OPEN ACCESS – CASE REPORT

An Incidental Finding of Leydig Cell Tumor in a 36-year-old Southeast Asian Male who presents with Infertility: A Case Report and Literature Review

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ABSTRACT

Leydig cell tumor is a rare testicular neoplasm that can present as a non-palpable small testicular nodule. Here we present a case of a 36-year-old Filipino male who initially came in for fertility work-up. Semen analysis showed azoospermia. However, an incidental finding on ultrasound showed a well-circumscribed round tumor. The patient underwent radical orchiectomy. On histopathologic examination, a Leydig cell tumor was identified and supported by immunohistochemical staining. We discuss the clinical features pathogenesis, treatment, diagnosis and prognosis of this uncommon entity.

Key words: Leydig cell, testis, orchiectomy, infertility

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INTRODUCTION

Leydig cell tumor (LCT) is a rare neoplasm representing 1% to 3% of testicular tumors and is the most common sex cord-stromal tumor in all ages.[1] The tumor can be seen at any age but is most common in prepubertal boys (5-10 years old) and young adults (30-60 years old).[2,3] Patients are usually asymptomatic except for the finding of a testicular mass.[4] Furthermore, the tumor can produce endocrine changes because of increased production of androgens and/or estrogens such as precocious puberty, breast tenderness, or gynecomastia.[4] However, in this case, the patient had no overt signs and symptoms.

LCT's are generally benign tumors with only 5% to 10% being considered malignant.[3,5] While surgical resection is currently the mainstay effective treatment,[6] some cases require adjuvant medication for fertility.[7] According to the GLOBOCAN 2020 database, 2,858 testicular cancer cases have been reported in Southeast Asia, 358 of which are registered in the Philippines. This database is limited in that it does not specify the histologic type of testicular tumor. To the best of our knowledge, there is no definite number of cases reported in the Philippines. Currently, there is still a lack of data as to the relationship of testicular LTC with male infertility.

CASE

We present a case of a 36-year-old married Filipino male who originally sought consultation for an infertility work-up after trying to conceive with his wife for one year. Physical examination revealed that the left testis was smaller than the right testis and the patient denied claims of any palpable scrotal mass or pain. Testicular sperm extraction was done and revealed no spermatozoa seen in both left and right testicular tissues. Hormone studies revealed the following results: Follicle Stimulating Hormone (FSH): 13.10 mIU/mL (Normal value: 1.55-9.74), Luteinizing Hormone (LH): 5.12 mIU/mL, Testosterone (TT): 356 ng/mL (Normal value for ages 20-49: 132-813 ng/mL),

Estradiol (E2): 21.8 pg/mL (Normal value: 5.37-65.9 pg/mL), and Prolactin: 12.8 ng/mL (Normal value: 3.7-17.9 ng/mL). Only FSH was noted to be elevated. Scrotal ultrasound was done which revealed a hypoechoic nodular focus with minimal to absent vascularity in the left testicle (Figure 1). Also seen in the epididymal head is a cyst measuring 0.2 x 0.1 cm.

The patient underwent radical orchiectomy of the left testis with an uneventful postoperative course. Onco-testicular sperm extraction was done on both testes which yielded no sperm cells. The specimen was submitted for histopathologic examination. Gross examination revealed an unremarkable testis, epididymis and attached spermatic cord. Serial sectioning of the testis revealed brown tan, soft to spongy cut surfaces with no areas of hemorrhage or necrosis. There were no grossly identifiable lesions or masses as well. The entire testis was submitted for processing.

Microscopic examination revealed a tumor in small solid nests separated by delicate fibrovascular septae, composed of tumor cells with round to ovoid nuclei, some with prominent nucleoli, and eosinophilic, granular cytoplasm (Figures 2 and 3). Immunohistochemical studies showed positive staining with Inhibin, Melan-A, Calretinin, and AR (Figure 4). Hence, a diagnosis of Leydig Cell Tumor was made.

Unfortunately, there was no post-surgical update as the patient was lost to follow-up.

