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Immunohistological examination of thyroid hormone receptor expressions in placenta in maternal hypothyroidism

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Abstract

Thyroid hormones are necessary for the development of a healthy fetus and placenta. In our study, we evaluated the thyroid hormone receptor expressions in the placentas of pregnant women with hypothyroidism. We evaluated term placentas in two groups: control (n=5) and hypothyroidism (n=5). THRα and THRß were expressed in the placenta, especially in syncytiotrophoblasts, and also in placental macrophages. THRα expression was decreased in the hypothyroid group compared to the control group, but it was statistically non-significant (p=0,850) . THRß expression was decreased in the hypothyroid group compared to the control group at a statistically significant level (p=0,004). The results showed that thyroid hormone receptors are associated with maternal hypothyroidism.

Keywords: maternal hypothyroidism, placenta, thyroid hormone receptors

1. Introduction

Thyroid hormones have a wide range of effects, specifically on metabolism, growth, development, and different organs. From a metabolic perspective, thyroid hormones are calorigenic and cause oxygen consumption and body heat generation. They increase protein catabolism, promote gluconeogenesis, increase glucose utilization, and regulate lipid metabolism (1–3). In some cases, thyroid hormones act by binding to proteins such as integrin αvβ3 on the plasma membrane, but most of their effects are usually achieved by binding intracellularly to thyroid nuclear receptors (THRs). T3 has a higher affinity for binding to THRs, while T4 is more effective at binding to integrin αvβ3. THRs act as ligand-dependent transcription factors by acting directly on thyroid hormone response elements (TREs) on gene promoters (canonical signaling). THRs can also act by non-canonical signaling by activating molecules such as phosphoinositide-3-phosphate kinase (PI3K), protein kinase B (AKT), and mitogen-activated protein kinases (MAPK). THRs are encoded by two different genes (α and β) located on chromosomes 17 and 3. THRα1, THRβ1, and THRβ2 are the major hormone-binding isoforms (4–6). Particularly T3 plays an important role in trophoblast differentiation and fetal neurodevelopment (5). THRα and ß are expressed in the nuclei of syncytiotrophoblasts, cytotrophoblasts, and extravillous cytotrophoblasts at increasing levels with advancing pregnancy (7).

Hypothyroidism is common during pregnancy and is seen between 3.5% and 18% (8–11). In general, the main cause of hypothyroidism is iodine deficiency. The American Thyroid Association (ATA) and the Turkish Endocrinology and Metabolism Association have determined TSH levels according to pregnancy periods according to Table 1 below (12). In the case of untreated hypothyroidism during pregnancy, the risk of miscarriage, placental rupture, premature birth, pre-eclampsia, and neonatal deaths may be observed (8,13,14).

Table 1. TSH Reference Ranges in The Evaluation of Thyroid Functions During Pregnancy in the First, Second, and Third Trimesters

Trimester	TSH Reference Range
First Trimester	0,1 – 2,5 mIU/ml
Second Trimester	0,2 – 3,0 mIU/ml
Third Trimester	0,3 – 3,0 mIU/ml

In our study, we aim to evaluate THR expressions in placentas with hypothyroidism during pregnancy from an immunohistological perspective.

2. Materials and Methods

2.1. Group design

Placentas that were discarded after delivery were taken from pregnant women who came to the Gynecology and Obstetrics Clinic of DEU Application and Research Hospital. While placentas taken from healthy pregnant women were used for the Control group (n=5), placentas from pregnant women diagnosed with hypothyroidism were taken for the Hypothyroidism group (n=5).

Patients in the hypothyroidism group were selected from pregnant women diagnosed with subclinical hypothyroidism based on TSH serum concentration and free T3 and T4 reference values (TSH>2.5 mIU/L in the first trimester and TSH>3.0 mIU/L in the second trimester)(15,16). Pregnant

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women were selected between the ages of 20-35. Consent forms were obtained from the patients before the study. Based on the patient's medical records and their responses to the health status questionnaire, patients with established risk factors such as congenital/acquired heart disease, obesity, multiple pregnancy, diabetes mellitus, hypertension, and smoking were excluded from the study (17,18).

2.2. Immunohistological examination

For immunohistochemical staining from placental samples, approximately 1 mm^3 tissue samples were placed in 10% formalin to fix the tissues and embedded in paraffin blocks. A microtome was used to cut the paraffin tissue blocks at 5 µm thickness on positive charged slides.

