ANO-GENITAL DISTANCE IN HEALTHY NIGERIAN FULL-TERM NEWBORN INFANTS

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ABSTRACT

Background/Aim: In humans, anogenital distance (AGD) appears to provide a reliable guide to androgen exposure during fetal life. The aim of the present study is to determine the normal values for AGD in Nigerian newborns of both genders and to determine its association with birth weight and length.

Methods: For this descriptive cross-sectional study, 895 (448 males and 447 females) healthy full-term (37 to 42 weeks' gestation) infants between the age of 6 to 72 hours delivered in two Nigerian hospitals were recruited for this evaluation. A standardized anthropometric protocol was used to measure AGD, ano-scrotal distance (ASD) and ano-fourchette distance (AFD). The birth weight and the length of all the subjects were also measured and recorded.

Results: There was a total of 895 (448 males and 447 females) subjects for the study. The mean birth weights were 3288 ± 472 g (95% CI = 3244 - 3332) and 3125 ± 552 g (95% CI= 3074 - 3176) for male and female infants, respectively; $p < 0.01$. The mean AGDs ($\pm$ SD) at birth were 28.3 ± 3.9 mm (95% CI = 27.9 - 28.7) in males and 15.2 ± 2.4 mm (95% CI= 15.0 - 15.4) in females; $p < 0.001$. The ratio of the male-to-female ano-genital distance (ASD/AFD) was 1.7. The adjusted AGD [$\text{AGD}_{\text{ASD}} / \text{birth weight (mm/kg)}$] and [$\text{AGD}_{\text{AFD}} / \text{birth weight (mm/kg)}$] was 8.7 and 4.9 for male and female newborn infants, respectively.

Conclusion: The results of this study provide reliable normative values that can be used as a standard with regard to AGD in Nigerian male and female newborns at birth.

Keywords: Ano-genital distance, newborn, Nigerian

INTRODUCTION

Measuring anogenital distance (AGD) in newborns has been suggested as a noninvasive method of determining male feminisation and female masculinization that could be utilized to predict neonatal and adult reproductive disorders (1). AGD is an easy-to-measure, reliable and a sensitive marker of the effects of fetal androgens and the related endocrine-disrupting chemicals (EDCs) (2). The development of both the perineum and the external genitalia are determined by dihydrotestosterone and as a result, males have a longer AGD compared

to females (3). Numerous animal studies have demonstrated that AGD is a sensitive indicator of prenatal androgen exposure and can be used as a means of retrospective measurement of fetal androgen exposure during the window of programming and masculinization of the reproductive tract (3, 4). In humans, AGD tracks from birth to early childhood and therefore, measurements at birth may be linked to later reproductive disorder (5, 6). In a report from the international panel of experts suggests that AGD is an important clinical measure to address endocrine-sensitive endpoints in the first year of life and recommended more in-depth examination of the

measurement in humans (7). The availability of normative data provides a means of utilizing this biological marker of androgen action in population studies of the effect of environmental chemicals on genital development (6). Measurement of AGD may enable us to distinguish newborns with androgen deprivation from those with other causes, but this could only be done convincingly by comparison with normative values (8). Similar considerations may apply to adult men with gonadal problems and when trying to discern for example, whether a low sperm count had its origins in fetal life or not (8). In contrast, a longer AGD has been demonstrated in females with

congenital adrenal hyperplasia (9) and multi-follicular ovaries (10). All these, reflect the usefulness of AGD measurements in both males and females.

Although AGD measurements sound straightforward, determining the actual measurement that needs to be taken and taking accurate measurements are 2 difficult tasks (10). Maintaining a newborn in the measurement position without moving, while the measurements are made, is difficult (10). In order to overcome this problem, several anthropometric protocols have been developed for the measurement of AGD. Romano-Riquer et al (11) have developed an anthropometric protocol for the measurement of AGD in human males. Similarly, Salazar-Martinez et al (12), have developed an anthropometric protocol for both male and female newborns. They measured the distance from the centre of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum in male newborns and the centre of the anus to the posterior convergence of the fourchette in female infants. There were two previous studies done in Nigeria using the same measurements. However, these did not adequately describe the steps taken to minimize measurement errors (13, 14).

AGD is morphologically a small distance and the reliability of anogenital measurement (AGM) is heavily influenced by measurement errors. The apparatus used for the measurements, the number of measurement taken, the experience of the person taking the measurements, availability of an assistant during the measurement and the number of investigators involved are some of the important factors that could influence the measurements (15). Although there were 2 studies done on AGD, smaller sample size and the coverage of a small geographical area of the country were major limitations of these studies (13, 14). The above considerations prompted the present study involving western and mid-

western regions of Nigeria. The purpose of the present study is to determine the normal values for AGD in Nigerian newborns of both genders and to determine its association with birth weight, length and occipito-frontal circumference.

