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ORIGINAL ARTICLE

Anogenital distance and anal position index in cadaveric human fetuses

Hakan Taşkınlar[1] · Özlem Elvan[2] · Caner İsbir[1] · İsa Kıllı[1] · Ali Naycı[1]

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Abstract

The aim of this study is to contribute to the determination of the normal values of human anogenital distance (AGD) and anal position index (API) in the antenatal period. 59 formalin-fixed human fetuses were examined. AGD was measured by the distance between the center of the anus and the posterior fourchette in females, and the distance between the center of the anus and the posterior scrotal raphe in males. API in female fetuses was determined with the formula API = fourchette–center of anus/fourchette-coccyx formula, and API = posterior scrotal raphe-center of anus/posterior scrotal raphe-coccyx in males. The mean AGDs of the female and male fetuses in the second trimester were 5.60 ± 1.60 mm and 9.64 ± 2.75 mm and 12.88 ± 4.14 mm and 17.26 ± 5.55 mm in the third trimester, respectively. The AGD values were found to be significantly higher in the males ($p = 0.002$). While the API values detected in the female and male fetuses were 0.43 ± 0.085 and 0.55 ± 0.072 in the second trimester, they were 0.46 ± 0.079 and 0.55 ± 0.058 in the third trimester. The API values were found to be significantly higher in the male fetuses ($p < 0.001$). When the distribution of API values of the fetuses in the second and third trimesters was examined, no significant difference was found ($p = 0.499$). In addition, no significant correlation was found between API and AGD values and percentile groups of fetuses ($p > 0.05$). The AGD and API differed significantly between female and male fetuses starting from the antenatal second trimester, and the difference was preserved independently of the fetal percentile in the later stages of pregnancy.

Keywords Fetus · Anogenital distance · Anal position index · Anomalies

Introduction

The distance between the anus and the genitals (AGD) is a criterion that shows dimorphism between the sexes starting from the genital development period, where androgens and their receptors are dominant; this is called the "masculinization programming window" in the intrauterine period (Dean and Sharpe 2013). Although AGD measurement was first used for fetal sex determination in animals, it has also been used to investigate fetal androgen activity, androgenic or anti-androgenic environmental exposure in the antenatal period (Chan et al. 2009). Today, indices such as AGD measurement and anal position index (API) derived from these measurements are associated with genital and anorectal anomalies such as undescended testis, short penis length, and constipation, in addition to determining gender (Suryana and Makhmudi 2018). In addition, AGD has been used clinically by being associated with diseases such as congenital adrenal hyperplasia seen in the neonatal period and endocrinopathies in adulthood, female and male fertility problems, and polycystic ovary syndrome seen in women (Gilboa et al. 2017; Romano-Riquer et al. 2007).

Although there are antenatal studies with fetal ultrasonography, neonatal, adolescent, and adult clinical studies in the literature regarding the measurement of these parameters, according to our knowledge, there is no study in the literature that includes anthropometric measurements in human cadavers during the antenatal period (Aydin et al. 2019; Dean and Sharpe 2013; Papadopoulou et al. 2013). Therefore, our aim in this study was to contribute to the

✉ Hakan Taşkınlar
hakantaskinlar@gmail.com

1 School of Medicine, Department of Pediatric Surgery, Çiftlikköy Campus, Mersin University, 33116 Yenişehir, Mersin, Turkey

2 School of Health, Department of Anatomy, Mersin University, Çiftlikköy Campus, 33116 Yenişehir, Mersin, Turkey

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determination of the demographic norms for AGD and API in fetuses in the second and third trimesters.

Materials and methods

Fifty-nine formalin-fixed fetuses (34 females and 25 males) without any structural deformity on the lower extremities or genital region, and which belonged to the collection of the Department of Anatomy in the School of Medicine, Mersin University, were evaluated. This study was performed in line with the principles of the Declaration of Helsinki. The Clinical Research Ethics Committee of University where the study was conducted approved the research (2020/526). Fetal development was assessed by femur length as an antenatal period percentile identifier. Femur length was specified by measuring femur diaphysis between the greater trochanter distal and lateral condyle proximal. Following the determination of gestational age in weeks through foot length, percentiles of fetuses were calculated by fetal biometry graph using measured femur lengths (Mackanjee et al. 1996; Vocel and Marková 1978). Fetuses were divided into four groups: "0<percentile<5", "5<percentile<50", "50<percentile<95", and "95<percentile<100" according to the calculated percentile values. Fetuses were divided into two gestational age groups: second trimester (14-26 weeks of gestation) and third trimester (27-40 weeks of gestation) (1, 31).

