CLINICAL-GENETIC DIAGNOSIS IN *TURNER SYNDROME*

Mariana Sprincean[1,2] – dr. în şt. psihol., conf. univ., Natalia Barbova[1,2] – dr. în şt. med., conf. univ., Elena Halabudenco[1] – dr., Ludmila Eţco[1,2] – dr. hab. în şt. med., prof. univ., Ana Mişina[1] – citogenetician, Tatiana Samoilenco[1] – citogenetician, Elena Paladi[1] – neuropediatru, Oxana Ghiluţă[2], Vladimir Egorov[1] – dr. în şt. med.,
[1]IMSP Institutul Mamei şi Copilului,
[2]IP USMF „Nicolae Testemiţanu”

Rezumat

Studiul desfăşurat a presupus analiza particularităţilor polimorfismului clinic şi citogenetic în sindromul Turner, care reprezintă o anomalie cromozomială, o aneuploidie caracterizată prin pierderea totală sau parţială a cromozomului sexual cu sau fără mozaicism. În lotul de studiu au fost incluşi 105 de copii cu sindrom Turner în vârstă de până la 18 ani diagnosticaţi clinic şi confirmaţi citogenetic prin cariotipare standard. În sindromul Turner cariotipul este foarte variabil înregistrând în circa 51,4% forma omogenă.

Cuvinte-cheie: sindromul Turner, consult medico-genetic, diagnostic citogenetic, cariotip

Summary. Clinical-genetic diagnosis in Turner syndrome

In the study are analyzed peculiarities of clinical manifestations and cytogenetic features in Turner syndrome, which is a sex chromosomal abnormality, characterized by loss of gonosome (total or partial) with or without mosaics. A group of 105 children with Turner syndrome was investigated during medical genetic counseling in the Center for Reproductive Health and Medical Genetics. Karyotype in Turner syndrome is highly variable and different, in 51,4% of cases being the homogeneous form or X monosomy - 45, X.

Key words: Turner syndrome, medical genetic counseling, diagnosis, karyotype

Резюме. Клинико-генетическая диагностика синдрома Тернера

В статье рассматриваются клинический полиморфизм и цитогенетические особенности при синдроме Тернера. Обследовано 105 детей с синдромом Тёрнера. Цитогенетическая диагностика и медико-генетическое консультирование проведены в Центре Репродуктивного Здоровья и Медицинской Генетики. Преобладающий кариотип при синдроме Тёрнера – гомогенная форма, моносомия X – 45,X – встречается в 51,4% случаев.

Ключевые слова: синдром Тёрнера, медико-генетическое консультирование, цитогенетическая диагностика, кариотип

Introduction. Turner syndrome (synonyms: Ullrich-Turner, Shereshevski-Turner, Bonnevie-Ullrich, gonadal dysgenesis) is a chromosomal abnormality, a single monosomy aneuploidy and viable in infants; its prevalence is one case per 2000 to 2500 births [1]. This represents about 10% of the causes of miscarriage in the first trimester. Unlike Down syndrome, age of the mother is not correlated with an increased risk for Turner syndrome. Risk factors are not clearly established, although it is considered that young age (18 years) increased genetic risk for congenital chromosomal pathology.

X Monosomy was initially described by Ullrich in 1930, who reported clinical case of a girl of 8 years with short stature, lymphedema, short neck with skin excess on the neck, facial dysmorphism, and in retrospect it was reported that the first case described in the literature [3]. At same time, the American endocrinologist Dr. Henry Turner, described in 1938 the same pathology, and showed that over 95% of women with Turner syndrome (naming the disease after his name) have short stature, and over 90% have ovarian failure [2].

Turner syndrome is a gonosomal chromosomal abnormality characterized by loss sex chromosome with or without mosaicism which characterized phenotypically by low waist, short neck, pterygium colli, peripheral lymphedema, sexual infantilism, primary amenorrhea, gonadal dysplasia, renal abnormalities and cardiovascular and behavioral disorders with some socio-emotional and sometimes mental deficiency. Most girls with Turner syndrome have a favorable prognosis for life [5]. Diagnosis as early as possible allows rapid evaluation of the clinical picture and analysis of karyotype, contribute to improving the physical as well as psycho-emotional status during teenage period, prevent or remedy the failure of growth, and medical genetics counseling for the family can solve the problems for children with Turner syndrome in order to enable proper treatment of cardiovascular and kidney malformations [4]. Though the phenotypic manifestations and physical characteristics can suggest the early neonatal diagnosis period, about 60% of girls with Turner syndrome are not diagnosed early in childhood [6].

