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Selection of Hormonal Reference Values for Undescended Testicle Case in 8-Year-Old Boy with Abnormal Chromosome

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ABSTRACT

Establishing the diagnosis of undescended testicles requires appropriate hormonal laboratory reference values based on age and gender. An 8-year-old boy with an undescended testicle, mental retardation, and stunting had a blood test that was carried out at the Clinical Pathology Laboratory, dr. Cipto Mangunkusumo (RSCM) Hospital on February 6, 2020, with testosterone levels of 0.69 nmol/L (N male: 4.94-32.01 nmol/L) indicating decreased testosterone levels. The patient was consulted from urological surgery to pediatric endocrinology to determine the presence or rudiment of the patient's testicles. Using the reference range of testosterone values assists clinicians in determining the diagnosis, monitoring therapy, and prognosis of a disease. There are some testosterone reference values, which are currently available, including Canadian Laboratory Initiative on Pediatric Reference Intervals Database (CALIPER) and the Tanner stage reference value. Later is more applicable because it is based on chronological age and secondary sexual development in assessing puberty development. A case of an 8-year-old boy with a clinical diagnosis of an undescended testicle, the laboratory test results showed normal-low testosterone levels using the CALIPER and Tanner stage ranges according to the patient's age. No increase of testosterone levels after the second HCG stimulation test might be due to differences in the HCG administration protocol; therefore, the diagnosis of anorchia had not been established, and chromosome abnormalities of 46 XY, +6 Mar, 17 dmin on chromosome analysis suggested the suspected syndrome. These findings were consistent with the suspicion of primary hypogonadism in children with suspected syndrome caused by bilateral cryptorchidism with a suspected seminiferous tubular defect.

Keywords: Hypogonadism, undescended testicle, anorchia, testosterone

INTRODUCTION

The primary reproductive organs or gonads for males consist of a pair of testes. The function of the testes in males is for spermatogenesis (spermatozoa formation) at puberty and to secrete the testosterone. Testosterone is mainly synthesized by the Leydig cells of the testes through Luteinizing Hormone (LH) stimulation as much as 95% and the rest is through the conversion of the dehydroepiandrosterone (DHEA) as a precursor steroid, and androstenedione, which is synthesized in the adrenal glands (Figure 1).[1]

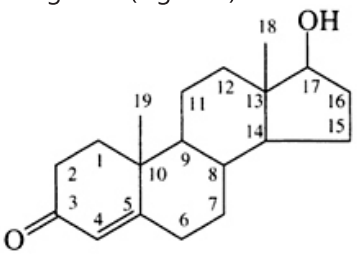

Figure 1. The biochemical structure of testosterone[1]

Testosterone will provide negative feedback that inhibits LH secretion in two ways, via reduced production of gonadotropin-releasing hormone (GnRH) in the hypothalamus, which indirectly reduces the release of Follicle Stimulating Hormone (FSH) and LH from the anterior pituitary and via direct action on the anterior pituitary to decrease LH secretion. The last process explains that testosterone exerts a greater inhibitory effect on LH secretion than that on FSH as shown in Figure 2.

The effect of testosterone before birth is for the maturation of the reproductive tract and external genitalia supports the testicular descent into the scrotum, growth, and maturation of the reproductive system at puberty play an important role in spermatogenesis and maintains the reproductive tract into adulthood.[2]

Testosterone circulates in 3 main forms as follows: 40% of testosterone, which is strongly bound to Sex Hormone Binding Globulin (SHBG); 60% of testosterone, which is weakly bound to albumin; and 2% of free of unbound testosterone

(Figure 3). Only free and weakly bound testosterone is bioavailable or can bind to androgen receptors.[3]

