Original article

Immunohistochemical study of IL-10 and CD46 in placental tissues in recurrent pregnancy loss

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Abstract

Purpose: The is to clarify the role of IL-10 and CD46 in recurrent pregnancy loss (RPL), via an immunohistochemical case-control study in placenta tissues.

Materials and Methods: This is a case-control study in which the experimental group consisted of 20 women, 30 to 42 years old, who miscarried during the first trimester of pregnancy for unknown reasons, while the control group consisted of 20 women, 27 to 39 years old, who had voluntarily performed in termination of pregnancy in the first trimester. An immunohistochemical study was performed on histological samples of decidua basalis, decidua parietalis and trophoblast, using the immunohistochemical markers IL-10 and CD46. The results were statistically analyzed by the Mann-Whitney test.

Results: A statistically significant difference in IL-10 expression was detected between the control group and the miscarriage group on decidual cells (p-value <.0001). There was increased immunohistochemical staining on the decidua of the control group in comparison to the miscarriage group. Regarding CD46, the immunohistochemical analysis did not reveal a statistically significant difference between the two groups in either the decidual or trophoblastic cells.

Conclusion: IL-10 seems to be associated with the RPL phenomena. It is necessary to further investigate the involvement of immune factors with RPL.

Keywords: RPL, IL-10, CD46, placenta, decidua, trophoblast

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Introduction

Recurrent pregnancy loss (RPL) constitutes a common complication of early pregnancy and is defined as three or more pregnancy losses occurring prior to the 20th week of gestation (Kolte, 2014). It affects 2-5% of couples, although the incidence of RPL varies widely between reports because of the differences in the definitions and criteria used. Known established causes of RPL are limited to 50% of cases and mostly include genetic, immunological and hormonal factors (El Hachem, 2017). Studies suggest a correlation between RPL of unknown cause and idiopathic-immunological factors. Given that fetal tissues are semi-allogenic and commonly cause development of antibodies, preventing the rejection of the fetus requires a tightly regulated association of immune mechanisms (Zhang, 2017). So, RPL cases of unknown cause may be a result of alterations regarding the number of immune cells in the decidua or/and the balance of cytokines.

IL-10 is an immunosuppressive cytokine that is essential to maintaining the balance between pro-inflammatory and anti-inflammatory effectors during gestation. By inhibiting the activity of Th1-cells and the production of TNF-α and IFN-γ, it protects the fetus from the maternal immunological reaction (Cheng, 2014). The majority of studies found a statistically significant association between RPL and IL-10. Its ability to inhibit specific immune responses and the fact that it has been found in areas of interest, like the cytotrophoblast, suggests that IL-10 is linked to a successful pregnancy outcome (Zammiti, 2006 & von Wolff, 2000). However, there is a number of studies that do not agree with this correlation (Bombell, 2008). The aim here is to clarify the implication of IL-10 with RPL occurrences.

The regulatory mechanism that makes the fetomaternal interface possible, without unwanted immune responses, also includes the complement system. CD46 is a membrane bound protein, secreted by trophoblastic cells, that acts as a cofactor to factor I. Its main role is to inhibit complement components C4b/C3b, thus inhibiting excessive complement activation (Mohlin, 2013). CD46 has been vaguely associated to RPL (Zhigang, 2006). There are not many data about CD46 expression in decidual and trophoblastic cells. The aim of this study also is to clarify the role of CD46 in RPL and normal pregnancies.