According to these arguments, the purpose of the present study is to assess the clinical features and cytogenetic polymorphism forms in girls with Turner syndrome.

In order to achieve the defined purpose, were the following objectives:

1. Earliest possible diagnosis of subjects with Turner syndrome in the period of early development, i. e. neonatal, prepubertal and pubertal;
2. Identifying the cytogenetic diversity of Turner syndrome and its correlations with phenotypic manifestations;
3. Determination of prevalence of different cytogenetic forms of Turner syndrome;
4. Develop genetic algorithm for evaluating patients with Turner syndrome in order to improve prevention methods and genetic diagnosis.

Material and method: The study consisted of a retrospective analysis and foresight of a sample of over 342 girls with short stature, hypogonadism, and primary amenorrhea, as well as newborn girls with craniofacial dysmorphism plus physical retardation, pterigium colli, and peripheral lymphedema, which passed the medico-genetic counseling in the Center for Reproductive Health and Medical Genetics (CRHMG) in 2007 – 2015.

During the study the group included 105 children with Turner syndrome aged up to 18 years clinically diagnosed and cytogenetic confirmed by standard karyotyping. For the earliest possible diagnosis of subjects with Turner syndrome and their evaluation

during the development stages the questionnaire was developed and used aimed to genetic diagnostic evaluation, that facilitated the investigations. This included data on the assessment of medical and family history provided mostly by mothers and/or other family members of these children. During the medical consultation on subjects suspected to Turner syndrome was collected necessary information from historical data that allowed compiling pedigree in each family. On the basis of historical data, laboratory data, and results of genetic tests (cytogenetic analysis and Barr test) the final diagnosis was made in the study group. Cytogenetics investigation in cultured peripheral blood lymphocytes with karyotype analysis revealed five different cytogenetic types of Turner syndrome: 1. homogeneous form or monosomy X (45,X); 2. mosaic form (45,X/46,XX; 45,X/46,XY); 3. structural abnormalities of sex chromosomes (46,XiX, 46,XdelX, 46,XdicX, 46,XrX); 4. mosaicism with structural abnormalities of the X chromosome (46,XX/46XiX/45,X); 5. other rearrangements (dysgenesis) – 46,XY.

All girls suspected to Turner syndrome have undergone a complex diagnostic procedure, including Barr test, ultrasound visualization of uterus with annexes, hormonal profile, karyotyping. We are revealed the following:

– The ultrasound examination of the uterus and annexes indicated what in all the girls in the studied group were developmental abnormalities including changes of size of uterus and changes in internal and external sexual organs (aplasia, hypoplastic uterus, rudimentary uterus etc.)

– Barr Test information in the first stage of examination and diagnosis was negative in most cases, with the exception of those with mosaics and sex chromosome structural abnormalities; We stated that cytogenetic method (karyotyping) was applied using metaphase chromosome preparations of peripheral blood lymphocytes in line with the standard and the study of chromosomes by G banding method.

Results and discussion: Girls with Turner syndrome have a specific clinical phenotype: small waist, craniofacial dysmorphism, triangular face, antimongoloid eyelid slits, epicanthic fold, ptosis, hypertelorism, dry eye, strabismus, amblyopia, low set ears, narrow jaws, micrognathia, arched palate, delaying of teeth's' erupting, additional teeth, palmate neck (pterigium coli), low-hair implanting on the nape, cutis laxa (loss of skin folds), shielded chest, cubitus valgus, short metacarpal IV, hypoplastic nails and convex, pigmented nevi, sometimes mental deficiency etc.

Clinical and genealogical data evaluation exam and phenotypic manifestations allowed identifying clinical features of polymorphism to the children with Turner syndrome in different periods of ontogenetic development: neonatal period, pre-pubertal and pubertal period. Clinical manifestations of the subjects with Turner syndrome (X monosomy) varies depending on the age. Thus, in the neonatal period phenotypic main features are: low birth weight, short neck, pterygium colli (excess skin on the neck), congenital lymphedema or peripheral edema, renal and cardiovascular abnormalities. In pre-pubertal phenotype is determined short stature, characteristic face, short neck, sometimes with excess skin on the neck, underdeveloped mammary glands with increased distance between nipples, broad chest, cubitus valgus, behavioral problems etc. During puberty short stature, undeveloped female secondary sexual characteristics, developmental delaying, primary amenorrhea, obesity, social vulnerability, psycho-behavioral disorders, rarely learning disabilities etc. becoming increasingly apparent.

