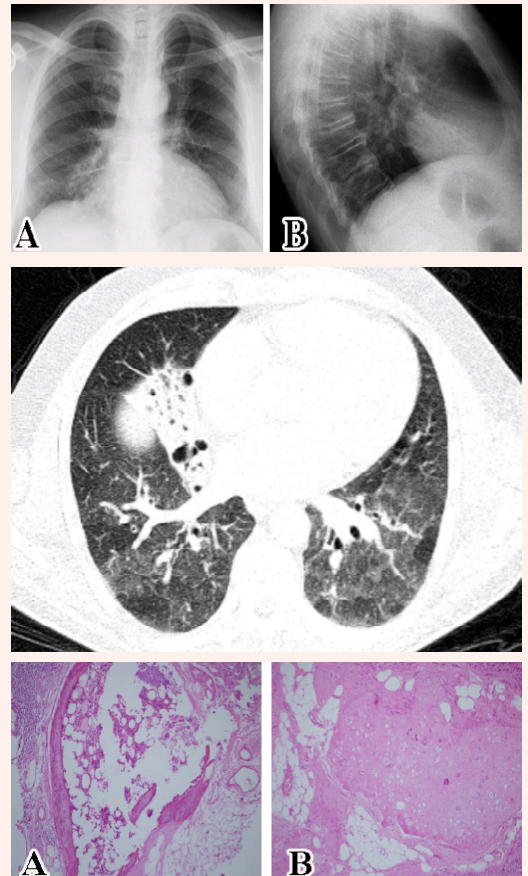




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Changes in antral follicle count and ovarian volume with age

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ABSTRACT

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We aimed to ascertain the chronologic age at which these sonographic measurements begin to show a significant difference. The study included 100 women (78, aged 19-44 and 22, aged 45-50) menstruated spontaneously and without thyroid disease, diabetes mellitus, hyperprolactinaemia, hypogonadotropic hypogonadism or polycystic ovary syndrome and any gynaecologic surgery. They all underwent sonographic measurement of total antral follicle (TAF) count and mean ovarian volume (MOV) on the second or third days of menstrual cycle. Then, the women were assigned to 5 groups: group 1, 19–24 years; group 2, 25–29 years; group 3, 30–37 years; group 4, 38–44 years; and group 5, 45–50 years. There was a significant association between chronological age and TAF number ($r^2 = 0.328$, $p < 0.001$). No significant difference was noted in TAF number among the first three groups ($r^2 = 0.58$, $p > 0.05$). TAF number was significantly different between groups 3 and 4 ($Z: -3.463$, $p < 0.001$), but not between groups 4 and 5 ($Z: -1.698$, $p > 0.05$). There was a significant relationship between chronological age and MOV ($r^2 = 0.149$, $p < 0.001$). MOV values did not show a significant difference among the first 4 groups ($r^2 = 0.58$, $p > 0.05$; $r^2 = 7.87$, $p > 0.05$ respectively). The MOV of group 5 was significantly different from those of groups 1, 2, 3, and 4 ($Z: -2.75$, $p < 0.01$; $Z: -4.351$, $p < 0.01$; $Z: -2.722$, $p < 0.01$; and $Z: -2.829$, $p < 0.01$ respectively). In conclusion, the TAF count decreases significantly beginning at 38 years of age, while MOV decreases significantly beginning at 45 years of age. Thus, there is no significant decrease in the ovarian follicular reserve until the age of 38 years, or in the ovarian volume until the age of 45 years.

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1. Introduction

Oocyte numbers progressively reduce during the reproductive years by ovulation and atresia. Therefore, concentration of follicles in women > 35 years is only one-third of that in younger women. At a mean age of 45 years, menstrual cycles become irregular and the number of wasting follicles decrease below a few thousand and then 1000 at menopause (Lass et al., 1997; Johnson et al., 2006; Kwee et al., 2007; Hansen et al., 2011).

A mathematical linear model has been developed that predicts the rate of follicular decline with advancing age. A biexponential decline is noted in follicular depletion, with

an acceleration in oocyte loss when the remaining oocyte number equals approximately 25,000. In this model, the decline starts at the age of 37 years and menopause occurs in approximately 10 years. At this point, the rate of follicular atresia accelerates. The number of follicles diminishes to a few thousand at approximately 45 years of age and menopause, the median age of which is 51 years in western countries, occurs when the number reaches 1000 follicles. This number is reported to be the critical threshold for maintaining the menstrual cycles (Faddy et al., 1992; Johnson et al., 2006; Kwee et al., 2007; Cedars and Evans, 2008; Hansen et al., 2011).

Antral follicle (AF) count and mean ovarian volume (MOV) measurement by ultrasound are frequently used to estimate ovarian reserve. AF count has been reported to be the most reliable sonographic test for estimating the primordial follicle cohort (Domingues et al., 2010; Hsu et al., 2011). It is better to interpret these tests in conjunction with chronological age of the women in order to identify the group who have diminished ovarian reserve (Sills et al., 2009). However, there are conflicting reports about whether there are significant age-related differences in sonographically measured ovarian volume during the period before the usual age of menopause (Andolf et al., 1987; Merz et al., 1996; Christensen et al., 1997; Pavlik et al., 2000; Oppermann et al., 2003).

In this prospective clinical study, which examines changes in AF number C and ovarian volume in women ranging from early reproductive to perimenopausal age, we aimed to ascertain the chronologic age at which these sonographic measurements begin to show a significant difference.

2. Materials and methods

The study was conducted at the Medical School Hospital of Ondokuz Mayıs University, approved by the Local Ethics Committee. Informed consent was obtained from each subject prior to enrolment in the study.

Healthy, non-pregnant and non-menopausal women who visited to our outpatient clinic for routine gynaecologic control examinations were considered to be eligible for this study. The study included 100 women; 78 women were aged between 19 and 44 years and had regular spontaneous menstrual cycles, whereas 22 women were aged between 45 and 50 years and had regular or irregular spontaneous menstrual cycles. All the women menstruated spontaneously; had 2 intact ovaries; showed no evidence of thyroid disease, diabetes mellitus, significant hyperprolactinaemia, hypogonadotropic hypogonadism, or polycystic ovary

syndrome; and had no history of any gynaecologic surgery.

The women underwent sonographic measurement of total antral follicle (TAF) count and MOV. Following these measurements, the women were assigned to 5 groups based on their age: group 1, 19–24 years; group 2, 25–29 years; group 3, 30–37 years; group 4, 38–44 years; and group 5, 45–50 years.

Sonographic measurements were performed on the second or third days of the menstrual cycle. For the AFC, round- or oval-shaped echo-free structures from 2 to 6 mm in diameter within the ovaries were considered to be follicles. TAF count was estimated as the sum of the number of antral follicles measured in both ovaries. Ovarian volume measurement and AFC were performed by using a two-dimensional (2D) abdominal probe (in virgin women) and an endovaginal probe of 6.5 MHz frequency (Siemens Medical Solutions, Issaquah, WA, USA). For each ovary (n = 200), a set of 2D images was stored to facilitate measurement of the standard 3 planes used for volume calculation. An ellipsoid formula ($D1 \times D2 \times D3 \times \pi/6$) which is composed of measurement of three perpendicular directions is used to calculate the volumes of the ovaries. The mean of the volumes of both ovaries was used as the MOV. All ultrasound scan procedures and measurements were performed by a single operator.

Statistical analysis was performed using the Student's t-test, Kruskal-Wallis variance analysis, the Mann-Whitney U Test, regression analysis. Receiver operating characteristic (ROC) analysis was used to determine the sensitivity and specificity and the data were presented as the mean \pm standard deviation (SD). A p value of <0.05 was considered to be significant.

3. Results

A comparison of some demographic properties of the study groups was shown in Table 1. The TAF number was 10.0 ± 3.89 in group 1, 11.09 ± 4.30 in group 2, 10.38 ± 4.37 in group 3, 6.30 ± 2.97 in group 4, and

Table 1. Comparison of some demographical properties of the study groups (Mean \pm Standard Deviation). The different letters (a, b, c, d, e) show a statistically significant difference ($p < 0.05$) between the groups.

Demographic Characteristics	Group-1 (n:13) (age:19-24)	Group-2 (n:21) (age:25-29)	Group-3 (n:21) (age:30-37)	Group-4 (n:23) (age:38-44)	Group-5 (n:22) (age:45-50)
Age (year)	22.0 \pm 1.73 (a)	26.61 \pm 1.35 (b)	34.04 \pm 2.08 (c)	41.39 \pm 1.80 (d)	47.54 \pm 1.71 (e)
BMI (kg/m ²)	22.0 \pm 3.24 (a)	22.23 \pm 3.12 (a)	25.52 \pm 5.10 (b)	26.60 \pm 4.96 (b)	28.81 \pm 4.61 (b)
Cycle length (day)	30.23 \pm 6.09 (a)	27.71 \pm 3.06 (a)	29.52 \pm 5.90 (a)	31.52 \pm 9.07 (a)	38.54 \pm 14.42 (a)
Bleeding duration (day)	5.84 \pm 1.28 (a)	5.76 \pm 1.67 (a)	5.71 \pm 2.81 (a)	6.21 \pm 2.85 (a)	10.13 \pm 4.43 (b)
Gravida	--	0.46 \pm 1.12 (a)	2.0 \pm 1.65 (b)	3.04 \pm 1.70 (b)	4.19 \pm 1.96 (c)
Parity	--	0.46 \pm 1.12 (a)	1.45 \pm 0.99 (b)	2.40 \pm 1.25 (c)	3.0 \pm 1.37 (c)
Basal FSH mIU/ml	7.12 \pm 5.52 (a)	6.47 \pm 1.60 (a)	5.68 \pm 2.50 (a)	9.22 \pm 5.05 (b)	17.84 \pm 13.64 (c)
MLP (ng/ml)	12.23 \pm 5.36 (a)	11.98 \pm 4.41 (a)	8.21 \pm 4.07 (b)	4.38 \pm 2.95 (c)	0.66 \pm 0.77 (d)

4.77 ± 2.11 in group 5. The MOV value was 6.16 ± 1.90 cm³ in group 1, 6.71 ± 3.77 cm³ in group 2, 6.28 ± 3.77 cm³ in group 3, 4.84 ± 1.62 cm³ in group 4, and 3.77 ± 0.76 cm³ in group 5.

There was a significant association between chronological age and TAF number ($r^2 = 0.328$, $p < 0.001$). As is seen in Fig. 1, TAF number began to decrease at approximately 25 years of age. Although a significant correlation was not found between chronological age and TAF number in women between the ages of 19 and 37 years ($r^2 = 0.002$, $p > 0.05$), there was a significant correlation between age and TAF number in women between the ages of 38 and 50 years ($r^2 = 0.179$, $p < 0.05$).

