Effect of Clipping of Spermatic Vessels in Laparoscopic Assisted Orchiopexy for Treatment of Intra-abdominal Testes

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Background/purpose: A controversy exists regarding the effect of clipping of the spermatic vessels on the testis during staged Fowler-Stephens orchiopexy. This study was conducted to evaluate the histological alterations in human intra-abdominal testes after dividing its main blood supply during staged Fowler-Stephens orchiopexy. Also to show whether hormonal treatment by Human chorionic gonadotrophin (HCG) would affect the outcome.

Patients & methods: The histology of 13 intra-abdominal testes in 13 patients between 7 months and 18 years old at stages 1 and 2 of the Fowler-Stephens procedure was studied. The patients were divided into 2 groups: group A (no hormonal treatment) and group B (received hCG in the interval between stages 1 and 2).

Results: Stage 2 biopsy showed decrease in the total number of germ cells per tubule in both groups; the percentage of degenerating tubules increased in group A, but decreased in group B. the mean diameter of the seminiferous tubules decreased in group A but increased in group B.

Conclusion: Division of the spermatic vessels is not free of some detrimental effect on testicular histology. Giving hCG in the interval between stages 1 and 2 of the staged Fowler-Stephens orchiopexy may decreases this detrimental effect.

Index words: Abdominal testis, histopathology, clipping, orchiopexy.

INTRODUCTION

The intra-abdominal testis can be managed by either an "open" surgical or laparoscopic orchiopexy procedure or by orchiectomy. The orchiopexy can either be staged or performed with complete mobilization of the testis into the scrotum in one procedure. The choice of repair technique depends on the viability of the testis, the anatomy of the para testicular structures, the distance of the testis from the scrotum, the status of the contra lateral testis, and, most importantly, the surgeon's experience and ability. A microsurgical autotransplantation of the intra-abdominal testis is an extreme measure with few indications. Ligation of the testicular vessels (Fowler-Stephens orchiopexy) occasionally becomes a necessary consideration, especially in the management of the high inguinal or intra-abdominal testis. The testicular artery and veins often limit the distal mobility of these testes. When the spermatic vessels are divided, blood supply to the testis is dependent on collateral circulation from the deferential artery, a branch of the inferior vesical artery, and the cremasteric system, a branch of the inferior epigastric artery. Although successful outcomes evaluating the macroscopic aspect of the testicle have been reported, there is paucity of information regarding the histological repercussion of the ligation of the spermatic vessels of an intra-abdominal testicle during a staged Fowler-Stephens orchiopexy. The aim of this study was to evaluate the histological alterations in human intra-abdominal testes after dividing its main blood supply during staged Fowler-Stephens orchiopexy and to investigate
whether hormonal treatment by hCG would affect the outcome.

**MATERIAL AND METHODS**

From October 2002 to June 2005, laparoscopy was done for 58 boys with 74 impalpable testes revealing 39 abdominal testes in 30 boys. Out of the 30 boys with intra-abdominal testes, 7 were excluded from the study because of relatively long spermatic vessels of their testes (Ain-Shams classification type II) and so; orchiopexy was done without division of the spermatic vessels. Another 7 were excluded for receiving hCG before surgery. One was excluded because of inadequate testicular biopsy material, one was lost to follow up, and another one who had haemophilia, division of spermatic vessels and orchiopexy were done in one stage. The remaining 13 boys were included. Their age ranged from 7 months to 18 years (mean age 4.1 years). In case of bilateral intra-abdominal testes, only one testis was included in the study, so we had a total of 13 testes in the study. Patients in the study were divided into 2 groups: group A (8 boys) did not receive hormonal treatment and group B (5 boys) those who received hormonal treatment between stages 1 and 2 of the Fowler-Stephens procedure. Hormonal treatment was hCG (Pregnyl) given by intra-muscular injection twice weekly for 3 successive weeks (from 1-2 years=1000 i.u / injection; above 2 years =1500 i.u / injection).

The parents were informed about the prognosis of impalpable testes, the procedure to be done, and that testicular biopsies would be taken to assess histology of the testis and the effect of the operation.

**Operative technique.**

Laparoscopy was initiated with an open technique, using 5mm cannula placed supra- or infra-umbilically. The peritoneal cavity was insufflated with carbon dioxide, with pressure between 8 and 12 cm water. The 0- degree laparoscope was introduced through the 5mm umbilical port, and the testis, internal ring, spermatic vessels and vas deferens were identified. With the patient in the Trendelenburg position, two accessory working trocars were placed on either side of the abdomen under direct vision. The contra-lateral trocar was 5mm, and was placed at the lateral margin of rectus abdominal muscle at the level of the umbilicus. The ipsilateral trocar was 10 mm, and was placed slightly lower than the umbilicus at the lateral margin of rectus abdominal muscle (used to deliver the testis out the abdomen for biopsy). The spermatic vessels were mobilized and freed from the posterior peritoneal wall, and then clamped and divided as high as possible. The testis was then grasped through the ipsilateral 10 mm port, abdomen deflated, and the testis delivered out the abdomen for biopsy, (Fig.1). The testis was held by 2 fingers; a longitudinal incision (5 mm) was made in the tunica albuginea; by gentle pressure on the testis more tissue would bulge through the incision of the tunica, and tissues were taken for biopsy by fine scissors. The incision in the tunica was closed by continuous 6-0 vicryl suturing. Re-insufflation of the abdomen helps the testis to be pushed back into the abdomen. Re-inspection of the abdomen by laparoscope making sure the testis not hanging to the anterior abdominal wall.