Table 1

Particularities of clinical polymorphism in Turner syndrome

Characteristics	**Newborn**	**Prepubertal**	**Puberty**
Low birth weight	+		
Short stature		+	+
Gonadal failure	+	+	+
Cranio-facial dysmorphism	+	+	+
Short neck	+	+	+
Pterygium colli	+	+	+
Peripheral lymphedema (hands and feet)	+/-	+/-	+/-
Cubitus valgus	+	+	+
Improper food, weight deficiency	+		
Heart murmur	+/-	+/-	+/-
Absent or incomplete puberty		+	+
Failure of secondary sexual characteristics		+	+
Hearing loss, deafness		+/-	+/-
Learning difficulties, social vulnerability		+/-	+/-
Endocrine disorders, obesity		+	+
Mental deficiency		+/-	+/-

Of all phenotypic manifestations, short stature is the most characteristic, short stature is the feature that offered in 97% cases, insufficient gonadal development in 95% cases, cubitus valgus – in 48%, low hair insertion in 44%, short neck in 42%, arched palate in 37%, multiple nevi in 23%, lymphedema

in 24%, nail dysplasia in 13%, scoliosis in 12%, Madelung deformities in 6% cases. Although the particularities of clinical picture may suggest the diagnosis in early neonatal period, about 60% of girls with Turner syndrome are not diagnosed during childhood early due to lack of complaints from family and addressing geneticist lately. Definitive clinical diagnosis was established by geneticist based on the results of laboratory investigations (cytogenetic research).

The results of investigation of 105 children with Turner syndrome examined the medical-genetic counseling in CSRGM are shown in Table 2. Postnatal diagnosis of children with Turner syndrome was performed in the cytogenetic laboratory of CSRGM.

Table 2 showed that in 54 of 105 children diagnosed with Turner syndrome were there monosomy X (45,X). The frequency of Turner syndrome cytogenetic types were as follows: 1. homogeneous monosomy X (45,X) in 51,4% of cases; 2. mosaicism (45,X/46,XX; 45,X/46,XY) in 11,4% of cases ; 3. sex chromosome structural abnormalities (46,XiX, 46,XdelX, 46,XdicX, 46, XrX) in 15,2% of cases; 4. mosaicism with structural abnormalities of the X chromosome (46,XX/46XiX/45,X) in 5,7% cases, and other cytogenetic forms (46,XY) in 16,3% of cases. Thus, the most frequent cytogenetic (in more than half of cases) returns to the X monosomy, resulting in a single cell line with the karyotype 45,XO. In total, 18 girls were diagnosed with Turner syndrome mosaic form constituting 17,1%. Mismatch genetic sex was found in 16,2% of subjects.

When comparing the clinical manifestations of monosomy X (45,X) (n = 54) with other cytogenetic types of Turner syndrome were commonly diagnosed 45,X/46,XX (n = 8), 46,XiX (n = 7), 45,X/46,X,Y, (n = 3), 46,XdelX (n = 3), 45,X/46,XX/46,XiX (n = 2), 46,XrX (n = 2), there were no statistically significant differences, except for intellectual development. In X monosomy, IQ was usually normal, and in Turner syndrome with structural abnormalities of X chromosome, X ring chromosome there was a mild to moderate mental deficiency. Average height in 16 children with Turner syndrome was 135 cm without growth hormone deficiency. In 17 patients with Turner syndrome have found a pure gonadal dysgenesis, the karyotype is normal as in male individuals – 46,XY.

