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Original Article CHILD SURGERY

Expression of androgen, estrogen and progesterone hormone receptors in penile tissues of children with different types of hypospadias

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ABSTRACT

OBJECTIVE: Androgen (AR), Estrogen (ER) and Progesterone (PR) hormones play an important role in the prenatal and postnatal development of urogenital tract and especially the penis. The expressions of AR, ER and PR receptors in penile tissues in children with hypospadiases had also been shown previously. In this leading study, to demonstrate of the sex hormone receptor expression in cases with different types of hypospadias were aimed.

METHODS: This study was designed in children operated due to hypospadiases without DSD. Biopsy samples of 3 mm's were obtained from three different sytes as the lateral parameatal tissue and the anterior corner of the prepuce, and inner layer of posterior prepuce. The presence of AR, ER and PR receptors was investigated immunehistochemically.

RESULTS: Mean age was 5.4 years in 18 children with hypospadiases; in totally 33 specimens were taken in 5 subcoronal as 5 specimens, and 7 penile as 15 specimens, and 6 penoscrotal as 13 specimens. According to sytes of samples; 13 samples were from lateral para-meatal tissues, and 13 were from anterior corners of prepuces, and 7 were from inner layers of posterior prepuces. In regard to receptor expression; ER and AR receptors were positive in 29 (87.8%) and 12 (36.4%) respectively; PR receptors were negative.

CONCLUSION: This study emphasized the dominant expression of estrogen receptors in penile tissues of children with hypospadias. Although there was not a manifest correlation of androgen receptors absence in regard to the severity of hypospadias patients, there was a marked estrogen receptors presence in penile tissues. These findings suggest that the disrupted androgen and estrogen receptor interaction and/or balance could play a role during the development of external genitalia in hypospadias patients. Progesterone receptor was not present and therefore the active role in the postnatal development of hypospadias is still debatable.

Keywords: Androgen; estrogen; hormone; hypospadias; penis; progesterone; receptor.

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In humans, like as in other mammals, the gonads, the internal genital ducts, and the external genital structures all develop from bi-potential embryologic tissues. Male or female phenotype develops through a cascade of processes which initiate with sex determination and follow with sex differentiation [1]. The karyotype of the embryo determines whether primordial gonad differentiates into a testis or an ovary, respectively as known gonadal differentiation. It is the Y chromosome that is essential for the development of the male reproductive organs, and with no Y chromosome, an embryo will develop into a female. In both males and females, the sex organs consist of three structures: the gonads, the internal genitalia, and the external genitalia [1, 2]. However, a Y-related gene, the SRY gene on the Y chromosome triggers the development of male sex-related characteristics in the embryonic period, particularly testis formations. Testis development process also involves several steps controlled by other non-OY-linked genes.

Over the five weeks, the fetus begins producing hormones that cause its sex organs to grow into either male or female organs. Differentiation of external male genitalia requires the transformation of testosterone to di-hydro-testosterone by 5-alpha reductase type 2 expressed in genital skin and urogenital sinus [2]. The effects of androgens occur in presence of functional androgen receptor protein. Mutations of genes coding for steroidogenic enzymes, anti-Mullerian hormone, anti-Mullerian hormone receptor, androgen receptor and 5-alpha reductase are all associated with impairment of sex differentiation [1, 2].

Androgen (AR), Estrogen (ER) and Progesterone (PR) hormones are effecting and play an important role in the prenatal and postnatal development urogenital tract and especially the penis [3–8]. Effects and role of these sex hormon receptors on pathogenesis of hypospadias were shown in previous studies, and the expressions of androgen, estrogen and progesterone receptors of penile tissues in children with/without hypospadias were demonstrated [9–11]. In this leading study, investigate of the sex hormones receptors expression (AR, ER and PR) in different types of hypospadias were aimed.

MATERIALS AND METHODS

Materials

This study was designed in patients operated due to hypospadias. Patients were investigated in regard to different types of hypospadias (subcoronal, penile and penoscrotal) and the site of biopsy. All biopsy specimens were obtained from excision materials during the hypospadias surgery. Sites of the biopsy are a specimen from nearby the parameatal tissues next to the urethral sulcus (A), a specimen from corner of the anterior prepuce (B), and a specimen from inner surface of the posterior prepuce (C); locations of the biopsy were shown in Figure 1. The existence and density of Androgen (AR), Estrogen (ER) and Progesterone (PR) hormone receptors were investigated. The ethical committee of our hospital approved this study (2012/18537) and the families gave consent to investigate about the biopsy specimens, which were taken during the hypospadias surgery from the excision materials.

