Journal of Reproductive Immunology 156 (2023) 103823

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Short communication

Tryptophan degradation enzymes expression in the placenta and the Kynurenine/Tryptophan ratio in maternal plasma after elective cesarean section

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ARTICLE INFO

Keywords:
Indoleamine 2,3-dioxygenase 1
Tryptophan 2,3-dioxygenase 2
Kynurenine Pathway
Labor
Cesarean section

ABSTRACT

Indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) metabolize tryptophan in the kynurenine pathway. We evaluated these enzymes' mRNA expression in maternal and fetal sides of the placenta of uncomplicated, unlabored full-term pregnancies after elective cesarean section and compared it with that of placentas obtained from vaginal delivery. Tryptophan and kynurenine plasmatic levels after cesarean section were measured, to investigate their possible correlation with IDO1 and TDO mRNA (TDO2) expression. The results suggested that IDO1 and TDO2 expression was higher in the maternal side of the placenta and that labor significantly affects TDO2 expression and the plasma Kynurenine/Tryptophan ratio.

1. Introduction

Indoleamine 2,3-dioxygenase 1 and tryptophan 2,3-dioxygenase catabolize tryptophan (Trp) into kynurenine (Kyn), regulating the first step of the kynurenine pathway (KP) (Yeung et al., 2015). The KP contributes to maternal immune regulation during pregnancy also in the placenta (Silvano et al., 2021). Indeed, Kyn and its metabolites induce regulatory T cells and other immune-regulatory cells' recruitment, inhibiting the immune response (Mellor et al., 2017).

During physiological pregnancy, IDO1 is expressed in maternal-fetal interface cells, such as T cells, decidual Natural Killer cells, dendritic cells, trophoblasts, and placental vascular epithelial cells (Chang et al., 2018). IDO1 in placental tissue has been previously observed in healthy and pathological pregnancies in the first and second trimesters (Ban et al., 2013; Ligam et al., 2005; Sedlmayr and Blaschitz, 2012). Few information is available on IDO1 expression in the maternal side of the placenta at term after cesarean section. One study showed that IDO1 mRNA expression was significantly lower in the maternal side of placentas from pregnancies complicated by preeclampsia compared to healthy pregnancies delivered by cesarean section (Broekhuizen et al., 2020). Recently, TDO mRNA (TDO2) expression has also been studied in the placentas of uncomplicated term pregnancies delivered vaginally, showing that TDO2 was expressed both in the maternal and fetal side of the placenta (Silvano et al., 2022). However, until now, no study has compared IDO1 and TDO2 expression between the placentas of pregnancies delivered vaginally and those of pregnancies delivered by elective cesarean section in the absence of labor.

Selecting placentas from unlabored, cesarean-delivered term pregnancies allows studying mRNA expression when the biochemical, hormonal, and mechanical processes inducing labor have not yet occurred (Irani and Foster, 2015). The labor, indeed, is a powerful inducer of oxidative stress, inflammatory cytokines, and angiogenic regulators in the placenta (Cindrova-Davies et al., 2007). Conversely, these mediators are not released in the placentas from cesarean section performed in the absence of labor (Cindrova-Davies et al., 2007).

Kyn/Trp plasmatic ratio is a suggested indicator of the rate of Trp degradation. It has been previously reported that plasmatic Kyn and Trp levels were significantly higher in the umbilical vein of women

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https://doi.org/10.1016/j.jri.2023.103823
Received 30 November 2022; Received in revised form 19 January 2023; Accepted 30 January 2023
Available online 1 February 2023

undergoing the cesarean section compared to those delivering vaginally after labor (Kazda et al., 1998). It has also been observed that in uncomplicated pregnancies Trp concentration decreases in maternal plasma as pregnancy advances and that, after delivery, its concentration returns to physiological levels (Schröcksnadel et al., 1996). However, a clinical study showed that maternal plasma Trp levels measured within a few days after vaginal delivery remain low (Maes et al., 2002).

