ISSN: 0067-2904

Chromosomal and Hormonal Analysis of Women with Primary Amenorrhea in Iraq

Majeed Arsheed Sabbah, Halah Khalid Ibrahim Al-Sammarraie, Abdulqader Abdulkareem Merie Alrawi

[1]*Department Of Gene Bank, Forensic DNA Center for Research and Training, Al-Nahrain University, Jadriya, Baghdad, Iraq*

[2]*Al-Fallujah teaching hospital for maternity and children, AL-Anbar, Iraq*

Received: 4/2/2023 Accepted: 1/10/2023 Published: 30/5/2024

Abstract

Primary amenorrhea is one of common gynecological diseases among adolescent girls in community which is characterized by absence of menstrual cycles and normal sexual characteristics. Chromosomal abnormalities and hormonal disturbance play a crucial role in developing this disease. However, due to social stigma, many people with sex-related abnormalities even in certain provincial regions of Iraq do not ask for medical help resulting in an unclear true incidence of sex abnormalities among amenorrhea patients. Thirty-four patients were referred to Genetic Consultation Clinic in the Medical city of Baghdad to determine the possible causes of primary amenorrhea. Ultrasound findings of the current study revealed that 5 (14.72%) patients had normal internal genital organs, 18 (52.94%) patients had hypoplastic uterus and ovaries, 4 (11.76%) patients had infantile uterus, 3 (8.82) patients suffered from rudimentary organs, and 4 (11.76%) patients had no visualized organs (absent). There was a substantial increase in the serum levels of hormones in the patients' group (p-value <0.05) particularly follicle stimulating hormone (FSH) and luteinizing hormone (LH), when compared to the control group.

Results also revealed that 26 patients had normal karyotype, 4 patients had Turner syndrome (TS) (45, XO, mosaic 45, XO/ 46XX, 46,XX/isoXq , 46 XO, ring Xq), two cases had (46, XX/isoXq and 46 xx, t (15q; 11q)), one case had 46 xx, t (1p;13q), and one case had 46 xx, t (1p;13q). It can be said in the end that each of these clinical examinations was crucial for making a correct diagnosis of the patient's condition and for identifying the important causes of primary amenorrhea in female patients from Iraq.

Keywords: Primary amenorrhea**,** Hormonal analysis**,** Chromosomal abnormalities.

تحليل الكروموسومات والهرمونات عند النساء المصابات بانقطاع الطمث الأولي

مجيد ارشيد صباح [1] ،هالة خالد ابراهيم السامرائي [1]، عبد القادر عبد الكريم مرعي الراوي [2]

[1]قسم بنك الجينات، مركزالدنا العدلي للبحث والتدريب ، جامعة النهرين ، بغداد ، العراق