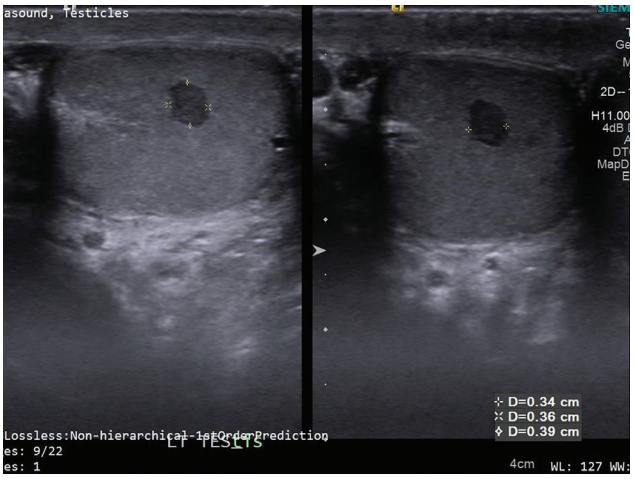

Figure 1. A well-circumscribed round hypoechoic focus with minimal to absent vascularity on scrotal ultrasound measuring 0.34 x 0.36 x 0.39 cm.

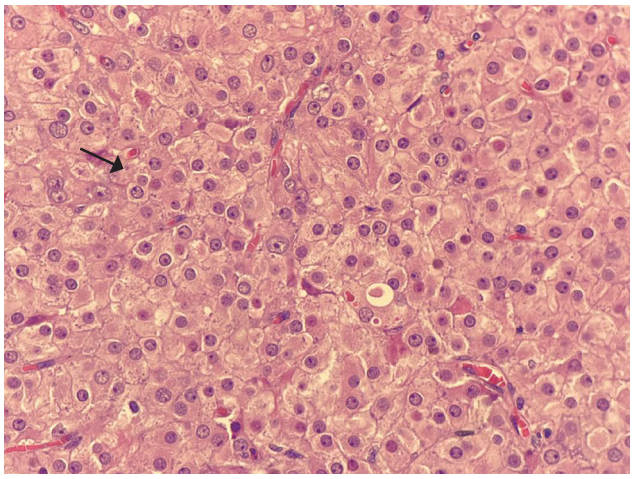

Figure 3. Polygonal cells with uniform round nuclei and eosinophilic granular cytoplasm (H&E, 400x).

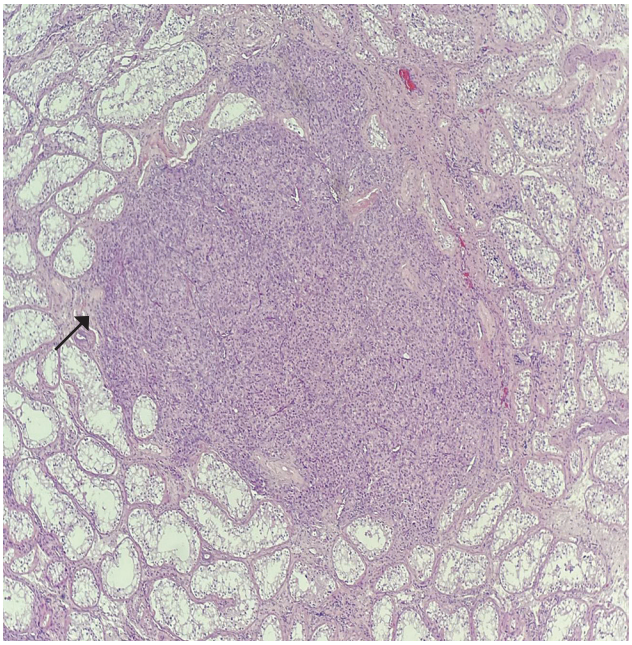

Figure 2. Tumor is composed of nests separated by delicate fibrous septa. The tumor is surrounded by seminiferous tubules showing severe hypospermatogenesis (H&E, 100x).

