Nig. J. Physiol. Sci. 26(June 2011) 023 – 028
www.njps.physocnigeria.org

Nig. J. Physiol. Sci.

Anogenital distance and umbilical cord testosterone level in newborns in Zaria, Northern Nigeria

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Summary: The anogenital distance (AGD) is the distance between the anus and the base of the penis in males and anus to fourchette (AF) distance in females and is a sexually dimorphic index that, on average, is twice as great in males as in females, so it is used as an indicator of appropiate masculine development. In this study, the anogenital distance (AGD) and anthropometric measurements such as birth weight, birth length, head circumference and placenta weight of 200 newborns (100 male, 100 female) were taken and umbilical cord serum was assayed for testosterone concentration using Radioimmunoassay (Microwell). Data obtained were analysed using Student t-test and Pearson's Correlation Analysis as applicable. Results revealed that mean total anogenital distance was 22.53±0.70mm, and it was significantly higher in males: 31.11±0.64mm than in females: 13.89±0.26mm and we observed that there was positive correlation between birth weight and AGD in females. In males head circumference correlated positively with AGD. The mean cord testosterone concentration was 2.78±0.30ng/ml in males and 2.09±0.22ng/ml in females and did not have any significant correlation with anogenital distance. It was concluded that AGD of the population studied, though high was not significantly higher than AGD in other parts of the world and umbilical cord testosterone level did not have any significant effect on AGD.

Keywords*:* Anogenital distance, Ethnicity, Umbilical cord testosterone, Anthropometry.

Abbreviations: *AF (Anus to Fourchette), AGD (anogenital distance), TDS (testicular dysgenesis syndrome)*

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Manuscript Accepted: April, 2011

INTRODUCTION

Anogenital distance (AGD) is the distance between the anus and the base of the penis in males and anus to fourchette (AF) distance in females. It is a sexually dimorphic index that, on the average, is twice as great in males as in females and it serves as a marker of proper male development. (Callegari *et al*, 1987; Salazar-Martinez *et al*, 2004 and Wiesse, 2006).

The utility of Anogenital Distance (AGD) measures in humans is supported by experimental data in primates showing that *in utero* exposure of females to androgenic agents increased AGD (Hendrickx *et al*., 1987). Measuring the anogenital distance in neonatal humans has been suggested as a non-invasive method to predict neonatal and adult reproductive disorders such as cryptorchidism and hypospadias (Breyer, 2008) and also can be associated with premature breast development in young girls (Colón *et al*., 2000). Other factors such as birthweight, race, genetics and endocrine disruptors are reported to affect AGD (Olorunshola *et al,* 2007 and Callegari *et al*, 1987)

The mechanism by which androgens increase AGD in females is by inducing "labioscrotal fusion" (Salazar-Martinez *et al*, 2004). In animal studies, measurement of anogenital distance (AGD) is now routine, and serves as a bioassay of fetal androgen action. In rodents, perineal growth is dihydrotestosterone-dependent, males have a greater AGD than females, and the use of AGD to differentiate between males and females is now standard (Gallavan *et al*, 1999).

Measurement of AGD using a flexible ruler which conformed to the natural curves of the perineum and measurement under anesthesia improved accuracy by eliminating any motion artefact. (Breyer, 2008).

From available literature, there is no report on AGD in newborns in Nigeria, consequently, this study was designed to determine if there is correlation between umbilical cord testosterone and anogenital distance and possible causes of variation.

MATERIALS AND METHODS

Study Design

This study is a cross sectional study conducted between June and August 2010. Questionnaires were used to obtain pregnancy history and sociodemographic parameters of the paturients. Anthropometric measurement (birth weight, birth length, head circumference and anogenital distance) were taken within six hours after delivery. Five mls of blood was collected from the umbilical cord for testosterone assay in the laboratory of the Chemical Pathology Laboratory of the Ahmadu Bello University Teaching Hospital, Shika, Zaria.

Demography of Study Area

Zaria is a cosmopolitan city in Kaduna State, Nigeria inhabited by about 408,198 people as stated by the 2006 census report (National Bureau of Statistics, 2011). Zaria occupies a portion of the high plains of Northern Nigeria, 652.6 meters above sea level and some 950 kilometers from the coast at 11^{o}04N, 7^{o}42E. Zaria is the second largest city in Kaduna State of Nigeria with many tertiary institutions of learning and research including Ahmadu Bello University Zaria.

