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Clinical Significance of Histomorphological Changes in Prepubertal and Postpubertal Cryptorchid Testis

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Abstract

Introduction: *Cryptorchidism i.e. undescended testis (UDT) is the most common disorder of male endocrine glands in children. At birth, approximately 4.5 % of the boys have undescended testis.*
The exact cause is poorly understood. Congenital, hereditary or hormonal imbalance appears to be related to gonadal maldevelopment. Despite successful relocation of the testis, long term sequels of cryptorchidism may occur, including infertility, sub-fertility, injury,, and malignancy.

Purpose/Aim: *To study gross and histopathological changes in prepubertal and postpubertal cryptorchid testes and correlate with clinical presentation and outcome.*

Study Design: *A total of 75 testicular specimens were evaluated in the current study. Participants were categorized into prepubertal and postpubertal groups. The patient's clinical and surgical details were retrieved. Histopathological diagnosis, gross and microscopic features of testicular and paratesticular tissues were recorded. The diagnosis of testicular regression syndrome was made according to Susan E. S. et al.*

Results: *The median age at presentation is 2 years in prepubertal and 29 years in postpubertal subjects. Secondary testicular parenchymal changes comprising thick capsule, atrophic testicular architecture, hyalinised basement membrane, abundant extracellular matrix, interstitial calcification, inflammation, edema, and fibrosis are more common in postpubertal subjects compared to prepubertal group (excluding TRS) and are statistically significant.*

Conclusion: *In >60% of the cases, the diagnosis of the undescended testis is made on clinical grounds.Mean tubular diameter (MTD) and tubal fertility index (TFI) decreases with the duration of the undescended testis and are independent predictors of future fertility, the severity of architectural change and abnormal spermatogenesis.*

Keywords: *Testis, cryptorchidism, undescended testis, atrophic testis, prepubertal testis, postpubertal testis.*

Introduction

Cryptorchidism i.e. undescended testis (UDT) is the most common disorder of male endocrine glands in children. At birth, approximately 4.5 % of the boys have undescended testis. Because complete testicular descent occurs late in gestation, 30% of

premature male infants have undescended testes, whereas the incidence is 3.4% at term. It is noted that the majority of undescended testes descend spontaneously during the first 3 months of life, hence the percentage falls to 1% and by 6 months the incidence decreases to 0.8 %. If the testes have not descended by 4 months, it will remain undescended. Cryptorchidism is bilateral in 10% of cases.[1]

The exact cause is poorly understood. Congenital, hereditary or hormonal imbalance appears to be related to gonadal maldevelopment. An increasing number of older boys are being diagnosed with UDT. Typically, these boys have a scrotal testis that "ascends" to a low inguinal position (retractile testis) and may require surgical intervention. A few have secondary cryptorchidism after an inguinal hernia.[2]

The main reasons advocated for treatment of UDT are due to increased risk of developing infertility, testicular malignancy, torsion, injury against the pubic bone, and the possible psychological stigma of an empty scrotum. Despite successful relocation of the testis, long term sequels of cryptorchidism may occur, including infertility, sub-fertility, injury, and malignancy. Despite the widespread frequency of UDT, there is a persistent lack of knowledge about many aspects of it'setiology, natural history, treatment, and outcomes.

Aim & Objective

- To study gross and histopathological changes in prepubertal and postpubertal cryptorchid testes and correlate with clinical presentation and outcome.

Research Design & Participant

A retrospective and prospective study of cryptorchid testes were done in the Department of Pathology, Kasturba Medical College (KMC), Manipal. All cryptorchid testes operated during a period of 7 years from 2007 to 2013 were selected. UDT was unilateral in 63 patients (right side in 32 & left side in 31) and bilateral in 8. Out of 16 testicular specimens from bilateral UDT, only 12 were available for study, as unilateral orchidopexy was performed in 4 patients. A total of 75 testicular specimens were evaluated in the current study.

Participants were categorized into prepubertal and postpubertal groups. Prepubertal subjects were defined as individuals with age <14 years and postpubertal subjects included patients age ≥ 14 years. Out of 71 patients selected in the current study, 27 patients were in the prepubertal and 44 patients were in postpubertal category.

The patient's clinical and surgical details were retrieved. Histopathological diagnosis, gross and microscopic features of testicular and paratesticular tissues were recorded. Haematoxylin & eosin stain, PAS (Periodic Acid Schiff) & Masson's trichrome special stains were used.

