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ORIGINAL ARTICLE

Effect of labor pain on placental gene expressions

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Abstract

A close relationship between labor and the placenta is known. The study of gene expression profiles describing the effects of labor on placental tissue has accelerated in recent years. This study aims to compare the expression levels of *GDF15, ADM, SERPINE1, NOS3, IL-6, TNF-α* genes in placental tissues discarded after vaginal deliveries with labor pain and elective cesarean deliveries before the onset of labor pain. For this purpose, placental tissues of vaginal deliveries with labor pain (n=9) and elective cesarean deliveries without labor pain (n=9) were collected immediately after births. RNA extracted from tissues (umblical cords and deciduas). Gene expression analyzes were performed by Real-time PCR method. *TNF-α* gene expression levels in decidua and umblical cord tissues in vaginal deliveries with labor pain were higher than in elective cesareans without labor pain, and *NOS3, ADM, SERPINE1* gene expression levels were low. *GDF15* and *IL-6* gene expression levels were high in the umblical cord tissues and were low in the decidua in vaginal deliveries with labor pain compared to elective cesareans without labor pain. The high level of *GDF15* gene expression in the vaginal deliveries with labor pain umblical cord tissues was significant ($p<0.05$). Changes in gene expression between different types of birth may help us to understand how labor pain affects gene expression levels. These results suggest that labor pain affects different tissues in different ways. The "birth experience" of a placenta that has experienced pain stress is absolutely different to others process. Since birth pain is a natural stress, it can be a light in determining the effects of the differences in cesarean section on the baby and the mother. Gene expression alterations may cause labor starting and progressing, or just be an result of labor.

Keywords: Labor pain, *SERPINE1*, *GDF15*, *ADM*, *NOS3*, *IL-6*, *TNF-α*

Abbreviations: Adrenomedullin (*ADM*); glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); growth differentiation factor 15 (*GDF15*); interleukin 6 (*IL-6*); nitric oxide synthase 3 (*NOS*); serpin family e member 1 (*SERPINE1*); tumor necrosis factor-α (*TNF-α*).

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Introduction

The onset of labor is characterised by changes in the uterus, including softening and ripening of the cervix, activation of the amniotic and decidual membranes, and transformation of the uterine smooth muscle from quiescent to contractile [1]. Wang et al., reported that there was a significant differences in gene expression between vaginal and cesarean placentas [2]. On the contrary to this Churchill [3] and Cui and Churchill [4] have suggested that there was a slightly difference in gene expression at birth compared with nonlaboring placentas.

Hypoxia of the placenta caused by uterine contractions during labor may be the reason for the differences of gene expression in the placenta depending on the mode of delivery. Labor is in connection with deep uterine contractions that may result in intermittent blood flow to the placenta in contrast to elective cesarean delivery [5,6].

The processes that initiate spontaneous labor may explain the differences between laboring and non-laboring placentas. Noting that these processes are not fully understood, inflammation is one of the important pathways proposed to initiate labor [7,8]. In addition, it is also possible that the conditions leading to the choice of elective cesarean delivery over vaginal delivery are related to gene expression in placental tissue alone. It is believed that labor and its associated pain affect a pregnant woman in a special way.

Women experience a lot of stress during pregnancy and childbirth, and the biggest stressor is labor pain [9]. On average, labor before birth (including the first and second stages) can last 9-18.8 hours for nulliparous women and 6-13.5 hours for multiparous women [10]. At the peak of contractions, the uterine pressure can reach 80-100 mmHg. There can be up to 5-6 contractions in 10 minutes [11]. It has been suggested that labor, in conjunction with uterine contractions and labor pain, has important physical and biochemical effects on the mother, baby and placenta [12].

It is reported that, during physiological or pathological events, gene expression profiling provides comprehensive information about the molecular mechanisms of cellular function in specific tissues. Human labor is a complex biological process with interactions between neurological, hormonal, mechanical stretch and inflammatory factors. In general, it has been reported that alterations of genes expressions involved in exacellular matrix elements, immune pathways, inflammatory processes and hormones is important during labor [12].

