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Anogenital distance: A longitudinal evaluation of its variants and indices in boys and girls of Sonora, Mexico

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ABSTRACT

There is no consensus on which anogenital distance (AGD) variant to use and how to adjust it by body size in humans. This study quantitatively evaluated AGD variants and body size adjustments to determine which would be the best choice. AGD variants, height, and weight were measured on five occasions during the first year of life of 307 infants. The ratio of anoscrotal distance (ASD) in boys and anofourchette distance (AFD) in girls increased from 1.9 at birth to 2.3 at 12 months of age. Each AGD variant was divided by each body size variable to generate different indices. Such indices were standardized to make them comparable when analyzing their performance through mixed models. ASD and AFD adjusted by height generated precise ($p < 0.05$) AGD indices: 0.4–0.5 and 0.2, respectively. Results suggest that the best body size adjustment for all AGD variants in the first year of life is height.

1. Introduction

The anogenital distance (AGD) is an anthropometric parameter determined by prenatal androgens and predictive of the feminized or masculinized phenotype, both in animals and humans [1–5]. In animals, AGD is defined as the distance from the center of the anus to the genital tubercle [6]. In humans, given the structure of the genitals, there are different male and female AGD variants. The AGD variants in girls are: from the center of the anus to the posterior commissure of the labia minora (fourchette) [7–11], from the center of the anus to the clitoris [8–10,12], and from the fourchette to the clitoris [8,13]. In boys, AGD variants are: from the center of the anus to the anterior base of the penis [7,9–11,14], from the center of the anus to the posterior base of the penis [11,14], and from the center of the anus to the anterior base of the scrotum [7,9–11,14,15].

The AGD is a dimorphic measure, being approximately twice as large in boys as in girls, from birth up till 24–30 months of age [1,13,16]. In a mature age (>18 years), the difference in AGD by sex is slightly less than the ratio observed in infants (<30 months) [2,17]. AGD variants from the center of the anus to the posterior commissure of the labia minora (AFD) in girls, and the distance from the center of the anus to the anterior base of the scrotum (ASD) in boys, are anatomically comparable measures used to determine sexual dimorphism (ASD/AFD) [10,11,13,18].

The structure and size that the sexual organs will reach is determined in the embryonic stage of the sexual differentiation, between weeks 7 and 12 of a woman's pregnancy. The sexual organs, and the AGD accordingly, grow subsequently only in relation to body growth [17,19–21]. While the normal development of the male sexual organs depends on the action of androgens, female organ development is the result of the absence of androgens [21].

In research on prenatal exposure to endocrine disruptors, AGD has been considered a reliable marker of fetal exposure to agonists and antagonists of androgen and estrogen receptors, and of their effects on the reproductive system [12,22–26]. However, using AGD in humans has limitations: different variants are used in different epidemiological studies, there are still no reference values for the measurements, and AGD is influenced by the body size [17].

The need to adjust the AGD by body dimensions is well established due to the wide range of body sizes that can be observed in

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human study populations. Previous studies suggest that body size and age could be the main confounding factors when using AGD as an endocrine disruptor marker, since they strongly affect its dimensions [7,17,27]. Unlike studies in animals [21], there is no consensus on the best way to account for body size in human beings AGD studies. Various investigations have proposed different ways to adjust AGD by body dimensions [17,27]. Among the adjustments used is the generation of AGD indices, which consist of dividing this measure by the body size, represented by the anthropometric variables of height [12] or weight (or its cube root) [7,12,24]. Likewise, an index of anal position [14], which is not dependent on age, has been tested; API = AGD/distance from the posterior base of the scrotum or the fourchette to the coccyx [25]. Other researchers have used the AGD without adjusting, but have controlled by weight, height, and/or z-scores in the predictive statistical models to adjust for body size [13,14,26].

Given the various forms of measuring AGD and adjusting it by body size, it is difficult to compare the results of epidemiological studies that use this marker. Furthermore, there is no numerical assessment comparing empirical human AGD data. In this study, the different variants of the AGD and their indices are evaluated to determine which are the most appropriate to use in epidemiological studies.

