Immunohistochemical study of Inhibin A and B expression in placentas from normal and pathological gestations

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Summary

Objective: The aim of the study was to examine, by an immunohistochemical method, the distribution of Inhibin-A and -B, in placentas from normal and pathological gestations. *Materials and Methods:* Sixty-two specimens of placental tissue were examined: i) ten cases from early gestations, ii) 28 cases from mature placentas, iii) six cases associated with intrauterine growth restriction, iv) four cases associated with diabetes mellitus and v) 14 placentas from gestations with fetal chromosome abnormalities. The expression of Inhibin A and B was studied by automatic Ventana method. *Results:* i) Early gestation specimens: Inhibin A (+) immunoreaction was observed in the syncytiotrophoblast (8/10 cases) and in the intermediate trophoblast (6/10 cases). Inhibin B (+) immunoreaction was observed in the syncytiotrophoblast (10/10 cases) and in the intermediate trophoblast (4/10 cases). ii) Normal mature placentas: Inhibin A (+) immunostain was observed in 2/28 cases in the syncytiotrophoblast and in 7/28 cases in the intermediate trophoblast. Inhibin B (+) immunostain was observed in 28/28 cases in the syncytiotrophoblast and in 18/28 cases in the intermediate trophoblast. iii) Placentas associated with intrauterine growth restriction: Inhibin A (+) immunostain was observed in the intermediate trophoblast in 2/6 cases. Inhibin B (+) immunostain was observed in 5/6 cases in the syncytiotrophoblast and in 4/6 cases in the intermediate trophoblast. iv) Placentas associated with gestational diabetes mellitus: Inhibin A (+) immunostain was observed in 2/4 cases in the intermediate trophoblast. Inhibin B (+) immunostain was observed in 2/4 cases in the syncytiotrophoblast. v) Placentas from gestations with fetal chromosome abnormalities: no Inhibin A immunoreaction was observed. Inhibin B (+) immunostain was observed in 13/14 cases in the syncytiotrophoblast and in 9/14 cases in the intermediate trophoblast. The cytotrophoblast, the umbilical cord, and the membranes do not participate in the production of Inhibins. *Discussion:* Inhibin A and B are located in the syncytiotrophoblast and the intermediate trophoblast of the placenta, during early pregnancy (Inhibin A) and present throughout pregnancy (Inhibin B). No remarkable findings in placentas of pathological gestations support the evidence that Inhibins do not participate in processes that affect the development of the placenta or the fetus, but may participate in the mechanism of labor.

Key words: Inhibins; Placenta; Syncytiotrophoblast; Trophoblast; Diabetes mellitus; Intrauterine growth restriction.

Introduction

Inhibins are heterodimeric glycoprotein hormones of the transforming growth factor-b super family and consist of one a-subunit and two b-subunits with main action in the suppression of follicle-stimulating hormone (FSH) secretion [1, 2].

The placenta is the main source of Inhibins in the maternal circulation and all Inhibins isoforms are present in extracts of term human placenta and towards the end of human pregnancy, increasing concentrations of immunoreactive and bioactive Inhibins are present in maternal serum [1-4]. The exact source of the specific inhibin isoforms from the placental components is yet unclear. Several studies report that Inhibins are involved in the paracrine regulation of prostaglandin, human chorionic gonadotropin (hCG), progesterone (P) release, and the maintenance of pregnancy and subsequent initiation of labor [1, 5-8].

In this study the distribution of Inhibin-A and Inhibin-B was investigated in normal placentas by an immunohistochemical method, as well as in placentas from cases diagnosed with intrauterine growth restriction (IUGR), or gestational diabetes mellitus (GDM), or fetal chromosome abnormalities.

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Materials and Methods

The authors examined Hematoxylin and Eosin-stained (H&E) sections from formalin-fixed and paraffin-embedded tissues from 62 placentas: i) ten from first trimester gestations, ii) 28 from normal full-term gestations, iii) six from pregnancies associated with IUGR, iv) four from pregnancies associated with GDM, and v) 14 associated with fetal chromosome abnormalities.

