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Estrogen receptors in hip joint capsule and ligamentum capitis femoris of babies with developmental dysplasia of the hip

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Objective: The aim of this study was to detect the incidence of estrogen receptors in human hip joint capsule and ligamentum teres.

Methods: The study included biopsies of the ligamentum capitis femoris (LCF) and hip joint capsule from 15 patients undergoing hip surgery for developmental dysplasia of the hip (DDH) and from the control hips of 15 cases of intrauterine fetal death. Mean age was 10.3 (range: 6 to 18) months at the time of surgery. Full-thickness 1x1 cm anterior capsule and LCF portions were taken as biopsy specimens. An immunohistochemical study using monoclonal antibody against estrogen receptors was performed to identify the rate of target estrogen cells in the hip joint capsule and LCF.

Results: Estrogen receptor (ER) staining rates were 1.6±0.2% for the LCF and 1.3±0.2% for the hip joint capsule in the control groups, and 2.5±0.3% for the LCF and 2.0±0.3% for the hip joint capsule in the DDH groups. Estrogen receptor staining rates in the LCF and hip joint capsule control groups were significantly lower than that in the DDH groups ($p<0.001$). In both groups, ER rates were significantly lower in the hip joint capsule than in the LCF ($p<0.01$).

Conclusion: The high rate of ERs in the LCF and hip joint capsule appears to support the effect of estrogen in the etiology of the DDH.

Key words: Developmental dysplasia of the hip; estrogen receptor; hip capsule; ligamentum capitis femoris.

Developmental dysplasia of the hip (DDH) is a neonatal condition with various causes. Within the outline provided by the genetic code, embryonic, fetal and childhood development of the hip continue to a variety of environmental and biomechanical factors.[1-5] Currently, only few laboratory studies have provided information on hormonal factors effective on the etiology of DDH.[6] Undue laxity of the hip joint capsule and ligamentum teres at the time of birth is the most important structural deformity that permits the initial dislocation.

Estrogen has been shown to affect the composition and structure of a variety of tissues.[7,8] Estrogen receptor (ER) proteins in target cells are necessary for hormone action.[9] It is very likely that estrogen may influence the

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structure and composition of the hip capsule and ligamentum teres as it does in other tissues. The exact pathophysiology of the etiology of DDH is unclear. Therefore, the aim of this study was to analyze the incidence of ERs in the ligamentum capitis femoris (LCF) and hip capsule in order to explore the hormonal effect on the development of DDH.

Materials and methods

Approval was obtained from the local scientific department of our hospital and consent to study participation from the families prior to surgery. The study included LCF and hip joint capsule biopsies from hips of 15 patients undergoing hip surgery for DDH (open reduction through medial or anterolateral approaches, Salter's osteotomy) and from 15 cases of intrauterine fetal death. All dislocated hips were unilateral. Fetal hips were examined and found to be normal. Mean age of the subjects was 10.3 (range: 6 to 18) months at the time of surgery. The 60 specimens were divided into DDH and control groups and further divided into LCF and hip joint capsule groups. Full-thickness 1x1 cm anterior capsule and LCF portions were taken as biopsy specimens. All babies undergoing surgery for hip dysplasia were female.

Commercial monoclonal estrogen receptor antibody Estrogen Receptor Ab-11 (Clone 1D5) MW: 67kDa (Fisher Scientific, Loughborough, UK) was obtained. A sample of breast carcinoma specimen was used for both positive and negative controls. For the negative controls, IgG1 immunoglobulin was substituted for the anti-estrogen receptor antibodies. One positive and one negative control sample were included in all assays.

Tissue sections were deparaffinized in xylene and hydrated in a graded series of alcohol. Slides were then placed in Tris-buffered saline for 15 minutes followed by washing in 0.1% Tween/phosphate-buffered saline (pH: 7.4) solution. Blocking of nonspecific antibody binding was achieved with non-immune serum for 30 minutes at room temperature. The slides, including positive and negative controls, were incubated with the respective primary antibodies for 16 hours at room temperature in a humidity chamber. Subsequently the secondary link antibody (LabVision; Biotinylated Goat Anti-polyvalent) followed by streptavidin-biotin peroxidase complex were separately applied in a humidity chamber. The substrate diaminobenzidine (DAB) tetrahydrochloride containing %0.024 hydrogen peroxidase in phosphate-buffered saline (pH: 7.4) was applied. Sections were dehydrated in graded alcohol and coverslips were applied with Entellan (Merck, Darmstadt, Germany).

Estrogen receptor staining was quantified as the rate of positive stained cells in 100 connective tissue cells. Statistical analyses were performed by independent samples t-test and paired samples t-test. P values of less than 0.05 were considered statistically significant. Data were analyzed using the SPSS for Windows v.11.5 computing program (SPSS Inc., Chicago, IL, USA).

Results

Estrogen receptor positive staining was located in nuclei of some connective tissue cells of all categorized tissue samples (Figs. 1 and 2). Estrogen receptor positive staining values are shown in Table 1. Estrogen receptor positive staining for the LCF and hip capsule control groups were significantly lower than those of the DDH groups ($p<0.001$). Estrogen receptor positive staining rate was 1.6±0.2 per 100 connective tissue cells for LCF and 1.3±0.2 for hip capsule in the control group, whereas it was 2.5±0.3 for the LCF and 2.0±0.3 for the hip capsule DDH groups. In both groups, we found ER positive staining significantly lower in the hip capsule specimens when compared to LCF samples ($p<0.01$).