The climate is Savannah with annual rainfall ranging from 0.0 to 816.0mm/month and minimum and maximum temperature 15.3C^{o} and 36.25C^{o} respectively.

Ethical Consideration

Approval for this study was obtained from the Ethical Committee on Human Research of the Ahmadu Bello University Teaching Hospital, Shika, Zaria. The study recruited parturients only after detailed explanation as to the nature and benefit of the study had been given, and verbal and written consent obtained.

Study Population

The study population used was 200 consecutive consenting paturients in labour at the Ahmadu Bello University Teaching Hospital, Shika – Zaria; Hajiya Gambo Sawaba General Hospital, Kofan Gayan, Zaria; Major Ibrahim B. Memorial General Hospital, Sabon Gari, Zaria and Salama Infirmary, Kwangila, Zaria

Exclusion Criteria

Non - consenting parturients and newborns with obvious anogenital anomalies such as imperforate anus and ambiguous genitalia were excluded from the study.

Subjects

A total of two hundred consecutive newborns (100 male and 100 female) were recruited for the study, within 6 hours of birth, using a structured questionnaire. Pregnancy history and socio-demographic data were obtained from the parturients about their family history and diet, parity, ethnicity (tribe), pregnancy order, drug history, alchohol and cigarette use by mother, marriage order, educational status and gestational age at delivery.

Collection and Storage of Cord Blood

On delivery, 5ml of umbilical cord blood was collected from the umbilical cord after cutting it from the placenta, into plain serum bottles and allowed to clot undisturbed for 1 hour at room temperature (18-25^{o}C) (Lewis *et al*, 2001).

The clot was then gently removed from the container wall by means of a glass rod to avoid lysis. The bottles were then closed and centrifuged for 10 minutes at 1200*g*. The supernatant serum was pipetted into another set of bottles and centrifuged for 10 minutes at 1200*g*. The serum was transferred into new serum bottles and stored in a freezer at -4^{o}C for analysis as described by Lewis *et al*, 2001 with some modification (according to Lewis *et al*, 2001 serum should be stored at -20^{o}C, but due to facilities available, the serum was stored at -4^{o}C).

Serum Testosterone Concentration

The serum obtained from the umbilical cord of the newborns (100 male, 100 female) was assayed for testosterone at the Chemical Pathology Laboratory of the Ahmadu Bello University Teaching Hospital. Enzyme based immunoassay (EIA) system was used to measure the testosterone level in the serum samples collected. The reagent diagnostic kit was obtained from Syntron Bioresearch Inc. (Carlifornia, U.S.A.).

Placenta Weight

The placenta was tied in polythene bag and weighed on a basinet scale. Placenta weight was taken to the nearest 10g. Other anthropometric measurements such as birth weight, birth length and head circumference were taken all in accordance with the method described by Athreya (1980).

Birth Weight

Without clothing the newborns were weighed on a basinet weighing scale. Weight was taken to the nearest 10g (Athreya, 1980).

Birth Length

Birth length was measured in the supine position, the feet were held against a fixed foot piece at the 0 mark

and a movable head piece was brought to touch firmly against the vertex. The readings were recorded to the nearest 1cm (Athreya, 1980).

Head Circumference

Head circumference was measured using a non-stretchable tape over the maximum point of occipital protuberance and above the the superior orbital ridges anteriorly (Athreya, 1980)

Anogenital Distance Measurement

The newborn infant was kept in the dorsal decubitus position; with the aid of an assistant, both hips were flexed and light pressure was exerted on the newborn's thighs until the assistant's hand touched the subject's abdomen. Measurements were made with flexible tape. Distance was measured from the center of the anus to the posterior convergence of the fourchette (where the vestibule begins) in female infants (Callegari *et al*, 1987) and from the center of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum in male infants. Results were recorded in millimetres.

Gestational age was estimated according to the Dubowitz scoring system (Dubowitz *et al*, 1970). This was done by examining the newborn using eleven physical (external signs) and ten neurologic characteristics. The physical and neurological criteria are each scored, recorded and added together and gestational age was then plotted against the total score. Gestational age (± 2 weeks) was read off this graph. The physical characteristics are edema, skin texture, skin color, skin opacity, lanugo, plantar creases, nipple formation, breast size, ear form, ear firmness and genitalia while the neurological characteristics include posture, square window, ankle dorsiflexion, arm recoil, leg recoil, popliteal angle, heel to ear, scarf sign, head lag and ventral suspension.

