

THE FREQUENCY AND THE TYPE OF DIFFERENT ETIOLOGICAL FACTORS IN PRIMARY AMENORRHEA

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ABSTRACT

Aim: Primary amenorrhea (PA) is defined as the absence of menarche by the age of 14 without the development of secondary sexual characteristics or lack of menstruation by the age of 16 despite the existence of normal growth with the appearance of secondary sexual characteristics. We carried out a retrospective study, with the purpose of establishing the frequency and the type of different etiological factors among patients with primary amenorrhea.

Material and method: A total of 108 subjects, age ranged from 14 to 33 years were included in the study. A complete physical examination, blood tests for hormonal profile, pelvic ultrasonography and magnetic resonance imaging were performed to all patients. Besides, genotypic evaluations were also performed for the patients who got the indication.

Results: Out of the 108 patients presenting with primary amenorrhea, 40 (37,0%) had gonadal dysgenesis, 25 (23,1%) had Mullerian agenesis and 14 (12,9%) patients had hypogonadotropic hypogonadism. The genotypic evaluation revealed that 77.5 % (n=31) of cases had normal chromosome composition whereas 22.5% (n=9) had chromosomal abnormalities.

Conclusion: In conclusion, we have determined the 3 most common causes of primary amenorrhea are ovarian dysgenesis, Mullerian agenesis and hypogonadotropic hypogonadism; this data is compatible with the literature. Abnormalities in chromosomal analysis were determined in 22,5% of patients, which is also compatible with the literature. Determining etiology, in this large range of diseases may be confusing in clinical practice. During evaluation of patients with primary amenorrhea; these results should be kept in mind in order to establish an algorithm.

Key words: Primary amenorrhea, etiological factors, chromosomal anomaly, genital anomaly.

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Introduction

Primary amenorrhea (PA) is defined as the absence of menarche by the age of 14 without the development of secondary sexual features or lack of menstruation by the age of 16 despite the existence of normal growth with the appearance of secondary sexual features⁽¹⁾. In fact, amenorrhea is a symptom rather than a disease that results due to several different causes. The main reasons include improper functioning of ovaries, absence of uterus and vagina, hormonal imbalance, excess of male testosterone, and endometritis⁽²⁾.

In United States, the prevalence of amenorrhea is reported to be less than 1%⁽³⁾. The main etiological factors of PA can be categorized as gonadal dysfunction (50.4%); pituitary/hypothalamic disorders (27.8%) and outflow tract abnormalities (21.8%)⁽³⁾.

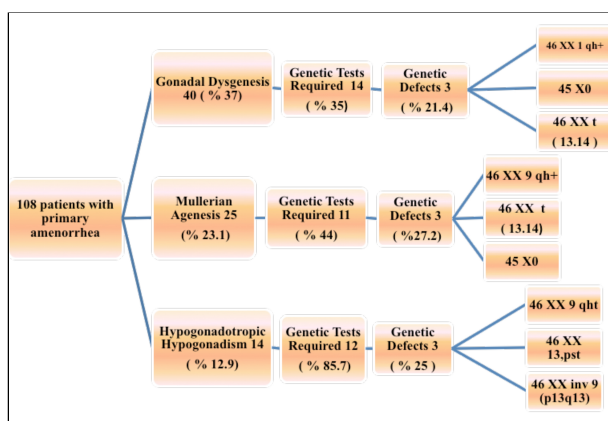


Table 1: Etiological factor distributions of primary amenorrhea in 108 patients.

Even if this is not a very common problem, it may completely change the life of patients depending on the underlying cause of it. Prompt diagnosis and treatment of primary amenorrhea, if possible,

prevent negative physical and psychological sequelae, preserve normal bone mass peak, and restore the fertility in affected patients. In that aspect, every patient should be evaluated carefully, being aware of the etiological factors of this condition.

We carried out a retrospective study, with the purpose of establishing the frequency and the type of different etiological factors among patients with primary amenorrhea (Table 1). By this way we aimed to increase the awareness of causative factors in primary amenorrhea, some of which are treatable.

Material and methods

This study was carried out between June 2011 and September 2013, in Obstetric and Gynecology Department of Dicle University Hospital. A total of 108 subjects, whose ages ranged from 14 to 33 years, were included in the study. None of the subjects has any known systemic diseases.

