

## Clitoral Length and Anogenital Ratio in Indian Newborn Girls

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**Objectives:** To generate normative data on clitoris length, anogenital distance and anogenital ratio in Indian newborns.

**Design:** Cross-sectional study.

**Setting:** Neonatal unit of a tertiary care teaching hospital in Kolkata.

**Participants:** 378 female neonates, who were hemo-dynamically stable without critical illness or chromosomal anomaly, and without any vulval hematoma or genital abnormalities.

**Interventions:** Measurements were recorded using a digital vernier caliper between 24–72 hours. Infant was held in position by an assistant, while the investigator measured clitoral length by gently retracting the labia majora. Anogenital distance (centre of the anus to posterior convergence of the fourchette) and anogenital ratio (anogenital distance divided by the distance from centre of the anus to base of the clitoris) was also measured.

**Main outcome measures:** Gestational age- and birthweight-wise

normative values of clitoral length, anogenital distance and anogenital ratios.

**Results:** Mean clitoral length was 3.1 (1.54) mm for the whole cohort while anogenital distance and anogenital ratio were 10.2 (2.78) mm and 0.34 (0.07) mm, respectively. The gestation age-wise percentile charts of clitoral length, anogenital distance and anogenital ratio have been generated. There was no correlation between clitoral length and gestational age, body length, head circumference and birth weight. Correlations were also weak for anogenital distance.

**Conclusions:** The normative values generated can serve as reference standard in the assessment of clitoromegaly, ambiguous genitalia, virilizing effects and suspected *in utero* androgen exposure.

**Keywords:** Ambiguous genitalia, Disorders of sexual differentiation, Dysmorphology, Neonate.

Assessment of external genitalia is important in newborns to diagnose ambiguous genitalia, and as a pointer to some other disorders. Clitoromegaly in the neonatal period is an important parameter to be evaluated, and may indicate inappropriate androgenic exposure *in utero* [1-3]. The anogenital distance (AGD) is a sexually dimorphic feature of genital development, and is a sensitive marker of *in utero* exposure to androgens and chemicals with anti-androgen effects [4,5]. Data of the normal AGD in human newborns is necessary to serve as a baseline to assess endocrine effect on newborn genitalia. Although studies on neonatal clitoral length and AGD [6-8] are reported in literature, there is no such data from India. The present study aimed to establish the normative values for clitoral length and AGD in newborn Indian girls in a neonatal unit of Eastern part of India.

### METHODS

This cross-sectional study was conducted in a tertiary care teaching hospital from September 2013 to February

2014. Institutionally-born female babies were recruited on two days of the week (Tuesday and Thursday), in the neonatal unit between 24 to 72 hours of birth, after obtaining written informed consent. The study received prior approval of the Institutional Ethics Committee.

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Both term and preterm singleton babies who were appropriate for gestational age were included. Newborns with antenatal history of serious maternal illness, medication affecting development of fetal genitalia, and fetal growth restriction either by ultrasound report or by modified Fenton's chart were excluded. Gestational age was estimated by New Ballard scoring [9,10]. Babies born by breech delivery, those with vulval hematoma or genital malformations, those known to have chromosomal anomalies, and those critically ill were excluded.

All measurements were taken by one investigator

with the same set of instruments. The weight was measured with a digital weighing scale (Phoenix) with resolution of 1g, supine length by an infantometer (Narang Medical Ltd.) with resolution of 1 mm, and head circumference and chest circumference were measured by a non-stretchable tape. The clitoris length and AGD was measured using a digital vernier calliper (Aerospace Digimatic Vernier Caliper), with resolution of 0.01 mm (accuracy 0.02 mm). During these measurements, the baby was placed in dorsal decubitus position by an assistant (neonatology staff nurse) with both hips flexed and light pressure exerted on the infant's thighs and the restraining hands resting on the baby's abdomen. The investigator gently retracted the labia majora with one hand while measuring the clitoral length with the other hand. AGD was measured from the centre of the anus to the posterior convergence of the fourchette and anogenital ratio (AGR) was calculated as AGD divided by the distance from the centre of the anus to the base of the clitoris. For all the parameters, three readings were taken and the mean value was recorded to the nearest millimetre.