Statistical Analysis

All data were recorded as mean ± SEM and was subjected to statistical analysis using Student t- test, Analysis of Variance and Pearson's Correlation test where applicable. A p value of equal to or less than $0.05(p \leq 0.05)$ was considered statistically significant. The statistical package for the Social Science (SPSS) for Windows Version 17.0 was used for all calculations and statistical analysis.

RESULTS

Anogenital Distance

The mean AGD of the newborns was 22.50±0.70mm, with males having AGD of 31.11±0.64mm, it was significantly higher than AGD of females who had AGD of 13.89±0.26mm ($p>0.001$). (Table 1)

Testosterone Concentration and AGD

The mean testosterone concentration was 2.78±0.30ng and 2.09±0.22ng/ml in males and females respectively. There was no statistically significant difference observed between the sexes (P value>0.05) and there was also no correlation between AGD and cord testosterone in both sexes as shown in table 2 and 3.

Anthropometric Measurements and AGD

There was positive correlation between birth weight

Table 1.

Mean Anogenital Distance, Testosterone Concentration, Placenta Weight and some Anthropometic Measurements of Newborns

	Male (n=100)	Female (n=100)	Total (n=100)
AGD (mm)	31.11±0.64	13.89±0.26	22.50±0.70
T. Conc (ng/ml)	2.78±0.30	2.09±0.22	2.44±0.19
Placenta weight(kg)	0.88±0.26	0.69±0.02	0.78±0.10
Birth length (cm)	50.13±0.40	49.24±0.37	49.19±0.27
Head circumference(cm)	34.45±0.21	34.23±0.21	34.34±0.15
Birth weight (kg)	3.23±0.06	3.04±0.05	3.03±0.04

AGD- Anogenital Distance, T.Conc- Testosterone Concentration

Table 2.

Correlation Matrix of AGD, Testosterone, Birth weight, Head Circumference, Birth Length and Placenta Weight (n=100) in females.

Variables	TC	PW	AGD	HC	BL	BW
TC	1	0.12	-0.05	0.25	-0.09	0.27
PW		1	-0.01	0.04	0.20	0.11
AGD			1	0.17	0.10	0.23[a]
HC				1	0.382[c]	0.52[c]
BL					1	0.44[c]
BW						1

TC- Testosterone Concentration, PW- Placenta Weight, AGD- Anogenital Distance, HC- Head Circumference, BL- Birth Length, BW- Birth Weight. a= p value<0.05, b= p value<0.01, c= p value<0.001

Table 3.
Correlation Matrix of AGD and Testosterone, Birth weight, Head Circumference, Birth Length and Placenta Weight (n=100) in males.

Variables	TC	PW	AGD	HC	BL	BW
TC	1	-0.06	0.26	-0.09	0.14	0.08
PW		1	0.07	0.45[c]	0.23	0.53[c]
AGD			1	0.23[a]	0.04	0.15
HC				1	0.48[c]	0.56[c]
BL					1	0.43[c]
BW						1

TC- Testosterone Concentration, PW- Placenta Weight, AGD- Anogenital Distance, HC- Head Circumference, BL- Birth Length, BW- Birth Weight. a= p value<0.05, b= p value<0.01, c= p value<0.001

and AGD in the females ($p<0.05$), in addition head circumference, and birth length also correlated positively with birth weight; there was also positive correlation between head circumference and birth length (Table 2).

In males there was positive correlation between head circumference and AGD, placenta weight, and birth weight; also there was positive correlation between birth weight and placenta weight, head circumference and birth length (table 3).

