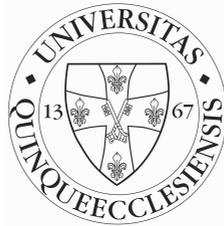# University of Pécs

Faculty of Health Sciences

Doctoral School of Health Sciences

Theses of the PhD Dissertation

The role of placental gene expression patterns of growth factors in the pathophisiology of intrauterine growth restrictions

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Introduction

The intrauterine growth restriction has always been one of the most challenging issues and the most important gestational diseaseses in obstetrical practice. The neonatal mortality and morbidity related to IUGR are significantly higher than that of in normal pregnancy. The etiology is complex, the pathological mechanisms of intrauterine growth restriction may be connected to maternal factors such as medical illnesses, socio-economic environment, maternal age, fetal factors wie chromosomal abnormalities, structural anomalies, intrauterine infections, or environmental factors. However, the most common etiology of intrauterine growth restriction is based on abnormal placental function.

As the genetical background of intrauterine growth restriction is the least commonly researched area, our study and my Ph.D work aims to provide this field with more data.

Study aims

The aim of my research was to study the placental gene expression patterns of TGF-ß1 and EGF genes in cases of intrauterine growth restriction and normal pregnancies with eutrophic fetuses, therefore contributing data regarding to the role of these genes in IUGR pathophisiology. The results were evaluated in the light of the available clinical data such as maternal age, gender distribution, maternal weight gain and BMI changes, adding information for clinical practice.

The following set of research questions were asked:

1. Is there a difference in the placental expression patterns of the TGF-ß1 gene in the cases of intrauterine restriction versus normal pregnancies with eutrophic fetuses?

2. Is there any correlation between the placental TGF-ß1 gene expression and fetal gender?

3. Is there a correlation between placental TGF-ß1 gene expression activity and the degree of intrauterine restriction?

4. Is there a difference in the placental expression patterns of the EGF gene in the cases of intrauterine restriction versus normal pregnancies with eutrophic fetuses?

5. Is there any correlation between the placental EGF gene expression and fetal gender?

6. Is there a correlation between placental EGF gene expression activity and the degree of intrauterine restriction?

7. How was the gender distribution of the fetuses with intrauterine growth restriction?
8. Is a significant difference detectable in median maternal age in the cases of intrauterine growth restriction versus control group with eutrophic fetuses? Can a significant difference be found if different age-subgroups are examined?

9. How do maternal weight gain and BMI change in the cases of intrauterine growth restriction versus control group with eutrophic fetuses?

Materials and methods

Patients, clinical characteristics
During the period between January 1, 2010 and January 1, 2011, we obtained placental samples postpartum for genetic studies from 101 pregnant mothers treated in our clinic at Semmelweis University, Budapest for SGA. SGA was defined as fetal birth-weight below 10 percentile per fetal gender and gestational age. In order to confirm the diagnosis, we measured fetal abdominal circumference (AC) fetal head (BPD, HC, OFD), femur lenght (FL) and compared it to standard sizes of AC/BPD/HC/FL for gestational age. SGA cases were considered to be due to placental dysfunction whenever intrauterine infections, chromosomal abnormalities, other fetal developmental disorders, maternal nutritional deficiencies, multiple pregnancy or other placental pathology could be excluded. Cases within the SGA group were subdivided into two categories based on percentile scores: those with

fetal birth-weight falling between 0–5 percentile were considered severely growth restricted, whereas those with 5–10 percentile birth-weight were defined as moderately growth restricted. During the same period 140 placental samples were taken postpartum from normal pregnancies to serve as the control group.

We extracted several clinical parameters for analysis from both group of patients. These included maternal age, obstetric history, maternal birth-weight, gestational age at delivery,

fetal birth-weight, Apgar score, gestational weight gain and gestational increase in Body Mass Index (BMI).

We made no distinction among cases with different delivery methods; both groups included cases with both vaginal delivery and cesarean section.

Method

Placental sampling, RNA preparation, cDNA synthesis Placental sample sizes were approximately 2_2_2 cm (8 cm3) in all cases. Samples were immediately frozen and stored at _70 _C until genetic testing. Subsequently, whole RNA content was extracted from the placental samples, total RNA concentration was then determined by spectrophotometer Reverse transcription was performed, Primers were constructed using the Primer Express Software (Applied Biosystems). All real time PCR reactions were performed using the same MX3000 Realtime PCR (Stratagen) equipment.

A two-sample t test was used to compare relative EGF and TGF-ß1 gene expression between groups (confidence interval, CI 95%). For the purpose of determining degree of freedom, the Welch-Satterhwaite correction was performed. Relative gene expression was expressed using three categories as follows: (1) over-expression if calculated Ln value was >1, $p<0,05$; (2) under-expression if calculated Ln value was<-1, $p<0,05$; (3) no change in expression if calculated Ln value was <1,>-1, $p<0,05$. Clinical and demographic data was analyzed using SPSS software. Level of significance was set at p50.05 in all cases.