[2]مستشفى الفلوجة التعليمي للولادة والأطفال ، الأنبار ، العراق

*Email: hala_kh76@nahrainuniv.edu.iq

الخلاصة

يعد انقطاع الطمث الأولي أحد الأمراض النسائية الشائعة بين الفتيات المراهقات في المجتمع ، ويتميز بغياب دورات الحيض والخصائص الجنسية الطبيعية. يلعب شذوذ الكروموسومات والاضطرابات الهرمونية دورًا حاسمًا في الإصابة بانقطاع الطمث الأولي . بسبب الوصمة الاجتماعية ، فإن العديد من الأشخاص الذين يعانون من تشوهات مرتبطة بالجنس خاصة في مناطق معينة من محافظات العراق لا يطلبون المساعدة الطبية ، مما يؤدي إلى عدم تسجيل التشوهات الجنسية بصورة واضحة بين مرضى انقطاع الطمث. تمت إحالة 34 مريضاً إلى عيادة الاستشارات الوراثية في مدينة بغداد الطبية لتحديد الأسباب المحتملة لانقطاع الطمث الأولي. أظهرت نتائج الموجات فوق الصوتية أن هناك 5 (14.72٪) مرضى بأعضاء تناسلية داخلية طبيعية ، 18 (52.94٪) مرضى يعانون من نقص تنسج الرحم والمبايض ، 4 (11.76٪) مرضى رحم طفولي ، 3 (8.82) مريض بأعضاء بدائية و 4 (11.76٪) مرضى ليس لديهم أعضاء مرئية (غائبة). كشفت الدراسة ان هناك زيادة كبيرة في مستويات الهرمونات في الدم في مجموعة المرضى (قيمة $p < 0.05$) وخاصة الهرمون المنبه للجريب (FSH) والهرمون اللوتيني (LH) ، عند مقارنتها بمجموعة السيطرة. كذلك كشفت نتائج التنميط الكروموسومي (تحليل الكروموسومات) أن 26 حالة من المرضى لديهم النمط النووي الطبيعي للإناث (46XX ،يوجد 4 حالات يعانون من متلازمة تيرنر (TS) ،وتشمل التغيرات : (فسيفساء XO 46XX / 45) ، XX / isoXq، 46 ، XO ، الحلقة Xq) ، بما في ذلك حالتان مع isoXq ، وحالتان فيها (46 ، XX / isoXq و 46 xx، t (15q ؛ q11) ، وحالة واحدة م t (1p;13q) ,xx 46 . والخلاصة أن كل هذه الفحوصات السريرية ضرورية لإجراء تقييم دقيق للحالة الطبية ، مما يشير إلى العوامل المهمة التي تسهم في تطور انقطاع الطمث الأولي لدى المريضات العراقيات.

1. Introduction

It is a gynecological condition characterized by the loose of menstrual cycles in the presence of normal sexual characteristics [1, 2] and is common in female conditions such as pregnancy and menopause [3]. To diagnose amenorrhea in females of reproductive age, one must first exclude out pregnancy as the cause. The difficulty will be in recognizing the specific cause of absent menstruation when there is no pregnancy [4, 5].

According to the World Health Organization (WHO), amenorrhea is the sixth leading cause of female infertility worldwide, affecting 2-5% of all women [6]. According to 2017 and 2018 data from the Iraqi Ministry of Health, 9.68% and 17.78% of women were affected by primary amenorrhea and menstrual disorders [7].

There are two types of amenorrhea: primary and secondary. Primary amenorrhea is characterized by absence of menstruating in teenagers (14-16 years old), while secondary type has sexual traits either present or absent. [8]. The cessation of menstruation after it has started is referred to as secondary amenorrhea [9].

Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus during a typical female period. which activates the pituitary to release FSH and LH. These two female hormones then work on the ovaries to secret estrogen and progesterone which then take job on the uterus to carry out the ovulation and signaling cascade process of the menstrual periods. Amenorrhea can result from any malfunction or anatomical problems disrupting female body's regular functions at any exocrine and endocrine gland level. It has been shown that elevated (FSH) levels play an essential role as major causes of menstrual irregularities [10, 11]. In primary amenorrhea several studies have reported that gonadal dysfunction represents 50.4% and frequency of cytogenetic abnormalities ranged from 15.9% to 63.3% [12].

Studies have revealed that patients with primary amenorrhea have male chromosome components [karyotype, 46, XY] but feminine phenotypes (Swyer syndrome) such chromosomal abnormalities are related with sex transformation, also some time the anomalies are related to X chromosomes, several forms of primary amenorrhea such as 47/XXX and 45/XO [13, 14].

The purpose of this study was to investigate the chromosomal factors that may relate to primary amenorrhea in patients from Iraq with inspect female hormones LH and FSH levels.

2. Materials and Methods

The thirty-four selected women patients attended to the Genetic Consultation Clinic in the Medical city of Baghdad and Kamal Al-Samarai IVF Hospital, Ministry of Health, Baghdad, Iraq for diagnosis by gynecologists with primary amenorrhea. The individuals committed in this study were authorized by Ethics Committee of the Iraqi Ministry of Health. The control group included 20 healthy women with constant menarche cycles.

An ultrasonic GE device (GE Healthcare, USA) which made use of 7.5 (MHz) linear converter turn energy from one form to some other, with a high determination IV real-time detector, was used for scanning the size, thickness and any abnormal appearance that could be identified. Alcohol was sprayed on the sites before applying ultrasonic gel to the areas being inspected as it's important to moisturize the scan area of examination with gel to minimize the air between the skin and the transducer causing sound waves being effectively converted to image through the air. It, therefore, needs a medium for better diagnosis [15].