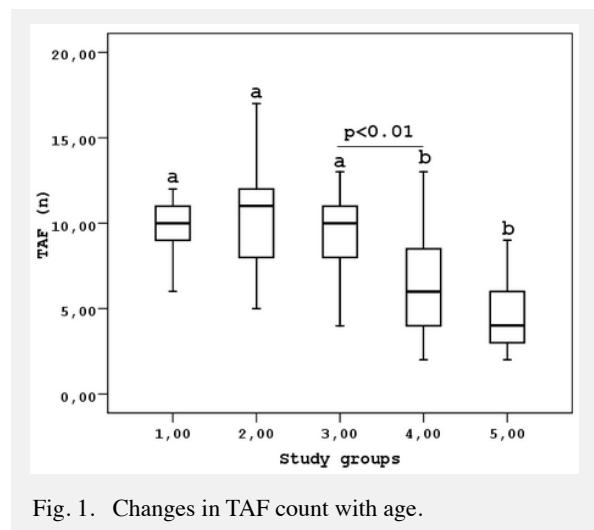


Fig. 1. Changes in TAF count with age.

No significant difference was noted in TAF number among the first three groups ($r^2 = 0.58$, $p > 0.05$) (Table 2). TAF number was significantly different between groups 3 and 4 ($Z: -3.463$, $p < 0.001$), but not between groups 4 and 5 ($Z: -1.698$, $p > 0.05$). The optimal cut-off value of the AF number for discrimination between groups 3 and 4 was determined through ROC analysis. The optimal cut-off value for the TAF count for discrimination between groups 3 and 4 was found to be 7. Based on this cut-off value, Table 3 shows the discriminative values for TAF number between groups 3 and 4.

Overall, there was a significant relationship between chronological age and MOV ($r^2 = 0.149$, $p < 0.001$). MOV began to decrease at 30 years of age. Although no significant correlation was noted between age and MOV in women between 30 and 44 years of age ($r^2 = 0.081$, $p = 0.175$), the correlation in women between 30 and 50 years of age was significant ($r^2 = 0.146$, $p = 0.01$) (Fig. 2).

MOV values did not show a significant difference among the first 4 groups ($r^2 = 0.58$, $p > 0.05$; $r^2 = 7.87$, $p > 0.05$ respectively) (Table 2). The MOV of group 5 was significantly different from those of groups 1, 2, 3,

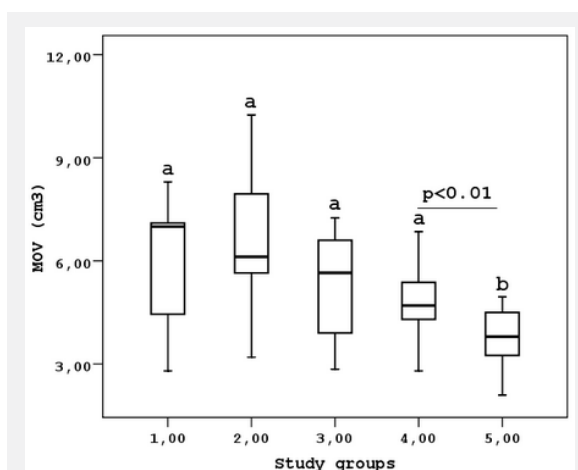


Fig. 2. Changes in MOV values with age.

and 4 ($Z: -2.75$, $p < 0.01$; $Z: -4.351$, $p < 0.01$; $Z: -2.722$, $p < 0.01$; and $Z: -2.829$, $p < 0.01$ respectively). The optimal cut-off value of MOV to discriminate between groups 4 and 5 was found to be 4 cm³. With regard to this cut-off value, the discriminative features of MOV between groups 4 and 5 are shown in Table 3.

Table 2. Comparison of TAF count and MOV among the study groups.

Sonographic measurements	Study Groups	Mean Rank	X ²		Mean Rank	X ²	
			P			P	
TAF	1	64.00			26.15		
	2	70.29			30.02	0.58	NS
	3	65.79	42.84	<0.001	27.12		
	4	36.41					
	5	23.77					
MOV	1	60.46			43.27		
	2	68.10			48.57	7.87	NS
	3	54.93	23.51	<0.001	38.60		
	4	46.33			29.91		
	5	27.95					

TAF: Total antral follicle, MOV: Mean ovarian volume, NS: Not significant ($p > 0.05$).

Table 3. Sensitivity, specificity, PPV, NPV and AUC values of the TAF count and MOV according to their optimal cut off values for the discrimination of the study groups.

Sonographic measurements	Cut off value	Group discrimination	Sensitivity %	Specificity %	PPV %	NPV %	ROC AUC	P Value	95% CI
TAF (count)	7	Between 3 and 4	77.8	65.4	60.9	81	0.803	0.001	0.675 - 0.932
									0.598 - 0.894
MOV (cm ³)	4	Between 4 and 5	82.6	63.6	70.4	77.8	0.746	0.005	-

TAF: Total antral follicle count MOV: Mean ovarian volume PPV: Positive predictive value, NPV: Negative predictive value, ROC-AUC: Area under the roc curve.

4. Discussion

Ovarian reserve has been found to be correlated with ovarian volume and antral follicle number measured by ultrasonography in the early follicular phase. However, interobserver variations regarding AFC could occur (Coccia and Rizzello 2008; Younis et al., 2010). Measurement of ovarian volume can be easily performed using ultrasound. Individual estimates of ovarian volume using 2D and three-dimensional (3D) ultrasound techniques do not differ in their precision (Brett et al., 2009). Real-time 2D pelvic ultrasonography is accepted more reliable method for determining ovarian volume and morphology. Transvaginal ultrasonography is shown to decrease the inter and intraobserver measurement differences (Kwee et al., 2007). Stored 3D data for predictive value of AFC which was calculated by 2D equivalent technique and two 3D techniques (multiplanar view, rendered inversion mode) was used in a study by Jayaprakasan et al. AFC measured by any of the 3 techniques was reported to be good predictor for poor ovarian response with optimal cut-off value of 6 or 7 follicles. In the present study, 2D pelvic ultrasonography was used.

There is reportedly a positive correlation between AFC and ovarian primordial follicle count; this is shown to remain significant even after adjustment for chronological age (Scheffer et al., 2003; Kline et al., 2005; Lutchman Singh et al., 2007; Hansen et al., 2011). It has been shown to be sufficient for predicting ovarian reserve if there are ≥ 5 antral follicles with a diameter of 2 to 5 mm on day 1 to 2 (Sills et al., 2009). The methodology for AFC differs among centres. Follicles of 2 to 5 mm or 2 to 10 mm predominantly are counted. When production of anti-Mullerian hormone (AMH) or inhibin B are compared according to the follicle sizes, pre-antral and smaller antral follicles of sizes up to 4 to 6 mm produce AMH, whereas inhibin B is produced by the follicles of sizes up to 13 mm. There is significant relationship between the number of small antral follicles (2–6 mm) and woman age; it is also significantly related to all endocrine ovarian reserve tests that we assessed, independent of age (Haadsma et al., 2007; Kwee et al., 2007). It was shown that functional ovarian reserve could be shown by the number of small antral follicles measuring 2 to 6 mm, independent of age. Therefore, it was proposed, only counted antral follicles of sizes 2 to 6 mm should be used to evaluate the outcome of endocrine ovarian reserve (Haadsma et al., 2007). Transvaginal ultrasonography can easily detect these follicles containing small amount of antral fluid in early antral phase (Kwee et al., 2007). Therefore, in our study, we counted the follicles between 2 and 6 mm.

In the present study, a significant difference was not noted among the TAF counts of the first 3 groups ($p > 0.05$) (Table 2 and Fig. 1). Optimal sensitivity and specificity values occurred at a cutoff TAF number of 7

to distinguish women younger than 37 years from those 37 years or older ($p < 0.01$) (Table 3). It was observed that the decrease in TAF count could be sonographically established beginning at age 25 years, and that this decrease was found to be significant beginning at the age of 38 years ($p < 0.05$) (Fig. 1).

MOV increase from 0.7 ml to 5.8 ml between 10 to 17 years. During reproductive period, there is no significant change in ovarian volume, however after 40 years old, a dramatic decline in ovarian volume which appears to be related with decreased oestrogen because when oestrogen is replaced decline in ovarian volume is not decreased (Kwee et al., 2007). In the present study, none of the participants underwent any oestrogen treatment and all had spontaneous menstruation.

An earlier study, no parity or age-related change was observed in ovarian volume of 155 premenopausal women aged 16 to 52 years (Merz et al., 1996). Moreover, the ovarian volume was measured in 428 women, aged 14 to 45 years, who visited a family planning clinic. It was reported that no correlation between the age and ovarian volume was found (Christensen et al., 1997). It was stated that, ovarian volume significantly decreases every decade from 30 to 70 years (Pavlik et al., 2000). A negative relationship between ovarian volume and age-not menopausal age- was found in a study including 337 women aged 40 to 70 (Andolf et al., 1987). In another study, ovarian volume was found to decrease beginning from 40 years and suggested to reach maximal value at the age of 39 years. It was found that ovarian volume was significantly less in all age groups over 40 years compared with the 35–39 years age group (Oppermann et al., 2003). In another former study, it was found that MOV was significantly less in women aged 35 years or older than in women younger than 35 years (Lass et al., 1997). In the present study, ovarian volume was found to reach a maximum value at approximately 30 years of age, and decrease became significant beginning at 45 years of age ($p < 0.05$) (Fig. 2). These results differ from the findings of Lass et al. Moreover, the fact that no significant difference was noted among the MOV of the first 4 groups strengthened the finding that a significant decrease in volume is noted after the age of 44 years (Table 2). In the present study, optimal sensitivity and specificity values were found at a cut-off MOV of 4 cm³ to distinguish the ovarian reserve between women younger than 45 years and those aged 45 years or older ($p < 0.01$) (Table 3).

There are several strengths of the present study. First, all participants younger than 45 years (78% of total) had regular spontaneous menstrual cycles. Second, the basal FSH level was less than 10 mIU/mL in 90% of the participants younger than 45 years. Third, none of the participants had any ovarian or endocrine disorder. Fourth, except for age, none had any factors that might affect the ovarian reserve. Fifth, sonographic

measurements were assessed without knowledge of the woman age, which eliminated the possibility of bias.

In conclusion, we found that the TAF count decreases significantly beginning at 38 years of age,

while MOV decreases significantly beginning at 45 years of age. Thus, there is no significant decrease in the ovarian follicular reserve until the age of 38 years, or in the ovarian volume until the age of 45 years.

