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Implication of Testosterone in Human Puberty Attainment

Olufemi Ebenezer Akinluwade[1,2], Alphonsus Ekpe Udoh[1,2] and Kunle Joseph Akinluwade[3,4,5*]

[1]Department of Chemical Pathology, University of Calabar Teaching Hospital (UCTH), Calabar 540213, Nigeria.
[2]Department of Medical Laboratory Science, Chemical Pathology Unit, University of Calabar, 540213, Nigeria.
[3]Department of Chemical, Materials and Biomolecular Engineering, University of Connecticut/University of Connecticut Health Center (UCHC), Farmington, CT 06032, USA.
[4]Department of Materials Science and Engineering, Obafemi Awolowo University (OAU), Ile-Ife, 220282, Nigeria.
[5]Department of Research and Development, Prototype Engineering Development Institute, [National Agency for Science and Engineering Infrastructure, NASENI], Ilesa, 233036, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OEA designed the study, collected samples, did laboratory/clinical tests, wrote the protocol and wrote the first draft of the manuscript. Author KJA performed the statistical analyses, did confirmatory laboratory/clinical tests and managed literature search. Author AEU supervised the study and interpreted results. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated and compared the serum levels of testosterone in male and female subjects aged 6—10 years. It also assessed the number of children among the subjects who could be potential candidates for precocious puberty based on their hormone levels. Male subjects in the

**Corresponding author: E-mail: jakinluwade@yahoo.com;*

study were 78 (44.1%) with a mean age of 8.3±1.4 years, height of 1.31±0.08 m, mean weight of 26.20±4.83 Kg, and Body Mass Index (BMI) of 15.30±1.71 kg/m^2. Female subjects were 99 (55.9%) with a mean age of 8.55±1.37 years, height of 1.34±0.10 m, weight of 27.78±5.36 Kg, and BMI of 15.38±1.68 kg/m^2. Results from statistical and clinical analyses showed that female subjects were significantly heavier and taller than male subjects ($p < 0.05$) but the BMIs for both sexes were identical ($p > 0.05$). Male subjects had mean testosterone of 0.49±0.38 ng/ml compared with mean testosterone of 0.57±0.59 for the female subjects. There is no significant age dependent differences in the levels of testosterone in both sexes. Based on the levels of testosterone of the children, only one of the children was adjudged qualified as candidate for precocious puberty.

Keywords: Testosterone; precocious puberty; children; age; hormone; BMI; blood.

1. INTRODUCTION

Testosterone, estradiol and prolactin are important hormones produced in humans. They are involved in reproduction and development of secondary sexual characteristics including attainment of puberty. The levels of prolactin, estradiol, progesterone and testosterone, among others, have been implicated in prompt attainment of puberty, delayed puberty and precocious puberty [1].

For boys, testosterone is the principal sex hormone. While testosterone produces all of boys' changes characterized as virilization; a substantial product of testosterone metabolism in males is estradiol. The conversion of testosterone to estradiol depends on the amount of body fat but estradiol levels in boys are typically much lower than in girls [2].

Testosterone is a steroid hormone from the androgen group. It is found in mammals, reptiles [3], birds and other vertebrates. In mammals, testosterone is primarily secreted in the testicles of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. Like other steroid hormones, testosterone is derived from cholesterol [4].

The present study aims at evaluating and comparing the levels of testosterone in male and female subjects; and to ascertain the number of children among the subjects who may be potential candidates for precocious puberty based on their ages and hormone levels.

2. EXPERIMENT

2.1 Study Scope

A total of 177 male and female apparently healthy metropolitan residents aged between six (6) and ten years (10) are the subjects in this study.

Informed consent was obtained from the parents/guardians as well as authorities in the schools attended by the subjects. Ethical clearance for the study was also obtained from relevant State Ethical Committee.

2.2 Sample Collection

Five milliliters (5 ml) of blood was collected from each subject by clean venous puncture into a plain sample container. The blood samples were allowed to clot, and then centrifuged at 2500 rpm for 5 minutes. The resulting sera were collected in plain bottles using sterile Pasteur pipettes and stored frozen for analysis.