METHODS

Study area

The study was conducted in two hospitals in Benin City, namely, the University of Benin Teaching Hospital (UBTH), a tertiary healthcare level institution and in St Philomena Catholic Hospital (SPCH), a secondary healthcare level institution. SPCH is located at the center of Benin City and is the second largest maternity unit in Benin City. In both hospitals, as a policy, the mothers usually kept for 2-3 days postpartum before discharge. This ensured the availability of the newborn infants for physical examination including AGD measurements during the first 72 hours of life. The study was conducted over a period of 4 months from October 2013 to January 2014. The study was conducted after obtaining ethical approval from the Ethics and Research Committee of the University of Benin Teaching Hospital. Permission was obtained from the authorities of the two hospitals. After informing the specific objectives of the study, written informed consent was obtained from the mothers for the study. A questionnaire was used in obtaining data from the mothers, regarding the obstetric history such as maternal age and parity.

Study population

Eight hundred and ninety-five (895) consecutively delivered healthy full-term (37 to 42 weeks of gestation) newborn infants aged between 6 and 72 hours whose parents gave consent for the newborn physical examination and AGD measurement were recruited for the study. The stillborn, premature babies and babies born with congenital illness or congenital

anomalies of the external genitalia or imperforate anus were excluded from the study.

Anthropometric measurements

A female nurse was trained in the method of holding and positioning the newborn infant for examination of the external genitalia and measurement of AGD. She also acted as a chaperone during the newborn physical examination. In order to minimize ano-genital measurement errors, we measured ano-scrotal distance in males and ano-fourchette distance in females, which are the easily identifiable soft tissue landmarks, as suggested by Thankamony et al (6). The AGD was measured with the newborn infant placed in the dorsal decubitus position on a table. The assistant stood at the infant's head end and held the infant's thighs in a flexed position with her hands, while placing her forearm on the baby's arm to minimize movement of the newborn (9). The anthropometrist positioned himself in front of the baby and using a flexible inelastic tape, Ano-scrotal distance (ASD) was measured from the centre of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum in male newborns (9). In female infants, Ano-fourchette distance (AFD) was measured from the centre of the anus to the posterior convergence of the fourchette, where the vestibule begins (11). All the measurements were recorded in millimetres. To minimize measurement errors, three consecutive measurements were taken at each assessment and the average was recorded as the final value. In addition, all measurements were performed by a single examiner where the proficiency was validated before the beginning of the study.

The adjusted ano-genital distance was computed as follows:

$AGD_{ASD}/\text{birthweight (mm/kg)}$ for male infants.

$AGD_{AFD}/\text{birthweight (mm/kg)}$ for female infants.

Other anthropometric parameters such weight, length and occipito-frontal circumference were measured, using standardized techniques.

Statistical analysis

The statistical analysis was performed using the SPSS software package version 15.0 (SPSS, Inc. Chicago, IL, USA). Descriptive statistics such as means, standard deviations, confidence intervals were used, as appropriate, to describe the variables. Student's t-test was used to assess the significance of differences between means. A p-value less than 0.05 was considered statistically significant.

RESULTS

There was a total of 895 (448 males and 447 females) newborn infants and their gestational ages were ranging from 38 to 42 weeks. The mean maternal age and parity in this general obstetric population were 27.8 ± 4.6 years and 1.5 ± 1.2 , respectively. The mean birth weight was 3193 ± 512 g. It was 3288 ± 482 g for male infants and 3125 ± 552 g for female infants. The mean birth weight was significantly higher in males than females; $p < 0.01$ (Table 1). The mean AGD ($\pm$ SD) at birth was 28.3 ± 3.9 mm in males and 15.2 ± 2.4 mm in females; p -value < 0.001 (Table 2). The ratio of male-to-female AGD_{ASD}/AGD_{AFD} was 1.7. The AGD/Birth weight (mm/kg) in male and female infants was 8.7 and 4.9, respectively (Table 3). The ratio of the AGD_{ASD}/Birth length and AGD_{AFD}/Birth length were 0.58 for males and 0.32 for females, respectively. A comparison of mean anogenital distances according to the

measuring apparatus used and country of study is depicted in Table 4.

DISCUSSION

The programming of the genital organs occurs during 8-14 weeks of gestation (16). Development of perineum and external genitalia is determined by the dihydrotestosterone

and the animal studies have demonstrated that exposure of rodent fetuses to androgens during fetal masculinization programming window resulted in longer AGD (16, 17). This effect of dihydrotestosterone explains the development of a longer AGD in males compared to females (2, 14, 15). Our study also demonstrated that AGD is significantly longer in male than female newborns.

