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REVIEW

Aromatase Inhibitors May Increase the Risk of Cardiometabolic Complications in Adolescent Boys

Özge Besci[1] · Yağmur Damla Akçura[2] · Kübra Yüksek Acinikli[1] · Gözde Akın Kağızmanlı[1] · Korcan Demir[1] · Ece Böber[1] · Mustafa Kır[2] · Ayhan Abacı[1]

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Abstract

Introduction Aromatase inhibitors (AIs) are increasingly used in children and adolescents to augment adult height. The aim of this study was to investigate the effects AIs have on cardiac morphology, functions and their relation to several metabolic parameters in adolescent boys.

Methods Three groups matched for sex (boys, n = 67), age (median age 13.5 years), weight, height, body mass index, and puberty stages were enrolled: (i) Group 1: 23 patients using AIs (only AI (n = 6) or in combination with growth hormone (GH) (n = 17)) for at least 6 months; (ii) Group 2: 22 patients using only GH, and (iii) Group 3: 22 healthy boys. Two-dimensional, M-mode conventional Doppler and tissue Doppler examinations of the left ventricle (LV) were performed. Bioelectrical bioimpedance analyses was conducted and follicle-stimulating hormone, luteinizing hormone, total testosterone, lipid, and hemogram parameters were obtained.

Results Patients in Group 1 had significantly higher serum total testosterone ($p < 0.001$) and hemoglobin ($p < 0.001$) levels, fat free mass ($p = 0.005$), LV mass (LVM) ($p = 0.002$), as well as increased LV posterior wall diameter (LVPWD) ($p = 0.002$), interventricular septum diameter (IVSD) ($p = 0.019$), and myocardial systolic wave velocity (Sm) ($p = 0.020$) compared to the two other control groups. No significant differences were observed in terms of diastolic and systolic functions and lipid profiles ($p > 0.05$). There were positive correlations between total testosterone, hemoglobin levels, LVM, LVPWD and IVSD ($p < 0.05$).

Conclusion Increased LVM, LVPWD, IVSD and Sm of patients receiving AI therapy in comparison to the control groups, and the significant correlations of these parameters with total testosterone and hemoglobin levels were determined as potential side effects of AIs. These findings emphasize the need of routine cardiac follow-up in patients using AIs.

Keywords Echocardiography · Anastrozole · Letrozole · Cardiovascular risks · Side effects · Complications

Introduction

Aromatase is a microsomal cytochrome enzyme that catalyzes the aromatization of androgens from estrogens in numerous tissues, such as the ovaries, placenta, brain, fat, and bone [1, 2]. The aromatase enzyme regulates the testosterone-to-estrogen balance, preserves bone mineral structure and maturation, and aids in the maintenance of normal body composition [3]. Loss of function of aromatase causes increased testosterone levels with delayed epiphyseal closure and tall stature, whereas an excess of the enzyme may be associated with elevated estrogen levels and accelerated bone maturation, resulting in short stature [1].

Aromatase inhibitors (AIs) bind irreversibly (type I, steroidal) or reversibly (type II, non-steroidal) to aromatase enzyme to block its activities [2]. They inhibit the conversion of testosterone to 17-β estradiol, resulting in an increase in serum total testosterone levels, and a subsequent increase in 5-α-dihydrotestosterone (DHT), both of which act through androgen receptors [4]. They were initially introduced for the treatment of estrogen-receptor-positive

✉ Ayhan Abacı
ayhanabaci@gmail.com

[1] Division of Pediatric Endocrinology, Faculty of Medicine, Dokuz Eylül University, Inciraltı-Balcova, İzmir 35340, Turkey

[2] Division of Pediatric Cardiology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

breast cancer, but have since been used off-label for a variety of conditions; including short stature, gynecomastia, both precocious and delayed puberty (pubertal induction), congenital adrenal hyperplasia, aromatase excess syndrome, Peutz-Jeghers syndrome, and testotoxicosis [5, 6]. The non-steroidal reversible inhibitors, anastrozole and letrozole, are often prescribed to adolescent boys with short stature or precocious puberty, with the goal of minimizing the estrogens' effects on growth by slowing bone maturation [7]. They are commonly used with growth hormone (GH) in order to increase the predicted adult height of adolescents [3, 7].

Since AIs have not been approved by FDA for use in children, there is no pharmacological data available on the optimal dosages, hence the dosage recommendations for adults with potentially maximum effects are being used [3]. Despite high efficacy, there are several reports on the side effects of AIs. Increased testosterone-to-estrogen ratios have been associated with increase in hemoglobin levels, abnormalities in lipid metabolism, decrease in bone mineral density, derangements in bone maturation, and infertility [3, 6, 8, 9]. Fatal cardiovascular problems, including ischemic heart disease, arrhythmias, venous thromboembolism, heart failure, angina, and stroke have been linked to AI treatment among large cohorts of women with breast cancer [10]. However, its adverse effects on cardiovascular system in children and adolescents is yet to be elucidated [11–13].

In light of the mounting evidence on the potential risks of these drugs in women with breast cancer [11–13], detailed cardiovascular examinations in adolescents using these treatments are needed to ensure their safety in the pediatric group [14]. In this study, we aimed to compare the cardiac morphology and functions and metabolic states of children who had been using AIs for at least six months to those of healthy boys of comparable age, body mass index, and puberty stage. Since AIs are commonly prescribed with GH, we also compared these children to boys with similar characteristics, who had been treated with GH in order to ensure their safety.