According to the serological investigation, peripheral blood samples were collected and transferred into a 6 ml gel clot activator tube using a sterile disposable syringe (3 ml). Once the samples had coagulated in the water bath at 37°C, the sera were centrifuged for 5 minutes at 1500 rpm. Hundred µl was then taken to measure serum concentrations of LH and FSH by using mini-vidus machine (Biomerieux, France). The principle used is called Enzyme Linked Fluorescent Assay (ELFA). The kits were provided from the same company (Biomerieux, France) as it’s closed system [16].

Blood samples (0.5ml) were cultured in 5 ml of RPMI-1640 media in a sterile tube enriched by 10% bovine albumin and 2% phytohaemagglutinin (PHA) for karyotyping. The tubes were incubated at 37°C for 72hr and then 100 µl of colchicine (0.45mg/ml) was added to each culture. The cultures were harvested by centrifugation at 2000rpm for 10min after 20 minutes. The cells pellet was dissolved in the remaining solution after discarding the supernatant . Cells were treated with a moderate hypotonic solution, KCL (0.075M), at 37 °C. Subsequent centrifugation allowed the nucleus to precipitate. After the supernatant had been separated, the cells were treated with a fixative solution with a ratio of 3:1 from methanol and glacial acid for 20 minutes. Fixed cells were placed on the clean slides and Giemsa stain was applied to the dry slides before examining them under a microscope [17].

3. Results and Discussion

The age of patients with primary amenorrhea in our study ranged from 13 to 35 years with a mean age of 24.2 ± 3.7 years. Majority of patient’s ages range 16 to 20 years as it's usually the age for marriage. According to the present study, it can be concluded that patients with primary amenorrhea compared to control (fertile women) subjects had substantial differences in several variables. The serum FSH and LH values were among those that demonstrated substantial variations (Table 1).

Table 1: Descriptions of FSH and LH levels in each group under study, patients and the controls.

	Controls (n = 20)	Amenorrhea (n =34)	*p*-value
Age	22.5 ± 2.4 (13–33)	24.2 ± 3.7 (16-35)	0.00
FSH (IU/l)	5.37 ± 2.56	71.88 ± 15.8	0.00
LH (IU/l)	4.54 ± 2.70	25.35 ± 5.75	0.00

Mean with standard deviation between control subjects and patients. *p*-value is less than 0.05 meaning that there was a less than 5% chance that the observed results could have come about by chance; this is often regarded as statistically significant. (FSH) follicle stimulating hormone, (LH) luteinizing hormone.

The normal baseline of serum FSH levels about (20 IU/l), compared to patient's serum FSH levels reached 71.88 ± 15.8 IU/l. In the clinical conditions of such hyper-gonadotropic hypogonadism, the serum FSH and LH levels sounded acceptable. These results implied that the observed amenorrhea and hormonal abnormalities could have been the result of a disruption in the normal hormonal control such as elimination of deleterious estrogen feedback [18]. Premature ovarian failure or gonad dysgenesis is the main cause of high secretion of ovarian hormones levels [19, 13]. The patients of amenorrhea displayed specific traits such as hormonal abnormality and/or genital tract anatomical problems like uterus, vaginal and ovarian agenesis. This structural abnormality is related to X- chromosome as in TS and mosaicism. The female normal pubertal uterus is about 5-8 cm long, 3 cm wide and 1.5 cm thick with a pear shape. The ratio of fundus to cervix is about 2/1 to 3/1 and can be easily detected by ultrasonography examination [20].

Ultrasound (US) medical imaging, due to its many advantages, is one of the commonly applied methods. Ultrasonography examination is inexpensive, practical, radiation-free, rapid, painless method, non-invasive, and has a high percentage of reliable detection which makes it beneficial in the diagnosis. All this has made it an advantageous tool for showing accurate information about medical soft tissue [21]. Pelvic ultrasonography can find structural problems connected to primary amenorrhea which could be used as the preliminary indicators as an imaging test. Pelvic ultrasonography is advised for women with primary amenorrhea but normal secondary sexual characteristics to look for any underlying anatomical explanations [10].