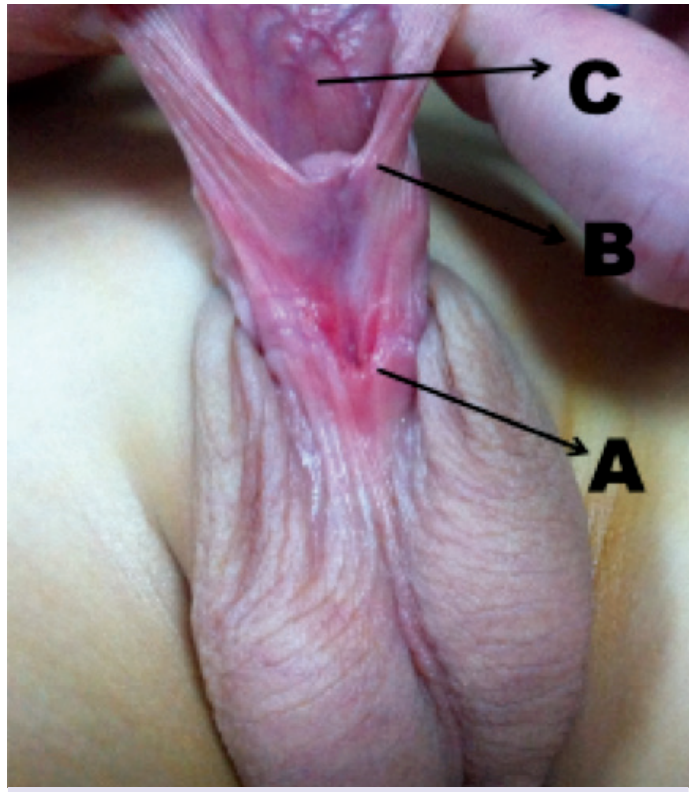

Figure 1. Biopsy specimens were taken from excision materials in patients during the hypospadias surgery. Sites of the biopsy specimens are a specimen from the lateral parameatal tissue next to the urethral sulcus, a specimen from corner of the anterior prepuce, and a specimen from inner surface of the posterior prepuce.

Surgical technique

All patients operated with single stage repairing of hypospadias with circumcision. ONLAY procedure was used in two patients with penoscrotal hypospadias; the others were operated with technique of Tubularized

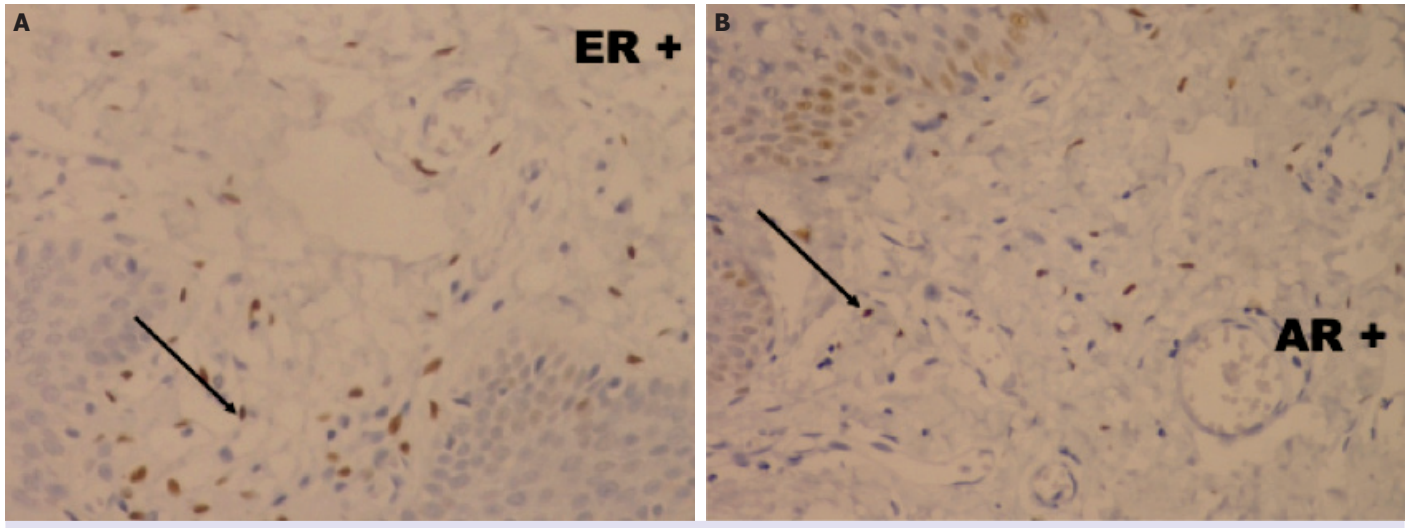

FIGURE 2. Reactions were interpreted as positive based on nuclear staining as brown color for estrogen (in shown **A**) and androgen (in shown **B**) receptors; reactions were interpreted as negative if there was not neclear staining like as progesteron receptors.

