

CLINICAL DILEMMAS AND SURGICAL TREATMENT OF PENOSCROTAL, SCROTAL AND PERINEAL HYPOSPADIAS

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ABSTRACT

Hypospadias is the most common congenital malformation of the urinary tract. It is a malformation with the opening of the urethra proximally from the usual site. The meatal opening can be anywhere along the shaft of the penis, or in more severe forms, within the scrotum, or in the perineum. Consequently the hypospadias can be distal, medial and proximal. The proximal ones can be penoscrotal (PS), scrotal (SC) and perineal (PE). The cause of hypospadias is largely unknown; however, current epidemiology and laboratory studies have shed new light into the etiology of hypospadias. With recent advancements in molecular biology, microarray technology, it appears that hypospadias is potentially related to disrupted gene expression. Currently, the only available treatment is surgery. The aim of this study was to present our results of the surgical correction of hypospadias and methods used to answer the clinical dilemmas about the gender. Authors have used two methods for a surgical resolution of the hypospadias – one-step operation suggested by Snodgrass and two-step operation, employing free graft suggested by Bracka. Clinical dilemmas regarding the gender were answered using cytogenetic assessment through lymphocyte cultivation method, suggested by Seabright. The cytogenetic assessment was carried in 23 patients with proximal hypospadias (penoscrotal, scrotal and perineal). Characteristic male karyotype (46, XY) was found in 22 patients. In one patient, with scrotal hypospadias, we found the characteristic female karyotype. This patient had testicles. The patient with female karyotype had a TDG gene that determines the differentiation of the testicles. Although surgery remains the only therapy for the treatment of the hypospadias, better understanding of the molecular and hormonal mechanisms behind the diseases may contribute to the prevention and the decrease in the incidence of the malformation. Cytogenetic testing in patients with unclear gender is important in planning further treatment.

KEY WORDS: proximal hypospadias, gender chromosomes.

INTRODUCTION

The frequency of hypospadias is 1:200-300 in boys and the disease is very rare in girls (1,2,3). It is a congenital abnormality in the development of urethra. This abnormality can be manifested in the different development phases, starting from the less severe one, with the opening of the urethra at the level of the head of the penis (glans), through the one with the opening on the body of the penis (penile), to the most severe ones, with the opening of the urethra at the penoscrotal border, scrotum and perineum. These more severe forms are complex. They are associated with other abnormalities of the sexual organ, making a determination of the gender very difficult (Figure 1.- a, b, c). The abnormality has familiar predisposition. This predisposition is based on a polygenetic multifactorial mechanism. In some occasions it is possible to find abnormalities at the sexual chromosomes (Figure 2.- a, b). (4,5). But it has to do with an interaction with the environment as

well (6,7). Research with genes responsible for hypospadias have enlighten the role of genetic factors in the development of hypospadias (8-14). A working hypothesis is that hypospadias is caused by a genetic susceptibility along with maternal exposure to endocrine disruptors in the first trimester of pregnancy. Specifically, some of the environmental agents are acting as antiandrogens and directly interfering with the action of testosterone. Morphologically, hypospadias is a result of double locked development of urethra – the regression in the closure of the channel, responsible for the hypospadiac meatus, and regression in the longitudinal development of urethra, responsible for the aplasia of the entire ventral surface of the penis. Hypospadias of the PS, SC and PE type, especially of the last two types, is associated with the dilemmas about the gender of the patient. Therefore, patients with these types of hypospadias should be subjected to the cytogenetic (6) testing for the determination of the gender, prior to the surgery. The aim of the study is to asses the suitability of the