Using the abdominal ultrasound to identify the uterus length that is classified into three categories: normal long if its > 6 cm, hypoplastic if its 6-3 cm, infantile (3 cm) and rudimentary if its < 3 cm (Table 2). There were 5 patients with normal internal genital organs, 18 patients with hypoplastic uterus and ovaries, 4 with infantile uterus, 3 had rudimentary organs and 4 patients with no visualized organs (absent).

Table 2: Karyotyping of the cytogenetic variations and ultrasound findings.

Cytogenetic Variations	Karyotyping	Number and Frequencies (%)	Ultrasound Findings with and Frequencies (%)
Normal karyotype	46XX	26 (76.48%)	Normal uterus 5 (14.72%)
Numerical abnormality Mosomy X Turner's mosaic Structural abnormalities	45, XO 45 XO, ring Xq. 45, XO/ 46XX 46 xx/ ring 5	4 (11.76%)	Hypoplastic uterus 4 (11.76%)
Structural abnormalities Isochromosome	46, XX/isoXq 46 xx, t (15q; 11q)	2 (5.88%)	Infantile uterus 3 (8.82)
Structural abnormalities	46 xx, t (1p;13q).	1 (2.94%)	Rudimentary uterus 4 (11.76%)
Structural abnormalities	46 xx, del (10q) t(10q;14q)	1 (2.94%)	absent uterus 4 (11.76%)

The ultrasound findings in Table 2 show that the vast majority of amenorrhea patients corresponded to female reproductive system hypoplasia which is comparable with the chromosomal variations, mentioned earlier (Table 2). The consistency and validity of the observed relationships are supported by these findings which are consistent with earlier studies in the area of interest. The most frequent reason of amenorrhea, about 30%–40%, is gonad dysgenesis which is the term used for the defective or inadequate development of the gonads [22].

Our findings revealed that TS, a condition which is known to be one of the common causes of female gonadal dysgenesis, was responsible for a many cases of amenorrhea patients reported in this case study and as amenorrhea is related to the number and quality of viable oocytes that are distorted by hormones beginning with the development of female reproductive system [23]. Genetic testing and evaluation are required for a correct diagnosis and to identify the precise chromosomal structure of each patient. Turner syndrome is typically linked to structural abnormalities in the X chromosome (ring chromosome X) (Figure 1). The features of TS could be noticed as physical abnormalities such as short human length, cause of ovarian failure, amenorrhea, ptosis (drooping of the superior eyelid), protrude lower jaw, web necks (loose neck folds and short or no neck), kidney anomalies and other congenital abnormalities are all common in TS [24].

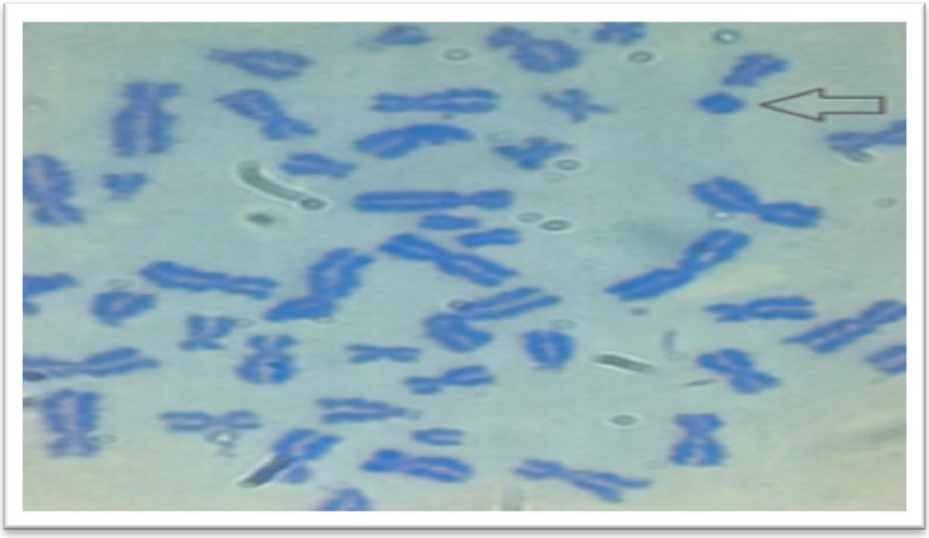

Figure 1: Female Karyotype of turner monosomy X: 46 XO, r Xq.