Fetuses were placed in the lithotomy position. In the female fetuses, posterior fourchette–center of anus (AGD) and posterior fourchette–coccyx distances were measured. In the female fetuses, API was determined with the formula API = posterior fourchette–center of anus/posterior fourchette–coccyx (Fig. 1). Posterior scrotal raphe–center of anus (AGD) and posterior scrotal raphe–coccyx distances were measured in the male fetuses (Fig. 2). In the male fetuses, API was determined with the formula API = posterior scrotal raphe–center of anus/posterior scrotal raphe–coccyx (Hernández-Peñalver et al. 2018). Morphometric measurements were recorded with digital calipers (0.01 mm precision). All the measurements were taken under the same environmental conditions by two researchers (HT as a pediatric surgeon and ÖE as an anatomist), and twice by each researcher.

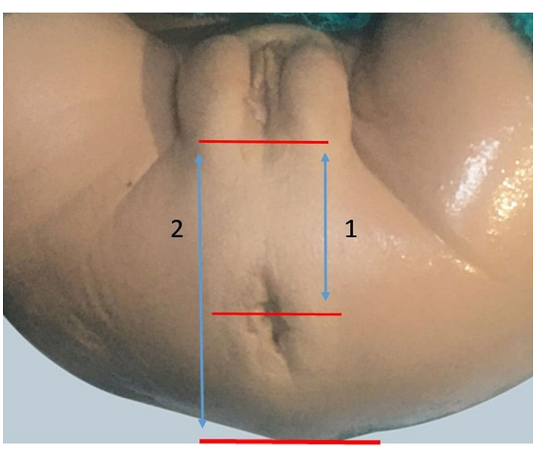

Fig. 1 35 Week old female fetus. Parameters were measured in dorso-lithotomy position distance between posterior fourchette and center of anus (1), distance between posterior fourchette and tip of coccyx (2), anogenital distance (AGD):1, anal position index (API): 1/2

Statistical analysis

The SPSS (Statistical Package for the Social Sciences) 23.0 package program was used for statistical analysis of the data. Categorical measurements were determined as numbers and percentages, and continuous measurements as mean and standard deviation. The Chi-square and Fisher's exact tests were used to analyze categorical expressions. The Shapiro–Wilk test was used to determine whether the parameters in the study showed normal distribution. The distribution of the API and AGD values of the fetuses in the second and third trimesters, which were the gestational age groups, were analyzed by the Spearman correlation test. The distributions of the API and AGD values in each gender were analyzed with the Independent Student's t test and the Mann–Whitney U test. The distribution of the API and AGD values in percentile value groups was analyzed by the Mann–Whitney U test and the Spearman correlation test. In addition,

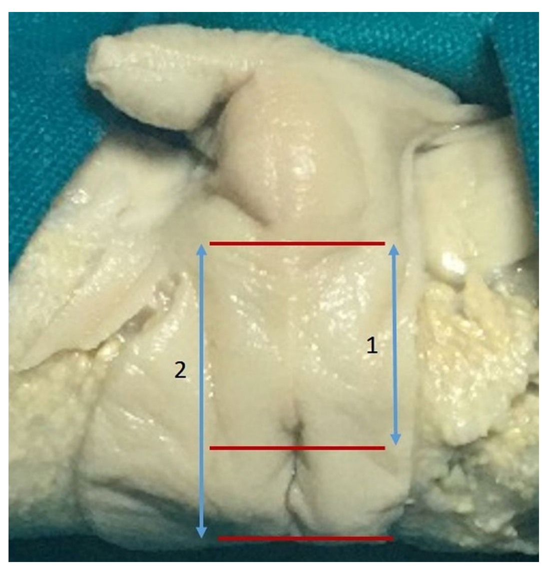

Fig. 2 26 Week old male fetus. Parameters were measured in the dorso-lithotomy position Distance between posterior scrotal raphe and center of anus (1), distance between posterior scrotal raphe and tip of coccyx (2), anogenital distance (AGD):1, anal position index (API): ½

the intraclass correlation coefficient (ICC) was calculated to compare two measures of researchers. The statistical significance was taken as 0.05 in all tests.