Incised Plate Urethroplasty. During The Tubularized Incised Plate Urethroplasty or Onlay Urethroplasty, the edges of urethral plate below the glanular level are usually very thin, devoid of corpus spongiosum and required a little trimming in order to perform tubularization of urethral plate; and these are the specimens from urethral plate in same cases. A glanular tissue was removed during the closure of glans wings from the inside of the glans penis and prepuce was sampled after the circumcision. Samples from corner of the anterior prepuce and posterior inner layer of the prepuce were sampled in all patients.

Immunohistochemistry

An Avidin-Biotin Horseradish Peroxidase Technique was used to lokalize expressions of Estrogen, Progesterone and Androgen in the different samples using Estrogen Receptor (SP1) (Dacopatts, Accurate NY, USA), Progesterone Receptor (SP2) (Dacopatts, Accurate NY, USA) and Androgen Receptor (SP107) (Sigma, MO, USA) antibodies [12]. Briefly, sections were dewaxed, dehydrated in alcohol and treated with 2% hydrogen peroxide for 20 minutes in methanol to block endogenous peroxide. Sections used were pre-trated in an 850-Watt domestic microwave oven in 0.01 M citrate buffer (pH 6.0) for 5 minutes. Sections were incubated for 30 minutes with normal horse serum (Vector Laboratories, Burlingham, CA, USA) and then incubated with the primary antibodies over night at 4 Celcius. The following day the sections were washed with 20mM phosphate buffered saline (pH 7.3, saline concentration is 20%) and then incubated with 1:200 biotinylated horse anti-mouse secondary antibody for 30 minutes. After a further wash step, all sections were incubated with the avidin-biotin peroxide complex ELITE system (Vector Laboratories, Inc., Burlingham, Ca, USA) for 30 minutes and then subsequently with 3,3-diaminobenzidine (Vector Laboratories, Burlingham, CA,USA) for 10 minutes. Sections were washed in tap water, counter stained with Gill's haematoxylin, then dehydrated in a series of graded ethanol, cleared in xylene and mounted.

All prepared sections were examined under the light microscope. Reactions were interpreted as positive based on nuclear staining with brown color and negative if there was not nuclear staining for Estrogen, Progesteron and Androgen receptors respectively (Figure 2A, B).

RESULTS

This study compromised findings of 18 patients with different grade of the hypospadias. 16 patients underwent TIPU and two patients underwent Onlay Urethroplasty. The mean age was 5,39 years old (ranges 2 and 10 years). There was no patient with Disorder of the Sex Development in this selected group, only one patient had undescended testes.

Total 33 biopsy specimens were taken from 18 patients. Five biopsy specimens were taken in 5 patients with subcoronal hypospadias, 15 biopsy specimens were taken in 7 patients which had penile hypospadias, and

Table 1. Distribution of the receptor positivity in total specimens

Receptor	Biopsy	%
Estrogen	28/33	87.8
Androgen	12/33	36.4
Progesteron	0/33	0

13 biopsy specimens were taken in 6 patients which had penoscrotal hypospadias. In 33 biopsy specimens, 13 specimens were from lateral parameatal tissues, 13 were from anterior prepuce and 7 were from inner layer of posterior prepuce. According to results of the Avidin-Biotin Horseradish Peroxidase Immunohistochemical Technique Staining, estrogen receptors were found positive in 29/33 biopsy specimens (87.8%). Androgen receptors were found positive in 12/33 biopsy specimens (36.4%). Progesterone receptors were found negative in all specimens. Results of the avidin-biotin horseradish peroxidase technique staining in all biopsy specimens were summarised in Table 1.

