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Unexpected effect of testosterone in hypergonadotropic hypogonadism and nodular Leydig cell hiperplasia. Report of one case

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ABSTRACT

We report a 27 -year-old male referred because of hypergonadotropic hypogonadism with low testosterone and azoospermia. At 23 years of age, he underwent an excision of a hypoechoic 0.7 cm nodule of the left testicle. The pathological diagnosis was a Leydig cell tumor. In the right testicle, there were three nodules at ultrasound, the biggest measuring 0.6 cm. Four years later, the nodules in the right testicle were still present and the larger nodule was excised. The biopsy showed tubules with only Sertoli cells in the perinodular zone. Diffuse and nodular hyperplasia of the Leydig cells was found in the interstitium. The pathological diagnosis was Sertoli syndrome with severe hyperplasia of the Leydig cells. With testosterone therapy, LH decreased, and the nodules disappeared. Thereafter, upon interrupting therapy, LH increased, and the nodules reappeared in two occasions. Resuming testosterone treatment, the nodules disappeared again, suggesting a Leydig cell hyperplasia dependent on chronic LH stimulation.

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Key words: *Azoospermia; Hyperplasia; Hypogonadism; Leydig Cells.*

Efecto inesperado de la testosterona en un hipogonadismo hipergonadotrófico e hiperplasia de las células de Leydig. Informe de un caso

Presentamos un varón de 27 años referido por hipogonadismo hipergonadotrófico con testosterona baja y azoospermia. El paciente tenía el antecedente de un nódulo sólido hipoecogénico de 0,7 cm en el testículo izquierdo, extirpado los 23 años de edad en el año 2002 y diagnosticado patológicamente como tumor de células de Leydig. En ese año se encontraron tres nódulos en el testículo derecho por ultrasonografía, el mayor de 0,6 cm. Cuatro años después, en 2007, los micronódulos del testículo derecho seguían presentes. El mayor de ellos fue extirpado. En la biopsia, había túbulos con solo células de Sertoli en la zona perinodular. En el intersticio había hiperplasia difusa y nodular de las células de Leydig. El diagnóstico patológico fue un síndrome de Sertoli con severa hiperplasia de células de Leydig. La terapia con testosterona disminuyó la LH y

los nódulos inesperadamente desaparecieron. En dos ocasiones, al interrumpir esta terapia, la LH aumentó y los nódulos reaparecieron. Este proceso revirtió nuevamente con el uso de testosterona, sugiriendo una hiperplasia de células de Leydig dependiente del estímulo crónico de LH.

Palabras clave: *Azoospermia; Células de Leydig; Hiperplasia; Hipogonadismo.*

In experimental animals, Leydig cells proliferate when they are stimulated for long periods with chorionic gonadotropin (HCG) or due to chronic luteinizing hormone (LH) elevations caused by medications. Leydig cell hyperplasia (LCH) and occasionally Leydig cell tumors (LCT) are reported in humans with hypergonadotropic hypogonadism[1]. The pathogenic mechanism could be a LH elevation and stimulation of its receptor in the Leydig cell.

Activating mutations of LH receptors or of protein G, generate Leydig cell proliferation independent of LH, exclusively involving the testicle or forming part of genetic syndromes such as McCune-Albright, Carney y Peutz Jeghers[2].

Ninety five percent of testicular tumors derive from germinal cells, namely seminoma and non-seminoma. The other 5% are Cord - stromal tumors (LCT and Sertoli)[3]. One to 3% are LCT. With good quality ultrasonography, the figure increases to 15%[4]. A symptomatic or asymptomatic testicular mass during physical examination or testicular ultrasonography supports the diagnosis. The latter shows a well delimited hypoechogenic solid nodule that is generally unilateral and uni or poly-focal but it can be bilateral in 5 to 10% of cases. The higher incidence of these tumors occurs between 5 and 10 years or between 30 and 60 years of age. They can be non-functioning or they can secrete testosterone, causing precocious puberty in children. If produce estrogens, gynecomastia can develop in children or adults. Ten percent of LCT can become malignant[5]. Their differential diagnosis is with other testicular tumors, LCH and testicular adrenal rest tumors (TARTs) caused by elevated adrenocorticotropic hormone (ACTH)[6].