**Statistical analysis:** Genital dimensions were compared between term and preterm babies by Mann-Whitney U test with two-sided  $P < 0.05$  as the cut-off for statistical significance. Linear correlation between parameters was quantified as Spearman's rank correlation coefficient  $\rho$  ( $r$ ). Statistica version 6 (Tulsa, Oklahoma: StatSoft Inc., 2001) and MedCalc version 11.6 (Mariakreke, Belgium: MedCalc Software 2011) softwares were used for statistical analysis.

## RESULTS

Out of the 415 neonates enrolled for the study, 37 were excluded due to various reasons such as genital abnormalities (*e.g.* vulval hematoma), serious morbidity (*e.g.* septicemic shock) and incomplete data. Of the 378 neonates whose data were analyzed, 93 (24.6%) were preterm.

The various characteristics are detailed in **Table I**. The mean (SD) clitoral length was found to be 3.1 (1.54) mm for the whole cohort while the corresponding values for AGD and AGR were 10.2 (2.78) mm and 0.34 (0.07) mm, respectively.

The gestation age-wise percentile charts of clitoral length, AGD and AGR are presented in **Table II**. The difference in mean clitoris length between term and preterm neonates was 0.35 mm ( $P=0.008$ ), and in AGD and AGR, it was 1.05 mm ( $P=0.002$ ) and 0.012 ( $P=0.058$ ), respectively.

There was no correlation between clitoral length and gestational age ( $r=-0.086$ ,  $P=0.09$ ), and between clitoral length and head circumference ( $r=-0.096$ ,  $P=0.06$ ). Weak correlations were found between the clitoris length and birth weight ( $r=-0.148$ ,  $P=0.004$ ) and body length ( $r=-0.144$ ,  $P=0.02$ ). The AGD had weak positive correlation with gestational age ( $r=0.189$ ,  $P < 0.001$ ), birth weight ( $r=0.232$ ,  $P < 0.001$ ), body length ( $r=0.165$ ,  $P=0.008$ ) and head circumference ( $r=0.225$ ,  $P < 0.001$ ).

## DISCUSSION

In this study on 387 Indian neonates, mean values of clitoral length, AGD and AGR were 3.1 mm, 10.2 mm and 0.34 mm, respectively. The clitoral length showed no correlation with gestational age, with the median length remaining almost constant at 3 mm. Thus normative data can be considered to be relatively independent of gestational age. There was also no correlation between clitoral length and anthropometric parameters like body length, head circumference and birth weight. Although a clitoral length greater than 10 mm is traditionally taken as criteria for clitoromegaly [11,12]; we suggest a clitoral length cut-off of 6 mm.

The study had a few limitations. This was a single institution-based study, and even all institutionally delivered babies during the study period were not

**TABLE I** DESCRIPTIVE SUMMARY OF THE STUDY POPULATION

Measurement	Mean (SD)	95% CI of mean	5th percentile	10th percentile	25th percentile	Median percentile	75th percentile	90th percentile	95th percentile	99th percentile
Birthweight (kg)	2.61(0.61)	2.55-2.67	1.25	1.75	2.30	2.70	3.00	3.25	3.50	4.00
Head circumference (cm)	32.8(2.03)	32.6-33.0	29.0	30.0	31.5	33.0	34.0	35.0	35.5	36.0
Body length (cm)	45.9(3.25)	45.5-46.3	39.5	40.0	45.0	47.0	48.0	49.0	49.0	51.0
Clitoris length (mm)	3.1(1.54)	3.0-3.3	1.0	2.0	2.0	3.0	4.0	5.0	6.0	9.0
Anogenital distance (cm)	1.02(0.28)	0.99-1.04	0.7	0.8	0.8	1.0	1.1	1.5	1.5	2.0
Anus to clitoris distance (cm)	2.96(0.52)	2.91-3.02	2.5	2.5	2.7	2.8	3.0	3.75	4.0	4.5
Anogenital ratio	0.342(0.07)	0.336-0.349	0.25	0.29	0.29	0.33	0.39	0.43	0.46	0.60