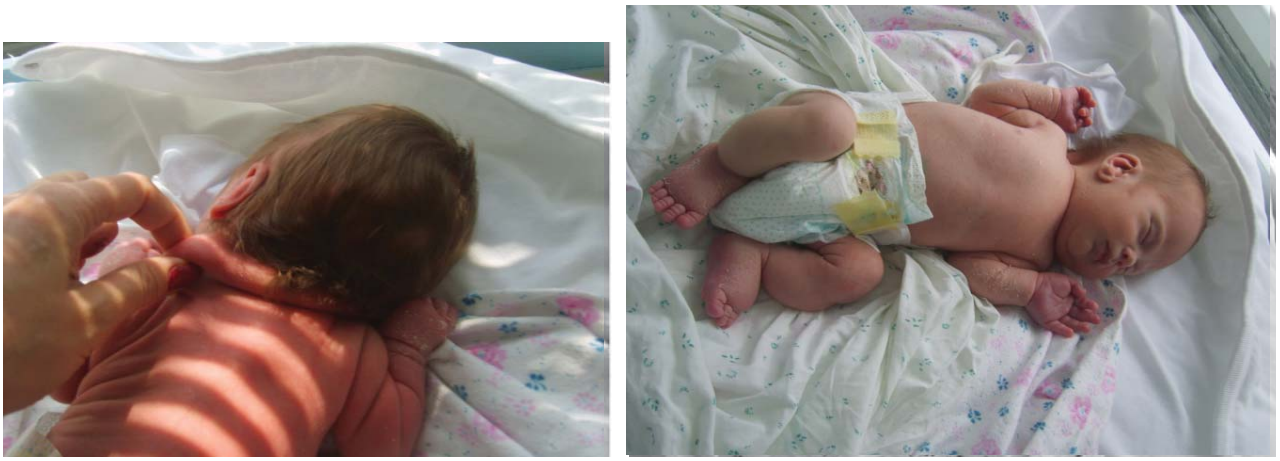
Particularities of clinical polymorphism in the neonatal period. We present below the clinical case of a newborn infant with classic signs of Turner syndrome.

Case report: A. baby, newborn, female, presents with low birth weight, craniofacial dysmorphism, antimongoloid eyelid slots, hypertelorism, low-setted ears, micrognathia, bilateral lymphedema in dorsal feet and hands, short neck, pterygium colli (excess skin on the neck), low implantation of hair on neck, wide chest, V hands bilateral

Table 2

Distribution Turner syndrome cases diagnosed in the years 2007 - 2015

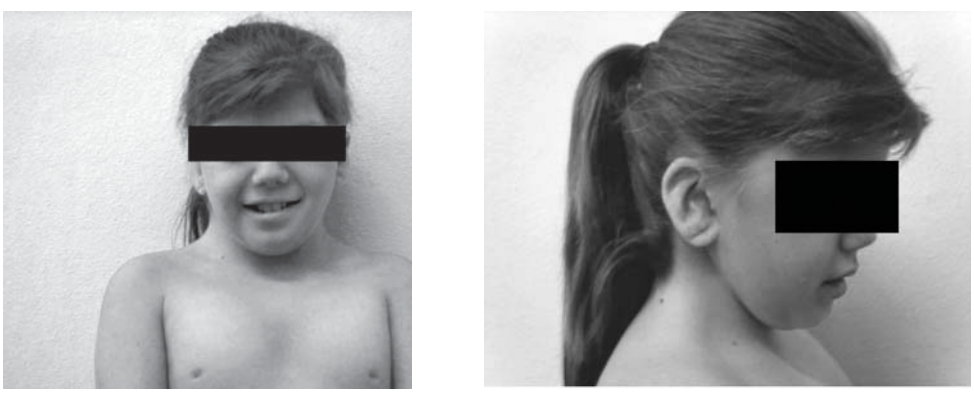
Turner syndrome	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total,%
	abs., (%)	abs., (%)	abs., (%)	abs., (%)	abs., (%)	abs., (%)	abs., (%)	abs., (%)	abs., (%)	
Monosomy X (45,X)	7(6,6)	7(6,6)	10 (9,5)	3(2,9)	9(8,6)	7(6,6)	4(3,8)	4(3,8)	3(2,9)	54 (51,4)
45X/46XdelX						1(0,9)				1 (0,9)
46,XX/45,X		1(0,9)		3(2,9)	1(0,9)		2(1,9)		1(0,9)	8 (7,6)
46,XY/45,X	1(0,9)		1(0,9)	1(0,9)						3 (2,9)
46,XiX	1(0,9)	3(2,9)	1(0,9)		1(0,9)	1(0,9)				7 (6,6)
46,XiX(q)								1(0,9)		1 (0,9)
46,XiX(p)								1(0,9)		1 (0,9)
45X/46,XiX						1(0,9)	1(0,9)			2 (1,9)
46,XrX		1(0,9)		1(0,9)						2 (1,9)
46,XdelX				2(1,9)	1(0,9)					3 (2,9)
46,XdicX			1(0,9)							1 (0,9)
46,XX/46,XiX/45,X			2(1,9)							2 (1,9)
45/46,XY/46,X+mar									1(0,9)	1 (0,9)
45/46,XX/47,XXX									1(0,9)	1 (0,9)
45,Xinv9									1(0,9)	1 (0,9)
Mismatch genetic sex 46,XY	2(1,9)	2(1,9)	1(0,9)	3(2,9)	2(1,9)	3(2,9)	1(0,9)	2(1,9)	1(0,9)	17 (16,2)
Total	11 (10,5)	14 (13,3)	16 (15,2)	13 (12,4)	14 (13,3)	13 (12,4)	8 (7,6)	9 (8,6)	7 (6,6)	105



