Hepatocellular Carcinoma, Virilization, and Hilus Cell Hyperplasia in a Girl With Turner Syndrome

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Patients with Turner syndrome (TS) are known to be at risk for excess androgen production and virilization associated with gonadoblastoma and Y chromosome mosaicism, and excess androgens are a risk factor for the development of hepatocellular carcinoma. However, virilization and hepatocellular carcinoma have not been described in a patient with TS. A 10-year-old with non-mosaic 45,X TS presented with clitoromegaly, accelerated linear growth velocity, advanced bone age, and elevated testosterone levels as well as a second occurrence of hepatocellular carcinoma. Gonadectomy was performed, and pathology revealed hilus cell hyperplasia. Immunohistochemical staining of both the original and recurrent hepatocellular carcinoma tissues was diffusely positive for androgen receptors. After gonadectomy, testosterone levels were measurable but normal, with no further virilization; however, the liver mass continued to grow. Ovarian hilus cell hyperplasia should be considered a potential etiology for virilization in the TS population. Excess endogenous testosterone exposure in girls and women with TS may be associated with hepatocellular carcinoma expressing the androgen receptor, though normalizing testosterone levels may not lead to tumor regression in these cases.

Turner syndrome (TS), a condition affecting females, is caused by a missing or incomplete sex chromosome. Excess androgen production with resultant virilization is known to occur in TS, most frequently in association with gonadoblastoma and mosaicism for a cell population containing Y chromosomal material [1].

Excess androgen exposure is well described as an important risk factor for the development of liver tumors, yet hepatocellular carcinoma (HCC) and hepatic adenoma have been reported only rarely in children with TS [2–4]. Unlike with exogenous testosterone (T) exposure, the association between liver tumors and virilization secondary to excess androgen exposure in TS is not well known and is likely uncommon.

1. Case Presentation

A biracial (African American/white) female aged 7 years and 11 months with 45,X TS and a history of HCC post right hepatectomy at age 5 years 8 months presented with new parental concerns of clitoromegaly and hirsutism. At initial evaluation for clitoral growth, the clitoris appeared normal to the examiner (1.2×0.3 cm), and no other signs of virilization were apparent to the examiner.

At age 9 years 3 months, the patient and her family returned for further concerns about clitoromegaly, which was then appreciated on examination (2.5×0.8 cm), as was the presence of darkening facial hair. History was negative for the use of androgen supplements in the patient or family members. The patient never received growth hormone, oxandrolone, or estrogen replacement. Her annualized growth rate was accelerated at 7.1 cm per year, and her bone age was advanced by ~2 years. Laboratory evaluation indicated gonadal failure with T excess: follicle-stimulating hormone, 95 mIU/mL (normal prepubertal female, 1.0 to 4.2 mIU/mL); luteinizing hormone, 21 mIU/mL (normal prepubertal female, 0.02 to 0.18 mIU/mL); estradiol, 4.40 pg/mL (normal prepubertal female, 5 to 20 pg/mL); T, 63 ng/dL (normal prepubertal female, <7 to 20 ng/dL); dehydroepiandrosterone sulfate, 32.9 μg/dL (normal prepubertal female, 16 to 96 μg/dL); and 17-hydroxyprogesterone, <40. Androgens had not been measured previously, though at age 2 years 0 months, the patient had a follicle-stimulating hormone level of 23.4 mIU/mL and luteinizing hormone level of 1.7 mIU/mL. Pelvic ultrasonography showed poor visualization of the ovaries but no evidence of malignancy. Magnetic resonance imaging (MRI) of the abdomen and pelvis with and without contrast material showed no gonadal, adrenal, or hepatic masses.

Multiple cytogenetic studies performed on different tissues were negative for Y chromosome material, including karyotype of cells from the original hepatic biopsy, the original hepatic tumor resection, and peripheral white blood cells (WBCs); fluorescence *in situ* hybridization of WBCs; and microarray of WBCs.

Her virilization progressed such that by age 10 years 1 month, she exhibited onset of pubarche, further darkening of facial hair, onset of acne, and further clitoral growth (3.5×1.4 cm). At that time, serum total and free T levels were elevated at 96 ng/dL (normal prepubertal female, <7 to 20 ng/dL) and 0.96 ng/dL (normal prepubertal female, <0.04 to 0.59 ng/dL), respectively, and antimullerian hormone was undetectable. Repeated MRI of the abdomen revealed a well-circumscribed 4.4×3.5–cm mass in the right hepatic lobe. Computed tomography of the chest was normal. Serum liver chemistries included elevated aspartate aminotransferase level of 77 U/L (normal, <40 U/L) and alanine aminotransferase level of 56 U/L (normal, <30 U/L) with normal bilirubin, alkaline phosphatase, and albumin levels and an α-fetoprotein value of 1.14 ng/mL (normal, <7.51 ng/mL). Hepatitis studies showed negative hepatitis C antibody, hepatitis B surface antigen, hepatitis B surface antibody, and hepatitis B core antibody. These studies were also negative along with other infectious disease and rheumatologic studies at the time of the original hepatic mass evaluation.

