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Juvenile Granulosa Cell Tumor with Elevated Peripheral Interleukin-6 Level Shows Prolonged Fever and Delayed Puberty

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ABSTRACT

Juvenile granulosa cell tumor (JGCT), classified as a sex cord-stromal tumor, is a rare neoplasm. This is an instructive case report of JGCT accompanied by augmented interleukin (IL)-6 secretion. A 13-year-old girl with prolonged fever and delayed puberty was diagnosed with JGCT of the left ovary based on an imaging study and pathological investigation. Although it was not clear whether IL-6 was secreted from the tumor cells, her serum level of IL-6 was very high. After tumorectomy, the patient's symptoms immediately disappeared, her IL-6 level decreased, and she entered puberty. Therefore, augmented IL-6 secretion production induced by tumors should be considered a potential cause of prolonged fever and/or delayed puberty.

Key words delayed puberty; interleukin-6; juvenile granulosa cell tumor; prolonged fever

Granulosa cell tumor (GCT) is a type of ovarian neoplasm categorized as a sex cord-stromal tumor. GCT is very common in adults, particularly in peri- and postmenopausal females[1]; however, juvenile GCT is extremely rare, with only 5% of GCT cases occurring during childhood.[2] GCT is classified as borderline malignant tumor. A majority of juvenile GCT (JGCT) cases have localized lesions and take a benign course[3–5]; however, a few JGCT cases have been reported to be invasive and fatal despite intensive chemotherapy.[3–5]

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Abbreviations: CA, cancer antigen; CRP, C-reactive protein; CT, computed tomography; FSH, follicle stimulating hormone; GCT, granulosa cell tumor; GnRH, gonadotropin releasing hormone; IBD, inflammatory bowel disease; IL, interleukin; JGCT, juvenile granulosa cell tumor; LCA, leukocyte common antigen; LH, luteinizing hormone; MRI, magnetic resonance imaging; SAA, serum amyloid A

Common symptoms of JGCT include an abdominal mass and/or pain and precocious puberty due to elevated estrogen secretion.[3–5] Herein, we report a case of JGCT accompanied by augmented interleukin (IL)-6 secretion, resulting in continuous fever, general fatigue, and delayed puberty.

PATIENT REPORT

A 13-year-old girl visited a hospital due to low-grade fever and general fatigue for 5 months. Later, she was referred to Tottori University Hospital because she was diagnosed with an abdominal tumor through computed tomography (CT). Her body temperature was 38.6 °C, and physical examination revealed a mass without tenderness in her lower abdomen. She had facial and conjunctival pallor. Her breasts and pubic hair were in the prepuberal state (Tanner developmental stage 1).

The results of a blood test revealed remarkably augmented inflammatory reaction; that is, the white blood cells and platelet count increased to 16,000/μL and 723,000/μL, respectively, and microcytic hypochromic anemia was detected. The erythrocyte sedimentation rate was 140 mm/h. Blood levels of C-reactive protein (CRP: 18.7 mg/dL), serum amyloid A (SAA: 1750 μg/mL, normal < 8 μg/mL), fibrinogen (732 mg/dL), ferritin (429 ng/mL), haptoglobin (665 mg/dL), and immunoglobulin G (2,982 mg/dL) were elevated. Moreover, the serum inflammatory cytokine levels of soluble IL-2 receptor and IL-6 were elevated to 1,465 U/mL (normal, 145–519) and 348 pg/mL (normal, < 4), respectively. In contrast, the levels of serum albumin (1.8 g/dL), iron (12 μg/dL), and unsaturated iron binding capacity (192 μg/dL) decreased. Serum cancer antigen (CA)-125, a tumor marker, was elevated (6,020 U/mL, normal < 35). Serum alpha-fetoprotein, beta-human chorionic gonadotropin, CA19-9, and carcinoembryonic antigen were within normal limits. Serum estradiol, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were not elevated (14.5 pg/mL, < 0.1 mIU/mL, and 0.33 mIU/