**TABLE II** DESCRIPTIVE SUMMARY OF NEWBORN GENITAL DIMENSIONS STRATIFIED ACCORDING TO THE GESTATIONAL AGE AT BIRTH

	No.	Mean (SD)	PC5	PC10	PC25	PC50	PC75	PC90	PC95	PC99
<i>Clitoral length (mm)</i>										
Week 32-33	28	3.7 (1.31)	2.0	2.0	3.0	3.0	4.5	6.0	6.0	7.0
Week 34	19	3.8 (1.40)	1.0	2.0	3.0	3.0	5.0	6.0	6.0	6.0
Week 35	14	3.3 (2.33)	1.0	1.0	2.0	2.5	4.0	5.0	6.0	7.0
Week 36	32	3.0 (1.33)	1.0	2.0	2.0	3.0	3.5	4.0	6.0	7.0
Week 37	10	4.8 (3.05)	2.0	2.0	3.0	3.0	8.0	8.0	8.0	8.0
Week 38	47	2.9 (1.48)	1.0	1.0	2.0	3.0	3.0	5.0	6.0	8.0
Week 39	80	2.8 (1.29)	1.5	2.0	2.0	2.0	3.0	5.0	5.5	7.0
Week 40	136	3.1 (1.47)	1.0	2.0	2.0	3.0	3.0	5.0	6.0	9.0
Week 41-42	12	3.2 (1.27)	1.0	2.0	2.5	3.0	4.0	4.0	6.0	6.0
<i>Ano-genital distance (cm)</i>										
Week 32-33	28	0.8 (0.20)	0.6	0.6	0.8	0.8	0.9	1.3	1.3	1.3
Week 34	19	1.0 (0.31)	0.8	0.8	0.8	1.0	1.2	1.5	2.0	2.0
Week 35	14	0.8 (0.11)	0.6	0.8	0.8	0.9	1.0	1.0	1.0	1.0
Week 36	32	0.9 (0.19)	0.7	0.7	0.8	0.9	1.0	1.2	1.2	1.5
Week 37	10	0.9 (0.23)	0.7	0.7	0.8	0.9	1.0	1.3	1.5	1.5
Week 38	47	0.9 (0.26)	0.7	0.7	0.8	1.0	1.0	1.5	1.5	2.0
Week 39	80	1.0 (0.26)	0.8	0.8	0.8	1.0	1.2	1.5	1.5	1.8
Week 40	136	1.0 (0.30)	0.7	0.8	0.8	1.0	1.2	1.5	1.5	2.0
Week 41-42	12	1.1 (0.36)	0.7	0.8	0.9	1.0	1.3	1.5	2.0	2.0
<i>Anus to clitoris distance (cm)</i>										
Week 32-33	28	2.7 (0.30)	2.0	2.5	2.5	2.8	2.8	3.0	3.1	3.2
Week 34	19	3.0 (0.52)	2.5	2.5	2.8	3.0	3.0	4.0	4.5	4.5
Week 35	14	2.8 (0.25)	2.5	2.5	2.7	2.8	2.8	3.0	3.5	3.5
Week 36	32	2.7 (0.44)	2.3	2.5	2.5	2.7	3.0	3.5	3.5	4.0
Week 37	10	2.7 (0.57)	1.5	1.9	2.5	2.8	2.9	3.4	3.8	3.8
Week 38	47	2.9 (0.59)	2.5	2.5	2.6	2.8	3.0	3.5	4.5	4.5
Week 39	80	2.9 (0.42)	2.5	2.6	2.8	2.8	3.0	3.6	4.0	4.5
Week 40	136	3.1 (0.57)	2.5	2.5	2.8	2.9	3.2	4.0	4.5	4.5
Week 41-42	12	3.1 (0.72)	2.5	2.5	2.7	2.9	3.5	3.7	5.0	5.0
<i>Ano-genital ratio</i>										
Week 32-33	28	0.3 (0.06)	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.5
Week 34	19	0.3 (0.06)	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.5
Week 35	14	0.3 (0.04)	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.4
Week 36	32	0.3 (0.09)	0.2	0.2	0.3	0.3	0.4	0.5	0.5	0.7
Week 37	10	0.4 (0.11)	0.3	0.3	0.3	0.3	0.4	0.5	0.6	0.6
Week 38	47	0.3 (0.08)	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.7
Week 39	80	0.3 (0.06)	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.5
Week 40	136	0.3 (0.06)	0.2	0.3	0.3	0.3	0.4	0.4	0.4	0.5
Week 41-42	12	0.3 (0.07)	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.5

