Cross-sectional observational study of nipple and areola changes during pubertal development and after menarche in 313 Italian girls

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Summary. *Objective:* To describe the characteristics of nipple development during puberty in Italian females. In addition this is compared with Tanner criteria for breast and pubic hair development at different stages and correlated with body mass index (BMI) and the menstrual age. Design: Cross-sectional observational study. Setting: Italian children and adolescents consecutively assessed at the Endocrine and Adolescent Outpatient Clinic of Private Accredited Quisisana Hospital of Ferrara. Partecipants: Healthy females 2-20 years, belonging to Italian middle socio-economic class. Study duration: This study was conducted for a total period of 39 months from September 2012 to December 2015. Methods: Demographic characteristics, age at menarche and menstrual duration, anthropometry (weight, height, BMI) and pubertal status (breast and pubic hair) were evaluated. The greatest diameters of the erect nipple and areola sizes were measured in all subjects in supine position using a plastic template with graduated circular cut-outs or a transparent graduated ruler. Results: Significant increments in nipple diameter were noted between Tanner's stages B1, B2, B3, B4 and B5 but the differences between stages B1 vs B2, B3 vs B4, B4 were more significant (p<0.001) than the difference between B4 vs B5 (p<0.004). The same statistical pattern was documented between Tanner's stage PH1 and PH2, PH2 and PH3 and PH4 and PH5. The nipple diameter increased significantly in relation to BMI in all Tanner's stage groups (p: 0.021 for B1 vs B2; and p<0.001 for B3 vs B4 and B4 vs B5) but one (B2 vs B3=p: 0.518). A significant correlation was observed between duration of menses, nipple and areola sizes (r: 0.490; p=<0.001 and r: 0.252; p=0.012, respectively). Conclusions: The assessment of sexual maturation is important in population studies as well as for daily clinical encounter. In our subjects the mean age of onset of secondary sexual characteristics was 10.3±1.4 years and mean age at menarche was 12.02±1.5 years. The study provides the normative data for nipple and areola sizes in Italian girls. Change in nipple and areola sizes may represent an early marker for transition to puberty. (www.actabiomedica.it)

Key words: puberty, breast development, nipple size, areola size, Body Mass Index

Introduction

A myriad of biological changes occur during puberty including sexual maturation, increases in height and weight, completion of skeletal growth accompanied by a marked increase in skeletal mass, and changes in body composition.

Development of breast tissue is the first clinical sign of the onset of central puberty (CP) in girls (1-4). Whilst in boys, the first physical indication is enlargement of the testis and reddening of the scrotal skin (1,2). While the first menstruation is the most commonly assessed pubertal marker in large-scale epidemiological studies, it is a rather late event in puberty. It occurs on average 2 years after the onset of breast development, and its timing varies between populations and ethnic groups (1,2).

Sexual Maturity Rating (SMR), also known as Tanner Staging, is based on an assessment scale of development of secondary sexual characteristics. This helps health professionals to gauge the degree of pubertal maturation that has occurred among adolescents, regardless of chronological age. SMR is based on the appearance of pubic hair, the development of breasts, and the occurrence of menarche in females. However, difficulties exist in determining breast stage development in the obese children (4). In teens self reporting of breast stage, using this method, is associated with considerable inaccuracies (5-7).

The availability of an easy, cost-effective, reliable, non-invasive method to assess the onset of puberty is useful in both clinical and research settings. Very little attention has been paid to development of nipple and areola maturation characteristics during pubertal development and after menarche (8-11).

The primary purpose of this project was to describe the characteristics of nipple development during puberty in Italian females. In addition this is compared with Tanner criteria for breast and pubic hair development at different stages and correlated with body mass index (BMI) and the menstrual age.

Subjects and Methods

Target population

This cross-sectional observational study was conducted on children and adolescents correlating the nipple and areola diameters with the SMR stages described by Tanner et al. Our target population was healthy females 2-20 years, belonging to Italian middle socio-economic class (12).

Study duration

This study was conducted for a total period of 39 months from September 2012 to December 2015.

Inclusion criteria

1. Italian children and adolescents consecutively assessed at the Endocrine and Adolescent Outpatient Clinic of Private Accredited Quisisana Hospital of Ferrara were enrolled; 2. Children and adolescent whose consent was obtained.

