

# Association between Anogenital Distance and External Genital Anomalies in Nigerian Male Newborn Infants

Onyiriuka AN<sup>1</sup>, Ikeanyi EM<sup>2</sup>

<sup>1</sup>Endocrinology and Metabolism Unit, Department of Child Health, University of Benin Teaching Hospital, PMB 1111, Benin City, Nigeria, <sup>2</sup>Department of Obstetrics and Gynaecology, Niger Delta University, Wilberforce Island, PMB 071, Bayelsa State, Nigeria.

Received: 23-Jan-2017; Accepted: 30-Mar-2017

**Aims:** Animal studies regarding endocrine dysfunction have linked anomalies of male external genitalia with reduced anogenital distance (AGD). Human studies have associated shorter AGD with genital anomalies in males. The aim of the present study is to report the AGD measurement among Nigerian newborn infants with undescended testis and/or hypospadias and compare the results with those of infants with normal external genitalia.

**Methods:** In this case-control study, the AGD of 17 newborn infants with abnormal external genitalia (undescended testis (UDT)/hypospadias) was measured and the result compared with those of 34 newborn infants with normal external genitalia (descended testes and no hypospadias, epispadias or chordee). Each subject recruited was matched for birth weight and length with two controls delivered consecutively. Thus, a total of 51 (cases and controls) newborn infants were ultimately studied. The differences in AGD between infants with abnormal external genitalia compared with that of infants with normal external genitalia were examined, using two-tailed Student's t-test, with p-value set at < 0.05. In addition, the AGD in infants with hypospadias was compared with the AGD in infants with UDT.

**Results:** The mean AGD was 27.6±4.9mm (95% CI = 26.0-29.2), 26.3±5.4mm (95% CI = 23.0-29.6) and 25.1±6.2 mm (95% CI = 20.1-30.1) in infants with normal external genitalia, UDT and hypospadias, respectively; p > 0.05. The mean AGD was shorter in infants with hypospadias compared with infants with UDT, p > 0.05.

**Conclusions:** The AGD in Nigerian newborn infants with either UDT or hypospadias tended to be shorter than that of their counterparts with normal genitals and this reduction was more in infants with hypospadias compared with infants with UDT.

**Keywords:** anogenital distance, hypospadias, undescended testis, newborn, Nigeria

DOI: 10.3126/njog.v12i2.19942

## INTRODUCTION

The formation of the male external genitalia is a complex developmental process involving genetic programming, cell differentiation, hormonal signaling, enzyme activity and tissue remodeling. The masculinization of the external genitalia commences in the 10<sup>th</sup> -12<sup>th</sup> weeks of gestation under the influence of testosterone converted to 5 $\alpha$ -dihydrotestosterone in response to a surge of leutinizing hormone from the pituitary gland.<sup>1</sup> This masculinization is time-specific and occurs during a period called the masculinization programming window (MPW) of the reproductive tract.<sup>1-4</sup> One of the first signs of masculinisation is an increase in the distance between the anus and the genital structures i.e., the length of the perineum.<sup>1</sup> Any disruption of

the process during the MPW of the reproductive tract could result in hypospadias or cryptorchidism and a shorter perineal length.<sup>2-4</sup> Animal models have shown that such a disruption may be caused by both known and putative endocrine-disrupting chemicals (EDCs) present in the environment.<sup>2,3,5</sup> Welsh et al, concluded that the MPW in humans is likely to occur between 8-14 weeks gestation.<sup>2</sup> The concept of testicular dysgenesis syndrome (TDS) proposes that various male reproductive disorders, such as hypospadias, cryptorchidism, testicular germ cell cancer and low sperm count may have a common origin in early fetal life caused by abnormality in the development of the testis.<sup>6,7</sup> The phase of transinguinal testicular descent is mediated by androgens via its effect on the genitofemoral nerve and subsequent release of guiding neurotransmitters.<sup>8,9</sup> Undescended testis (UDT) together with hypospadias, is the most common congenital external genital anomaly in male infants, affecting 1.6% to 9.0% live-births.<sup>10</sup>