Primary amenorrhea was described as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older or aged 16 or older if secondary sexual characteristics were present. The diagnosis of primary amenorrhea was determined at the patient's first visit and physical examination was performed to distinguish any secondary sexual characteristics or syndrome features. Laboratory examination and clinical information were obtained from hospital records. Informed consent was obtained from all patients prior to the study. Ethics approval was obtained from the local institution.

Stage	Definition	Frequency (n)	Percentage (%)
Breast stage B1	The elevation of the papilla only	13	12 %
Breast stage B2	Breast bud stage with palpable glandular breast tissue and elevation of the papilla	23	21.3%
Breast stage B3	Further enlargement of the breast and areola with no separation of the contours	12	11 %
Breast stage B4	The areola and papilla form a secondary mound above the level of the breast	8	7.4 %
Breast stage B5	The mature breast with projection of the papilla	52	48.1%

Table 2: Tanner staging of breast and frequency in our patients.

All patients were subjected to a detailed history, a thorough clinical examination, and relevant biochemical, hormonal, and radiological investigations. The physical examination was performed at the first visit, caring particular interest to secondary sexual features. Hirsutism scores were assessed using the Ferriman and Gallwey system⁽⁴⁾. The pubertal stages were evaluated using the criteria and definitions described by Marshall and Tanner⁽⁵⁾. Accordingly, breast stages 1-5 were determined by both inspection and palpation (Table 2). The onset of puberty was measured as the age at breast development at Tanner stage 2 (B2).

Hormonal profile (estradiol, luteinizing hormone, follicle-stimulating hormone, and testosterone), thyroid function, and prolactin levels were tested at first consultation. To assess possible gynecological abnormalities, transvaginal or transabdominal ultrasound (USG) was performed as appropriate using a Voluson 730 expert sonography 1.8 GHZ probe. When available, the surface area of the ovaries was calculated as: $S=L$ (length) \times W (width) \times 0.8. The normal surface area of the ovary ranges between 2 and 6 cm²⁽⁶⁾.

Magnetic resonance imaging (MRI) of reproductive system was obtained for all patients with a 3.0T MR imager (Philips, Netherlands), using a pelvic phased-array coil. An experienced gynecologic radiologist analyzed the MR images.

The chromosomal analysis was performed in the Department of Medical Biology- Genetic. blood samples were drawn in a heparinized vacutainers for cytogenetic analysis and the lymphocyte cultures were set up in duplicates⁽⁷⁾. Two set of slides were prepared from each culture. Karyotyping was performed on routine peripheral blood lymphocyte cultures using G-banding after Trypsin and Giemsa staining (GTG)⁽⁸⁾. At least 30 GTG-banded metaphases were scored for each patient. Three cells were karyotyped according to International System for Human Cytogenetic Nomenclature (ISCN) criteria⁽⁹⁾. Usually, the total chromosome count was determined in 30 cells, but if mosaicism was suspected then 50 or more cell counts were undertaken⁽¹⁰⁾.

Statistical analyses were performed using the Statistical Package for the Social Sciences (Windows version 18; SPSS Inc, Chicago [IL], US). The data were expressed as mean \pm standard deviation. Frequency tables were obtained for the different etiological factors. The significance level was set at 0.05.

Results

At the time of AIT thyroid remnants were preseA total of 108 subjects, whose ages ranged from 14 to 33 years with a mean age of 18.1 ±3.9 years were included in the study. One hundred and two (94.4%) of our patients were single while 6 (5.6%) were married. The physical examination of 94 (87,0%) of patients were unremarkable, whereas 14 (13,0%) patients had hirsutism. Gynecological examination of patients revealed that; in 97 (89.3%) of them vagina was normal while in 10 (9.3%) of them vagina was blind and 1 (0.9 %) patient had hemi-vagina. Imperforate hymen was viewed in 5 (4.6 %) patients. In breast examination; according to Tanner scoring system, 13 (12%) patients were at stage B1, 23 (21.3%) were at stage B2, 12 (11.1%) were at stage B3, 8 (7.4%) were at stage B4, 52 (48.1%) were at stage B5 (Table 2). In 3 (2.7%) patients, galactorrhea has been determined.