Stage 2 was done after 6 weeks. The laparoscope was reintroduced through umbilical 5 mm port.

The patient was positioned in a moderate degree of Trendelenburg with the ipsilateral side turned upward. A peritoneal pedicle was created using sharp and blunt dissection starting lateral to the internal ring and spermatic vessels and then extending medially as far as 1 cm from the vas deferens. The testis thus remained pedicle on a peritoneal flap attached to the perideferential peritoneum. A dartos pouch was created through a scrotal incision. Artery forceps were gently moved from the scrotum to the superficial inguinal ring and then into the peritoneal cavity medial to the epigastric vessels under camera control. The forceps were opened once or twice to dilate the point of entry and to establish a track that would allow the un-resisted passage of the testis. Carefully the artery forceps were withdrawn and replaced by Babcock forceps to grasp the testis and pull it down to the scrotum to be fixed in the dartos pouch. Continuous observation during this maneuver was essential to detect and avoid excessive tension or torsion. At this stage it was also possible to visualize any remaining peritoneal attachments and to divide them, reducing the tension and increasing the length. A testicular biopsy was taken by the same method described in stage one.

**Histological Analysis**
Testicular biopsies were fixed in 3% glutaraldehyde, embedded in Epon, sectioned at one micron thickness, and stained with toluidine blue. Histomorphometric analysis was performed by light microscopy at a total magnification of 400X. The number of seminiferous tubule cross-sections in the specimen, total number of germ cells per tubule (GC/T), percentage of degenerating (damaged) tubules (DT), and mean diameter of seminiferous tubules (MTD) were calculated using image analyzer Leica Q 500 MC programme. By the damaged (degenerating) tubules, we mean those tubules with all or almost all its cells are showing signs of degeneration (small, irregular, deeply stained nuclei).

Statistical analysis using SPSS software was done. The statistical analysis was performed on paired samples using the Wilcoxon test. The Mann-Whitney test was used to compare the results between different groups.

Fig 1. Testis delivered out the abdomen for biopsy.

Fig 2. Testicular biopsies from left abdominal testis of 15 months old boy in group A during stages a&b of Stephens-Fowler orchiopexy showing sections of seminiferous tubules. Stage 2 biopsy (right) showed decreases in mean tubular diameter. Toluidine blue (x 400).

Fig 3. Testicular biopsies from left abdominal testis of 2 years old boy in group B during stages 1&2 of Stephens-Fowler orchiopexy showing sections of seminiferous tubules. Stage 2 biopsy (right) showed survival of germ cells (arrows) and increase in mean tubular diameter. Toluidine blue (x 400).
RESULTS

The mean number of seminiferous tubule cross-sections in the specimens was 55.3 in stage 1, and 42.8 in stage 2. As regard to the total number of germ cells per tubule (GC/T), germ cells were absent in biopsies of 4 boys at stage-1 (30.8%). These were excluded from the statistical analysis as no further reduction in the germ cell count could be detected. Biopsy findings at stage 2 orchiopexy revealed a non significant reduction in the total number of germ cells per tubule in group A (p=0.08) and in group B (p=0.14). There was non significant statistical difference between the 2 groups using the Mann-Whitney test (p=0.62). The percentage of damaged (degenerating) seminiferous tubules (DT) in biopsy findings at stage 2 revealed a non significant increase in the percentage of damaged seminiferous tubules in group A (p=0.06), while there was a non significant reduction in the percentage of damaged seminiferous tubules in group B (p=0.8). There was non significant statistical difference between the 2 groups (Mann-Whitney test; p=0.09) (Fig2). The mean diameter of seminiferous tubules (MTD) in biopsy findings at stage 2 revealed a non significant decrease in the mean tubular diameter in group A (p=0.58), while there was a non significant increase in group B (p=0.35). There was non significant statistical difference between the 2 groups (Mann-Whitney test; p=0.17) (Fig3).

DISCUSSION

In 1957, Fowler and Stephens popularized the concept of spermatic vessel ligation for orchiopexy. They described the vascular configuration in which the vas deferens was long and redundant, extending distal to the testis with anastomotic vascular arcades to the testis directly into the most distal segment of the spermatic artery (the “long-loop vas”). This technique has been extrapolated for use in the intra-abdominal testis without a long looping vas. In 1984, Ransley et al reported a variation of the Fowler-Stephens orchiopexy in which this approach is divided into 2 stages. The spermatic vessels are ligated and the testis remains in situ (stage 1). Theoretically, this method allows for the development of collateral blood flow in time. Subsequently, stage 2 entails testicular mobilization as well as its enhanced collateral blood flow from the vasal artery, thereby improving its survival rate.

