age 25.6 ± 3.8 years, mean gestational age at birth time 33.2 ± 4.0 weeks). The diagnosis of suspected IUGR was based on ultrasound measurements (foetal biometry: biparietal diameter, head and abdominal circumference, femur length). Birth weight at or below the 10$^{th}$ percentile for gestational age and sex according to the centile chart for Polish standards for birth weight. Fifty healthy pregnant women (mean age 26.6 ± 3.3 years, mean gestational age at birth time 39.9 ± 1.2 weeks) were evaluated as a control group. The statistically differences were noted in average gestational age at birth between the groups. However, the differences in maternal age between the groups were not statistically significant. Gestational age was confirmed by an ultrasound examination performed in early pregnancy. All subjects were singleton pregnancies. Multiple pregnancies, pregnancies with chromosomal abnormalities and fetal anomalies, women with diabetes mellitus, cardio-vascular-, and renal diseases were excluded from the study. All the patients were Caucasians of Polish origin. Each subject was informed about the goal of the study and gave their written consent. The regional Ethics Committee approved the study.

## METHODS

*Genetic analysis:* From each woman, 5-6 ml of venous blood sample was collected and stored in –20$^{o}$C until further analysis. Genomic DNA was extracted by phenol-chloroform method from blood leucocytes. In both groups, single nucleotide polymorphism (*-238G/A*) in the regulatory region of the gene coding for TNF-α was evaluated using PCR/RFLP (polymerase chain reaction/restriction fragment length polymorphism) assay. For detection *-238G/A* point mutation 131 bp fragment of DNA was amplificated with specific oligonucleotides (TNF1: 5'AAA TGG AGG CAA TAG GTT TTG AGG GGC TTG and TNF2: 5'TAC CCC TCA CAC TCC CCA TCC TCC CTG ATC) (Tib MolBiol, Poland). After the digestion, the PCR fragment was incubated with *Alw*I restriction enzyme (New England BioLabs Inc, USA). Two DNA fragments (110 and 21 bp long) indicated homozygote wild-type, whereas lack of cutting place was diagnosed as the presence of mutated *A* allele.

*Biochemical analysis:* TNF-α level in maternal serum in both investigated groups (IUGR and control) was determined using enzyme immunoassay (CytElisa Human TNF-α, Cytimmune Sciences Inc, USA), sensitivity 4.8 pg/ml, range of detection 15.6 – 1000.0 pg/ml.

*Ultrasound Doppler analysis:* Doppler examination was performed with Aloka SSD 5500 duplex scanner (Aloka Corp., Japan) using 5 MHz transducer with color

**Table 1.** The frequency of genotypes and alleles of *-238G/A* of TNF-α polymorphism.

| | IUGR | | | Controls | | |
|---|---|---|---|---|---|---|
| | n (%) | Expected (%) | 95% CI | n (%) | Expected (%) | 95% CI |
| **Genotypes** | | | | | | |
| **GG** | 32 (76.2) | 75.0 | 60.0–88.0 | 43 (86.0) | 73.9 | 73.2–94.8 |
| **GA** | 9 (21.4) | 23.0 | 10.3–36.8 | 7 (14.0) | 24.1 | 5.8–26.7 |
| **AA** | 1 (2.4) | 2.0 | 0.06–12.6 | 0 (0.0) | 2.0 | 0–7.1 |
| **Alleles** | | | | | | |
| **G** | 73 (86.9) | – | 77.8–93.3 | 93 (93.0) | – | 86.1–97.1 |
| **A** | 11 (13.1) | – | 6.7–22.2 | 7 (7.0) | – | 2.9–13.9 |