In hormonal evaluation, 3 (2.8%) patients had hypothyroidism with elevated serum thyroid stimulating hormone (TSH) levels (TSH>5 g/dL) but normal serum free thyroxin levels. In one of these 3 patients, ovaries could not be visualized by MRI and in another one the ovaries were hypoplastic. When the prolactin levels were evaluated, 5 (4.6%) patients had elevated serum prolactin levels (PRL> 20 mcg/L). Among those 5 patients, uterus was not present in 2 patients and uterus was hypoplastic in other 2 cases. Whereas in 3 cases; ovaries were not present.

Demographic parameters	Frequency (n)
Age	18.1 ±3.9
Married	6 (5.6%)
Hirsutizm	14 (13 %)
Blind Vagina	10 (9.3%)
Tanner Stage 0	13 (12 %)
Imperfore Hymen	5 (4.6%)
FSH	20.5± 30.9 IU/l
LH	12.2±17.6 IU/l
E2	29.4±51.5 pg/ml
TSH	2.7±2.2 IU/ml
PRL	12.9±8.6 ng/ml

Table 3: Demographic characteristics of the patients.

The mean serum follicle stimulating hormone (FSH) level was 20.5± 30.9 (normal range (n), 3.5-12.5IU/l) and the mean serum luteinizing hormone (LH) was 12.2±176 IU/l (n: 2.4-12.6IU/l). The mean serum estradiol (E2) level was 29.4±51.5 pg/ml (n:10–880). Elevated testosterone levels were not established in any of the cases (Table 3).

Lower than normal FSH and LH levels were determined in 14 (12.9%) patients. Genotyping has been performed to 12 of these 14 cases and in 3 (% 25) cases 46, xx, 9qht; 46,xx,inv 9 (p13q13) and 46,xx,13pst abnormalities were determined. In one of these 14 patients, hyperprolactinemia was present.

In USG evaluation of reproductive system of our patients; uterus was not present in 27 (% 25) cases while hypoplastic in 31 (% 28) cases. On the other hand, ovaries could not be visualized in 41 (% 38) cases and were hypoplastic in 36 (% 33) cases. In 15 (% 13.8) cases were observed neither uterus nor ovaries.

In MRI evaluation of reproductive system of our patients; uterus was not present in 25 (23 %) cases while hypoplastic in 39 (36 %) cases. On the other hand, ovaries could not be visualized in 40 (37%) cases and were hypoplastic in 37 (34%) cases. In 11 (10%) cases were observed neither uterus nor ovaries (Table 4).

Radiological imaging	Absent	Hypoplastic
Uterine size on ultrasound	27 (25%)	31 (28%)
Right over size on ultrasound	40 (37%)	37 (34%)
Left over size on ultrasound	41 (38%)	36 (33%)
Uterine size on MRI	25 (23%)	31 (28%)
Right over size on MRI	40 (37%)	37 (34%)
Left over size on MRI	40 (37%)	37 (34%)

Table 4: The radiological findings of the patients.

Out of the 108 patients presenting with primary amenorrhea, 40 (37.0 %) had gonadal dysgenesis. Fourteen of these 40 cases had genotyping and in 3 (21.4 %) of them a genetic abnormality with chromosomal arrangements of 46 XX 1 qh+, 46 XX t (13, 14), 45 X0 has been determined. Moreover one (2.5%) of the patients with gonadal dysgenesis was diagnosed with Turner syndrome.

Twenty five (23.1%) of patients had Mullerian agenesis with MRI. Genetic testing has been deter-

mined in 11 of them and in 3 (27.2%) of them the chromosomal arrangement was as 46 XX 9qh+, 46 XX t^(13,14), 45 XO. In 2 of the cases with Mullerian agenesis; Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome was diagnosed.

The chromosomal analysis and karyotypes of the patients are summarized in table 5. The genotypic evaluation revealed that 77.5 % (n=31) of cases were having normal chromosome composition and 22.5% (n=9) have demonstrated chromosomal abnormalities.

Chromosomal arrangement	Frequency (n)	Percentage (%)
46,xx	31	77.5
46,xx,9qht	4	10.0
46,xx,inv9(p13q13)	1	2.5
46,xx,1qht	1	2.5
46,xx,t(13,14)	1	2.5
45,x,turner	1	2.5
46,xx,13pst	1	2.5
Total	40	100.0

Table 5: The chromosomal analysis and karyotypes of the patients.