Materials and Methods

Patients and Study Design

This retrospective observational case-control study was conducted at our pediatric endocrinology and cardiology clinics between January 2021 to January 2023. The study group comprised of 23 boys who had been treated with AI (anastrozole or letrozole; one tablet per day) for at least 6 months (Group I, only AI-treated (n = 6) and both AI- and GH-treated (n = 17)) aged ≥ 9 and < 16 years with bone ages ≤ 14 ½ years who were in puberty and sex- and age-matched boys using only GH (Group II, n = 22) and healthy subjects (Group III, n = 22) with normal puberty served as controls. Individuals with hypertension were not included in the study. Systolic blood pressure and diastolic blood pressure were measured twice at the right arm after a 10 min rest in the supine position using a calibrated sphygmomanometer. Hypertension was defined when blood pressure was ≥ 95th percentile for age, sex, and height on ≥ 3 occasions in children < 13 years of age and ≥ 130/80 for adolescents ≥ 13 years of age [15]. Two patients with conduction rhythm abnormalities (Wolf Parkinson White syndrome (n = 1, Group 2) and complete atrioventricular block (n = 1, Group 2) and one patient (licensed athletic trainer, Group 1) with athlete's heart) were not included in the study. Individuals with obesity (body mass index (BMI) standard deviation (SD) scores ≥ 2), genetic anomalies, scoliosis, chronic diseases (such as diabetes mellitus or celiac disease), low birth weight, or neoplasia were also excluded from the study. Data on age, gender, anthropometry, body composition, cardiac measurements, and biochemical analysis were collected. All measurements and examinations, including anthropometric evaluations, Pulsed Tissue Doppler Echocardiography, bioelectrical impedance analysis and laboratory evaluations were performed in the morning after an overnight fasting.

AI treatment was prescribed to patients with pubertal gynecomastia (n = 2) and to patients (n = 21) for height gain (GH deficiency (n = 17), idiopathic short stature with rapid pubertal progression (n = 3), and constitutional pubertal delay and growth (n = 1)) [3, 7]. GH deficiency was defined in the evidence of either; short stature or growth deceleration of velocity < 25% of corresponding chronological age with serum peak GH concentration less than 7 ng/ml in two different GH stimulation tests (i.e., clonidine, insulin tolerance test, and levodopa) [16]. Each child with GH deficiency received 25–35 mcg/kg/day of rhGH [17].

Data Collection

Anthropometric Measurements

Participants were weighted with light clothes and without shoes. Height was measured with a sensitivity of 0.1 cm, using a Harpenden stadiometer (cm). Weight was measured using a scale with a sensitivity of 0.1 kg (kg). BMI was calculated as weight per height (kg/m^2). The respective SD scores were calculated according to Turkish standards [18]. The following clinical parameters were recorded: age (years); gender; pubertal status [according to Tanner [19]; bone age [calculated according to Greulich and Pyle Atlas [20]], and weight (kg), height (cm), BMI (kg/m^2) and the respective SD scores.

Cardiac Evaluation

Conventional Two-dimensional Doppler Echocardiography

The same pediatric cardiologist (YDA), under the supervision of MK, performed all M-mode and two-dimensional echocardiograms and Doppler analyses on all subjects using a commercially accessible device (IE 33, Philips Ultrasound system, Bothell, Washington, USA; equipped with a S 5 – 1 standard sector probe) [21]. Before imaging, all individuals rested for 10 min and echocardiographic examinations were conducted in left lateral decubiti's position.

Two-dimensional parasternal long-axis and short-axis views and apical two-chamber and four-chamber images were acquired using standard transducer positions. The following parameters were measured: (i) Left ventricle (LV) morphological parameters (interventricular septum (IVS) thickness, LV posterior wall diameter, LV end diastolic diameter (LVEDD), LV end-systolic diameter (LVESD)), (ii) systolic parameters (LV ejection fraction, per cent fractional LV shortening, ejection time (ET)) and (iii) diastolic parameters (Mitral early diastolic velocity (peak E), late diastolic velocity (peak A)).

Indicative of diastolic function, the E/A ratio, was computed by dividing peak E by peak A. Peak A and peak E transmitral peak flow velocities were measured using pulsed wave Doppler in an apical four-chamber view. LV mass (grams) was measured using M-mode echocardiography [22]. Devereux's formula was used to determine LVM index, by which LV mass was indexed to height raised to allometric power of 2.7 (grams/meters$^{2.7}$) [22, 23]. Mitral DT was measured in milliseconds. The LV dimensions were reported in centimeters, and z-scores were computed using published normal values [24]. The fraction of shortening was computed as (LVEDD - LVESD)/LVEDD. Myocardial performance index (MPI), demonstrating overall ventricular (systolic and diastolic) function, was calculated by measuring mitral inflow Doppler tracings (interval a = isovolumic relaxation time (IVRT) + isovolumic contraction time (IVCT) + ET and LV outflow (interval b = ET). Interval 'a' defined the time interval between two consecutive mitral inflow velocity tracings (from cessation to onset of mitral inflow). The LV outflow velocity trace (ET, or interval 'b') was acquired from apical 5-chamber view with the Doppler sample volume positioned just below the aortic valve. MPI was calculated using the method MPI = a-b/b [25]. All of these time intervals represented the average of five measurements taken during consecutive cardiac cycles [21].