Particularities of clinical polymorphism during puberty

clinodactilia, finger deformity, hyperteloritic nipples (both nipples were outside line means collarbone). General and biochemical analyzes: normal data from blood and urine analysis. Were determined fever, cough, poor nutrition, behavioral disorders. Family history: the child was born from first birth, non-sibling marriage. Birth weight was 2400 g, 46 cm length, head circumference 34 cm. Maternal age at birth was 25 years. On examination the child was active, normal body temperature, normal voice and physiological neonatal reflexes. Skin was without the signs of pallor, cyanosis or jaundice. There was a sign of lymphedema on the dorsal region of the feet and hands, bilateral edema which was not present on other parts of the body. There were no signs of anomalies of the cardiovascular, respiratory and gastrointestinal systems. The child was hospitalized and completely examined: General and biochemical analysis of blood and urine were normal, TORCH infectious agents were not revealed. Chest and skull X-rays were not determined any abnormalities. Ultrasound examinations of the abdominal organs were normal, except that the uterus and the ovaries could not be viewed. Echocardiography was normal. The child was seen by a geneticist who indicated cytogenetic analysis by karyotype study. The result of this examination indicated an abnormal karyotype 45, XO, which corresponds to the X monosomy or Turner syndrome. Given the clinical manifestations and laboratory data was established, the diagnosis of Turner syndrome was made. The child was supervised and counseled by medical genetic.

Clinical manifestations for girls with Turner syndrome during puberty is characterized by: short stature, undeveloped mammary glands, hypertelorism nipples, delayed puberty, infertility, primary amenorrhea, lymphedema of the hands and feet; gonadal dysgenesis, abnormal kidneys (horseshoe kidney), obesity, diabetes type I and II in childhood and adulthood, gastrointestinal bleeding due to vascular malformations, cardiovascular malformations, i. e. aortal stenosis, bicuspid aortic defect, aortic dissection; Hypothyroidism due to the presence of antithyroid antibodies, changes in Turkish saddle; Congenital hip dislocation; blurred vision, glaucoma, cataracts, retinitis, dyschromatopsia; ear infections (otitis media frequently), hearing loss (deafness); IQ normal, sometimes mental deficiency.



The study conducted allowed us to adapt and use within medical-genetic consultation diagnostic algorithm of Turner syndrome.