LCH can be an intertubular diffuse proliferation or nodular. Nodular LCH can appear as microscopic nodules only observed in pathology (more than 25 cells), ultrasound detected micronodules (ranging from 5 to 10 mm of diameter) and palpable nodules. Nodular and non-nodular LCH may coexist[7].

The aim of this communication is to report a male with hypergonadotropic hypogonadism with testicular nodules excised that were a LCT and LCH with unexpected response to testosterone treatment.

Case report

A 27 years old male was referred to endocrinology due to a hypergonadotropic hypogonadism. At the age of 23 (2002), consulted urologist for left testicular pain. Testicular palpation disclosed an epididymal cyst confirmed for ultrasound beside a testicular nodule of 0.7 cm. In the right testicle three well delimited hypoechogenic nodules, the bigger measuring 0.6 cm.

The left nodule was excised and the pathological diagnosis was a benign LCT. We didn't have the biopsy to be reviewed.

In 2006 a ultrasonography showed persistence of three solid nodules in the right testicle measuring 0.3, 0.4 and 0.8 cm compatibles con LCT and left epididymal and sub-albugineous cysts persisted in all subsequent ultrasonographs.

Tumor markers such as alpha-fetoprotein, chorionic gonadotropin beta subunit and lactate dehydrogenase were negative.

The bigger nodule of the right testicle was excised. The final pathological study reported a 0.8 cm nodule composed by polyhedral cells with eosinophilic cytoplasm, Reinke crystals, positive inhibin, a KI-67 of 3% and a mitotic index on 0-10 in 10 high power fields. No vascular or lymphatic proliferation was observed. There was displacement of peri-nodular testicular tissue. In the non-nodular tissue, there was thickening and hyalinization of the tubular walls, tubules only with Sertoli cells and few with scarse germinal epithelium. The interstice had a nodular and diffuse LCH. The pathological diagnosis was a LCH without intratesticular neoplasia (Figure A).