Pulsed Tissue Doppler Echocardiography

To evaluate systolic and diastolic mitral annular velocities, tissue Doppler echocardiography (TDE) was used [21]. The sample volume was deposited on the septal and lateral mitral annulus locations in the apical four chamber view. Each subject's systolic (S_m, $IVCT_m$, ET) and diastolic (E'_m, A'_m, and $IVRT_m$) velocities and intervals were recorded from both the mitral annulus and the interventricular septum. E'_m/A'_m ratio suggesting LV diastolic function and ventricular filling was calculated by dividing E'_m by A'_m. By dividing the sum of IVCT and IVRT by ET, the MPI was computed. LV diastolic function, calculated by pulsed wave Doppler-derived E-wave velocity/TDE-derived E'_m velocity ratio was calculated as well.

Bioelectrical Impedance Analysis data

Bioimpedance analysis was performed using Body Composition Analyzer TANITA BC-418 MA model single frequency (50 Hz) (Tanita Corporation, Tokyo, Japan). The eight electrode bioelectrical impedance analysis was performed by the same person following the operating principles of the device [26]. Body fat (FM, kg), body fat mass percentage (FMP, %), fat free mass (FFM, kg), total body water (TBW, kg), and appendicular skeletal muscle mass (ASMM, the sum of muscle mass of four limbs, kg) were recorded. Muscle-to-fat ratio was also calculated [MFR = (ASMM (kg)/ FM (kg)] [27].

Laboratory Data

All previous biochemical and hormonal evaluations were obtained from patient files. Serum triglyceride (mg/dL), total cholesterol (mg/dL), high density lipoprotein (mg/dL), low-density lipoprotein (mg/dL) levels were recorded. Serum levels of luteinizing hormone (LH, mIU/mL), follicle-stimulating hormone (FSH, mIU/mL), total testosterone (ng/dL) and hemoglobin (g/dL) levels were also recorded.

Sample Size Calculation

An a priori power analysis was conducted using G*Power program version 3.1.9.4 for Windows to determine the minimum sample size required to test the study hypothesis [28]. There were no previous similar cross sectional analyses to inform our power calculations. Using Cohen's standard effect sizes [29], in order to detect a large effect size (f = 0.4), the total sample size required was calculated to be 64 participants (21 in each of the three groups) to have 80% power at the 5% alpha level.

Ethical Approval

The study was approved by the local ethics committee of Dokuz Eylül University Faculty of Medicine (Ethical approval number: 2023/04–18). Written informed consents of all the parents were obtained prior to the study.

Statistical Analysis

Statistical analyses were performed using SPSS v.24 for Windows. Clinical variables were tested for normality using the Kolmogorov-Smirnov test. All variables complied with nonsymmetrical distribution. Continuous variables were expressed as median with interquartile ranges (IQR). In order to compare the three groups, Kruskal-Wallis analysis was performed and Dunn's multiple comparison post-hoc test was used for nonparametric pairwise multiple comparisons. The associations between total testosterone, hemoglobin and cardiac parameters were performed with Spearman's correlation. For all tests, $p < 0.05$ was considered statistically significant.

Results

The study included 23 boys [Group I, median (IQR) age of 13.5 (1.9) years] using AI (anastrozole (n = 16) or letrozole (n = 7)), 22 boys [Group II, median (IQR) age of 13.3 (2.7) years] using GH, and 22 healthy controls [Group III, median (IQR) age of 13 (1.5) years]. The duration of treatment for boys treated only with AI (n = 6) and both AI + GH (n = 17) were 14 (22.3) and 12 (13.5) months, respectively. Overall the duration of AI for Group I (n = 23) and GH for Group II (n = 22) groups were 12 (15) and 24 (60) months respectively. There was statistically no significant difference between the groups regarding age, puberty stages, SD scores for weight, height and BMI ($p > 0.05$) (Table 1). None of the participants have reported any adverse cardiac events or symptoms, including palpitations, fluttering, chest pain, shortness of breath, or dizziness. None of the participants had hypertension (Table 1) and the participants did not report to be involved in extensive physical activity.

Serum levels of FSH, LH, total testosterone, and hemoglobin were significantly higher in Group I compared to control groups ($p < 0.001$) (Table 1). The median (IQR) level of total testosterone was 531 (512) ng/dL, five of whom had

