St PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

CLITORAL SIZE, ANOGENITAL DISTANCE AND NORMATIVE DATA IN TERM FEMALE NEWBORNS AT SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE, ADDIS ABABA ETHIOPIA, 2021.

BY: ANILEY ASSAB (MD, PEDIATRIC RESIDENT)

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A THESIS TO BE SUBMITTED TO DEPARTMENT OF PEDIATRICS AND CHILD HEALTH, SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE CERTIFICATE IN PEDIATRICS AND CHILD HEALTH

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Abbreviations

AFD - Ano-fourchette distance

ACD – Ano-clitoris distance

AGD – Anogenital distance

AGR – Anogenital ratio

CL- Clitoral length

CW - Clitoral width

DSD – Disorder of sexual development

FCD – Fourchette-clitoris distance

SPHMMC- Saint Paul hospital millennium medical college

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Abstract

Background: Significant enlargement of clitoris during birth reflects virilization and suggesting the female foetus exposure to androgens during the intrauterine period. Measurement of clitoral size and anogenital distance helps early detection of intrauterine androgen exposure in female newborn neonates. This study may contribute baseline information on anogenital distance and clitoral size in female newborns in Ethiopia which can help as entry point for further evaluation before assigning their sexes to avoid long term sequelae including embedment of scarred self and embarrassment to the entire family and relatives.

Objective: To assess clitoral size, anogenital distance and generate normative data in term female newborns in Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2021.

Method: Institution based descriptive cross-sectional study design was used and sample was determined using single population mean formula with final sample size of 284. Data was collected by means of standard questioner and direct measuring anogenital distance and clitoral size. Data entry was done using Epidata version 3.1 and analyzed by Statistical package for social sciences version 26.

Result: The cutoff point for reference value in term female neonates using mean ± 2standard deviation of clitoral length and clitoral width were 6.06 – 12.47mm and 2.17 – 6.05mm respectively while anus-fourchet distance 7.44 – 13.76mm , anus-clitoris distances 23.29 – 39.73mm and anogenital ratio of 0.2-0.34.Normogram of clitoral size and anogenital distance was generated based on gestational age. There were weak positive correlation of anus-clitoris distance with birth weight (r=0.218, p= 0.00) and birth length. (r =0.222, p= 0.00) and anus-fourchet distance with head circumference (r = 0.146, p =0.014) and with birth weight (r =0.145, p =0.014).

Conclusion: provided overall mean±2SD reference values and generated normogram of clitoral size and anogenital distance using mean, standard deviations and gestational age. This simple, affordable and non-invasive evaluation could help early identification and intervention of female newborns with potentially harmful conditions like congenital adrenal hyperplasia, particularly where newborn screening is not available.

Key words: *Clitoral size, anogenital distance, Ethiopia.*

1. *Introduction*

1.1 Background

It is a common trend that after the delivery of a baby to identify the sex although there is prenatal information about the sex of the new born by using a prenatal obstetric ultrasound imaging. Delaying in assigning the sex could be distressing for the involved health professionals largely to the family (1). Although it is not usually easy to determine the sex of a newborn, early assignment is recommended (2). Virilization of the external genitalia requires a testosterone derivative, dihydrotestosterone (DHT) in which masculinization or feminization of these structures is determined by the presence or absence of DHT which affects sex assignment (3).

External genitalia virilization is caused by endogenous or exogenous androgen excess from which the most common forms of genotypicaly female infants (46, XX DSD) are virilizing forms of congenital adrenal hyperplasia requiring biochemical characterization of possible steroidogenic enzymatic defects in each patient with genital ambiguity to diagnose it(4).

Early detection of genital anomalies in the female infant is crucial. Significant enlargement of clitoris during birth usually reflects virilization and suggests that the female foetus has been exposed to androgens during the intrauterine period (5). Clitoromegaly due to androgen exposure in a 46, XX newborn can be caused by virilizing congenital adrenal hyperplasia, ovotesticular or testicular DSD and maternal androgen exposure (6).

Clinically; the most important measurement is the length of the clitoris, which is not routinely performed in many settings. This simple, affordable and non-invasive evaluation could aid early diagnosis and treatment of female infants with potentially harmful conditions such as congenital adrenal hyperplasia (7).

Normal clitoral width ranges from 2 to 6 mm and mean clitoral length less than 9 mm in newborn infants (8, 9).Standard endocrine books states that clitoral length greater than 10mm is considered to be clitoromegaly (10).

Although clitoral length is traditionally defined as greater than 10 mm there might be racial differences between clitoral length values, and there is suggestion that each country define its own normative data and the definition of clitoromegaly is indefinite (11).

Other parameters used for assessment of newborn infants intrauterine androgen exposure is anogenital distance (AGD) including ano-fourchette distance (AFD) and ano-clitoral distance (ACD) which are longer in females who had intrauterine androgen exposure (12).

In these tissues, the process seems largely governed by dihydro-testosterone (DHT) acting by binding to and activating the androgen receptor stimulating the growth of the perineal muscles levatoraniand bulbocavernosus resulting in increased AGD in the presence of high androgen level during intrauterine period (13).

1.2 Statement of the problem

Many jurisdictions have newborn screening programs that include the measurement of 17-hydroxyprogesterone (17-OHP) and allow for early identification of infants with 21-hydroxylase deficiency, their justification lies in the increased mortality that occurs in infants who are not recognized at birth and die of salt-losing crisis or sudden infant death as well as the increased morbidity that results from adrenal insufficiency, incorrect gender assignment, and pre-mature virilization (14).

It may be difficult to screen all newborns especially in developing countries using biochemical parameters but there are physical parameters used as entry point for further screening of a newborn such as clitoral size anogenital distance and anogenital ratio by creating normal value distribution. Even though there is difference in different ethnicity, a normal full-term female infant has a clitoris <1 cm in length and <0.5 cm in width and posterior fusion of the labioscrotal folds is assessed by determining the anogenital ratio, which is the distance between the anus and the posterior fourchette divided by the distance between the anus and the base of the clitoris in which an anogenital ratio >0.5 indicates first-trimester androgen exposure (15).

Failure to identify newborns with disorder of sexual development (DSD) early and informing parents about condition may affect later life of individual. A study done in Bangladesh to assess barriers in access to healthcare services for individuals with disorders of sex differentiation revealed that around 80% of DSD population sought healthcare services from government healthcare facilities, where the overall success rate in getting healthcare services was less than 50% and the DSD population reported a number of reasons for failures in getting healthcare services, including non-friendly interaction by non-clinical hospital's staff, non-friendly interaction by physicians, public fright as general people do not want to mingle with a DSD person, undesirable excess public interest in DSD individuals, and limitation of the treatment opportunities of hospitals to merely male or female patients(16).So early detection and making the family as well as the affected individuals ready for future plan is very important.

Before assigning sex of children with DSD there should be careful evaluation using combination of history taking, physical examination, laboratory evaluation, and radiologic assessment which can assist

with the diagnosis (17). This can help to avoid psychological impact on the affected individual in later life.

Studies showed in developing countries with poor level of awareness many female children with even major aberrations of external genitalia are being missed at birth with affected children reared along wrong gender line. A study done in Nigeria on female Children with ambiguous genitalia in awareness-poor sub-region showed that a total of 40 female children were seen with congenital aberrations of external genitalia in the age of 3 months and 16 years who were not diagnosed at birth or during neonatal period presented with clitoromegaly (18).