IUGR – intrauterine growth restriction, CI – confidence limit
Expected values were calculated according to Hardy-Weinberg equilibrium

and pulsed Doppler options. In each case Doppler recordings in umbilical (UA) and middle cerebral (MCA) arteries have been performed. Recordings from the UA were obtained from a free-floating central part of the umbilical cord and MCA approximately 1 cm from the origin of the vessel. The Doppler recordings were only performed during the periods when fetal breathing and body movements were absent. Three consecutive blood velocity waveforms were analyzed for pulsatility index (PI) and correlated to normal reference values [12, 13]. Doppler examination was considered as pathological when the PI exceeded 2 standard deviations above the mean value for gestational age. The ultrasound examinations in the both groups were performed repeatedly every second week. The result of last examination before delivery in IUGR group was considered in our study. All the Doppler examinations were performed by same operator (M.D.). All results were plotted on the normal chart for UA (PI range in UA for 33 week of gestation 0.75–1.25) [12] and for MCA (PI range in MCA for 33 week of gestation 1.50-2.35) [13]. PI values of middle cerebral and umbilical arteries were compared in both groups at a similar gestational age (33.2 ± 4.0 *vs.* 33.5 ± 0.9 weeks in IUGR and control groups, respectively, non-statistically significant). Fetal brain sparing was defined as an increase in blood flow of the middle cerebral artery demonstrated by a lower value of the pulsatility index (< mean -2 SD).

*Perinatal outcome:* In each case the perinatal outcome was evaluated by Apgar score at 5 min., fetal birth weight, arterial and venous pH, the need of operative delivery for fetal distress (ODFD), admissions for the neonatal intensive care unit (NICU), neonatal ventilation (the need of using the mechanical system for support the bodily process of inhalation and exhalation in neonate) and neonatal mortality (the death rate during the first 28 days of life).

*Statistical analysis:* The statistical analysis was performed using SPSS v. 11.5 for Windows. The results were calculated as the mean, standard deviation, and range value. The inter-group comparisons have been done using the chi-squared test. Continuous variables between groups were compared using the Mann-Whitney test. In genetic analysis the odds ratio and 95% confidence limit has been calculated. Expected genotypes frequency was from calculated applying Hardy-Weinberg equation and compared with observed frequency by chi-squared test. The results were considered as statistically significant if the $p$ value was below 0.05.

## RESULTS

*Genetic analysis:* In the IUGR group a higher frequency of the genotypes containing at least one mutated *A* allele (homozygous *-238A/A* and heterozygous *–238G/A* genotypes) was found (23.8% *vs.* 14.0%, OR = 1.92, 95%CI 0.6–6.6, $p$ = ns). Underrepresentation of *-238G/-238G* genotype (wild-type) was also found in the IUGR group (76.2% *vs.* 86.0%, OR = 0.5, 95%CI 0.2 – 1.7, $p$ = ns). Heterozygous genotype *-238G/A* was detected in 21.4% in IUGR and 14.0% in controls, respectively (Table 1). These proportions were consistent with the Hardy-Weinberg equilibrium. Related to the presence of alleles, the overrepresentation of *-238A* mutated allele was observed in IUGR group (13.1% *vs.* 7.0% in the IUGR and controls, respectively, OR = 2.0, 95% CI 0.67 – 6.39, $p$ = NS) (Table 1).

*Doppler velocimetry:* A statistically significant differences were observed for PI value in umbilical artery between the pregnancies affected with IUGR and the controls (1.91 *vs.* 0.89, $p < 0.0001$). This observation related also to the subgroups with *GG* genotype (2.00 *vs.* 0.88, $p < 0.0001$) and *GA + AA* genotypes (1.63 *vs.* 0.93, $p < 0.0001$) comparing IUGR and the controls. However, no differences were detected for mean PI values considering particular genotypes (*GG vs. GA + AA*) separately in IUGR (*GG vs. GA + AA* = 2.00 *vs.* 1.63) and control (*GG vs. GA + AA* = 0.88 *vs.* 0.93) group. PI value in MCA was not correlated with presence of mutated *A* allele in IUGR group (*GG vs. GA + AA* = 1.53 *vs.* 1.56) and in the controls (*GG vs. GA + AA* = 1.62 *vs.* 1.64).