Table 1 Clinical and laboratory characteristics

	All n = 67	Group I n = 23	Group II n = 22	Group III n = 22	p-value[p]
Clinical characteristics					
Age (years)	13.5 (1.8)	13.5 (1.9)	13.3 (2.7)	13 (1.5)	0.16
Weight (SDS)	-0.6 (1.9)	-0.5 (2.2)	-1.1 (1.9)	-0.8 (1.8)	0.38
Height (SDS)	-1.1 (1.4)	-1.0 (1.3)	-1.4 (1.6)	-0.9 (1.3)	0.87
BMI (SDS)	-0.1 (1.9)	-0.1 (1.9)	-0.7 (2.0)	-0.2 (1.7)	0.19
Testis volumes, mL	10 (12)	10 (12)	10 (10)	12 (16)	0.77
SBP (mm/Hg)	112 (17)	112.5 (9)	109.0 (15)	111.0 (15)	0.10
DBP (mm/Hg)	71 (11)	74.0 (12)	73.0 (12)	70.0 (8)	0.27
Heart rate (beats/minute)	81 (17)	85 (22)	79 (12)	78 (14)	0.35
Bioelectrical impedance analysis data					
Fat (%)	17 (10)	16.5 (8.6)	17.5 (12)	20 (10.4)	0.15
Fat mass, kg	7.4 (6.2)	7.8 (8.7)	7.3 (6.4)	7.6 (6.5)	0.55
FFM, kg	37.3 (15.4)	40 (13.9)[a.b]	32.6 (17.8)[a]	33.4 (12.1)[b]	0.005
Appendicular mass, kg	15 (7.1)	17 (6.6)[c.d]	13.2 (8.4[c]	13.6 (5.7)[d]	0.003
TBW, kg	27.3 (10.8)	29 (10.2)[e,f]	22.8 (13.1)[e]	24.5 (9.5)[f]	0.005
MFR	1.9 (1.7)	2.2 (2)	1.8 (2.3)	1.8 (1)	0.58
Laboratory data					
FSH, mIU/mL	3 (3)	5 (5.7)[g,h]	2 (2.5)[g]	2.6 (2.6)[h]	< 0.001
LH, mIU/mL	1.8 (2.4)	3.2 (2.8)[i,j]	1.1 (1.8)[i]	1.2 (1.5)[j]	0.001
Total testosterone, ng/dL	290 (485)	531 (512)[k,l]	187 (323)[k]	207 (308)[l]	< 0.001
Total cholesterol, mg/dL	159 (51)	163 (48)	159 (61)	149 (43)	0.56
HDL, mg/dL	48 (11)	47 (7)	49.5 (9.8)	53.5 (17)	0.22
LDL, mg/dL	91 (38)	98 (41)	91.3 (39)	83 (40)	0.24
TG, mg/dL	90 (56)	78 (45)	82 (42)	97 (75.5)	0.09
Hemoglobin, g/dL	13.6 (1.4)	14.4 (1.3)[m,n]	13 (1.6)[m]	13 (1)[n]	< 0.001

Data are presented as median (interquartile range)

[p]Kruskal-wallis test, [a-n]Dunn's test (nonparametric pairwise multiple comparison), [a,c,e,g,i,k,m]Group I vs. Group II, [b,d,f,h,j,l,n]Group I vs. Group III.

(a) $p = 0.003$, (b) $p = 0.007$, (c) 0.002, d = 0.006, e. $p = 0.003$, f. $p = 0.008$, g. $P < 0.001$, h. $p = 0.002$, i. $P = 0.001$, j. $p = 0.003$, k. $p < 0.001$, l. $p = 0.003$, m. $p < 0.001$, n. $p = 0.001$

Abbreviations: Group I, patients using only aromatase inhibitor or both aromatase inhibitor + growth hormone; Group II, patients using growth hormone; Group III, healthy controls; FFM, fat free mass; TBW, total body water; MFR, muscle-to-fat ratio; FSH, follicle stimulating hormone, LH, luteinizing hormone; HDL, high density lipoprotein; LDL, low density lipoprotein, TG, triglyceride, SBP, systolic blood pressure; DBP, diastolic blood pressure

levels greater than 1000 ng/dL in Group I (n = 23); while in the control Groups of I and II the medians were 187 (323) and 207 (308) ng/dL, respectively, with levels ranging from 9 to 694 ng/dL (Fig. 1). The median testosterone levels were similar between patients using anastrozole (n = 16) and letrozole (n = 7) (514 (319) vs. 1205 (927) ng/dL, p = 0.082).

All parameters (FM, FMP, FFM, TBW, MFR, and ASMM) of bioelectrical impedance analysis were similar between Groups I and II, while patients in Group I had significantly higher FFM, TBW, and ASMM than both Groups II and III (Table 1). The levels of total testosterone were positively associated with FFM, TBW, and ASMM (Spearman's correlation (r_s) = 0.621, (r_s) = 0.626, (r_s) = 0.604; p < 0.001), while there was no association with FM ((r_s) = 0.161, p = 0.19). FFM was positively correlated with LVEDD (rs = 0.651, p = < 0.01), LVESD (r_s = 0.499 p < 0.01), IVSD (r_s = 0.651, p < 0.001), LVPWD (r_s = 0.674, p < 0.001) and LVM (r_s = 0.815, p < 0.001), while other parameters (FS, EF, Peak E, Peak A, E/A, MPI) were not found to have any correlations.