In experimental studies, ligation of the spermatic vessels generated controversial results depending on the animal model. In Sprague-Dawley rats clipping of the spermatic vessels reduced testicular blood flow by 80% at 1 hour but it was restored to normal at 30 days without loss of testicular integrity. Histological examination revealed intact parenchyma and stroma, normal Leydig and Sertoli cell numbers, and mild tubular disturbances. However in Wistar rats division of the principal spermatic vessels resulted in atrophy of previously normal testes, interruption of spermatogenesis and interstitial cell dysfunction. In experimental studies, ligation of the spermatic vessels generated controversial results depending on the animal model. In Sprague-Dawley rats clipping of the spermatic vessels reduced testicular blood flow by 80% at 1 hour but it was restored to normal at 30 days without loss of testicular integrity. Histological examination revealed intact parenchyma and stroma, normal Leydig and Sertoli cell numbers, and mild tubular disturbances. However in Wistar rats division of the principal spermatic vessels resulted in atrophy of previously normal testes, interruption of spermatogenesis and interstitial cell dysfunction.

The effect of division of the spermatic vessels on the histology of intra-abdominal testes in humans has not been adequately addressed. Division of the main testicular pedicle and reliance on the development of collateral blood flow have been cited as major causes of reduced spermatogenesis in patients undergoing orchiopexy. Controversially, it has also been reported that sufficient germ cells can survive clipping and division of the spermatic vessels of the intra-abdominal testis allowing the possibility of future paternity. It has also been shown that high clipping of the spermatic vessels causes no damage to spermatogenesis in scrotal testes, and may even increase fertility in patients with varicocele. Rosito et al (2004), demonstrated that volumetric structure of intra-abdominal testes is maintained after ligation of spermatic vessels but leads to reduction in the number of spermatogonia.

Here, we studied the effect of dividing the spermatic vessels on testicular histology in 13 patients with intra-abdominal testes and short vessels, treated by two-stage Stephens-Fowler orchiopexy. By studying the histological changes in the same testis, we tried to overcome the difficulties of matching patients’ age, and the variable degrees of dysgenesis in cryptorchidism. In our study, germ cells were absent in 30.8% of patients with abdominal testes at stage-1 biopsies. Saito and Kumamoto found that the higher the testes were located the worse the ratio of spermatogonia per seminiferous tubule. They found absent spermatogonia in 60% of abdominal testes. In a similar study, Rosito et al found diminished number of spermatogonia per tubular cross section at stage 1 in 45.7% of patients, making it difficult to interpret further reduction following ligation of spermatic vessels. However, Thorup et al found that even in abdominal testes spermatogonia are always
present, and sometimes the number is even normal when the patient is younger than 15 months at operation, and that it is optimal to operate before that age. Here, the difference in results may be partially explained by the difference in size of the testicular biopsy specimens. Thorup et al examined 100 tubular transverse sections, while in our study and that of Rosito, up to 50 tubular transverse sections were examined. Our biopsy findings at stage 2 revealed reduction in the total number of germ cells per tubule which was statistically non significant in both groups. Rosito et al (2004) found a reduction in the number of spermatogonia at stage 2 orchiopexy compared to their number at stage 1. This reduction was significant in younger patients with higher number of spermatogonia. Thorup et al found slight decrease in median number of spermatogonia per tubular cross-section at stage 2 of the operation but the difference was not significant.

Beside the total number of germ cells per tubule, alterations in the diameter of seminiferous tubules and the percentage of damaged (degenerating) tubules were used to study the effect of dividing the spermatic vessels. The tubular diameter is a useful indicator of the trophic stage of the seminiferous cells. Before puberty, the tubules are devoid of lumens, and thus the tubular diameter depends exclusively on the number and trophism of the Sertoli cells.

Use of hCG in the treatment of cryptorchidism has been both widespread and debated since the 1930s. Reviewing the literature, the effect of hormonal treatment with hCG on the outcome of the two-stage Stephens-Fowler orchiopexy has not been studied. In this study, we divided the patients in 2 groups: group A (8 boys) those who did not receive hormonal treatment; group B (5 boys) those who received hormonal treatment after division of spermatic vessels. In those who received hCG after stage 1 (group B), biopsies at stage 2 showed better histology than in group A regarding all parameters (alterations in mean tubular diameter, germ cell survival and percentage of damaged tubules). Although the difference did not reach statistical significance, yet this may be explained by the small number of patients. However, the hormonal treatment might have helped the testis salvation after dividing its main blood supply.

CONCLUSION
Division of the spermatic vessels is not free of some detrimental effect on testicular histology. Giving hCG in the interval between stages 1 and 2 of the staged Fowler-Stephens orchiopexy may decrease this detrimental effect.

REFERENCES