**Tab. 2.** PI values in umbilical and middle cerebral arteries and TNF-α level in maternal serum correlated with genotypes in the investigated groups (IUGR and controls).

| | Genotypes GG + GA + AA | | Genotype GG | | Genotypes GA + AA | |
|---|---|---|---|---|---|---|
| | IUGR | Controls | IUGR | Controls | IUGR | Controls |
| **Patients number** | **42** | **50** | **32** | **43** | **10** | **7** |
| **Mean gestational week of examination ± SD** | 33.2 ± 4.0 | 33.5 ± 0.9 | 33.2 ± 4.1 | 33.5 ± 0.9 | 33.3 ± 3.8 | 33.6 ± 1.0 |
| | *p* = ns | | *p* = ns | | *p* = ns | |
| **PI in umbilical artery** | | | | | | |
| **Mean value ± SD** | 1.91 ± 0.80 | 0.89 ± 0.09 | 2.00 ± 0.80 | 0.88 ± 0.09 | 1.63 ± 0.80 | 0.93 ± 0.07 |
| **Median** | 1.76 | 0.89 | 1.83 | 0.88 | 1.39 | 0.92 |
| **Range** | 0.78–3.79 | 0.69–1.15 | 0.84–3.79 | 0.69–1.15 | 0.78–3.22 | 0.85–1.05 |
| ***p*** | *p < 0.0001* | | *p < 0.0001* | | *p < 0.0001* | |
| **PI in middle cerebral artery** | | | | | | |
| **Mean value ± SD** | 1.54 ± 0.45 | 1.62 ± 0.11 | 1.53 ± 0.45 | 1.62 ± 0.12 | 1.56 ± 0.48 | 1.64 ± 0.11 |
| **Median** | 1.49 | 1.62 | 1.49 | 1.61 | 1.56 | 1.62 |
| **Range** | 0.96–2.65 | 1.47–1.87 | 0.98–2.65 | 1.41–1.87 | 0.96–2.12 | 1.49–1.77 |
| ***p*** | *p = ns* | | *p = ns* | | *p = ns* | |
| **Brain sparing efect (n)** | 16 | 0 | 12 | 0 | 4 | 0 |
| **TNF-α level** | | | | | | |
| **Mean level ± SD (pg/ml)** | 225.9 ± 187.2 | 34.08 ± 19.8 | 174.1 ± 145.6 | 32.0 ± 18.1 | 258.9 ± 231.3 | 46.9 ± 26.6 |
| ***p*** | *p < 0.0001* | | *p < 0.0001* | | *p < 0.05* | |

IUGR – intrauterine growth restriction, TNF-α – tumour necrosis factor alpha, PI – pulsatility index, UA – umbilical artery, MCA – middle cerebral artery

**Table 3.** PI values of umbilical and middle cerebral arteries correlated with the presence of abnormal velocimetry blood flow effects and genotypes in the IUGR group (n = 42).

| | Genotypes | | |
|---|---|---|---|
| | **GG** | **GA + AA** | ***p*** |
| **Normal PI in umbilical artery (n = 16)** | | | |
| **n** | 10 | 6 | |
| **Mean value ± SD** | 1.18 ± 0.25 | 1.14 ± 0.23 | *p = ns* |
| **Median** | 1.13 | 1.13 | |
| **Range** | 0.84 – 1.57 | 0.78 – 1.44 | |
| **Abnormal PI in umbilical artery (n = 26)** | | | |
| **n** | 22 | 4 | |
| **Mean value ± SD** | 2.37 ± 0.67 | 2.35 ± 0.80 | *p = ns* |
| **Median** | 2.20 | 2.31 | |
| **Range** | 1.61 – 3.79 | 1.57 – 3.22 | |
| **PI in middle cerebral artery correlated with brain sparing effect – not present (n = 26)** | | | |
| **n** | 20 | 6 | |
| **Mean value ± SD** | 1.75 ± 0.43 | 1.89 ± 0.31 | *p = ns* |
| **Median** | 1.65 | 1.98 | |
| **Range** | 1.02 – 2.65 | 1.30 – 2.12 | |
| **PI in middle cerebral artery correlated with brain sparing effect – present (n = 16)** | | | |
| **n** | 12 | 4 | |
| **Mean value ± SD** | 1.16 ± 0.15 | 1.08 ± 0.12 | *p = ns* |
| **Median** | 1.15 | 1.08 | |
| **Range** | 0.98 – 1.55 | 0.96 – 1.18 | |