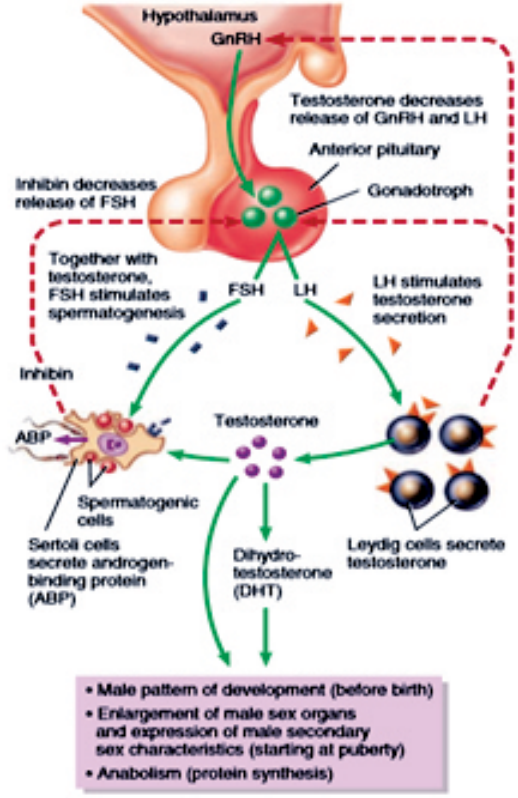

Figure 2. Hypothalamic-pituitary-testicular axis[1]

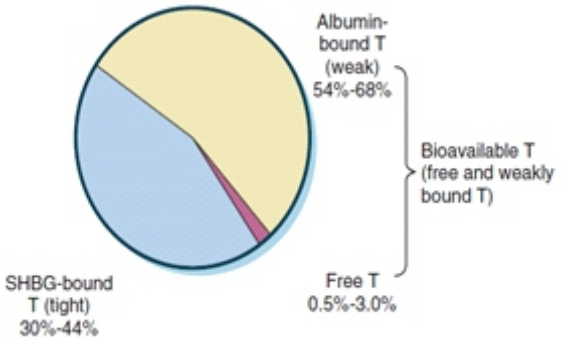

Figure 3. Fraction of circulating testosterone[4]

Hypogonadism is a condition of reduced testosterone levels in male patients, which may originate from the central (hypothalamus or pituitary) or testicular, or a combination of both. Hypogonadism in male patients with testicular failure due to genetic disorders (e.g., Klinefelter syndrome), orchitis, trauma, radiation, chemotherapy, or undescended testes is known as hypergonadotropic hypogonadism or primary hypogonadism. Hypogonadism in male patients with gonadotropin deficiency or dysfunction due to disease or damage to the hypothalamic-pituitary axis is known as hypogonadotropic hypogonadism, central hypogonadism, or secondary hypogonadism. This may be due to Kallmann syndrome, tumor, trauma, radiation, sarcoidosis, or tuberculosis that attacks the hypothalamus or pituitary.[5]

The diagnosis of hypogonadism in men should include identification of the lowest serum testosterone level, making it necessary to provide a normal testosterone reference value for a healthy population of all ages that will assist in the clinical assessment of hypogonadism in males.[6]

Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)

Gonadotropic hormones consist of 2 glycoproteins known as LH and FSH. Both have the same subunit as Thyroid Stimulating Hormone (TSH) and Human Chorionic Gonadotropin (HCG) but with different β subunits.

Follicle-stimulating hormone receptors in testicular Sertoli cells mediate FSH stimulation in the development of spermatogenesis in the testes while LH stimulates Leydig cells in the testes to produce testosterone. Luteinizing hormone secretion is inhibited by androgens and estrogens and FSH secretion is suppressed by the production of inhibin, a 31 kDa glycoprotein produced by Sertoli cells. Inhibin consists of α and β subunits joined by disulfide bonds.[7]

Laboratory tests

The reference value for the Tanner stage is better because it uses the basis for the development of secondary sexual characteristics other than chronological age in assessing pubertal development. The age of puberty in males ranges from 9 to 14 years, with the first visible secondary sexual characteristic being gonadarche i.e. when the testicular volume reaches more than or equal to 4 mL (or the long axis is greater than or equal to 2.5 cm) until it enters the tanner stage 2. During Tanner stage 3 development, males undergo peak growth rates. Spermarche, the female equivalent of menarche, is the development of sperm in males and usually occurs during Tanner stage 4. The range of normal testicular size according to some references is as follows (Table 1).