The slides were kept in an oven at 60ºC overnight. The next day, deparaffinization was carried out by passing through three series of xylene, the first of which was in the oven. The sections were passed through increasing alcohol series and brought to distilled water. Antigen retrieval was performed with citrate buffer (pH 6, 15-M103, Bio-Optica, Italy) to open antigenic epitopes that were closed during fixation. Slides were boiled in the microwave at 850W for 15 min and allowed to cool for 20 min. UltraVision Large Volume Detection System (TR-125-AL, Thermo Fischer, USA) was used for histochemical immunostaining. Slides were incubated with H_2O_2 for 15 min to block endogenous peroxidase activation and washed with PBS. Slides were incubated with Ultra V Block for 7 min. Slides were incubated with primary antibody [THRα (1:50, bs-6221R, Bioss, USA) and THRß (1:50, bs-11440, Bioss, USA)] overnight at +4°C in a humidified chamber. Slides were incubated with Streptavidin Alkaline Phosphatase for 10 min. The slides were washed with PBS. Slides were incubated with HRP Polymer for 15 min. Washed with PBS. Coloring of bound antibodies was achieved with a DAB chromogen (11718096001, Roche, Swiss) solution. The slides were washed with distilled water. Counterstaining was done with hematoxylin and covering was done with mounting medium (107960, Merck, Germany).

2.3. Positive area density measurement

After immunohistochemistry staining, 10 random fields were photographed from each section using a microscope (Euromex, IS.3153-PLFi/3) with a 40x objective. (1) The photos were opened in ImageJ and the photo was separated according to color channels with the color deconvolution plugin (2) Color channel windows were closed except for the brown color channel representing DAB staining (3) threshold was applied and brown stained areas were selected and (4) area measurement of the selected areas was performed.

2.4. Statistical analysis

All data were loaded into the GraphPad Prism 10 statistical program and the results are shown as mean ± standard error. After evaluating the normality and homogeneity of the data statistically, a nonparametric Mann-Whitney U analysis was performed to compare the two groups. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

In the control group, both THRα and THRß showed significant expression in syncytiotrophoblasts, while labeling was observed to be lower in the Hypothyroidism group. In addition, Hofbauer cells, which are placental macrophages, also gave a positive immunoreaction both THRα and THRß.

After ImageJ analysis, the THRα positive area density in the Control group was 3481584.50±260138.79, while in the Hypothyroidism group, it was 2921985.7±286587.20, and this decrease was found to be statistically non-significant ($p=0.416$). The THRß positive area density in the Control group was 3069027.90±404848.09, while in the Hypothyroidism group, it was 1817939.2±260711.31, and this decrease was found to be statistically significant ($p=0.013$).

4. Discussion

Thyroid hormone receptors show differences in hypothyroid placentas. In our study, THRα and THRβ showed different expressions in the control and hypothyroidism groups. While THRα and THRß gave strong immunoreactivity in the Control group, a weaker signal was observed in the Hypothyroid group.

In the study conducted with trophoblast cells, it was shown immunohistochemically that thyroid hormone receptors were expressed in cytotrophoblasts and syncytiotrophoblasts (19).

In a similar study on placentas with GDM, THRα expression was found to be at the strongest level in healthy placentas, while THRß expression was found to be weaker. THRα expression was also found to be lower in GDM placentas compared to healthy placentas. THRß expression showed similar expression in GDM placentas to healthy placentas (6).

In a study examining the relationship between IUGR and THR, similar to the GDM study, the strongest expression was for THRα. When they compared healthy and IUGR placentas, both THRα and THRß expression decreased significantly in IUGR placentas (7).

In a study conducted on spontaneous and recurrent miscarriages, THRα and THRß expressions in both placenta and decidua were significantly decreased compared to healthy pregnant women (20).

There is an important relationship between thyroid hormones and the immune system. Abnormal thyroid hormone secretion can affect immune functions. Hormones and endocrine transmitters bind to immune cells, leading to the production of factors that alter immune functions and regulate the intensity of the immune response. Studies have shown that THR is expressed in macrophages (21–23). In our study, we showed that THRα and THRß are expressed in Hofbauer cells.

As a result, it was observed that the effect of THRs changed in hypothyroidism as in different pregnancy complications. This change may pave the way for the development of

pregnancy complications caused by hypothyroidism.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: G.B., S.C.M.; Design: G.B., S.C.M.; Data Collection or Processing: G.B.; Analysis or Interpretation: G.B., S.C.M.; Literature Search: G.B., S.C.M.; Writing: G.B., S.C.M.

Ethical Statement

The study was approved by 'Non-Interventional Research Ethics Committee' of Dokuz Eylül University (2023/20-15). The investigation was carried out according to the Declaration of Helsinki. A consent form was taken from all volunteers.

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