*PI – pulsatility index, UA – umbilical artery, MCA – middle cerebral artery*

**Tab. 4.** The pregnancy outcome correlated with genotypes in investigated groups (IUGR and controls). Numbers given are mean ± SD.

| | Genotype GG + GA + AA | | Genotype GG | | Genotypes GA + AA | |
|---|---|---|---|---|---|---|
| | **IUGR** | **Controls** | **IUGR** | **Controls** | **IUGR** | **Controls** |
| **Patients number** | **42** | **50** | **32** | **43** | **10** | **7** |
| **Mean maternal age (years)** | 25.6 ± 3.8 | 26.6 ± 3.3 | 25.4 ± 3.9 | 26.4 ± 3.5 | 26.3 ± 3.7 | 27.3 ± 2.6 |
| **Week of delivery** | 33.2 ± 4.0 | 39.9 ± 1.2 | 33.3 ± 4.1 | 39.9 ± 1.1 | 33.7 ± 3.9 | 40.0 ± 1.3 |
| **Birth weight (g)** | 1367.9 ± 565.5 | 3284.2 ± 275.6 | 1329.7 ± 569.9 | 3304.9 ± 289.2 | 1490.0 ± 562.1 | 3157.1 ± 114.1 |
| **range** | 600–2410 | 2530–3920 | 600–2410 | 2530–3920 | 720–2250 | 3000–3340 |
| **Apgar score at 5′** | 7 | 10 | 7 | 10 | 6.5 | 10 |
| **median range** | 0–9 | 7–10 | 0–9 | 7–10 | 3–8 | 8–10 |
| **Arterial pH** | 7.16 ± 0.09 | 7.23 ± 0.07 | 7.16 ± 0.09 | 7.24 ± 0.07 | 7.15 ± 0.07 | 7.21 ± 0.06 |
| **Venous pH** | 7.21 ± 0.08 | 7.27 ± 0.06 | 7.22 ± 0.09 | 7.28 ± 0.07 | 7.21 ± 0.08 | 7.26 ± 0.04 |
| **ODFD** | 34 | 2 | 26 | 2 | 8 | 0 |
| **NICU** | 37 | 3 | 28 | 3 | 9 | 0 |
| **Ventilation** | 26 | 2 | 20 | 2 | 6 | 0 |
| **Mortality** | 8 | 0 | 7 | 0 | 1 | 0 |

IUGR – intrauterine growth restriction, ODFD – operative delivery for fetal distress, NICU – neonatal intensive care unit

No difference was found in PI value of MCA between IUGR and control group, either (Table 2).

Statistically significant difference were found between the presence of brain sparing ($p < 0.0001$) in the whole IUGR group and the controls. In the IUGR group (42 subjects) the UA PI value were analysed in correlation with genotypes and normal and abnormal blood flow velocimetry in UA and no differences observed. MCA velocimetry was also evaluated in correlation with particular genotypes. No statistical differences were observed between genotypes neither in terms of PI or signs of brain sparing (Table 2 and 3).

*TNF-α level:* The average TNF-α level was 225.9 pg/ml in IUGR group and 34.08 pg/ml in controls ($p < 0.0001$). Statistically differences were also found between IUGR group and controls for *GG* ($p < 0.0001$) and *GA + AA* subgroups, respectively ($p < 0.05$). TNF-α level was higher in IUGR group with the presence of least one mutated *A* allele (*GA + AA vs. GG* = 258.9 *vs.* 174.1 pg/ml, $p$ = ns) (Table 2).

