

The incidence and histological characteristics of intratubular germ cell neoplasia in postpubertal cryptorchid testis

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Purpose: It is well known that testicular germ cell tumors arise with increased frequency in patients with cryptorchidism. In addition, intratubular germ cell neoplasia (ITGCN) is a precursor lesion to testicular germ cell tumor. Approximately 50% of patients with ITGCN will develop an invasive of testicular germ cell tumors within 5 years. Therefore, we evaluated that the incidence of ITGCN in postpubertal cryptorchidism.

Materials and Methods: Between January 2002 and August 2012, orchiectomy specimens from 31 postpubertal patients (aged 12 or over) with cryptorchid testis were reviewed. The specimens were evaluated for ITGCN using immunohistochemical stains of placental-like alkaline phosphatase and Oct 3/4 with routine hematoxylin-eosin stain. Additionally, the degree of spermatogenesis was assessed using the Johnsen score.

Results: Mean age was 34 years (range, 17 to 74 years) at surgery. All patients were diagnosed as unilateral cryptorchidism. One patient (3.2%) of 20-year-old had ITGCN in surgical specimen with all positive markers. Histological assessment of spermatogenesis showed that mean Johnsen score was 3.42 (range, 1 to 9). Majority of patients (27 of 31) presented impaired spermatogenesis with low Johnsen score lesser than 5.

Conclusions: Considering the risk of malignancy and low spermatogenesis, we should perform immunohistochemical stains and discuss preventative orchiectomy for the postpubertal cryptorchidism.

Keywords: Cryptorchidism; Germ cell and embryonal neoplasms; Puberty

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INTRODUCTION

Cryptorchidism (undescended testis) is the most common congenital malformation in newborn boys, occurring in approximately 3% of full-term infants. In most cases, the undescended testes seen after birth descend normally

within a few months, and only 1% of patients with a persisting cryptorchid condition require medical or surgical treatment [1]. It is well known that undescended testes, if untreated, lead to an increased risk of testicular malignancy, usually seminomas that arise from mutant germ cells [2]. There is also an increased risk of infertility in patients

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with undescended testis. Urry et al. [3] reported that azoospermia was evident in 13% of patients with unilateral cryptorchidism; this rate increases to 89% in untreated bilateral cryptorchid patients.

Intratubular germ cell neoplasia (ITGCN) is the most common precursor of testicular malignancy [4]. Dieckmann and Skakkebaek [5] reported that approximately 50% of patients with ITGCN will develop an invasive testicular germ cell tumor within five years, and Pourkeramati et al. [1] found that 23.08% of infertile men with intra-abdominal testis had ITGCN based on examinations of orchiectomy specimens. Furthermore, the incidence of ITGCN has markedly increased over the past decade, and hence, it is essential that it is promptly and accurately diagnosed in patients with cryptorchidism [1,2]. In the present retrospective study, we aimed to determine the incidence of ITGCN in postpubertal cryptorchidism, and the usefulness of immunohistochemical staining in its diagnosis. In addition, we analyzed the degree of spermatogenesis to evaluate the fertility of these patients.

MATERIALS AND METHODS

Between January 2002 and August 2012, we performed orchiectomy in 31 postpubertal patients (aged 12 years or over) with unilateral cryptorchidism after counseling them about the risk of malignancy, androgenic dysfunction, and male infertility. Cryptorchidism was defined as a condition where testis that was not descended into the scrotum. All patients had a normal contralateral testis and no apparent phenotypic alterations were observed. This study was approved by the Institutional Review Board of Yonsei University Wonju College of Medicine (YWMR-12-0-027).

Each surgically removed testicle was examined histological in multiple sections. One pathologist (M.E.) reviewed all the slides to confirm the pathologic diagnosis. The specimens were evaluated for ITGCN using immunohistochemical staining with antibodies against placental-like alkaline phosphatase (PLAP) and Oct 3/4, as it is difficult to recognize ITGCN based on hematoxylin-eosin (H&E) staining alone.

Malignancy was defined cytologically as the presence of atypical germ cells that showed a significant increase in size, were clearly pleomorphic, had hyperchromatic nuclei, and were arranged in layers attached to the basal tubular membrane. They also contained a clear, vacuolized cytoplasm. None of the cases involved infiltration of the interstitium or showed signs of inflammatory lymphocytic infiltration. To confirm the diagnosis of ITGCN, sections from the testis

were stained with Oct 3/4 and PLAP antibodies.