Results

The age of the female fetuses was 24.44±6.53 (min: 16, max: 40) weeks, and the age of the male fetuses was 23.24±4.27 (min: 17, max: 33) weeks. 24 of the female fetuses were in the second trimester and 10 were in the third trimester. 19 of the male fetuses were in the second trimester and six of them were in the third trimester.

The AGD values in the female (posterior fourchette–center of anus) and male (posterior scrotal raphe–center of anus) fetuses were 5.60±1.60 mm and 9.64±2.75 mm in the second trimester, and they were 12.88±4.14 mm and 17.26±5.55 mm in the third trimester, respectively. While the posterior fourchette–coccyx measurements in the female fetuses were 13.10±4.02 (min: 4.23, max: 20.81) mm in the second trimester, they were 27.70±7.52 mm (min: 14.29, max: 38.90) mm in the third trimester, and while the posterior scrotal raphe–coccyx measurements in the male fetuses were 17.08±3.77 (min: 10.13; max: 25.30) mm in the second trimester, they were 30.21±6.58 (min: 24.49, max: 40.84) mm in the third trimester. API values detected in the female and male fetuses were 0.43±0.08 and 0.55±0.07 in the second trimester, and they were 0.46±0.07 and 0.55±0.05 in the third trimester, respectively. The distribution of the distances between posterior fourchette–center of anus (AGD), posterior fourchette–coccyx, posterior scrotal raphe–center of anus (AGD), posterior scrotal raphe–coccyx, API, and femur length measurements in the male and female fetuses according to gestational age is shown in Table 1.

The AGD values measured in the second and third trimesters in the fetuses were found to be significantly higher in the male fetuses than in the female fetuses ($p = 0.002$). When the distribution of API values in the fetuses was examined, the API values of the male fetuses were found to be significantly higher than the API values of the female fetuses ($p < 0.001$). The Student's t test was used to analyze the differences between the two means of the AGD and API values, and the differences were shown using Error Bar graphs (Fig. 3). In addition, the linear relationship between AGM and API was examined using the correlation coefficient. The correlation coefficient was found to be statistically significant in the same direction at a moderate level ($r = 0.440$, $p < 0.001$). The relationship between the variables was shown in a scatter plot diagram according to gender (Fig. 4). When the distribution of API values in female and male fetuses in the second and third trimesters was examined, it was determined that the ratio of API values with increasing gestational age was preserved with gestational age, and there was no significant increase or decrease with advancing gestational age ($p = 0.499$), ($p = 0.928$). In addition, when the distribution of AGD values in the second and third trimesters was examined, it was found that the values increased significantly in both female and male fetuses with increasing gestational age ($p < 0.001$).

In terms of the distribution of fetuses in percentile values calculated according to femur lengths, 7 fetuses were found in the 0-5 percentile (11.9%), 13 fetuses were found in the 5-50 percentile (22%), 27 fetuses were found in the 50-95 percentile (45.8%), and 12 fetuses were found in the 95–100 percentile (20.3%). When the distribution of API and AGD values of

