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CASE REPORT

PUBERTAL VIRILIZATION IN AN ADOLESCENT WITH 46, XY DISORDER OF SEXUAL DEVELOPMENT: A NOVEL MUTATION IN NR5A1 GENE

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Abstract

Background. NR5A1 [Steroidogenic factor 1 (SF1)] is a nuclear receptor that is essential for the development of gonads and adrenal glands as well as the establishment of steroidogenesis in these organs. The clinical findings of the mutations of NR5A1 gene in 46, XY individuals are variable. Virilization at puberty can be seen in some of the 46, XY children who have a female phenotype and are raised as female.

A girl aged 13 years and 10 months old was brought by the family for deepening of her voice. On physical examination, her breast development was Tanner stage 2, axillary hair (+) and pubic hair was Tanner stage 4. She had labioscrotal fusion and 4.4 cm phallus (External Masculinisation Score was 6). Hypergonadotropic hypogonadism, low AMH and high testosterone levels were detected in laboratory tests. Uterus was not visualized in pelvic ultrasonography. Karyotype analysis was reported as 46, XY. Sequence analysis of the NR5A1 gene revealed a novel heterozygote c.1075_1089del (p.Leu359_Leu363del) variant. The patient was raised as a female and oestrogen replacement was started following gonadectomy.

Conclusion. It should be kept in mind that virilization may develop at puberty in individuals with 46, XY disorder of sexual development due to NR5A1 mutation.

Keywords: NR5A1, pubertal virilization, novel mutation, disorder of sexual development.

INTRODUCTION

NR5A1 [Steroidogenic factor 1 (SF1)] is a nuclear receptor which regulates many genes that have a critical role in endocrinological functions and normal reproductive physiology. It is essential for the development of gonads and adrenal glands as well as establishment of steroidogenesis in these organs. There are many possible different clinical presentations with its mutations; it can be seen as complete female external genitalia, ambiguous genitalia (bifid scrotum and/or micro penis), hypospadias or infertility in 46, XY individuals (1, 2). Mutations in NR5A1 can cause incomplete virilization at birth, pubertal virilization, and gonadal malignancy in 46, XY individuals due to gonadal dysgenesis. The patients may develop adrenal insufficiency (3-5). Partial androgen insensitivity syndrome, 5-alpha reductase deficiency, 17-beta hydroxysteroid dehydrogenase deficiency and partial gonadal dysgenesis (NR5A1 mutation) should be considered in children with 46, XY disorder of sexual development (DSD) presenting with virilization at puberty (6). We present this case with 46, XY DSD, who was brought with pubertal virilization, in which we detected a novel mutation in NR5A1 gene.

CASE DESCRIPTION

A 13 years and 10 months old girl presented with deepening of the voice. Her voice has started to coarsen 18 months ago, and she has been having increased hair on her upper lip, chin and sideburns for the last year. In the past medical history, she had an inguinal hernia repair when she was 7 years old and had a surgery due to labial synechiae when she was 9. In the family history, her mother had no history of medication use during pregnancy, virilization or any other illness, and no consanguinity between the parents. On physical examination; her weight was 0.55 SDS, height -0.31 SDS, BMI 0.8 SDS; blood pressure 100/60 mmHg, pulse 88 bpm, she had acne on the face, hirsutism on upper lip, chin and sideburns. Breast development was Tanner stage 1 on the right and stage 2 on the left, pubic hair was

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Tanner stage 4. Genital examination revealed a posterior labial fusion, and 4.4 cm phallus. No gonads were palpable on labioscrotal folds. External Masculinization Score (EMS) was 6 and Sinnecker Stage 3 (Fig. 1).

Laboratory results

Serum sodium, potassium, chloride, glucose, cortisol, adrenocorticotropic hormone (ACTH) and adrenal androgen levels were all in normal range. There were hypergonadotropic hypogonadism, low AMH and high testosterone levels. Serum testosterone/dihydrotestosterone and testosterone/androstenedione ratios were also normal (Table 1). Bone age was compatible with 15 years according to Greulich & Pyle Atlas.

