10.4274/jcrpe.galenos.2022.2022-8-20

Case report

Mitotically Active Follicular Nodule in Early Childhood: A Case Report with A Novel Mutation in the Thyroglobulin Gene

Kizilcan Cetin S et al. Thyroglobulin Gene Mutation Related Mitotically Active Nodule

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Abstract
Dyshormonogenesis is the failure of thyroid hormone production due to a defect in thyroid hormonogenesis. Loss-of-function mutations in the thyroglobulin(TG) gene are a cause of dyshormonogenesis, leading to gland stimulation by thyroid-stimulating hormone (TSH), resulting in goiter. We report a mitotically active follicular nodule in an 11-year-old female with a novel mutation in the TG gene. The patient had been under follow-up due to congenital hypothyroidism since the neonatal period, and she had normal TSH levels. Genetic test revealed a novel compound heterogeneous mutation [c.2149C>T (p.R717*) (P.Arg717Ter) / c.5361_5362delCCinsG (p.H1787Qfs*3) (p.His1787GlnfsTer3)] in TG gene. She underwent total thyroidectomy for a thyroid nodule that was reported as Bethesda IV on FNAB and noted as suspicious for noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Pathological examination revealed a 16 mm well-demarcated follicular nodule with a solid/insular pattern. Mitotic activity and Ki67 proliferation index were unusually high (10 mitoses/2mm2 and 10% respectively). Marked cellular pleomorphism and nuclear atypia are well-known diagnostic pitfalls in patients with dyshormonogenetic goiter. However, high mitotic activity is a feature that is less emphasized in dyshormonogenetic goiter and may raise suspicion of poorly differentiated carcinoma when observed together with a solid pattern. The absence of signs of invasion, history of congenital hypothyroidism, and awareness of the presence of mutations compatible with dyshormonogenetic goiter can prevent the overinterpretation of such lesions. The risk of cancer development in the dyshormonogenetic thyroid gland is possible in childhood. The close follow-up is life-saving and prevents morbidities and mortalities.
Keywords: Congenital hypothyroidism, Thyroglobulin synthesis defect, thyroglobulin (*TG)*

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25.08.2022
08.11.2022

Published: 01.12.2022

Introduction
Dyshormonogenesis (DG) is the failure of thyroid hormone production by a structurally intact gland due to a defect in thyroid hormonogenesis, leading to congenital hypothyroidism (CH). DG is a rare but significant risk factor associated with developing thyroid cancer [1, 2]. Thyroglobulin (*TG)* gene is on chromosome 8q24.2-8q24.3. TG is essential in promoting thyroid hormone synthesis, storage of iodine, and inactive thyroid hormones. The incidence of thyroid DG due to *TG* mutations is approximately 1 in 100,000 newborns[3]. TG synthesis defect causes the chronic stimulating effect of thyroid-stimulating hormone (TSH)[2]. Long-standing TSH stimulation leads to goiter. Cancer development is mostly in adulthood[4].
The first mutation identified in TG was g.IVS3–3C>G in a family with congenital goiter in 1991[5]. Since then, 52 more mutations (11 splice site mutations,11 nonsense mutations,23 missense mutations, six deletions, and one single nucleotide insertion) have been identified[4]. All patients with mutations in *TG* had a similar phenotype, such as low/absent serum TG, high levels of serum TSH, low levels of thyroid hormones, and enlarged thyroid gland[4].
This paper presents clinical, biochemical, and pathological characteristics and an eleven-year follow-up of a case of primary CH with TG synthesis deficiency. Additionally, the patient had a novel mutation in the *TG* gene, which caused the early development of a mitotically active follicular nodule.

Case Report
An 11-day-old female patient was admitted to our outpatient clinic due to elevated TSH (75.5µIU/mL), which was detected by a neonatal screening test on the 7th day of life. Her medical history showed that she was born to non-consanguineous parents at 38 weeks of gestation, with a birth weight of 3820 g. A physical examination revealed a weight of 3820 g (68%), a length of 53 cm (85%), and a head circumference of 35.5 cm (51%). The anterior fontanelle was 3X3cm, and the posterior fontanelle was 1X1cm. . Laboratory tests showed normal hemogram and liver and kidney function and blood glucose. Thyroid function test (TFT) confirmed primary hypothyroidism [free (f)T4:5.58 pmol/lt (7-16pmol/lt), TSH:>100mIU/ml (0.34-5.36 mIU/ml)]. The urinary iodine level was 159 µg/L (normal value 100-200µg/L). TG level was <0.1ng/ml (1.15-50.03ng/ml). Her thyroid volume was 2.07 ml (4.45SD), which excluded thyroid agenesia. Treatment with L-thyroxine (L-T4), 10 µg/kg per day, was initiated on the 10th day of life. One month later, the TSH,