Although there is no study done on DSD in Ethiopia there were 3 case reports related to DSD and sex assignment. Two siblings of 4 and 6 years of age were assigned female sexes, named and raised so far as girl children and dressed like girls even though upon deep interrogation, it was found out that they had their reservations and preferred to be treated like males and the genetic test showed XY karyotype and later diagnosed to have Complete Androgen Insensitivity Syndrome and the second case a 20 years old diploma graduate referred for the purpose of clarification of the sexual ambiguity with a complaint of unduly enlarging bilateral breast tissue since the age of 14 despite having a phallus of appreciable size. He was assigned a male sex and named accordingly later the karyotype showed XX but he preferred to be treated as male (19).

This study may contribute baseline information on anogenital distance and clitoral size in female newborns in Ethiopia which can help as entry point for further evaluation before assigning their sexes to avoid long term sequelae including embedment of scarred self and embarrassment to the entire family and relatives.

1.3 Significance of the study

Many countries have newborn screening programs for early identification of phenotypic or genotypic sex abnormalities to decrease the increased mortality and morbidity that occurs in infants who are not recognized at birth and incorrect gender assignment and pre-mature virilization. For example congenital adrenal hyperplasia and intrauterine androgen exposure require early detection. These screening programs involve hormonal and genetic analysis which is inapplicable in developing countries related to cost (14).

It may be difficult to screen all newborns especially in developing countries using biochemical parameters but there are physical parameters used as entry point for further screening of a newborn such as clitoral size anogenital distance by creating normal value distribution.

Countries are creating their own normal reference values using these physical parameters (clitoral size anogenital distance) because there are reports of normal value difference among races and ethnicities.

In Ethiopia, there is no study done to assess clitoral size and AGD. This study hopes to create a huge impact on the early detection and management of female neonates who are virilized.

So the significant of this study provided reliable normative values that can be used as a baseline with regard to anogenital distance and clitoral size in female neonates in Ethiopia particularly in Saint Paul hospital millennium medical college.

1.4. Literature review

1.4.1 Clitoral size

The clitoral length measured from the pubic ramus to the tip of the glans clitoris and clitoral width measured by taking the transverse diameter at the middle of the clitoris can be as references for diagnosing genital abnormalities although examining the genitalia is an important aspect of newborn evaluation to diagnose external genital abnormalities (8).

A cross sectional study done in Iran on Nomogram of Clitoral Length and Width in Iranian term and Preterm Neonates delivered at 28-42 weeks of gestation and measured on the first 72 h of birth with a total of 580 female newborns (187 term and 393 preterm) showed that the Mean ±SD values of clitoral length and clitoral width in term neonates were 6.11± 0.39 mm and 4.22 ±0.43mm respectively. Regression analysis showed that clitoral length was correlated with gestational age, birth length, birth weight &head circumference; and clitoral width was associated gestational age, birth length, and birth weight.(20).

A hospital based cross sectional study was conducted in Turk in 2008 on clitoral length in female newborns total of 325 term newborn female neonates with uncomplicated pregnancy spontaneous vaginal delivery showed that the mean ± SD of clitoris length was 4.93 ± 1.61mm. There was no correlation between clitoral size and anthropometric parameters(25).

A study done in Nigeria in 2009 on country-based reference values and international comparisons of clitoral size in healthy Nigerian newborn infants involving a total of 244 healthy newborns delivered at 28–43 weeks gestation clitoral lengths and widths were measured at <72 hours and showed that the mean clitoral length was 7.7 mm with a standard deviation of ±1.37 mm, while the mean clitoral width was 4.40 with a standard deviation of ± 0.89 mm. Clitoral length and clitoral width did not correlate significantly with birth length, birth weight or gestational ages (21).

A cross-sectional study in Ghana in 2014 on Clitoral size in term newborns apparently well full-term newborns of postnatal age < 48 hours and delivered at Komfo Anokye Teaching Hospital including a total of 612 newborn females neonates and the result showed that the mean ±SD) clitoral length and the mean ±SD clitoral width were 4.13 ± 1.6 mm and 4.21 ± 1.1 mm, respectively. Mean clitoral length was inversely related to birth weight while mean clitoral width was inversely related to both gestational age and birth weight (22).

Multi-centered cross-sectional descriptive study was conducted in Sagamu town in Southwest Nigeria state-owned university teaching hospital , primary health centers and private hospitals on Clitoral sizes and anogenital distances in term newborns involving 317 female term neonates within the first 72 hours

of life. The result showed that the mean clitoral length 6.7 ± 1.6mm, the mean clitoral width 5.6 ± 0.8 mm. There was no correlation between clitoral measurements and anthropometry parameters (7).

1.4.2 Anogenital distance

Several studies showed that AGD is used as a biomarker of fetal androgen exposure in which AGD measure is longer in males than in females due to high androgen level in male's fetus (23).

A study done in United Kingdom on assessment ofanogenital distance from birth to 2 years of age by measuring from the center of the anus to the base of the scrotum in males and to the posterior fourchette in females at birth and at 3, 12, 18, and 24 months of age enrolling 463 male and 426 female full-term infants and the Mean ±SD AFD of female neonates at birth was 9.1±2.8 mm (24).

Another study which was conducted in Turk on anogenital Distance in Turkish Newborns in 2009 involving 115 female and 135 male newborns born at term. The result in female newborns showed that anus-fourchette distance (AFD) 10.3±0.2 and anno- clitoris distance (ACD) 30±0.2 (25).

A Multi-centered cross-sectional descriptive study was conducted in Nigeria on Clitoral sizes and anogenital distances in term newborns involving 317 female term neonates within the first 72 hours of life. The result was the mean ACD 35±2.5mm, AFD 17.0±2.6mm and anogenital ratio of 0.48 ± 0.05. There was significant correlation in the anus-clitoris and anus-fourchette distances with the anthropometric parameters (7).

Other descriptive cross-sectional study was also done in Nigeria among 448 male and 447 female healthy full-term neonates between 6 to 72 hours of age to assess ano-genital distance. The result in female neonates showed that the mean ±SD AFD was 15.2±2.4 mm (26).

In 2010 other Cross sectional study was conducted on anogenital distance and umbilical cord testosterone level in newborns in Zaria, Northern Nigeria involving 200 newborns (100 male, 100 female) and result revealed that the mean±SD of AFD in females was13.89±0.26mm.There was positive correlation between birth weight and AFD (27).

3. Objectives

3.1. General objective

- To assess clitoral size, anogenital distance and generate normative data in term newborns at Saint Paul hospital millennium medical college Addis Ababa, Ethiopia, 2021.

3.2. Specific Objective

- ✓ To determine clitoral size of term newborns at Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021.
- ✓ To determine anogenital distance of term newborns at Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021.
- ✓ To determine anogenital ratio of term newborns at Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021.
- ✓ To generate normative data on clitoral size and anogenital distance in term newborns at Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021.

4. Methods and materials

4.1 Study Area and Period

This study was conducted from July 1 to August 31, 2021 at Saint Paul's hospital millennium medical college. Saint Paul millennium medical college is the leading teaching and tertiary referral hospital under the Federal Ministry of Health Ethiopia with a catchment population more than 5 million. It was built in 1961 by Emperor Haileselase in collaboration with German evangelical church, as a source of medical care for underserved populations. In addition to an inpatient capacity of more than 700 beds, on average 1200 emergency and outpatient clients are seen daily in the hospital.

Gynecology and obstetrics department, in which the study was conducted, is among the major departments serving the population. One year prior to data collection for this research, at emergency and labor wards average live birth per month was 909 with a total of live birth per year 10910. Out of 10910 total live births, female births account about 45% (4909). From these 4909 female live births 3829 were term female. Monthly term female live birth was about 320 (2012 annual reports of SPHMMC health information and management system unit).

4.2 Study Design

Institutional based descriptive cross-sectional study design was conducted to assess clitoral size, anogenital distance, and generate normative data in term female newborns at SPHMMC Addis Ababa, Ethiopia, 2021.