Pelvic imaging and diagnostic laparoscopy

Transabdominal ultrasonography showed absence of the uterus, and the presence of both gonads in proximal inguinal canal. Pelvic magnetic resonance imaging revealed one gonad on each side adjacent to inguinal rings and close to the anterior abdominal wall, sized 18x12x10 mm (1.2 mL) on the right side and 24x18x16 mm (3.7 mL) on the left side (Fig. 2).

Diagnostic laparoscopy showed both gonads, consistent with testicular tissue in the entrance of inguinal canals with left inguinal hernia. The cord was entering to the structure from normal anatomical localization and seen to be tracking towards suprapubic area. No Mullerian structures were seen in the pelvic area (Fig. 3). Chromosome analysis was planned with a provisional diagnosis of partial gonadal dysgenesis relying on the clinical and laboratory data.

Genetic analysis

The cytogenetic analysis of the DNA derived from peripheral blood lymphocytes was reported as 46,XY. At the second stage, 46,XY Disorder of Sexual

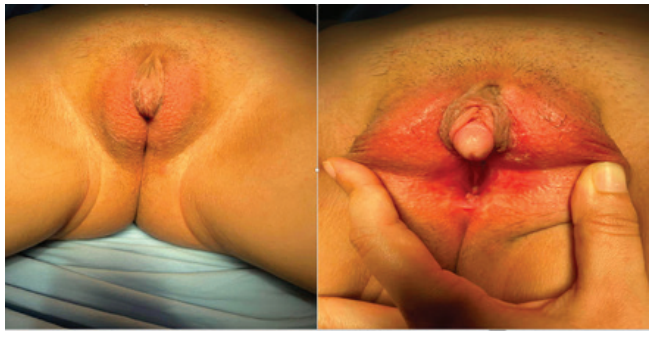

Figure 1. External genitalia of the patient. On genital examination, she had labioscrotal fusion and a 4.4 cm phallus. No gonads were palpable on labioscrotal folds. 154x78mm (72 x 72 DPI).

Table 1. Laboratory test results of the patient 110x86mm (236 x 236 DPI)

Test	Result	Reference range
ACTH (pg/mL)	28.3	0-46
Cortisol (ug /dL)	12.2	3-21
17 OH progesterone (ng/mL)	2.54	0.1-1.7
AFP (ng/mL)	1.39	0-13.4
HCG (mlU/mL)	<1.20	0-5
DHEAS (pg/dL)	198,2	8.6-169.8
FSH (mlU/m1)	62.98	3.35-21.63
LH (mlU/m1)	15.22	2.39-6.60
AMH (ng/mL)	0.49	1.3-83.9
Oestradiol (pg/mL)	20	5-11
Total testosterone (nmol/L)	10.76	0.38-1.97
Free testosterone (pg/mL)	11.8	0-4.2
Dihydrotestosterone (nmol/L)	1.3	<0.29
Androstenedione (nmol/L)	2.98	0.31-2.86

ACTH, adrenocorticotropic hormone; AFP, alpha-fetoprotein; HCG, beta human chorionic gonadotropin; DHEAS, dehydroepiandrosterone sulphate; FSH, follicle stimulating hormone; LH, Luteinizing hormone; AMH, anti-mullerian hormone.

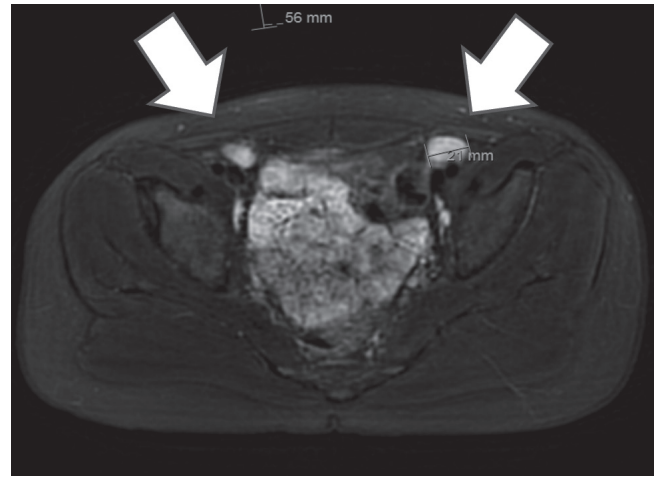

Figure 2. Pelvic Magnetic Resonance Imaging of the patient. The arrows show one gonad on each side adjacent to inguinal rings and close to the anterior abdominal wall. 119x74mm (236 x 236 DPI).