The diagnosis of testicular regression syndrome (TRS) was determined by the presence of the following diagnostic criteria according to Susan E. S. et al.[3]:

- A vascularized fibrous nodule with calcification and/or hemosiderin deposit or
- A minimum of vascularized fibrosis with cord element (s) in proximity.

20 - 50 cross-sectioned seminiferous tubules from each testis were quantitatively evaluated for mean tubular diameter (MTD), Sertoli cell number (SCN), and tubular fertility index (TFI –The percentage of tubules with germ cells).

Exclusion Criteria

- Inadequate/no testicular, and paratesticular tissue
- Infarction/ torsion of testicular parenchyma
- Ambiguous genitalia
- Testicular tumors

SPSS version 20 was used to analyse the results. Frequency and percentage were used to summarize values in variables. Specific data were expressed as the mean ± standard deviation (SD) to summarize continuous values and median (Quartile 1, Quartile 3) to summarize non-continuous values. Chi-Square test was used to test the association between two categorical variables. Significance was defined as 'P' value <0.05.

Results

Clinical & Gross Features:

Table 1 Clinical & surgical details of all the cases

Features		Prepubertal	Postpubertal	Total	
Age range (mean)		**1-12 yrs. (3.9)**	**15-62 yrs. (22.7)**	**1-62 (22.7)**	
Clinical diagnosis	Undescended testis	21 cases	40 cases	61 cases	75
	Atrophic testis	4 cases	8 cases	12 cases	
	Hypoplastic testis	2 cases	0	2 cases	
Histopathological diagnosis	Cryptorchidism	12 cases	34 cases	46 cases	75
	SCOS/ FHT	0	10 cases	10 cases	
	TRS	15 cases	4 cases	19 cases	
Position of testis	High scrotal	1	6	7 (9.3%)	75
	Inguinal	22	39	61 (81.3%)	
	Abdominal	4	2	6 (8.0%)	
	Ectopic	0	1	1 (1.3%)	
Laterality	Right side	9	23	32 (45.1%)	
	Left side	14	17	31 (43.7%)	
	Bilateral	4	4	8 (11.2%)	
Gross appearance	Normal	6	23	29 (38.7%)	75
	Atrophic	17	23	40 (53.3%)	
	Rudimentary	4	2	6 (8.0%)	

SCOS, Sertoli cell-only syndrome; FHT, Fully hyalinised tubules; TRS, Testicular regression syndrome

Comorbidities: In the prepubertal group, 2 cases had hernias and one case each of associated underdeveloped scrotum, phimosis, hydrocele, anorectal malformation with intestinal obstruction and persistent Mullerian duct syndrome was also documented. Among the postpubertal group, the hernia was identified in 12 cases, infertility and orchitis in 2 cases and varicocele, ectopic kidney with pelvic-ureteric obstruction, andpenoscrotal lymphedema in one case each.

Microscopic features:

Table 2 Distribution of changes in testicular parenchyma

Features		Prepubertal	Postpubertal	Total		P value (<0.05)
Testicular architecture	Normal	6(22.2%)	22(45.8%)	28	75	0.042*
	Atrophic	21(77.8%)	26(54.2%)	47		
Capsular thickness	Normal	6(50.0%)	8(18.2%)	14	56	
	Increased	6(50.0%)	36(81.8%)	42		0.024
Extracellular matrix	Normal	6(22.2%)	6(12.5%)	12	75	
	Increased	21(77.8%)	42(87.5%)	63		0.270
Seminiferous tubule basement membrane	Normal	11(91.7%)	5(11.4%)	16	56	
	Increased	1(8.3%)	39(88.6%)	40		