Exclusion criteria

1. Subjects with history of endocrine and growth disorders; 2. Subjects with physical disability, which prevented accurate anthropometric measurements; 3. Subjects with dysmorphic features suggestive of syndromic diseases or bone dysplasia known to affect height of the child and/or sexual maturation; 4. Subjects with congenital and acquired pediatric breast anomalies (13-15); 5. Unwillingness of the parent or guardian to participate in the study; 6. Obese children and adolescents with BMI >30 Kg/m².

Clinical data collection

Demographic characteristics, age at menarche and menstrual duration, anthropometry (weight, height, BMI) and pubertal status (breast and pubic hair) were evaluated.

Anthropometry and definitions

Height and weight were measured according to international recommendations (16,17). Body weight was measured, wearing minimal underclothes, to the nearest 100 g on properly calibrated scales. Body mass index (BMI) was calculated (weight in Kg/height in m²). A subject was considered overweight when the BMI was between 25 and 30 and obese above 30 (18).

Assessment of pubertal stage

A pediatric endocrinologist (VDS) assessed breast development (inspection and palpation) and pubic hair using SMR described by Tanner et al as following:

Sexual Maturity Rating (SMR)

I. Preadolescent; elevation of papilla only; II. Breast bud stage; elevation of breast and papilla as a small mound and/or enlargement of areola diameter; III. Further enlargement of breast and areola, with no separation of their contours; IV. Projection of areola and papilla to form a secondary mound above the level of the breast; and V. Mature stage; projection of papilla only, owing to recession of the areola to the general contour of the breast.

Onset of puberty was defined as Tanner's breast stage 2.

Techniques for nipple and areola size measurements

The greatest diameters of the erect nipple and areola sizes were measured in all subjects in supine position using a plastic template with graduated circular cut-outs (8) or a transparent graduated ruler (11). When the areola diameter was greater than the graduated circular cut-outs of plastic template, the greater horizontal or vertical midpoint diameter of areola was recorded to the nearest millimetre with the transparent ruler pressing it against each areola until the areolar margin contacted the ruler.

Ethical Approval

Informed verbal consent was taken from the person or the guardian for each study subject. All procedures were carried out with the adequate understanding and consent of parents or patients in accordance with the Declaration of Helsinki (<u>http://www.wma.net</u>).

Statistical analysis

Standard computer program SPSS for Windows, release 13.0 (SPSS Inc, Tulsa, IL, USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in the two groups was made using unpaired - Student t-test and Mann-Whitney test for normal and nonparametric variables respectively. Kruskal-Wallis and McNemar-Bowker tests were used to study correlations between variables with parametric and non-parametric distributions . p < 0.05 was considered as significant.

Results

There were 313 eligible girls for this study. Table 1 shows the age and auxological parameters of our children and adolescents in relation to their breast Tanner stage. The youngest subject was 2 years old and the oldest 20 years old. The mean age of onset of secondary sexual characteristics was 10.3±1.4 years and mean age at menarche was 12.02±1.5 years.

A statistical chronological age difference was observed in the five studied groups (p<0.001 for Tanner's stage B1 vs B2; B3 vs B4 and B4 vs B5 and a p value <0.015 for Tanner's stage B2 vs B3).

Significant increments in nipple diameter were noted between Tanner's stages B1, B2, B3, B4 and B5 but the differences between stages B1 vs B2, B3 vs B4, B4 were more significant (p<0.001) than the difference between B4 vs B5 (p<0.004).The same statistical pattern was documented between Tanner's stage PH1 and