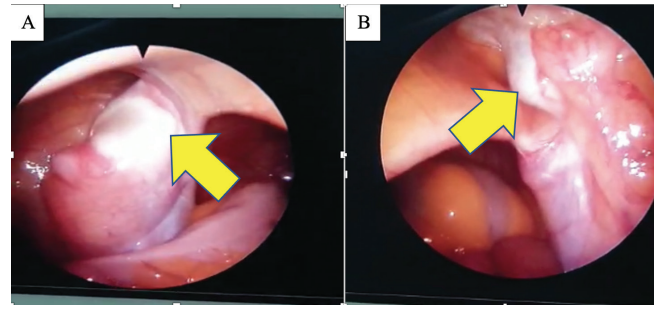

Figure 3. Pictures of gonads of the patient at the timepoint of laparoscopic gonadectomy. The arrows show the dysgenetic gonads in the entrance of the inguinal canals of the patient. (A) Laparoscopic view of the dysgenetic gonad on the left. (B) Laparoscopic view of the dysgenetic gonad on the right. 154x72mm (236 x 236 DPI).

Development Panel work up was planned. AMH, AMHR2, AR, ARX, ATRX, CYB5A, CYP11A1, DHCR7, DHH, GATA4, HCCS, HSD17B3, LHCGR, MAMLD1, MAP3K1, NR5A1, OPHN1, SOX9, SRD5A2, SRY, WTI, ZFPM2 genes were scanned with Targeted Next Generation Sequencing (NGS) Analysis. Heterozygote c.1075_1089del (p.Leu359_Leu363del) variant was detected in NR5A1 gene (Fig. 4). The result confirmed with Sanger sequencing. Pathogenicity prediction of identified germline NR5A1 mutation was analyzed using Franklin (https://franklin.genoox.com/clinical-db/home) and VarSome (https://varsome.com). The mutation is located in the ligand-binding domain region of the NR5A1 gene. The region is an exonic hotspot region and several pathogenic/likely pathogenic reported variants were found surrounding this variant in exon 6 without any missense benign variants. It's an inframe deletion and not in a repetitive region. Allele frequency is 0.0% in all databases. Since the amino acid sequence changes, protein functions are expected to be affected. Parents were also analyzed for the same variant and the variant was neither detected in father nor mother. Therefore, the mutation was accepted as a "de novo mutation" (Fig. 5).

Gender affirmation MDT and treatment decision

The sexual characteristics of the patient were compatible with female gender and it was seen in repeated psychiatric reviews that she adopted the female gender. It was decided unanimously by the multidisciplinary team (MDT) that it would be in her