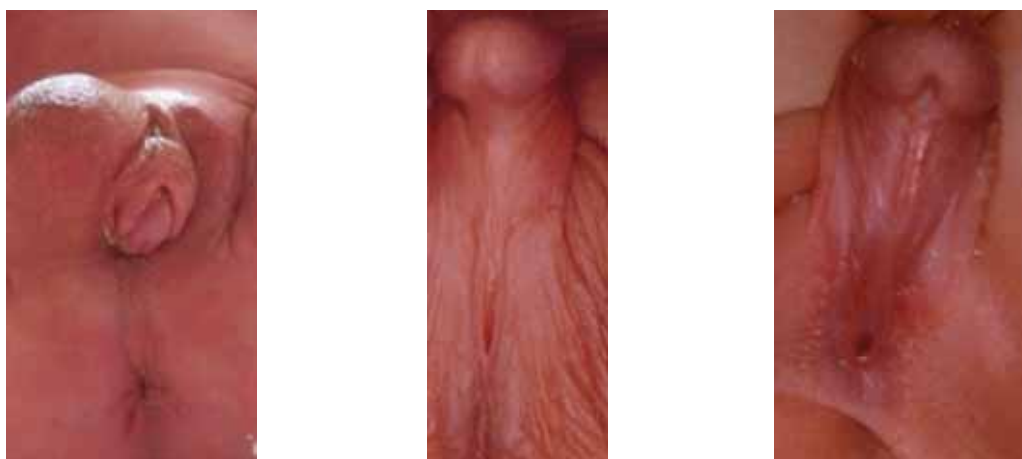


FIGURE 1. Types of hypospadias

a. penoscrotal hypospadias

b. scrotal hypospadias

c. perineal hypospadias

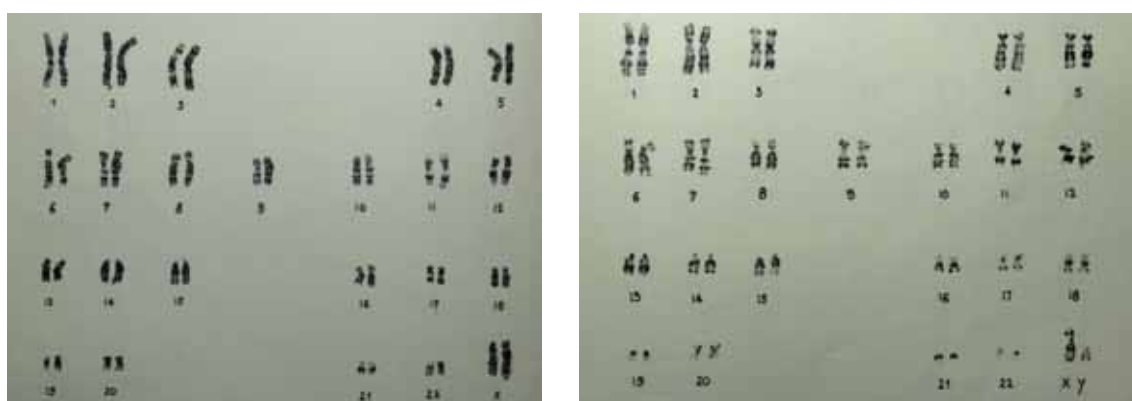


FIGURE 2. Abnormalities at chromosomes

a. one patient with karyotype 46 xx and male phenotype

b. 22 patients with karyotype 46 xy

techniques that we use for surgical treatment of the hypospadias PS, CR and PE, as well as cytogenetic assessment for the determination of gender in these patients.

MATERIAL AND METHODS

We employed two surgical methods to treat 22 cases with PS, SC and PE hypospadias. Employed techniques were one-step technique, suggested by Snodgrass (9,15) and free transplant technique, suggested by Bracca, using a dorsal prepuce or buccal mucosa (15, 16). Patients treated employing one-step technique underwent procedure suggested by Snodgrass. Incision of the urethral plate was possible even when the plate has been mobilized off the penis without dividing it into independent strips. Tubularization was done in 2 layers, the 1st using 6.0 PDS interrupted subepithelial sutures while the 2nd as a running stitch of 6.0 PDS. Spongioplasty brought the laterally displaced strips of corpus spongiosum together over the neourethra, which was then additionally covered with a dorsal dartos flap. With patients treated employing two-steps technique, we used free graft placing dorsal prepuce at the ventral surface of the penis. Genital skin (dorsal prepuce) and buccal mucosa have been used as a source for grafts. The ventral suture line approximated tissues with an inherent blood supply while the dorsal graft provided epithelial coverage for the gap created by midline strip incision. After waiting 6 months the graft becomes palpably supple, the second operation is scheduled. In this procedure the buccal or skin tissues are tubularized and the penis reconstructed. A "U" shaped incision then extends from the proposed neomeatus proximally around the cutaneous urethrostomy, taking care to minimize incorporation of hair-bearing skin into the urethra. The neourethra was tubularized in 2 layers using absorbable subepithelial suture to invert all epithelium. Derivation of urine was done using transurethral silicone Foley catheter. Following surgery patients were on broad spectrum antibiotics for 7 days. Prior to the surgery patients were subjected to cytogenetic testing for the determination of the gender. Cytogenetic testing was performed on the chromosomes derived from the cultivated peripheral blood lymphocytes as suggested by Moorhead. To achieve better determination of the chromosomes we used the standard bands methods suggested by Seabright (17, 18). Cytogenetic assessment has been done on chromosomes derived from blood lymphocytes cultivated in Eagle Minimum Essential Medium, employing Moorhead's technique. 20% of human serum of two donors with "o" blood

group and phytohemagglutinin were added to the Medium. After 68 hours 0.3mL of 0.04% colchicine were added to stop the culture. Then 0.075M KCL hypotonic solution was added. The hypotonic effect was disrupted by addition of the mixture of methanol and glacial acetic acid in 3:1 ratio. By washing the culture of lymphocytes several times with a fixating substance the suspension of the cells was derived. To exactly identify chromosomes the standard G-bands techniques suggested by Seabright. According to this technique prepared chromosomes are incubated in the weakened solution (0.25%). Chromosomes are then colored with 20% solution of Giemsa in M/17 buffer phosphate having pH 6.8. Analysis and photographing of the metaphases was done using immersion objective of the "Polyvar" microscope and 1600 times enlargement. For each person 50 metaphases were analyzed. Metaphases with best chromosomes were photographed and from these photos karyograms were presented.