Discussion

The possible effect of maternal hormones on the hip capsule leading to joint laxity has been previously described in the literature.[3,6,10] Prior studies have hypothesized that a newborn's response to maternal hormones may explain the higher incidence of DDH in females. However, maternal hormones affect both male and female fetuses. As detection of specific receptors on tissue can prove hormonal activity, we decided to explore estrogen receptors in the hip joint.

Hormone action at the cellular level begins with the association of the hormone and its specific receptor. Estrogen receptor is a nuclear protein synthesized in cytoplasm and then rapidly transferred to the nucleus.[8] Estrogen and progestins bind their intracellular receptors and cause conformational changes of the latter.

Wynne-Davies described hereditable ligamentous laxity as one of two major mechanisms for the inheritance of DDH.[4] Peripheral joint laxity produced in the fetus is attributed to the maternal hormones entering fetal circulation during the second and third trimesters.[3]

Collagen content is negatively influenced by estrogen. We believe that this might be the major cause behind the relaxation of connective tissue. Studies have shown the laxity producing effect of estrogen over connective tissue.

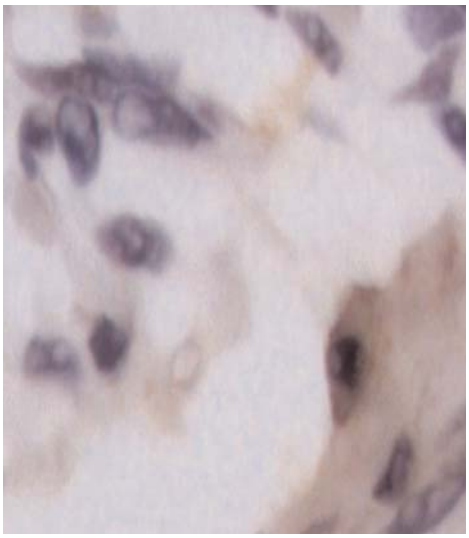

Fig. 1. Localization of estrogen receptor in paraffin-embedded sections of the LCF. Receptors were detected in the nuclei of fibroblasts within the connective tissue stroma of the ligament. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

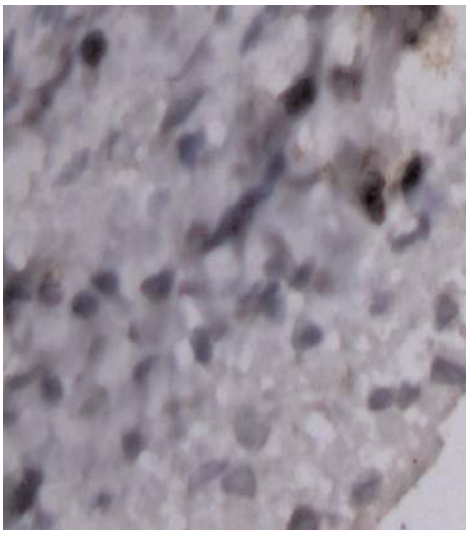

Fig. 2. Localization of estrogen receptor in paraffin-embedded sections of the human hip capsule. Receptors were detected in the nuclei of fibroblasts within the connective tissue stroma of the hip capsule. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 1. Estrogen receptor positive staining for the LCF and hip capsule of the DDH group and the control group.

	DDH group	Control group	p value
LCF ER (%)	2.5±0.3	1.6±0.2	p<0.001
Hip capsule ER (%)	2.0±0.3	1.3±0.2	p<0.001
p value	p<0.01	p<0.01	

Liu et al. reported that the laxity producing effect of estrogen on anterior cruciate ligament caused female athletic injury and reduced collagen synthesis with increasing amounts of 17beta-estradiol levels.[7] In another study, Akeson et al.[6] showed that administration of 17beta-estradiol administration decreased the content of soluble collagen fractions in periarticular tissue. They proposed that the therapeutic use of 17beta-estradiol in the short-term treatment of injuries or diseases results in enforced immobilization manifested as joint contractures.

Andren et al. analyzed the excretion of conjugated estriol, conjugated estrone and 17beta-estradiol of newborns with DDH and found higher levels in the DDH group than the controls.[10] In another study, the association between 17beta-estradiol from umbilical cord blood and neonatal hip instability was evaluated and high levels of 17beta-estradiol tended to be associated with an increased risk of neonatal hip instability in girls.[11]

Nakamura et al. studied TGF-beta1 and reported that TGF-beta1 induced a dose-dependent contraction of collagen gels containing bovine trabecular meshwork cells.[12] Previous studies suggested that estrogens suppress TGF-beta-induced gene expression.[13] We believe that a decrease in collagen contraction due to the effect of estrogen through TGF-beta on collagen may increase the laxity of tissues around the hip.

Shynlova et al. reported that the levels of collagen Type 1 and Type 3 in rat uterine tissue correlated with gestational changes in the plasma estrogen/progesterone ratio.[14] Administration of estrogen causes a decrease in the total amount of collagen in the rat hip joint capsule, skin, aorta, and tail tendon.[15-18]

Estrogen receptors were found in all samples. Estrogen receptors in the LCF and hip capsule specimens of the control group were significantly lower than in the DDH group. This result may be attributed to the receptor up-regulating effect of estrogen. As the positively stained ER concentration was higher in LCF samples than hip joint capsule samples, LCF may be affected more by estrogen than the hip joint capsule. This difference in receptor rates may not be limited only to the hip joint but may also be valid for other joints. Therefore, similar receptor studies should be performed for other tissues as well.

A disadvantage of our study was that the mean ages of the control and the DDH group were different, which may have affected the receptor rates.

In conclusion, the high rate of ERs in LCF and hip joint capsule appears to support the effect of estrogen in the etiology of DDH.

Conflicts of Interest: No conflicts declared.

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