Table 1 Demographic data of fetuses

Gestational age Mean ± SD (min–max)	Number of fetuses (*n*)	Foot length (mm) Mean ± SD (min–max)	Femur length (mm) mean ± SD (min–max)	0–5 Percentile (*n*)	5–50 Percentile (*n*)	50–95 Percentile (*n*)	95–100 Percentile (*n*)
Female							
2nd trimester 20.47 ± 2.87 (16–25 weeks)	24	32.30 ± 7.45	33.90 ± 5.95 (18.28–44.77)	4	5	9	6
3rd trimester 31.58 ± 4.77 (26–40 weeks)	10	58.74 ± 10.44	55.50 ± 10.09 (41.06–74.02)	3	4	3	0
Male							
2nd trimester 20.94 ± 2.58 (16–24 weeks)	19	33.82 ± 6.60	36.32 ± 5.94 (24.35–43.55)		3	13	3
3rd trimester 28.22 ± 2.48 (26–33 weeks)	6	51.33 ± 5.28	50.81 ± 3.36 (45.06–53.79)		1	2	3

mm millimeter, *n* number, *SD* standard deviation, *min* minimum, *max* maximum

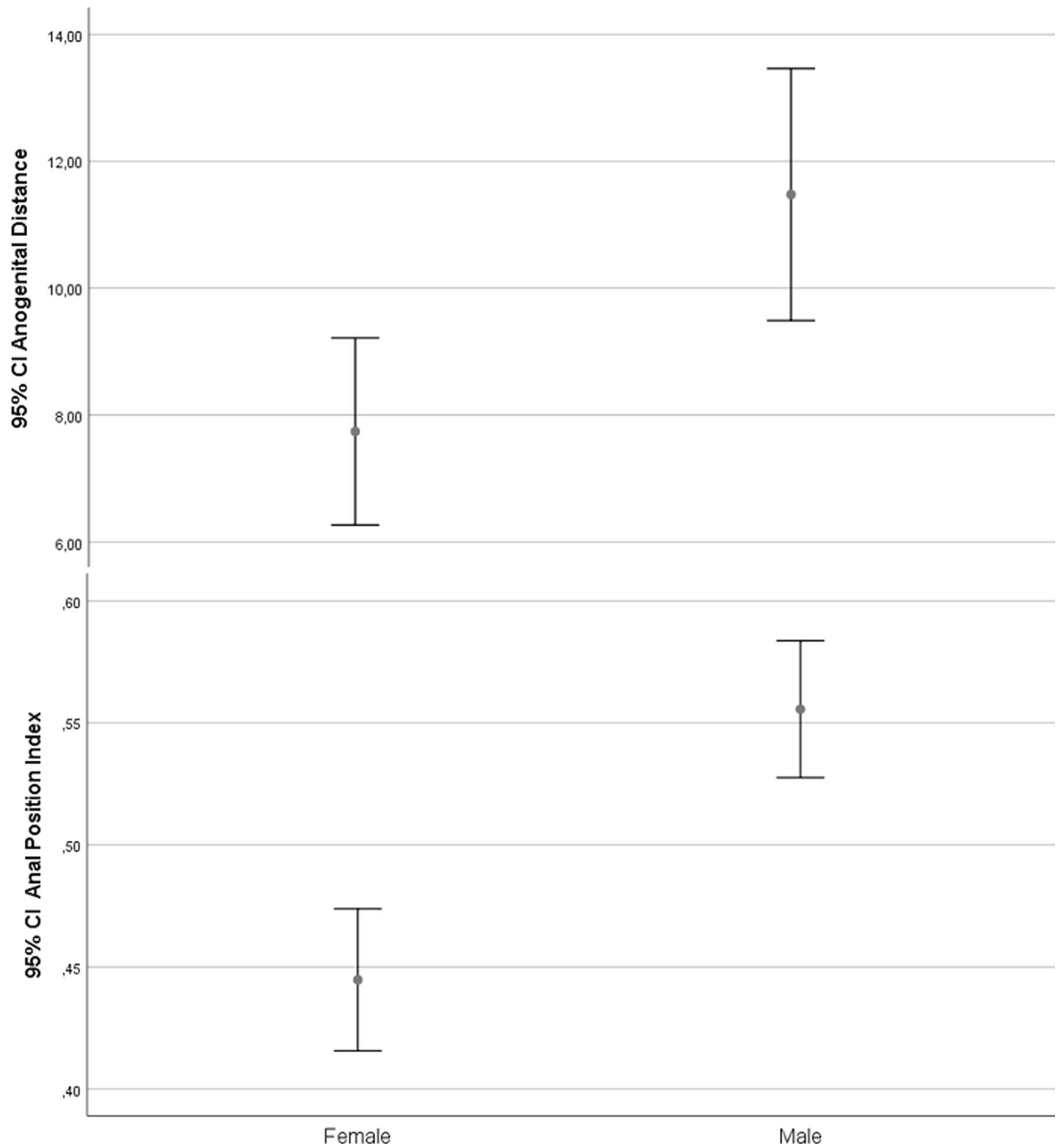

Fig. 3 Representation of the mean and standard deviation of AGD and API values in female and male fetuses using bar graphs