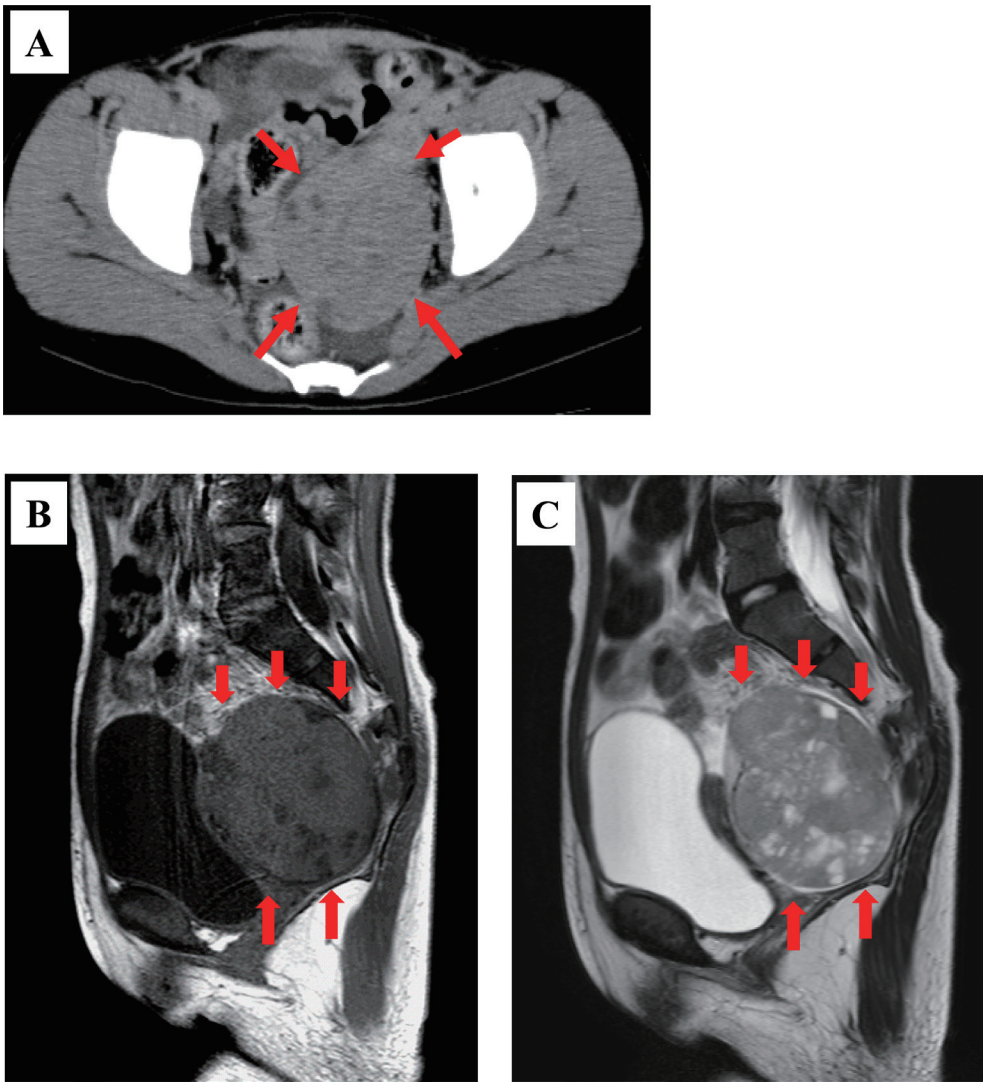

Fig. 1. Imaging of the tumor. **A**, **B**, and **C** are plane CT, MRI T1 weighted image (WI), and T2WI, respectively (*red arrows*). The tumor showed iso-intensity in T1WI and high intensity in T2WI compared to the muscle. The size of the tumor was estimated to be 67 × 61 × 74 mm.

mL, respectively). This data was in accordance with the prepuberal pattern.

CT and magnetic resonance imaging (MRI) examination revealed that the tumor was located in the left side of the pelvic cavity (Figs. 1A–C). No metastatic lesions were detected.

The patient underwent laparoscopic left salpingo-oophorectomy 6 days after admission. The tumor was excised by piecemeal resection. The gross image is presented in Figs. 2A and 2B. Histologically, the tumor was mainly composed of sheet-like growth of neoplastic cells with abundant eosinophilic cytoplasm and round nuclei (Figs. 2C and 2D). A pseudopapillary pattern and follicles containing eosinophilic secretions were focally observed. Tumor cells lacked the nuclear grooves characteristic of adult granulosa cell tumors. Immunohistochemically, the tumor was focally positive for inhibin-α (Fig. 2E), CD56, CD99 and cytokeratin AE1/AE3, and negative for calretinin, PAX8, CD10, estrogen receptor, progesterone receptor, chromogranin A, synaptophysin, c-kit, and podoplanin/D2-40. The Ki-67 index was approximately 3%. These findings were consistent with a diagnosis of juvenile granulosa cell tumor.