Cryptorchidism is the failure of one or both testes to descend normally from the abdomen through the inguinal canal into the scrotum. It is the most common congenital disorder of the genital tract of children. This case is more common in premature

Table. Testicular size based on Tanner stage[8]

Tanner Stage (genital)	Length (cm) (mean±SD)	Volume (mL)
I	2.0 ± 0.5	2
II	2.7 ± 0.7	5
III	3.4 ± 0.8	10
IV	4.1 ± 1.0	20
V	5.0 ± 0.5	29

babies, low birth weight, and babies small for gestational age. Spontaneous descent of the testes occurs during the first year in most infants (induced by the neonatal gonadotropin and testosterone surge); therefore, the prevalence of cryptorchidism in boys and adults is lower, by 0.3% to 1.0%. Both unilateral and bilateral cryptorchidism is associated with impaired sperm production and infertility and an increased risk of testicular cancer. Unilateral or bilateral cryptorchidism of unknown cause usually results in primary hypogonadism, leading to impaired sperm production (low sperm count), normal testosterone concentrations, selectively elevated FSH levels, and, occasionally, high LH.[5]

CASE

On January 19, 2020, an 8-year-old patient with a clinical description of undescended testicle, mental retardation and stunting went to the Urology Outpatient Clinic with complaints of missing both testicles. Both testes were still palpable when the patient was born. The patient was consulted to the Pediatric Endocrine Outpatient Clinic to determine the presence or rudiment of the patient's testicles (Figure 4).

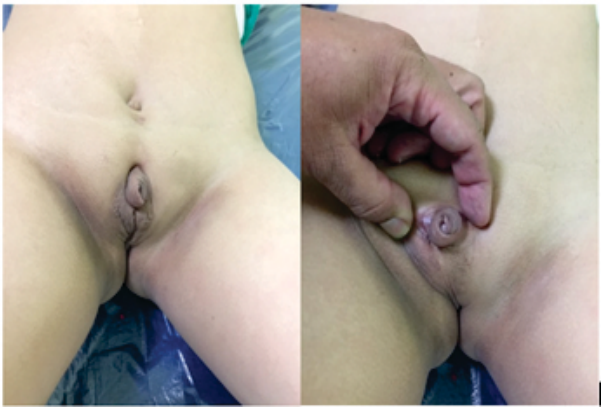

Figure 4. Genital of the patient (January 2020)

Testicular ultrasound test results on February 6, 2020, showed an oval testicular-like lesion, heterogeneous hypoechoic lesion in the bilateral inguinal as shown in Figure 5.

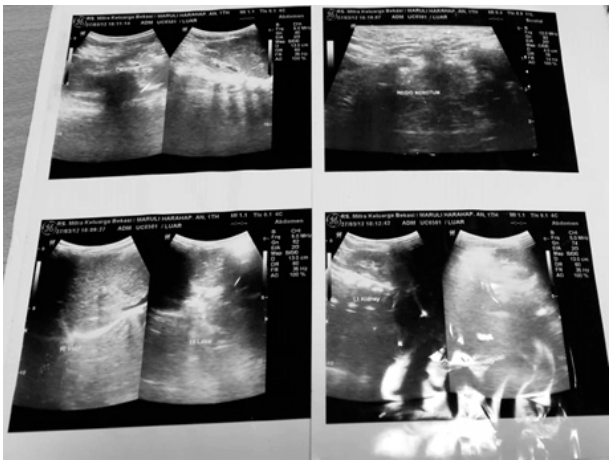

Figure 5. USG of patient's testicular on February 6, 2020

The results of the HCG stimulation test showed an increase in testosterone levels from 0.49 nmol/L (reference value for males: 4.94-32.01 nmol/L) to 0.69 nmol/L.

Medical History

The patient was born on March 27, 2011, with a gestational age of 30 weeks (premature gestational age), birth weight of 1200 g, birth length of 46 cm, and spontaneous birth. Bodyweight when returning from Hospital A was 1400 g. Unclear genital history of male or female, unpalpable testes, and small penis from birth until the patient was one year old. Abdominal ultrasound results at hospital showed no testes in the abdomen or inguinal.

Physical examination (August 1, 2018, chronological age of 7 years and 4 months)

General condition: alert; Height: 113 cm (height age for age 3 years); Weight: 25.2 kg; Body mass index: 19.8 kg/m2 (normal weight); Nutritional status: good nutritional status with short stature; Head and neck: head circumference 39 cm (microcephaly). Additional laboratory tests: Chromosome analysis in Laboratory of Biology, Faculty of Medicine, UI (June 28, 2012, with the chronological age of 1 year 3 months) 46 XY, +6 Mar, 17 dmin; Diagnosis: suspected syndrome.