female and male fetuses in percentile groups was examined, it was seen that the distribution was homogeneous and there was no significant difference ($p > 0.05$) (Table 2). The measurements made by the independent researchers proved that the reliability of the data was significantly compatible (ICC=0.986, $p<0.001$). Previous studies that included values for API and AGD are depicted in Table 3.

Discussion

The main finding of this study performed on human fetal cadavers was that the AGD and API differed significantly between female and male fetuses starting from the antenatal second trimester, and the difference was preserved

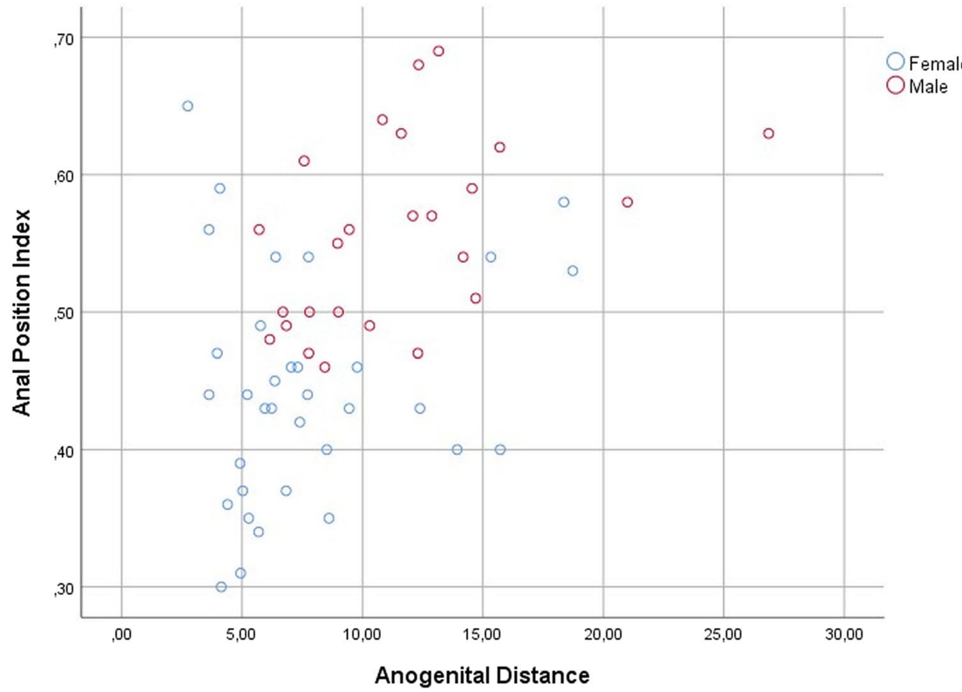

Fig. 4 Representation of the relationship of the variables between AGM and API in the scatter plot diagram taking sex into account

Table 2 Distribution of API and AGD values of female and male fetuses in percentile groups

	Percentile 0–5 5–50 50–95 95–100				*p*
	Mean ± SD(mm)	Mean ± SD (mm)	Mean ± SD (mm)	Mean ± SD (mm)	
API (female)	0.45 ± 0.069	0.43 ± 0.106	0.44 ± 0.089	0.45 ± 0.061	0.949
API (male)		0.51 ± 0.051	0.57 ± 0.066	0.53 ± 0.066	0.135
AGD (female)	8.66 ± 3.18	9.44 ± 5.97	7.14 ± 3.80	5.31 ± 1.49	0.267
AGD (male)		9.46 ± 3.84	10.78 ± 2.96	14.54 ± 7.84	0.181

API anal position index, *AGD* anogenital distance, *mm* millimeter, *SD* standard deviation, *min* minimum, *max* maximum

independently of fetal percentile in the later stages of pregnancy. It is believed that the determination of the normal values of these measurements can not only support the morphological sex determination in the antenatal period but also provide parameters for detecting anomalies associated with AGD and API in the antenatal period.

