Bahrain Medical Bulletin, Vol.28, No.3, September 2006

The Morphology of the Testicular Tissue of Unilateral Inguinal Cryptochid Testes in Children : Electron Microscopic Study

Ali Abalkhail, DCP, FRCPath* Ibrahim Bani Hani, MD,FRCS**
Fakhri Al-Baghdadi, PhD*** Younis Abu-Ghalyun BSc****

Objective: The aim of this study is to review the ultrastructural changes of the inguinal cryptochid testes of children at different ages.

Method: Children between one and sixteen years old with inguinal cryptochid testes, referred for surgical correction were biopsied. The testicular tissue obtained was processed for electron microscopical examination.

Setting: Princess Basmah Teaching Hospital in Irbid-Jordan.

Design: Prospective study.

Result: The process of cellular differentiation in the seminiferous tubules was slow or embryologically arrested. There were progressive degenerative changes and increased thickening of the basement membrane as the duration of testicular retention in the inguinal region increased.

Conclusion: The ultra-structural changes of the inguinal cryptochid testes were advanced time wise. Further studies to define the time needed for irreversible changes to take place to help in better timing of the surgical correction without losing fertility.

Bahrain Med Bull 2006; 28(3):

---

* Department of Pathology
Arabian Gulf University
College of Medicine and Medical Sciences
Kingdom of Bahrain.

** Department of Surgery
Faculty of Medicine
Jordan University of Science and Technology
Irbid – Jordan.

*** Department of Comparative Biomedical sciences
Louisiana State University
Baton Rouge LA, USA

**** Department of Biology
Yarmouk University, Irbid – Jordan

Cryptorchidism or failure of normal testicular descent is most frequently seen in horses, swine and man[1,2]. It is associated with greatly reduced or absent spermatogenesis in humans and in experimental animals[3,4]. Cryptorchidism causes changes in the interstitial cells and in the cellular elements of the testicular seminiferous tubules of the testis of the pig, rat, goat, horse and man[2,5-9].

Ten percent of testicular cancer in humans are associated with cryptorchidism[10,11]. Most of the reported observations on the morphological changes in cryptochid testis did not differentiate between inguinal and abdominal location of the undescened testis. Few reports have demonstrated some differences in the histological and ultra structural changes between inguinal and abdominal cryptochid testis. The changes were more severe in the abdominal cryptochid testis[12-14].

The purpose of this study is to evaluate and report the changes that take place in inguinal cryptochid testis in relation to age. This might influence the timing of the surgical repair to avoid loss of fertility.

METHOD

The study included seven children, between the ages of 1 and 16 years, who were diagnosed as unilateral cryptochid testis and biopsied during surgical correction. Testicular biopsies for electron microscopy were obtained from each of cryptochid testis. Tissue was placed in a labeled vial containing 2% of cold glutaraldehyde in sodium cacodylate buffer at pH 7.4. Each biopsy was minced in a fixative and kept for 90 minutes. The specimen was then washed in the same buffer and post fixed in 1% osmium tetroxide for 60 minutes. After processing one micron thick sections were stained with methylene blue for orientation. Silver sections were then cut and stained with uranyl acetate and lead citrate. The sections were examined by Zeiss – 10 electron microscope and photographed by black and white Kodak plate film.

RESULT

Sections of testicular tissue taken from children aged 1, 3, and 5 years showed seminiferous tubules containing mainly undifferentiated cells, having irregular nuclei with thinly marginated hetrochromatin. The cytoplasm had abundant mitochondria and scattered ribosomes. The spermatogonia were large having few perinuclear mitochondria. The sertoli cells were primitive with irregular nuclei and distinct nucleoli. The tubular basement membrane had normal even thickness. The peritubular tissue contained mainly collagen with scattered fibroblasts and several myoid cells (Fig. 1).

Testicular tissue taken from children aged 7, 10, and 13 years showed tubules containing differentiated epithelial cells with more subcellular organelles. The nuclei were of variable shapes and some were irregular. The chromatin was marginated at the rim of the nuclear membrane with few scattered clumps. The cytoplasm of the epithelial cells contained large number of vacuoles most of which contained electron dense granular material. Similar vacuoles were also noted in the peritubular connective tissue. The basement membranes were undulated and moderately increased in thickness. Moderate numbers of mast cells in the peritubular tissue were noted. Several dilated blood vessels engorged with red cells were also seen in the vicinity (Fig. 2).