2.3 Principle of Testosterone Assay by ELISA

The DRG Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding [5]. The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site on the testosterone molecule. Endogenous Testosterone of a patient sample competes with a Testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of testosterone in the sample. After addition of the substrate solution, the intensity of the color developed is inversely proportional to the concentration of testosterone in the patient's sample.

2.4 Assay Procedure

All reagents and specimens were allowed to attain room temperature. Ninety six (96) microtiter wells were secured in the holder. Twenty five microliters (25 µL) of each standard solution (control solution) and subject sample was dispensed with new disposable tips into the

respective microtiter wells. Two hundred microliters (200 µL) of Enzyme Conjugate was then added to each well. This was thoroughly mixed for 10 seconds. It was then allowed to incubate at room temperature for 60 minutes without covering the plate.

The contents of the wells were briskly shaken out, and the wells rinsed three times with dilute Wash Solution. The wells were sharply struck against absorbent paper to remove residual droplets.

Two hundred microliters (200 µL) of substrate solution was then added to each well and incubated at room temperature for 15 minutes. At the end of 15 minutes, 100 µL of Stop Solution was added. The absorbance was read at 450 nm with a microtiter plate reader.

2.5 Calculation of Results

The subjects' and control's quantitative values were derived automatically using a 4 Parameter Logistics curve fit of the micro plate reader.

2.6 Statistical Analysis

One way analysis of variance (ANOVA) was used to determine if there were age related differences in the profiles of testosterone levels in this age range.

Student t-test was used to compare hormone values of male and female subjects. Pearson Correlation was used to evaluate the association between each and every one of the parameters measured.

3. RESULTS AND DISCUSSION

Results obtained are tabulated in Tables 1—10. The study measured and compared the levels of testosterone in children aged 6 to 10 years. Male subjects in the study were 78 (44.1%) with a mean age of 8.32±1.40 years, mean height of 1.30±0.08 m, mean weight of 26.14±4.90 Kg, mean BMI of 15.25±1.73 kg/m^2, mean testosterone of 0.49±0.38 ng/ml. Table 1 pointed out that although the ages of the female and male subjects are the same (NS), the females have significantly higher height and weight. This suggests that females are steps ahead in their growth spurt. Regarding testosterone, Soldin [6] and Meites [7] already suggested that at this age bracket, it has little or no difference in value.

Female subjects were 99 (55.9%) with a mean age of 8.56±1.39 years, mean height of 1.34±0.10 m, mean weight of 27.84±5.45 Kg, mean BMI of 15.43±1.71 and mean testosterone of 0.57±0.59 ng/ml.

Tables 2 and 3 disclosed that testosterone is higher in 10 years old subjects than in 8 years old but with no differences in age group 6—10 years. In terms of size, 8 years old subjects are significantly taller and heavier than 6 & 7 years; so are 9 years old compared with 6-8 years old and so are 10 years old compared with 6-9 years old (Table 3).

A comparison of the means between the male and the female using student t-test showed that the females have statistically significant higher height ($p \leq 0.01$) and weight ($p=0.05$). Females were significantly taller, meaning that at age 10 years, the early growth spurt has set in in females ahead of their male counterpart (Table 8). Age, BMI and testosterone values showed no statistically significant difference in means between the male and female subjects.

Findings recorded in Tables 4-7 indicated that testosterone levels from age 6 to 10 showed no significant difference. Hence, same reference ranges can be used to interpret testosterone results for this age group. There was also no significant difference in parameters, they maintained the same rate of development in all parameters at those age stages. Tables 9 and 10 present the ranges of parameters in male and female subjects. The range in both male and female are almost the same, meaning that, the same reference range can be used in interpreting the testosterone values of male and female children at this age group.