According to the hypospadias degree: In subcoronal hypospadias, estrogen receptors were positive in 4/5 specimens (80%), and androgen receptors were positive in 2/5 specimens (40%). In penile hypospadias, estrogen receptors were positive in 13/15 specimens (86.7%), androgen receptors were positive in 5/15 specimens (33.3%). In penoscrotal hypospadias estrogen receptors were positive in 11/13 (84.6%), androgen receptors were positive in 5/13 (38.5%) respectively (Table 2).

According to the biopsy location: In lateral parameatal tissues, estrogen receptors were positive in 9/13 specimens (69.2%), Androgen receptors were positive in 5/13 specimens (38.5%). In biopsy specimens of the anterior prepuce, estrogen receptors were positive in 13/13 specimens (100%), androgen receptors were positive in 4/13 specimens (30.8%). In biopsy specimens of the posterior inner preputium estrogen receptors were positive in 7/7 (100%) and androgen receptors were positive in 3/7 (42.8%) respectively (Table 3).

Table 2. Receptor positivity according to type of hypospadias

		%
Subcoronal hypospadias		
Estrogen receptor	4/5	80.0
Androgen receptor	2/5	40.0
Penil hypospadias		
Estrogen Receptor	13/15	86.7
Androgen receptor	5/15	33.3
Penoscrotal hypospadias		
Estrogen receptor	11/13	84.6
Androgen receptor	5/13	38.5

Table 3. Receptor positivity according to biopsy site. Sites of the biopsy specimens are A: 13 specimens from lateral parameatal tissue near urethral sulcus, B: 13 specimens from the corner of the anterior prepuce, and C: 7 specimens from inner surface of the posterior prepuce

		%
Parameatal tissue		
Estrogen receptor	9/13	69.2
Androgen receptor	5/13	38.5
Anterior preputium		
Estrogen receptor	13/13	100
Androgen receptor	4/13	30.8
Posterior preputium		
Estrogen receptor	7/7	100
Androgen receptor	3/7	42.8

DISCUSSION

Human male fetuses become externally distinct between 8 and 12 weeks, as androgens enlarge the phallus and cause the urogenital groove and sinus to fuse in the midline, producing an unambiguous penis with a phallic urethra, and a thinned, curled scrotum. The testes begin to secrete three hormones (anti-Mullerian hormone, testosterone, and di-hydro-testosterone) that influence the male internal and external genitalia. Anti-Mullerian hormone causes regression of the paramesonephric ducts. Testosterone, which is secreted and converts the mesonephric ducts into male accessory structures, such as epididymis, vas deferens and seminal vesicle. Testosterone will also control the descending of the testes from the abdomen into the scrotum. The di-hydro-testosterone, also known as will differentiate the remaining male characteristics of the external genitalia. A sufficient amount of androgen can cause external masculinization.

The most potent is di-hydro-testosterone, generated from testosterone in skin and genital tissue by the action of 5α-Reductase [1–8].

Current theories of mechanisms of sexual differentiation in humans are based primarily on three sources of evidence: Animal research involving manipulation of hormones in early life, observation of outcomes of small numbers of individuals with disorders of sexual development [13–17]. In mammals, like as in human, the gonads, the internal genital ducts, and the external genital structures all develop from bi-potential embryologic tissues [13]. It is known that the effects of hormones were by receptors/mediators. There are a lot of hormone-receptor studies about the sexual differentiation in mammals. Their results suggest that androgens may play an important role in the development of the male genitalia at a much earlier stage than that indicated by previously published work and that scrotal development in this species may not be androgen-independent [14, 15]. Findings were discussed with respect to similarities and differences between marsupials and eutherians in hormonal environment during the perinatal period and with respect to the possible role of androgens in sexual differentiation of the gray opossum brain [16]. Advances in understanding frequently come from studying experimental findings from animal models. In review of Hutson and his colleagues, the key issues that urologists need to understand in order to link animal studies to clinical practice were discussed; that review used two common disorders such as hypospadias and undescended testes [13].

Androgens contribute to testicular descent and, differentiation of male external genitalia [1–4]. Estrogen is known mainly as a female hormone and effecting female sex differentiation. However, the demonstration of estrogen receptors in both sexes and especially in male tissues raised the questions in regard the role of female hormones in male urogenital system development. Cunha and his friends also showed that estrogens played a critical role in penile and clitoral development, specifying the position of the urethral orifice, determining elasticity of the urethral meatus, and facilitating epithelial-epithelial fusion events required for proper formation of the distal urethra/urogenital sinus and prepuce [17]. Accordingly, prenatal inhibition of estrogen synthesis via administration of Letrozole as an aromatase inhibitor leads to malformations of the glans as well as the prepuce or hypospadias. The effects of prenatal androgens, anti-androgens and impaired estrogen synthesis correlated with the tissue expression of androgen and estrogen receptors [17, 18]. These data demonstrated that estrogen plays an active role in prenatal penile development as androgen does. Up to date, the relevance of estrogen receptors in the development of urethra is not clearly understood [5–8, 17, 18]. There is a hormonal balance between androgen and estrogen hormones during the development of external genitalia [1–5].