Discussion

We have studied all etiological aspects of primary amenorrhea in this study and determined that the most common etiological causes of primary amenorrhea are gonadal dysgenesis (37.0%), Mullerian agenesis (23.1%) and hypogonadotropic hypogonadism (12.9%) in this study group. Genotyping has been performed to 40 cases who got the indication, and revealed abnormalities in 9 (22.5%) cases.

In 1981, the ranges of etiological factors of delayed sexual development has been described approximately as gonadal dysgenesis in 50 % of patients, hypothalamic diseases in 20 %, uterine anatomical defects in 20 %, pituitary abnormalities in 5 % and other causes including some hormonal deficiencies or enzyme defects in 5 %⁽¹¹⁾. In fact, from this time on, the most common causes of primary amenorrhea has not changed much. Timmreck et al described the four most common causes of primary amenorrhea as ovarian failure (48.5 %), con-

genital absence of the uterus and vagina (16.2 %), GnRH deficiency (8.3 %), and constitutional delay of puberty (6.0 %)⁽¹²⁾. More recently, in a study done in Thailand, the 3 most common causes of primary amenorrhea were established as Müllerian agenesis (39.7 %), gonadal dysgenesis (35.3 %), and hypogonadotropic hypogonadism (9.2 %) on 295 cases⁽¹³⁾. Interestingly, in this study amongst 88 cases of gonadal dysgenesis, 59 cases (67.0 %) incurred abnormal karyotype including 45X (n = 21), mosaic (n = 31), and others (n = 7). Although the results of this study were not very different from our study, in regards to the first 3 etiological factors of primary amenorrhea; the ratio of abnormal karyotypes was about 3 times more than that of our results.

Gonadal dysgenesis, the single most common cause of primary ovarian failure in female, is defined as absent or insufficient development of ovaries⁽¹⁴⁾. The main feature of female gonadal dysgenesis is the primary ovarian failure with streak gonads that result in primary amenorrhea and lack of development of secondary sexual characteristics. Although the karyotype in patients with gonadal dysgenesis can be 45XO, mosaicism or deletion of a certain part of X chromosome, a normal karyotype of 46XX may also be present. In our study, 3 of the cases with ovarian dysgenesis had genetic abnormalities while 11 had normal genotypes.

Congenital anatomical lesions of uterus and vagina are the second most common cause of primary amenorrhea. Vaginal agenesis, imperforate hymen and transverse vaginal septum are the common types of these anatomical lesions. One of the most important points is the differential diagnosis of Mullerian agenesis with androgen insensitivity syndrome and increased testosterone levels. None of our cases had male range testosterone levels. In 10 of our patient vaginal agenesis was found, and one presented hemivagina .

On the other hand hypogonadotropic hypogonadism is a large group of diseases causing abnormalities in secretion or pulsatility of gonadotropin releasing hormones including eating disorders, exercise, stress, hyperprolactinemia, tumors and infiltrative diseases of hypothalamus and pituitary and congenital GnRH deficiency. Among our cases, 12,9 % of patients had hypogonadotropic hypogonadism and 3 of them also had accompanying genetic abnormalities and 1 of them had hyperprolactinemia.

In fact the role of chromosomal abnormalities reported is greatly variable, from 15.9 % to 63.3 % for primary amenorrhea⁽¹⁵⁻¹⁷⁾. Twenty two point five

percentage of chromosomal abnormalities detected in our patients with PA is compatible with many studies in literature that has determined this ratio in between 20-27.8%⁽¹⁸⁻²⁰⁾. Male karyotype was observed in a significant percentage of patients with primary amenorrhea in previous studies, ranging from 8.4% to 18.2%⁽¹⁵⁾; however, we did not determine any male karyotype in our study. This may be due to the low number of patients (n: 40) who got the indication for genotypic evaluation in our study.

In conclusion, we have determined the 3 most common causes of primary amenorrhea as ovarian dysgenesis, Mullerian agenesis and hypogonadotropic hypogonadism; which is compatible with the literature. The abnormalities in chromosomal analysis of patients with the indication were determined in 22.5% of patients which is also compatible with the literature. Determining etiology, in this large range of diseases may be confusing in clinical practice. During evaluation of patients with primary amenorrhea; these results should be kept in mind in order to establish an algorithm.

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