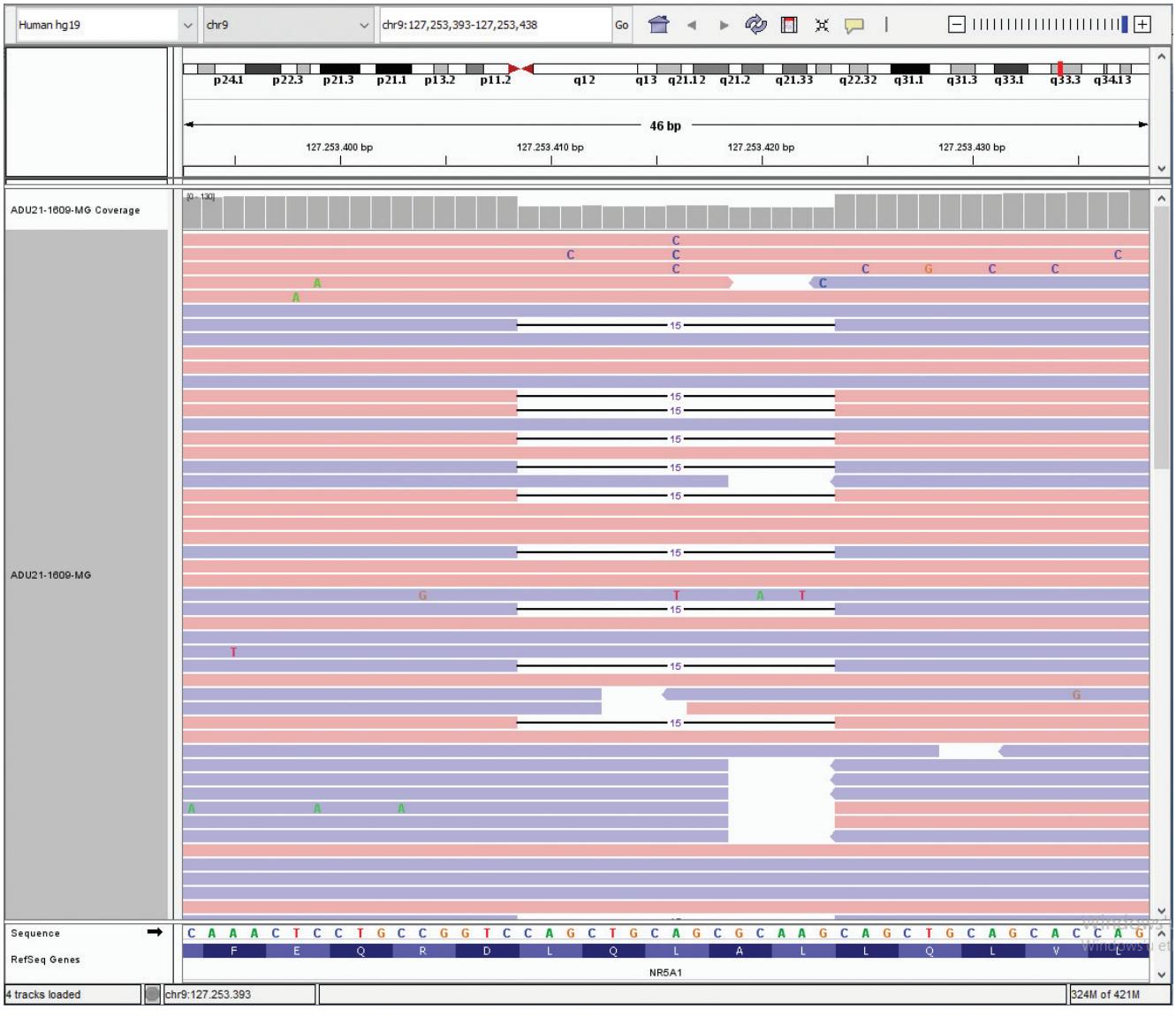

Figure 4. The 15-base (CTTGCGCTGCAGCTG) denovo novel in-frame mutation causing the deletion of 5 amino acids (leu-ala-leu-gln-leu) in the NR5A1 gene located at 9q33.3. 141x165mm (236x236 DPI).

best interest to be raised as a female as she already adopted to the female gender. The family and the child agreed on the same decision. Dysgenetic gonads were removed, and estrogen replacement treatment was initiated.

Pathological evaluation

Testis, epididymis, and spermatic cord were seen in cross-sections of both gonads. Germ cell aplasia was seen in testis. Seminiferous tubules were formed of Sertoli cells only and did not contain germ cells and therefore this was taken as "Sertoli-cell only" pattern (Fig. 6). There was no hyperplasia or decrease in number of Leydig cells. Immunohistochemical staining with OCT3/4 was not shown and no Carcinoma in Situ (CIS) was seen.