fT4, and fT3 levels were normal. Molecular analysis revealed a novel compound heterogeneous *TG* mutation [c.2149C>T(p.R717*) (P.Arg717Ter) / c.5361_5362delCCinsG (p.H1787Qfs*3) (p.His1787GlnfsTer3)]. The mutation was likely pathogenic[6].
She was followed up every three months. TSH was carefully targeted in the lower part of the normal range (Table 1). She had normal growth and puberty. Neurological evaluation revealed normal language, cognitive, social, and fine motor development.
She underwent periodical ultrasound (US) investigation once a year. At age 10, thyroid US revealed a hypoechoic, well-defined nodule of approximately 7x6 mm in size, with high internal hypervascularity on the homogenous parenchyma of the left inferior thyroid lobe without any sign of calcification. Lymphadenopathy was not observed. She was on L-T4, 2 μg/kg per day. Her TFT was as follows: serum TSH:6 μIU/mL (0.6-4.64 μIU/mL), fT4:21.8 pmol/L (11-22 pmol/lt). Fine-needle aspiration biopsy was performed and interpreted as "suspicious for follicular neoplasm". Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was suggested as a possible diagnosis. The patient underwent a total thyroidectomy six months later when the family agreed on the operationPathological examination revealed a 16 mm well-demarcated follicular nodule with an insular and solid pattern on the left lobe. Mitotic activity and Ki67 proliferation index were unusually high (10 mitoses/2mm^2 and 10%, respectively). The vascular or capsular invasion was not observed in the comprehensive examination of the nodule, and nuclear features were not adequate for a diagnosis of papillary carcinoma. The case was reported with a descriptive diagnosis as a "mitotically active follicular tumor".
The patient is now 11 years of age and on 100 μg of L-T4 daily (2.3 μg/kg/day). She weighs 42.7 kg (0.55 SD) and her height is 154.3 cm (1.24 SD); BMI is 17.9 kg/m^2 (-0.05 SD). Her growth, pubertal and mental development are normal. Her TFT is as follows: serum TSH: 2.64 μIU/mL (0.6-4.64 μIU/mL), free T4: 22 pmol/L (11-22 pmol/lt), anti-TPO antibody (Ab): 2.3 IU/ml (0-34 IU/ml), anti TG: 10 IU/ml (0-115 IU/ml). Due to lack of TG, she is under close follow-up by physical examination every three monthly, with periodic neck ultrasound. No lymphadenopathy or metastases were observed.