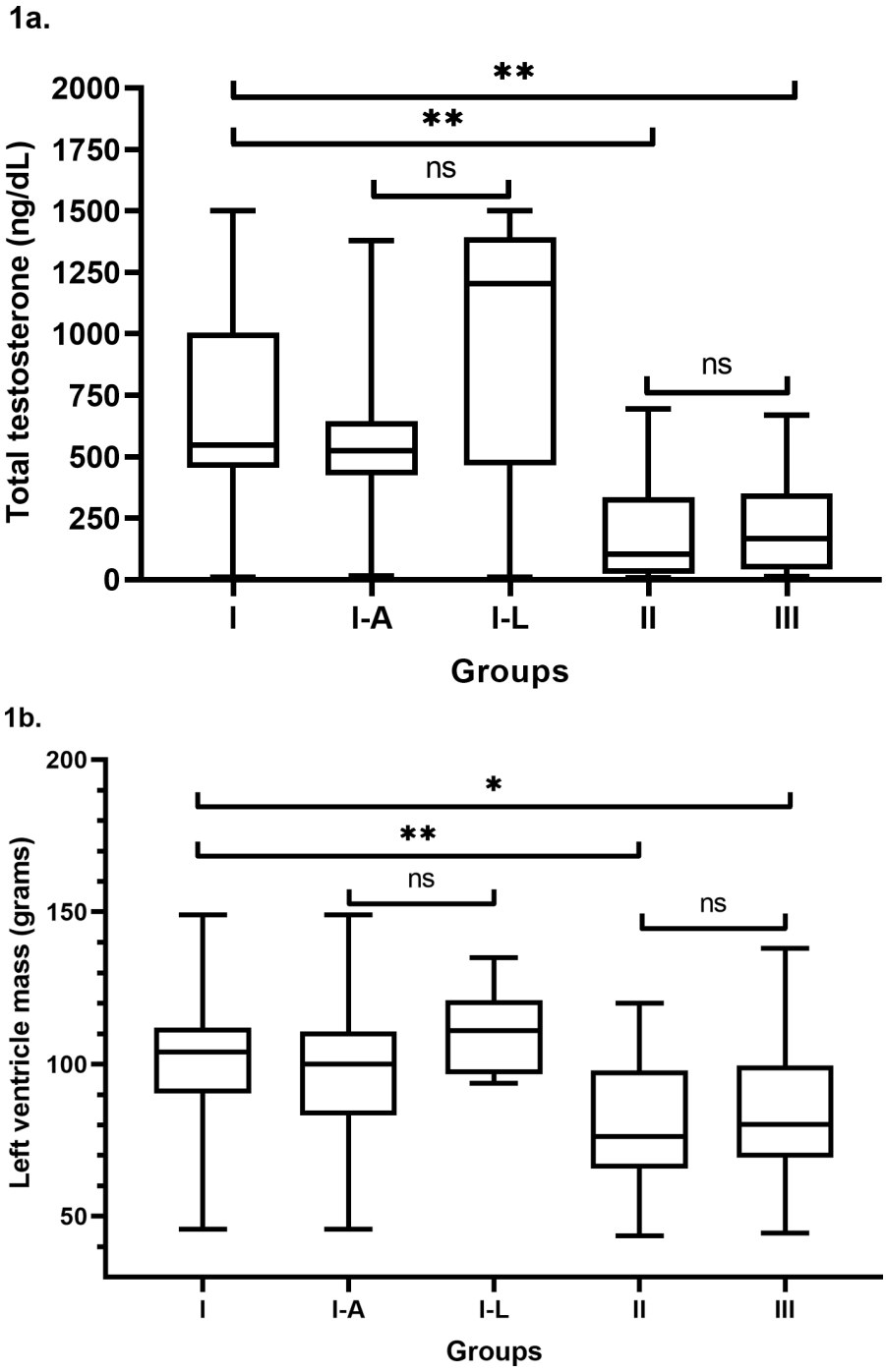

Fig. 1 The comparison of the levels of total testosterone (ng/dL) (Fig. 1a) and left ventricle mass (grams) (Fig. 1b) measurements between groups. The whiskers in boxes indicate minimum to maximum levels. Group I, patients using only aromatase inhibitor (n = 6) or both aromatase inhibitor + growth hormone (n = 17); Group I-A, patients using anastrozole (n = 16), Group I-L, patients using letrozole (n = 7), Group II, patients using growth hormone (n = 22); Group III, healthy controls (n = 22). ns: not statistically significant (p > 0.05); **p < 0.01; *p = 0.01

Patients in Group I, had significantly higher LVM and LVMI compared to both groups of II and III, while IVSD and LVPWD of Group I were also higher than those in Group II (Table 2). LVM of 13% (n = 3; anastrozole (n = 2), letrozole (n = 1)) of the patients who received AI treatment were above the reference limits for age and height, while the parameters of all other patients were within the reference ranges. The median LVM were similar between patients using anastrozole and letrozole (100 (27) vs. 111 (24) grams, p = 0.133). Other parameters (LVEDD, LVESD, FS, EF, Peak E, Peak, E/A, MPI) of M-mode and Doppler echocardiography were similar in all groups (Table 2). Total testosterone levels were positively correlated with LVEDD, LVESD, IVSD, LVPWD, LVM ((r_s) = 0.408, p = 0.001; (r_s) = 0.335, p = 0.006; (r_s) = 0.517, p < 0.001; (r_s) = 0.481, p < 0.001, respectively). Total testosterone was strongly associated with Hb levels ((r_s) = 0.635, p < 0.001). Hb levels were also correlated with LVEDD, IVSD, LVPWD, and LVM. ((r_s) = 0.266, p = 0.03; (r_s) = 0.410, p = 0.001; LVPWD (r_s) = 0.429, p < 0.001; 0.475, p < 0.001). There was no correlation between the duration of treatment and total testosterone levels, LVM, and LVMI (p = 0.26, p = 0.64, p = 0.66).