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Correlation of phenotype with the CYP21 gene mutation analysis of classic type congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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ABSTRACT

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Ambiguous genitalia is seen as the most common phenotypic reflection of sexual development disorders. Congenital adrenal hyperplasia (CAH) is the most common cause of ambiguous genitalia, while the most common cause of CAH is a 21-hydroxylase deficiency with a rate of 90-95%. The disease is caused by mutations in the CYP21A2 gene located at 6p21.3. It is inherited in an autosomal recessive manner. Seven previously identified point mutations, an 8-bp deletion and large deletions, have significant role in the etiology of the disease. In this study, we aimed to report CYP21 molecular genetic evaluation by RFLP and MLPA methods in classic CAH patients with 21-hydroxylase deficiency. In this study, 26 patients with pre-diagnosis of Classic Type Congenital Adrenal Hyperplasia due to 21-Hydroxylase deficiency were reported. Seven previously identified point mutations, an 8-bp deletion, and large deletions were analyzed by PCR-RFLP methods in the patient group. For the MLPA study, SALSA MLPA KIT P050-B2 CAH (Lot0408) kit which was produced by MRC Holland was used. In 21 (80.7%) of 26 patients analyzed, causative mutations were found. The most frequent mutation was the large deletions (6 patients, 12 alleles), accounting 23% of the patients. In 21 (80.7%) of 26 patients, the causative mutations were found by using PCR (8-bp del. and large deletions) and RFLP (7 known point mutations) methods. MLPA analysis confirmed all of the deletions detected by PCR-RFLP, and the 83% of the detectable point mutations with MLPA. A complete genotype-phenotype relationship could be established in all patients in whom mutation could be detected in the study group.

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1. Introduction

Ambiguous genitalia is seen as the most common phenotypic reflection of sexual development disorders. Congenital adrenal hyperplasia (CAH) is the most common cause of ambiguous genitalia, while the most common cause of CAH is a 21-hydroxylase deficiency with a rate of 90-95% (White and Speiser, 2000). 21-Hydroxylase enzyme allows the production of 11-Deoxycortisol from progesterone and

11-Deoxycorticosterone from 17 α -OH-Progesterone. The shift of the resulting precursors to androgenic pathways results in an androgenic burden that begins in the prenatal period for a patient with 21-hydroxylase deficiency (21-OHD) (Ohlsson et al., 1999). While this androgen excess does not have a serious effect on the sexual development of male fetuses, it causes virilization in the female fetus. Fetal adrenal glands start the enzyme synthesis in 6-8th weeks; in this

period urogenital sinus is in the vaginal and urethral canalization stage and because of this androgen burden, urogenital sinus may not be able to separate, clitoral tissue may enlarge and the labioscrotal folds may fuse. Since the vagina in the 12th week is separated from the urogenital sinus, androgen exposure after this period can cause only the clitoromegaly. The affected female fetus is typically born with Ambiguous genitalia or with external genitalia that is similar to a male with hypospadias and undescended testes (Rimoin, et al., 2007).

Because of the defect in the synthesis pathway leading from the progesterone to Aldosterone, 3 out of 4 patients with “**Classic CAH**” do not produce sufficient levels of aldosterone. Patients with both androgen excess and mineralocorticoid deficiency are associated with the “**Salt-wasting Type CAH**” phenotype. The concentration of potassium in the extracellular fluid is significantly increased, the concentrations of sodium and chlorine are reduced. Total excretion of fluid and blood volume is significantly reduced. On the Clinical side the manifestations could be so severe that shock status may develop. In 21-OHD the newborns with salt wasting, are in the risk of rapid progression to mortal stage because of aldosterone and cortisol deficiency. In untreated cases, hyponatremia-hyperkalemia and hypovolemic shock may cause the newborn's death with Adrenal Crisis within 1-4 weeks after delivery (Rimoin et al., 2007).

The phenotype is defined as “**Simple Virilizing Type CAH**” in cases where the production of mineralocorticoids is not affected very much and androgen excess are at the forefront. In men, accelerated linear growth, pubic hair growth, increased penis size, and testicular enlargement may occur. In females, clitoral enlargement, occurring of facial acne, ovarian dysfunction and hirsutism can be seen.

The patients who are not born with ambiguous genital but are affected by postnatal androgen excess constitute “**Non-classical CAH**” patients. Females are mostly affected by irregular menstruation, hirsutism and occurring of facial acne (Kelestimur et al., 2009). Males are usually asymptomatic, if they are affected, acne and infertility can be seen. The frequency of the disease is estimated at 0.1% in the general population (Torresani and Biason-Lauber, 2007; Concolino et al., 2009).

The disease is caused by mutations in the CYP21A2 gene located at 6p21.3. It is inherited in an autosomal recessive manner. The frequency of the disease is 1 in 15.000 live births in the classical form and 1 in 1000 live births in milder forms Steroid 21-Hydroxylase (P450c21) is encoded by the CYP21 (CYP21B or CYP21A2) gene. There is a pseudogene (CYP21P, CYP21A1P or CYP21A) in the close proximity of the CYP21 gene. Studies in families where CYP21P

has been completely lost have proven that this gene is completely dysfunctional. As CYP21 and CYP21P have approximately 98% sequence homology and settle at a distance of approximately 30 kb, the “Unequal Crossing-over” events may occur between these genes. As a result, deletions and duplications may occur in the functional gene. Previously, “Gene Conversion” mechanism of transmission of mutations from the CYP21P gene to the CYP21 gene was suggested. Seven previously identified point mutations, an 8-bp deletion and large deletions, which are mediated by these mechanisms, have a significant role in the etiology of the disease (Torresani and Biason-Lauber, 2007).

The clinical effects of these mutations are associated with the loss of enzyme activity they produce. The mutations are classified according to the degree to which the enzyme activity is affected (Wilson et al., 1995):

- **Type-A:** Genotypes with complete loss of enzyme activity: (Large deletions, wide conversions, 8-base pair deletion, E6 cluster mutations, Q318X and R356W)

- **Type-B:** Genotypes leading an enzyme activity around 1% (IVS2 mutations and I172N)

- **Type-C:** Genotypes leading an enzyme activity around 20-60 % (V281L and P30L)

The CAH phenotypes are associated with these genotypes are defined as follows:

- **Salt-wasting Type CAH:** Type-A/ Type-A genotype

- **Simple virilizing type CAH:** Type-A/ Type-B or Type-B/ Type-B genotypes

- **Non-classical type:** Type-A / Type-C, Type-B / Type-C and Type-C/ Type-C genotype

Previous studies about the relevant genotype-phenotype association, has shown that it brings about 80% accuracy of prediction (White and Speiser, 2000).

In this study, we aimed to report the result of the molecular genetic evaluation of CYP21 gene by two different methods, RFLP and MLPA, in classic CAH patients with 21-OHD and to determine the frequency of the mutations and deletions, correlate the results with the phenotypes and to compare the effectiveness of the two molecular analysis methods used.

2. Material and methods

Patient group

This study involved 26 patients with pre-diagnosis of Classic Type Congenital Adrenal Hyperplasia due to 21-OHD. The patients were followed by the Departments of Pediatric Endocrinology and Medical Genetics of Ondokuz Mayıs University, between 2005-2010. Informed consent forms have been signed by the patients or their parents. This study was approved by Ondokuz Mayıs University research ethics committee.

Chromosome analysis

Chromosomes were obtained by peripheral venous blood lymphocyte culture using modified synchronization method (Rooney and Czepulkowski, 2001). GTG banding were used to determine karyotypes.

Isolation of DNA from peripheral blood

Peripheral blood samples were taken from patients and DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche, Germany).

Cyp21 RFLP analysis

Seven previously identified point mutations, an 8-bp deletion, and large deletions were analyzed by PCR-RFLP methods in the patient group. For the PCR amplifications, 10 different primers were used (Sadeghi et al., 2008). Four different PCR amplifications (Fragments 1, 2, 3, 4) were performed for 7 point mutations. For the PCR amplifications of the CYP21 gene, specific primers were used that did not amplify pseudogene. The four DNA fragments specific to alleles of the 7 point mutations were amplified by PCR. Two additional PCR amplifications (Fragment 5 and 6) were performed to detect 8-bp deletion or large deletions. The resulting PCR products were digested with the appropriate restriction enzymes. The restriction products were visualized on 3% agarose gel prepared with Ethidium Bromide. The DNA fragments analyzed after the enzyme cut-up was normal to the normal allele, mutant allele, or both, and allowed the wild-type, homozygous mutant or heterozygous expression to be assessed, respectively. All PCR reactions and Restrictions were carried out according to the methods that were previously reported (Sadeghi et al., 2008).

In the case of the presence of mutations E6 Cluster (I236N / V237E / M239K), V281L, Q318X, and R356W in the amplified fragment 1, the recognition site of the MboI, Alw21I, PstI, and AciI enzymes were lost respectively. In this case, a single fragment consisting

of their total lengths was detected instead of the two fragments in the enzyme-cut products. Similarly, an enzyme recognition site of AciI enzyme was lost in Fragment 4 in the presence of the mutation.

In Fragment 2 and Fragment 3, the corresponding mutations create a novel enzyme recognition site for the AluI and BseII enzymes, respectively, where a fragment at that point was divided into two smaller fragments.

The amplified PCR products (Fragments), analyzed mutations and the expected fragment lengths after the restriction reactions were summarised in Table 1.

MLPA

For the MLPA study, SALSA MLPA KIT P050-B2 CAH (Lot0408) kit which was produced by MRC Holland was used. The kit included probes for CYP21A2, CYP21A1P, TNXB, C4A, C4B, and CREBL1 genes. The reactions were carried out according to the manufacturer's protocols.

The PCR products obtained as a result of MLPA reaction were run on the Beckman Coulter CEQ8800 capillary electrophoresis device. As a result of the process, peak images and peak areas of the probes of each sample were obtained in the CEQ program. Excel based Coffalyser 9.4 program was used for the analysis.

3. Results

Patients

Of the 26 patients, 15 were consulted for ambiguous genitalia, 3 were consulted for problems such as genital hyperpigmentation and hirsutism. 8 of the patients presented with complaints such as feeding problems, vomiting, weakness, and prolonged jaundice.

In 8 of 26 patients, consanguinity was defined between parents.

The preliminary phenotype was identified as virilized female genitalia in 16 of 26 patients and all 16 of them were karyotyped as 46, XX.

Table 1. Mutations, fragments, the expected fragment lengths and detected allele frequencies.

Fragment	Mutation	Protein Change/ Other name	Rest. Enz.	Wild Type Frag. (bp)	Mutation Frag. (bp)	Allele Frequencies
F1	c.[710T>A;713T>A;719T>A]	p.[Ile237Asn;Val238Glu;Met240Lys] (E6 Cluster)	MboI	349.336	685	3.5%
F1	c.844G>T	p.Val282Leu (V281L)	Alw21I	99.17	1161	1.7%
F1	c.955C>T	p.Gln319Ter (Q318X)	PstI	298.154	452	16%
F1	c.1069C>T	p.Arg357Trp (R356W)	AciI	189.3	219	7.1%
F2	c.293-13A>G(659A>G)	(IVS2)	AluI	51	34,17	14.2%
F3	c.518T>A	p.Ile173Asn (I172N)	BseII	231	217,14	14.2%
F4	c.92C>T	p.Pro31Leu (P30L)	AciI	153.43	196	3.5%
F5	8-bp deletion	-	-	64.56	56	-
F6	Large deletions	-	-	789	-	23%

Additional laboratory findings

Serum levels of 17-hydroxyprogesterone were found to be high in all 26 patients.