RESULTS

At the Pediatric Surgery Clinic of the Prishtina University Hospital, in period 1999-2007 we treated 352 patients with hypospadias of all types. Of this number in 23 patients, 18 with penoscrotal hypospadias, 4 with scrotal and 1 with perineal hypospadias we had difficulties in clinical determination of gender. Cytogenetic assessment that we performed to do so has found characteristic male chromosome (46, XY) in 22 patients. Only one patient had characteristic female sex chromosome (46, XX). Cytogenetic assessment found no alterations in the structure of the autosomal and gender chromosomes. All patients with male chromosomes (46, XY) underwent surgery. 17 patients underwent surgical treatment using two-steps technique and 5 patients using one-step technique. The patient with female chromosomes (46, XX) refused proposed surgery (vaginoplasty). In group treated using two steps technique only one urogenital fistula developed. In the other group, treated using one step technique suggested by Snodgrass two urogenital fistula developed.

DISCUSSION

Anatomical anomalies in hypospadias are an abnormal ventral opening of the urethral meatus, abnormal ventral curvature of the penis and abnormal distribution of the foreskin around the glans with a ventrally deficient hooded foreskin. The prevalence of hypospadias varies widely between countries and populations, ranging

from 0,37 to 41 per 10 000 infants (19); the prevalence in Japan, e.g. is 3,9 per 10 000 infants (20). In 1999, the International Clearinghouse for Birth Defects Monitoring Systems, a nongovernmental organization of the World Health Organization, reported an increase rate of hypospadias in seven European countries, including Norway, Sweden, England and Wales, Hungary, Denmark, Italy, and France in the years 1960, 70's, and 80's (21). Independently, the Centers for Disease Control and Prevention (CDC) report their findings of a doubling of hypospadias from 1968 to 1993 in the United States (21). The data was based on collective analysis from two independent surveillance systems. Specifically, data from the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based registry that uses active case studies in 22 hospitals and clinics in the Atlanta, Georgia area, indicates that the rate of total hypospadias almost doubled from 1968 to 1993 at an annual rate of 2,9%. Concurrently, the Birth Defects Monitoring Program (BDMP), a program that gathered discharge diagnoses of newborn across the country, also reports an increase in hypospadias; 20,2 per 10 000 live births in 1970 to 39,7 per 10 000 live births in 1993. Overall, these longitudinal studies suggest an almost doubling of hypospadias in the United States over a 14-year period. Tubularized incised plate repair has been the mainstay for distal hypospadias. In cases of proximal hypospadias, one-stage repairs such as the Duckett repair or the Koyanagi repair have been well established, while two-stage repairs remain important alternatives. Whether dorsal plication or ventral lengthening should be used to correct penile curvature is still controversial, and long-term results are required. Efforts have been made in this decade to improve cosmetic appearance, constructing a slit-like meatus or performing foreskin reconstruction, and to prevent onerous complications. Corrective surgery is done preferably between the ages of 6 and 12 months. The aim of surgery is to obtain a functionally and cosmetically normal penis while limiting the psychological burden on the child as much as possible. In general, two-steps technique is preferable (6,7,22). There are authors however, that prefer one step technique and are reporting very good results (23-27). Fistula is one of the complications in each method. But, having in mind the severity of malformation that we dealt with, we think that these three fistulas (13,6%) represent satisfactory performance comparing with other authors (28, 29). Many studies carried recently have found existence of gene TDF, responsible for the differentiation of the testicles. The second gene subjected to the studies is gene Hy found at the proximal part of the chromo-