Anogenital distance (AGD) is an anthropometric

## CORRESPONDENCE

Alphonsus N. Onyiriuka  
Endocrinology and Metabolism Unit, Department of Child Health, University of Benin Teaching Hospital, PMB 1111, Benin City, Nigeria.  
E-mail: alpndiony@yahoo.com

measure which has been accepted as a sensitive reproductive end-point of masculinization in animal models. Animal studies regarding endocrine dysfunction has shown that reduced anogenital distance (AGD) is associated with congenital anomalies of the male external genitalia. In that report, the authors stated that their findings strongly encouraged measurement of AGD in humans as a reflection of androgen action, specifically within the MPW, as AGD may be predictive of adult-onset TDS disorders.<sup>2</sup>

Some cross-sectional studies have reported a shorter AGD in newborn infants as well as in older infants with undescended testis or hypospadias.<sup>5,11-14</sup> To the best of our knowledge, there is no report of the association of AGD with undescended testis and/or hypospadias from Nigeria. The two previous studies in Nigeria that reported on AGD measurements in the newborn did not examine its association with undescended testis and/or hypospadias.<sup>15,16</sup> The purpose of the present study is to report the AGD measurement among Nigerian male newborn infants with undescended testis and/or hypospadias and compare the result with those of infants with normal external genitalia.

## METHODS

### Study area

A case-control study was conducted over a four-month period (October 2013 to January 2014) among newborn infants delivered in two hospitals in Benin City, namely, the University of Benin Teaching Hospital (UBTH), a tertiary level healthcare institution and St Philomena Catholic Hospital (SPCH), a secondary level healthcare institution. SPCH is located at the centre of Benin City and is the second largest maternity unit in Benin City. As a policy, in both hospitals, mothers usually stay for 2-3 days postpartum before discharge, forming the basis for the selection of these two hospitals for the study. This ensured availability of the newborn infants for physical examination including AGD measurements in the first 72 hours of life.

The research protocol was approved by the Ethics and Research Committee of the University of Benin Teaching Hospital. Permission was obtained from the authorities of the two hospitals. Consent was obtained for examination of the newborn infants from their mothers, after informing them of specific objectives the study.

### Study population

In this study, 465 consecutively delivered healthy full-term (37 to 42 weeks gestation) male neonates aged between 6 and 72 hours were examined and 17 of them were found to have abnormal external genitalia (undescended testis (UDT)/hypospadias). For each of these 17 neonates with abnormal external genitalia, two infants with normal external genitalia were recruited as controls. Together, a total of 51 infants constituted the study population. The AGD of 17 newborn infants with abnormal external genitalia was measured and the result compared with those of 34 newborn infants with normal external genitalia (descended testes and absence of hypospadias, epispadias or chordee). We excluded infants with imperforate anus (which could interfere with identification of the anatomical landmarks for AGD measurement) and infants with genital anomalies which were a part of a known malformative syndrome. A questionnaire was used in obtaining data (e.g., maternal age and parity) from the mothers.

### Examination of the external genitalia

All the infants were examined within the first 72 hours of birth by a single observer (ANO) and in the presence of the infant's mother. The examination began with infant lying in supine position and held in position by an assistant, a female nurse, who also acted as a chaperone. The penile shaft was inspected for hypospadias (and other anomalies) and the testicular position was determined. The testicular examination of the infant involved a two-handed technique as described by Docimo et al.<sup>17</sup> The palpation took place in anxiety-free environment and with warm hands to avoid cremasteric reflex retracting the testes. The key to distinguishing a retractile from an UDT is success of delivery and stability of the testes within the scrotum. The retractile testis will remain intrascrotal after overstretching of the cremaster muscle, whereas a low UDT will return to its undescended position after being released.<sup>18</sup> The position of each testis was categorized into two major group as normal (if they were either scrotal or normal retractile) or undescended. The undescended group was sub-classified into prescrotal (if they were high scrotal or suprascrotal), inguinal (if the testes are palpable somewhere along the line of descent in the inguinal region but above the scrotum) or non-palpable (testes not palpable on external physical examination).<sup>19</sup>