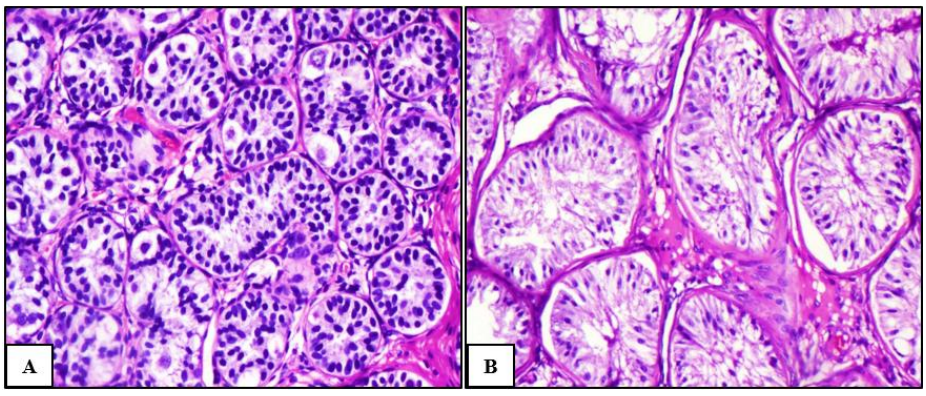


Fig. 1 (A & B) Microscopic features of testicular parenchyma in prepubertal (A) & postpubertal (B) cases.

Table 3 Distribution of Changes in testicular stroma

Features	Prepubertal	Postpubertal	All cases	Significance
Inflammation	10(37.0%)	2(4.2%)	12(16%)	
Edema	10(37.0%)	36(75.0%)	46(61.3%)	0.001
Calcification	12(44.4%)	7(14.6%)	19(25.3%)	0.004
Fibrosis	19(70.4%)	14(29.2%)	33(44.0%)	0.001
Haemorrhage	3(11.1%)	11(22.9)	14(18.6%)	

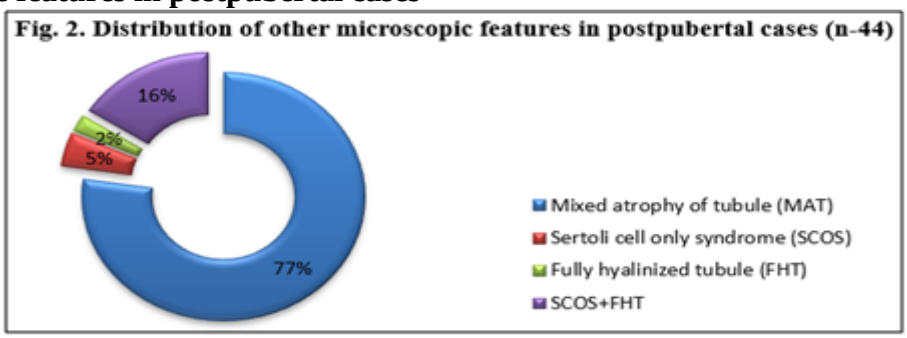
Table 4 Distribution of cellular changes

Cell type	Variables	Prepubertal	Postpubertal	Total
Leydig cell	Nodular hyperplasia	0	21(43.8%)	21(28.0%)
	Diffuse hyperplasia	3(11.1%)	6(12.5%)	9(12.0%)
	Vacuolation	0	5(10.4%)	5(6.7%)
	Intracytoplasmic pigment	0	5(10.4%)	5(6.7%)
Sertoli cell	Normal in number	13(48.1%)	22(45.8%)	35(46.7%)
	Decreased in number	4(14.8%)	21(43.8%)	25(33.3%)
	Vacuolation	0	13(27.1%)	13(17.3%)
	Eosinophilic granules (EG)	0	5(10.4%)	5(6.7%)
	Vacuolation& EG	0	2(4.2%)	2(2.7%)
Other finding	Pick's Adenoma	0	8(16.7%)	8(10.7%)
	AHRT	1(3.7%)	7(14.6%)	8(10.7%)

Changes in Paratesticular Tissue: Only 65 cases had attached paratesticular structures in the study, inflammation was noted in 7 cases, of which, only 1 case was from postpubertal and rest from the prepubertal group. Edema was present in 21 cases (8 from prepubertal and 13 from the postpubertal group) and calcification in the only 1case (prepubertal). Haemorrhage was seen in 8 (12.3%) cases (3 from prepubertal and 5 from postpubertal group) and necrosis was noted in 2 cases each from prepubertal and post pubertal groups. Necrosis was intraluminal in all 4 cases (6.1%).

Fibrosis was observed in 35(53.8%) cases of which, 18 were from prepubertal and 17 from the postpubertal group. Statistically, significant correlation was noted for fibrosis, between prepubertal and postpubertal group (p - 0.003).