Table 1. Comparison of some physical parameters between male and female subjects using t-test

Parameters	Male (76)	Female(96)	Cal-t	Crit-t	P-Val	Sig
Age (yrs)	8.34±1.381	8.55±1.368	0.994	1.96	0.322	NS
Height (m)	1.305±0.082	1.338±0.096	2.490	1.96	0.015	S
Weight (kg)	26.20±4.833	27.78±5.357	2.034	1.96	0.044	S
BMI (kg/m^2)	15.30±1.71	15.38±1.68	0.314	1.96	0.754	NS
Testosterone (ng/ml)	0.49±0.38	0.57±0.59	0.862	1.96	0.376	NS

Table 2. Comparison of some physical parameters and sex hormones of female subjects between different age groups

Parameter	6 years	7 years	8 years	9 years	10 years	Calc-F	Crit-F	p-value
Height (m)	1.22±0.05	1.23±.04	1.30±0.06 ^{xy}	1.35±.04 $^{xy\#}$	1.43±0.07 $^{xy\#*}$	37.440	3.027	0.000
Weight (kg)	22.0±2.80	22.9±5.52	26.4±3.91 ^{xy}	27.8±4.56 ^{xy}	32.2±4.56 $^{xy\#*}$	19.48	3.027	0.000
BMI (kg/m^2)	14.8±1.71	14.9±2.69	15.4±1.56	15.2±1.76	15.8±1.42	0.971	3.027	0.427
Testosterone (ng/ml)	0.55±0.43	0.50±0.37	0.37±0.34	0.48±0.27	0.64±0.37 $^{\#}$	2.041	3.027	0.095
n	13	7	21	24	31			

*X = higher than that of six years, Y= higher than that of seven years, #= higher than that of eight years, *=higher than that of nine years*

Table 3. Comparison of some physical parameters and sex hormones of male subjects between different age groups

Parameter	6 years	7 years	8 years	9 years	10 years	Calc-F	Crit-F	p-value
Height (m)	1.19±0.028	1.23±0.06	1.30±0.059 ^{xy}	1.33±0.056 $^{xy\#}$	1.37±0.06 $^{xy\#*}$	25.71	3.036	0.000
Weight (kg)	20.9±2.17	23.2±4.17	25.5±2.81 ^{x}	27.0±3.98 ^{xy*}	30.7±4.55 $^{xy\#*}$	14.84	3.036	0.000
BMI (kg/m^2)	14.6±1.06	15.3±1.53	15.0±1.23	15.1±2.02	16.3±1.85 $^{x\#*}$	2.355	3.036	0.062
Testosterone (ng/ml)	0.38±0.37	0.40±0.35	0.47±0.39	0.47±0.36	0.56±0. 34	0.545	3.036	0.703
n	13	6	17	22	18			

*MEAN ± SD, X = higher than that of six years, Y= higher than that of seven years, #= higher than that of eight years, *=higher than that of nine years*

Table 4. Comparison of some physical parameters between male and female subjects aged 6 years old

Parameters	Male(13)	Female(13)	Cal-t	Crit-t	P- Val	Sig
Height (m)	1.19±0.03	1.22±0.047	1.56	1.96	0.131	NS
Weight (kg)	20.92±2.18	22.00±2.80	1.10	1.96	0.284	NS
BMI (kg/m^2)	14.63±1.06	14.84±1.71	0.37	1.96	0.713	NS
Testosterone (ng/ml)	0.38±0.37	0.55±0.43	1.07	1.96	0.294	NS

Table 5. Comparison of some parameters between male and female subjects aged 7 years

Parameters	Male (6)	Female (7)	Cal-t	Crit-t	P- Val	Sig
Height (m)	1.23±0.06	1.23±0.040	0.22	1.96	0.829	NS
Weight (kg)	23.17±4.17	22.86±5.52	0.11	1.96	0.913	NS
BMI (kg/m^2)	15.30±1.53	14.91±2.69	0.31	1.96	0.763	NS
Testosterone (ng/ml)	0.40±0.35	0.50±0.37	0.50	1.96	0.623	NS