The next step was the measurement of the anogenital distance. The hypospadias was classified into mild (defined as glandular or coronal); moderate (defined as subcoronal on penile mid-shaft) and severe (defined as opening in the scrotum, penoscrotal or perineal).<sup>20</sup>

#### Anthropometric measurements:

With the infant placed in the dorsal decubitus position on a table and held in position by the assistant, the AGD was measured, using a flexible inelastic tape. In order to minimize ano-genital measurement errors, we measured ano-scrotal distance because of the presence of easily identifiable soft tissue landmarks, as suggested by Thankamony et al.<sup>21</sup>

From the infant's head end, the assistant 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. The anthropometrist positioned himself in front of the baby and using a flexible inelastic tape, measured the distance from the centre of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum, representing the ano-scrotal distance (ASD). In some cases, the scrotum was raised (without stretching) to be able to identify the landmark of the perineoscrotal junction.<sup>22</sup>

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 order to eliminate inter-observer variability, all measurements were performed by a single examiner (ANO) whose proficiency was validated before the beginning of the study. The ASD of the control group was also measured by the anthropometrist in a similar manner. Other anthropometric parameters such as weight, length and occipito-frontal circumference were measured, using standard techniques.

**Statistical analysis:** The differences in AGD between infants with abnormal external genitalia compared with those of infants with normal external genitalia

were examined, using two-tailed Student's t-test, with p-value set at < 0.05.

## RESULTS

Among a total of 465 consecutively delivered full-term male live-born infants, 17(3.7%) had disorder of sex differentiation (anomaly of the external genitalia). The mean maternal ages of neonates with anomaly of the external genitalia and normal external genitalia were  $28.2 \pm 4.4$  years and  $28.6 \pm 4.7$  years, respectively ( $t = 0.780$ ;  $p > 0.05$ ). The mean maternal parity of neonates with and without anomalies of the external genitalia was  $1.5 \pm 1.4$  versus  $1.4 \pm 1.6$  ( $t = 0.438$ ;  $p > 0.05$ ). The anthropometric characteristics of the studied neonates is shown in Table 1. Eleven (2.4%; 95% Confidence Interval, CI= 2.0-2.8) and 6(1.3%; 95% CI=0.3-2.3) of the 465 male neonates had undescended testes (UDT) and hypospadias, respectively. Of the 11 neonates, 9(81.8%) were unilateral and the remaining 2(18.2%) were bilateral. The two cases of bilateral UDT had abdominal-pelvic ultrasonography performed and no Mullerian structures such a uterus, fallopian tubes were present. Our hospital does not have facility for determining genetic sex. Of the six neonates with hypospadias, 4(66.7%) were glandular or coronal (mild) and the remaining 2 subcoronal on penile mid-shaft (moderate). None of the neonates had penoscrotal or perineal hypospadias (severe) and there was no fistula. The mean AGD in infants with normal external genitalia and abnormal external genitalia (UDT and hypospadias combined) was  $27.6 \pm 4.9$ mm (95% CI=26.0-29.2) versus  $25.4 \pm 5.2$ mm (95% CI= 22.9 -27.9) and ( $t = 1.452$ ;  $p$ -value > 0.05). As shown in Table 2, the AGD was shorter in newborns with UDT compared with infants with descended testis but it was not statistically significant. The mean AGD was shorter in the hypospadiac newborn infants than in their non-hypospadiac counterparts but this was not statistically significant (Table-2). The AGD was shorter in infants with hypospadias compared to infants with UDT.

