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Original Research

# Leukemia inhibitory factor, glycodelin, and HOXA-10 in gestational diabetes

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**Summary**

*Purpose of investigation:* The authors aimed to localize glycodelin, leukemia inhibitory factor (LIF), and HOXA-10 in term placentas and study the relationship between these markers and maternal gestational diabetes. *Materials and Methods:* Following labor, placentas from 12 pregnant women with gestational diabetes and 15 healthy pregnant women were collected, fixed, and paraffin-embedded, and immunohistochemical studies were performed. Two independent researchers examined the specimens under light microscopy. Immunoreactive scores were calculated and compared. *Results:* Glycodelin and HOXA-10 were stained in the decidua, and LIF was stained both in the trophoblast and in the decidua of term placentas. The three markers' immunoreactive scores were not different between pregnant women with gestational diabetes and the healthy controls. *Conclusion:* Glycodelin and HOXA-10 were produced by maternal decidual cells; LIF was produced by both trophoblasts and maternal decidual cells. Glycodelin, HOXA-10, and LIF are not related to the pathophysiology of gestational diabetes.

*Key words:* Gestational diabetes; Glycodelin; HOXA-10; Leukemia inhibitory factor.

## Introduction

Leukemia inhibitory factor (LIF) is a cytokine involved in the regulation of multiple processes that allow implantation. LIF expression makes the uterus receptive and facilitates decidualization and blastocyst growth and development. LIF plays a role in embryo-endometrial interaction, trophoblast invasion, and immune modulation [1]. Decidualization is essential for successful reproduction, and LIF is an important regulator of this process.

Research has shown that the endometrium of LIF-negative mice could not decidualize even after various well-established stimuli were applied in an attempt to induce this process [2] Glycodelin, a progesterone-regulated glycoprotein, is abundantly expressed in the human endometrium during the secretory phase [3]. The concentration of glycodelin gradually increases in the endometrial glands 4-5 days after ovulation, and reaches its peak on day 10, coinciding with the implantation window [3, 4]. Expression of these adhesive and anti-adhesive factors is associated with an increase in glycodelin expression during the implantation window [3, 5].

Decreased concentrations of glycodelin are associated with unexplained infertility and early pregnancy loss [6]. Aberrant glycodelin secretion is also associated with ovarian and breast cancers [7, 8].

HOXA-10 is a member of the homeobox multigene family of transcription factors [9]. Null mutation of HOXA-10 in mice causes defective implantation and decidualization, which in turn leads to female infertility. HOXA-10 mutant mice also exhibit partial oviductal transformation of the proximal uterus; however, this has been shown to be unrelated to fertility defects [10]. HOXA-10 expression in endometrial epithelial and stromal cells increases during the midsecretory phase of the menstrual cycle [11, 12]. In non-human primates, HOXA-10 protein expression during the luteal phase appears to be different in the functionalis and basalis zones of the endometrium [13]. Studies have shown that HOXA-10 is primarily regulated by progesterone and have suggested that the signals generated by the implanting embryo indicate that HOXA-10 also plays an important role in the early pregnancy phase in non-human primates. Overall, these studies suggest that the endometrial expression of HOXA-10 plays an important role in the control of uterine receptivity, implantation, and decidualization [14, 15]. Thus, these three markers play very important roles in the achievement and maintenance of early pregnancy. However, their roles in advanced pregnancy remain unclear. Thus, in this study, the authors first examined whether these three markers were expressed in term placentas and attempted to locate their expression. Then, they investigated the relationships between these markers with gestational diabetes.

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## Materials and Methods

Twelve pregnant women who were diagnosed with gestational diabetes and 15 healthy pregnant women who gave birth in the Obstetrics and Gynecology Department of Cerrahpasa Medical Faculty were included in this case control study. After the term deliveries, the placentas from both the study and control groups were immediately collected and fixed in 5% paraformaldehyde solution for 24 hours. The samples were then washed with PBS and soaked in a series of 50%, 60%, 70%, 80%, and 90% ethanol (30 minutes per solution) and then in 95% and 100% ethanol for one hour each. The samples were then held in a solution of 100% ethanol and xylene at a 1:1 ratio for 30 minutes and embedded in paraffin at 60°C for one hour to form paraffin blocks.

Next, 5-µm-thick cross-sections were cut from the aforementioned blocks and prepared for immunohistochemical staining. These samples were first incubated at 60°C overnight and then held in xylene for 30 minutes. After washing them with serial concentrations (95%, 80%, 70%, and 60%) of ethanol for two minutes per concentration, the sections were washed with distilled water, followed by the same using phosphate buffer saline (PBS) for 10 minutes. They were then held in 2% trypsin in Tris buffer at 37°C for 15 minutes and subsequently washed with PBS three times for five minutes. The limits of the sections were then drawn with a pen and incubated with 3% hydrogen peroxide for 15 minutes to inhibit endogenous peroxidase activity. The tissues were subsequently washed with PBS three times for five minutes each and stained with primary antibodies, specifically, polyclonal anti-LIF (1/100), monoclonal antiglycodelin (1/100), and monoclonal anti-HOXA-10 (1/100) for 18 hours.

Following staining, the samples were washed with PBS three times for five minutes each, a step that was followed by incubation with biotinylated anti-rabbit IgG. Streptavidin–peroxidase was then administered. The incubation steps were interspersed by three washing steps. After washing the secondary antibody with PBS three times for five minutes, the sections were washed using an AEC substrate system containing 3-amino-9-ethylcarbazole (for five minutes to detect immunoreactivity) and then with Mayer's hematoxylin. They were covered with a medium and observed with a light microscope. Control samples were processed in an identical manner but in the absence of primary antibody.