4.3 Source and study population

4.3.1. Source Population

All term female newborns in Saint Paul Hospital Millennium Medical College gynecology and obstetrics department at labor and maternity wards Addis Ababa, Ethiopia in the study period.

4.3.2. Study Population

All term female newborns who fulfilled inclusion criteria in Saint Paul Hospital Millennium Medical College gynecology and obstetrics department at labor and maternity wards Addis Ababa, Ethiopia in the study period.

4.4. Inclusion & Exclusion Criteria

4.4.1. Inclusion criteria

Term female neonate (≥37wks of gestation)

Clinically stable

4.4.2 Exclusion criteria

Delivered via breech presentation

With any type of mass around the genitalia

Newborns with ambiguous genitalia

Dysmorphic feature

Small for gestational age

Maternal hormonal therapy during pregnancy (androgens)

Known maternal virilizing feature

Known maternal androgen producing tumors

History of unknown cause of newborn/infant death

Having a child with ambiguous genitalia

4.5. Sample Size Determination

Single population mean formula was used to determine the sample size. The standard deviations of clitoral size and anogenital distances were taken from previous studies conducted in other countries. Anus-fourchet distance standard deviation (SD) 0.26 from Nigeria's study, anus-clitoris distance SD 0.2 from Turk's study, clitoral length SD 0.39 and clitoral width SD 0.43 from Iran's studies were taken. Sample was calculated using all the three parameters with 5% marginal error (d) and 95% of confidence interval (Z-value of 1.96). The maximum sample size was taken.

The assumptions taken were: Level of confidence interval (95%), 5% degree of marginal error (d), with Standard deviations (δ) = (anus-fourchet distance = 0.26, anus-clitoris distance 0.2, clitoral length = 0.39 and clitoral width = 0.43).

n = Sample size

Za/2 = 1.96

$$n = \frac{(Za/2)^2 (\delta)^2}{(d)^2}$$

Using anus-fourchet distance SD (0.26): n=104

Using anus-clitoris distance SD (0.2): n=62

Using clitoral length SD (0.39): n = 234

Using clitoral width SD (0.43): n=284

The maximum estimated sample size was determined from clitoral width standard deviation. So the final sample size was **284.**

4.6. Sampling procedure

All term female newborns who fulfilled inclusion criteria up to the age of 72 h were included in the study period until the required sample size was achieved.

4.7. Data Collection Tool and Procedures

Structured interviewer-based questionnaire was used for collecting maternal demographic, medical, gynecologic and obstetric factors. Maternal information was collected from mothers and their charts. Anthropometric parameters of newborns were measured from each study participants. Adjustable sliding digital caliper was used to measure clitoral size and anogenital distance (figure 1). Non-stretchable tape meter for head circumference and length measurement and infantogram for weight measurement were used to collect neonatal anthropometry parameters.

To determine the gestational age of the neonate maternal last regular menstrual period an ultrasound scan taken from the first trimester was used. If gestational age difference between maternal last regular

menstrual period and ultrasound scan was greater than 2 weeks or there was no 1st trimester ultrasound scan, the New Ballard score was used. Two general practitioners were involved in data collection. Adequate training was given to data collectors on how to measure clitoral size, anogenital distance and how to use the data collecting tools. The same equipments were used to measure clitoral size and anogenital distances. Two measurements were taken, once by each data collectors, for clitoral size and anogenital distances and the average value was taken. Measurement procedure was done as follows:

Each baby was exposed and placed in supine position with the assistant gently flexing the Knees while applying light pressure on the hips. Using adjustable sliding caliper the distances from the centre of the anus to the beginning of the mucosa of the posterior fourchette(anus-fourchette distance, AFD) and from the centre of the anus to the base of the clitoris (anus-clitoris distance, ACD) were measured.

The clitoral length was measured from the pubic ramus to the tip of the glans clitoris after parting the labia majora and gently retracting the hood of the clitoris and clitoral width measured by taking the transverse diameter at the middle of the clitoris(7) see figure 2 and 3 below.

Figure 1 Anogenital distance and clitoral size measuring adjustable digital sliding caliper

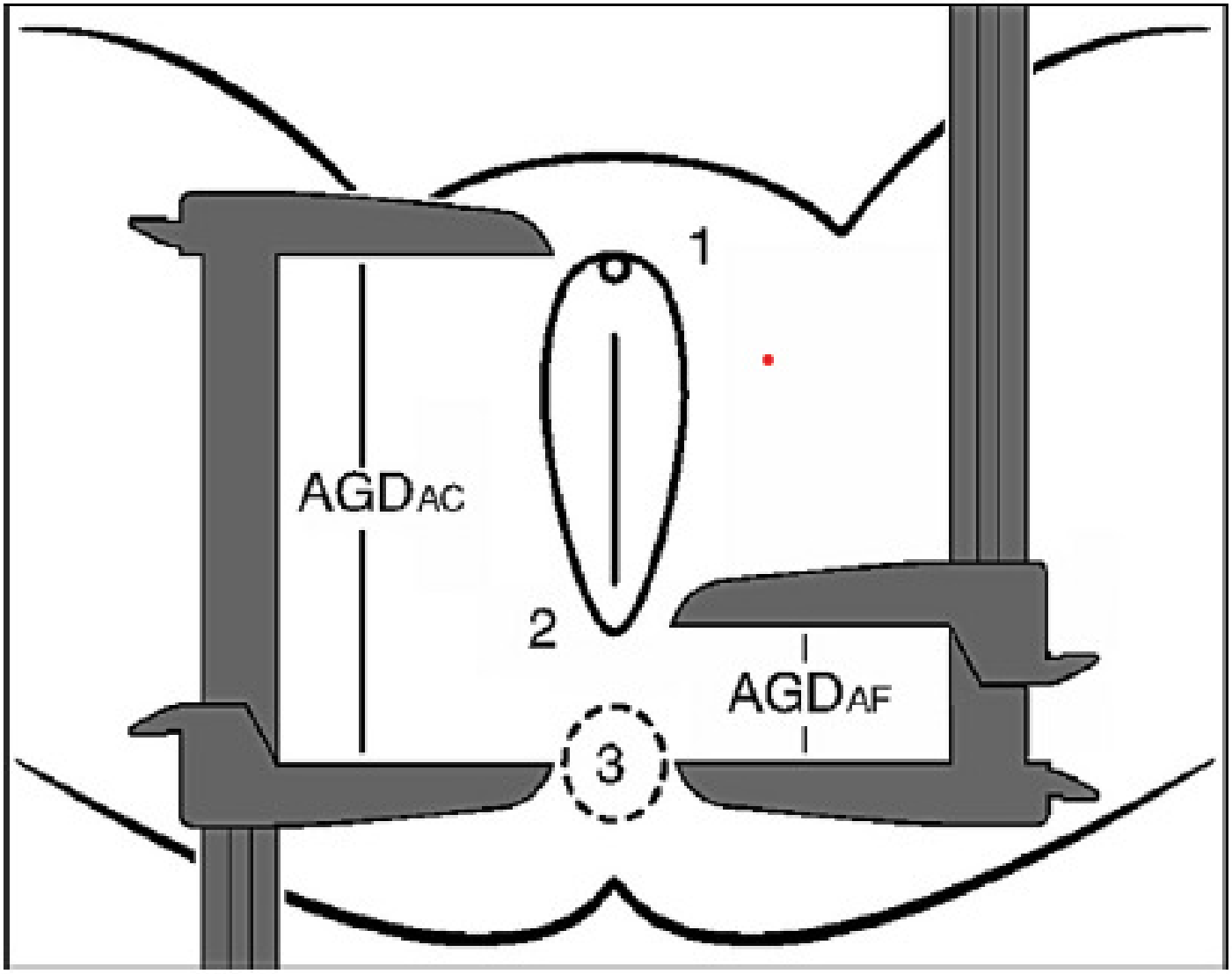

Figure 2 Landmarks for measurements of anogenital distance (AGD) .Anus-Clitoris distance (AGDAC), from the anterior clitoral surface to the centre of the anus (point 1 to point 3); and anus-fourchette distance (AGDAF), from the posterior fourchette to the centre of the anus (point 2 to point3). (Adapted from Sathyanarayana et al. 2010 with modification**).**