GDF15 protein acts as a pleiotropic cytokine. It is involved in cellular damage stress response. It is reported that, protein levels increased in case of tissue hypoxia, acute injury, inflammation and oxidative stress [13]. *SERPINE1* encodes a member of the serine proteinase inhibitor superfamily. The protein also functions as a component of innate antiviral immunity and high concentrations of the gene product are related to thrombophilia [13]. *ADM* is a pre-hormone and involved in vasodilation, regulation of hormone secretion, promotion of angiogenesis and antimicrobial activity. In fetoplacental tissues, *ADM* has been proposed to control vascular tone at the local level to regulate uteroplacental-fetal circulation [14]. *IL-6* is a marker and its expression is known to be increased in preterm labor, premature rupture of membranes and labor. *TNF-α* accumulation has been associated with an increase in markers of inflammation, fibrotic response, vascular remodeling and proteins that facilitate lipid deposition in the placenta [15]. *NOS3*, nitric oxide, is a reactive free radical that acts as a biological mediator in various processes including neurotransmission and antimicrobial and antitumoral activities. It has been reported that ovarian excretion also occurs in the placenta [13].

During normal labor, uterine contractions may create stress for the fetus by affecting fetal oxygen saturation and may lead to slow fetal heart rate, hypoxia and even death [16]. Other pathways that are differentially expressed between birth types include immune pathways and inflammatory. Given the stress and inflammation known to occur with both childbirth [12] and major surgery [17], these pathways warrant further investigation due to their importance. Epigenetic changes in the genome that occur during labor have been

described in one study. More specifically, there is increased DNA methylation in leukocytes from cesarean-born babies compared to vaginally-born babies. Whether these epigenetic changes have lasting effects on both mother and child is not yet known [18]. Kothiyal et al., identified differential gene expression between different birth delivery modes. Enrichment of antimicrobial peptide (*AMP*) pathways in vaginal delivery is of particular interest. It is reported that, the PI3 gene (*AMP*), showed a twofold increase in vaginal delivery compared to cesarean section [19].

It is suggested that, contractions and labor can influence oxidative and inflammatory stresses that may trigger changes in placental gene expression. The effects of labor may therefore be the cause of some of the changes observed in the placenta. The contractions of the uterus during labor affect the uteroplacental blood flow, which temporarily reduces the maternal perfusion of the placenta. This intermittent perfusion may be the cause of ischaemia-reperfusion injury to the placenta. Therefore, delivery may serve as an in vivo model of acute ischaemia and reperfusion injury [20]. There is strong evidence that intrauterine cytokine upregulation is related to both on-time labor, preterm labor, and a number of key aspects of labor [21-23].

Peng et al. identified novel genes and several signaling pathways involved in inflammatory and immune pathways with additional diverse biological functions, in addition to several known genes regulated in the maternal-fetal-placental compartments during birth. Interestingly, overexpressed genes were found to be different when compared maternal blood, placental tissues and cord blood [12].

In this study, the expression levels of *GDF15, ADM, SERPINE1, NOS3, IL-6, TNF-α* genes in placental tissues discarded after vaginal deliveries with labor pain and non-painful elective cesareans before the onset of labor were determined.

Materials and Methods

Sample Collection and Ethics

This study has been approved by Afyonkarahisar Health Sciences University Ethical Board with decision number 33 of 07/01/2022/1. All patients have given their written consent for the use of their placentas.

Eighteen patients who underwent giving birth between January-March 2022 were chosen for the study. All the placentas from the 9 mothers with vaginal delivery (with labor pain) and from 9 mothers with elective cesarean delivery (without labor pain) (aged 18-45) were collected from the polyclinic of the Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Obstetrics and Gynecology.