The parameters ($IVCT_m$, ET, E'_m, A'_m, IVRT, MPI) regarding the lateral localisations of the mitral annulus or the interventricular septum were not different between all three groups (p > 0.05, Table 3). Patients in Group I had longer S_m time than those in Group III (11.4 (2.9) vs. 9.6 (2.2) cm/s, p = 0.02). Total testosterone levels were positively correlated with S_m, MPI, $IVRT_m$ ((rs) = 0.434, p < 0.001; (rs) = 0.279, p = 0.019; (rs) = 0.305; p = 0.012), but not with $IVCT_m$, ET, E'_m, A'_m.

Table 2 M-mode left ventricular echocardiographic parameters of the groups

	Group I n = 23	Group II n = 22	Group III n = 22	p-value[p]
Morphological parameters				
LVEDD (cm)	4.5 (0.6)	4.1 (0.5)	4.3 (0.4)	0.56
LVESD (cm)	2.6 (0.6)	2.5 (0.4)	2.5 (0.4)	0.37
IVSD (cm)	0.8 (0.1)[a]	0.6 (0.2)[a]	0.7 (0.1)	0.019
LVPWD (cm)	0.7 (0.1)[b]	0.6 (0.1)[b]	0.6 (0.1)	0.016
LVM (g)	104 (21)[c,d]	76 (32)[c]	80 (30)[d]	0.002
LVMI ($g/m^{2.7}$)	31.5 (6.7)[e,f]	27.3 (7.1)[e]	26.9 (5.8)[f]	0.007
Systolic parameters				
FS (%)	40 (3)	39 (3.5)	41 (5.3)	0.57
EF (%)	71 (4)	69.5 (5)	72 (7.3)	0.72
Diastolic parameters				
Peak E (cm/s)	101 (28.2)	104.5 (13.7)	100.4 (24.2)	0.42
Peak A (cm/s)	60.2 (19)	58 (11.9)	55.2 (17.4)	0.71
E/A ratio	1.6 (0.7)	1.8 (0.7)	1.7 (0.6)	0.69
Global ventricular performance				
MPI	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.48

Data are presented as median (interquartile range)

[p]Kruskal-wallis test, [a–f]Dunn's test (nonparametric pairwise multiple comparison),

[a,b,c,e]Group I vs. Group II, [d,f]Group I vs. Group III.

a. p = 0.008, b. p = 0.006, c. p. = 0.001, d. p = 0.01, e. p = 0.005, f. p = 0.008

Abbreviations: Group I, patients using only aromatase inhibitor or both aromatase inhibitor + growth hormone; Group II, patients using growth hormone; Group III, healthy controls. LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; IVSD, thickness of the interventricular septum; LVPWD, thickness of the left ventricle posterior wall; LVM, left ventricle mass; LVMI, left ventricle mass index; FS, fractional shortening; EF, ejection fraction; ET, ejection time; peak E, early transmitral flow velocity; peak A, late transmitral flow velocity; MDT, mitral deceleration time; MPI, myocardial performance index

Discussion

This study evaluates the effects of AIs by comparing the data on conventional two-dimensional and pulsed tissue Doppler echocardiography, bioelectrical bioimpedance and laboratory analysis of 67 age-matched adolescent boys with similar characteristics. The results demonstrate that adolescents on AI treatment show significantly increased levels of serum total testosterone and hemoglobin in association with significant changes in cardiac morphology in comparison to controls.

In our cohort, total testosterone levels were significantly elevated in patients using AIs, compared to those patients not receiving AIs. A quarter of patients' testosterone levels, who were using AIs, had reached levels above 1000 ng/dL, and despite not reaching statistical significance, patients using letrozole had higher median levels in comparison to patients using anastrozole. Testosterone levels exceeding the upper limits of normal have been reported as a potential side effect of aromatase inhibitors [30, 31]. However, these exceedingly high androgen levels are particularly noteworthy, since various tissues and systems express androgen receptors; including bone, muscle, adipose tissue, reproductive, immune, neural, hematopoetic and cardiovascular systems [32]. While increased testosterone levels, and subsequently elevated DHT, both act through androgen receptors; the interactions between androgens and androgen receptors are complex with diversities in ligand-receptor

Table 3 Pulsed Tissue Doppler Echocardiography parameters of the groups

	Group I n = 23	Group II n = 22	Group III n = 22	p-value[p]
Lateral localisation of the mitral annulus				
Systolic parameters				
S_m (cm/s)	11.4 (2.9)[a]	10.1 (2)	9.6 (2.2)[a]	0.02
$IVCT_m$ (ms)	46 (7)	46 (7)	46 (8.8)	0.75
ET (ms)	270 (42)	289 (24)	283.5 (29.8)	0.19
Diastolic parameters				
E'_m (cm/s)	17.2 (5.2)	17.7 (4.8)	17.2 (4.7)	0.92
A'_m (cm/s)	6.1 (2.6)	5.3 (2)	6 (2.2)	0.22
$IVRT_m$ (ms)	56 (10)	56 (8.8)	56 (5)	0.58
Global ventricular performance				
MPI	0.4 (0.1)	0.3 (0.1)	0.4 (0.1)	0.14
Interventricular septum				
Systolic parameters				
S_m (cm/s)	7.6 (1.3)	7.6 (1.3)	7.7 (1.3)	0.4
$IVCT_m$ (ms)	46 (4)	47.5 (7)	46 (7)	0.24
Diastolic parameters				
E'_m (cm/s)	11.8 (2)	13.2 (3)	12 (2.3)	0.17
A'_m (cm/s)	6.1 (1.4)	6.1 (1.8)	5.9 (1.6)	0.55
$IVRT_m$ (ms)	56 (10)	54.5 (11)	56 (7)	0.28