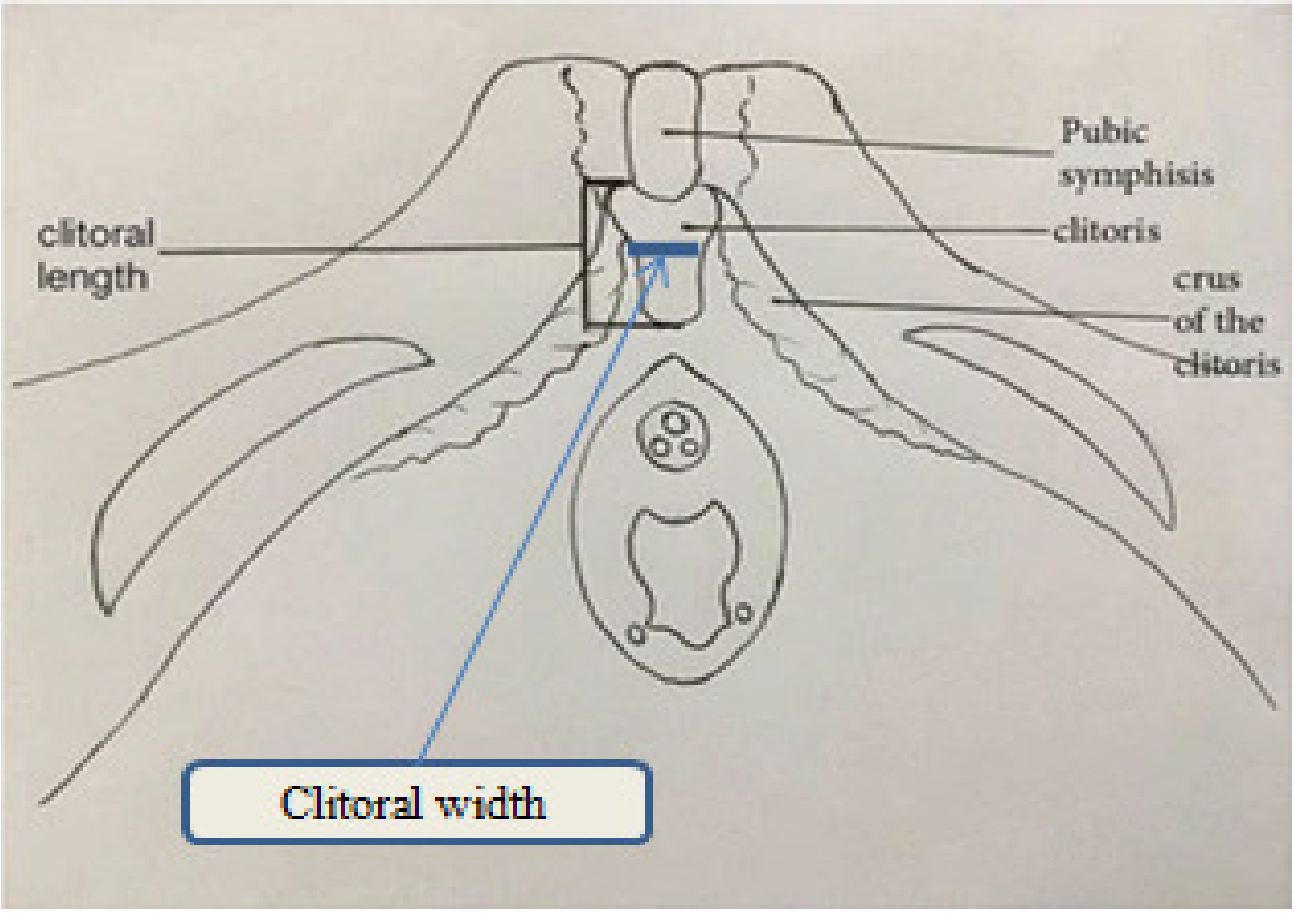

Figure 3 Schematic diagram of clitoris and surrounding structures showing the anatomic landmarks. utilized for clitoral length and width measurement.(Taken from Clitoral size in term newborns in Kumasi, Ghana 2014 with modification).

4.8 Study Variables

4.8.1 Dependant variables

Clitoral length (CL)

Clitoral width (CW)

Anus-fourchet distance (AFD)

Anus-clitoris distance (ACD)

Anogenital ratio

4.8.2 Independent variables

Maternal age

Maternal ethnicity

Number of parity

Number of pregnancy

Gestational age

Anthropometric parameters (birth weight, birth length and head circumference)

4.9. Operational definitions

- **Anus-fourchette distance (AFD)**: The distance from the centre of the anus to the beginning of the mucosa of the posterior fourchette
- **Anus-clitoris distance (ACD):** the distance from the centre of the anus to the base of the clitoris
- **Anogenital ratio:** The ratio of AFD and ACD
- **Clitoral length**: The distance from the pubic ramus to the tip of the glans clitoris
- **Clitoral width**: Transverse diameter at the middle of the clitoris
- **Cutoff point for normal reference value:** Between -2SD and +2SD for both anogenital distance and clitoral size were taken.

4.10. Data Processing and Analysis

Each questionnaire was checked for completeness based on the code given during data collection. The data was entered into Epidata and then exported to SPSS version 26 and analyzed.

Descriptive statistics was used. Data was presented using tables, charts and mean ± standard deviation of the data was determined for each dependent variables. Pearson's correlation was determined among dependent and independent variables. Normogram was also generated for each dependent variable.

4.11. Data Quality Control

To ensure the quality of data adequate training was given for data collectors about techniques of measurements, data collection and documentation. Pretest was conducted using 5% (n =15) of study population in health facilities out of the actual data collection (Ras Desta Damitew Memorial hospital) using the already developed standard tools. Based on the pretest, tools for data collection were modified accordingly. The collection process and completeness of data was closely monitored by the principal investigator so as to provide feedback to correct when necessary. Data collectors and the principal investigator were communicating each other every time about data collection and documentation activities. After data collection, each questionnaire was coded. Variables in the questionnaire were also followed by coding. Data entry was done using Epidata to assure the quality of data.

4.12. Ethical clearance

The ethical clearance was obtained from Ethical Review Board of SPHMMC. Written consent was provided to the participating mothers prior to their involvement in the study that clearly stated their right to refuse to answer any question and withdraw from the study at any time. It was made clear to them that there was no benefit in terms of item for the mothers or the neonates for participating in the study. They were informed that everything they provided and examined on their newborns during the study would be treated with utmost confidentiality and used for study purpose only.

5. Result

5.1. Maternal socio-demographic characteristics

A total of 284 term female newborns were recruited. Majority of maternal ethnicity were Oromo 107(37.7%) and Amhara 106(37.3%) followed by Gurage 46 (16.2%). Mean maternal age was 28.28±4.92 with minimum and maximum of 18 and 41 years respectively.

5.2. Obstetric Characteristics of study participants

Ninety three point three percent (265/284) of pregnancy was singleton and most of participating mothers were Para I (41.5%) and II (31%). Mean gestational age at delivery was 38.95 ±1.21 with minimum and maximum gestational age of 37 and 42 weeks respectively. Majority of neonates (84.2%) were born by cesarean section and 15.8 % through spontaneous vaginal delivery.