Hypospadias is a spectrum of disease and the ultimate cause is probably a defect in the androgen stimulation of the developing penis. But the increased risk of hypospadias in the sons of women exposed to di-ethyl-stilbestrol and higher incidence of hypospadias in the last decade have raised the questions about the effects of estrogen in the etiology of hypospadias [10, 11]. Expression of estrogen and androgen receptors in human fetal penile tissue were demonstrated in some studies previously [1–5, 7, 8, 10, 11, 18, 19]. The estrogenic effects on target tissues are mediated mainly by two types of estrogen receptors; classical estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) [20, 21].

The presence of androgen and estrogen receptors in the bladder trigon of boys have been demonstrated previously by immunohistochemistry and the possible role/effects of androgen and estrogen hormones in children with lower urinary tract dysfunction were implemented [9]. In recent study, biopsy samples were taken circumcision materials from intact prepuce in children, and progesterone receptors in addition to rule out the distribution of three hormone receptors in penile tissues of patients without hypospadias were also investigated; an equal distribution in both androgen and estrogen receptors in the prepuce were demonstrated, however progesterone receptor was not present [22]. In regard to hypospadias patients regardless of the degree of hypospadias, positive results of the androgen and estrogen receptors were present but progesterone receptors were found negative in all tissues. It is a fact that in children with hypospadias, all specimens from prepuce and neighborhood of urethral plate and inner layer of prepuce had either only estrogen or both estrogen and androgen receptor expression as in our study. No expression of only androgen receptor in any sampled penile tissue was found. Additionally, ER and AR receptors did not have a special side of expression among the prepuce, glans and urethral plate. According of our results, the dominant expression of estrogen receptors (87.8%) can assume when compared to androgen (36.4%) and progesterone receptors (0%) in penile tissues of children with hypospadias; these results may be demonstrated the postnatal finding of disrupted

estrogen and androgen receptor interaction during the intrauterine development of external genitalia. However, it's hard to speculate that the reasons of negative progesterone results as the progesterone is a hormone for pregnancy, the role could be pregnancy related.

This study is a preliminary report consisted of a limited number of patients with distal and middle penile type of hypospadias. This study took our knowledge in regard to the expression and role of sex hormone receptors to a further point as this study have a larger number of patients with different degree of hypospadias control group. However, the weak side of this study is absent of a real control group and the estrogen-beta hormone receptor expression in regard to functionality. The clue for the question "hypospadias development is related to the absence of the androgenic receptor, and/or to the imbalance of the estrogenic/androgenic receptor; or receptors are not present because there is no tissue" is partly answered in our recent published corresponding study looking to AR and ER receptors in circumcised patients, where were demonstrated that both AR and ER receptors had been present in all intact prepuce tissues [16]. Effects of the ER during the intrauterine development of normal urogenital tract were known [17, 18]. As this present study emphasized the dominant expression of estrogen receptors in penile tissues of children with hypospadias, the theory of disrupted androgen and estrogen receptors interaction and/or balance could be play a role during the development of external genitalia in patients with hypospadias. Some questions as following are still waiting for answers: If a balance between ER and AR receptors in the development of penile tissues is present, should topical sex hormones especially testosterone before the hypospadias surgery used routine or should it be combined with topical estrogen in a certain percentage? Further studies "if exists" in regard to the possible inhibitory effect of estrogen in penile tissues during the intrauterine and/or postnatal development in hypospadias will help to solve the clue in regard to the relation of sex hormone distribution and these hormonal functions in the urogenital tract. To investigate and demonstrate the exact mechanism of this hormone receptors function/dysfunction leading to certain pathology were still need.

In conclusions; this study showed that "progesterone receptor was not present in penile tissues and prepuce, and therefore progesterone has not an active role in the postnatal period, for the urethral plate and/or hypospadias development were assumed that". There was not a manifest correlation of androgen receptor absence in regard to severity of the hypospadias; and there was a marked estrogen receptors presence in penile tissues.

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