Other microscopic features in postpubertal cases



MAT, Mixed atrophy of tubule;
SCOS, Sertoli cell only syndrome
FHT, Fully hyalinised tubule

TRS

Table 5: Distribution of Microscopic Features in TRS Cases (n=19)

	PREPUBERTAL	POSTPUBERTAL	ALL CASES
Calcification	11(73.3%)	3(75.0%)	14(73.7%)
Hemosiderin deposit	11(73.3%)	2(50.0%)	13(68.4%)
Fibrosis	15(100.0%)	4(100.0%)	19(100.0%)
Increased blood vessels	15(100.0%)	4(100.0%)	19(100.0%)
Fibrovascular nodule	2(13.3%)	0	2(10.5%)
Discrete vascular fibrosis	13(86.7%)	4(100.0%)	17(89.5%)

Out of 19 TRS cases, characteristic epididymis was present in 17 cases, pampiniform plexus in 12 cases, rete testis in 6, and ductus (vas) deferens or spermatic cord in 3 cases. Also, ductuli efferents were reported in 2 cases and dominant or feeding vein in only 1 case. Major combinations include epididymis with pampiniform plexus (PP) in 5 cases, rete testis (RT) with epididymis in 3 cases, and rete testis with pampiniform plexus in 2 cases. One case was reported in the remaining combination of paratesticular structures.

Mean tubular diameter (MTD) & Tubal fertility index (TFI)

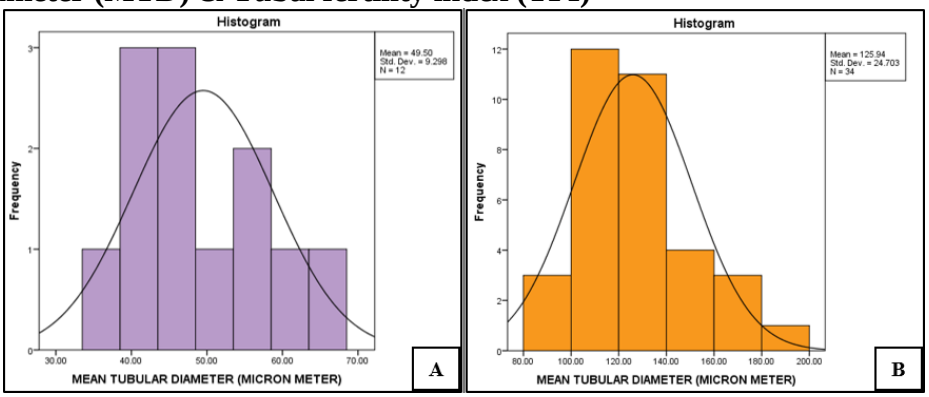


Fig. 3A & 3B. Distribution of frequencies of MTD (µm) in prepubertal and postpubertal cases

Table 6 Distribution of frequencies of tubular fertility index (TFI) among 46 cases

	Mean (%)	Median (%)(Q1,Q3)	Range (%)
Prepubertal	30.83	20(12.5,45.0)	10-90
Postpubertal	37.65	30(20.0,50.0)	10-90
Total	35.9	30(20.0,50.0)	10-90

Q1, 1st quartile25%; Q3, 3rdquartile 75%

Discussion

Cryptorchidism is the most common genital problem encountered in paediatrics affecting 0.8% of all boys at the age of 1 year. Cryptorchidism means hidden or obscure testis and generally refers to an undescended or maldescended testis. Despite more than 100 years of research, many aspects of cryptorchidism are not well defined and remain controversial. Untreated UDT has deleterious effects on the testis over time.

Age: Patients may present with absent testis at any time in life, ranging from birth to late in age. In the current study, the mean± SD and median age at presentation were 22.7±17.1 and 25 (5, 34) years respectively. Our results were in concordance with the previous study by D. Govinder et al.[4]

According to the literature, the majority of the patients in the prepubertal group come to the hospital with complaints of absent testis since birth, at the age of 2-4 years with a median age of 2 years. The late presentation may be due to a lack of knowledge and the prior trial of hormonal therapy. In the current study, mean ± SD and the median age was 3.9±3.6 and 2 years, which were comparable to data reported in previous studies.[5],[6]

Postpubertal individuals usually notice the absence of testis at later age between 27-35 years with a mean age at presentation of 33.2 (SD ±11.9) years. At this age, patients usually come with inguinal swelling or increasing inguinal pain (? due to torsion of UDT) with incidental detection of the ipsilateral empty scrotum. Also, infertility and hydrocele could be presenting complaints. Our study results for mean ± standard deviation and the median age of the postpubertal individuals at the time of presentation were incomparable to the previous studies.[7],[8]

Clinical Diagnosis and Comorbidities: In the current study, most (>60%) of the patients in both prepubertal and postpubertal groups were clinically

diagnosed as undescended testis. The rest of the cases were diagnosed as atrophic or hypoplastic testis based on a local examination, ultrasonography, or intraoperative findings.