Some Turner's patients had X-chromosome inversions, deletions; rings and rearrangement of X-chromosome (translocations). Figure 2 represents the constitutional translocation of (15; 11) which is the most common non-Robertsonian translocation. A specific sort of rearrangement of chromosomes occured when genetic material from chromosomes 15 and 11 was exchanged. Chromosomes 1 and 13 were involved in a unique chromosomal rearrangement called a translocation, is identified by the karyotype 46 XX, t (1p; 13q). The translocation in this instance took place between the long arm (q) of chromosome 13 and the short arm (p) of chromosome 1 (Figure 3).

Figure 4 indicates that a piece of the long arm (q) of chromosome 10 was absent or had deleted. The particular region or segments could have had an impact on the genes concerned, had they not been removed. In the same case another chromosomal aberration detected t (10q; 14q) indicated translocation of chromosome (short arm p) chromosome 13 (long arm q). Figure 5 shows the X monosomy (loose X-chromosome), also one of the most common genetic defects in amenorrhea patients [26, 27].

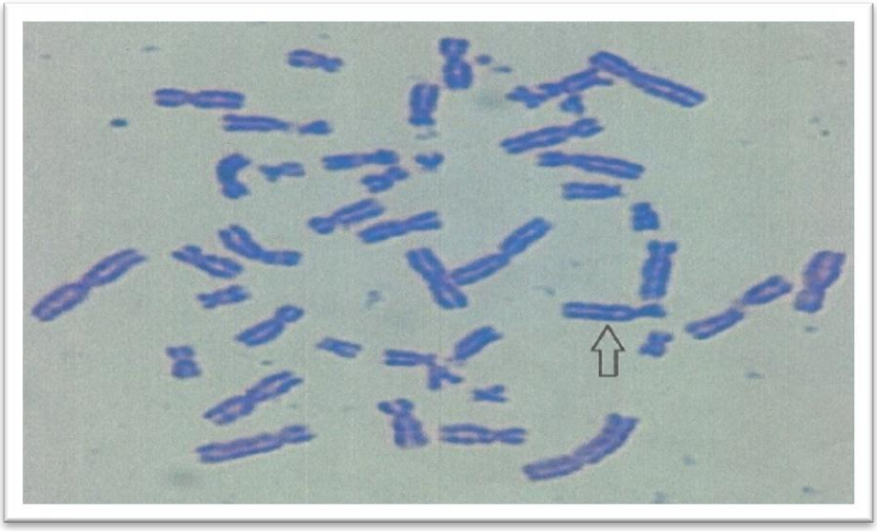

Figure 2: Female karyotype show 46 xx, t (15q; 11q) sometime refer as t (15; 11).