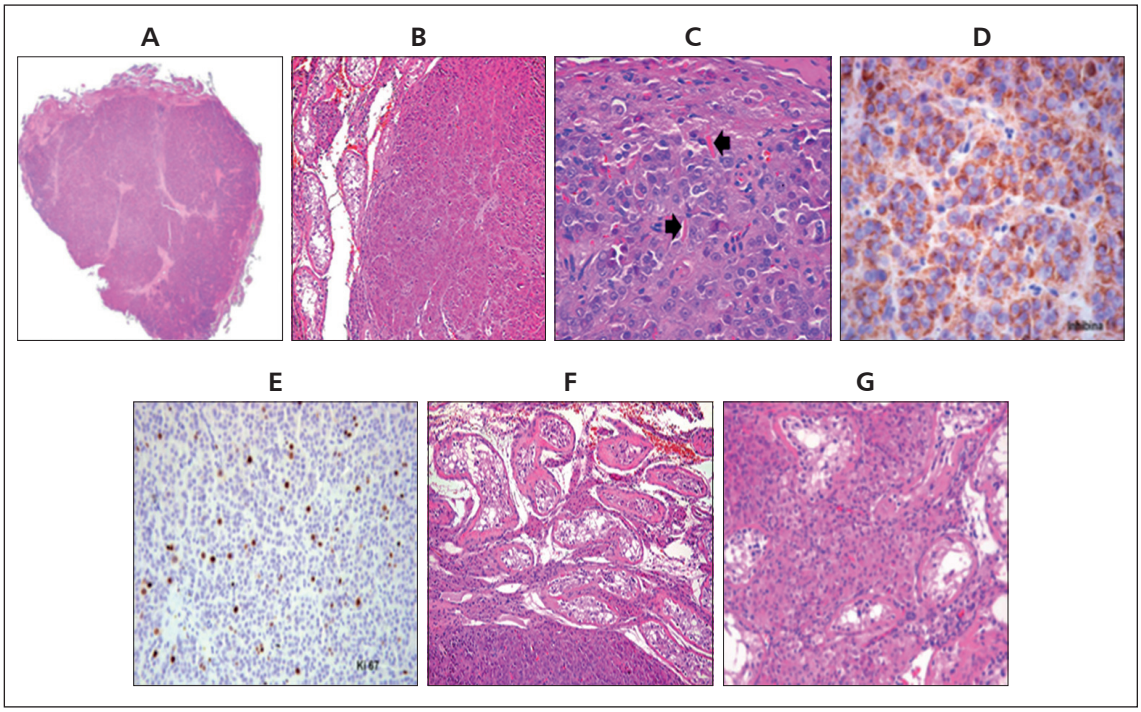

Figure 1. Pathological slides of right testicle nodule excised in 2006. (**A**) Eight mm nodule. (**B**) Well delimited nodule displacing peritumoral tubules. (**C**) Benign cytological aspect and Reinke crystals. (**D**) Positive inhibin staining. (**E**) Ki67 = 3%. (**F-G**) Leydig cell hyperplasia and tubule atrophy with basal membrane thickening and hyalinization and scarce germinal cells.

Referred to endocrinologist. No history of endocrine disease, genital, neither genetic. Normal puberty, without clinical hypoandrogenism.

The general physical examination was normal. The right and left testicular volumes assessed using a Prader orchidometer were 11 and 15 ml respectively (Normal 15 to 30 ml). They had a normal consistency and were devoid or palpable testicular nodules.

Laboratory showed FSH 28 (1-8) mIU/ml, LH 17 (2-12) mIU/ml, total testosterone of 255 ng/dl (n > 300 ng/dl) and a SHBG of 42 nmol/L. Calculated free testosterone was 4.2 ng/dl (n > 5) or 1.67% of the total. Calculated bioavailable testosterone was 99.8 ng/dl (n > 150) or 39% of the total. Estradiol was 20 pg/ml (n < 50). The karyotype was 46XY without chromosomal anomalies. A Fluorescence in situ hybridization (FISH) of the tumor showed 100% of XY in 377 examined cells.

An adrenal stimulation test showed basal and post ACTH 17-hydroxyprogesterone levels of 2.4 and 3.6 ng/ml, respectively (the normal rise should be less than 10 ng/ml).

The screening for an activating mutation of α subunit of the G protein (GNAS1) in genomic ADN of circulating leukocytes using salting out, enzymatic digestion and polymerase chain reaction for R201H mutation, was negative.[8]

Semen analysis showed azoospermia.

Testosterone undecanoate 1,000 mg IM every 12 weeks was started. Figure B shows the evolution of testicular ultrasonography, demonstrating a disappearance of non-palpable nodules when the patient used testosterone and their reappearance when he discontinued the treatment. FSH, LH and testosterone were normalized when receiving testosterone undecanoate, and relapse hypergonatropic hypogonadism when was withdrawal.

Orquiectomy was requested, further no control was done.

We have the informed consent of the patient.