Abbreviations: SD = standard deviation; PC = percentile.

**WHAT IS ALREADY KNOWN?**

- Limited data on clitoral length is available from some populations.

**WHAT THIS STUDY ADDS?**

- Normative data for clitoral length and anogenital ratios in Indian newborns with gestational age-wise percentile charts are provided.

included. Small sample size, especially for preterm infants was a major limitation. Longitudinal follow-up data of genital parameters were also not collected.

Newborn genital measurements including clitoral length seem to vary with ethnicity [13, 14]. Phillip, *et al.* [2] reported a mean value of 5.9 mm in babies of Jewish origin and 6.6 mm in Bedouin babies. Riley and Rosenbloom reported a mean clitoral length of 3.27 mm in white and 3.66 mm in black neonates [3]. In a study on Nigerien babies, the mean value was 7.5 mm [15]. Our results more closely resemble the values reported by Riley and Rosenbloom [3]. However, Callegari, *et al.* [13] did not record any ethnic variability despite having populations from Hispanic, black and white backgrounds. While Litwin, *et al.* [16] observed a strong negative correlation between birth weight and clitoral length, we did not find any correlation of clitoris length and gestational age. Our results of AGD are in consonance with most of the studies [6,8,13] It seems that the racial and ethnic variability is minimal for this parameter.

In most of the published studies [6,7,8,11], vernier calipers were used to measure clitoral length and AGD. We used a digital version of the instrument for our measurements. This demanded careful handling to avoid injury, and considerable practice because of difficulty of these procedures and indistinct soft tissue landmarks. The accuracy and standardization of other options like tape, wooden tongue depressor or dental floss is questionable [14].

Clitoromegaly may indicate intrauterine exposure to testosterone or other androgens [12]. Moreover, certain endocrine diseases have been reported in babies with apparently small genitalia [3]. AGD and AGR are anthropometric parameters that show sexual dimorphism. Variations in AGD may occur due to prenatal exposure to androgens and endocrine disrupting chemicals. As studies demonstrate the utility of AGD as a marker of *in utero* exposure to androgens and chemicals with antiandrogen effects, its measurement has been advocated by the United States Environmental Protection Agency guidelines for reproductive toxicity studies in humans [14].

We have generated normative data for the clitoral length and anogenital distance and ratio in selected Indian newborns. These values can be used as reference standard in the assessment of clitoromegaly, ambiguous genitalia, virilizing effects and suspected *in utero* androgen or antiandrogen exposure.

*Contributors:* RK: Conception or design of the work; analysis, interpretation of data, write up the draft, reviewed and corresponding author; KC: acquisition of data, drafting; MS: design of work and revising manuscript; AH: analysing of data, drafting, manuscript revision; SR: acquisition of data and drafting; TKS: interpretation of work and revising; BB: analysing of data and drafting; SD: design of work and drafting; DR: drafting and interpretation of data; RB: acquisition of data, drafting. All authors have agreement to be accountable for all aspects of the work including approval of the final manuscript.

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