Results

Gene expression results
TGF-ß1 (transforming growth factor-ß1)
The TGF-ß1 gene expression was compared based on the evaluation of the placental samples of 101 fetuses with intrauterine growth restriction and 140 placental samples of normal pregnancy eutrophic fetuses, cases representing the control group.

There was no significant difference between the two groups in TGF-ß1 gene expression ($Ln2\alpha$: 0.16; p=0.07).

Within the IUGR group no fetal gender-related differences were seen in placental expression of the TGF-ß1 gene ($Ln2\alpha$: -0.11; p=0.05).

Similarly, no significant differences could be detected within the IUGR group by growth restriction severity categories. In more severe growth restriction, represented by the subgroup of cases with fetal birth-weight falling between 0–5 percentile, TGF-ß1 gene activity was not significantly different from the subgroup of cases with fetal birth-weight between 5–10 percentile ($Ln2\alpha$: 0.32; p=0.06).

EGF (epidermal growth factor)
The EGF gene expression was compared based on the evaluation of the placental samples of 101 fetuses with intrauterine growth restriction and 140 placental samples of normal pregnancy eutrophic fetuses, cases representing the control group.

The placental expression of EGF gene in IUGR cases is under-expressed compared with control group ($Ln2^{\alpha}$: -1.54; p=0.04).

Within the IUGR group no fetal gender-related differences were seen in placental expression of the EGF gene ($Ln2^{\alpha}$: 0.44; p=0.06).

No significant differences could be detected within the IUGR group by growth restriction severity categories. In more severe growth restriction, represented by the subgroup of cases with fetal birth-weight falling

between 0–5 percentile, EGF gene activity was not significantly different from the subgroup of cases with fetal birth-weight between 5–10 percentile ($Ln2^{\alpha}$: -0.08; p=0.05).

Clinical results
The gender distribution of newborns with intrauterine growth restriction in our study group, related to male/female ratio was 0.58 (male: 37, female 64), while the same value in the control group was 1.09 (male: 73, female 67) which is a significant difference (p<0.05).

The median maternal age in the intrauterine growth restricted group was 30.82±4,34 years, not substantially different from median age in the control group 31,45±3,12 years (p>0,05).

Analysing the maternal age in subgroups, we found that the IUGR pregnancies were significantly more common in the maternal age group 35-44 years compared to other age groups.

Mean gestational weight gain was 14,8 kg in the normal pregnancy group versus 10,9 kg in the IUGR group. The corresponding changes in the maternal BMI were an increase of 5,3 in the control group and 4,1 in the intrauterine restricted group.

Conclusions

1. We found no difference in placental geneexpression of TGF-ß1 between the IUGR and normal pregnancy groups. This finding suggests that the stimulatory effect of TGF-ß1 on vascular endothelial cell proliferation observed in the early phase of gestation is no longer operational in later stages of pregnancy when IUGR tends to develop. We therefore conclude that TGF-ß1 does not play a direct role in the development of IUGR.
2. The placental gene expression of TGF-ß1 in female and male fetuses in pregnancies with intrauterine growth restriction cases did not show significant difference related to the gender. Based on above data, fetal gender has no importance in TGF-ß1 gene expression.

3. Placental gene expression activity of TGF-ß1 in the group of severe – 0 to 5 percentile and moderate – 5 to 10 percentile – IUGR groups showed no significant difference regarding the severity of intrauterine growth restriction.

4. Placental expression of EGF in our study was found to be reduced in IUGR pregnancies versus normal pregnancies. This may partly explain the smaller placental size and placental dysfunction commonly seen with IUGR and suggests that EGF may have a potential role in the development of intrauterine growth restrictions.

5. The placental gene expression of EGF in female and male fetuses in pregnancies with intrauterine growth restriction cases did not show significant difference related to the gender. Based on above data, fetal gender has no importance in EGF gene expression.

6. Placental gene expression activity of EGF in the group of severe – 0 to 5 percentile and moderate – 5 to 10 percentile – IUGR groups showed no significant difference regarding the severity of intrauterine growth restriction.

7. In our IUGR study group there was a significant difference regarding fetal gender, the intrauterine growth restriction proved to be significantly more common in girls than is boys. That seems to be only the result of the small sample size from epidemiological aspect rather than a potencial pathophysiological correlation.

8. There was no found significant difference between median maternal age in cases with intrauterine growth restriction versus the control group. Analising the maternal age in subgroups, wie found that the IUGR pregnancies were significantly more common in the maternal age groups of 17-24 and 35-44 years compared to 25-31 and 32-34 years age groups.

9. In our study intrauterine growth restriction is more commonly correlated with either very low (3-9 kg) or higher than average (14-17 kg) weight gain compared to average weight gain (10-13 kg). Increase in BMI was also significantly less in IUGR cases compared to control group with eutroph fetuses.

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