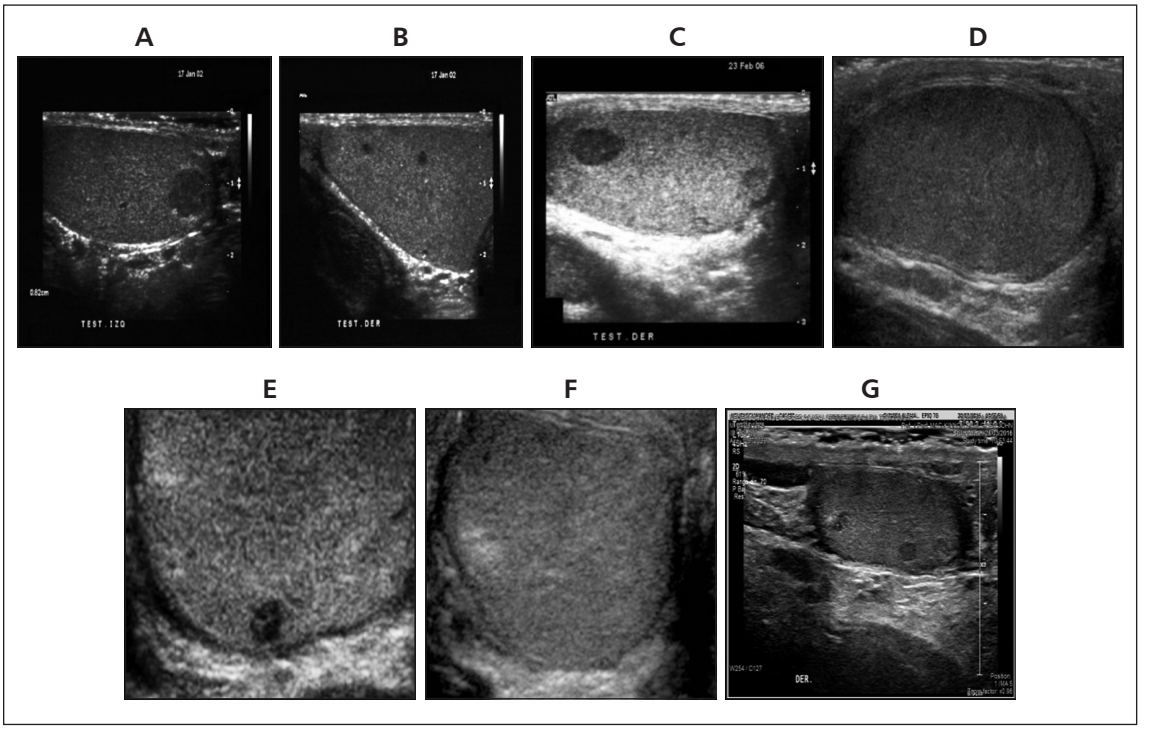

Figure 2. Evolution of testicular ultrasonography. Left **(A)** and right **(B)** testicular ultrasound prior to tumorectomy in 2003. Right testicle ultrasound in 2006 prior to tumorectomy **(C)**. Right testicle nodule disappearance with testosterone replacement on 2009 **(D)**. Right testicle nodule relapse after one year without testosterone in 2010 **(E)**. Right testicle without nodules while on testosterone replacement in 2012 **(F)**. Right testicle nodule relapse after two years without testosterone in 2016 **(G)**.

Discussion

The patient had a hypergonadotropic hypogonadism, left LCT excised in 2002 and right small ultrasonographic micronodules. In 2006 the pathology showed the co-existence of LCH and tubular damage with severe derangement of spermatogenesis. The initial diagnostic workup was directed to diagnose the clinical conditions that could explain these findings.

The excised nodules were compatible with LCT on ultrasonography. The differential diagnosis between nodular LCH and LCT with this procedure is difficult. The presence of multiple bilateral nodules supports a LCH, since this finding is uncommon in LCT.

The second nodule excision showed a benign proliferation of Leydig cells that did not comply with malignancy criteria (more than 5 cm, margin infiltration, hemorrhagic necrosis, extra testicular invasion, cytological atypia, a high mitotic activity (more than 3 mitoses per high power fields), lymphatic or vascular permeation and a high KI 67 index. The most specific malignancy criterion is the presence of systemic metastases[5,7].