Biopsy of the right hepatic lobe mass was performed, and the result was compared with the previous specimen from the right hepatectomy; the two specimens showed similar morphology. Histologic examination of the previous right hepatectomy specimen revealed a well-differentiated hepatocellular neoplasm with overall bland nuclei with focal necrosis and hemorrhage (Fig. 1A). The tumor cells were arranged in thin and thick trabeculae (Fig. 1B). The reticulin stain showed extensive reticulin loss and wide cell plates, supporting the diagnosis of a well-differentiated HCC in the right hepatectomy specimen. The recent liver biopsy specimen demonstrated a well-differentiated hepatocellular neoplasm with pseudoacinar architecture (Fig. 1C). The reticulin stain showed thick trabeculae and focal reticulin loss. An immunohistochemical stain for the androgen receptor (AR) was performed. Both the tumor and the background liver parenchyma were diffusely positive for AR (Fig. 1D). The archival tumor tissue of the previous right hepatectomy specimen was also retrospectively examined by immunohistochemistry and also was diffusely positive for AR.
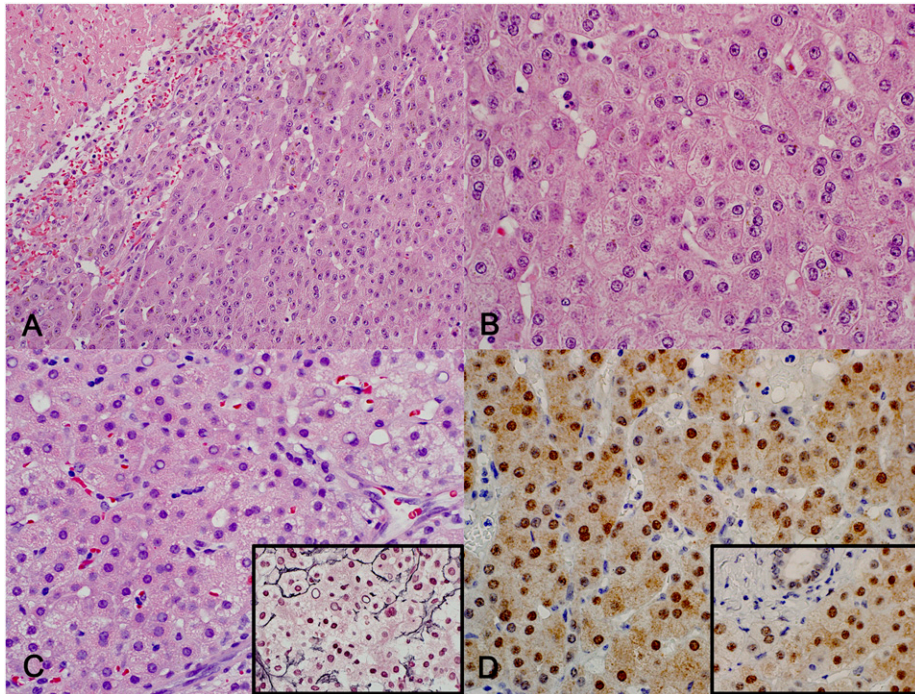
Figure 1. Histologic features of the liver tumor. (A) Right hepatectomy specimen revealed a well-differentiated hepatocellular neoplasm with overall bland nuclei with focal necrosis and hemorrhage [hematoxylin and eosin (HE), ×100]. (B) The tumor cells were arranged in thin and thick trabeculae (HE, ×400). (C) The most recent liver biopsy demonstrated a well-differentiated hepatocellular neoplasm with pseudoacinar architecture (HE, ×400). The reticulin stain showed focal reticulin loss (inset). (D) Both the tumor cells and the nonneoplastic hepatocytes (inset) in this biopsy were diffusely positive for androgen receptor (×400).

Because the patient exhibited gonadal failure and the source of the T was still unknown, at age 10 years 2 months she underwent a bilateral gonadectomy. Gonadal pathology showed bilateral scant residual ovarian parenchyma with hilus cell hyperplasia and fibrosis, adjacent mesonephric duct proliferation, benign fallopian tube, and no evidence of malignancy (Fig. 2). Eight days after gonadectomy, serum total and free T levels decreased to the normal prepubertal female ranges (20 and 0.22 ng/dL, respectively).
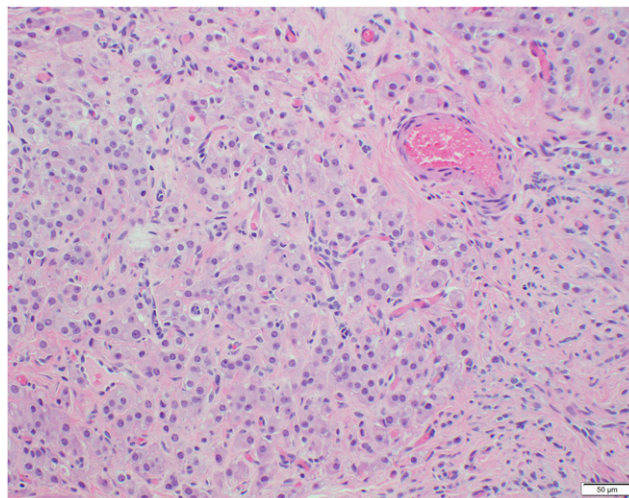