5.3. Anthropometric measurements and age of study participants

Mean postnatal age of neonates was 27.1±15.8 hrs and mean ± SD for head circumference, birth weight and birth length were 34.4 ± 1.19 cm, 3074.44 ± 482.45gm and 48.64± 1.9 cm respectively (Table 1).

Table 1 Anthropometric measurements and age of term female newborns at SPHMMC, Addis Ababa Ethiopia, 2021 (n= 284).

	Mean ±SD	Minimum	Maximum	Range
Postnatal age	27.10±15.80	1.00	72.00	71.00
Head circumference (Centimeter)	34.40±1.19	33.00	37.00	4.00
Birth weight (Gram)	3074.44±482..45	2300.00	5000.00	2700.00
Birth length(Centimeter)	48.64±1.90	44.00	55.00	11.00

5.4. Neonates' anogenital distance and clitoral size (*in millimeter*)

The overall mean ±SD of clitoral length, clitoral width, anus-fourchet distance and anus-clitoris distance of the neonates were 9.27 ±1.60, 4.11 ±0.97, 10.60 ±1.58 and 31.51 ±4.11, respectively (Table 2). The mean ±SD of clitoral length, clitoral width, anus-fourchet distance and anus-clitoris distance based on gestational age is displayed below (table 3).

Table 2 Clitoral size and anogenital distance of term newborns at SPHMMC, Addis Ababa Ethiopia, 2021 (n= 284).

	Mean±SD	Minimum	Maximum	Range
Clitoral length (CL)	*9.27±1.60*	3.50	15.30	11.80
Clitoral width(CW)	*4.11±0.97*	1.40	9.90	8.50
Ano-fourchet distance (AFD)	*10.60±1.58*	7.10	18.00	10.90
Ano-clitoris distance (ACD)	*31.51±4.11*	14.30	42.00	27.70
Anogenital ratio (AGR)	*0.34±0.07*	0.2	0.66	0.075

Table 3. Clitoral size and anogenital distance of term newborns based on gestational age at SPHMMC, Addis Ababa Ethiopia, 2021 (n= 284).

Gestational age(weeks)	No. of cases	Variables	Mean ±SD	Minimum	Maximum	Range
37	14	Clitoral length	9.18 ±1.22	6.30	10.70	4.40
		Clitoral width	4.44 ±0.67	3.10	5.50	2.40
		Anus-fourchet distance	10.37 ±1.96	8.40	16.00	7.60
		Anus-clitoris distance	30.16 ±5.63	14.30	37.00	22.70
		Anogenital ratio	0.36 ±0.12	0.24	0.66	0.43
38	104	Clitoral length	9.15±1.65	4.70	15.30	10.60
		Clitoral width	4.05±1.09	1.40	9.90	8.50
		Anus-fourchet distance	10.67±1.69	8.00	18.00	10.00
		Anus-clitoris distance	31.50±3.67	14.80	42.00	27.20
		Anogenital ratio	0.34 ±0.07	0.26	0.58	0.32
39	100	Clitoral length	9.45±1.53	4.00	13.50	9.50
		Clitoral width	4.26±0.83	2.60	8.40	5.80
		Anus-fourchet distance	10.36±1.34	8.00	15.0	7.00
		Anus-clitoris distance	31.49±4.16	17.20	39.30	22.10
		Anogenital ratio	0.33 ±0.06	0.24	0.66	0.42
40	33	Clitoral length	9.20±1.82	3.50	13.1	9.60
		Clitoral width	3.90±0.99	1.80	5.20	3.40
		Anus-fourchet distance	10.59±1.53	7.90	15.30	7.40
		Anus-clitoris distance	32.29±4.46	22.20	42.00	19.80
		Anogenital ratio	0.34 ±0.08	0.24	0.56	0.32
41	15	Clitoral length	9.07±1.49	6.80	11.90	5.10
		Clitoral width	3.85±1.07	2.20	5.60	3.40
		Anus-fourchet distance	11.40±2.16	8.30	16.30	8.00
		Anus-clitoris distance	30.36±4.71	21.00	39.00	18.00
		Anogenital ratio	0.39 ±0.10	0.26	0.57	0.31
42	18	Clitoral length	9.41±1.72	3.50	12.00	8.50
		Clitoral width	3.94±0.91	1.50	5.10	3.60
		Anus-fourchet distance	11.00±1.27	7.10	12.40	5.30
		Anus-clitoris distance	32.23±3.84	21.10	39.00	17.90
		Anogenital ratio	0.35 ±0.07	0.20	0.54	0.34

5.5. Normogram of clitoral size and anogenital distance

The overall 2 standard deviation above and below the mean were determined for the study variables. For Clitoral length 12.47mm and 6.06mm, clitoral width 6.05mm and 2.17mm, anus-fourchet distance 13.76mm and 7.44mm, anus-clitoris distance 39.73mm and 23.29mm and anogenital ratio 0.34 and 0.2 respectively.

Normogram of clitoral length, clitoral width, anus-fourchet distance and anus-clitoris distance were determined using mean, standard deviations and gestational age. (Figures 4, 5 and 6 below).

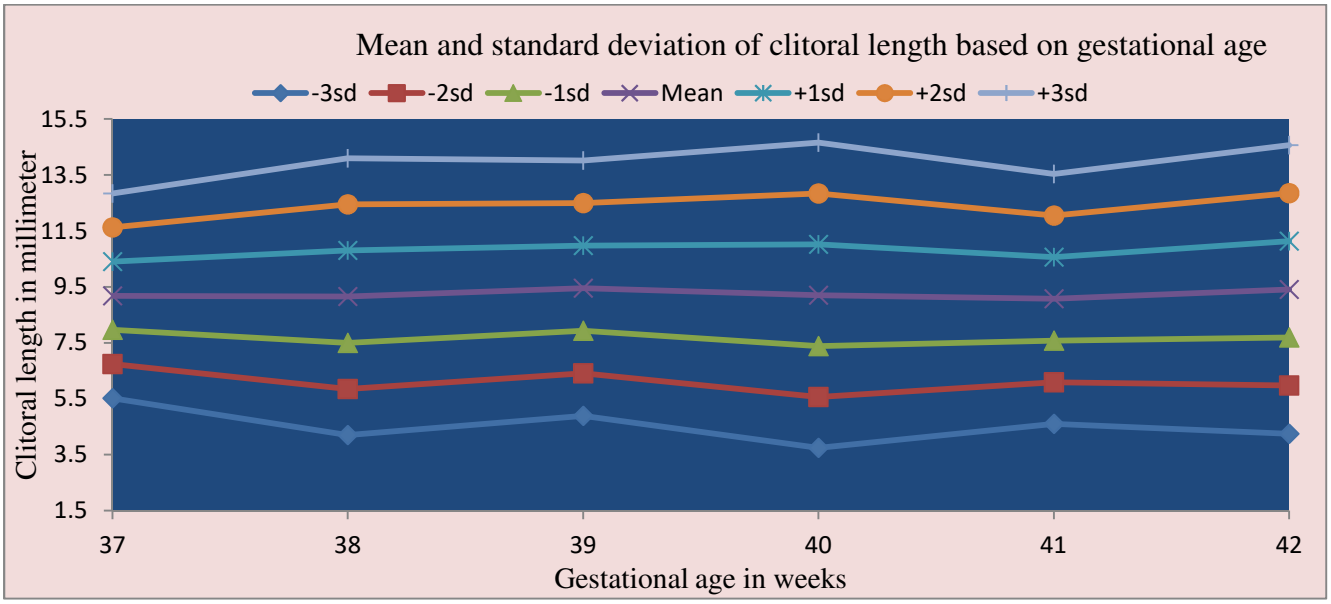

Figure 4 Normogram of clitoral length based on gestational age in the assessment of clitoral size, anogenital distance and generate normative data in term female neonates at SPHMMC Addis Ababa Ethiopia 2021 (n=284).