Comorbidities like hernia, hydrocele, small scrotum, varicocele, infertility, hypospadias, etc. are frequently reported in various studies in association with undescended testis. They are more common in postpubertal subjects. Congenital genital anomalies and infantile syndromes are more frequently associated with UDT in prepubertal subjects. According to the literature, the hernial sac is present in 20% to 90% of the cases of UDT.[9]

JRHCSG (John Radcliffe Hospital Cryptorchidism Study Group) (1992) reported small scrotum as the most frequent co-morbidity associated with UDT followed by hypospadias, hydrocele, and hernia[10]. In our study, the most common co-morbidity associated with UDT was hernia followed by infertility and orchitis. In addition, the less frequent co-morbidities like a small scrotum, hydrocele, persistent Mullerian duct syndrome, phimosis, and anorectal malformation with intestinal obstruction were more common in the prepubertal group. Varicocele, ectopic kidney with pelvic-ureteric obstruction, and penoscrotal lymphedema were commonly detected in the postpubertal group.[7], [10], [11]

Laterality: C.G. Scorer[12] found a high proportion of left testis involvement among unilateral UDT at birth. In the current study, an almost equal proportion of cases presented with right and left testis involvement in unilateral UDT. There was no difference in laterality in pre and postpubertal cases. These results were in concordance with previous studies.[7]

Age at the assessment of testis has a vital role. A high incidence of UDT was reported when examined immediately after birth, whereas the incidence decreases by 3 months. This is due to the testosterone that is released in intrauterine life will also be secreted immediately after birth to enhance testicular descent[13]. According to JRHCSG, testis that does not reach scrotum by 3months remains undescended thereafter.[10]. High proportions of cases were diagnosed as B/LUDT when examined at birth and the proportion decreased by 3 months[13].

In the current study, bilateral UDT was noted in 12% of cases, which was within the range of 10-20% reported in the previous studies.[5],[6],[9]

Position/ Location of Cryptorchid Testis: Arrest of the testis can occur anywhere along its route of descent. Abnormal localization of unilateral or B/L testes above the scrotum including the inguinal canal, intra-abdominal and ectopic has been reported in the literature. In the current study, the most common location of the arrest of testis was inguinal/ supra-scrotal in 61 (81.3%) followed by high scrotal in 7 (9.3%), intra-abdominal in 6 (8.0%) and ectopic testis in 1 case (1.3%).

The trend of the arrest of testis along its route of descent was the same in both study groups (M/C being inguinal). Similar results were demonstrated by previous studies.[8],[14],[15]

Gross Appearance: In the current study, the majority (40 cases - 53.3%) demonstrated gross atrophy of the testis followed by the normal testis in 29 cases (38.7%) and rudimentary testis in 6 cases (8.0%).

In prepubertal specimens, atrophic testes (63.0%) constituted major bulk of the cases. This in due to the high proportion of TRS cases (15/27). Among the postpubertal cases, an equal proportion of normal (47.9%) and atrophic testis (47.9%) were observed. Similar results are seen in the prepubertal group without TRS cases.

Changes in Testicular Parenchyma: In the current study, a high proportion of prepubertal cases had atrophic testicular architecture, oedema calcification, and fibrosis compared to postpubertal, which was statistically significant ($P < 0.05$). This is attributed to >50% of TRS cases in the prepubertal group.

In cases without TRS, features like a thick capsule, hyalinised BM, and abundant ECM were more frequently observed in postpubertal compared to prepubertal cases, which are statistically significant.

Leydig cells and Sertoli cell changes are frequently reported in UDT. In normal individuals, Sertoli cell number per transverse ST section diminishes gradually from birth (40 ± 3) to adulthood (10 ± 1).

As puberty progresses, marked changes both in the cytoplasm and in the nucleus havebeen observed as a result of the functional maturation process. According to the literature, SCN in undescended testes tends to vary greatly in patients of the same age, and the degree of maturation is not reached in the majority of the patients.