The aim of this study was to characterize IDO1 and TDO2 mRNA expression in the maternal and fetal side of the placenta after elective cesarean section and to compare it with the expression in placentas from vaginal delivery. Moreover, maternal plasmatic Trp and Kyn concentrations and Kyn/Trp ratio after elective cesarean section without labor were also investigated.

2. Materials and methods

2.1. Sample collection

This observational study was conducted between March 2021 and October 2021 at Careggi University Hospital in Florence, Italy. A nonconsecutive series of 13 singleton uncomplicated pregnancies delivered by elective cesarean section between 38 and 40 weeks were included. All women gave written informed consent. Fresh placental tissue and maternal blood samples were collected immediately after cesarean section. The control group consisted of 20 gestational age-matched women with uncomplicated pregnancies who delivered vaginally, previously enrolled in another study (Silvano et al., 2022).

2.2. Real-Time PCR for IDO1 and TDO2 mRNA expression

Samples were obtained from the maternal surface (decidua basalis) and fetal surface (chorion and thin layer of villi) of placentas. One μg of total RNA was used for the reverse transcription reaction with Prime Script RT reagent Kit Takara (Otsu, Japan), the cDNAs were amplified with specific primers described below, as previously reported (Silvano et al., 2022). The SYBR Premix Ex Taq (Takara) was used for Quantitative Real-Time PCR (qRT-PCR), according to the instructions on a Rotorgene RG-3000A cycle system (Qiagen) platform. The 18 S rRNA housekeeping (Silvano et al., 2022) was used and the difference between CT values of the target genes and 18 S gene was used to calculate the delta CT. IDO1 primers were: IDO1 fw: 5'-AGTTCTGGGATGCATCACCA-3' and rev: 5'-CAGTTTCTTGGAGAGTTGGCA-3'. The cycle was set at 95 °C for 5 s, 55 °C or 52 °C for 30 s and 72 °C for 30 s, repeated 35 times.

2.3. Plasma tryptophan and kynurenine determinations

Blood samples were collected after elective cesarean section and are centrifuged at a 2000 RPM for 10 min. One ml of plasma was withdrawn. The concentrations of Trp and Kyn plasma were measured by ELISA immunoassay (ImmuSmol, Bordeaux, France) and their ratio was calculated.

2.4. Ethics approval

Ethics approval for this study was granted by the local ethics committee (Area Vasta Centro, protocol number 16022_bio).

2.5. Statistical analysis

Continuous variables were expressed by mean and standard error or standard deviation of the mean. The statistical significance was set at $p < 0.05$. To evaluate the correlation between continuous variables, Pearson's correlation coefficient and its 95% confidence interval was used. To compare the mRNA expression of the enzymes studied between different placental areas in the same group of women, a simple Generalized Estimating Equation (GEE) linear regression model was used, in addition, to compare the mRNA expression of these enzymes in the two groups of enrolled women (vaginal delivery or cesarean section) the Mann-Whitney or Student's t-test was used. To evaluate the correlation between Kyn/Trp ratio in two groups of enrolled women, the Welch-Satterthwaite t-test was used.

3. Results and discussion

Our study shows that IDO1 and TDO2 mRNAs were expressed on both the maternal and fetal side of the placenta of uncomplicated full-term pregnancies after elective cesarean section or vaginal delivery (Fig. 1a, b). However, a higher IDO1 mRNA expression was observed in the maternal side compared to the fetal side ($p < 0.05$ and $p < 0.01$ for placentas from cesarean and vaginal deliveries, respectively). The higher expression of IDO1 mRNA in the maternal side is consistent with the existence of a positive gradient of IDO1 protein, from the maternal to the fetal interface, observed by Blaschitz et al. (Blaschitz et al., 2011) and it could depend on the higher amount and variety of immune cells localized in the decidua (Trundley and Moffett, 2004).

No difference in IDO1 mRNA expression was found between samples from vaginal delivery and cesarean section on either the maternal or fetal side of the placenta. TDO2 was higher in the maternal side compared to the fetal one after cesarean section, although the difference was not statistically significant ($p = 0.06$), as we also previously observed in placentas from vaginal delivery (Silvano et al., 2022). Interestingly, the mode of delivery seemed to affect TDO2 expression in the maternal side of placentas, as it was more expressed in samples from elective cesarean section than in those from vaginal delivery ($p < 0.01$).