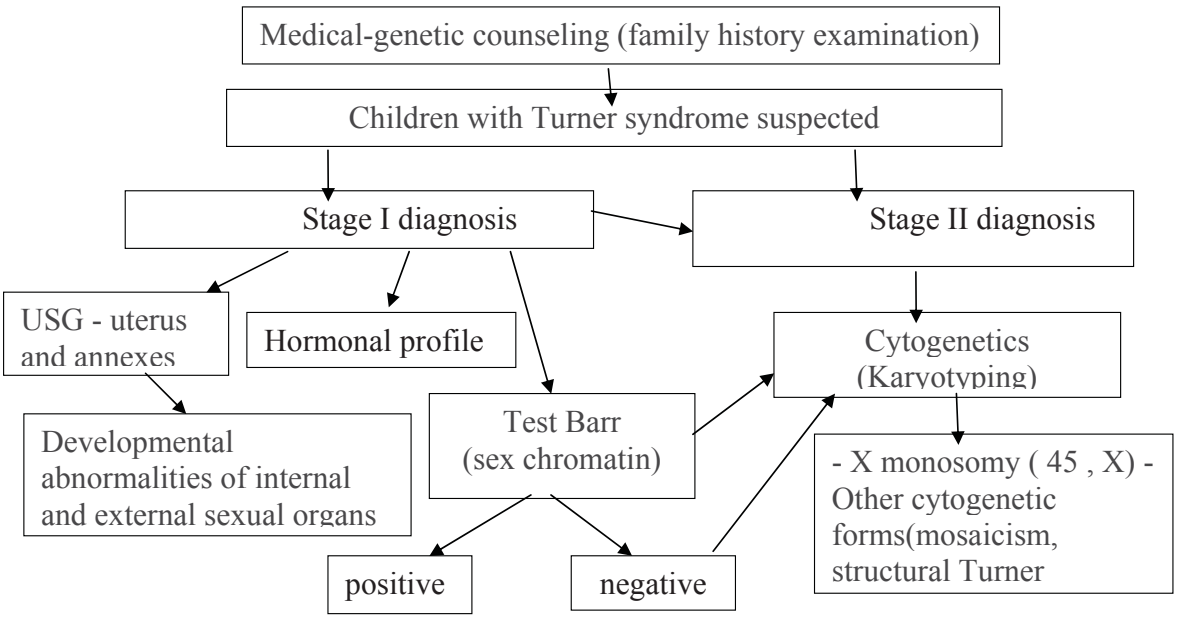


Fig. 1. *Genetic diagnostic algorithm in Turner syndrome*

The steps of the presented algorithm facilitated diagnosing children with Turner syndrome who were sent for medical-genetic counseling.

Conclusions:

1. Particularities of the phenotypic manifestations of the girls with Turner syndrome can suggest the early neonatal period diagnosis, however about 60% of patients are not diagnosed during early childhood due to lack of family and late addressing accusations to the geneticist.

2. In Turner syndrome the karyotype is highly variable; 51.4% registering a homogenous abnormalities. Thus, the most frequent cytogenetic form is X monosomy, resulting in a single cell line with the karyotype 45, XO.

3. Frequency of other cytogenetic variants of Turner syndrome are: mosaicism (45,X/46,XX; 45,X/46,XY) in 11,4% of cases; Structural abnormalities of sex chromosome (46,XiX,46,XdelX, 46,XdicX, 46,XrX) in 15,2% of cases; mosaicism with structural abnormalities of the X chromosome (46,XX/46XiX/45,X) in 5,7% cases, and other cytogenetic forms (46,XY) in 16,3% of cases.

4. In X monosomy IQ is usually normal, and Turner syndrome with structural abnormalities of chromosome X chromosome X ring there is a mild to moderate mental deficiency.

Bibliography

1. Collaer M.L. *Cognitive and Behavioral Characteristics of Turner Syndrome: exploring a Role for Ovarian Hormones in Female Sexual Differentiation* / M.L.Collaer, M.E.Geffher, F.R.Kaufman et al. // Horm. Behav. 2002. - Vol.41, №2 - P.139 - 155.

2. Partsch C.J. *Screening for Turner's syndrome by chromosome analysis of all girls with short stature* / C.J.Partsch, U.Raffenberg, W.G.Sippell // J. Pediatr. 2012. - Vol. 140, №l.p. 140.

3. Rivkees S.A., Hager K., Hosono S., et al. *A highly sensitive, high-throughput assay for the detection of Turner syndrome.* J Clin. Endocrinol. Metab. 2011. P. 696-699.

4. Torre R. *Genotypic sex and phenotypic sex: clinical, biochemical and molecular aspects in a patient with male hypogonadism and 46XX-45XO karyotype* / R.Torre, A.Savino, P.Venturi et al. // Recenti. Prog. Med.- 2001 -Vol.9, № 12. P. 747 - 750.

5. Вяткина С.В. *Цитогенетический и молекулярный анализ хромосомного мозаицизма у пациенток с синдромом Шерешевского-Тернера*/С.В.Вяткина, И.И. Нагорная, Ю.А.Логинова и др. // Мед. генетика. 2003. - Т.2, № 3. - С.124 - 129.

6. Гоготадзе И.Н. *Алгоритм обследования и принципы терапии при задержках полового развития у девочек: метод, пособие* / И.Н.Гоготадзе. СПб., 2003. - 19 с.