Pathologically, the differentiation between LCT and nodular LCH is complicated when nodules are of less than 1 cm[2]. This could be the case in this patient in whom all the nodules may be nodular LCH; if he first excised nodule was LCH mimicking LCT or was actually LCT.

Bilateral testicular nodulectomy, instead of radical orchiectomy was possible thanks to the frozen section biopsy negative to malignancy[9] but final pathologycal study is always necessary.

The interpretation of the right residual nodules after tumorectomy was complicated. They could be: small LCT, isolated nodular LCH or LCH as part of Sertoli and Klinefelter syndrome, TARTs, in situ germinal carcinoma or small seminomas[6].

TARTs was ruled out due to the lack of con-

genital adrenal hyperplasia history or situations in which ACTH increases (Nelson or Addison syndrome)[10]. Also, the patient had a negative 21 hydroxylase deficiency stimulation test. Activating mutations of LH receptors (LHR) or of protein G, reported in McCune Albright syndrome and isolated LCT could not be investigated; we only had available the screening for GNAS1, which was negative[8,11].

Azoospermia, elevated gonadotropins, tubulopathy and LCH can be seen in Klinefelter and Sertoli cell syndrome. The former was ruled out since the patient had a testicular volume higher than that observed in the syndrome, a normal karyotype and FISH. In Sertoli syndrome there is a complete absence of germinal epithelium or scarce spermatogenesis foci. The classic form has tubulopathy, elevated FSH and normal testosterone levels. There is a subgroup with a severe spermatogenesis disturbance, elevated LH, decreased testosterone and a higher frequency of micronodular LCH on pathology. The features of this patient coincide with such subgroup[12,13,14].

The unexpected disappearance of residual ultrasonographic nodules during testosterone substitution coincide with the reduction in LH and FSH. Their reappearance when testosterone was discontinued and subsequent disappearance when reinstated, suggest that nodular LCH was dependent on the chronically elevated LH levels. We do not have pathological evidence to support this transition, although this case looks like to reported by Smith et al in a patient with Klinefelter syndrome. During testosterone replacement, gonadotropin levels decreased and microscopic LCH disappeared[15].

LCT and LCH can be independent entities, coexist or evolve from LCH to LCT. This is analogous to the thyroid C cell hyperplasia that precedes medullary thyroid carcinoma and adrenal medullary hyperplasia that precedes pheochromocytoma[16].

In transgenic mice harboring the simian oncogene SMV4OT, Quintana studied the proliferation of Leydig cells during mice life cycle and noted the progression from hyperplasia to tumor formation[16]. This observation supports the biological plausibility of LCH to LCT transition in the development of first excised nodule.

This case could be: 1) Left LCT that coexists with bilateral LCH; 2) Transition of LCH to LCT in left testicle with right LCH; 3) Only bilateral LCH mimicking LCT in left testicle.

Mutations that activated the LH receptor have been communicated in animals (murines)[19]. In children with gonadotropin-independent precocious puberty, with autonomous secretion of testosterone (Testotoxicosis), cases of mutations that activate the same receptor are also reported[20]. These aspects are relevant to understand the etiopathogenesis of these cases.

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References

1. Clegg E, Cook J, Chapin R, Foster P Daston G. Leydig cell hyperplasia and adenoma formation: mechanism and relevance to humans. Reprod toxicol. 1997; 11: 107-21.
2. Maqdasy S, Bogenmann L, Batisse-Lignier M, Roche B, Franck F, Desbiez F, et al. Leydig cell tumor in a patient with 49, XXXXY karyotype: a review of literature. Reprod Biol Endocrinol. 2015. https//: doi.org/ 10.1186/ s12958-015-0071-7.
3. Cheville J. Classification and pathology of testicular germ cell and sex cord - stromal tumors. Urol Clin North Am. 1999; 26: 595-609.
4. Leonhartsberger N, Ramoner R, Aigner F, Stoehr B, Pichler R, Zangerl F. Increased incidence of leydig cell tumours of the testis in the era of improved imaging techniquest. BJU Int 2011, https//: doi.org/10.1111/j.1464-410X.2011.10177.
5. Kim I, Young R, Scully R. Leydig Cell tumor of the testis. A clinical pathological analysis of 40 cases and review of literature. Am J Surg Pathol. 1985; 9: 177-92.
6. Naughton C, Nadler R, Basler J, Humphrey P. Leydig Cell Hyperplasia. Br J Urol. 1992; 81: 282-9.
7. Georghisan-Galateanu A. Leydig cell tumors of the testis: a case report. BMC Res Notes 2014. https //: doi.org / 10.1186/1756-0500-7-656.
8. Román R, Johnson C, Codner E, Cattani A, García H, Mericq V, et al. Estudio clínico-molecular de pacientes