In 11 of 26 patients (1,3,9,11,14,15,18,21,24,25,26), hyponatremia and hyperkalemia were detected at admission.

Phenotype-karyotype

In our patient group, the initial determination of sex revealed that the dominant phenotype was male in 11 patients and the dominant phenotype was female in 4 patients. The remaining 11 patients were defined as ambiguous genitalia. Chromosome analysis of 16 of the 26 patients was determined as 46, XX, 10 of the 26 patients was determined as 46, XY. All the patients with XY karyotype had male genitalia.

CYP21 RFLP results

In 21 (80.7%) of 26 patients analyzed, causative mutations were found. In 4 more patients the mutation was detected (25 patients; coverage, 96.2%) in the heterozygous state. In one patient, no mutations were detected.

Of the 56 alleles evaluated, 46 (82%) had mutations. The most frequent mutation was the large deletions (6 patients, 12 alleles), accounting 23% of the patients. The most frequent point mutations were **c.955C>T** (Q318X) (9 alleles, 16%), **c.293-13A>G** (IVS2) (8 alleles, 14.2%), **c.518T>A** (I172N) (8 Allels, 14.2%), **c.1069C>T** (R356W) (4 Allels, 7.1%), **c.92C>T** (P30L) (2 Allels, 3.5%) and **E6 cluster** (2 Allels, 3.5%) and **c.844G>T** (V281L) (1 Allel, 1.7%).

The 8-bp deletion was not detected.

MLPA results

All the deletions detected by RFLP (6 patients) were confirmed by MLPA method.

In 10 of our 12 patients who had at least one of the I172N, E6 cluster, and Q318X mutations, MLPA analysis showed RPR values lower than 0.7, supporting the presence of mutations.

All the clinical findings, karyotype, RFLP and MLPA results were summarised in Table 2.

4. Discussion

The cause of more than 90% of cases in virilizing CAH is a 21-OHD In classical disease, females may be born with virilized external genitalia due to prenatal excessive androgen exposure. Since three-quarters of the cases cannot synthesize enough aldosterone, they experience lethal sodium and potassium imbalances if not treated. The disease is the result of mutations in CYP21, a steroid 21-hydroxylase gene. More than 90% of mutations result from intergenic recombinations between CYP21 and pseudogene CYP21P (Wilson et al., 1995; White and Speiser, 2000; Dolž et al., 2005; Torresani and Biason-Lauber, 2007).

Several studies have been conducted in different populations to determine the frequency of mutations in classical CAH due to 21-OHD In 2005, Dolzan et al. performed CYP21 genotyping with PCR-SSP, PCR-SSO, Southern Blotting and sequence analysis in patients with CAH (Classic and Non-Classical) from middle European region (Austria, Czech Republic, Hungary, Slovakia, Slovenia). As a result, 98% of genotyped patients had mutations. The most frequent detected alleles were **IVS2 (31%)**, **deletions (28%)**, **I172N (14.5%)**. It was suggested that these 7 known mutations and deletions cover 85.9% of the mutant alleles and that the remaining alleles were formed by rare mutations and minor conversions (Dolž et al., 2005). In 1999, a study was performed by Ohlsson et al. in Denmark. CYP21 genotyping was performed using the sequence analysis method in 68 patients with CAH (Classical and Non-Classical). As a result of the study, deletions (36%) were found to be the most frequent mutations. **The IVS2 mutation (33.8%)**, **I172N mutation (10.3%)**, Q318X mutation (8.8%), V281L mutation (4.4%) were listed as other frequent mutations (Ohlsson et al., 1999). In 2004, Kharrat et al. Performed CYP21 genotyping with PCR, RFLP and sequence analysis in 25 Tunisian patients with classic CAH. **35.3% of the patients had Q318X, 19.6% had deletions, 17.6% had IVS2** and 10.8% had I172N. They found that 5.9% had no mutations.

Similar to the ones in other countries, studies have also been conducted to determine the frequency of mutations in Turkey. In 2008 Sadeghi et al. released the mutation analysis results of 100 patients with classic CAH. In this study, they have reported that the most frequent mutation were IVS2 (28.5%), deletions (17%), Q318X (11.5%), I172N (4%), V281L (3.5%), R356W (3.5%) and 8-bp deletion (3%)(Sadeghi, et al., 2008). In 2009, Baş et al., analysed the eight most common point mutations in 56 patients. They have introduced that the most common mutation were: IVS2 (22.0%), large conversion (14.3%), I172N (9.9%) R356W (8.8%), and large deletion (6.6%) (Baş et al., 2009). In 2013, Toraman et al., published the CYP21A2 mutation analysis results of 48 CAH patients (Toraman et al., 2013). They have reported that among identified mutations, previously described IVS2, large rearrangements and Q318X mutations were the most common mutations (Toraman et al., 2013). In 2014, Kirac et al. characterized the mutations in 124 Turkish CAH patients. They have reported that IVS2, 8-bp deletion, and large rearrangements were the most frequent homozygous mutations in the salt wasting form (Kirac et al., 2014).

In our study, the most frequent mutation was a **large deletion (23%)**. The most frequent point mutations were **c.955C>T (Q318X) (9 alleles, 16%)**,

Table 2. Ages, admission cause, phenotype, karyotype results, molecular genetic results and clinical diagnosis.

Patient #	Age	Admission Cause	Genital phenotype	Salt Wasting	Karyotype	CYP21 RFLP	CYP21 MLPA	Diagnosis
#1	11 day-old	Jaundice	Virilised female genitalia	(+)	46, XX	Homozygous c.293-13A>G (IVS2)	Normal	Salt Wasting Type Classical CAH
#2	7 day-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	-	Normal	Simple Virilizing Classical CAH ?
#3	26 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous Large Deletion	CYP21 Exon 2, 3 Deletion	Salt Wasting Type Classical CAH
#4	9 year-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Homozygous c.518T>A (I172N)	Homozygous c.518T>A	Simple Virilizing Classical CAH
#5	2 year-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Comp. Het. c.955C>T/ c.518T>A	Heterozygous c.518T>A (c.955C>T:ND)	Simple Virilizing Classical CAH
#6	1 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous c.955C>T (Q318X)	Homozygous c.955C>T	Salt Wasting Type Classical CAH
#7	2 year-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Heterozygous c.518T>A (I172N)	Heterozygous c.518T>A	Simple Virilizing Classical CAH
#8	3 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous Large Deletion	CYP21 Exon 3, 4 Deletion	Salt Wasting Type Classical CAH
#9	16 month-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Homozygous c.518T>A (I172N)	Homozygous c.518T>A	Simple Virilizing Classical CAH
#10	5 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous c.955C>T (Q318X)	Homozygous c.955C>T	Salt Wasting Type Classical CAH
#11	5 month-old	Vomiting	Male with hyper pigment.	(+)	46, XY	Homozygous c.1069C>T (R356W)	Normal	Salt Wasting Type Classical CAH
#12	1 month-old	Genital Hyperpigment.	Male with hyper pigment.	(-)	46, XY	Comp. Het. c.844G>T/c.92C>T	Normal	Simple Virilizing / Non Classical?
#13	1 month-old	Vomiting	Male with hyper pigment.	(+)	46, XY	Heterozygous c.955C>T (Q318X)	Heterozygous c.955C>T	Salt Wasting Type Classical CAH
#14	5 month-old	Vomiting	Male	(+)	46, XY	Homozygous c.293-13A>G (IVS2)	Normal	Salt Wasting Type Classical CAH
#15	2 year-old	Hypervirilisation	Male with hyper pigment.	(-)	46, XY	Heterozygous c.518T>A (I172N)	Heterozygous c.518T>A	Simple Virilizing Classical CAH
#16	1 day-old	Vomiting	Male	(-)	46, XY	Comp. Het. c.955C>T/c.92C>T	Normal (c.955C>T:ND)	Simple Virilizing Classical CAH
#17	2 month-old	Vomiting	Male	(+)	46, XY	Homozygous c.1069C>T (R356W)	Normal	Salt Wasting Type Classical CAH
#18	2 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous Large Deletion	CYP21 Exon 1-3 Deletion	Salt Wasting Type Classical CAH
#19	1 month-old	Vomiting	Male with hyper pigment.	(+)	46, XY	Homozygous E6 Cluster	Homozygous E6 Cluster	Salt Wasting Type Classical CAH
#20	17 year-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Heterozygous c.518T>A (I172N)	Heterozygous c.518T>A	Simple Virilizing Classical CAH
#21	1 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous c.955C>T (Q318X)	Homozygous c.955C>T	Salt Wasting Type Classical CAH
#22	18 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous Large Deletion	CYP21 Exon 1-3 Deletion	Salt Wasting Type Classical CAH
#23	1 day-old	Ambiguous Genitalia	Virilised female genitalia	(+)	46, XX	Homozygous Large Deletion	CYP21 Exon 1-8 Deletion	Salt Wasting Type Classical CAH
#24	1 month-old	Jaundice	Male	(+)	46, XY	Homozygous Large Deletion	CYP21 Exon 1-8 Deletion	Salt Wasting Type Classical CAH
#25	10 year-old	Hirsutism	Male	(-)	46, XY	Homozygous c.293-13A>G (IVS2)	Normal	Simple Virilizing Classical CAH
#26	2 day-old	Ambiguous Genitalia	Virilised female genitalia	(-)	46, XX	Homozygous c.293-13A>G (IVS2)	Normal	Simple Virilizing Classical CAH

c.293-13A>G (IVS2) (8 alleles, 14.2%), c.518T>A (I172N) (8 Alleles, 14.2%), c.1069C>T (R356W) (4 Alleles, 7.1%), c.92C>T (P30L) (2 Alleles, 3.5%) and E6 cluster (2 Alleles, 3.5%) and c.844G>T (V281L) (1 Allele, 1.7%). The 8-bp deletion was not detected. These results were consistent with the previously reported studies.

The genotypes of **15 patients with salt wasting phenotype** were associated with Type-A mutations. Of these 15 patients, 6 had Homozygous large deletions, 3 had Homozygous Q318X mutations, 2 had Homozygous R356W mutation, 2 had homozygous IVS2 mutation, 1 had Homozygous E6 Cluster mutation and 1 had heterozygous Q318X mutation.

The 6 patients with homozygous large deletions (#3,#8,#18,#22,#23,#24) were showing Salt wasting severe phenotype as expected. **The IVS2 mutation** disrupts the splice site of intron 2 and causes a shift in the translational reading frame. Because of this, almost all of the enzyme was abnormally spliced, whereas in the cultured cells there can be little normal enzyme activity. For this reason, the IVS2 mutation is associated with both a salt-wasting and simple virilizing types of the phenotype. In our study, 2 patients (#1,#14) with homozygous IVS2 mutation were found to have a salt wasting phenotype, while the other 2 (#25,#26) showed the simple virilizing phenotype. This situation was appropriate for the nature of the mutation (Forsham and Greenspan, 1983; Goossens et al., 2009).