Data are presented as median (interquartile range)

[p]Kruskal-wallis test [a]Dunn's test (nonparametric pairwise multiple comparison), [a]Group I vs. Group III, p = 0.007

Abbreviations: Group I, patients using only aromatase inhibitor or both aromatase inhibitor + growth hormone; Group II, patients using growth hormone; Group III, healthy controls. S_m, myocardial systolic wave; $IVCT_m$, isovolumetric contraction time; ET, ejection time; E'_m,early diastolic wave; A'_m, atrial diastolic wave; $IVRT_m$, isovolumetric relaxation time; MPI, myocardial performance index

reactions [32]. It has been demonstrated that increased levels of androgens may upregulate androgen receptor levels; both by increasing receptor half-lives and rates of receptor synthesis [33]. Further, there are ligand dependent and independent pathways regarding androgen and androgen receptor interactions, along with DNA binding and non-DNA binding mechanisms; each unique to different tissue types [32]. Hence, the impacts of androgens on different tissues may vary unpredictably. Given that the patients using letrozole had comparably higher median testosterone levels, anastrozole seems to be a safer treatment option for adolescent boys.

We also demonstrated increases in FSH, LH and hemoglobin levels compared to control groups. Even though the levels did not exceed the upper limits of normal, these modest increases were strongly positively correlated with testosterone levels, similar to previous studies [14, 31]. The increases in FSH and LH have not been found to be detrimental to future spermatogenesis capacity; on the contrary, Kohva et al. [34] had even suggested that it could actually improve Sertoli cell proliferation. On the other hand, erythrocytosis caused by increased testosterone levels [6], may pose risks due to potentially increased rates of thrombocytosis [35]. Therefore, the increases in hemoglobin levels should be cautiously monitored.

Our results have shown that adolescent males in Group 1 had higher FFM, TBW, and ASMM compared to control groups. Further, levels of testosterone were positively correlated with ASMM and FFM in adolescents. Several parameters in bioelectrical bioimpedance analysis have been associated with serious conditions in adults, including cardiovascular risks, mortality, coronary calcification, and heart failure, among others[36]. Although, MFR were similar between groups in our analysis, patients in Groups 1 had higher FFM and ASMM, indicating a relatively increased muscle mass compared to other groups. However, none of these patients had levels greater than the previously reported reference ranges for this age group [37]. The associations between sex hormones and muscle mass has been primarily studied in older men and women, but scarcely explored in adolescents [38–40]. While a meta-analysis by Sasha et al. [41] found no association between testosterone and muscle mass in older women, Auyeung et al. [42] reported that testosterone was related to both muscle mass and strength among 1489 older man. Similar to our results, a study by Xu et al. [39] reported a strong association between total testosterone and muscle strength in male adolescents.

In our study, in comparison to control groups, patients receiving AIs had a significantly higher LVM, LVMI, as well as thicker LVPWD, and IVSD. These measures were also strongly correlated with total testosterone levels. Even though, all participants in our study had normal levels of blood pressure and had reported not to be involved with extensive physical activities, it is important to note that the anatomy of heart can be influenced by blood pressure and physical activity, as shown by the correlations with LVM in previous studies [43, 44]. The effects of sex hormones on both the anatomy and functions of heart is still a matter of debate [45–48]. Testosterone and its active metabolite DHT have direct anabolic effects on myocytes, which may lead to myocardial hypertrophy [49, 50]. Oral testosterone administration was not found to have any adverse effects on cardiac functions in 16 pediatric individuals treated over a median duration of 2.5 years [51]. However, among healthy

men aged 25–84 years, a negative association with testosterone levels and LVM was reported [47], while a positive association was demonstrated with RVM [46]. In contrast, Subramanya et al. [52] demonstrated among 1941 women and 2221 men aged 45–84 years, higher testosterone levels were associated with a greater increase in LVM. However, these studies investigated the relationship of sex hormones with cardiac profiles in healthy men and postmenopausal women; while a different spectrum of changes had been demonstrated with supra-physiological testosterone doses. In consistent with our findings, supra-physiological androgen levels, mostly reported in male athletes, have been linked to left ventricular hypertrophy with systolic and diastolic dysfunctions in several studies [48, 53–55]. Similarly, Pirgon et al.[56] demonstrated a rapid growth of LV, due to increased total testosterone levels, after human chorionic gonadotropin injection in boys with cryptorchidism. Further, at the extreme ends of the spectrum, the patients with aromatase deficiency were reported to be at risk for the development of cardiovascular diseases and clinical follow-up was recommended [57].