All cases were retrieved from archival material of the Aretaieion University Hospital Pathology Laboratory. Additional sections of each case were obtained for immunohistochemical investigation by Automated Ventana Immunostainer. Slides were incubated with primary mouse monoclonal anti-Inhibin $\beta_{a/b}$ subunit IgG2b antibody (Serotec, Oxford, England; batch 0694, clone E4, dilution 1:100) and monoclonal anti-Inhibin α subunit IgG2a antibody (Serotec, Oxford, England; batch 1097, clone R1, dilution 1:50). Positive controls (sections from normal human testis which showed typical strong immunostain of Leydig cells for both Inhibin-α and Inhibin-β subunit) and negative controls were also included with each staining procedure.

The percentage of Inhibin-immuno-positive cells was determined by two independent observers and subsequently graded as "focal" (< 25% of cells positive), "intermediate" (25% to 75% of cells positive), or "diffuse" (> 75% of cells positive) (Table 1).

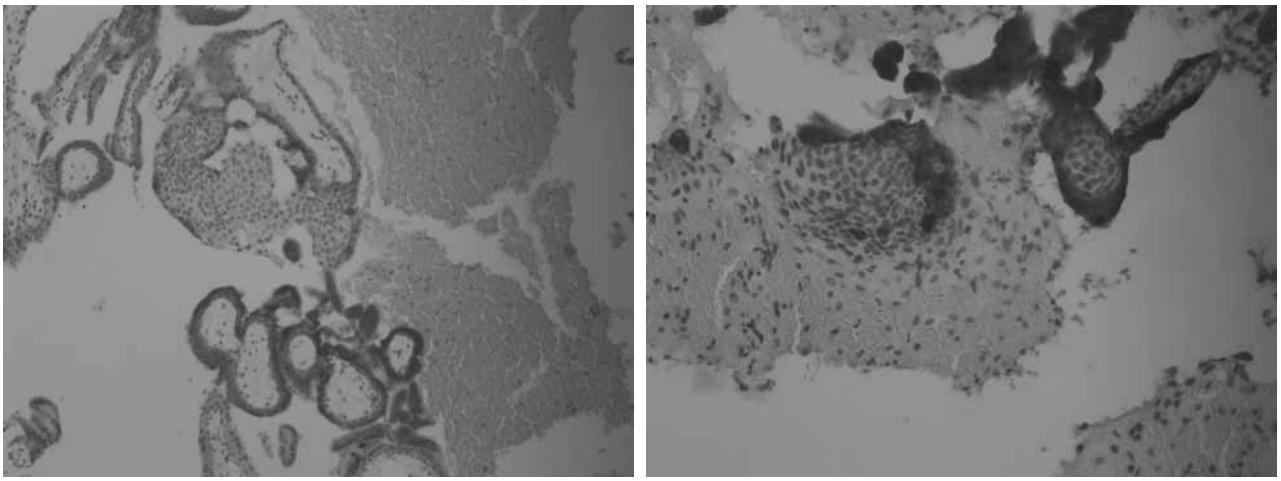

Figure 1. — Inhibin-A (+) immunoreaction of the villous syncytiotrophoblast. Cytotrophoblast does not express Inhibin-A (immunostain x 25).
Figure 2. — Inhibin-B (+) immunoreaction of the syncytiotrophoblast (immunostain x 120).

Results

Group A: Placentas from first trimester gestations - ten cases

A diffuse Inhibin-A positive immunostain was observed in 8/10 specimens located in the villous syncytiotrophoblast, and in 6/10 specimens located in placental intermediate trophoblast (Figure 1).

Inhibin-B positive immunostain of the syncytiotrophoblast was observed in all specimens (10/10) (Figure 2), and of intermediate trophoblast in 4/10 specimens. The cytotrophoblast and the various cellular components of the decidua, presented negative immunoreaction.