RNA Extraction and Real-Time PCR Analyses

Total RNA from placenta tissues (umblical cords and deciduas) was isolated according to the PureZole isolation kit protocol steps (Biorad, USA). cDNA was obtained from 1 µg of total RNA by using iScript Reverse Transcription Supermix (Biorad, USA). Real-time PCR analysis was performed using Rotor Gene-Q (Qiagen, Hilden, Germany) by using iTaq Universal SYBR Green Supermix (Biorad, USA) to analyse *SERPINE1*, *GDF15*, *ADM*, *NOS3*, *IL-6* and *TNF-α* mRNA levels (Table 1).

Oligonucleotide primers were designed Oligomere Biotechnology (Ankara, TURKEY). PCR protocol for *SERPINE1, GDF15, ADM, NOS3, IL-6, TNF-α* and *GAPDH:* 95°C for 30s initial denaturation followed by 40 cycles of 95°C for 5s and 60°C for 30s.

Statistical Analysis

REST 2009 V2.0.13 and SPSS v.19 Software [24] were used for assessing the relative expression results.

Results

We evaluated the gene expression changes between diferent delivery birth modes. Changes in related mRNA levels of placenta tissues with labor pain were analysed according to the mRNA levels of laboring painless placenta tissues.

mRNA Analyses of *GDF15, ADM, SERPINE1, NOS3, IL-6,* and *TNF-α* Genes

TNF-α mRNA levels were increased in umblical cord and decidua tissues of vaginal deliveries with labor pain compared to those of elective cesarean deliveries without pain (1.126-fold

and 1.424-fold, respectively) (p>0,05). *NOS3*, *ADM*, *SERPINE1* gene expression levels were decreased. *NOS3* mRNA level decreased 0.352 fold in umblical cord tissue and 0.355 fold in decidua tissue. *ADM* mRNA level decreased 0.933 fold in umblical cord tissue and 0.562 fold in decidua tissue. *SERPINE1* mRNA level decreased 0.768 fold in umblical cord tissue and 0.691 fold in decidua tissue (p>0,05). *GDF15* and *IL-6* gene expression levels were increased (2.009-fold p<0.05, 1.153-fold, respectively) in umblical cord, whereas they were decreased (0.623-fold, 0.619-fold, respectively) in decidua compared to elective cesarean section related tissues (Figure 1, and Figure 2) (fold changes are shown in Log10 level).

Discussion

A close relationship between labor and the placenta is known. But little is known about the gene expression profiles describing the effects of labor on placental tissue. Gene expression profiling is a powerful tool that enables the study of changes in gene expression to study complex biological processes such as the labor process [25].

In this study, the expression levels of *GDF15*, *ADM*, *SERPINE1*, *NOS-3*, *IL-6*, *TNF-α* genes were determined in placentas discarded as a result of vaginal delivery with labor pain and placentas discarded as a result of elective cesarean delivery without labor pain before the onset of labor and contractions. The expression of the related genes differed between the two modes of delivery. Lee et al., suggesting a large diversity of gene expression changes and an overall complexity in the birth process [20].

In addition, a surgical procedure can induce relevant gene expression changes that can cause adverse outcomes. For example, a study examining changes in gene expression after major thoracoabdominal surgery showed that genes related to innate immunity and inflammation

Table 1. Primer sequences.