some Y considered responsible for the spermatogenesis. At the short arm of the chromosomes X and Y there are homologous sites that approach each other during the zygonema of the myosis. This means that the genetic material is exchanged between X and Y chromosomes during the cross-over process. Since TDF gene, localized at the short arm of the chromosome Y, is close to the homologue place of the X chromosome, it is possible, that in some occasions, moves from one to other chromosome (Y to X). This way chromosome X become carrier of the TDF gene responsible for the differentiation of the male gender. Nowadays, thanks to the specific cytogenetic tests, it is found that at the short arm of the chromosome X, inherited from father, is one sequence of the DNA of the chromosome Y. This sequence may be of different length, but always hold in TDF "locus". Almost 80% of XX males assessed up to now have this sequence at one of the X chromosomes, as a consequence of incorrect crossover between short arms of the X and Y chromosomes during the meiosis in father. We think that this was the case with one patient with 46, XX karyotype in our study. In the past, environmental effects were generally ruled out as causes for hypospadias. However, in light of the growing number of endocrine disruptors reported in human tissue and the probability of shared exposure, environmental contaminants are now being evaluated in familial clusters studies. Reports of increase rates of hypospadias have paralleled reports of other adverse effects observed in male reproductive health, include increased rates of testicular cancer, cryptorchidism, and decreasing semen and sperm quality (30,31). Cheng et al. (32) found that 8% of patients (n = 252) with undescended testes also had other urogenital anomalies including hypospadias. A separate study reports an increase in testicular cancer risk with undescended testicles and hypospadias (33). There are several synthetic chemicals that have been consistently shown to induce hypospadias in laboratory animals. Vinclozolin, a commonly used fungicide, induces female-like AGD (anal genital distance), retained nipples, reduced sperm count and fertility, cleft phallus, and hypospadias in 100% of male offspring that were exposed to the chemical in utero (34,35). Procymidone, another antiandrogenic fungicide, induces hypospadias in all male rat offspring when mothers are exposed during gestation (36). It has been shown that procymidone exerts its effects by inhibiting DHT-induced transcriptional activities (37). Mice exposed to vinclozolin have less robust and atrophic urethras when compare to controls (38,39). Phthalates, ubiquitous chemicals found in plastics, have gained attention because of their hormon-

al effects in laboratory animals. Male rodents exposed to dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) have reduced AGD, retained nipples, epididymal agenesis, undescended testes, and hypospadias (40). As mentioned previously, DES provides an excellent model for studying interrupted genital development due to exogenous hormone in humans. Longitudinal studies have shown that DES daughters have a 2,5 fold increase in breast cancer after age 40, in addition to an increased risk for vaginal and cervical cancers (41). Sons of DES exposed mothers are at an increased risk for feminization of the male fetus; a study noted a 20-fold increase in the development of hypospadias (42). A separate study confirmed this risk: 10 out of 225 DES-exposed male offspring developed penile malformations, include strictures/ stenosis and hypospadias, versus zero in patients who were unexposed (43). The exact molecular events that lead to normal urethral development and hypospadias remain largely unknown; however, recent studies have revealed possible involvement of sonic hedgehog (SHH), fibroblast growth factors 8 and 10 (FGF8 and FGF10), and homeobox A13 and D13 (HOXA13 and HOXD13) genes in early genital tubercle outgrowth and patterning (44). As discussed earlier, antiandrogen might plays a role in the development of hypospadias. It has been shown that pregnant female mice exposed to synthetic estrogen compounds

gave birth to offspring with hypospadias (38, 46, 47). Microarray analysis of hypospadias tissue samples have identified additional genes that are both estrogen responsive and upregulated (48). One of such gene that was found in these human hypospadias tissues/foreskin, which also demonstrated significant up regulation in the presence of estrogen, is activating transcription factor-3 (ATF-3). A recently published study utilizing human foreskin fibroblast treated with estrogen showed an increase in staining for ATF-3 within two hours of treatment. This was further substantiated by an increase in protein expression, and ATF-3 promoter activity (48). Similar results were also noted in mouse genital tubercle, where quantitative RT-PCR showed that ATF-3 mRNA is up regulated in estrogen exposed specimen when compare to control. A study of human tissues from 28 children who underwent hypospadias repair versus 20 tissue samples from children who underwent elective circumcision, demonstrated a significant increase in immunohistochemical staining for ATF-3 in the hypospadias population (86% versus 13%) (48,49). Studies have shown that ATF-3 protein suppresses cellular growth, we can hypothesize that its up regulation induces an arrest in urethral development as a potential cause of hypospadias. Further studies of ATF-3 and other estrogen related genes would perhaps provide a link between exogenous hormones and the development of hypospadias.

CONCLUSION

The techniques of hypospadias surgery continue to evolve. The current standard of care for hypospadias repair includes not only a functional penis adequate for sexual intercourse and urethral reconstruction offering the ability to stand to urinate, but also a satisfactory cosmetic result. We have found two steps technique advantageous over the one-step one. Although surgery remains the only therapy for the treatment of the hypospadias, better understanding of the molecular and hormonal mechanisms behind the diseases may contribute to the prevention and the decrease in the incidence of the malformation. Cytogenetic testing in patients with unclear gender is important in planning further treatment.

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