Bone age (February 28, 2012, with the chronological age of 11 months)

Skeletal age of 6 months with an impression of retardation

IQ Test Results (January 5, 2020)

IQ score of 71 – mental retardation

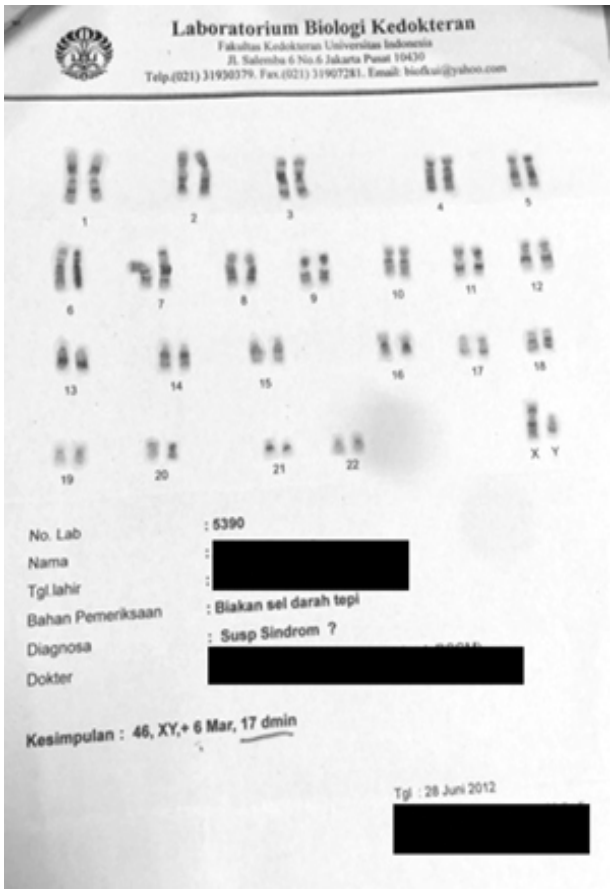

Figure 6. Report of chromosome analysis on June 28, 2012

Laboratory tests

Table 2. Laboratory test results (May 8, 2012)

Parameter	Results	Unit	Reference value
FT4	1.26	ng/dL	0.85- 1.75
TSHs	2.63	IU/mL	0.54 – 4.53

Table 3. Laboratory test results on July 18, 2012 (laboratory P) before HCG stimulation test and on July 21, 2012, after HCG stimulation test of 1500 IU for 3 days

	July 18, 2012	July 21, 2012	Unit	Reference Value
Reproductive hormone				Males (1 year old)
LH	3.61		mIU/mL	< 4.1
FSH	**20.53**		mIU/mL	0.58 – 2.4
Testosterone	4.58	**152.7**	ng/dL	3 -32 ng/dL
	0.2	**5.3**	nmol/L	

Table 4. Laboratory test results on February 5, 2020 (RSCM) before HCG stimulation test and on February 6, 2020 post-administration of HCG 5000 IU/m^2

Parameter	Feb 5, 2020	Feb 6, 2020	Unit	Reference Value
Reproductive hormone				Males
LH	**1.6**		mIU/mL	1.7 – 8.6
FSH	7.2		mIU/mL	1.5 – 12.4
Testosterone	**0.49**	**0.69**	nmol/L	4.94 – 32.01

The Reference value of testosterone

There are several testosterone references values available that can be used to determine normal testosterone ranges, such as the Canadian Laboratory Initiative on Pediatric Reference Intervals Database (CALIPER) study and Tanner stage.

The CALIPER study used 1459 samples from Outpatient Clinic pediatric patients (Dental Outpatient Clinic, orthopedics, and plastic surgery) The Hospital of Sick Children, Toronto, Ontario, Canada, which were considered metabolically stable and the analyte measurement in this study used the ARCHITECT ci8200 analyzer.[9]

Tanner staging based on the physical examination of adolescents is considered the gold standard for measuring the puberty stage. There are five stages separated by definition for boys and girls, determined by pubic hair growth and testicular development for boys.[8]

Table 5. Reference range of testosterone level according to CALIPER study[10]

Testosterone (nmol/L)	Median	Male Percentile 2.5 to 97.5
Newborn-12 months	1.24	0.36 -15.08
1-5 years old	0.65	0.28-1.48
6-10 years old	0.94	0.47-1.96
11-14 years old	2.2	0.65-19.29
15-20 years old	15.45	4.70-41.68