Mean birth length for male newborns was 50.13±0.40cm, while in females 49.24±0.37cm. There was no statistically significant difference between the mean values of head circumference, birth length, head circumference, birth weight and placenta weight between the males and the females (P value> 0.05). (See table 1)

DISCUSSION

A worldwide increase in testicular dysgenesis syndrome (TDS) consisting of testicular cancers, low and declining semen quality, high frequency of undescended testis and hypospadias have been reported by several authors (Skakkeback *et al.,* 2001, Skakkeback *et al.,* 2003, Giwercman *et al*, 2006 and Aschim *et al.,* 2006). This increase has been reported to be due to adverse environmental influences resulting in disruption of early embryonal programming and gonadal development during early fetal life (Aschim *et al.,* 2006: Asklund *et al.,* 2004 and Wohlfart-Veje *et al*., 2009). Animal experiments have shown that all TDS symptoms except testicular cancer can be induced by foetal exposure to anti-androgenic chemicals (Wohlfahrt-Veje *et al*., 2009).

AGD is a sexually dimorphic index and a biomarker of proper male development and androgenisation. In addition, it could be used as a screening tool for TDS, especially in resource poor countries. The anogenital distance obtained in this study of 31.11±0.64mm in males and 13.89±0.26mm in females respectively, showed that male values are significantly higher than female values (Swan *et al,* 2005, Salazar- Martinez *et al.,* 2004, Callegari *et al,* 1987), for this reason, AGD is a variable of sexual dimorphism in males and females. The significant sex differences noted confirmed that it is a sexual dimorphic index and may be helpful as a preliminary sexing diagnostic tool in situations of ambiguous genitalia (Weiss *et al.,* 2006).

The mean anogenital distance for this population study was 22.52mm, higher than 18mm which was obtained by Salazar- Martinez *et al.,* (2004) in Morelos, Mexico. Values obtained by Salazar-Martinez *et al* (2004) for males and females were 21mm and 11mm respectively. The difference in the AGD from values in our study may be associated with genetic and or /racial differences. Birth weight and racial differences are known to affect AGD (Callegari *et al*, 1987., Philips *et al*, 1996 and Salazar- Martinez *et al*, 2004) but we found positive correlation with birth weight only in female newborns. The highest value for AGD in males in this study was 44.00mm in a newborn that weighed 2.20kg while the lowest was 17mm in a newborn that weighed 1.45kg. Probably this shortened AGD could be attributed to the low birth weight. The highest anus to fourchette (AF) distance was 30mm in a newborn that weighed 3.0kg while the lowest AF distance was 8.00mm in a newborn weighing 2.0kg. This high AF distance value was obviously not related to birth weight as revealed by the study.

It is also reported that endocrine disrupting chemicals (EDC) such as phthalate exposure in utero could lead to decreased AGD (Barlow and Foster, 2003; Foster *et al* 2000; Hendrickx *et al*., 1987 Farr, 2003 Hauser *et al* 2005 and Swan et al 2005). There is high unregulated use of insecticides for agricultural purposes and they are also contained in cosmetics and other household products. There is paucity of information about the average daily intake (ADI), Minimum tolerable dose (MTD) and Residual Level (RL) of these chemicals in food and drinking water in our environment (Olorunshola *et al*., 2007).

The mean umbilical cord testosterone concentration in this study was 2.44±0.19ng/ml. There was no significant difference in the mean testosterone concentration 2.78±0.30ng/ml in males and 2.09±0.22ng/ml in the females ($p>0.05$). There was no correlation between umbilical cord testosterone and AGD in this study. Umbilical cord testosterone in the newborn may not be a true reflection of serum testosterone.

For the first time probably, we documented that AGD correlated positively with head circumference in males. This may be explained due to the possible effects of testosterone on brain size/weight and skull size or decreased exposure to anti-androgens. Parameters such as birth length, head circumference, birth weight and placenta weight showed no significant correlation with AGD. Conclusively, results from this study indicate that umbilical cord testosterone level at birth did not affect anogenital distance and the mean AGD of the newborns in Zaria showed a greater value than obtained by Salazar-Martinez *et al*,(2004)., Philips *et al.* (1996) and Callegari *et al.,* (1987). Cord testosterone concentration at birth was higher in male newborns though not statistically significant. AGD positively correlated with head circumference in males. Other parameters such as birth weight, birth length and placenta weight did not affect AGD.

The authors acknowledge the assistance of the Medical Director and staff of Salama Infirmiry, Zaria, Nigeria, Maternity Wards of St. Luke's Hospital, Wusasa, and Hajiya Gambo Sawaba General Hospital, Kofan Gayan, Zaria, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria and Dr. Olugbenga Oguntunde of the department of Community Medicine, Ahmadu Bello University, Zaria for the statistical analysis.

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