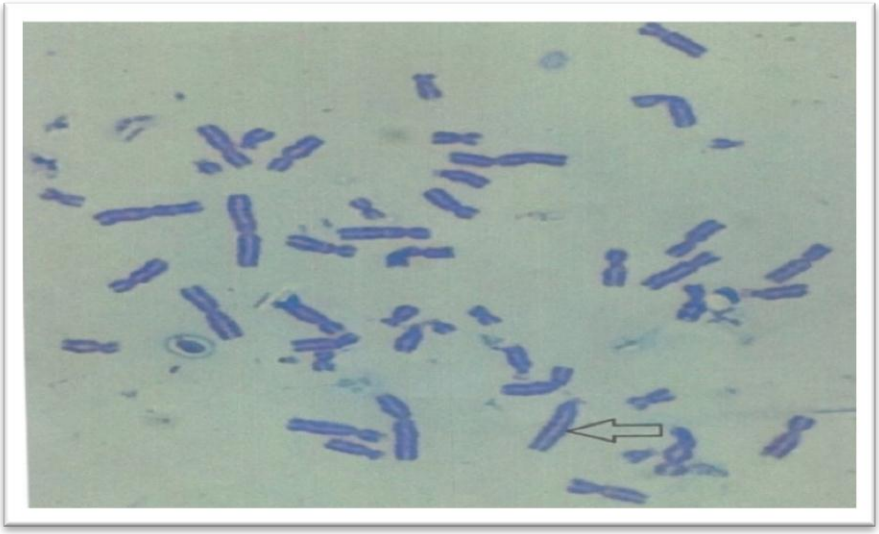

Figure 3: Female karyotype show 46 xx, t (1p; 13q). In this case, a translocation occurs between the short arm (p) of chromosome 1 and the long arm (q) of chromosome 13.

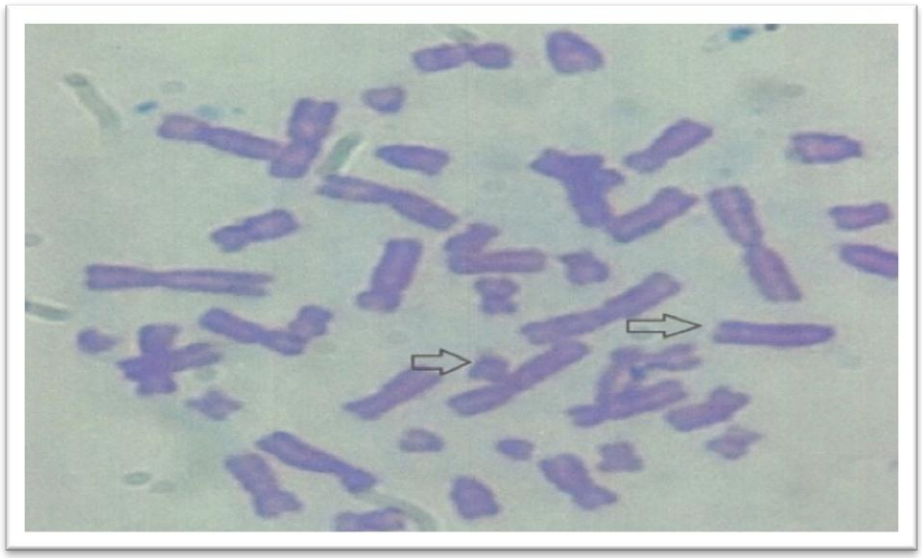

Figure 4: Female karyotype show 46 xx, del (10q), deletion in q part of chromosome 10 T (10q; 14q) translocation between the short arm (p) of chromosome 1 and the long arm (q) of chromosome 13.

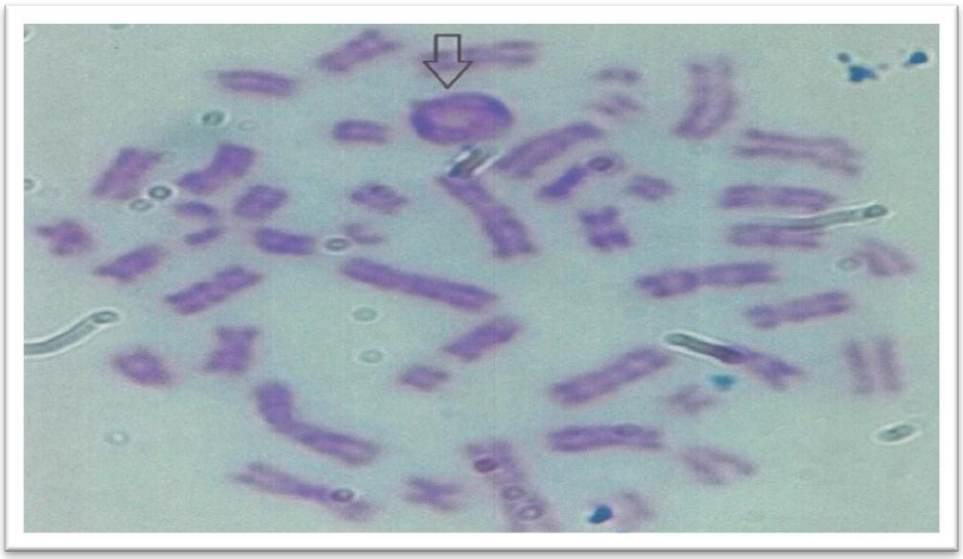

Figure 5: female karyotype shows 46 xx, r, 5 (ring chromosome 5).