Variables expressed as mean and standard deviation	Tanner's stage 1 (n. 55)	Tanner's stage 2 (n. 56)	Tanner's stage 3 (n. 70)	Tanner's stage 4 (n. 49)	Tanner's stage 5 (n. 83)
Age (years)	7.0 ± 2.3	10.3±1.4	11.4±1.6	12.6±1.6	14.4±2.0
95% Confidence Interval for Mean					
Lower Bound	6.4	10.0	11.1	12.1	14.0
Upper Bound	7.7	10.7	11.8	13.0	14.9
Median	7.6	10.1	11.4	12.6	14.6
Minimum- Maximum	2.0-11.0	8.1-13.8	8.9-15.5	9.4-16.1	9.1-20.0
Weight (Kg)	21.8±6.8	29.4±5.0	35.7±7.2	43.6±7.6	52.4±9.4
Height (cm)	118.2±16.0	136.6±7.3	144.6±7.5	152.1±8.8	158.8±8.2
BMI (kg/m²)	15.2±1.8	15.7±1.7	16.9±2.4	18.7±2.1	20.7±2.9

Table 1. Auxological parameters in 313 girls in relation to breast stage development (Tanner's stage 1-5)

Legend: In brackets the number of children and adolescents

Nipple and areola sizes (mm) in	Breast	Breast	Breast	Breast	Breast
relation to Tanner's breast stage	stage 1	stage 2	stage 3	stage 4	stage 5
Mean and Standard Deviation	(n. 55)	(n. 56)	(n. 70)	(n. 49)	(n. 83)
Nipple size	2.9±0.5	3.3±0.6	4.4±0.8	6.3±1.3	8.0±1.3
Areola size	14.1±2.7	18.0±3.0	22.1±3.4	29.3±5.8	36.6±9.0
Nipple and areola sizes (mm) in	Pubic hair				
relation to Tanner's pubic hair stage	stage 1	stage 2	stage 2	stage 2	stage 2
Mean and Standard Deviation	(N. 90)	(N. 40)	(N. 52)	(N. 54)	(N. 74)
Nipple size	3.1±0.6	3.7±0.7	4.6±1.1	6.7±1.5	7.9±1.4
Areola size	16.0±3.7	19.5±4.1	22.9±4.9	31.2±7.9	35.9±9.0

Table 2. Auxological parameters in Italian girls in relation to breast and pubic hair stage development (Tanner's stage 1-5)

Legend: In brackets the number of children and adolescents

PH2, PH2 and PH3 and PH4 and PH5 (Table 2). No significant changes in the nipple and areola sizes were observed in girls with Tanner's stage B1 and PH 2 vs Tanner's stage B1 and PH 1.

The mean BMI is reported in table 1. The BMI range values in the 5 studied group of subjects were as follows: BMI 1: 12.0-21.2 Kg/m²; BMI 2 : 12.4-22.9 Kg/m²; BMI 3: 13.0-24-8 Kg/m²; BMI 4: 13.9- 26.2 Kg/m²; BMI 5: 16.0-29.3 Kg/m². The nipple diameter increased significantly in relation to BMI in all Tanner's stage groups (p: 0.021 for B1 vs B2; and p<0.001 for B3 vs B4 and B4 vs B5) but one (B2 vs B3= p: 0.518)



Figure 1. Correlations of nipple sizes in relation to the months after menarche (r=0.490; p<0.001)

A significant correlation was observed between duration of menses, nipple and areola sizes (r: 0.490; p=<0.001 and r: 0.252; p=0.012, respectively) (Figures 1 and 2).

Conclusions

Breast development is the first manifestation of puberty in approximately 85% of girls. The normal age for initial breast development is 8 to 13 years. Menarche generally occurs within 2 years after the onset of breast development at breast Tanner's stage of 4 (2).



Figure 2. Correlations of nipple sizes in relation to the months after menarche (r=0.252; p=0.012)

The assessment of sexual maturation is important in population studies and in clinical care. Pubertal staging allows doctors to assess the maturation of adolescents, to correlate several pubertal phenomena such as age at menarche, growth spurt and final height, to advise and manage patients appropriately, and to have sensitive "sensors" for the effects of environmental exposure on human populations.

The most widely used scale, the Tanner scale, was developed by Marshall and Tanner in the late 1960's based on British girls and boys participating in the Harpenden Growth Study who were followed every 3 months during adolescence. The researchers took photographs of secondary sex characteristics (pubic hair in both sexes, breasts in girls, and genitals in boys) and classified each trait into five developmental stages, ranging from pre-adolescent child like morphology to fully mature (2). This assessment may be performed by a physician with expertise in adolescents or by self-assessment, in which the adolescent identifies his or her stage of maturation based on Tanner's photographs/ illustrations. However, obesity significantly increases the difficulty of detecting the appearance of breast buds. Obese girls have worse concordance with trained personnel and tend to over-diagnose their mammary development due to the presence of lipomastia (19,20). A study of 9,132 Chinese girls found that the Kappa

statistic for evaluating Tanner breast stage 2 was 0.62 among non-obese girls, while it was only 0.53 among obese girls; moreover, almost one in five girls in the latter group over-diagnosed their mammary development (20).