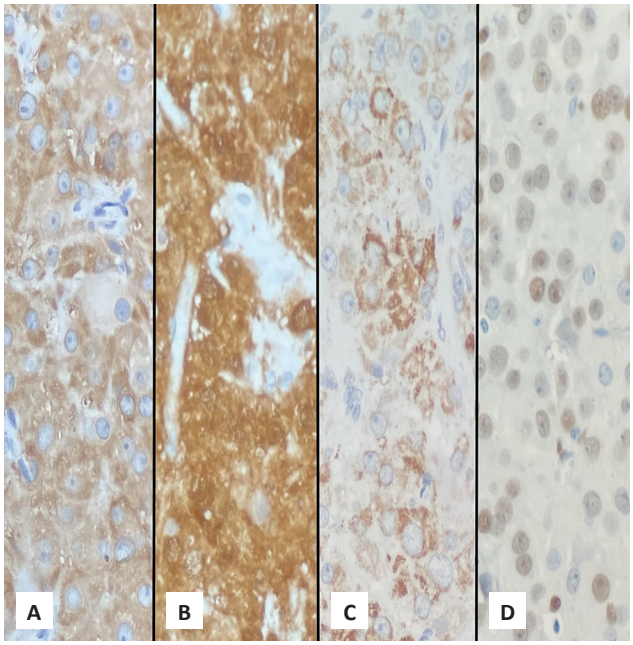

Figure 4. Positive immunohistochemical staining for Inhibin **(A)**, Calretinin **(B)**, Melan-A **(C)**, and AR **(D)**, 1000x.

DISCUSSION

Testicular tumors can be grouped into five (5) general categories: germ cell tumors arising from the germinal epithelium of seminiferous tubules; sex-cord stromal tumors; mixed germ cell-sex cord-stromal tumors; primary tumors not specific to the testis; and metastatic tumors.[4] Sex cord–stromal tumors are derived from 2 types of somatic cells: the Leydig cells, and the Sertoli cells. LCTs are derived from Leydig cells, which are interstitial cells located between the seminiferous tubules and they produce androgens, mainly testosterone when stimulated by luteinizing hormone.[5,7] They are thus involved in the development of secondary male characteristics and maintenance of spermatogenesis. A hormonal imbalance with increased production of estradiol instead of testosterone in adults can then lead to disruption of spermatogenesis, although with regards to our case, the patient had normal estradiol levels.[5,8] In our case, the patient sought consultation for a fertility work-up after trying to conceive with his wife for a year.

The exact pathogenesis underlying the development of these tumors are still unknown. However, it was suggested that activating mutations of the G protein genes, specifically *gsp*, may play a significant role in the pathogenesis of these neoplasms.[9]

Although scrotal ultrasound is not routinely requested in the initial evaluation of male infertility cases, its widespread use has led to a marked increase in the number of incidentally detected small testicular nodules, described by Fabiani et al. 2014 as a non-palpable (<10 mm), asymptomatic solid lesion with normal levels of oncological testicular markers.[10] Most of these lesions are hypoechoic and vascularity is variable but usually increased.[11] Up to 6% have a final histopathologic finding of LCT.[6] It was found that a considerable number of patients who were diagnosed with LCT initially sought consultation for infertility work-up.[6,12] Other common referrals were for general andrologic screening and varicocele.[12] This is similar to our patient who initially sought consultation for an infertility work-up and tried to conceive with his wife for a year.

The tumor is typically well-circumscribed, often lobulated by fibrous septa and is generally uniformly solid. It is generally yellow, yellow-tan, brown or red-brown.[2] Microscopically, tumor cells have well-defined borders, eosinophilic, occasionally clear cytoplasm and a round or oval nucleus. Marked variation in the size and shape of cells can occur. It generally presents as solid but other patterns of growth such as trabecular, myxoid, pseudofollicular and microcystic formation can occur.[4] In the case of our patient, a solid growth pattern was appreciated with tumor cells predominantly in nests separated by delicate fibrous septa. These tumor cells have fairly uniform round nuclei and ample eosinophilic, granular cytoplasm. A differential diagnosis would be Leydig cell hyperplasia which represents an overgrowth of normal Leydig cells. However, this would usually present multifocally. Another differential would be Granular cell tumors. These tend to have nesting or diffuse growth patterns and the cells are organized in a swirl pattern, giving a distinct appearance.[4] A combination of clinical, imaging, and histological evaluation is crucial for accurate diagnosis and appropriate management.