Discussion/Conclusion

We shared our case with a novel compound heterogeneous mutation in the *TG* gene. Her pathological findings were unusual. We observed a mitotically active follicular nodule on a dyshormonogenetic goiter. To our knowledge, these pathological features were not previously defined in the literature. Mitotic activity and Ki67 proliferation index were unusually high in the present case. Marked cellular pleomorphism and nuclear atypia are well-known diagnostic pitfalls in patients with DG. However, high mitotic activity is a feature that is less emphasized in DG and may be more alarming for the pathologist, raising suspicion of poorly differentiated carcinoma, especially when observed together with a solid insular trabecular (STI) pattern. In the recent WHO classification of thyroid tumors, two types of high-grade follicular-derived carcinomas are described. Differentiated high-grade thyroid carcinoma is a papillary or follicular carcinoma with increased mitotic counts (≥ 5 mitosis/ 2 mm^2) or tumor necrosis. The second category is poorly differentiated thyroid carcinoma (PDTC), and it is defined as a malignant tumor of follicular cells with a STI pattern, without typical papillary carcinoma nuclei, and with the presence of convoluted nuclei or necrosis or high (≥ 3 mitosis/2 mm^2) mitosis [7]. Although the present nodule shows a much higher mitotic activity and a STI pattern , it does not show any clear-cut histopathological features of malignancy. Although high mitotic activity is a worrisome histopathologic feature in a STI patterned follicular nodule, the diagnosis of PDTC should not be made, especially in a pediatric patient, based solely on the presence of a STI pattern and high mitotic activity in a completely well-circumscribed nodule without capsular or vascular invasion. The absence of signs of invasion, history of congenital hypothyroidism, and awareness of the presence of mutations compatible with DG can prevent the overinterpretation of such lesions. Although it is not possible to diagnose such a nodule as a malignant tumor in the present state, the definitive nature of the present tumor remains to be characterized by follow-up of similar cases. Since thyroidectomy was performed in our case, it is not possible to comment on whether this particular nodule would have developed an invasive character. The high mitotic activity and high Ki67 index observed in the present case may be related to the novel TG mutation. We think that RAI or lymph node dissection is unnecessary. However, since follow-up information on similar cases has not been reported so far, it will be safer to keep the patient under close follow-up, for early detection of a recurrence, albeit with a low probability.
Thyroid US and TG levels are defined as the most valuable tools to determine the etiology of CH, since all present with low/absent serum TG, high levels of serum TSH, low levels of thyroid hormones, and enlarged thyroid gland Although TG synthesis defect can be easily diagnosed with enlarged thyroid in the US, the clinical presentation of mutations in the *TG* gene is variable.
Few patients develop a fetal goiter, diagnosed by antenatal ultrasound, and need intrauterine hormone replacement. However, others present with goiter at a later age [4]. In our case, we showed a mitotically active follicular nodule at an early age, which might be related to the novel mutation.
Dyshormonogenetic goiter is a rare risk factor for developing thyroid cancer. In a study, 56 cases of dyshormonogenetic goiters with ages ranging from newborn to 52 yr were evaluated. Ten cases of all (18%) were diagnosed with thyroid cancer. Follicular type thyroid cancer was mainly seen, and almost all ten patients were diagnosed with thyroid cancer in adulthood. None of them had TG synthesis defects [8]. Long-term elevated TSH causes the growth of thyroid follicular cells, and it might have a role in developing malignant tumors. However, cancer occurs after a long time under TSH stimulation, especially in adulthood[9]. The most exciting thing in our case is that her TSH level was normal from the beginning of her life except for the last three years. However, it was never markedly elevated under treatment (Table 1). This situation is significant in considering the genotype effect on tumorigenesis. Defining underlying genetic mechanisms will be more helpful in understanding the progression of the disease.
It can be speculated that we may have detected the nodule before the development of an aggressive tumor in adulthood. We want to emphasize two points about our case: Firstly, an annual thyroid US examination is significant and lifesaving. We believe that serial thyroid ultrasound examination was our advantage for the early diagnosis of this nodule. Secondly, in the last three years of the follow-up, we realized that patient had elevated TSH levels, although fT4 was in the normal range (Table 1). TSH stimulation might have initiated tumor development.

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

Medical Practices:SC, ZA, ZS, EO , Concept:SC, ZA, SS, SC, MB, Design: SC,ZA, ZS, MB, Data collection: SC, ZA, SS, SC, EO , Analysis: : SC, ZA, SS, SC, EO, MB, Literature Search: SC, ZA, ZS, MB , Writing: SC, ZA, ZS, EO, MB.

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Table. 11 year follow-up of the patient

Age at screening	TSH (0.6-4.64 µIU/mL)	free T4 (11-22 pmol/lt)	free T3 (3.8-6 pmol/lt)	LT4 dose (µg/kg)	Thyroid volume (SD) on US*
11 days	74.3	5.58	6.27	10	2.07 ml (4.45 SD)
1 month	5.28	14.9	5.3	10	-
1 year	1.3	18.35	6.2	3.5	2 ml (2.14 SD)
2 years	2.9	18.5	6.8	3.2	-
3 years	2.3	18	7	3	5.53 ml (2.9 SD)
4 years	5.4	17.04	-	2.8	-
5 years	2.8	19	-	2.5	6.1 ml (3.5 SD)
6 years	1.3	21.8	-	2.4	-
7 years	5.3	18	-	2.3	7.5 ml (2.6 SD)
8 years	5.2	16.6	6.8	2.4	7.1 ml (2.4 SD)
9 years	6.8	18	7.3	2.2	9.7 ml (4.1 SD)
10 years	6	21.8	7.2	2.2	10.2 ml (4.4 SD)**
11 years	2.64	22	6.3	2.3	total thyroidectomy

*Thyroid SD measurements were calculated using age and gender for the Turkish population and an online calculator then available online at CEDD online [10,11].
** a nodule of 7x6 mm in size, hypoechoic, well-defined with high internal hypervascularity on the homogenous parenchyma of the left inferior thyroid lobe **.**