4. Conclusion

A substantial percentage of uterine anomalies seen by ultrasound imaging, such as aberrant size and shape, could act as preliminary indications of amenorrhea. Despite having a normal karyotype (chromosome structure), the occurrence of phenotypic abnormalities in uterus size, length, or width raised the possibility of underlying molecular genetic variants. In addition to ultrasound imaging and in order to corroborate the results, cytogenetic study should be performed as chromosomal abnormalities are one of the main causes of primary amenorrhea. For a precise diagnosis, risk assessment and genetic counselling, it is essential to look at the genetic component. It is crucial to assess the outcomes of chromosomal analysis and ultrasonography to determine the most appropriate treatment approach.

Acknowledgements

For conducting this study and chromosome analysis of the potential causes of primary amenorrhea, we are grateful to the working staff of Genetic Consultation Clinic in Baghdad.

Ethical Clearance

All individuals taking part in the study provided their informed consent.

Conflict Of Interest

There is no conflict of interest, according to the authors.

References

[1] W.A, Balwan, S. Neelam and K.S. Jamwal., "Cytogenetic investigation of cases with primary amenorrhea in Jammu region of Jammu and Kashmir". *International Journal of Zoology and Research*, 10(2), p: 1-8, 2020.

[2] S. Basak, A. Prakash, "Investigation and treatment of primary amenorrhoea". *Obstet Gynaecol Reprod Med*. 23, p: 364-369, 2013.

[3] L. Gaspari, F. Paris, N. Kalfa, and C. Sultan, "Primary Amenorrhea in Adolescents: Approach to Diagnosis and Management ", *Endocrines*, 4, p: 536–547, 2023.

[4] G. Shanoli, R. Sanchita, P. Pritha, D. Atreyee, and H. Ajanta, "Cytogenetic analysis of patients with primary amenorrhea in Eastern India". *Journal of Obstetrics and Gynaecology*, 38:2, 270-275, 2018.

[5] C. J. Hayden, and A. H. Balen, "Primary amenorrhoea: investigation and treatment". *Obstetrics, Gynaecology & Reproductive Medicine*, 17(7), 199-204, 2007.

[6] M. G. Madziyire, T. L. Magwali, V. Chikwasha and T. Mhlanga, "The causes of infertility in women presenting to gynaecology clinics in Harare, Zimbabwe; a cross sectional study". *Fertility research and practice*, 7(1), 1, 2021.

[7] R. A. Nijeeb, A. Alkazaz and R.A. Yaseen, "Follicle –Stimulating Hormone Receptor Polymorphisms in Iraqi Women with Primary Amenorrhea". *Indian Journal of Forensic Medicine & Toxicology*, 14 (1), 2020.

[8] V. Sarathi, R. Reddy, S. Atluri and C. Shivaprasad, " challenging case of primary amenorrhoea". *BMJ Case Rep*. Jul 11; 2018.

[9] P. Tanmahasamut, M. Rattanachaiyanont, C. Dangrat, S. Indhavivadhana, S. Angsuwattana and K. Techatraisak, "Causes of primary amenorrhea: a report of 295 cases in Thailand". *Journal of Obstetrics and Gynaecology Research*, 38(1), p: 297-301, 2012.

[10] DA. Klein, SL. Paradisea and RM. Reeder, "Amenorrhea: A Systematic Approach to Diagnosis and Management". *Am Fam Physician*. Jul 01;100 (1), p:39-48, 2019.

[11] M. I. Ibrahim, and J. M. Al-Saffar, "Serum level evaluation of interleukin-18 in obese women with polycystic ovary syndrome". *Iraqi Journal of Science*, 59(4), p: 1989–1994. 2018.

[12] S. Ghosh, S. Roy, P, Pal, A. Dutta and A. Halder, "Is There any role of metal toxicity in amenorrhea?" *Malays J Med Res*. Vol 1, Issue 4, p: 40-4, 2017.