To investigate the mechanism underlying the elevation of serum IL-6 level, we evaluated tumoral IL-6 expression through immunohistochemistry. A rabbit polyclonal anti-IL-6 antibody (ab6672, Abcam, Cambridge, UK) was used as a primary antibody at dilution of 1:400. Antigen retrieval was performed by

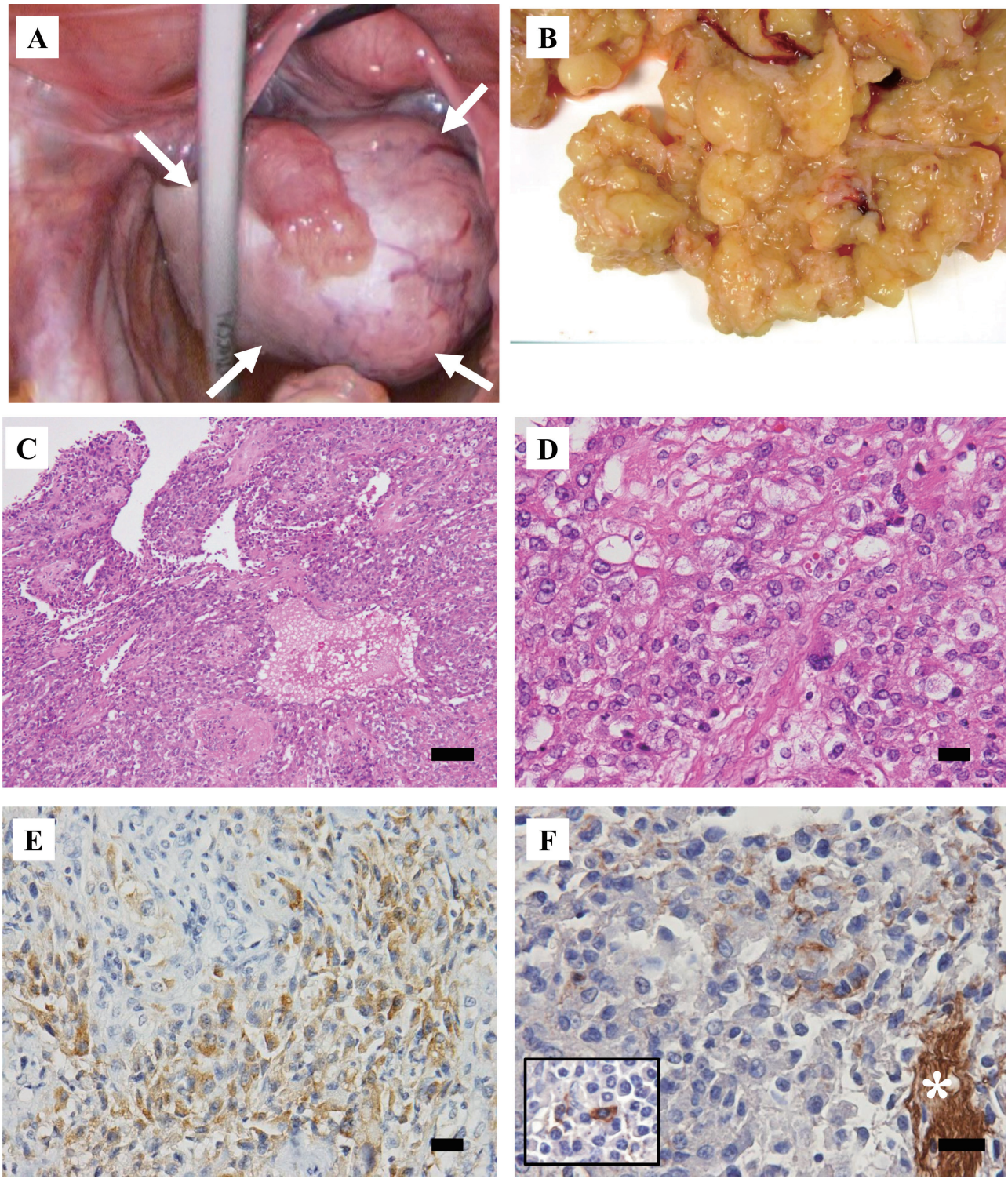

Fig. 2. Gross image and histology of the ovarian tumor. (**A**) The gross image of the tumor before (**A**, white arrows) and after (**B**) the resection. The tumor surface was smooth and white while whole. The cut surface was solid and yellow in color. (**C**) The tumor is mainly composed of sheet-like growth of neoplastic cells, focally showing a pseudopapillary pattern (top left) and follicle formation (bottom right) (hematoxylin and eosin stain). (**D**) High-power view of the tumor (hematoxylin and eosin stain). (**E**) Immunohistochemistry for inhibin-α. (**F**) Immunohistochemistry for IL-6 showing positive staining in a subset of tumor cells as well as non-neoplastic stromal tissue (white asterisk). Note IL-6-positive immune cells from human tonsil used as positive controls (inset). Bars; (**C**) 100 μm, (**D**–**F**) 20 μm.