Table 6. Reference range of testosterone level according to Tanner stage[11]

Male	Age	Total Testosterone (ng/dL)
Tanner stage 1	7 months-9 years old	<30
Tanner stage 2	10-13 years old	<300
Tanner stage 3	14-15 years old	170-540
Tanner stage 4,5	16-19 years old	250-910
	20 years old and above	280-1080

DISCUSSIONS

The use of an appropriate range of testosterone reference values helps clinicians in determining the diagnosis, therapy, and prognosis of a disease. Reference values of testosterone, which is currently available include the CALIPER and are based on the Tanner stage. The reference value of the Tanner stage is more applicable because it is based on chronological age and secondary sexual development in assessing pubertal development.

Chromosomal abnormalities of 46 XY, +6 Mar, 17 dmin were most likely related to the patient's condition, mental retardation, stunting, and undescended testicular. Unfortunately, the patient did not undergo further chromosomal analysis to determine the type of syndrome. The patient did not follow-up for further control to the Pediatric Endocrinology Outpatient Clinic.

The patient has been tested for HCG stimulation twice, in July 2012 and February 2020. The HCG stimulation test (July 2012) as shown in Table 3 showed an increase in testosterone levels more than twice or the peak value of testosterone after stimulation was 5.3 nmol/L, that the suspicion of anorexia can be ruled out. However, eight years later the results of the second HCG stimulation test (February 2020) (Table 4) showed the opposite result. This might be due to differences in the way HCG was administered. The HCG stimulation test protocol in the Davenport study alone provided three intramuscular injections of hCG (Pregnyl) on 3 consecutive days. The results of this study showed that the hCG stimulation test could correctly predict all cases of anorchia (100% sensitivity) using either a plasma testosterone doubling threshold or a stimulated peak value of 5 nmoI/L.[12]

The results of the patient's laboratory test on July 18, 2012 (Table 3) showed an increase in FSH hormone levels, while LH and testosterone levels were still within the normal range. This might be because the patient's bilateral cryptorchidism is primary hypogonadism with seminiferous tubular defects, which results in impaired sperm production or function resulting in increased FSH levels but normal LH levels as shown in the schematic Figure 6.[5,12]

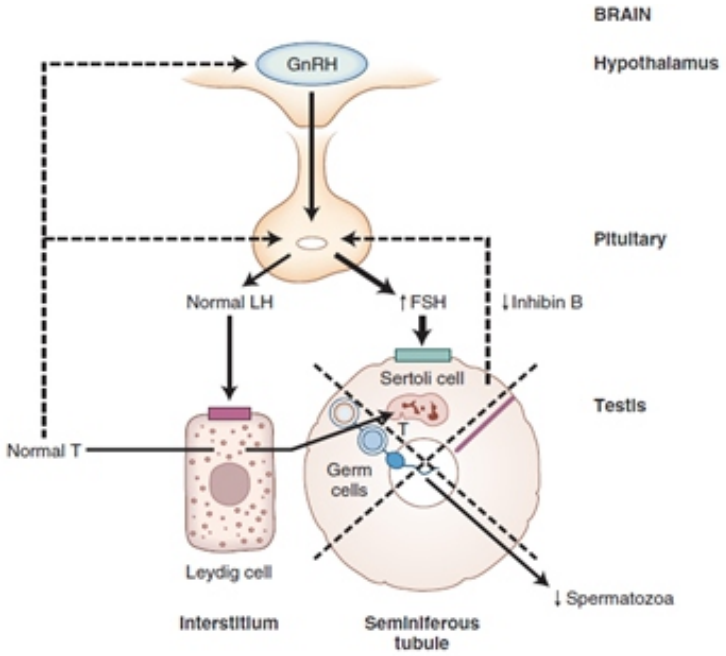

Figure 7. Mechanism of primary hypogonadism via selective increase of FSH[4]

Measurement of testosterone levels using the Architect i2000SR ABBOTT analyzer uses the Chemiluminescent Microparticle Immunoassay (CMIA) method for the quantitative determination of testosterone levels in serum or plasma. The one-step delayed immunoassay method was used for the measurement of testosterone levels. The testosterone hormone in the sample will bind to anti-testosterone, which is coated on the microparticles. After the incubation period, an acridinium-labeled testosterone conjugate was added and then washed to remove the unbound component, a pre-trigger solution and a trigger were added and those would produce chemiluminescence. The chemiluminescence, which appears will be measured and reported as Relative Light Units (RLUs). The testosterone level in the sample will be inversely proportional to the detected RLU.[13]