Testicular biopsies taken from 16 year old child showed similar findings to that seen in the second group with more vacuoles in the cytoplasm of the epithelial cells and marked uneven thickening of the basement membrane. Large numbers of mast cells at different stages of maturation were seen in the peritubular tissue (Fig. 3).

DISCUSSION

The sertoli cells of the seminiferous tubules of the inguinal cryptochid testis did not show morphological differentiation at the age of 5 years. This indicates that the process of cellular differentiation of the seminiferous tubules is slow or embryologically arrested in the inguinal cryptochid testis. This confirms the findings previously reported that inguinal cryptochid testes are under partial embryological arrest[12]. Another feature noticed is enlargement of the spermatogonia which was observed before though without mention as to whether the cryptochid testis was inguinal or abdominal[15]. Cytoplasmic vacuoles in the epithelial cells of the seminiferous tubules and peritubular tissue progress with age. Similar vacuoles were described in testicular biopsies from men with varicocele and from testes of rats that had been prenatally irradiated[16-18].

The normal basement membrane of the seminiferous tubules has a regular profile and even thickness[19]. The basement membrane had its highest thickness in cryptochid testes at puberty[20]. The increased production of basement membrane material and its invagination into tubules might lead to the formation sertoli cell nodules containing basement membrane material[21]. In this study, the basement membrane of the seminiferous tubules showed progressive increase in thickness and undulation as the age of the child increased. The most severe was at the age of 16 years (the oldest in this study). Thickened basement membrane of the seminiferous tubules had been reported in men with infertility and in cryptochid testis[22].

Mast cells are normally present in the connective tissue of the human testis. An increase in their number was reported in testes of patients with idiopathic infertility[23]. In this study, the number of mast cells increased in the 13 and 16 years old children. Mast cells have been reported to increase in number as a response to allergic inflammation[24]. Other workers also noted dilated and engorged blood vessels in addition to mastocytosis in inguinal cryptochid testes[25]. The presence of both features in this study suggests that an inflammatory process might contribute to the changes noted in cryptochid testes. This study demonstrated that the ultra structural pathological changes of inguinal cryptochid testes progress with age.

The morphological changes in this study are less extensive than those of abdominal cryptochid testes studied in experimental animals with comparable periods of testicular retention in inguinal or intra abdominal sites[13]. This might indicate that timing of surgical repair of inguinal cryptochid testes is not as critical as in abdominal undescended testes.

Further experimental studies designed to find out the time needed for irreversible changes in the seminiferous tubules in both inguinal and abdominal cryptochid testes are suggested. This might be a good guide to time the surgical repair without loss of fertility in cryptochid testes.

CONCLUSION

The ultra-structural changes in the testicular tissue of inguinal cryptochid testes increase progressively with time. Irreversible changes start after the age of five years. The changes are more severe in the abdominal cryptochid testes retained for the same time. Further study is needed to define the time length for the irreversible changes in both types of cryotochid testes and to time surgical correction.

REFERENCES

1. Marshall F. Anomalies associated with cryptorchidism. *Urol. Clin. North Am* 1982*;* 9, 339-47.
2. Pinart E, sancho S, Briz MD,et al. Ultrastructural study of the boar seminiferous epithelium:changes in cryptorchidism. Morphol 2000;244(3):190-202.
3 Mieusset R, Bujan L, Massat G, et al. Clinical and biological characteristics of infertile men with a history of cryptorchidism. *Hum. Reprod* 1995; 10: 613-9.
4. Van Staaten H, Wensing C. Histomorphological aspects of testicular morphogenesis in the naturally unilateral cryptorchid pig. *Biol. Reprod* 1977; 17: 473-9.
5. Kerr J, Rich K, Des Kretser D. Alteration of the fine structure and androgen secretion of the interstitial cells in the experimentally cryptorchid rat testis. *Biol. Reprod* 1979; 20: 409-22.
6. Risbridger G, Kerr J, Des kretser D. Evaluation of Leydig cell function and gonadotropin binding in unilateral Leydig cell function by seminiferous tubule. *Biol. Reprod* 1981; 24: 534-40.
7. Minninburg D, Rodger J, Bedford J. Ultra structural evidence of the onset of testicular pathological conditions in the cryptorchid human testis within the first years of life. *J. Urol* 1983 ; 128: 87-9.
8. Jegou B, Risbridger G, DeKretser D. Effects of experimental cryptorchidism on testicular function in adult rats. *J. Androl* 1983; 4: 88-9.
9. Dzeasor F. Light and electron microscopical observations on the Leydig cells of the scrotal and abdominal testes of rat. *J. Anat* 1987; 141: 27-40.
10. Brender H., Cryptorchidism and cancer progress in Clinical and biological Research. New York,Alan R, Liss 1985; 189-96.