All of the **11 patients with a simple virilizing phenotype** had Type-A / Type-B or Type-B / Type-B genotypes. Of these 11 patients, 2 had Homozygous I172N mutation, 3 had Heterozygous I172N mutation, 2 had homozygous IVS2 mutation, 1 had compound heterozygous Q318X/I172N mutations, 1 had compound heterozygous V281L/P30L mutations, 1 had compound heterozygous Q318X/P30L mutations. One patient had no mutations.

The Q318X mutation is classified as a Type-A mutation and this mutation leads to complete loss of enzyme activity in the homozygous state. However, Kharrat et al. suggested that Q318X mutation with I172N mutation resulted in a simple virilizing phenotype in the compound heterozygous state (Kharrat et al., 2004). Our patient #5 with Q318X / I172N Compound Heterozygote genotype had also had a simple virilizing phenotype. **The I172N mutation** leads to up to 1% of normal enzyme activity and is the most common cause of simple virilizing phenotype. I172N allele was detected in 6 patients. 2 of them (Patient #4 and #9) were in homozygous state and they were showing a simple virilizing phenotype. In patient #12, compound heterozygous V281L / P30L mutations were found. These two mutations were defined as Type-C mutations which are leading an enzyme activity around 20-60%. Our patient was removed from the follow-up after

receiving medical treatment for a while. This mild phenotype (Non-Classical?) was consistent with the genotype. In patient #12, compound heterozygous Q318X / P30L mutations were detected. The Q318X mutation was classified as Type-A (null group) and P30L was classified as Type-C. Our patient (#12) was showing genital hyperpigmentation and followed-up as simple virilizing/Non-Classical CAH Phenotype. This mild phenotype was consistent with the genotype (Delague et al., 2000; Gonçalves et al., 2007).

In conclusion, all patients with a salt wasting phenotype participating in our study had Type-A/ Type-A genotype and all patients with a simple virilizing phenotype had Type-A / Type-B or Type-B / Type-B genotype. All these results show that in CAH a good genotype-phenotype correlation can be established.

RFLP and MLPA methods were used to determine the CYP21 genotypes of 26 patients. In many studies, 7 known point mutation, 8-bp deletion, and large deletions were detected in more than 90% of cases. In the literature, the deletions in most studies have been investigated by genomic blot hybridization but nowadays, they are replaced by PCR based methods that require less labor and provide more information. This data obtained by PCR is limited in determining the extent of the deletion and not being able to detect duplications frequently. The MLPA method has been identified as a sensitive method for detecting deletions and duplications. Concolino et al. (2009) reported in their study that, of the 7 known subjects with CYP21A2 deletions and 2 with gene duplications previously characterized by Southern Blot, all were successfully identified by the MLPA). Researchers have argued that the MLPA method may be a highly informative method in the molecular diagnosis of CAH but, due to the complex nature of the CYP21A2 gene, which is one of the most known polymorphic genes, these studies require profound experience in the genetics of CYP21A2. In our study, MLPA confirmed the deletions in all 6 cases with deletions detected by PCR. Additionally, the four probes had been designed for the detection of 4 common mutations (8-bp deletion, I172N, E6 cluster, and Q318X). In our study, in 10 of 12 patients who had at least one of the I172N, E6 cluster, and Q318X mutations, MLPA analysis showed decreased peaks, supporting the presence of mutations.

Conclusion

Major results of the study;

1. The study was conducted on 7 known point mutations and 2 deletions. Although small in number, these 9 mutations did cover more than 96% of the mutant alleles.
2. The most frequent mutation was the large deletion (23%). The most frequent point mutations were c.955C>T (Q318X) (9 alleles, 16%), c.293-13A>G

- (IVS2) (8 alleles, 14.2%), c.518T>A (I172N) (8 Allels, 14.2%), c.1069C>T (R356W) (4 Allels, 7.1%), c.92C>T (P30L) (2 Allels, 3.5%) and E6 cluster (2 Allels, 3.5%) and c.844G>T (V281L) (1 Allel, 1.7%). The 8-bp deletion was not detected.
3. Order of mutation frequency did not differ in between our cohort which included 9 mutations and 26 families and between the cohort of New and Rosenwaks (2019), which included 113 known mutations and 1507 families. Briefly, in both deletions and Q318X were the most frequent mutations followed by I172N and IVS2 (New and Rosenwaks, 2019).
 4. In 21 (80.7%) of 26 patients, the causative mutations were found by using PCR (8-bp del. and large deletions) and RFLP (7 known point mutations) methods.
 5. MLPA analysis confirmed all of the deletions detected by PCR-RFLP, 83% of the detectable point mutations with MLPA.
 6. A complete genotype-phenotype relationship could be established in all patients in whom mutation could be detected in the study group.

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Comparative investigation of some liver enzyme functions considering age and gender distinctions in healthy Akkaraman sheep

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ABSTRACT

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Enzymes are proteins that possess catalytic properties, convert substrates into products and are biocatalysts of all biological and metabolic reactions in the body. Biochemical blood variables, mainly enzymes, are critical health and disease status parameters, which vary based on a living organism's conditions (metabolic and physiologic periods), age, gender, breed and diet as well as seasonal changes and regional and geographical differences. It has been well established that the measurement of serum levels of various enzymes is a worthy part of diagnosis. Some enzyme levels, which can serve as biochemical parameters, were measured in serum samples obtained from 220 healthy Akkaraman sheep breed, composed of ewes, rams, and female and male lambs. In the current study, which compared the values of biochemical variables, a significant difference ($p < 0.05$) between the four groups was observed in the concentrations of evaluated alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase and creatine kinase. Differences in the values of aspartate aminotransferase were not found to be statistically significant.

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1. Introduction

Enzymes are biocatalysts in specialized protein structures with catalytic properties. A sensitive enzyme analysis can give a sense of overall health or of pathological changes and the nature of any diseases present. Low or high amounts of these enzymes in serum or plasma can indicate the presence of damage

in the cells and the state of the disorders in a living organism (Center, 2007; Hoffmann et al., 2008; Kaneko et al., 2008). In clinical enzymology, enzymes such as transaminases (AST and ALT), creatine kinase (CK), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are very important for diagnosis (Center, 2007; Srivastava et al., 2007; Hoffmann et al.,

2008). Alanine aminotransferase (ALT) is a cytosolic enzyme found in many vertebrate species, especially homo sapiens, with the highest concentration in the liver and smaller concentrations in the heart, skeletal muscle and kidneys. This enzyme has variable ranges that depend on tissue and animal breeds, species, sub-species, regional changes, age and gender differences. Pathological increases in serum ALT levels are detected in the inflammation or destruction of any of the tissues where the enzyme is highly concentrated (Hoffmann et al., 2008; Kaneko et al., 2008). Aspartate aminotransferase (AST) activity is found in the liver in amounts similar to that in skeletal and cardiac muscle. The serum AST level can be about 1.5 times higher in newborns than in adults. In myocardial infarctions (MI), viral hepatitis, toxic liver necrosis, shock and hypoxia-associated circulatory failure, the serum AST level can increase by 10 ~ 100 times more than normal (Calbreath, 1992; Kaplan et al., 1996; Bishop et al., 2000; Srivastava et al., 2007; Hoffmann et al., 2008; Burtis et al., 2012). Gamma glutamyl transferase (GGT), which transfers amino acids from cell membranes, is used as a diagnostic test because it is affected by tissue specificity. The tissue distribution of GGT has been found especially in the liver, spleen, lung, pancreas and intestines as well as in the mammary glands of dogs, cattle, goats and sheep (Goldberg, 1980; Calbreath, 1992; Kaplan et al., 1996; Bishop et al., 2000; Srivastava et al., 2007; Hoffmann et al., 2008; Burtis et al., 2012). The highest GGT activity in the liver is found in cattle, horses, sheep and goats (Hoffmann et al., 2008). Alkaline phosphatase (ALP) has a catalytic effect in bone mineralization. ALP is found in all tissues (especially bones, placenta, intestines, the spleen and the kidneys). However, it is present in higher amounts in the liver and bones. The most abundant are bone ALP iso-enzymes in children and adults and liver ALP iso-enzymes (Calbreath, 1992; Kaplan et al., 1996; Hoffmann et al., 2008; Burtis et al., 2012). ALP activity is elevated physiologically in young animals (regardless of sex) where bone development is active, and is raised pathologically as a result of liver and heart disease (Hoffmann et al., 2008). During the contraction of the muscle, creatine kinase (CK) catalyzes the transfer of phosphate by providing the formation of creatine phosphate-ATP. While the height of serum CK activity in healthy individuals is affected by age, sex, race, body mass, obesity and physical activity, other genetic differences have less effect. Children have higher CK values than adults, and males have higher CK levels than females (Calbreath et al., 1992; Anderson et al., 1993; Kaplan, 1996; Bishop et al., 2000; Burtis et al., 2012).

To accurately evaluate metabolic profiles, it is essential to compare with reference range values appropriate for the region and the breed. Accordingly,

it is crucial to determine and standardize the specific values for each breed and region (Braun et al., 2010). The main purpose of this study was to determine the reference intervals for some important enzyme variables for male and female Akkaraman sheep of different ages.

2. Materials and methods

Ethical scope

This study was directed in accordance with the principles of the "Local Ethics Committee" in the framework of the ethics confirmed by the "Bahri Dagdas International Agricultural Research Institute" Directorate of Local Ethics Committee of Animal Experiments (14.01.2015 / 35 and 0088).

Sample collection

The blood samples were collected within the framework of ethical rules for animals in private enterprises located in Aksaray, Turkey. The animal samples consisted of 220 healthy Akkaraman sheep, which came from Aksaray and its nearby environment. The animals were separated into four groups based on gender and age, each group including 55 sheep. 220 totally healthy sheep, composed of females (n=55 lambs and 55 ewes) and males (n=55 lambs and 55 rams), were used as animal samples. 15 mL of blood was taken from the vena jugularis and blood samples were centrifuged and (Coles, 1986) used for analysis.

Enzyme assays

Enzymatic parameters identified in each sample were ALT, AST, GGT, ALP and CK. Analyses of these enzymes were carried out with a commercial assay kit (Assel, Italy) and a Humalyzer-3000 (Germany) biochemical analyzer according to the method of the commercial kit procedure.