Measurement of low testosterone levels in these patients using the normal range values in the RSCM laboratory indicates that the determination of testosterone deficiency should consider clinical signs and symptoms together with laboratory values. The rapid biochemical and physiological changes that occur during childhood and adolescence require a specialized set of Reference Intervals (RIs) to accurately interpret androgen status in growing children. However, the determination of an appropriate pediatric RI can be hampered by several factors such as the limited sample size, especially in newborns <1 year, and the lack of use of retrospective laboratory data or hospitalization.[11] Adjustment of laboratory reference values of hormonal parameters based on age and gender plays an important role in terms of diagnosis, monitoring of therapy, and prognosis of a disease course.

CONCLUSIONS

A pediatric patient with an initial MH and age of 8-years-old was reported with a clinical description of undescended testicular. Laboratory test results showed normal-low testosterone levels using the normal range of Caliper and Tanner stages according to the patient's age. No increase in testosterone levels after the second HCG stimulation test might be due to differences in HCG administration protocols that a diagnosis of anorchia has not been established, and abnormalities were found. Chromosome 46 XY, +6 Mar, 17 dmin on chromosome analysis led to a suspected syndrome. This finding was consistent with the suspicion of primary hypogonadism in a child with suspected syndrome caused by bilateral cryptorchidism with suspected isolated seminiferous tubule defects.

REFERENCES

1. Mari L. DeMarco ML, Gronowski AM. Reproduction -related disorders. In: Carl A. Burtis, David E. Bruns, editor. Tietz fundamentals of clinical chemistry. 7th Ed., St.Louis, Missouri, Elsevier, 2015; 824-47.
2. Sherwood L. The reproductive system. In: Sherwood L, editor. Human physiology from cells to system. 7th Ed., Belmont, Brooks/Cole, 2010; 741-98.
3. Pagana KD, Pagana TJ. Testosterone. In: Mosby's manual of diagnostic and laboratory tests. 6th Ed., Philadelphia, Elsevier, 2018; 425-7.
4. Matsumoto AM, Bremner WJ. Testicular disorders. In: Shlomo Melmed KSP, Kronenberg HM, editors. Williams textbook of endocrinology. 13th Ed., Philadelphia, Elsevier Inc, 2016; 688-777.
5. Petering RC, Brooks NA. Testosterone therapy: Review of clinical applications. Am Fam Physician, 2017; 96(7): 441-9.
6. Mulhall JP, Trost LW, Brannigan RE, Kurtz EG, Redmon JB, *et al.* Evaluation and management of testosterone deficiency: AUA guideline. J Urol, 2018; 200(2): 423-32.
7. Felner EI, Parks JS. Hormones of the hypothalamus and pituitary. In: Kliegman RM, editor. Nelson textbook of pediatrics. 20th Ed., Philadelphia, Elsevier, 2016; 2635-7.
8. Jack J, Young SB. Endocrinology. In: Helen K. Hughes, MD M, Lauren K. Kahl M, editors. A manual for pediatric house officers the Harriet Lane handbook. 21st Ed., Philadelphia, Elsevier, 2018; 708-20.
9. Adeli K, Higgins V, Trajcevski K, White-Al Habeeb N. The Canadian laboratory initiative on pediatric reference intervals: A CALIPER white paper. Crit Rev Clin Lab Sci [Internet]. 2017; 54(6): 358-413.
10. Raizman JE, Quinn F, Armbruster DA, Adeli K. Pediatric reference intervals for calculated free testosterone, bioavailable testosterone and free androgen index in the CALIPER cohort. Clin Chem Lab Med, 2015; 53(10): e239-43.
11. Emmanuel M, Bokor BR. Tanner Stages. SAGE Encycl Lifesp Hum Dev [Internet]. 2018;6-9. Available from: www.closer.ac.uk (accessed August 7, 2020).
12. Kurniawan LB. Hypotetoteron in male with obesity. Indones J Clin Pathol Med Lab, 2021; 27(2): 217.
13. Food and Drug Administration report. 510(k) substantial equivalence determination decision summary assay only template. Chicago, 2018; 510.