Group B: Placentas from normal term gestations - 28 cases

Inhibin-A positive immunostain was observed in 2/28 specimens located in syncytiotrophoblast and in 7/28 specimens located in intermediate trophoblast. The cytotrophoblast, cellular components of the decidua, umbilical cord, and membranes presented negative immunoreaction.

Inhibin-B positive immunostain was observed in the syncytiotrophoblast in all 28 specimens and in the intermediate trophoblast in 18/28 specimens. The cytotrophoblast, cellular components of the decidua, umbilical cord, and membranes presented a negative immunostain reaction.

Group C: Placentas from IUGR gestations - six cases

Only the intermediate trophoblast showed a positive Inhibin-A immunostain, observed in 2/6 specimens. The other villous and placenta components were negative.

Inhibin-B positive immunostain was observed in syncytiotrophoblast in 5/6 specimens and in intermediate trophoblast in 4/6 specimens. All other placental components presented a negative immunostain reaction.

Group D: Placentas from pregnancies complicated with GDM - four cases

Inhibin-A positive immunostain was observed only in intermediate trophoblast in 2/4 specimens. All other placental components presented a negative immunostain reaction.

Inhibin-B positive immunostain was observed only in the syncytiotrophoblast in 2/4 specimens.

Group E: Placentas associated with fetal chromosomal abnormalities - 14 cases

All placental components presented a negative Inhibin-A immunostain reaction.

Inhibin-B positive immunostain was observed in syncytiotrophoblast in 13/14 specimens and in the cytotrophoblast in 5/14 cases. A positive focal immunostain was observed in intermediate trophoblast of the decidua in 9/14 cases.

Discussion

Inhibins are glycoprotein heterodimeric hormones that belong to the super family of the β transforming growth factor (TGF-β). They were initially detected by their effect of down-regulation on FSH.

Inhibins present two subunits: a common subunit α and two subunits β_A and β_B. The combination of these subunits gives rise to two different dimmers, Inhibin-A (α-β_A), and Inhibin-B (α–β_B), respectively. The two subunits are peptidic chains connected by double sulphide attachments. The molecular weight of subunit α and β is 18 kDa and 14 kDa respectively, and when combined, a mature Inhibin molecule of 32 kDa is created.

The two subunits are produced by two different genes and when separate, they do not present any biological activity. The heterogeneity of Inhibin can also be attributed to the action of proteases, to which this molecule is subjected during its passage through the vascular tree.

Table 1. — *Comparative study of the distribution of immunopositive cells (%) among the placentas of the five groups examined.*

	Inhibin-A		Inhibin-B	
	SC	IMT	SC	IMT
Group A	80%	60%	100%	40%
Group B	7%	25%	100%	64%
Group C	0%	33%	83%	67%
Group D	0%	50%	50%	0%
Group E	0%	0%	93%	64%

Group A: First trimester placentas, Group B: last trimester placentas, Group C: placentas from IUGR pregnancies, Group D: placentas from pregnancies with GDM and Group E: placentas from pregnancies with fetal chromosomal abnormalities.
SC: syncytiotrophoblast; IMT: intermediate trophoblast; Focal expression: < 25% of positive cells.
Intermediate expression: 25% to 75% of positive cells. Diffuse expression: > 75% of positive cells.

There is evidence that only a part of circulating Inhibin is biologically active as a dimmer [1-3].

Inhibin is produced by the feto-maternal unit during pregnancy. Its circulating levels reach their maximum during the first week of gestation. Beyond this gestational age they decline and remain stable until the 25th week from which they rise up again until the completion of gestation. Inhibin, detected in the maternal serum throughout this period, is produced exclusively by the placenta, and studies have shown that placental trophoblastic cells of term gestations do actually secret Inhibin.