This is also the first study to evaluate the ventricular functions using TDE in children treated with AIs. TDE, which quantifies myocardial velocities to assess segmental and global ventricular functions [58], has been extensively studied in several areas [21, 59–62]. We showed that other than systolic lateral mitral annulus velocity wave (S_m), all TDI parameters were similar in patients using AI as compared to the other groups. S_m, which measures the peak velocity of cardiac fiber shortening in the longitudinal direction [63], was significantly higher in patients using AI, despite being within reference ranges [64]. Significant differences in motion measurements evaluated at different regions, such as lateral, posterior, anterior walls have previously been documented [65, 66]. We hypothesize, that the comparable measurements of annulus motion of IVS between the three groups can be explained by the supporting role of the normal right ventricular functions. Given that TDE is able to detect abnormalities even at subclinical stages and increased peak mitral annulus velocity has been shown to be an independent predictor of mortality, different lateral mitral S_m measurements may be noteworthy [67]. Relatively increased S_m levels could be explained in light of the positive inotropic effects of testosteron, which are due to increased and rapid rates of calcium removal [68–70]. In line with these findings, Wadthaison et al. [68] demonstrated in vivo that after the initial beneficial state following testosterone treatment, cardiac pump function, myocyte contractility, and cardiac myofilament functions were depressed significantly after 12 weeks of treatment. This had been postulated to be the result of a maladaptive process, occurring due to reduced cardiac Troponin-I phosphorylation, which leads to decreased myofilament function [68]. Duration of testosterone administration have also been highlighted as a risk factor for the development of cardiac fibrosis [68, 71]. AIs, which are administered for longer durations, increase testosterone levels above reference intervals, with some exceeding 1000 ng/dL, resulting in increased S_m levels; therefore, it is reasonable to assume that these adolescents may also experience these adverse effects; it would be prudent to monitor their cardiac functions for longer durations.

Endogenous testosterone has several beneficial effects on cardiovascular system, and low levels are associated with an elevated risk of cardiovascular death [72]. Despite the independent causal relationship between DHT and cardiovascular disease, stroke, and all-cause mortality [73], significant associations have not been made with total testosterone levels [74]. Due to clinical heterogeneity and variable methodology, the association of cardiovascular risks with testosterone treatment have been considered to be controversial [53, 75, 76]. While previous studies and meta-analyses have found no significant differences in testosterone related cardiovascular events, a meta-analysis by Borst et al. [77] has revealed statistically significant cardiovascular risks linked with oral testosterone forms, demonstrating that not only the serum levels, but also the routes of administration may be associated with adverse outcomes. However, it should be noted that the majority of these studies included men aged 65 to 97 years, with modestly elevated testosterone levels [73]. Therefore, the supra-physiological levels created with an oral medication by inhibiting a crucial enzyme of testosterone-estrogen balance might influence the cardiovascular system to a greater extent, and uncertain cardiovascular risks might be observed.

Study Strengths and Limitations

The main strength of our study is that, to our knowledge, it is the first to evaluate and compare all parameters related to AI use, including cardiac structure, function, clinical features, and laboratory data, in a holistic way. The homogeneity of all groups in terms of age, sex, puberty and blood pressure, all of which are potential confounding factors influencing the anatomical parameters of heart, including the LVM, increases the strength of our findings in the study. However, there were some limitations too. Given that total testosterone exists both in inactive and active bioavailable forms; the measurement of free testosterone or DHT would have been more reliable markers in our study. Further, the patients in the Group I were receiving both AI and GH treatments, hence it could be argued that it would be impossible to designate whether AIs alone had caused these alterations or they were the consequences of the synergistic adverse effects of both treatments on heart. Moreover, we speculated

that the statistical significance was not attained between patients using letrozole and anastrozole, due to small numbers of patients in each subgroup. Homogenous studies with greater patient numbers comparing the effects of these drugs separately are needed. Finally, despite the statistically significant difference between several anatomical features, the findings should be interpreted carefully, since parameters such as LVMI were below the thresholds designated for a pathological increase. On the other hand, the probability that they might actually reach such high levels after longer terms of use cannot be ruled out among these pediatric cases using off-label medications; hence longer follow-up periods in future studies are warranted. Therefore, we have planned to monitor all our patients using AIs prospectively periodically for longer durations to document the reversibility of the changes in cardiac morphology, the progression of the increase in LVM, the correlation of these changes with respect to drug administration and dosage, and the occurrence of further cardiac events.

Conclusion

This is the first study to date evaluating the cardiac morphology and functions of adolescent boys using AIs. In this study, we found that (i) LVM, LVMI, LVPWD and IVSD were significantly higher in the patient group receiving AI therapy compared to the control groups and that (ii) these parameters were significantly correlated with serum total testosterone and hemoglobin levels. These significant differences and correlations were evaluated as potential side effects of AIs. Patients receiving AI should be regularly monitored for potential long-term cardiovascular morbidity and reversibility of the existing condition.

Author Contributions All authors contributed to the study conception and design. Conceptualization: [Ayhan Abacı]; Methodology: [Ayhan Abacı]; Material preparation, data collection and analysis were performed by [Özge Besci], [Yağmur Damla Akçura]; Writing - original draft preparation: [Özge Besci]; Writing - review and editing: [Korcan Demir, Ayhan Abacı, Ece Böber, Mustafa Kır] All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Statement of Ethics The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All participants' parents or legal guardians have given their written informed consent.

Study Approval Statement This study protocol was reviewed and approved by [Dokuz Eylül University], approval number [2023/04–18].

Consent to Participate Statement All participants' parents or legal guardians have given their written informed consent to participate in the study.

Conflict of Interest The authors have no conflicts of interest to declare.

Competing Interests The authors declare no competing interests.

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