Besides morphological images, studies conducted on both animals and humans have shown that more accurate results can be obtained by measuring the distances between the anus and the genitals (Callegari et al. 1987; Davari and Hosseinpour 2006). Najdi et al. showed that the distance between the anus and the genitals could be measured successfully by ultrasonography in the antenatal period, starting from the 11th week, during the evaluation of fetal sex in human fetuses. However, they also reported that the most reliable results were obtained in the measurements made in the 12th week. The AGD values measured in the second and third trimesters were found to be significantly higher in male fetuses than in female fetuses in this study ($P = 0.002$), consistent with the literature (Kluth et al. 1995). According to endocrinological developments, such as increased fetal linear development and androgen exposure in male fetuses, it was reported that sex determination was more accurate in the late stages of the second trimester than in the early periods (Aydin et al. 2019). In addition, Isbir et al. (2020) reported that the external genitalia in female fetuses showed different morphological features in the second and third trimesters that might affect the measurements of AGD and API (Thankamony et al. 2016). Thus, the morphometric measurements of AGD and API were analyzed by dividing

Table 3 Literature data of anogenital distance (AGD) and anal position index (API)

Authors	Year	Country	Method	Cases (*n*)	Gender	AGD (mm)	API
Reisner et al.	1984	Israel	Anthropometric	100 100	Female newborn Male newborn		0.44 ± 0.05 0.58 ± 0.06
Callegari et al.	1987	USA	Anthropometric	115	Female newborn	10.9 ± 3.5	
Genç et al.	2002	Turkey	Anthropometric	34 26	Female newborn Male newborn		0.46 ± 0.08 0.53 ± 0.05
Salazar-Martinez et al.	2004	USA	Anthropometric	42 45	Female neonate Male neonate	11 ± 2 21 ± 3	
Davari and Hosseınpour	2006	Iran	Anthropometric	200 200	Female neonate Male neonate		0.42 ± 0.08 0.54 ± 0.07
Romano-Riquer et al.	2007	Mexico	Anthropometric	781	Male newborn	19.1	
Chan et al.	2009	Taiwan	Anthropometric	100 100	Female neonate Male neonate		0.40 ± 0.04 0.54 ± 0.03
Nunez-Ramos et al.	2011	Spain	Anthropometric	267 262	Female newborn Male newborn		0.40 ± 0.05 0.53 ± 0.06
Ertürk and Kandemir	2017	Turkey	Anthropometric	143 124	Female newborn Male newborn		1.06 ± 0.04 0.90 ± 0.08
Gilboa et al.	2017	Israel	Ultrasound	23	Male newborn	16.90 ± 4.08	
Loreto-Gomez et al.	2017	Mexico	Anthropometric	154 153	Female newborn Male newborn	10.2 ± 1.2 19.1 ± 3.7	
Suryana and Makhmudi	2018	Indonesia	Anthropometric	33 29	Female newborn Male newborn		0.37 ± 0.07 0.46 ± 0.06
Najdi et al.	2018	Iran	Ultrasound	125 105	Female embryo Male embryo (first trimester)	> 4.9 > 4.5	
Aydın et al.	2019	United Kingdom	Ultrasound	107 101	Female fetus Male fetus (26–30 weeks of gestation)	9.61 ± 1.98 14.85 ± 2.18	
This study	2021	Turkey	Anthropometric	24 10 19 6	Female fetus 2nd trimester 3rd trimester Male fetus 2nd trimester 3rd trimester	5.60 ± 1.60 12.88 ± 4.14 9.64 ± 2.75 17.26 ± 5.55	0.43 ± 0.08 0.46 ± 0.07 0.55 ± 0.07 0.55 ± 0.05

n number, *mm* millimeter

the fetuses into two gestational age groups: the second and third trimesters.