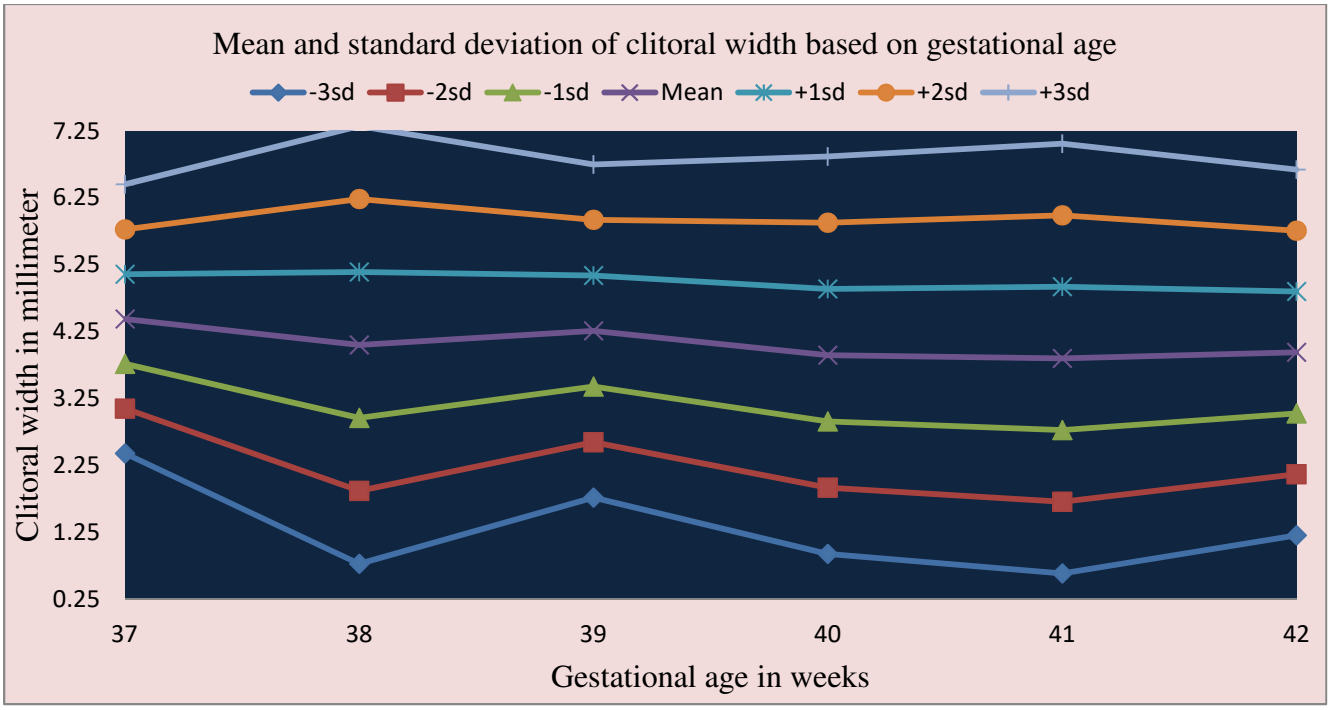

Figure 5 Normogram of clitoral width based on gestational age in the assessment of clitoral size ,anogenital distance and generate normative data in term female neonates at SPHMMC Addis Ababa Ethiopia 2021 (n=284).

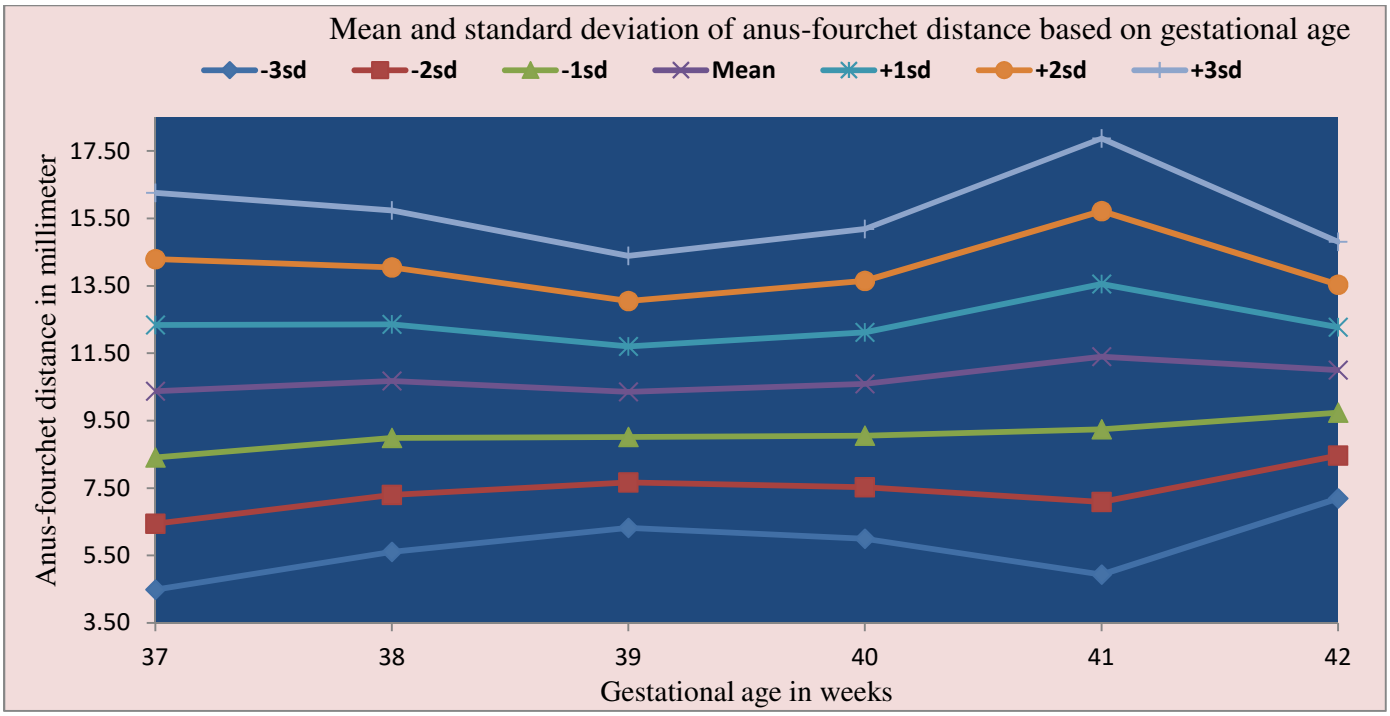

Figure 6 Normogram of anus-fourchet distance based on gestational age in the assessment of clitoral size, anogenital distance and generate normative data in term female neonates at SPHMMC Addis Ababa Ethiopia 2021 (n=284).