It has been reported that genes involved in placental Trp metabolism or transport increase their expression over the course of gestation (Karahoda et al., 2020). We can speculate that TDO2 expression increases during gestational age while it decreases during labor, although there are no studies that investigate the mechanism through which labor may affect such expression.

In addition, we found a strong positive correlation for both IDO1 mRNA expression and TDO2 expression between the maternal and fetal sides of the placentas from cesarean section (Pearson 0.65 and 0.90, respectively, $p < 0.01$). These positive correlations could be another expression of the closely related cross-talk between the mother and the feto-placental unit, which ensures the maintenance of pregnancy (Saito, 2001).

The selective expression of these enzymes in physiological pregnancies has never been investigated before and these data may be useful to clarify their involvement in pathological pregnancies, and eventually to provide the basis for future studies investigating if these enzymes or their products may be used as a target of therapeutic interventions. It was previously reported, for instance, that Trp levels decreased in placentas from women with pre-eclampsia compared with healthy controls (Keaton et al., 2019). Pre-eclampsia can be associated with fetal growth restriction (FGR) and both IDO1 and TDO2 and their protein have been shown to be less expressed in the placentas of FGR pregnancies compared to healthy ones (Murthi et al., 2017).

Plasma Kyn/Trp ratio is used to assess the activity of the extrahepatic Trp metabolism (Schröcksnadel et al., 2006), although plasma free Trp concentration may depend on its binding to albumin (Badawy and Guillemin, 2019). Therefore, we measured this ratio in the plasma of the enrolled patients at the time of the cesarean section, to assess whether there was a correlation between Trp and Kyn plasmatic concentration and placental IDO1 and TDO2 mRNAs expression. We found a mean plasmatic Trp concentration of 18.17 ± 3.20 μg/ml and a mean plasmatic Kyn concentration of 0.61 ± 0.20 μg/ml resulting in a mean Kyn/Trp ratio of 0.03 ± 0.01 (Fig. 1c). A moderate positive correlation was only found between the Kyn/Trp ratio and IDO1 mRNA expression in the maternal side of the placenta after cesarean section (Pearson 0.52, $p = 0.06$).