*Perinatal outcome:* The statistically significant difference was found between birth weigth ($p < 0.0001$) in IUGR group and the controls. The same observation was confirmed by comparison of the particular subgroups in IUGR and the controls. The statistically significant differences were found when the Apgar score at 5' ($p < 0.0001$), arterial ($p < 0.0001$) and venous ($p < 0.0001$) pH, and gestational age of delivery ($p < 0.0001$) in IUGR and control groups were compared. The same observation was found relating to ODFD presence ($p < 0.0001$), NICU ($p < 0.0001$), ventilation ($p < 0.0001$), and mortality ($p < 0.001$) between both investigated. If compared the genotypes containing at least one mutated *A* allele (*GA + AA*) in IUGR and the controls the statistically significant differences were found between Apgar score at 5' ($p < 0.001$), presence of ODFD ($p < 0.05$), NICU ($p < 0.0001$), and ventilation ($p < 0.05$) but not between arterial, venous pH and mortality. The perinatal outcome relating to the particular genotypes is listed in Table 4.

## DISCUSSION

IUGR belonging to maladaptation diseases is considered to be related to disturbances in uteroplacental circulation. This pregnancy-related pathological condition is correlated with maternal serum changes of inflammatory cytokines that are essential factors for fetal and placental growth [5, 14, 15]. The possible mechanism of this imbalance could be connected with the increased release of interleukin 2 and interleukin 12 from helper lymphocytes Th1 and activated macrophages, and decrease release of angiogenesis factors such as basic fibroblast and vascular endothelium growth factor. Activated macrophages produce the excessive value of TNF-α that could direct damage endothelial cells. Although the precise mechanism for IUGR mediated via cytokines is still unclear, both interleukin 1 and TNF-α along with interleukin 6, might induce the secretion of substances enhancing the vasoconstriction of the fetal placental vascular bed [15]. Cytokines may also influence in-

travascular cell adhesion, coagulation and thrombosis. They can activate the endothelium and stimulate its procoagulant properties. This might explain the changes of cytokines levels related to blood flow disturbances in several uteroplacental circulation such as umbilical and uterine arteries [16]. Therefore, the most common investigation method in the case of IUGR suspicion is a longitudinal Doppler velocimetry evaluation of placental and fetal circulation [2]. The early identification of the patients suspected with IUGR may be very useful in clinical practice. Research is therefore focused on early detection of this condition. At present, beside the Doppler velocimetry, the measurement of cytokines in amniotic fluid, maternal serum or in cervical secretion may be helpful in the evaluation of cases suspected for IUGR [5, 6].

Cytokines could also play an important role in many pathological processes and may act synergistically to induce inflammatory reactions. The clinical studies are aimed at elucidating the role of these immunomodulating factors in the processes mediating preterm labour [17], preterm rupture of membranes [18, 19], intra-amniotic infection [17], and IUGR [5]. TNF-α is a proinflammatory cytokine that acts against a variety of pathogens and tumour cells. TNF-α has recently been suggested to act as a transducer of cardiovascular diseases, namely coronary artery disease and congestive heart failure [10]. In recent years many investigations have also shown the role of TNF-α in the pathogenesis of numerous disorders like: obesity [20], diabetes [21], multiplex rheumatoid arthritis [22], osteoporosis [23], hyperandrogenism [24], Alzheimer [25] and Parkinson's diseases [26].

The genetic control of cytokines production could play a role in modulation of cytokines activity. It is widely accepted that molecular mechanisms may influence cytokine gene expression and at the same time immunological reaction [9]. Although the molecular regulatory mechanism of TNF-α gene expression is still unknown it has been suggested that the presence of point mutations in regulatory region of TNF-α gene is associated with susceptibility to IUGR development. Much attention was paid to TNF-α activity regulation, and genetic polymorphisms in the promoter region (*-163G/A, -238G/A, -308G/A, -376G/A* oraz *-863G/A*). These point mutations regulate the transcription process of TNF-α gene and influence TNF-α activity [27].