Gene	Primer sequences
GDF15-F	5'-GCAAGAACTCAGGACGGTGA-3'
GDF15-R	5'-TGGAGTCTTCGGAGTGCAAC-3'
ADM-F	5'-ATGAAGCTGGTTTCCGTCG-3'
ADM-R	5'-GACATCCGCAGTTCCCTCTT-3'
SERPINE1-F	5'-CCTCCAGCAGCTGAATTCCT-3'
SERPINE1-R	5'-GGGTTTCTCCTCCTGAAGTTCT-3'
NOS-3-F	5'-AACAGCATCTCCTGCTCAGA-3'
NOS-3-R	5'-CACTGAGCGGATTGTAGCCT-3'
IL-6-F	5'-ACAGCCACTCACCTCTTCAG-3'
IL-6-R	5'-CCATCTTTTTCAGCCATCTTT-3'
TNF-α-F	5'-CCCGAGTGACAAGCCTGTAG-3'
TNF-α-R	5'-GATGGCAGAGAGGAGGTTGAC-3'
GAPDH-F	5'-CATTGCCCTCAACGACCACTTT-3'
GAPDH-R	5'-GGTGGTCCAGGGGTCTTACTCC-3'

were upregulated and genes related to adaptive immunity were downregulated after surgery [17]. In placental tissue, delivery increases the expression of genes related to placental oxidative stress, inflammatory cytokines, angiogenic regulators and apoptosis [26]. It also regulates genes involved in placental hormone metabolism [27,28]. *TNF-a* and *IL-6* gene expressions were found to be higher in umblical cords in vaginal delivery compared to cesarean delivery. Similarly *TNF-α* gene expression was higher in decidua, whereas *IL-6* gene expression decrease. Placental and extraplacental membranes are also known to secrete many cytokines and chemokines [29]. The onset of labor at term causes an increase in amniotic fluid concentrations of interleukin *IL-1β*, *IL-6*, *IL-8* and tumor necrosis factor *TNF-α* [30]. Normal labor is associated with an up-regulation, although not significant, of cytokine expression in the amnion and chorionic villi, as shown by cDNA array studies [31].

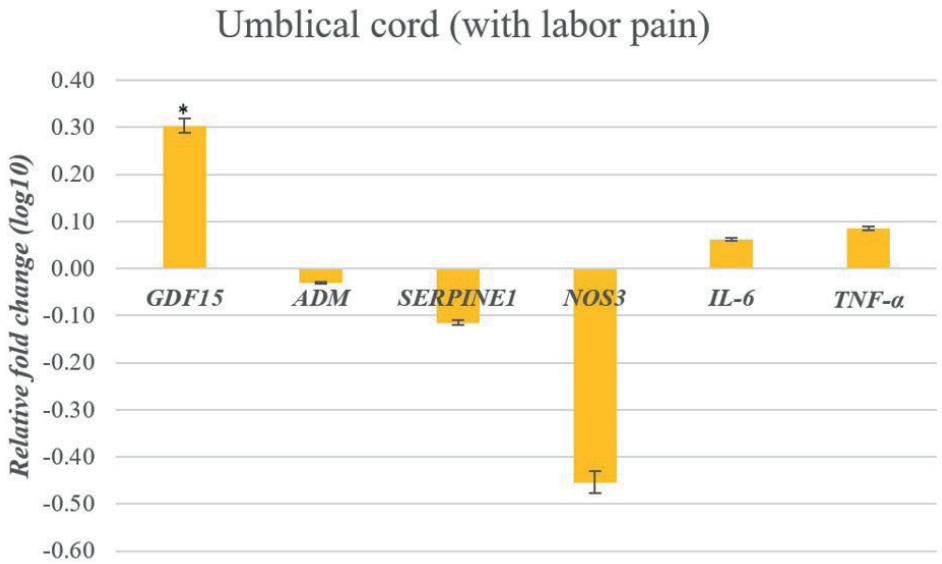

Figure 1. Umbilical cord gene expression alterations in vaginal deliveries with labor pain compared to elective cesarean deliveries without labor pain. *GAPDH* was used as a reference gene for normalization. * $p<0.05$.