2. Materials and methods

This study is part of a prospective longitudinal research study of the National Institute of Public Health, Mexico: Prenatal exposure to persistent organic pollutants, metals and their associations with neurodevelopment and endocrine disruption in the first year of life in Sonora, Mexico. The research was carried out between November 2012 and June 2014. Briefly, all women in the third trimester of pregnancy who attended follow-up prenatal examinations in the health centers of the Ministry of Health, in the municipalities of Benito Juarez, Huatabampo, Etchojoa and Navojoa in Sonora, Mexico, were invited to participate in this study.

Women recruited received information on the study objectives and procedures for performing the anthropometric and AGD measurements. Participants answered a questionnaire on sociodemographic variables and exposure to environmental pollutants. Also, maternal blood samples were drawn upon recruitment to determine serum concentration of persistent organic compounds. The women were followed-up to carry out AGD and body size measurements of their offspring since their birth and up till their first birthday.

The project was approved by the ethics, biosecurity and research committees of the National Institute of Public Health, Mexico. Those who agreed to participate were assured confidentiality of the information provided and asked to sign a letter of informed consent.

2.1. Participants

There were 307 mother-daughter/son pairs who fulfilled the inclusion criteria; namely women should be in good physical and mental health (reportedly and as far as we could assess), be able to read and write in Spanish, and have a normally developing pregnancy of a singleton product. Exclusion criteria of the infants included: preterm birth (gestational age <37 weeks), low birth weight (<2.5 kg), having any health problem or congenital malformation at birth, alterations in the newborn screening tests results, or neonatal hypoxia (considered as APGAR <8). After birth, 153 girls and 154 boys met the aforementioned pre-established criteria and had at least one AGD measurement.

2.2. Follow-up

At the time of birth, gestational age, (considered as the time interval between the first day of the last menstrual period, as reported by the mother and the date of birth of the product) was recorded. The infants' height, weight, head circumference, and AGD were measured at birth and when they were one, three, six and twelve months old. In boys, the width of the penis was also measured.

Monitoring of the infants was carried out through programmed monthly visits to their corresponding health center. Community health promoters in each locality visited the participants at their homes to remind them of the appointment on the previous day.

A single nurse did all the anthropometric measurements of the study. This nurse was personally trained in the measurement technique of the different AGD variants by Romano-Riquer, who participated in the development of a protocol for the measurement of anogenital distance in human males [6]. Such training was carried out in infants under one year old, who attended examinations at the Hospital del Niño Morelense in Cuernavaca, Morelos, one month prior to the beginning of the study (October 2012).

2.3. AGD measures

AGD was measured according to the recommendations of the Meeting report: measuring endocrine-sensitive endpoints within the first years of life and the measurement technique of the different AGD variants proposed by Romano-Riquer [6,28]. Three different AGD variants were measured for each gender, as shown in Fig. 1. A digital DigiMax branded Vernier caliper, with a measuring range of 0–150 mm and 0.01 mm accuracy, was used for all measurements. The AGD was measured in increments of 1 mm, placing the infants in a dorsal decubitus position, with abducted lower extremities and bent knees (Fig. 1). The girls were measured from the center of the anus to the fourchette (AFD), from the center of the anus to the clitoris (ACD) and from the fourchette to the clitoris (FCD), as shown in Fig. 1(a). The boys were measured from the center of the anus to the anterior base of the penis (AGD1), from the center of the anus to the posterior base of the penis (AGD2), from the center of the anus to the anterior base of the scrotum (ASD), and the width of the penis (PW) as shown in Fig. 1(b).

2.4. Body size measures

Body weight was measured in kilograms using a Tanita© portable electronic scale with a reading accuracy of 0.10 g for weights up to 10 kg and 0.20 g for weights between 10 and 20 kg. The scale was calibrated on each occasion before being used. The height was obtained in centimeters with Seca© portable infantometers, model 417, with a reading accuracy of 1 mm. The head circumference was measured in centimeters (cm) with a Futaba© (model R-280) measuring tape, with a reading accuracy of 1 mm.

2.5. Statistical analysis

Follow-up rates of participants were estimated as the percentage of assistance calculated between each visit. An exploratory analysis was conducted to observe the distribution of the different AGD measures at every age and of the anthropometric variables of weight, height, and head circumference by gender. The ASD/AFD ratio was calculated to determine the magnitude of AGD sexual dimorphism at each age of measurement.

The correlation of each AGD variant at different ages and between different AGD variants amongst each other at a specific age was evaluated using Pearson's correlation coefficient in both girls and boys, but separately. The association between the AGD and