Inhibin acts by down-regulating the secretion of FSH and by up-regulating the secretion of gonadotropin-releasing hormone (GnRH) from the central nervous system, of P by the placenta, and of hCG. In vitro experiments show that Inhibin drastically down-regulates the production of hCG by the placenta at the end of gestation, a fact that is not supported by other studies. In cases of molar pregnancy, high levels of circulating Inhibins are detected, followed by high levels of hCG. Furthermore, there is evidence that Inhibin seems to regulate the release of prostaglandins as well.

It is generally accepted that Inhibins enroll a regulating action of paracrine and endocrine functions during pregnancy. In the amniotic fluid, both Inhibin-A and B are found in high concentrations. In the maternal serum on the other hand, only levels of circulating Inhibin-A are high. In the umbilical cord Inhibin-B is detected in male embryos only, while Inhibin-A cannot be traced in either male or female embryos. Additionally, Inhibin-A cannot be traced in embryonic circulation. Finally, both isoforms can be traced in placental villi.

Serum concentration of Inhibin-A during the eighth to ninth gestational weeks in normal pregnancies is 550 pg/ml, and it is considered a predictive index for a good gestational outcome after the 16th to 18th week. It must be noted that Inhibins are the products of the placenta and of the ovarian follicle and thus not traceable in the circulation of a male patient. Considerable reduction of the levels of Inhibin-A in the amniotic fluid is noted, when comparing gravidas during active labor with gravidas subjected to Caesarian section. This fact may be explained by an alteration of the relationship between Inhibin and activin, that is believed to be of crucial significance for the proper function of the feto-maternal unit, and for good gestational and obstetric outcomes [4-6].

Trophoblastic tissues are considered the main sites of production of Inhibins. Placental syncytiotrophoblast is the main site of production of Inhibin-A, while chorionic trophoblast and membranes produce both Inhibin-A and B, that are then released in the amniotic fluid. Placenta secreted Inhibin-A in high concentrations in the maternal circulation remains invariable during active labor.

In the embryonic circulation, secretion of Inhibin-B is sex-dependent and its main sources are the testicles and probably the lungs. No Inhibin-A can be traced in the embryonic circulation.

The results of this investigation show that Inhibin-A is mainly located in the syncytiotrophoblast, and in certain cases, in the placental intermediate trophoblast during the initial stages of gestation (first trimester). There is a gradual decrease of the immunoreaction until the middle of the gestation, from which point and until the end of pregnancy, it is no longer traceable.

On the other hand, Inhibin-B is detectable throughout gestation and presents a characteristic distribution. It is detectable mainly in the syncytiotrophoblast and less in the intermediate trophoblast, in the chorion laeve, and decidua. The cytotrophoblast, umbilical cord, and membranes present a negative immunostain in all cases.

From the examination of the specimens of the other Groups (IUGR, GDM, and fetal chromosomal abnormalities), no significant findings were observed, and this may insinuate that Inhibins do not participate significantly in the normal growth of the embryo and placenta, while they play a major role in sustaining a normal gestation and initiating the phenomenon of labor [9, 10].

Conclusion

In normal pregnancies, Inhibin-A is present in the syncytiotrophoblast and in the intermediate trophoblast of the placenta during the first gestational weeks (first trimester), and it gradually decreases until the first half of pregnancy where it is no longer detectable.

In contrast, Inhibin-B can be traced by immunohistochemistry during gestation, presenting a characteristic distribution, and being located in the syncytiotrophoblast and in smaller amounts in the intermediate trophoblast of the chorion laeve and the decidua.

No significant changes of these patterns in placentas of pathological pregnancies were observed and this supports the theory that Inhibins do not participate in the mechanisms needed for the proper growth of the embryo or the placenta, having an action limited probably to the initiation of active labor.

The other components of the placenta, the cytotrophoblast, umbilical cord, and membranes, do not participate in the production of Inhibin-A or B, as observed by the immunohistochemical investigation.

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