11. Ellsworth PI, Ebb RG. The cryptochid testis.J Med liban.2004;52(4)227-33.
12. Al-Bagdadi F, Hoyt P, Karns P, et al. Morphological differences between abdominal and inguinal testes in stallions. *Proc 45th Annu. Meet. Electron Soc. Am.* 1987; 820-2.
13. Al-Bagdadi F, Hoyt P, Karns P, et al. The morphology of abdominal and inguinal cryptorchid testes in stallions: a light and electron microscopic study. *Int. J. Fertil.* 1991; 36:57-64.
14. Rajber J. Congenital Anomalies of the testes. Walsh P, Gittes R, Perlmitter A. et al .eds. *Campbell's Urology*.3rd edition. Philadelphia: WB Saunders Co, 1985: 1947-68.
15. Codesal J, Paniagua R, Queizan A, et al. Cytomorphometric DNA quantification in human spermatogonia of cryptorchid testes. *J. Urol.* 1992;382-5.
16. Cameron D, syndle F, Ross M, et al. Ultra structural alterations in the testes in men with varicocele. Fertil.steril 1980; 33:526-33.
17. Terguem H, Dadoune J. Morphplogical findings in varicocele. Int. J. Androl 1981; 4: 515-31.
18. Hatier R, Gringnon G, Touati F, et al.Ultrastructural study of seminefrous tubules in the rat after prenatal irradiation. Anat.Embryol. 1982; 165:425-35.
19. Holstein A.F, Mackawa M, Nagano T, et al. Myofibroblasts in the lamina propria of human seminiferous tubules are dynamic structures of heterogeneous phenotype. *Arch. Histol. Cytol.* 1996;59:109-25.
20. Cinti S, Barbatelli G, Pierleoni C, et al. The normal cryptorchid and retractile prepuberal human testis: A comparative morphometric ultra structural study of 101 cases. *Scan. Micro*. 1993;71:351-62.
21. Govender D , Sing Y , Chetty R. Sertoli cell nodules in the undescended testis:a histochemical,immunohistochemical,and ultrastructural study of hyaline deposits..J Clin Pathol.2004; 57(8):802-6.
22. Salomon F, Hedinger C. Abnormal basement structures of seminiferous tubules in infertile men. *Lab. I nves*.1982; 47(6): 543-54.
23. Maseki Y, Miyak K, Mitsuya H, et al. Mastocytosis occurring in the testes from patients with idiopathic male infertility. *Fert. Steril.* 198;36:814-7.
24. Nistal M, Paniagua R, Queizan A. Histologic lesions in undescended ectopic obstructed testes. *Fertil. Steril*. 1985; 43: 455-62 .
25. Abalkhail A, Bani Hani I, Al-Bagdadi F, et al. Observation of the ultra structure of mast cells in inguinal cryptorchid testes of thirteen-year old children. Micosc Microanal 2003; 9(suppl 2) :1396-8.

FIGURES

Figure 1

Part of seminiferous tubule containing a distinct spermatogonium (S) with an apical group of rounded mitochondria (M) and clear cytoplasm. Undifferentiated epithelial cells (UE), normal basement membrane (arrow) and peritubular collagenous connective tissue between the myoid cells (MC). Stained with uranyl acetate and lead citrate X 3180.(one- year- old case)

Figure 2

Part of seminiferous tubule (ST) and a wavy basement membrane (arrow). The peritubular connective tissue is mainly collagen fibers and a myoid peritubular cell (MC). Cross section of three dilated blood vessels (BV) filled with red blood cells RBC. Stained with uranyl acetate and lead citrate X 2650.(thirteen- year- old case)

Figure 3

Part of seminiferous tubule containing an undifferentiated epithelial cell (UE), with a nucleus resembling that of a Sertoli cell (N). Abundant cytoplasmic vacuoles (V), most of them contain granular material. The basement membrane (arrow) is thick bordered by peritubular collagenous connective tissue and a myoid cell (MC). Stained with uranyl acetate and lead citrate X 6860. (sixteen-year-old case) .