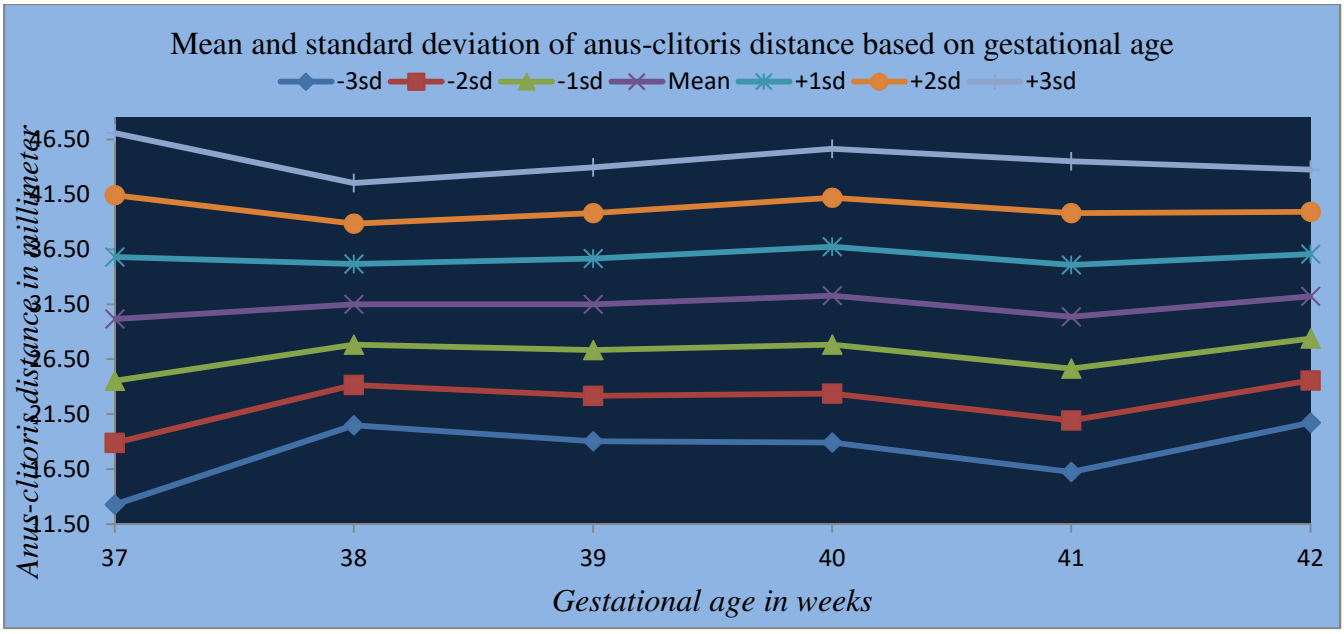

Figure 7 Normogram of anus-clitoris distance based on gestational age in the assessment of clitoral size, anogenital distance and generate normative data in term female neonates at SPHMMC Addis Ababa Ethiopia , 2021 (n=284).

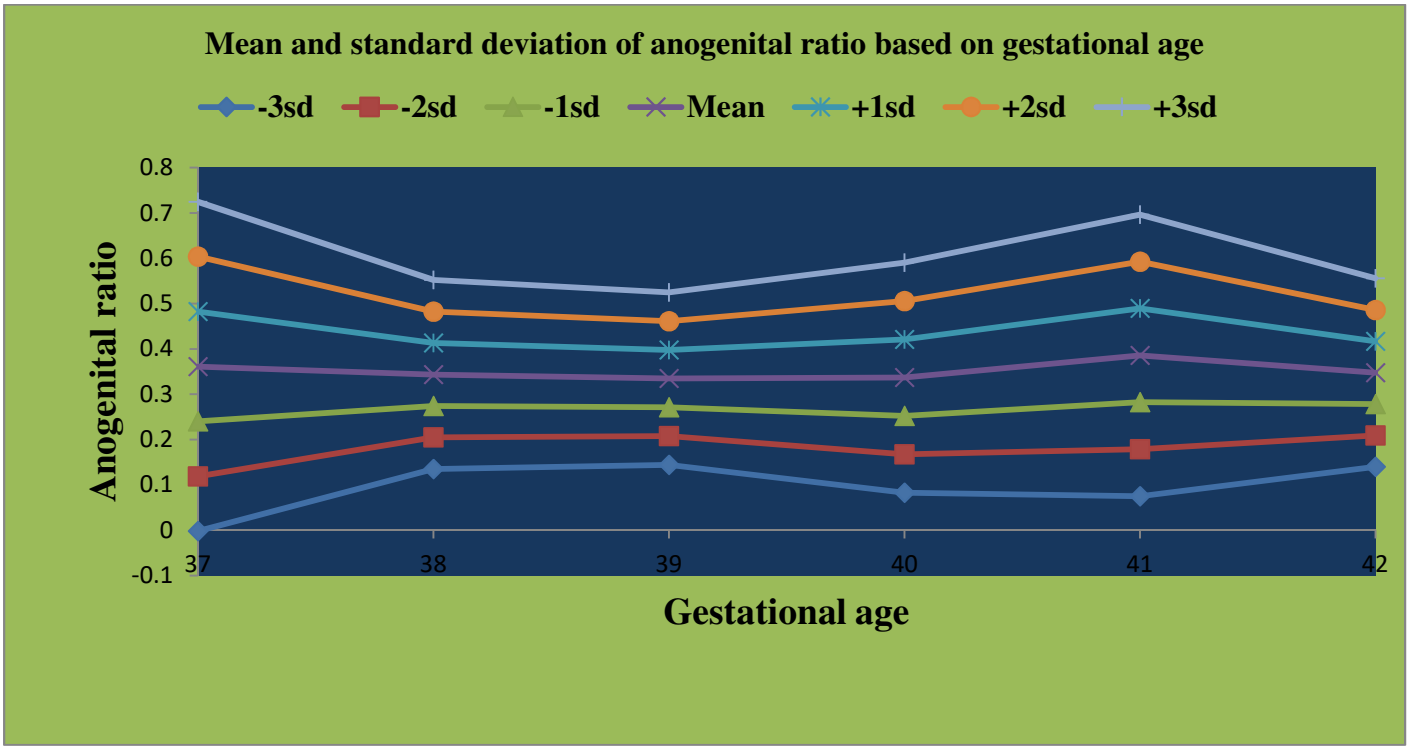

Figure 8 Normogram of anogenital ratio based on gestational age in the assessment of clitoral size, anogenital distance and generate normative data in term neonates at SPHMMC Addis Ababa Ethiopia , 2021 (n=284).

6. **Correlation**

There were weak positive correlation of anus-clitoris distance with birth weight (r=0.218, p= 0.00) and birth length. (r =0.222, p= 0.00) and anus-fourchet distance with head circumference (r = 0.146, p =0.014) and with birth weight (r =0.145, p =0.014). (Table 4). The Pearson's correlation showed no statistically significant relationship between the dependent and other independent variables.

Table 4 Pearson's Correlation of anogenital distance and anthropometric parameters at SPHMMC, Addis Ababa Ethiopia, 2021 (n= 284).

	Pearson's correlation values	Anus-fourchet distance	Anus-clitoris distance
Head circumference	r value	0.146	-
	P value	0.014	-
Birth weight	r value	0.145	0.218
	P value	0.014	0.00
Birth length	r value	-	0.222
	P value	-	0.00

7. Discussion

This study is thought to be the first in its kind to describe the clitoral size and anogenital distance in Ethiopia. Regardless of gestational age, the overall mean clitoral length and clitoral width were 9.27±1.6mm and 4.11±0.97mm respectively. There was no correlation of clitoral size with neonatal anthropometric parameters, parity, gestational age or number of pregnancy. Our finding of mean clitoral length was higher than studies done in different countries with similar methodology. For instance, study done in Iran female neonates showed mean clitoral length of 6.11±0.39mm (20).Other two studies conducted in Nigeria on clitoral size showed that the mean clitoral lengths were 7.7±1.37 mm and 6.7 ± 1.6mm and mean clitoral width 5.6 ± 0.8 mm, respectively (21, 7).This finding also higher than cross-sectional studies in Ghana (22) and Turk (25) which showed that the mean clitoral lengths were 4.13 ± 1.6 mm and 4.93 ± 1.61mm, respectively.