In the study of Callegari et al., measurements were made between the center of anus-posterior fourchette (AGD), center of anus–clitoris and posterior fourchette–clitoris in babies born between 25 and 42 weeks, and it was stated that all measurements increased in line with gestational age. In addition, they reported that there was a twofold difference in AGD measurements between the sexes in postnatal studies in healthy newborns and this difference occurs between postnatal weeks 17 and 20 (Broome et al. 1998). It has been reported that this condition is maintained until the 30th month, although it decreases slightly in adolescence and adulthood (Mackanjee et al. 1996; Pedreira et al. 2001). Salazar et al., in their study on newborns, reported that the AGD value is also a useful parameter for sex determination in the antenatal period (Papadopoulou et al. 2013). In the present study, in parallel with the literature, the AGD in female and male fetuses were 5.60 ± 1.60 mm and 9.64 ± 2.75 mm in the second trimester, and they were 12.88 ± 4.14 mm and 17.26 ± 5.55 mm in the third trimester. Also, it was observed that AGDs increased with gestational age and were significantly higher in the male fetuses than the female fetuses.

It has been reported that AGD is directly related to body mass (Cools et al. 2018; Pang 1993). Pedreira et al. reported that the rate of accurate sex determination decreased with antenatal ultrasonographic morphological evaluation, especially in male fetuses with intrauterine growth retardation (Najdi et al. 2019). Regarding the relationship between intrauterine growth retardation and AGD, no significant difference was found in the distribution of AGD values in the percentile groups in this study ($p > 0.05$). The publications that try to determine normal and pathological AGD values generally reflect the demographic values of a certain geographic location (Aydin et al. 2019; Genç et al. 2002). It has been reported that low AGD values in the intrauterine period may be associated with serum aromatization and intrauterine masculinization steps, apart from cryptorchidism, short penile length, hypospadias, and androgen deficiency (Aydin et al. 2019; Genç et al. 2002; Suryana and Makhmudi 2018). In addition, it has been reported that pathological AGD values may be associated with hormonal and neurodevelopmental disorders such as infertility in men, polycystic ovary syndrome, and autism in women (Baron-Cohen et al. 2015; Efrat et al. 1999; Ertürk and Kandemir 2017; Gilboa et al. 2017). In conclusion, the mean AGD measurement values of the fetuses can be used both with regards to sex determination and external genital pathologies in the second and third trimesters, regardless of developmental delay.

Ertürk et al. reported API values for newborns as "<1" in female fetuses and "<0.9" in male fetuses (Eisenberg et al. 2013). However, they used a different protocol for calculating the API values. The ratio of scrotum/fourchette—coccyx distance to anococcygeal distance was used to determine the API in previous studies. In contrast, the following formulas were used to determine the API in the present study: API = scroto-anal distance (cm)/anococcygeal distance (cm) in male neonates; API = fourchette–anal distance (cm)/anococcygeal distance (cm) in female neonates. Reisner et al. reported that the API values were higher in boys than in girls (Núñez-Ramos et al. 2011). In addition, Ramos et al. evaluated API values as close to each other in both sexes, but they reported that the anus was closer to the anus–coccyx midline in male fetuses (Loreto-Gómez et al. 2017). In the present study, the API values of the male fetuses were found to be significantly higher than the API values of the female fetuses in the second and third trimesters ($P < 0.001$). Thus, it is believed that API values can be used as a parameter for sex determination in the antenatal period, starting from the second trimester.

Reisner et al. reported API values as "<0.34" in female fetuses and "<0.46" in male fetuses, which remained constant regardless of age (Núñez-Ramos et al. 2011). Ertürk et al. examined the distribution of postnatal API values in term and preterm babies, and did not detect a significant difference (Eisenberg et al. 2013). Similarly, when the distribution of API values of female and male fetuses in the second and third trimesters and percentile groups was examined in the present study, no significant difference was found ($P > 0.05$). In addition, Efrat et al. reported that the rates of morphologically correct sex determination by ultrasonography decreased with a decrease in gestational age (Dean et al. 2012). Based on the findings of the present study, it is thought that the determination of API values, independent of gestational age and antenatal developmental status, may play a supportive role in sex determination.