In order to assess fertility, the degree of spermatogenesis was assessed using the Johnsen score [6]. This involved assessing 100 tubules and recording their heterogeneity by grading them between one and ten using the most advanced germ cell contained in the tubule. A key assumption is that the progressive degeneration of the tubule invariably features the loss of constituent cells in a defined order beginning with the most mature (spermatozoa), followed by the spermatogonia, and then the Sertoli cells. The most mature cell type present was recorded as an index of tubule quality. All tubules were classed from ten (normal) to one (no germ or Sertoli cells), with the midpoint on the scale represented by tubules that contained spermatocytes as the most advanced cell type.

RESULTS

The mean patient age at the time of surgery was 34 years (range, 17–74 years). All 31 patients were diagnosed as having unilateral cryptorchidism. The majority of the patients (21 of 31) presented with an empty scrotum, and the undescended testis was found on subsequent examination. Inguinal pain was the chief complaint amongst elderly patients. Others presented with an inguinal mass, small testis, and infertility. Abdominal cryptorchidism was present in four patients, while inguinal retention was found in 27 patients.

One patient (3.2%), a 20-year-old man with abdominal cryptorchidism, was confirmed to have ITGCN based on immunohistochemical nuclear staining for PLAP and Oct3/4 in the surgical specimen (Fig. 1).

Histological assessment of spermatogenesis revealed that the mean Johnsen score was 3.42 (range, 1–9). The most frequent (12 of 31 patients) Johnsen score was 2 (no germ cells present). The majority of patients (27 of 31) presented with impaired spermatogenesis with a Johnsen score of <5 (no spermatozoa or spermatids, but many spermatocytes present). There were two patients each with a Johnsen score of 6 (only a few spermatids present) and 9 (many spermatozoa present but the spermatogenesis is disorganized).

DISCUSSION

Several putative risk factors for testicular malignancy have been identified, although there is generally only limited evidence to support their prognostic value. One of the most intensively studied risk factors is undescended testis. Thorup

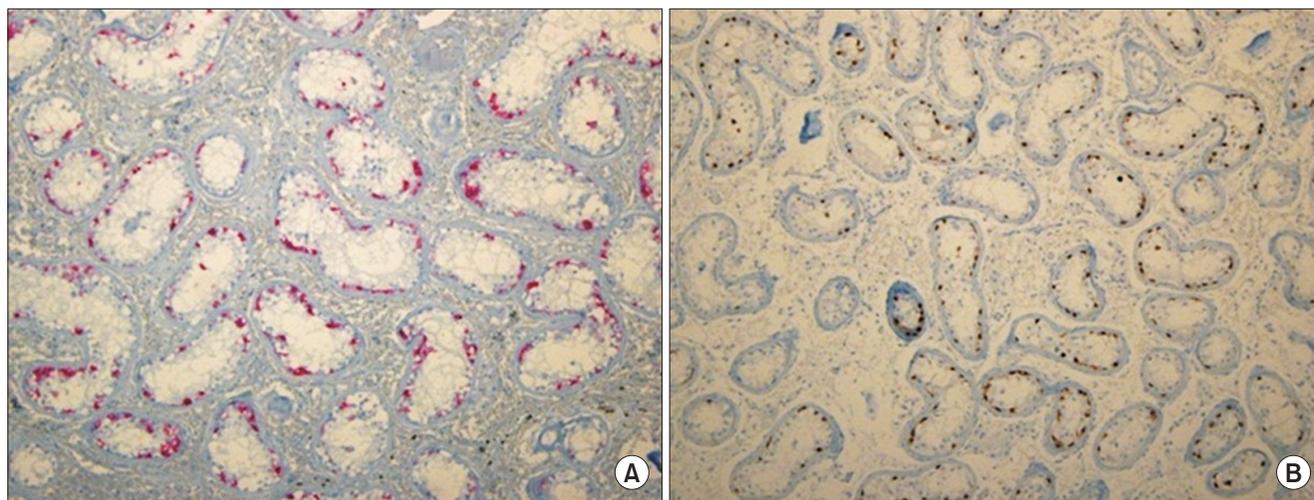


Fig. 1. The germ cells are positive with immunohistochemical staining of placental-like alkaline phosphatase (A) and Oct 3/4 (B) ($\times 100$).