LCT's are generally benign and an estimated 10% are malignant. Diagnostic criteria for malignancy have not been established except for metastasis.[3,13] However, features that are suggestive of aggressive behavior have been described such as large tumor size (greater than 5 cm.,), increased mitotic figures (>3 mitotic figures per 10 high power fields), overt cytologic atypia, vascular or capsular invasion, infiltrative borders and tumor necrosis.[1-3] Age was also found to be significantly associated with malignancy in several reports.[14,15] At least 2 of the features mentioned above are required to assume malignancy.[14] As there is limited data on having proper identification for malignant LCTs, we can only rely on current literature and available data in describing these tumors. In the study by Benarji et al., a combined census of 79,120 cases of testicular cancer between 1998 and 2011 was assessed. 250 of which were malignant Leydig cell tumors and races that were not African American or White comprised 48 out of the 250 cases (19%). In another case series from Fankhauser et al., a total of 101 out of 1,040 (10%) patients developed metastases at any point in time, 65 of which developed during follow-up and 82% of all metastases were diagnosed during the first 5 years. The diagnosis was primarily made clinically by the detection of metastases at the initial presentation or during follow-up.[14]

The immunohistochemical markers utilized for evaluating these tumors are Inhibin, Calretinin, Melan-A, and AR.

- **Inhibin alpha subunit protein (INHA)** is a member of the TGF-beta (transforming growth factor-beta) superfamily encoded by a gene located at 2q35.[16] It is currently used as an immunohistochemical marker for adrenocortical tumors and sex cord-stromal tumors of the testis and the ovary and is generally considered an important diagnostic feature for sex cord-stromal tumors of the testis. In a study by Iczkowski, it was shown that Inhibin was a sensitive marker showing 100% expression in LCTs. It is also the recommended stain to use to differentiate from germ cell tumors.[16]
- **Melan-A/MART-1** is a melanocytic differentiation marker, which is recognized as an antigen on melanoma cells by cytotoxic T-lymphocytes. One monoclonal antibody available against Melan-A is A103 which was made by the Ludwig Institute by Chen et al.[17] A103 has the unique property of staining many steroid hormone-producing cells, and immunoreactivity can be detected in Leydig cells of the testis and in tumors derived from.[17] This clonal antibody is specific for sex-cord stromal tumors. Thus, it is a good marker for steroid-secreting tumors, including LCT.
- **AR** expression has been described previously in reproductive tissues in the second trimester of human fetal development. It is specifically located in the nuclei of Sertoli cells and Leydig cells and is seen to be strongly positive.[18]
- **Calretinin,** also called calcium retinal protein 2 (calb2), is a hexa-EF-hand Ca^{2+} binding protein.[19] It participates in a variety of physiological functions, such as regulation of synthesis of sex hormones, cell proliferation and apoptosis. Calretinin was recently found to be expressed in steroidogenic cells, such

as adrenal cells and Leydig cells and expression was detected in many tumors derived from these cells (such as seminomas, Leydig cell tumors and Sertoli cell tumors).[19]

The management of benign LCT is primarily surgical with simple orchiectomy historically being the standard of care in these patients. If features suggestive of malignant behavior are present, a retroperitoneal lymph node dissection should be considered (as these tumors are chemo and radioresistant).[1] After surgery, hormone replacement treatment can be done in an attempt to establish spermatogenesis.[20] However, there have been several reports on testicular sparing surgery in conjunction with an intraoperative frozen section showing promising results especially those in small lesions (<2.8 cm^3) with normal tumor markers.[21,22] The advantages of which can include preservation of fertility and endocrine function, avoiding the risk of late-onset hypogonadism and preservation of male body image as well.[23] This was implemented to reduce the risk of overtreatment with radical orchiectomy.[23] Outcomes of *Testis Sparing Surgery* (TSS) can be comparable to that of radical orchiectomy in managing benign LCTs where there was an excellent prognosis as well as no tumor recurrence on follow-up.[8,22,23]

CONCLUSION

LCT is a rare, testicular neoplasm that can present clinically occult findings. Scrotal ultrasound evaluation can help detect scrotal pathologies especially when they are non-palpable. Immunohistochemical studies may be used to confirm the diagnosis. Treatment options include radical orchiectomy, testicular sparing surgery, excisional biopsy and active surveillance. Although no ultrasound appearances are entirely diagnostic, it is best to correlate clinical, imaging studies as well as histopathologic findings for the appropriate management.

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ETHICAL CONSIDERATION

Informed consent was not obtained despite all efforts and due diligence exerted to contact the patient. Efforts were undertaken to ensure that no personal identifying patient information was shared.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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