Statistical analysis

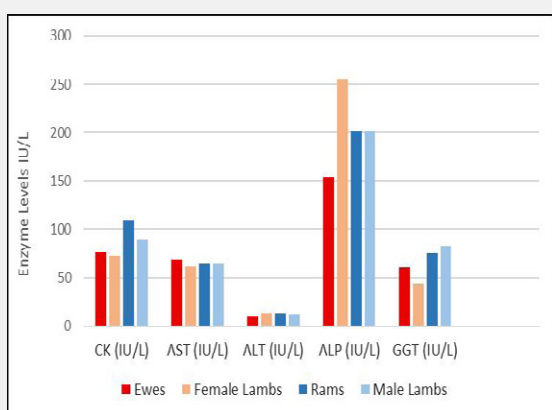
Descriptive statistics for the properties studied were mean, standard deviation, standard error, and minimum and maximum values. Data were analyzed using the statistical software SPSS 15.0 for Windows™ (SPSS Inc., Chicago, IL, USA). Differences among the groups were analyzed by student t-test. "One-way ANOVA" was performed to compare the group averages in terms of continuous variables. A Duncan multiple comparison test was used to identify the different groups following the analysis of variance. The data are given as the means \pm standard error ($X \pm SH$). Statistical significance was accepted as $p < 0.05$ level.

3. Results

The results and statistical mean values of enzymatic measurements of the sheep used in this study are represented in Table 1 and Fig. 1, respectively.

Table 1. Enzyme level findings of four groups of Akkaraman sheep.

Parameter	Mutation	Protein Change/ Other name	Rest. Enz.	Wild Type Frag. (bp)	Mutation Frag. (bp)
AST (IU/L)	69.05±3.65	61.23±4.32	64.38±4.45	64.08±3.64	>0.05
ALT (IU/L)	10.26±0.70b	13.48±1.21a	12.25±0.55ab	11.94±0.40ab	0.038
ALP (IU/L)	153.51±7.68b	255.20±30.34a	201.93±15.45ab	202.40±19.56ab	0.006
GGT (IU/L)	61.07±8.16bc	44.41±4.26c	75.09±6.70ab	82.79±5.92a	0.000
CK (IU/L)	76.27±4.63b	72.57±5.81b	109.67±11.69a	89.14±5.57b	0.002

**Fig. 1.** Levels of liver enzyme parameters in four groups of sheep.

AST levels were found to be relatively higher in ewes than in the other three groups. The results were not statistically significant. When the ALT values were examined, it was found that the values obtained from the ewes were lower than those of female lambs and male lambs and adults. The ALT values were highest in female lambs. The results were statistically significant ($p < 0.05$). In the current study, ALP values were statistically significantly higher in female lambs than in both the ewes and the male groups ($p < 0.05$). Overall, the female lambs had the highest values and the ewes had the lowest values; these results were statistically significant ($p < 0.05$). While no significant age-related differences were observed in males, ALP values in females increased as age decreased. GGT levels were found to be considerably lower in female lambs than in male lambs. In adults, the amount of GGT was higher in rams than in ewes. GGT values were higher in male lambs than in rams whereas in female lambs, they were lower than ewes; these findings were statistically significant ($p < 0.05$). The highest CK value measured was found in the rams and the lowest in the female lambs. It was found that the difference was not significant between ewes and female lambs whereas the large difference between rams and male lambs was statistically significant ($p < 0.05$).

4. Discussion

The values of some serum enzymes that were measured, studied and presented in this study are the basis for further research into indigenous sheep breeds. In this completed study, the activities of AST, ALT, ALP, GGT and CK were investigated to establish an extensive biochemical perspective for the investigation and thus health status was viewed in detail. The main purpose of liver tests in sheep is to clarify the liver functions that effect the total health of the organism. The serum values of these enzymes are often routinely used for assessing liver function (Braun et al., 2010). The principal utilization of clinical biochemistry based on enzymes in sheep are health management and the follow-up of health status, the diagnosis of diseases and the monitoring of treatment, for which selected examples are investigated in this study. Biochemical and enzymological profiles provide reliable information on the health status of animals and also reflect the responsiveness of an animal to its internal and external environments. Braun and co-workers recommended that, because there are many different breeds and breeding systems in sheep, each laboratory specify its own reference values and ranges. Thus, they claim that there must be different reference ranges (Braun et al., 2010). Some of the scientists also emphasized that the effect of seasonal change is often very hard to discern from many misleading determinants, such as feeding conditions and the reproductive condition of female animals (Yokus et al., 2004.; Braun et al., 2010). Measuring the activities of liver enzymes requires sensitive analysis involving the biochemical parameters of the relevant organism. In the current study, AST levels were higher in females than in males, there was a variation due to age difference in females and there was no difference between male age groups; these results were not statistically significant. In a study conducted in 2015, the AST and ALP values of Akkaraman sheep (gender and age not specified) in the healthy control group are consistent with the current study's total means results (Gunes et al., 2015). Some studies in the literature show enzyme amounts higher than the AST value and slightly lower than the GGT value of our

study (Stevanović et al., 2015). Compared with a study by Kurt et al. (Fartosi et al., 2010), our measured AST and ALT values were lower, our measured ALP and GGT values were higher and, the CK values of both studies were similar. In another study, values of AST, ALT and ALP in both males and females are in general much lower than the values we obtained (Durak et al., 2015). A study conducted by Durak et al. shows that Zom sheep did differ significantly ($p < 0.05$) between gender and age groups in terms of serum AST, CK, ALT and GGT levels. They found that ALP and GGT were affected by age, while ALP levels were significantly affected by sex. They also detected high GGT levels in adults as compared to young Zom sheep (Durak et al., 2015). If the results for Zom sheep are compared with our results without considering gender and age, the AST, ALT and CK levels were lower than in this study; ALP values were nearly the same and GGT values were higher than in this study. In another study, much lower GGT levels were found in Lika pramenka sheep (ewes) as compared to our GGT results (Vugroveci et al., 2017). A study presented in 2015 used healthy sheep of the Morada Nova breed and some biochemical data were monitored based on gender, age and body condition score. Carlos and colleagues claimed that age affected the serum ALT and AST levels in the Morada Nova sheep. The activity of ALT was found to be higher in males (Carlos et al., 2015). These situations could be connected to the differences in hepatic activity in different age periods. In the current study, apparent height in female lambs was significant for ALT and ALP ($p < 0.05$). In males, the effect on the ALT level is insignificant, whereas the ALP level is minimal but significant. Another study (Cruz et al., 2017) investigated the effect of age and gender on the biochemical parameters of Dorper sheep, measuring AST, ALP and GGT values among 15- and 121-day-old lambs. They showed that the highest AST value was in 3 different groups ($p < 0.05$) in ages ranging from 45 to 121 days, and they did not find any significant differences in ALT between the four groups. In a study linking these differences to developing muscle activity

and metabolic activity, they stated that gender did not affect biochemical variability. In a study managed by Yokus and colleagues of female adult sheep, ALT, AST, ALP, GGT and CK were studied as enzymatic parameters (Yokus et al., 2006). In this study, which was conducted in southeast Turkey in April, AST, ALT and CK were lower than our study's (ewes) results but ALP and GGT values were found to be higher. Despite the fact that both studies took place during the same season and had similar feeding conditions for animals, there were differences in enzyme values between different breeds (Sakiz-Awassi crossbreed sheep / Akkaraman sheep breed) based in different geographical regions (southeast Turkey / Mid-Anatolia) in the same country. In three different studies in similar tropical regions, the biochemical variables ALT, AST and LDH were investigated. Compared to the current study, ALT and AST levels were higher. Given that the working regions are tropical areas, it is not surprising that these three studies were similar and differ from our study. Factors including animal gender, geographical distribution, ecological and geological differences, nutritional properties and health conditions can influence hemoglobin levels (Kiran et al., 2012; Bhat et al., 2014; Pradhan, 2016). After comparing the current study with these other studies, the following conclusion can be reached: changeable ALT and AST concentrations indicate a great variation in ALT and AST levels among different ovine in different geographical regions. As can be clearly seen here, differences in enzyme activity depend on the animals, species and breeds as well as age, gender and environment.

As presented in the study, liver enzymes, which are vital to life, possess notable age and gender differences in ovine. The main purpose of this study was to determine the reference intervals of selected clinic enzymologic variables for males and females in different ages of Akkaraman sheep. This paper focused on the major application of enzymologic biochemistry in ovine in tracking health status, and on establishing regional reference values and ranges for Akkaraman sheep.

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Case Report

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Bilateral multivalvular polymicrobial endocarditis in an intravenous drug user

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ABSTRACT

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A 30-year-old man with a history of intravenous drug use presented to emergency room with fever, fatigue and altered mental status. He was admitted to intensive care unit with the diagnosis of sepsis, disseminated intravascular coagulation, multiple organ dysfunction syndrome and acute respiratory distress syndrome. On transthoracic echocardiography, there were big vegetations on both mitral and tricuspid valves. Consecutive blood cultures grew *Staphylococcus aureus* and *Staphylococcus hominis*. The diagnosis was bilateral, multi-valvular, polymicrobial infective endocarditis. The patient had a fulminant course and died on the fourth day of admission.

Keywords:

Infective endocarditis
Intravenous drug use
Sepsis
Transthoracic echocardiography

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1. Introduction

Intravenous (IV) drug use is a well-recognized predisposing factor for infective endocarditis (IE). According to, 'definitions of the terms used in the European Society of Cardiology 2015 modified criteria for the diagnosis of infective endocarditis, injection drug use represents a minor diagnostic Duke criterion for IE (Habib et al., 2015). Most infections are community-acquired, and *Staphylococcus aureus* is the leading causative microorganism, with methicillin-resistant strains becoming more prevalent (Leone et al., 2012). Also, due to high-risk behaviors, IV drug addicts are subjected to needle-borne infections by organisms that are usually non-pathogenic. Possibly due to the

habit of cleaning the needles with saliva and using it to dissolve the drug, polymicrobial infection is frequent in this setting (Miró et al., 2002). In fact, the main risk factor of polymicrobial IE is IV use (Sousa et al., 2012).