**Table 1. Anthropometric characteristics of the studied infants**

Category of Infants	Mean birth weight (min - max)	Mean birth length (min - max)	Mean OFC* (min – max)
Normal genitals (n=34)	3.2kg (2.3-4.3)	49.2cm (48.0-51.0)	34.5cm (34.0-35.5)
UDT** (n=11)	3.0kg (2.8-4.3)	49.0cm (48.5-50.0)	34.4cm (34.0-35.0)
Hypospadias (n=6)	2.7kg (2.3-3.1)	48.8cm (47.5-49.0)	34.2cm (33.5-34.5)

\*OFC = Occipitofrontal circumference; \*\*UDT = Undescended testis

**Table 2. Comparison of anogenital distance in newborn infants with normal undescended testes/hypospadias and normal external genitalia.**

Category of Infants	Mean AGD (mm)	95% CI	t-statistic	p-value
Normal external genitalia <sup>a</sup> (n=34)	27.6±4.9	26.0-29.2	a vs b: 0.777	> 0.05
Undescended testis <sup>b</sup> (n=11)	26.3±5.4	23.0-29.6	a vs c:0.569	> 0.05
Hypospadias <sup>c</sup> (n=6)	25.1±6.2	20.1-30.1	b vs c:0.467	> 0.05

## DISCUSSION

Our data show that newborn infants with undescended testis (UDT) tended to have a shorter AGD compared with infants with descended testis but this difference did not reach statistical significance. This finding is not surprising because a similar observation has been reported in two separate studies one involving Indian<sup>14</sup> newborns and the other Caucasian<sup>23</sup> newborns. We could not find any report on AGD in African newborn infants with UDT for comparison. The study population in the two other previous studies on the subject were post-neonatal infants.<sup>5,11</sup> However, animal studies have established that AGD is significantly shorter in cryptorchid males than non-cryptorchid males.<sup>2</sup>

The results of the present study show that the AGD tended to be shorter in hypospadiac newborn infants than in their non-hypospadiac counterparts. Our finding is in keeping with the report of the study among newborns in Cambridge, United Kingdom.<sup>23</sup> In another study in Denmark among children aged 4 to 84 months, the authors concluded that children with hypospadias have a shorter AGD than their counterparts without hypospadias.<sup>12</sup> We could not find any report on AGD in African newborn infants with UDT for comparison. Considering that AGD is a reflection of perineal length, a reduced AGD in infants with hypospadias might be a reflection of the important role testosterone plays in the development of both AGD and the formation of the penis including descent of the testes. A similar view was held by Hsieh et al in their report.<sup>13</sup> Li et al, have proposed a mechanism to explain the occurrence of hypospadias in newborns.<sup>3</sup> In that report, they stated that disruption of endocrine function by environmental chemicals

may delay testicular development and hence, secretion of testosterone during urethral formation. Such disruption may result in hypospadias.

In the present study, an intra-group comparison revealed that the AGD tended to be shorter in infants with hypospadias compared to infants with UDT. A similar observation was reported by Thankamony et al.<sup>23</sup> The shorter AGD in infants with hypospadias compared to infants with UDT may suggest the occurrence of a more severe and time-specific disruption of genital development in hypospadias. As proposed by Hughes and Acerini, this is inconsonance with androgen dysfunction or deficiency occurring during the proposed MPW in hypospadias whereas the androgen influence on testes descent occur during the later part of gestation.<sup>24</sup>

The major limitation of our study is the small sample size. This may explain the statistically non-significant difference between the two groups. Despite these limitations, this study revealed that there is a tendency for the AGD to be shorter in Nigerian neonates with either UDT or hypospadias compared with their counterparts with normal external genitalia. Ours is the first pilot study on this subject in Nigeria. A future study involving a larger sample size is being planned to confirm our findings.

## CONCLUSIONS

In conclusion, the AGD in Nigerian newborn infants with either UDT or hypospadias tended to be shorter than those of their counterparts with normal genitals and this observation was more in infants with hypospadias compared to infants with UDT. Although none of the trends reached statistical significance, we encourage other researchers who have the necessary data to follow up on our hypothesis.

## REFERENCES

- Baskin LS, Himes K, Colborn T. Hypospadias and endocrine disruption: Is there a connection? *Environ Health Perspect.* 2001;109:1175-83.
- Welsh M, Saunders PTK, Fiskens M, Scott HM, Hutchison GR, Smith LB, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest.* 2008;118(4):1479-90.
- Li N, Chen X, Zhou X, Zhang W, Yuan J, Feng J. The mechanism underlying dibutylphthalate induced shortened anogenital distance and hypospadias in rats. *J Pediatr Surg.* 2015;50(12):2078-83.
- Marty MS, Chapin RE, Parks LG, Thorsrud BA. Development and maturation of male reproductive system: Birth Defect Research Part B. *Develop Reprod Toxicol.* 2003;68:125-36.