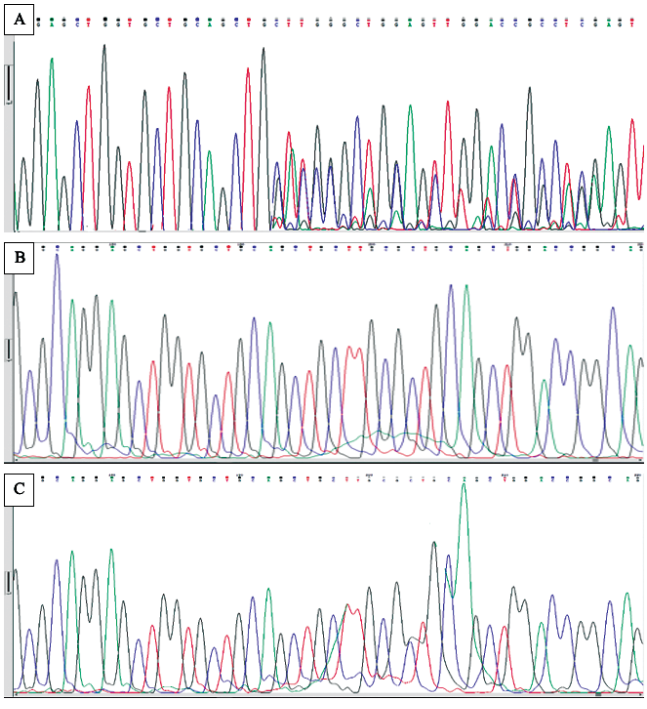

Figure 5. Electropherograms of the patient with heterozygote c.1075_1089del (p.Leu359_Leu363del) variant in NR5A1 gene (A), the mother (B) and the father (C). A peak is seen for each base in electropherograms of the patient`s mother (B) and father (C) whereas there is a heterozygote insertion-deletion view in the patient`s electropherogram (A) 15 nucleotide DNA fragment containing the 1075th and 1089th base intervals is deleted as heterozygous.71x76mm (236 x 236 DPI).

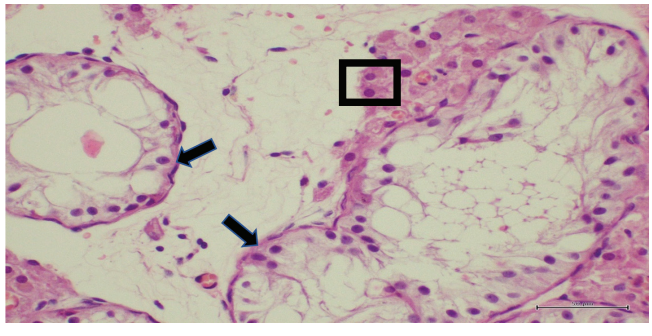

Figure 6. Histopathological view of the gonad tissue. Two seminiferous tubules are shown in the picture. The arrows show the Sertoli cells and the square shows the Leydig cells. The germ cells supposed to be seen together with Sertoli cells are not seen in seminiferous tubules (germ cell aplasia). 154x76mm (236 x 236 DPI).

DISCUSSION

The clinical spectrum of NR5A1 mutations is quite wide. It can cause female phenotype or ambiguous genitalia in 46, XY individuals in severe disease, whereas it could present only with infertility in mild disease. Adrenal insufficiency may accompany in some cases although this would be seen mostly with homozygote mutations (3). Amongst the reported NR5A1 mutations, there are 188 different mutations in 238 patient (7). The most frequently associated clinical manifestation with NR5A1 mutation is partial gonadal dysgenesis (7). The other clinical manifestations are 46,XY complete gonadal dysgenesis, infertility and primary ovarian insufficiency. Of 238 cases, only 3% were homozygote and the rest were all heterozygote (including 4 compound heterozygote cases with unknown position and 2 compound heterozygote cases at cis position). Our case's mutation is a novel mutation; never has been reported in the literature so far and allele frequency is 0.0% in all databases. The mutation is located in the ligand-binding domain region of the NR5A1 gene. The region is a exonic hotspot region and several pathogenic/likely pathogenic reported variants were found surrounding this variant in exon 6 without any missense benign variants. It's an inframe deletion and not in a repetitive region. In our case, it can be anticipated that the mutation which detected as deletion of 15 base in one allele of NR5A1 gene and caused loss of 5 amino acids in the protein synthesis; could change the structure of the new protein and disturb the function of the protein. The nature of the variation being de novo and partial gonadal dysgenesis in the clinical manifestation would support the pathogenicity of the change.