chilenas con síndrome de McCune-Albright. Rev Med Chile 2001; 129 (12): 1365-72.
9. Bozzini G, Rubino B, Maruccia S, Marenghi C, Casellato S, Picozzi S, et al. Role of frozen section examination in the management of testicular nodules: a useful procedure to identify benign lesions. Urol J. 2014; 11: 1687-91.
10. Claahsen-van der Grinten HL, Otten B, Hermus A, Sweep F, Hulsbergn-van de Kaa C. Testicular adrenal rest tumor in patients with congenital adrenal hyperplasia can cause severe testicular damage. Fértil Steri. 2008; 89: 59-601.
11. Boot AM, Lumbroso S, Verhoef-Post M, Richter-Unruh A, Looijenga L, Funaro A. Mutation analysis of the LH receptor gene in Leydig cell adenoma and hyperplasia and functional and biochemical studies of activating mutations of the LH receptor gene. J Clin Endocrinol Metab. 2011. https//doi.org / 10.1210/jc.2010-3031.
12. Cajaiba M, Reyes-Mujica M, Rios J, Nistal M. Non tumoral parenchyma in Leidig cell tumours: pathogenic considerations. Int J Androl. 2007; 31: 331-6.
13. Lardone MC, Piottante A, Valdevenito R, Ebensperger M, Castro A. Histological and hormonal testicular function in azo/oligozoospermic infertile men. Andrologia 2012, https://doi.org/10.1111/and.12026.
14. Holm M, Rajpert-De Mehits E, Andersson A, Skakkbeak N. Leydig cell micronodules are common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreased testosterone LH ratio. J Pathol. 2003; 199: 378-38.
15. Smith B, Leeson C, Bunge R. Microscopic appareance of the testis in Klinefelter´s síndrome before and after suppression of gonadotrophin production with testosterone. Investigative Urology 1967; 5: 58-72.
16. Quintana S, Venara M, Rey R, di Clemente M, Chemes HE. Origin and evolution of somatic cell testicular tumors in transgenic mice. J Pathol. 2010. https//doi.org/10.1002/path.2731.
17. Newman C, Connolly S, McEneaney O, O´Keane C, Mcquaid S. Leydig Cell Hyperplasia Mimiking a Testicular tumor in a patient with Klinefelter Syndrome. Eur J Case Rep Inter Med. 2019. Doi 10.12890/2019_001129.
18. Heintich A, de Falco T. Essential roles of interstitial cells in testicular development and function, Andrology 2020; 8: 903-14.
19. McGee S R, Narayan P. Precocious puberty and Leydig cell hyperplasia in male mice with a gain of function mutation in the LH receptor gene. Endocrinology. 2013. doi: 10.1210/en.2012-2179
20. Özcabı B, Tahmiscioğlu Bucak F, Ceylaner S, Özcan R, Büyükünal C, Ercan O, et al. Testotoxicosis: Report of Two Cases, One with a Novel Mutation in LHCGR Gene. J Clin Res Pediatr Endocrinol. 2015; 7: 242-8. doi: 10.4274/jcrpe.2067