Regarding cases where API values deviate from normal, it has been reported that API values are lower in studies conducted in fetuses with hypospadias, newborns with undescended testicles, and adult men with atypical external genitalia (Colmant et al. 2013; Salazar-Martinez et al. 2004). In addition, Ramos et al. reported that the determination of API value is important in the detection of anal canal location anomalies, and it can therefore be associated with anorectal malformations and intestinal motility problems (Loreto-Gómez et al. 2017). In the current study, while the API values detected in female and male fetuses were 0.43 ± 0.08 and 0.55 ± 0.07 in the second trimester, they were 0.46 ± 0.07 and 0.55 ± 0.05 in the third trimester. It is thought that the determination of normal values of API in the antenatal period may be a supportive parameter in the early diagnosis of genitourinary and anorectal anomalies.

Broome et al. reported that the primary central ossification process occurs between the antenatal 18th and 29th weeks, with the cauda spina starting to ossify at antenatal 10^{th}–12th weeks (Welsh et al. 2008). Congenital anomalies, such as caudal subluxation and hypoplasia, are reported to affect cauda spinal angulation (Woon and Stringer 2012). These results suggest that caudal abnormalities may affect posterior fourchette–coccyx and posterior scrotal raphe–coccyx measurements. It has been reported that 17% of anorectal malformations and 2.7% of Hirschsprung's disease may be accompanied by distal spinal and caudal abnormalities (Straaten et al. 2020). For this reason, introducing normograms for posterior fourchette–coccyx and posterior scrotal raphe–coccyx values may contribute to the detection of anorectal and caudal pathologies in antenatal scans.

A moderately significant relationship was found in the same direction when the correlation between API calculation and AGD measurements was evaluated ($r = 0.440$, $P < 0.001$). This result was compatible with the requirement that API should be directly proportional to AGD due to the calculation method. Antenatal factors such as androgen exposure and other environmental factors affecting AGD and urorectal septal development may change anus location. A moderate significance of correlation may be explained with embryological differences (Davari and Hosseinpour 2006; Vocel and Marková 1978). It is thought that the morphometric measurements made in the study might contribute to the early diagnosis of urogenital and anorectal anomalies that might occur due to pelvic and endocrinological developmental abnormalities. However, studies on this subject have reported differences in morphometric measurements due to environmental factors and hormonal, biological, and genetic diversity. Studies indexing AGD and API measurements in postnatal and antenatal periods by fetal ultrasonography are listed in Table 3. Our morphometric mean AGD values were lower than the third-trimester fetal ultrasonography mean AGD measurements according to Aydın et al. and Giboa et al. (Aydin et al. 2019; Genç et al. 2002). This could be attributed to different ethnicity or the positive/negative intolerance due to the ultrasonography techniques.

Usage of different techniques and different distances in the literature for AGD measurements is among the limitations of this study. Another factor limiting the reliability of such studies is the accuracy of the measurements. To overcome any memory bias, two different measurements were taken by two different experienced authorities, leaving time between two measurements. Another limitation is that there may be population-specific ethnic and regional differences in AGD values (Aydin et al. 2019; Hernández-Peñalver et al. 2018; Reisner et al. 1984). In addition, besides fetuses with anogenital and perineal anomalies, fetuses whose extremity anomalies could not be measured properly were excluded from the study, as the study aimed to determine standard API and AGD values. As a result, comparison of the API and AGM values in such cases with normal values, and an evaluation of the rates of deviation rates, could not be made. For the standardization of these measurements, there is a need for clinical studies to be carried out on larger multicenter study groups, including antenatal ultrasonographic measurements and morphometric measurements after preterm labor.

Conclusion

This study was novel in presenting unique data about the normal values of AGD and API in the antenatal period of human fetuses. These parameters can help in sex determination, independent of gestational age and antenatal development status. The creation of morphometric norms and different population-specific studies might contribute to the early diagnosis of genitourinary, anorectal, caudal, and other congenital anomalies in the future.

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Author contributions HT and OE: protocol development, data analysis, and manuscript writing; CI and IK: data collection, data analysis, and manuscript writing; AN: protocol development, manuscript writing, and editing.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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