2. Case

A 30-year-old man with a history of IV drug use presented to emergency room with fever, fatigue and altered mental status and he was admitted to intensive care unit (ICU) with the diagnosis of sepsis, disseminated intravascular coagulation (DIC), multiple organ dysfunction syndrome (MODS), acute respiratory distress syndrome (ARDS). On physical examination, the patient was confused. His vitals were:

Blood pressure 120/70 mmHg, pulse rate 110/minute, respiratory rate 29/minute and body temperature 40° C. A pansystolic grade 2/6 murmur was audible at the left sternal edge. The respiratory sounds were diminished in both lungs and there were rales and rhonchi in the right lung. The liver was enlarged. There were skin rashes on patella and on both lower extremities. Blood tests were as follows: White blood cell count: 16700/mm³ (82% neutrophils), hemoglobin: 8.1 g/dl, hematocrit: 24.2%, platelets: 15000/mm³, aPTT 33 seconds, prothrombin time 17.5 seconds, INR 1.58, D-dimer 5548 ng/ml (N: 0-243 ng/ml), fibrinogen 297 mg/dl (N: 195-410 mg/dl), fasting blood glucose 141 mg/dl, blood urea nitrogen 172 mg/dl, creatinine 2.58 mg/dl, glomerular filtration rate 45 ml/min, ALT: 38 U/L, AST: 45 U/L, GGT: 46 U/L, albumin: 1.7 g/dl, total bilirubin 11 mg/dl (N: 0.3-1.2 mg/dl), direct bilirubin 7.37 mg/dl (N: 0-0.2 mg/dl). Hepatitis and HIV markers were negative. Chest X-ray revealed bilateral diffuse pulmonary infiltrates. On transthoracic echocardiography (TTE), there were big vegetations on both mitral and tricuspid valves (Fig. 1 and 2). The structure and thickness of other heart valves were normal. On color Doppler there were moderate mitral and tricuspid regurgitation. No right-to-left communication was observed. Consecutive blood cultures grew both *Staphylococcus aureus* and *Staphylococcus hominis*. The final diagnosis was sepsis, DIC and MODS secondary to multivalvular, multipathogen infective endocarditis. He was treated with meropenem 1 gram every 12 hours, daptomycin 6 mg/kg/day and caspofungin 70 mg loading and then 50 mg maintenance dose. He was also given platelet and human albumin transfusions. However, the patient was unresponsive to treatment and on the fourth day of admission, cardiac arrest developed and the patient died.

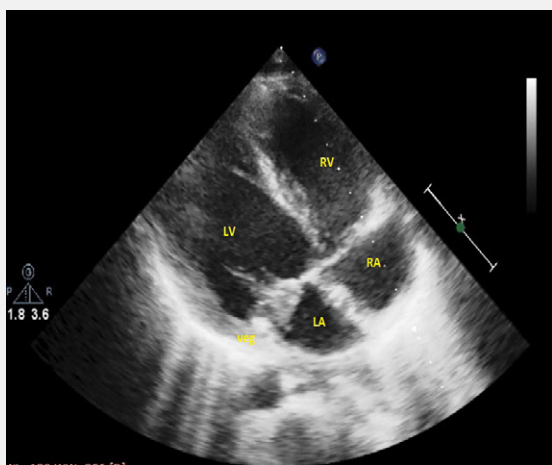


Fig. 1. Transthoracic echocardiographic image shows a large vegetation on the mitral valve (veg). RA: Right atrium, RV: Right ventricle, LA: Left atrium, LV: Left ventricle.

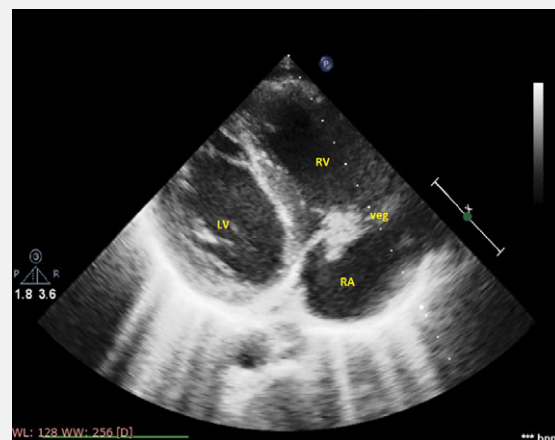


Fig. 2. Transthoracic echocardiographic image shows a large vegetation on the tricuspid valve (veg). RA: Right atrium, RV: Right ventricle, LV: Left ventricle.

3. Discussion

The majority of IE cases involve a single valve and the occurrence of multivalvular endocarditis is uncommon. The incidence of multivalvular endocarditis was reported between 13% and 18% in different series (Kim et al., 2000; Abid et al., 2010; Selton-Suty et al., 2010). In these series, the most common etiologic micro-organisms were staphylococci. However, in a review by Selton-Suty, the multivalvular location was often related to group D streptococci (Selton-Suty et al., 2010). Multivalvular IE includes both bilateral IE (affecting left and right heart valves at the same time) and IE affecting both mitral and aortic valves. The proposed mechanisms for multivalvular IE are: i-) Simultaneous seeding during the same bacteremia, ii-) Sequential seeding from a previously damaged valve, iii-) Creation of a new valvular lesion secondary to the infection of the first one, iv-) Spread of infection between left and right heart through congenital shunts. Multivalvular IE is frequently responsible for severe heart failure (Abid et al., 2010) and often needs aggressive and complex surgical therapy (Selton-Suty et al., 2010).

Our patient had bilateral endocarditis (affecting mitral and tricuspid valve), which is seen in only 5%-10% of patients (Sousa et al., 2012). Bilateral location usually occurs in patients with intracardiac devices and IV drug users (Duval et al., 2004). Our patient was an IV heroin addict and IE continues to be an important health problem among IV drug abusers. Injection drug users represent 5%-10% of all IE cases (Miró et al., 2002). *Staphylococcus aureus* is responsible for most of IE cases among these patients. Other agents are; staphylococci, pseudomonas and pathogenic fungi (Sousa et al., 2012). Remarkably, recurrent IE is more common in IV drug addicts, and the median time interval between episodes is shorter in addicts than in

non-addicts. This fact can be at least partly explained by the continuation of drug use in many of these patients (Vilacosta et al., 2016). Recurrent IE is also common in HIV positive injection drug users.

Right-sided endocarditis accounts for %5 - 10% of all IE cases and is common in IV drug abusers (Habib et al., 2015). In IV drug users, the right-sided endocarditis has a high recurrence rate and most of these patients develop sepsis, congestive heart failure, embolization, or other complications that lead to organ failure, intensive care unit admission and surgery (Brown et al., 2002). Among injection drug users, a new pattern is on the rise; infection on the left side of the heart with a severe clinical course (Mathew et al., 1995). Left- sided endocarditis, compared to right, and polymicrobial, compared to single organism are risk factors for increased morbidity and mortality in IV drug addicts with IE (Garcia-Granja et al., 2015).

In our patient, blood cultures grew *Staphylococcus aureus* and *Staphylococcus hominis*. The causative microorganism can be identified in roughly 90% of the episodes of IE. The isolation of more than one microorganism in patients with IE (polymicrobial IE) is quite uncommon, ranging from 1% to 6.8%. However, the frequency of polymicrobial endocarditis

is rising. In a recent study, among 1011 episodes of left-sided endocarditis, 60 were polymicrobial (5.9%) (Garcia-Granja et al., 2015). In 1991, Adler et al. (1991) reported an IV drug addict patient with tricuspid valve endocarditis involving 7 pathogens. Polymicrobial multivalve endocarditis is described in patients with prolonged IV infusion, in patients with congenital heart disease with shunts, and particularly in injection drug users. The most common combination of microorganisms are: *Staphylococcus aureus* and *Streptococcus pneumoniae* (second *Staphylococcus aureus* and *Pseudomonas aeruginosa* and third *Candida spp.* with bacteria). Polymicrobial endocarditis carries a very high mortality rate (greater than 30 %) and an uncommonly large number of patients (more than 50%) need heart surgery either to control the infection or to repair cardiac damage resulting from the infection. Combined therapy, medical and surgical, represents the standard of care in cases of polymicrobial endocarditis (Sousa et al., 2012).

In conclusion, polymicrobial multivalve endocarditis has a fulminant course and low survival rate in injection drug users. Patients with this type of endocarditis need to be identified and treated as soon as possible.

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Case Report

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Tracheobronchopatia osteochondroplastica case resulting with big airway obstruction

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ABSTRACT

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Tracheobronchopatia osteochondroplastica is an unusual non neoplastic abnormality with an unknown etiology. It is characterised with the improvement of one or both of osteoid or cartilaginous milimetric nodules in the submucosa of trachea and bronchial walls. It can be focal or diffuse. Trachea posterior wall conservation is characteristic. Even though most of the cases are asymptomatic, most frequent symptoms are cough, effort dyspnea, wheezing or recurrent respiratory tract infections. Diagnosis is coincidentally determined with autopsy or bronchoscopy. Recently, coincidentally diagnosed patients number is increased with the frequent CT usage. A 50 year old male applied our clinic with cough and phlegm whose respiratory function test indicated big airway obstruction. Thorax CT demonstrated a totaly atelectatic right middle lobe medial and lateral segment. Fiberoptic bronchoscopy indicated endobronchial lesions in the right middle lobe's medial and lateral segments. Biopsy proved uncertainty of carcinoid tumor and small cell tumor. In the result of right middle lobectomy, pathology informed TO.

Keywords:

Infective endocarditis
Intravenous drug use
Sepsis
Transthoracic echocardiography

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1. Introduction

Tracheobronchopatia osteochondroplastica is a very rare non neoplastic tracheobronchial disorder with an unknown etiology (Leske et al., 2001). It's characterised by bony or cartilaginous(or both) 1-8 mm. nodules in the submucosa of trachea and bronchial walls. It can be focal or diffuse (Ekinici et al., 2012). Trachea posterior wall conservation is characteristic (Martin, 1974). Even though most of the cases are asymptomatic, most

frequent symptoms are cough, effort dyspnea, wheezing or recurrent respiratory tract infections (Leske et al., 2001). Hemoptysis can be rarely seen because of a nodule ulceration or acute infection (Sevim et al., 2002). Diagnosis is coincidentally determined with autopsy or bronchoscopy. Recently, coincidentally diagnosed patients number is increased with the frequent CT usage (Mariotta et al., 1997).

2. Case

50 year old male referred to our clinic with cough and sputum continued for a long time. Nonsmoker patient was followed up with coronary artery disease, HT and asthma. In chest X-Ray, there was no pathology (Fig. 1A and 1B). In respiratory function test, obstructive pattern was detected. (FEV1 was 1970 ml (56%), FVC:2370 ml (69%), FEV1/FVC was 83%.) In Thorax CT right middle lobe lateral segment was atelectatic (Fig. 2). In fiberoptic bronchoscopy right middle lob lateral segment was obstructed with endobronchial lesion, right middle lobe medial segment was obstructed with a pulsating lesion. Biopsy was reported as carcinoid tumor? small cell tumor? (Fig. 3). In PET CT, density in the right hilar region reaching to thoracal pleura suv max was 2.8; dansity in the right hilar 1 cm region anterolateral to the right main bronchus suv max was 3.0. With these results, exploration was planned to the patient. In the exploration, right middle lobe was totally atelectatic and right middle lobectomy was performed. There was no postoperative complication. Right middle lobectomy pathology material was reported as TO. Follow up period was 6 days.

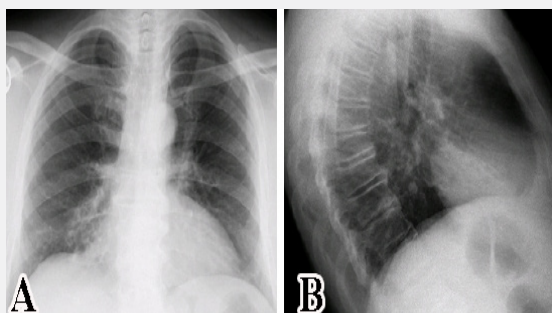


Fig. 1. A. Preoperative PA chest X-Ray; B. Preoperative lateral chest X-Ray.