Table 6. Comparison of some parameters between males and females aged 8 years

Parameters	Male (17)	Female (21)	Cal-t	Crit-t	P- Val	Sig
Height (m)	1.30±0.06	1.30±0.06	0.02	1.96	0.987	NS
Weight (kg)	25.53±2.81	26.38±3.91	0.75	1.96	0.456	NS
BMI (kg/m^2)	15.05±1.23	15.44±1.56	0.86	1.96	0.398	NS
Testosterone (ng/ml)	0.47±0.39	0.37±0.34	0.87	1.96	0.392	NS

Table 7. Comparison of some parameters between male and female aged 9 years

Parameter	Male(22)	Female(24)	Cal-t	Crit-t	P –Val	Sig
Height (m)	1.34±0.06	1.35±0.04	1.017	1.96	0.324	NS
Weight (kg)	27.00±3.98	27.83±3.38	0.768	1.96	0.447	NS
BMI (kg/m^2)	15.07±2.02	15.22±1.75	0.281	1.96	0.780	NS
Testosterone (ng/ml)	0.47±0.36	0.48±0.27	0.161	1.96	0.874	NS

Table 8. Comparison of some physical parameters between male and female subjects aged 10 years

Parameter	Male(18)	Female(31)	Cal-t	Crit-t	P – Val	Sig
Height (m)	1.37±0.06	1.43±0.08	2.64	1.96	0.011	S
Weight (kg)	30.67±4.55	32.23±4.56	1.16	1.96	0.254	NS
BMI (kg/m^2)	16.29±1.85	15.78±1.42	1.07	1.96	0.288	NS
Testosterone (ng/ml)	0.56±0.34	0.64±0.37	0.76	1.96	0.453	NS

Table 9. Range of some physical parameters of male subjects

Parameters	n	Mean±SD	Range
Age (yrs)	76	8.34±1.38	6.96–9.72
Height (m)		1.31±0.08	1.23– 1.39
Weight (kg)		26.20±84.83	21.37–31.03
BMI (kg/m^2)		15.30±1.71	13.59–17.01
Testosterone (ng/ml)		0.47±0.36	0,11–0.83

Table 10. Range of some parameters of female subjects

Parameters	n	Mean±SD	Range
Age (yrs)	96	8.55±1.37	7.18–9.92
Height (m)		1.34±0.10	1.24–1.44
Weight (kg)		27.78±5.36	22.42–33.14
BMI (kg/m^2)		15.38±1.68	13.70–17.06
Testosterone (ng/ml)		0.52±0.36	0.16–0.88

Significant finding in the study is that testosterone did not show any statistically significant difference in means for the population under consideration. This answers the research question which seeks to know whether there is a significant difference in the levels of testosterone between the male and the female subjects.

In male children, there is no statistically significant difference in the assayed testosterone hormone when compared among the age groups. This suggests the male and female subjects are in their normal stages of development.

Children require more than just diet to flourish. Critical to normal development, proper hormonal level in children controls many bodily functions including growth, sleep and secondary characteristics associated with puberty.

Testosterone is important for normal sperm development, it activates genes in serotoli cells, which promote differentiation of spermatogonia and regulate acute HPA (Hypothalamic-pituitary-adrenal axis) response under dominance challenge [8]. Testosterone regulates physical energy and cognitive function and maintains

muscle trophism. Testosterone regulates the population of thromboxane A2 receptors on megakaryocytes and platelets and hence aggregation of platelets in humans [9,10]. Elevated androgen levels are associated with menstrual cycle irregularities in healthy women and clinical population [11].

4. CONCLUSION

The study described above concludes as follows:

1. Age (the comparison of mean age of the female and male subjects under consideration in this study), BMI and testosterone values in healthy children aged 6—10 years showed no statistically significant difference in mean between male and female subjects.
2. A comparison of the means between male and female subjects using student t-test showed that the females have statistically significant higher height ($p<0.05$) and weight ($p<0.05$).
3. Significant finding in the study is that testosterone did not show any statistically significant difference in mean for the population under consideration.
4. The range obtained in this work can be a useful reference range in interpreting the testosterone results of children aged 6 -10 years.
5. This work also established that there is no need to apply age-specific or sex-specific reference ranges in interpreting the testosterone of children aged 6-10 years.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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