Table 1: Mean birth weight, length and occipito-frontal circumference according to gender

Parameter	Male (n=448) (95% CI**)	Female (n=447) (95% CI)	t-statistic (p-value)
Mean birth weight(g)	3288 ± 482 (3243-3333)	3125 ± 552 (3074-3176)	4.705 <0.01
Mean birth length(cm)	49.4 ± 0.9 (49.3-49.5)	47.7 ± 0.7 (47.6-47.8)	31.545 < 0.001
Mean OFC* (cm)	34.4 ± 0.6 (34.3-34.5)	34.2 ± 0.7 (34.1-34.3)	0.998 > 0.05

*OFC = Occipito-frontal circumference; **CI= Confidence interval

Table 3: Adjusted anogenital distance for birth weight, length and occipito-frontal circumference according to gender

Parameter	Adjusted AGD for body size
AGD _{ASD} */Birthweight (mm/kg) males	8.7
AGD _{AFD} **/Birthweight (mm/kg) females	4.9
AGD _{ASD} /Birth length (mm/cm) males	0.58
AGD _{AFD} /Birth length (mm/cm) females	0.32
AGD _{ASD} /Birth OFC (mm/cm) males	0.82
AGD _{AFD} /Birth OFC (mm/cm) females	0.44

*AGD_{ASD} = AGD-ano-scrotal distance; **AGD_{AFD} = AGD-ano-fourchette distance; OFC = Occipito-frontal circumference

Table 2: Comparison of ano-scrotal distance (male) and ano-fourchette distance (female) in newborn infants

Parameter	No of infants	Mean $\pm$ SD	95% CI	t-statistic (p-value)
AGD: Ano-scrotal (mm) males	448	28.3 ± 3.9	(27.9-28.7)	60.531
AGD: Ano-fourchette (mm) females	447	15.2 ± 2.4	(15.0-15.4)	(<0.001)

CI= Confidence interval

Table 4: Comparison of mean (SD) anogenital distance according to measuring apparatus used and country of study.

Study	Male AGD ASD*(mm)	Female AGD AFD** (mm)	Measuring apparatus used	Country of study
Thankamony et al [7]	19.8±6.1	9.1±3.8	Vernier caliper	UK
Sathyanarayana et al [16]	23.0±3.8	10.3±2.0	Vernier caliper	USA
Özkan et al [17]	23.0±6.0	10.3±2.0	Vernier caliper	Turkey
Papadopoulou et al [6]	23.6±5.1	13.8±2.5	Vernier caliper	Spain
Papadopoulou et al [6]	27.1±4.4	14.4±3.0	Vernier caliper	Greece
Oguz Kutlu [19]	25.8±4.7	13.4±3.2	Vernier caliper	Turkey
Ovidime et al [15]	31.1±6.4	13.9±2.6	Flexible inelastic tape	Nigeria
Present study	28.3±3.9	15.2±2.4	Flexible inelastic tape	Nigeria

*ASD = Ano-scrotal distance; **AFD = Ano-fourchette distance

Numerous studies have shown significant variations in AGD. It seems to be multifactorial and genetics, ethnicity, method of AGD measurement, birth weight, the age of the new born at the time of measurement are some of the factors (1, 5, 6, 15, 18). There have been numerous studies demonstrating the ethnic and geographical variation of AGD. Sathyanarayana et al (19), who reported a longer AGD in Caucasian male infants in comparison with Asian or Native Americans. A study done in Greece and Spain, where the same measuring apparatus and the same anthropometrists obtained different AGD measurements in their study population, reflecting inter-country variations in AGD (5). However, there were not data for African countries. The tool used for measurement contributes to the observed differences in AGD measurements. A study in the United Kingdom, where a vernier caliper was also used for measurement, has demonstrated a very much lower mean AGD (19.8mm) compared to most of the other studies (6). In our study, the mean AGD at birth in male newborns, is similar to previously published studies where the flexible inelastic tape was used for their measurements (14, 20). In addition, our AGD measurements in males were in total agreement with the measurements reported by Papadopoulou et al (27.1mm) in Greece, using a vernier caliper (6). In

females, our mean AGD was also in keeping with those reported in previous studies (5, 14, 21) but some differences were also observed (6, 13, 15).

The body size and several other factors account for the longer AGD observed in males than females. Animal studies have shown that body size influence AGD and needs to be adjusted in studies concerning the effect of environmental pollutants on AGD (22, 23, 24). AGD of newborn infants also has a correlation with the birth weight, the length and the occipito-frontal circumference (14, 15). Therefore, when analyzing AGD data, it is important that adjustments are done for these parameters before comparing AGD data. Our data showed that sexual dimorphism in AGD persisted even after correcting for birth weight, length and occipito-frontal circumference (OFC) by calculating the adjusted AGD (AGD/ birthweight; AGD/ birth length; AGD/birth OFC, respectively).

There were some limitations in our present study. Maternal urinary levels of metabolites of endocrine-disrupting chemicals, which has the potential to affect the ano-genital anthropometric measurements were not assessed. The use of an inelastic flexible tape, which is not the ideal, rather than vernier caliper for the

AGD measurements was another limitation. However, a tape has the advantage of following the curves of the perineum, which the vernier caliper cannot do, and could be taken as an equally accurate measurement. Despite this limitation, the normative data reported in the present study are probably the most reliable data available for Nigeria.

CONCLUSIONS

Despite the limitations of our study, the results of this study provide a more reliable and comparable normative data that can be used as a standard with regard to AGD in Nigerian male and female newborns at birth.

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