After the immunohistochemical studies were performed for glycodelin, LIF, and HOXA-10, the results were evaluated under light microscopy using the immunoreactive scoring system (IRS). Two observers blinded to the clinical information of the endometrial samples independently evaluated the staining scores. IRS score was calculated via the multiplication of optical staining intensity and the percentage of the cells stained. Optical intensity was graded as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). A percentage of the cells stained was also graded as 0 (the cells were not stained), 1 (less than 10% was stained), 2 (11% to 50% of the cells were stained), 3 (51% to 80% of the cells were stained), or 4 (more than 81% of the cells were stained). Mean IRS scores were calculated and compared using the Mann Whitney U test. Results were expressed as ± 2 SD at 95% confidence level. $P < 0.05$ was accepted as significant.

## Results

Demographic properties, mean maternal age, number of live births, number of aborted fetuses, maternal body mass indexes, and fetal weight were all similar between the study and control groups (Table 1).

Glycodelin was stained only in the decidua, not in the trophoblasts. Mean IRS scores for glycodelin were 3.4 ± 2.3 in the gestational diabetes group and 4.1 ± 2.9 in the control group, respectively; this difference was not statistically significant. HOXA-10 was also stained only in the decidua, not in the trophoblasts. Mean IRS scores for HOXA-10 were 2.5 ± 1.8 and 2.0 ± 1.5 in the gestational diabetes group and in the control group, respectively. Mean IRS scores for HOXA-10 were similar in both groups. LIF was stained in both the decidua and the trophoblasts. Mean IRS scores for LIF were 3.4 ± 2.4 and 3.8 ± 2.6 in the study and control groups, respectively, and there was no difference between the IRS scores of the groups (Table 2).

Table 1. — *Demographic properties of the groups.*

| | Maternal age | BMI kg/m² | Live birth | Abortus | Fetal weight(gr) |
|---|---|---|---|---|---|
| GDM | 28±6.5 | 30.4±3.3 | 2.3±1.3 | 1.8±1.1 | 3259±598 |
| Control | 27±5.3 | 0.09 | 1.8±1.2 | 1.6±0.9 | 2819±473 |
| *p* | 0.34 | 0.23 | 0.16 | 0.23 | 0.09 |

Table 2. — *Glycodelin, LIF and HOXA-10, imminoreactive scores (IRS) of the groups.*

| | Glycodelin | LIF | Hoxa-10 |
|---|---|---|---|
| GDM | 3.4± 2.3 | 3.4± 2.4 | 2.5±1.8 |
| Control | 4.1± 2.9 | 3.8± 2.6 | 2.0±1.5 |
| *p* | 0.08 | 0.13 | 0.11 |

## Discussion

Glycodelin levels are altered in certain pathological pregnancies. Maternal serum glycodelin levels tend to be lower than normal in pregnancies with intrauterine growth restriction [16]. In gestational hypertension, serum glycodelin levels have been shown to be higher than normal [17]. Although not confirmed by all studies, some studies have stated that some women with pre-eclampsia show an increased glycodelin concentration during advancing pregnancy, as opposed to the normal pattern of decreasing values [16, 18]. In pregnancies complicated by diabetes, serum glycodelin concentrations have been studied only in the first trimester when they did not deviate from normal [19]. Loukovaara *et al.* investigated umbilical cord glycodelin in diabetic pregnant women treated with insulin and found no difference in these specimens and those from healthy pregnant women. They also measured maternal glycodelin, and found that the glycodelin concentration in the serum of pregnant women was about 70-fold higher than that found by the present authors in cord serum [20]. This observed difference agrees with the theory that any glycodelin in cord serum is derived by transfer from the mother. This study proved this theory, in that the authors detected glycodelin only in the decidua and not in the trophoblasts, which indicates that glycodelin is expressed only in maternal tissue. This study is the first histopathologic

one to show glycodelin localization in term placentas. From its findings, we can conclude that the source of glycodelin is maternal decidual cells, and that glycodelin production is not different in patients with gestational diabetes mellitus compared with that in healthy pregnant women.

It is well known that LIF plays a role in regulating uterine receptivity. LIF is expressed in the endometrial glands and has a robust action on the uterine luminal epithelium. Induced by estrogen, LIF functions to convert the luminal epithelium from a non-receptive state to an embryo-responsive state [21]. However, the role of LIF in the pathophysiology of complicated pregnancies, such as preeclampsia and preterm birth, has been investigated recently [22, 23]. The role of LIF in gestational diabetes patients has also been unclear. This study demonstrated that LIF was found in trophoblastic cells as well as in decidual cells, which means that LIF is present in mature placentas, and plays some role during the gestation cycle, but is not related with gestational diabetes.

Currently, there is little-to-no knowledge about the role of HOXA-10 in complicated pregnancies. The role of LIF in implantation and decidualization has been investigated, and is well -understood However, this study is the first that showed that HOXA-10 continued to be expressed in term pregnancy decidua, and the authors noted that they did not recognize it in trophoblasts. HOXA-10 was stained similarly in both the term placentas of gestational diabetes patients and in control subjects, which suggests that it is not involved in the pathophysiology of gestational diabetes mellitus.

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