In our analysis we have observed the overrepresentation of *–238A* mutated allele in the IUGR group such as statistically significant increased level of TNF-α in maternal serum with IUGR (225.9 *vs.* 34.08 pg/ml). The determined values were also higher in the subgroups with the genotypes containing at least one mutated *A* allele (*GG vs. GA +AA* = 174.1 *vs.* 258.9 pg/ml). However, the results were not statistically significant, but might suggest a probable role of several cytokines in IUGR pathology. Additionally to our results it was shown that in fetal growth restriction TNF-α level was elevated in amniotic fluid and the others cytokines such as colony stimulating factors were reduced [4]. However, it were also suggested that these cytokines have been not raised in pregnancies complicated by fetal growth restriction [28].

Summarizing, we have shown that the presence of the *-238G/A* polymorphism in the gene coding for TNF-α could play a modulating role in the immunological answer of IUGR. It could be speculated that the presence of more active form of TNF-α together with the others disturbances in immune system and endothelium injury are the processes involved in IUGR development. The changes in the umbilical and fetal blood flow are secondary to the pathological changes in the placenta and in the uteroplacental circulation. Nevertheless, in our resent work the correlation between cytokines (TNF-α and interleukin 6) and disturbances in blood flow velocimetry has been detected, in the present study any influence of *Alw*I genetic variant of TNF-α to the Doppler velocimetry in umbilical and middle cerebral arteries and the perinatal outcome was observed. We have should, however, note that the number of the patients in our investigation was relatively small, and for making clear conclusion the subsequent investigations are necessary. We have concluded that the etiology of IUGR is probably composed of many elements that act together at the same time, thus the problem is more complicated and deserves further studies.

## CONCLUSION

The TNF-α polymorphism *Alw*I (*-238G/A*) could play a role in etiology of IUGR and influence TNF-α expression in maternal serum. However, in our study *Alw*I restriction polymorphism of TNF-α gene did not correlated with blood flow Doppler velocimetry in umbilical and middle cerebral arteries. The perinatal outcome did not correlate with the presence of mutated *A* allele of *Alw*I restriction polymorphism.

REFERENCES

1 Styne DM (1998). Fetal growth. Clin Perinatol. **25:** 917-938.
2 Marsal K (2002). Intrauterine growth restriction. Curr Opin Obstet Gynecol. **14:** 127-135.
3 Mandruzzato GP, Meir YJ, Maso G, Conoscenti G, Rustico MA (2003). Monitoring the IUGR fetus. J Perinat Med. **31:** 399-407.
4 Stallmach T, Hebisch G, Joller-Jemelka HI, Orban P, Schwaller J, Engelmann M (1995). Cytokine production and visualized effects in the feto-maternal unit. Quantitative and topographic data on cytokines during intrauterine disease. Lab Invest. **73:** 384-392.
5 Bartha JL, Romero-Carmona R, Comino-Delgado R (2003). Inflamatory cytokines in intrauterine growth retardation. Acta Obstet Gynecol Scand. **82:** 1099-1102.
6 Heyborne KD, Witkin SS, McGregor JA (1992). Tumor necrosis factor-alpha in midtrimester amniotic fluid is associated with impaired intrauterine fetal growth. Am J Obstet Gynecol. **167:** 920-925.
7 Old LJ (1985). Tumor nrecrosis factor (TNF). Science. **230:** 630-632.