In the current study, nodular Leydig cell hyperplasia was observed in 21cases (28%), which are comparable to 6 cases (27%) reported previously by M. Nistal et al. Author also demonstrated intra-cytoplasmic eosinophilic granules in Sertoli cells in 7cases (31%) and our study found eosinophilic granules in only 5 cases(6.7%). According to the literature, intra-cytoplasmic vacuolation, pigment deposits and eosinophilic granules in the cells are in response to insult and represent degenerative changes.[7],[8]

Testicular Regression Syndrome: Testicular regression syndrome (TRS) or 'vanishing testis' is a condition that is considered to be due to the subsequent atrophy and disappearance in foetal life of an initially normal testis.

According to literature, the testis is non-palpable in 10% to 20% of cryptorchidism cases, and of these, TRS accounts for 35% to 60%.[16]. In our study, TRS was reported in 55.6% (15/27) of prepubertal cases.

In the current study, calcification, hemosiderin deposit, and discrete vascular fibrosis were noted in >50% of cases and these findings were in concordance with results of Susan et al.[3] and previous literature.[17],[18]. TRS data of the current study can be retrieved from our published article.[19]

According to the diagnostic criterion, the presence of paratesticular structures, in addition to fibrosis of testicular tissue is required for the diagnosis of TRS.[3]. In the current study, epididymis was present in all cases.

Mean Tubular Diameter and Tubal Fertility Index: In normal individuals, MTD and TFI increase with age. At puberty, MTD is 200 ± 16µm and TFI reaches 100%. Thereafter, a decrease in TFI has been noted with increasing age. According to the literature, MTD and TFI decreases with duration of the undescended testis and are independent predictors of future fertility, and abnormal spermatogenesis. In the current study, MTD and TFI were decreased/ below the normal values when compared with normal controls of the same age.[5],[20]

Other Microscopic Features in Postpubertal Cases: Congenital UDT is usually associated with mixed atrophy of testis (MAT) in postpubertal age. M. Nistal et al.[5] reported MAT in 95.4% of postpubertal cases which confers the worst prognosis for future in vitro fertilization. In the current study, MAT was observed in 77.3% of postpubertal study subjects.

According to the literature, the SCOT could represent deficient colonization of the seminiferous cords by gonocytes or early degeneration of gonocytes. In our study, in addition to MAT, SCOT and /or FHT were identified in 10 (22.7%) cases. This finding was in concordance with previous study results by M. Nistal et al.[7], [8]

Conclusion

- Cryptorchidism is the most common disorder of male endocrine glands in children
- The median age at presentation is 2 years in prepubertal and 29 years in postpubertal subjects. Patients may present with absent testis at any time in life.
- In >60% of the cases, the diagnosis of the undescended testis is made on clinical grounds.
- The hernia is the most common comorbidity associated with UDT followed by infertility and orchitis. In addition, small scrotum, hydrocele, and varicocele are infrequently seen. Congenital malformations are frequently detected at prepubertal age.
- There is no difference in laterality in both groups (46.2% vs. 41.8%).
- The trend of the arrest of testis along its route of descent is the same in pre and postpubertal subjects, most common being inguinal followed by high scrotal and intra-abdominal.

- Gross atrophy of testis is common in both the study groups and TRS constitute the major bulk in prepubertal subjects.
- Secondary testicular parenchymal changes comprising thick capsule, atrophic testicular architecture, hyalinised basement membrane, abundant extracellular matrix, interstitial calcification, inflammation, oedema, and fibrosis are more common in postpubertal subjects compared to prepubertal group (excluding TRS).
- Degenerative changes in Sertoli cells and Leydig cells comprising intra-cytoplasmic vacuolation, pigment deposit, and eosinophilic granules are frequently noted in cryptorchid testes and more common in postpubertal patients.
- TRS is characterized by calcification, hemosiderin deposit, vascular fibrosis (discrete/nodular), residual seminiferous tubules, and dominant vein, in addition to the presence of paratesticular structures in various combinations.
- MTD and TFI decrease with duration of the undescended testis and are independent predictors of future fertility, the severity of architectural change,and abnormal spermatogenesis.

Limitation

The present study is based on a small number of cases and patients in the prepubertal and postpubertal groups are different. Ideally, testicular biopsy taken at prepubertal age has to be compared with a subsequent biopsy taken from the same patient after puberty has been attained.

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