Fig. 2. Preoperative Thorax CT of the patient.

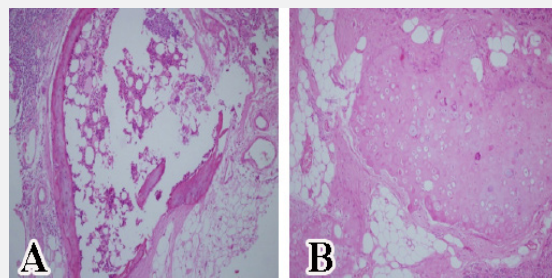


Fig. 3. A. Histopathologic view of bone nodules; B. Histopathologic view of submucosal cartilage.

3. Discussion

Tracheobronchopatia osteochondroplastica is a benign pathology submitted by Rokitansky in 1855 (Mariotta et al., 1997). Estimated prevalence is 0.7%. Typically there is a predisposition for 5. decade and males. Otopsy prevalence is 0.3%. Even though the exact pathology is unknown, endosytosis or eksosytosis of cartilaginous rings and bony or cartilaginous metaplasia of elastic tissue in the internal elastic fibrose membrane are blamed (Mariotta et al., 1997). Factors like chronic inflammation, infection, trauma, toxic material inhalation can have an important role. Atrophic rhinitis, cold, amiloidosis, silicosis and tbc are also suggested to be related to TO. In our case, there was no positive symptom related to these disorders (Tuncer et al., 2007).

Although patients are generally asymptomatic, dyspnea, cough, hemoptysis, recurrent airway infections and dysphagia are the probable symptoms. Our patient had cough and sputum. TO seems to be with narrowing and irregularity in the effected tracheal and bronchial segments. In Thorax CT, also, thickened tracheal cartilages with protected posterior membranous portions can be seen (Lundgren et al., 1981). Small calcific nodules aligned along trachea protruding to tracheal lumen are present. This image is more irregular than normal cartilage calcification. In bronchoscopy, white cartilaginous or bony mucosa covered nodules like a bead set in tracheobronchial tree are seen and TO is diagnosed with the nodules seen in bronchoscopy. As in our broncoscopic view, growing and inosculating nodules can cause obstruction (Tuncer et al., 2007). This view can interfere with benign diseases like amiloidosis, sarcoidosis. Like our case, differential diagnosis can be difficult beside malignities. Our patient's preoperative bronchoscopic biopsy result was uncertain to be small cell lung cancer or carcinoid tumor. Diagnosis was certain with pathologic diagnosis of surgical material. Prognosis is generally good and conservative approach is exhibited. (Willms et al., 2008). To decide the treatment protocol, airway obstruction has first degree importance (Simsek et al., 2006). In patients with symptomatic airway obstruction, both diagnostic and therapeutic surgical exploration and resection can be the exact solution.

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Case Report

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An unusual clinical presentation of isolated tricuspid valve endocarditis: Acute leukosis

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ABSTRACT

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Right-sided infective endocarditis (IE) is observed especially in patients using intravenous medications or illicit drugs, and who have right-sided pacemaker, central venous catheter, or congenital heart disease. We present a case of successful medical and surgical treatment for isolated tricuspid valve infective endocarditis with abnormal hematological and pulmonary findings in a young woman without predisposing risk factors.

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Keywords:

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Methicillin-sensitive staphylococcus
aureus (MSSA)
No risk factor
Transthoracic echocardiography

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1. Introduction

Right-sided infective endocarditis (IE) is observed especially in patients using intravenous medications or illicit drugs, and who have right-sided pacemaker, central venous catheter, or congenital heart disease (Weymann et al., 2012). Isolated tricuspid valve IE is the least common one with the incidence of 5 to 10% in the literature and is considerably rare in the absence of predisposing risk factors (Weymann et al., 2012, Chahoud et al., 2016). In this report, we present a case of successful medical and surgical treatment for

isolated tricuspid valve IE with abnormal hematological and pulmonary findings in a young woman without predisposing risk factors.

2. Case

A 31-year-old female patient was admitted to our hospital with the complaints of high fever, dyspnea, weight loss and night sweats. The patient had no known chronic systemic disease or substance abuse, and was not taking any medication or dental treatment. Physical examination revealed respiration rate of 25/minute, body

temperature of 39°C, heart rate of 110/minute, blood pressure of 100/60 mmHg, and hepatosplenomegaly, axillary lymphadenopathy. She had no infectious focus in the skin, joint, genitourinary tract, gastrointestinal tract and maxillofacial area. She only described small superficial skin erosion three week ago in clinical history. She had only a systolic murmur, grade 2 over 6, at the left sternal area. Laboratory findings at admission were as follows: hemoglobin 7.2 g/dl (normal range: 12-16), white blood cell count 25.3x10³ cells/ μ l (normal range: 4.0-10.5), platelet count 1.100x10³ cells/ μ l (normal range: 140-400), erythrocyte sedimentation rate 77 mm/hour, and C-reactive protein 6.8 mg/l (normal range 0-5). The patient was admitted to the hematology service with diagnosis of acute leukemia. The peripheral blood smear was performed. It was rich in functional platelets and showed dominance of reactive segmented cells. While planning the bone marrow biopsy, most important finding was determined during transthoracic echocardiography. There was a mobile mass with dimensions of 19x21 mm on tricuspid valve (Fig.1a). With the suspicion of tricuspid valve IE, antibiotic therapy with cefazolin (6 g/day) and gentamicin (240 mg/day) was started. All blood cultures were positive for methicillin-sensitive staphylococcus aureus which was sensitive to the given antibiotics. Meanwhile, thorax computed tomography

showed pulmonary infiltrates, pleural effusion, atelectasis, and hilar lymph nodes on the right side, all of which might indicate septic pulmonary embolism or pneumonia due to vegetation (Fig. 1b). Since fever continued and vegetation size increased at follow-up despite antibiotic therapy, surgical operation was decided. Bioprosthetic valve was implanted instead of necrotic and degenerated native tricuspid valve (Fig. 1c-d). The patient's hematological parameters improved. It thought reactive thrombocytosis and leukocytosis secondary to the infection. The same microbiological agent was identified in the cultures taken from tricuspid valve. After operation, antibiotic treatment was completed to six weeks and the patient was discharged from hospital without complication. The patient has been under medical follow-up for one year without symptoms.

3. Discussion

Symptoms and signs of right-sided IE are usually less prominent than the left-sided one, and septic pulmonary embolism or pneumonia is the leading clinical feature in some cases (Revilla et al., 2008; Wilczynska et al., 2010; Weymann et al., 2012; Chahoud et al., 2016). Right-sided IE is common in patients using intravenous drugs, and in those with indwelling cardiac devices or congenital heart problems (Weymann et al., 2012). These patients usually have severe comorbidities such as renal failure, diabetes mellitus, cancer, or HIV infection (Ortiz et al., 2014; Chahoud et al., 2016). Our case was unusual in that the patient did not have these predisposing risk factors and she had abnormal hematological (i.e., high platelet) and pulmonary (i.e., septic embolism, pneumonia) findings due to IE.

For diagnosis, high clinical suspicion is required in patients presenting with unusual clinical features and pyrexia of unknown origin. Infective endocarditis should be kept our mind (Wilczynska et al., 2010; Chahoud et al., 2016). These patients should be assessed without delay by transthoracic echocardiography. In tricuspid valve IE, the most common microorganism isolated is staphylococcus aureus, accounting for 50% to 80% of all cases (Revilla et al., 2008; Wilczynska et al., 2010; Ortiz et al., 2014). Streptococcal tricuspid valve IE is rare and is usually in combination with left-sided IE. Methicillin-resistant Staphylococcus spp. is frequently due to hospital-related causes (Revilla et al., 2008; Ortiz et al., 2014). Most cases are treated with appropriate antibiotics. Cardiac surgery is required in approximately 29% of patients (Revilla et al., 2008). These patients requiring operation usually have large vegetation, leaflet destruction, inability to eliminate bacteremia, or right-sided heart failure. Virulence of causative organism and vegetation size are the major determinants for prognosis (Revilla et al., 2008; Ortiz et al., 2014).

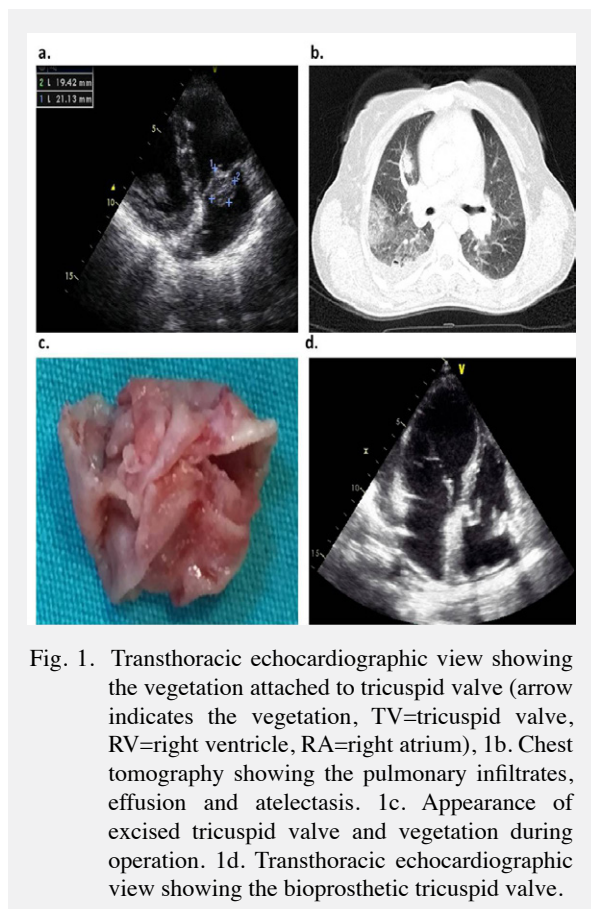


Fig. 1. Transthoracic echocardiographic view showing the vegetation attached to tricuspid valve (arrow indicates the vegetation, TV=tricuspid valve, RV=right ventricle, RA=right atrium), 1b. Chest tomography showing the pulmonary infiltrates, effusion and atelectasis. 1c. Appearance of excised tricuspid valve and vegetation during operation. 1d. Transthoracic echocardiographic view showing the bioprosthetic tricuspid valve.

Conclusion

We presented the isolated tricuspid valve IE with abnormal hematological and pulmonary findings in a young woman without predisposing risk factors. For

diagnosis, high clinical suspicion is required and when the patients with unusual clinical features, at pyrexia of unknown origin, infective endocarditis should be kept our mind.

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ORGANIZATION OF THE ARTICLE

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