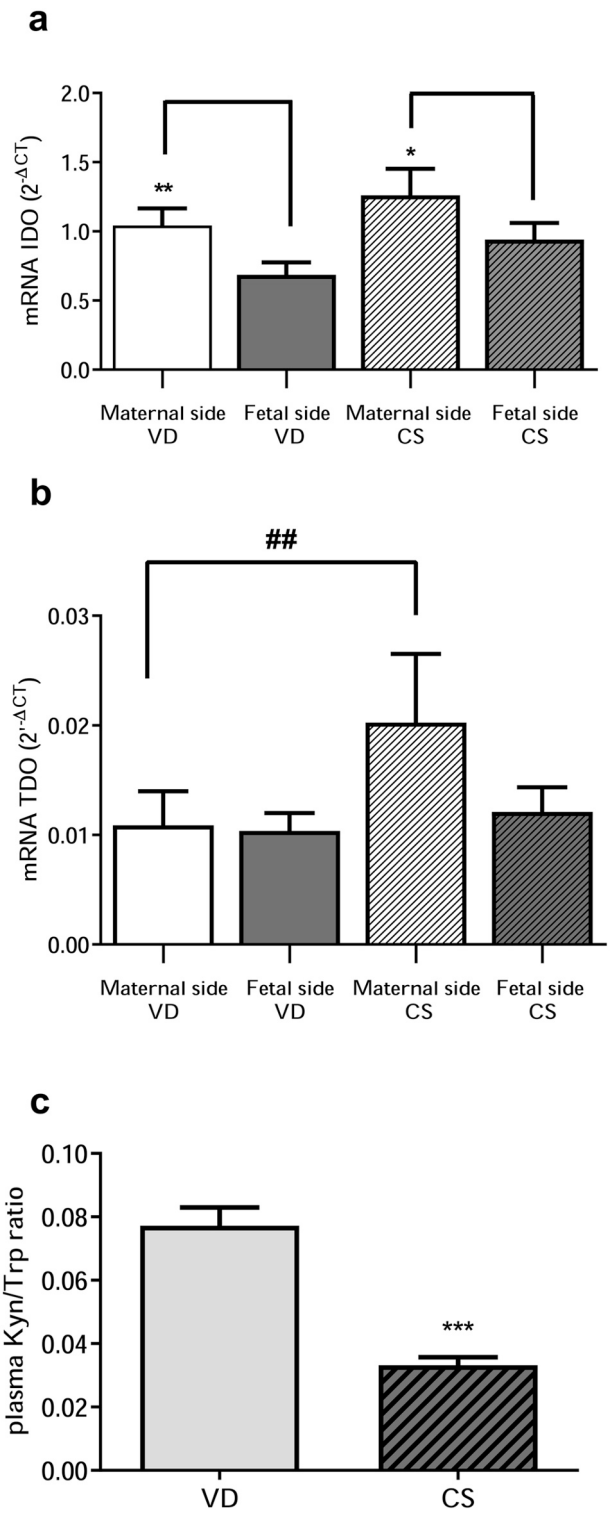

Fig. 1. Comparison of IDO1 mRNA (a) and TDO2 (b) expression between different areas of the placenta, after vaginal delivery (VD, n = 20) and after cesarean section (CS, n = 13), assessed by quantitative Real-Time PCR. Data are expressed as Mean ± SE. (a) IDO1 mRNA is more expressed in the maternal side compared to fetal side of placentas obtained from VD (**$p < 0.01$; Student's t-test) and of placentas obtained from CS (*$p < 0.05$; Student's t-test). (b) TDO2 is more expressed in the maternal side of the placenta after CS compared to the maternal side after VD (## $p < 0.01$; Mann-Whitney test). (c) Comparison of plasmatic Kyn/Trp ratio between women undergoing VD or CS. Data are expressed as Mean ± SD. The plasmatic Kyn/Trp ratio after CS was significantly lower than the ratio after VD (***$p < 0.001$; Welch-Satterthwaite t-test).

We also compared the plasmatic concentrations of Kyn and Trp of women undergoing cesarean section with those of women who had vaginal delivery (Silvano et al., 2022), and we observed that, while the plasmatic Kyn concentration was similar, the plasmatic Trp concentration was higher in the absence of labor, i.e. in women undergoing elective cesarean deliveries than in woman who delivered vaginally ($p < 0.001$). Consequently, the plasmatic Kyn/Trp ratio after cesarean section was significantly lower compared to the ratio after vaginal delivery (0.03 vs 0.08; $p < 0.001$, Fig. 1c). During pregnancy, besides the KP, other systems are involved in the maintenance of Trp plasma levels (Groer et al., 2017). Trp is also the precursor of serotonin, that is reported to stimulate contractions of human myometrium (Rudolph et al., 1993). This may explain the lower Trp levels detected in the plasma of women who delivered vaginally. To our knowledge, there are no studies that compare plasma levels of serotonin or measure Trp hydroxylase, the enzyme that metabolizes Trp to serotonin, before and after labor.

The plasmatic Trp and Kyn concentrations and the Kyn/Trp ratio reported in our study in uncomplicated, unlabored full-term pregnancies after elective cesarean section, and previously in women after labor (Silvano et al., 2022), may be used for comparison in future studies assessing variation of Trp metabolism in pathological conditions.

This brief research provides new insight into the role of Trp and the Kyn pathway in the human placenta, and how these can be affected by labor.

Acknowledgment

The research reported in this publication was supported by Fondazione CRF Firenze Grant number B15F21003630007 to M. Di Tommaso and by the University of Florence to AP (RICATEN 2022).

Conflict of Interest

The author have no conflict of interest to disclose.

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