Our cross-sectional study shows that nipple and areola diameters are feasible for sexual maturation staging. Both sizes show a significant increase during pubertal development and after menarche. Furthermore, comparing our data with previous studies reported in American and Turkish girls suggested that there are not substantial ethnic differences in nipple and areola size development during puberty (Table 3 and 4). However, validation of this simple method of diagnosis of pubertal progression is needed also in different populations.

In conclusions, the assessment of sexual maturation is important in population studies as well as for daily clinical encounter. SMR proposed by Tanner et al and age at menarche are the most widely used methods to assess sexual maturation. However, techniques for examining onset of breast development are often visual or descriptive and without physical palpation of apparent breast tissue. It is possible to confuse fat accumulation in overweight and obese girls for the appearance of a breast bud (3).

Breast Tanner's stage	Rohn's cross sectional study	Rohn's longitudinal study	Buyukgebiz and Kinik cross sectional study	Ayguun et al. cross sectional study	Our cross sectional study
B1	2.8 ± 0.8	3.0±0.7	1.8±0.8	2.5±1.5	2.9 ± 0.5
Mean ± SD	(NA)	(NA)	(18)	(163)	(55)
B2	3.28 ±0.8	3.3±0.9	4.2±2.1	3.3±1.4	3.3±0.6
Mean ± SD	(NA)	(NA)	(45)	(69)	(56)
B3	4.0±1.3	4.7±1.4	5.9±2.0	5.2±1.9	4.4±0.8
Mean ± SD	(NA)	(NA)	(59)	(152)	(70)
B4	7.7±1.6	7.2±1.4	7.1±2.2	6.2±1.7	6.3±1.3
Mean ± SD	(NA)	(NA)	(59)	(52)	(49)
B5	9.9±1.3	9.4±1.4	8.6±2.7	6.9±1.5	8.0±1.3
Mean ± SD	(NA)	(NA)	(48)	(61)	(83)

Table 3. Nipple diameter (mm) versus breast development: Comparison of different studies

Legend: In brackets the number of children and adolescents

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Breast Tanner's stage	Ayguun et al. cross sectional study	Our cros sectional study	Pubic hair Tanner's stage	Ayguun et al. cross sectional study	Our cross sectional study
B1	14.3± 5.0	14.1±2.7	PH1	15.3 ± 57	16.0±3.7
Mean ± SD	(170)		Mean ± SD	(170)	(55)
B2 Mean ± SD B3 Mean ± SD	20.2±5.8 (75) 28.8±6.3 (119)	18.0 ±3.0 22.1 ±3.4	PH2 Mean ± SD PH3 Mean ± SD	19.3±6.3 (75) 25.2±7.3 (119)	19.5±4.1 (56) 22.9±4.9 (70)
B4	32.0±8.8	29.3 ±5.8	PH4	32.5±9.3	31.2±7.9
Mean ± SD	(113)		Mean ± SD	(113)	(49)
B5	37.6±9.9	36.6 ±9.0	PH5	35.6±9.9	35.9±9.0
Mean ± SD	(24)		Mean ± SD	(24)	(83)

Table 4. Areola diameters (mm) versus breast and pubic hair development: Comparison of two different studies

Legend: In brackets the number of children and adolescents

Furthermore, problems in ascertainment of precise pubertal phenotype can be due to difficulty in obtaining permission to use the best measurement techniques available, which are sometimes thought to be invasive to one's privacy.

In our subjects the mean age of onset of secondary sexual characteristics was 10.3 ± 1.4 years and mean age at menarche was 12.02 ± 1.5 years. The study provides the normative data for nipple and areola sizes in Italian girls. Change in nipple and areola sizes may represent an early marker for transition to puberty.

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