5. Hsieh MH, Breyer BN, Eisenberg ML, Baskin LS. Association among hypospadias, cryptorchidism, anogenital distance and endocrine disruption. *Curr Urol Rep.* 2008;9:137-42.
6. Shakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod.* 2001;16:972-8.
7. Sharpe RM, Skakkebaek NE. Testicular dysgenesis syndrome: mechanistic insight and potential new downstream effects. *Fertil Steril.* 2008;89:e33-e38.
8. Ritzen EM, Bergh A, Bjerknes R, Christiansen P, Cortes D, Haugen SE, et al. Nordic consensus treatment of undescended testes. *Acta Paediatr.* 2007;96:638-43.
9. Hutson JM, Clarke MC. Current management of the undescended testicle. *Semin Pediatr Surg.* 2007;16:64-70.
10. Toppari J, Kaleva M, Virtanen HE. Trends in the incidence of cryptorchidism and hypospadias and methodological limitations of registry-based data. *Hum Reprod Update.* 2001;7(3):282-6.
11. Swan SH, Main KM, Liu F, Stewart SL, Kruk RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect.* 2005;113:1056-61.
12. Main KM, Skakkebaek NE, Virtanen HE, Toppari J. Genital anomalies in boys and the environment. *Best Pract Res Clin Endocrinol Metab.* 2010;24:279-89.
13. Hsieh MH, Eisenberg ML, Hittelman AB, Wilson JM, Tasian GE, Baskin LS. Caucasian male infants and boys with hypospadias exhibit reduced anogenital distance. *Hum Reprod.* 2012;27:1577-80.
14. Jain VG, Singal AK. Shorter anogenital distance correlates with undescended testis: a detailed genital anthropometric analysis in human newborns. *Hum Reprod.* 2013;28:2343-9.
15. Orish C, Didia B. Anogenital distance in human male and female newborns: A look at a cross section of a Nigerian population. *Internet J Biol Anthropol.* 2008;3(2):e1-4.
16. Ovidime OM, Olorunshola KV, Dikko AAU. Anogenital distance of newborns and causes of variation in Zaria, Northern Nigeria. *Asian J Med Sci.* 2011;3(2):74-8.
17. Docimo SG, Silver RI, Cromie W. The undescended testicle: Diagnosis and management. *Am Fam Physician.* 2000;62(9):2037-44.
18. Ashley RA, Barthold JS, Kolon TF. Cryptorchidism: pathogenesis, diagnosis, treatment and prognosis. *Urol Clin North Am.* 2010;37:183-93.
19. Virtanen HE, Bjerknes R, Cortes D, Jorgensen N, Rajpert-De Meyts E, Thorsson A, et al. Cryptorchidism: Classification, prevalence, and long-term consequences. *Acta Paediatrica.* 2007;96(5):611-6.
20. Ghirri P, Scaramuzza RT, Boldrini A. Prevalence of hypospadias in Italy according to severity, gestational age and birth weight: an epidemiological study. *Italian J Pediatr.* 2009;35:18-30.
21. Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. *Environ Health Perspect.* 2009;117:1786-90.
22. Callegari C, Everett S, Ross M, Brasel JA. Anogenital ratio: measure of fetal virilization in premature and full-term newborn infants. *J Pediatr.* 1987;111:240-3.
23. Thankamony A, Lek N, Carroll D, Williams M, Dunger DB, Acerini CL, et al. Anogenital distance and penile length in infants with hypospadias or cryptorchidism: Comparison with normative data. *Environ Health Perspect.* 2014;122:207-11.
24. Hughes IA, Acerini CL. Factors controlling testes descent. *Eur J Endocrinol.* 2008;159:(Suppl 1):S75-S82.