The clitoral width of this study finding was almost similar to study done in Iran female neonates which showed that the mean clitoral width of 4.22 ±0.43 (20).Other studies in Nigerian on clitoral size showed that the mean clitoral width was 4.40 ±0.89 mm (21). There were nearly comparable mean clitoral widths in studies in Ghana (22) and Turk (25) which showed that the mean clitoral width were 4.21 ± 1.1 mm and 4.93 ± 1.61mm, respectively. However, a multicenter cross sectional study in Nigeria showed that the mean clitoral width was 5.6 ± 0.8 mm which was higher than mean clitoral width in this study (7).

Possible contributing factors to this observed difference in clitoral length among different population might be racial, inter-observer, or genetic differences.

The overall mean AFD, ACD and AGR were determined and the values were 10.6±1.58mm, 31.51±4.11mm and 0.34±0.07 respectively. The AFD was consistent with studies conducted in in United Kingdom (24) on426 female neonates and Turk (25) showed that the mean ±sd of AFD was 9.1±2.8 mm and 10.3±0.2, respectively. The mean ACD also consistent with a study conducted in Turk on among 115 female neonates which showed mean of ACD 30±0.2 (25). However, the mean AFD in this study was much lower than studies conducted in Nigeria on 3 different study settings which showed the mean AFD were 13.89±0.26mm (27), 15.2±2.4 mm (26), 17±2.6mm(7), respectively. The mean ACD also lower than a study conducted in Nigeria with mean ACD of 35±2.5mm (7).AGR in this study was slightly lower than similar study in Nigeria which was 0.48 ± 0.05 *(7).*This difference might be genetic variations or environmental exposure that had androgenic effects because the studies were in the same country and exposure status was not mentioned in the study clearly.

In this study there was no correlation of clitoral size with neonatal anthropometric parameters, parity, gestational age, maternal ethnicity or number of pregnancy. Most of the previous studies also showed no correlations of clitoral size with anthropometric parameters or gestational age (7, 21, 25).

However, two studies showed controversial results. In Iran study on nomogram of clitoral length and width regression analysis showed clitoral length positively correlated with gestational age, birth length, birth weight & head circumference and clitoral width with gestational age, birth length, and birth weight (20). But in a cross-sectional study in Ghana on Clitoral size in term newborns showed the mean clitoral length was inversely related to birth weight and the mean clitoral width was inversely related to gestational age and birth weight (22).

These differences might require further multicenter studies to identify the contributing factors.

There was weak positive correlation of anus-clitoris distance with birth weight (r=0.218, p= 0.00) and birth length (r =0.222, p= 0.00) and anus-fourchet distance with head circumference (r = 0.146, p =0.014) and with birth weight (r =0.145, p =0.014). There was no correlation with parity, gestational age or number of pregnancy. In Nigerian study there were significant positive correlations of AFD and ACD with all anthropometric measurements (7).

These minor differences with this study might be genetic variations in the study populations.

The overall cutoff point of normal value (regardless of gestational age) were; clitoral length 6.06mm-12.47mm , clitoral width 2.17mm - 6.05mm,AFD 7.44 -13.76mm , ACD 23.29 - 39.73mm and AGR 0.2-0.34 . Using this cutoff point Clitoral length can be classified into normal, clitoromegaly or microclitoris. Standard textbooks show average normal value of clitoral length 9mm which is in the range of clitoral length in this study (10). Similarly using anogenital distance cutoff point anogenital distance can be classified into normal, long or short.

Normogram of clitoral length, clitoral width, anus-fourchet distance and anus-clitoris distance were determined using mean, standard deviations and gestational age. There was no previously determined published normogram to compare with this study.

8. Limitation of the study

Although this study contributed a clear insight into normative data for term babies, it was single center institutional study that limits its application outside the study area. Current study tried to include as many variables as possible but other variables like umbilical cord and serum testosterone level were not measured.

9. Conclusion

Mean±2SD reference values in this study were Clitoral length 6.06–12.74 mm, clitoral width 2.17–6.05 mm, anus-fourchet distance 7.44–13.76mm, anus-clitorisdistance 23.28– 39.72mm and anogenital ratio 0.2 -0.48. Normograms were also generated based on gestational age

This simple, affordable and non-invasive evaluation could help early identification and intervention of female infants with potentially harmful conditions like congenital adrenal hyperplasia, particularly where newborn screening is not available.

10. Recommendation

Clitoral size and anogenital distance should be measured appropriately to use this reference curve in the study hospital. We recommend multicenter study including preterm female neonates to make reference value more applicable as national level.

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List of Annexes

Annex 1. Consent form

St Paul Hospital Millennium Medical College Department of Pediatrics and Child Health

To assess clitoral size, anogenital distance and generate normative data in term female newborns in Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021.

Code _____

Hello, my name is …… and I am conducting a research on assessment of clitoral size, anogenital distance and generate normative data in term newborns in Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia, 2021. You are kindly requested to be involved in the study which will improve the quality of care that we are providing. The questionnaire will only take a few minutes of your time. I want to inform you that you have the right to skip any question that can make you uncomfortable, the information you give will be kept confidential and also you have the right to withdraw from participation at any time during the interview. The study has approval and ethical clearance from the department as well as from hospital's ethical committee. If you are willing to participate in the study, please confirm it with your signature on the space provided below.

I place my signature as a sign of agreement to participate_____.

Thank you for your cooperation!!

Data Collector_____ Signature_____

Date_____

Annex 2. Data collection questionnaire

Code----------

1. Maternal Socio-demographics

1.1 Maternal age ----------

1.2 Maternal ethnicity ----------

1.3 Number of Parity----------

1.4 Did you take any medication other than folic acid, Iron or antihelmnths during pregnancy?

1.4.1 Yes 1.4.2 No

1.5 If the answer is yes for 1.4, when?

1.5.1 First trimester

1.5.2 Second trimester

1.5.3 Third trimester

1.6 Name of the drug(s) ----------

1.7 Any known chronic diseases/neoplasm

If yes for 1.7, mention ----------

1.8 Do you have hirsutism?

1.8.1 Yes 1.8.1 No

1.9 Did you use IVF to conceive this baby?

1.9.1 Yes 1.9.1 No

2 Neonatal factors

2.1 Number of pregnancy

2.1.1 Singleton

2.1.2 Twin

2.1.3 Triplets and more

2.2 Gestational Age ----------

2.3 Mode of delivery ----------

2.4 Post-natal age (hours) ----------

2.5 Head circumference (cm) ----------

2.6 Birth weight in grams----------

2.7 Length (cm) ----------

2.8 Clitoral length (CL) in millimeter ----------

2.9 Clitoral width (CW) in millimeter ----------

2.10 Anus-fourchette distance (AFD) in millimeter ----------

2.11 Anus-Clitoris Distance (ACD) in millimeter ----------

Statement of Declaration

By my signature below, I declare and affirm that this thesis is my own original work in partial fulfillment of the requirements for the certification of pediatric and child health specialty. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All the sources of the materials used for this thesis and all people who gave support for this work are fully acknowledged.

I affirm that I have cited and referenced all sources used in this document. Every effort has been made to avoid plagiarism in the preparation of this thesis.
Requests for permission for extended quotations from, or reproduction of, this thesis in whole or in part may be granted by the Head of the Department or the adviser of the thesis when in his judgment the proposed use of the material is in the interest of scholarship and publication. In all other instances, however, permission must be obtained from the author of the thesis.

STUDENT

Dr. Aniley Assab Signature: _____ Date: _____

RESEARCH ADVISOR:

Dr. Fikir Messeret signature: _____ Date: _____