et al. [7] concluded that cryptorchidism is the most widely accepted risk factor for testicular cancer, and is associated with a relative risk of between 3.7 and 7.5 times that among the general population. Furthermore, 5% of all testicular cancers are associated with cryptorchidism. Granados Loarca and Esau Ortega [8] reported that one of the 25 (4%) postpubertal undescended testis patients they assessed had seminoma, and similarly, Ben Jeddou et al. [9] found that two of 81 patients aged >14 years with undescended testis had malignant disease. In the present study, one of the 34 men with untreated postpubertal undescended testis was diagnosed as having ITGCN. Interestingly, the overall incidence of ITGCN in intersex states was found to be higher than in the normal group (6%, six of 102 cases), and was significantly higher in the pubertal age group [10]. It is important to elucidate the frequency with which ITGCN occurs to appropriately monitor the at-risk groups. Periodic testicular self-examination, close follow-up by the physician, and periodic testicular ultrasound are mandatory. Subtle changes in internal testicular architecture, even in the absence of suspicious palpable findings, may warrant a biopsy [11]. (We attempted to contact the patient who was confirmed to have ITGCN to perform physical exam and check paternity but failed.)

Several immunohistochemical markers have been used to identify malignant germ cells in adult cases of ITGCN, the most common of which include PLAP and Oct 3/4 [12]. On the basis of multiple findings, it has been hypothesized that ITGCN originates early during fetal development [13]. This is illustrated by the presence of a number of markers common to ITGCN and immature germ cells, including PLAP and Oct 3/4 [11]. PLAP shows membranous positivity in 90%–100% of seminomas, and Oct 4 shows uniform

nuclear staining in all seminomas [14].

Given the risk of ITGCN or malignancy in postpubertal undescended testis, immunohistochemical staining for PLAP and Oct 3/4 was recommended as part of the diagnostic process, and preventive orchiectomy might be needed after informing patients of the consequences of testicular preservation and the likelihood of their removal in the therapeutic process [15]. In other studies, immunohistochemical study underwent in cases with germ cells did not show ITGCN on routine H&E staining. However, in our study, every specimens were evaluated for ITGCN using immunohistochemical staining for PLAP and Oct 3/4, along with routine H&E staining.

Ford et al. [16] reported that spermatogenesis is severely impaired in persistently undescended testis. Pryor et al. [17] found ITGCN in testicular biopsies with low Johnson's criteria scores and atrophic germinal epitheliums, and other studies have also shown that ITGCN and invasive testicular tumors occur at a high incidence in patients with undescended testis, which are characterized by atrophic germinal epitheliums [18,19]. In our study, the specimen that was positive for the germ cell tumor markers also showed a low Johnsen score, which is consistent with these other studies.

Oct 3/4 and PLAP have a high sensitivity and specificity for diagnosing ITGCN in adults [14]. However, these proteins are present in a very high proportion of germ cells in the testis of normal neonates and young infants with cryptorchidism. The presence of these markers is generally related to the early stages of fetal germ cell maturation that normally proceeds in the neonate, but requires a longer period in cryptorchid testes due to delayed maturation [20]. Therefore, in contrast to the situation in adolescents and adults, these markers can be unreliable for the detection

of ITGCN in very young children [12], and thus, care is needed when interpreting the results of Oct 3/4 and PLAP immunohistochemical staining in these patients.

This study had several limitations. First, it was retrospective in nature and was based on a review of medical records. Second, there may have been a selection bias, because only patients in a single center were enrolled. Third, paternity was not investigated, and therefore, we could only use the Johnsen score to assess fertility.

CONCLUSIONS

We suggest that preventive orchiectomy should be performed in cases of postpubertal cryptorchidism because of its malignant potential and subsequent risk of infertility in some patients. Before performing preventive orchiectomy, we should discuss the possibility of androgenic dysfunction and male infertility with the patient. After surgery, the pathologist should perform immunohistochemical staining for PLAP and Oct 3/4, which are frequently expressed in testicular malignancy, because ITGCN may be overlooked when using H&E staining alone.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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