[13] A.H. Soltani and F. Mirzaei, Cytogenetic Study of Patients with Primary Amenorrhea in the Northeast of Iran". *Iranian Journal of Pathology*, 16(1), p: 57-61,2020.

[14] L. Dhanlaxmi, Shetty, P. Akshay, Kadam, T. Neeraja, Rupa C. Dalvi, S. Deepak, R. Bibu. and M. Swarna, " X-autosome translocations in amenorrhoea: a report of a three-way translocation from Indian Population". *Gynecological Endocrinology*, 30(4), p: 302-30, 2014.

[15] S. Afzal, M. Zahid, Z.A. Rehan, H.M. Shakir, H. Javed, M.M.H. Aljohani, S.K. Mustafa, Ahmad, M. M.M. Hassan, "Preparation and Evaluation of Polymer-Based Ultrasound Gel and Its Application in Ultrasonography", *Gels*, 8(1), 42, 2022.

[16] B.A. Kanaan, M.T. Al-Ouqaili and R.M. Murshed, "In terms of the PCR-RFLP technique, genetic screening of Ala575Val inactivating mutation in patients with amenorrhea". *Journal of Emergency Medicine, Trauma & Acute Care.* (6):8, 2022.

[17] T. M. Malla, A. A Pandith and M. H. Zargar, " Frequency and pattern of cytogenetic alterations in primary amenorrhea cases of Kashmir North India", *Egyptian Journal of Medical Human Genetics*, 17(1), p: 25–31, 2016.

[18] A. Catteau, K, Bach-Ngohou, J. Blin, P. Barrière, T. Fréour and D. Masson "Abnormally elevated follicle-stimulating hormone (FSH) level in an infertile woman". *Case Rep Endocrinol*.,Sep 22, 2019.

[19] S.R. Howard, "Interpretation of reproductive hormones before, during and after the pubertal transition identifying health and disordered puberty". *Clin Endocrinol (Oxf)*, 95, p:702-715, 2020

[20] G.S. Anitha, K.K. Tejeswini, G. A. Shivamurthy, "Clinical Study of Primary Amenorrhea", *J South Asian Feder Obst Gynae*. 7(3), p:158-166 ;2015

[21] A. Al-jaburi and A. H. AL-sudani "Medical Ultrasound Image Quality Enhancement and Regions Segmentation" *Iraqi Journal of Science*, 63(10), p: 4518-4533, 2022.

[22] J. Y. Yoon, and C. K. Cheon," Evaluation and management of amenorrhea related to congenital sex hormonal disorders", *Annals of pediatric endocrinology & metabolism*, 24(3), 149–157, 2019.

[23] R. E. Ahmed and M.Q. Al-lami" Anti-Mullerian Hormone and Follicle Stimulating Hormone as Markers of Ovarian Aging in a Sample of Iraqi Women"*Iraqi Journal of Science,* Vol. 57, No.3A, p :1671-1679, 2016.

[24] P. Chauhan, , S. K. Jaiswal, A. R. Lakhotia, and A. K. Rai," Molecular cytogenetic characterization of two Turner syndrome patients with mosaic ring X chromosome". *Journal of assisted reproduction and genetics*, 33(9), 1161–1168, 2016.

[25] H. Kurahashi, H. Inagaki, T. Ohye, H. Kogo, M. Tsutsumi, T. Kato, M. Tong, and B. S. Emanuel, The constitutional t (11;22): implications for a novel mechanism responsible for gross chromosomal rearrangements. *Clinical genetics*, *78*(4), 299–309, 2010.

[26] N. Li, L. Zhao, J. Li, Y. Ding, Y. Shen, X. Huang, X. Wang and J. Wang, "Turner syndrome caused by rare complex structural abnormalities involving chromosome X". *Experimental and therapeutic medicine*, 14(3), 2265–2270, 2017.

[27] Z. Razavi and HE. Momtaz, "Balanced Reciprocal Translocation t(X;1) in a Girl with Tall Stature and Primary Amenorrhea" *Iran J Med Sci.* 42(2):210-214, 2017.