Differential diagnosis of pubertal virilization in patients with 46, XY DSD include partial androgen insensitivity, 5-alfa reductase insufficiency, 17-beta hydroxysteroid dehydrogenase insufficiency, and partial gonadal dysgenesis (6, 8). In cases with NR5A1 mutation, virilization can be seen in puberty during puberty due to sufficient androgen production despite dysgenetic gonads. It is still not clear why the 46, XY individuals

with NR5A1 mutation are born with insufficient virilization but they get virilization during puberty. There are various theories and one of them is that SF-1 is more prominent to reactivate the fetal Leydig Cells while luteinizing hormone (LH) is more prominent to reactivate the mature Leydig cells (9). Another explanation is that intrauterine virilization of the external genitalia structures requires significantly higher testosterone levels than the levels required for the stabilization of the Wolffian structures and 5-alpha reductase enzyme is regulated by SF-1 during the intrauterine period (9). It is thought that another member of a nuclear receptor family rather than SF-1 starts to regulate testosterone production in Leydig cells during puberty and this regulator thought to be Liver Receptor Homolog 1 (LRH-1) also known as NR5A2 (10). Studies to date shows that there is no correlation between the level of virilization at birth and the change during puberty (11). Pubertal virilization in patients with 46, XY DSD and gonadal dysgenesis should make us consider also about androgen secretion due to malign transformation of dysgenetic gonads (12). Therefore, the gonads should be moved down to scrotum if they are decided to be raised as male but if the gonads are dysgenetic and not functional they should be removed. It should not be omitted that the risk of malignancy remains even after the gonads are moved down to scrotum. Screening with physical examination, ultrasonography and tumour markers (beta-HCG, alpha-fetoprotein) should be arrenged in this cases. The gonads should be removed, and hormone replacement therapy should be started at puberty if the female gender were decided (13). In our case, high LH level was detected despite the normal testosterone levels acceptable for the male gender. A decrease in Leydig cell functions is an expected finding in patients with NR5A1 mutation or partial gonadal dysgenesis. High LH levels suggest that gonadotropins are high in order to keep the testosterone level in normal range as in our case (11).

In conclusion, partial gonadal dysgenesis due to NR5A1 mutations should be considered in the differential diagnosis of pubertal virilization of patients with 46, XY DSD. The clinical findings of the mutations of NR5A1 gene in 46, XY individuals are variable; completely female external genitalia or ambiguous genitalia in severe forms, infertility in mild forms and adrenal insufficiency (mostly with homozygote mutations) can be seen as clinical presentation. 46,XY DSD with partial gonadal dysgenesis is one of the most difficult group of disorder in terms of decision for the gender. The degree of virilization, functional capacity of the gonads, fertilization capacity, risk of the malignancy development and the choice of the patient and family should all be considered to decide for the gender. Permanent and irreversible surgical procedures should not be performed early, especially during infancy period, until the patient`s capacity to make own decision develops and they should be postponed until after pubertal ages. It should not be dismissed that significant number of them change their sexual identity after pubertal ages. Considering the socio-cultural characteristics of our patient and her family, as well as risk of malignancy it was decided that female gender and gonadectomy would be more appropriate despite partially functioning gonad.

Conflict of interest

The authors declare that they have no conflict of interest. Informed Consent Statement: The written informed consent from the minor legal guardian for the publication of many images or data included this article.

Institutional Review Board Statement

Ethical review and approval were waived for this study due to the reason that it is a case report. This paper is only a report about an adolescent who experienced pubertal virilization with a novel NR5A1 gene mutation and partial gonadal dysgenesis. The ethical approval is not applicable for the case reports.

Ethics Statement

The study of the human subjects was conducted in accordance with the guideline of the Institutional Review Board at the Aydın Adnan Menderes University, Faculty of Medicine, Aydın, Turkey, and informed consent was obtained from the participants.

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