Figure 2. Gonadal biopsy specimen showing hilus cell hyperplasia.

The new liver tumor was thought to be resectable, but with narrow margins. That concern as well as the recurrence or new tumor formation after original gross total resection suggested the possible need for more extensive resection with liver transplantation. However, with the likelihood that gonadal production of excessive T was driving liver tumor growth, we elected to observe the liver mass after gonadectomy. When the patient was 10 years and 5 months old, MRI revealed a mass measuring 5.7×4.1 cm and four smaller lesions, with the largest measuring up to 1.4 cm. One month later, MRI showed further tumor growth, with the mass measuring 6.1×4.5 cm, a new lesion measuring 1.7×1.4 cm, and stable to mild increase in size of the four smaller lesions.

2. Discussion

We describe a girl with TS, virilization, and hilus cell hyperplasia and an AR-positive liver tumor. Although prior case reports described virilization in patients with TS and mosaicism and gonadoblastomas, our patient did not show evidence of mosaicism after various cytogenetic evaluations of different tissues. We did not use polymerase chain reaction techniques to identify smaller pieces of Y chromosome, so it is possible that Y chromosome material was missed. However, our patient did not have gonadoblastoma. Virilization is also a well-known possible side effect of oxandrolone, which is sometimes used to promote height growth in patients with TS. However, our patient has never used oxandrolone or other growth-promoting agents.

The child did have bilateral ovarian hilus cell hyperplasia, which has been described in TS with and without Y mosaicism [1, 5]. Ovarian hilus cells are morphologically similar to Leydig cells. Therefore, they can produce T in response to elevated gonadotropin levels and can decrease T production after leuprolide administration [6]. Ovarian hilus cell hyperplasia is more commonly recognized as an etiology of excess T and virilization in postmenopausal women, though it is still an uncommon occurrence even in that population. The most recent case reports in patients without TS describe postmenopausal women with signs of substantial virilization, such as extensive hirsutism, clitoromegaly, male-pattern baldness, and markedly elevated T production [7–9]. Considering that patients with TS typically exhibit gonadal failure at a younger age than most menopausal women, it is reasonable to consider hilus cell hyperplasia as a possible cause of virilization in a girl or woman with TS of any age who has experienced gonadal failure. The assumption that the ovarian hilus cell hyperplasia was the nidus of T production in our patient is supported by the abrupt decrease of serum T levels after gonadectomy.

In addition, our patient manifested AR-positive HCC, which may predict a worse prognosis with greater recurrence rates and higher mortality than AR-negative HCC [10]. Clearly, our patient demonstrated pathologically elevated T levels, but the role of this abnormal T in the development and/or rapid growth of HCC can only be postulated. T activation of the AR may play a critical role in hepatocarcinogenesis, though the AR may also contribute to hepatocarcinogenesis through androgen-independent signaling pathways [11].

For our patient, we were faced with treatment options that included a surgically difficult lobectomy, orthotopic liver transplantation, or observation in the hope that removal of T as a driver of the liver pathology would result in tumor shrinkage or resolution. Scant literature allows us to confidently anticipate the natural history of the liver tumor after removal of the T source. One report included six male users of exogenous androgen with secondary liver tumors, none of whom developed metastatic disease after tumor resection [12]. This report further details the notion that androgen-driven hepatic adenomas can regress after cessation of androgen use. However, no patients in these series were said to have had HCC. In one girl with TS and hilus cell hyperplasia who was similar to our patient but without mention of liver tumors, gonadectomy did result in normalization of T over a 3-month period [13].

Although T levels in our patient did normalize shortly after gonadectomy, she still has measurable T, most likely from adrenal production. It is unclear whether the AR is contributing to hepatocarcinogenesis with normal T concentrations, independent of T levels, or if

the presence of AR is unrelated to this patient's hepatocarcinogenesis. Excess androgen production and exposure may be a possible driving factor for hepatocarcinogenesis in pediatric patients. Despite not fully understanding the role of excess T production and the presence of AR in the development of HCC in our patient, we can confidently conclude that hilus cell hyperplasia should be considered a potential etiology for elevated T levels and virilization in the population with TS.

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