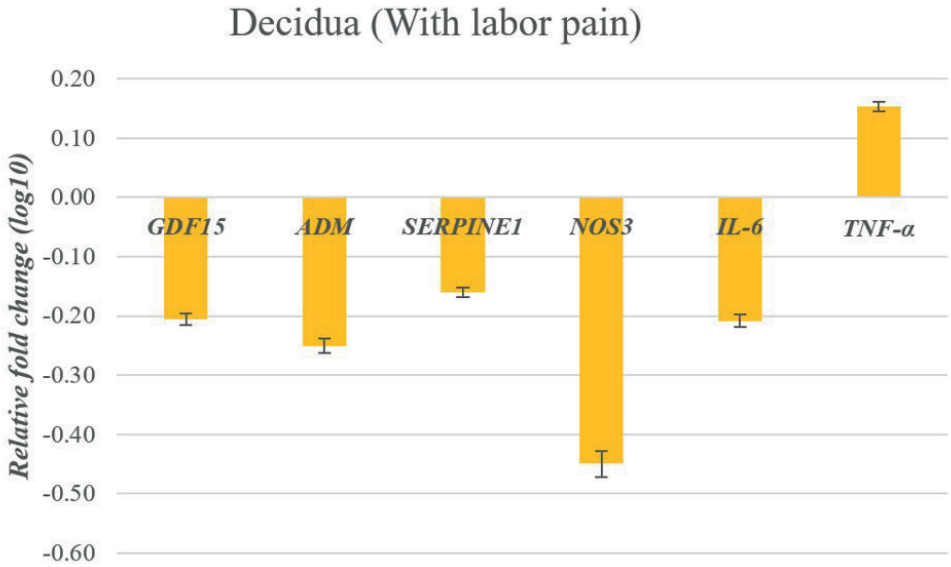

Figure 2. Decidua gene expression alterations in vaginal deliveries with labor pain compared to elective cesarean deliveries without labor pain. *GAPDH* was used as a reference gene for normalization.

NOS3 has been reported to be overexpressed in the placenta [13]. In our study, there was almost a 3-fold difference in *NOS3* gene expression levels in cesarean section tissues without labor pain compared to vaginal delivery tissues with lobor pain. This indicates that this free radical is triggered more during cesarean section operation.

ADM is involved in the biological process of fluid and electrolyte homeostasis [32]. In fetoplacental tissues, *ADM* has been suggested to control vascular tone at the local level to regulate uteroplacental-fetal circulation [33]. In our study, *ADM* gene expression level was found to be higher in both placental tissues in the cesarean group compared to vaginal delivery. Considering that anesthesia in cesarean section affects fluid and electrolyte homeostasis during the operation, this increase in *ADM* gene expression may be necessary to maintain the related homeostasis.

It has been reported that there was no significant difference in the distribution or intensity of *GDF15* staining in placentas with and without labor [34]. On the contrary, Peng et al. suggested that *GDF15* has a role in the regulation of this labor process in their observations by microarray and Western blot [20]. In our study, *GDF15* gene expression level in cord tissues of placentas from vaginal delivery with labor pain was significantly higher compared to cord tissues from cesarean delivery. To clarify the role of *GDF15*, further studies are needed.

SERPIN1 gene expression level was found to be lower in both cord and decidua tissues of placentas of vaginal delivery compared to cesarean section tissues. It is also high in tissues belonging to the cesarean section group. Considering that high concentrations of the *SERPIN1* gene product increase the risk of thrombophilia, the high level of gene expression in cesarean placenta draws attention to the risk. More detailed analyses are required to determine the relationship between this situation in cesarean with thrombophilia.

Conclusion

These results suggest that labor pain affects different tissues in different ways. The fact that we cannot fully disentangle the effect of labor, associated pain or stress on the different gene expression profiles in the maternal-fetal-placental compartment is a limitation of this study. However, it is clear that both labor pain/stress and labor itself underlie gene regulation during labor. The "birth experience" of a placenta that has experienced pain stress is absolutely different to others process. Since birth pain is a natural stress, it can be a light in determining the effects of the differences in cesarean section on the baby and the mother. These changes in gene expression may cause labor to start and progress, or simply be an effect of labor.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

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