8 Winkler M (2003). Role of cytokines and other inflammatory mediators. BJOG. **110:** 118-123.
9 Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarret-Nedwin J, Pennica D, et al (1985). Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res. **13:** 6361-6373.
10 Herrmann SM, Ricard S, Nicaud V, Mallet C, Arveiler D, Evans A, et al (1998). Polymorphisms of the tumour necrosis factor-alpha gene, coronary heart disease and obesity. Europ J Clin Invest. **28:** 59-66.
11 Knight JC, Udalova I, Hill AVS, Greenwood BM, Peshu N, Marsh K, et al (1999). A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. Nature Genet. **22:** 145-150.
12 Gudmundsson S, Marsal K (1988). Umbilical and uteroplacental blood flow velocity waveforms in pregnancies with fetal growth retardation. Eur J Obstet Gynecol Reprod Biol. **27:** 187-196.
13 Mari G, Deter RL (1992). Middle cerebral artery flow velocity waveforms in normal and small for gestational age fetuses. Am J Obstet Gynecol. **166:** 1262-1270.
14 Hayashi M, Ohkura T (2002). Elevated levels of serum macrophage colony-stimulating factor in normotensive pregnancies complicated by intrauterine fetal growth restriction. Exp Hematol. **30:** 388-393.
15 Holcberg G, Huleihel M, Sapir O, Katz M, Tsadkin M, Furman B, et al (2001). Increased production of tumor necrosis factor-alpha TNF-alpha by IUGR human placentae. Eur J Obstet Gynecol Reprod Biol. **94:** 69-72.
16 Dubiel M, Seremak-Mrozikiewicz A, Bręborowicz GH, Drews K, Pietryga M, Gudmundsson S (2005). Fetal and maternal Doppler velocimetry and cytokines in high-risk pregnancy. J Perinat Med. **33:** 17-21.
17 Arntzen KJ, Kjollesdal AM, Halgunset J, Vatten L, Austgulen R (1998). TNF, IL-1, IL-6, IL-8 and soluble TNF receptors in relation to chorioamnionitis and premature labor. J Perinat Med. **26:** 17-26.
18 Belady PH, Farkouh LJ, Gibbs RS (1997). Intra-amniotic infection and premature rupture of membranes. Clin Perinatol. **24:** 43-57.
19 Weiyuan Z, Li W (1998). Study of interleukin-6 and tumor necrosis factor-alpha levels in maternal serum and amniotic fluid of patients with premature rupture of membranes. J Perinat Med. **26:** 491-494.
20 Rosmond R, Chagnon M, Bouchard C, Bjorntorp P (2001). G-308A polymorphism of the tumor necrosis factor alpha gene promoter and salivary cortisol secretion. J Clin Endocr Metab. **86:** 2178-2180.
21 Zinman B, Hanley AJG, Harris SB, Kwan J, Fantus IG (1999). Circulating tumor necrosis factor-alpha concentrations in a Native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocr Metab. **84:** 272-278.
22 Mulcahy B, Waldron-Lynch F, McDermott MF, Adams C, Amos CI, Zhu DK, et al (1996). Genetic variability in the tumor necrosis factor-lymphotoxin region influences susceptibility to rheumatoid arthritis. Am J Hum Genet. **59:** 676-683.
23 Ota N, Hunt SC, Nakajima T, Suzuki T, Hosoi T, Orimo H, et al (2000). Linkage of human tumor necrosis factor-alpha to human osteoporosis by sib-pair analysis. Genes Immunity. **1:** 260-264.
24 Escobar-Morreale HF, Calvo RM, Sancho J, San Millan JL (2001). TNF-alpha and hyperandrogenism: a clinical, biochemical, and molecular genetic study. J Clin Endocr Metab. **86:** 3761-3767.
25 McCusker SM, Curran MD, Dynan KB, McCullagh CD, Urquhart DD, Middleton D, et al (2001). Association between polymorphism in regulatory region of gene encoding tumour necrosis factor-alpha and risk of Alzheimer's disease and vascular dementia: a case-control study. Lancet. **357:** 436-439.
26 Kruger R, Hardt C, Tschenscher F, Jackel S, Kuhn W, Muller T, et al (2000). Genetic analysis of immunomodulating factors in sporadic Parkinson's disease. J Neural Transm. **107:** 553-562.
27 Wilson A, Symons J, McDowell T, McDevitt H, Duff G (1997). Effects of a polymorphism in the human tumour necrosis factor α promoter on transcriptional activation. Proc Nat Acad Sci. **94:** 3195-3199.
28 Johnson MR, Anim-Nyame N, Johnson P, Sooranna SR, Steer PJ (2002). Does endothelial cell activation occur with intrauterine growth restriction? BJOG. **109:** 836-839.