Materials and Methods

The present study was undertaken by the laboratory of Histology and Embryology of the A.U. Th. Medical School. It is a case-control study: the miscarriage group consisted of 20 women, between the ages of 35 to 42 years, who miscarried during the 1st trimester of gestation and control consisted of 20 healthy women, between the ages of 27 to 39 years, who had electively terminated their pregnancies, during the 1st trimester of gestation. In order to exclude known RPL causes and focus on the cases whose cause is yet undetermined, pertinent medical tests and history of all participants were taken into consideration. Placenta specimens were first distinguished into three categories: decidua basalis, decidua parietalis, trophoblast and were then processed using the immunohistochemical markers IL-10 and CD46. Microscopic evaluation and immunohistochemistry were performed similar to Papamitsou et al. (Papamitsou, 2021). In total, endometrial specimens from decidua basalis, trophoblast and decidua
parietalis were examined by two independent researchers. Intensity of staining was qualitatively evaluated as negative (-), weak (+), moderate (++), and strong (+++) based on each researcher’s observations on the microscope. Every distinct granular brown stain was scored as positive. The results were statistically analyzed and confirmed for their significance using the Mann-Whitney U-test. Our data were divided into two independent groups and Mann-Whitney’s null hypothesis was checked. The level of significance was .05 (two-tailed hypothesis) and the $P$-Value was estimated for each study undertaken among the two groups. In order to evaluate each case study and perform the statistics, we used the SPSS Statistics software.

**Results**

There is a statistically significant difference between miscarriage and control group, regarding immunohistochemical staining for IL-10 in decidual cells ($p$-value < .00001). More specifically, moderate staining was detected in scattered cells of the decidua basalis and in the epithelium of the decidua parietalis in control group, whereas all section ($n = 20$) of decidua in miscarriage group were found to be negative (Table 1), (Figures 1, 2).

On the contrary, there was no statistically significant difference between miscarriage and control group, regarding immunohistochemical staining for IL-10 in trophoblastic cells ($p$-value = 0.42).

Concerning staining for CD46, very weak staining was presented in control group, while all sections ($n = 20$) of decidua in miscarriage group were found to be negative.

As for trophoblastic cells, both groups presented moderate staining, mostly in the villi, on the base of the syncytiotrophoblast and mostly at the villous cytotrophoblast. However, according to the statistical analysis, there was no significant difference between miscarriage and control group, regarding immunohistochemical staining for CD46 in decidual cells ($p$-value = 0.28) or in trophoblastic cells ($p$-value = 0.31).
Table 1. P-Values* Decidua (parietalis and basalis), Trophoblast (mostly villi)

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>p-values</td>
<td>0.28</td>
<td>0.31</td>
<td>&lt; .00001*</td>
<td>0.42</td>
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MG : Miscarriage Group, CG : Control Group, *Statistically significant difference for p<0.05

Figure 1. IL-10 in decidua basalis (4x4) A: Miscarriage Group (-), B: Control Group (+)

Figure 2. IL-10 in decidua parietalis (4x4) A: Miscarriage Group (-), B: Control Group (+)

Figure 3. CD46 in villi (4x4) A: Miscarriage Group (+), B: Control Group (+++)
Discussion

Based on the results of our study, low IL-10 production, coupled with increased inflammatory cells, may result in pregnancy loss. This suggests that IL-10 plays a crucial role during the first months of gestation. These observations give rise to the hope that IL-10-based therapy may someday become a reality for enigmatic immunological pregnancy maladies (Thaxton, 2010).

Concerning CD46 expression, results indicate no statistically significant difference between miscarriage and control group. Further research is needed in order to reveal possible implication of the lack of regulatory proteins in the placenta (results so far have been, for the most part, vague, this study included). Few genetic studies support the hypothesis that the lack of complement proteins is linked to RPL. More specifically, alteration in genes coding complement inhibitors, including CD46, were found in specimens from women suffering from RPL (Mohlin, 2013). The crucial role of these proteins is further underlined by the fetal fatality observed in mice with complement inhibitor Crry deficiency, given that Crry is similar to human CD46 (Mohlin, 2013). From an immunohistochemical point of view, in this study, some findings concerning difference in expression (Figure 3) are quite interesting, although we are in no position to generalize these single observations. More specifically, the relatively milder CD46 expression of the miscarriage group may suggest a correlation between RPL and non-regulated complement activity. Further research is needed in order to have more results concerning the role of CD46 in RPL cases.

Conclusion

The findings confirm the crucial role of IL-10 for the pregnancy in the first months, as its deficiency appears to be associated with recurrent pregnancy loss. In contrast, no clear association of CD46 with miscarriages was found in this study. It is necessary to further investigate the implication of immunological factors on RPL.

References