treating the section with proteinase K (Code: S3020, Agilent, Santa Clara, CA) according to the manufacturer's instructions. Immune cells from the human tonsil were used as positive controls.[6] We observed weak cytoplasmic staining for IL-6 in a subset of neoplastic cells of the ovarian tumor (Fig. 2F); however, the staining was also observed in non-neoplastic stromal tissue within the tumor, probably reflecting an increased IL-6 level in extracellular interstitial fluid.

After the surgery, the patient's body temperature rapidly lowered to normal range, and the fever did not recur. The serum IL-6 level decreased by 1.6 pg/mL 10 days after the surgery. Other elevated inflammatory indices and tumor marker levels immediately returned to normal. The levels of estradiol, LH, and FSH were low (< 10 pg/mL, 0.18 mIU/mL, and 3.66 mIU/mL, respectively) 3 days after the operation. One month after the operation, although the level of estradiol was still low (< 10 pg/mL), LH and FSH were elevated to 33.19 and 68.49 mIU/mL, respectively. The patient had her first menstrual period 2 months post operation. Six months after the operation, values of estradiol, LH, and FSH reached the puberal pattern (23.85 pg/mL, 3.92 mIU/mL, and 4.81 mIU/mL, respectively). One year after the operation, her breasts and pubic hair reached stage 4 and stage 3 of the Tanner developmental stages, respectively. No recurrence of GCT has been observed for 3 years after the operation, and she has presented a normal progression of puberty.

DISCUSSION

In this case, an ovarian GCT patient with prolonged fever was assessed. Although the major symptoms of GCT include abdominal distention, palpable tumor, or gonadotropin-independent precocious puberty, a few cases also presented with fever in a previous report.[4] As the serum IL-6 level of our patient was considerably high, we assumed that the IL-6 produced by GCT caused prolonged fever.

Certain IL-6 producing tumors such as atrial myxoma,[7] mesothelioma,[8] pheochromocytoma,[9] paraganglioma,[10] bladder cancer,[11] and renal cell cancer[12] have been reported. IL-6 is one of the major inflammatory cytokines produced by various kinds of cells including lymphocytes, monocytes, macrophages, neutrophils, fibroblasts, and endothelial cells.[13] Serum IL-6 induces the production of acute-phase proteins such as CRP, SAA, fibrinogen, and haptoglobin, whereas it reduces the production of albumin.[14] Moreover, IL-6 promotes IL-2 receptor expression and produces hepcidin, which causes anemia by increasing iron storage and inhibiting intestinal iron absorption.[14] Conceivably, augmented IL-6 secretion would have triggered the inflammatory sequence reactions in this case. Both the symptoms and the laboratory data were in line with this scenario. We performed immunohistochemical analysis for IL-6 in order to clarify whether it was produced by the tumor cells. However, it was not possible to determine whether the positive immunohistochemical staining results indicated true expression of IL-6 in the ovarian tumor cells or simply represented IL-6 in the interstitial fluid at the tumor cell surface. Additionally, we could not investigate gene expression of IL-6 in the GCT cells because neither fresh nor frozen samples could be obtained. Accordingly, we could not confirm IL-6 production by analyzing gene expression or by using flow cytometry.

Despite the age of 13 years, secondary sexual characteristics were not observed in the patient before the operation. Although the detailed mechanism is unclear, inflammatory cytokines are known to have adverse effects on the nutritional state and to inhibit gonadotropin releasing hormone (GnRH) secretion in chronic inflammatory diseases such as inflammatory bowel disease (IBD).[15] According to an experiment using an IBD mouse model, one of the possible mechanisms underlying delayed puberty in the inflammatory setting involves a decreased level of leptin, a hormone responsible for the secretion of GnRH.[16] We, therefore, speculate that the pubertal delay in this case was due to elevated IL-6 level associated with the GCT because the patient experienced pubertal progression in response to the normalization of IL-6 and acute phase proteins levels in the serum.

We reported a GCT case with elevated serum IL-6 level showing atypical symptoms of prolonged low-grade fever and delayed puberty. It is possible that juvenile GCT promotes IL-6 secretion, and this should be included in the differential diagnosis list for prolonged fever. Imaging tests such as CT and MRI should be made routinely for early detection. This case demonstrated that massive IL-6 secretion can suppress gonadal function and result in the delay of puberty.

The authors declare no conflict of interest.

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