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Menstrual Cycle Hormone Changes Associated with Menstrual Cycle Interval in 9-18-year-old girls

Naeimeh Tayebi (MSc)[1], Maryam Keshavarz (MSc)[2], Mohammad Hossain Dabbaghmanesh (MD)[3], Marzieh Akbarzadeh (MSc)[4,5*]

[1] MSc in Midwifery, Noncommunicable Diseases Research Center, Bam University of Medical Sciences, Bam, Iran
[2] MSc in Midwifery, Department of the Midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
[3] Professor of endocrinology, Endocrine and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[4] Assistant Professor, Maternal –fetal Medicine Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[5] Department of Midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Background & aim: Hormonal disorders may be associated with a variety of menstrual disorders that can have different health consequences and are an indicator women's gynecologic health. The aim of this study was to evaluate the pattern of sex hormones of the menstrual cycle related to the menstrual cycle intervals in girls aged 9-18 years.

Methods: In this two-stage cross-sectional study, 2000 girls aged 9-18 years old in first stage were selected conveniently from four districts of Shiraz, Iran in cluster form in 2015, and their pattern of menarche age (early, normal, late) was determined. In the second stage, 50 students with normal menarche and 12 students with late menarche were selected and each completed a questionnaire including demographic characteristics, Hygam chart and menstrual bleeding characteristics (using Smith-Di Giulio criterion) questionnaire. Their hormones including TSH, Prolactin, FSH, DHEAS and Testosterone were also measured.

Results: The highest frequency of age was 16 years (40.3%) and the lowest was 17 years old (8.1%). The results of one-way analysis of variance between different hormones and menstrual cycle interval showed that there is a significant relationship between TSH (P=0.03), Prolactin (P=0.002) and menstrual intervals. But no significant relationship was seen between LH (P=0.63), FSH (P=0.08), DHEAS (P=0.82), Testosterone (P=0.703) and menstrual intervals.

Conclusion: TSH and prolactin disorders are the most common hormonal disorders in girls with menstrual disorders. It is recommended to check these hormones in girls with various menstrual disorders after eliminating structural disorders and before starting different treatments for menstrual disorders.

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Introduction

Menstruation is one of the most natural processes occurring during a woman's life. It is a sign of the beginning of one of the most basic stages of human life, namely the period of fertility and reproduction .also The occurrence of menstruation is affected by the health status of individuals (1). Menstrual cycle is one of the most important experiences of adolescents during puberty and reproduction and its disorders can cause many problems in different stages of reproductive life and the endocrine system plays a key role in the rhythmic regulation of the menstrual cycle (2). The menstrual cycle is a complex physiological process (3) and menstrual irregularities in the early postmenopausal age can be considered as an indicator of psychosocial disorder in 13 - 19-year-old girls (4). Menstrual disorders such as abnormal uterine bleeding or disorders related to the duration of the menstrual cycle are

* *Corresponding author*: Marzieh Akbarzadeh, Assistant Professor, Maternal –fetal Medicine Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: 07116474250; Email: akbarzadm@sums.ac.ir

relatively common in adolescents due to hormonal disorders of the hypothalamus, pituitary gland, ovary and the development of anovulatory cycles (5). These disorders are often the source of anxiety for these patients and their families. Common adolescent disorders include amenorrhea, abnormal and excessive uterine bleeding, and premenstrual syndrome (6). Menstrual irregularities are one of the symptoms of damage to the reproductive system (7). The rate of menstrual irregularities and disorders in postmenopausal girls has been reported in different studies with different figures of 14.2% (8) and 27% (9).

In normal girls and women of reproductive age, any change in hormone levels may be associated with the metabolic syndrome index and cardiovascular risk (10). Hormonal imbalance has many consequences including hirsutism, hair loss and acne (11, 12). Polycystic ovary syndrome develops as a result of endocrine disorders such as increased serum levels of testosterone and beta estradiol, increased LH and decreased FSH to LH ratio. This leads to ovulation disorders and infertility (13). Hyperprolactinemia disrupts the gonadal response to gonadotropins and reduces the secretion of sex hormones (14). Secondary hyperthyroidism can increase serum prolactin and lead to sexual dysfunction (15). Menstrual disorders are one of the most common problems of adolescent girls. Considering the high prevalence of menstrual disorders in girls and the effects of these disorders on the effectiveness of women and girls in work, society and family, by considering the impact of individual and geographical factors on the prevalence of these disorders and the effects of menstrual disorders on physical and reproductive health students and ignoring it by families due to cultural factors. The aim of this study was to evaluate the pattern of sex hormones of the menstrual cycle related to menstrual cycle intervals in girls aged 9-18 years.

Materials and Methods

This study is a two- stage cross-sectional study conducted in 2015. The study population included all primary, middle and high school girls in four districts of Shiraz. According to previous studies (16) and the opinion of a statistician, the sample size with a 95% confidence interval, according to the formula, with a probability of a 20% drop, was 1625 people which was estimated as 2000 individuals.

$$n = \frac{Z^2 p q}{d^2}$$

P1= 0.35 P2=0.60 P3=0.05 1-α=0.95 d=0.01 q=1-p

The sampling method was multi-stage random sampling that was selected from all 4 education districts of Fars province. 500 students from each district (a total of 2000 students) were considered. Then 6-8 schools were randomly selected as clusters from each area and students of each school were selected by convenience sampling method. The study inclusion criteria in the first stage included: 9-18 years old girls, in one of the three mentioned education levels, willingness to participate in the study and completing the consent form, not taking any medication other than anti-allergies and painkillers (3 months before the study) and not having chronic physical and mental illness. The study exclusion criteria included: the occurrence of a crisis or stressful event, the student's willingness to leave the study and the parents' request for their child to leave. The present study was conducted after obtaining permission from graduate officials, approval of the ethics committee, coordination with the Department of Education, obtaining a letter of recommendation from the Security Department, presenting it to the officials of selected schools and coordinating the distribution time of the study tools. After reviewing the study inclusion and exclusion criteria and stating the objectives of the study, the researcher asked the subjects to complete a 3-part questionnaire including personal characteristics, family, health behaviors, 10-point visual pain scale, Hygam chart and menstrual bleeding profile questionnaire (duration of menstruation bleeding, the length of the menstrual cycle, the menstrual regulation using the Smith-Dijulio criteria) related to menstrual periods during the

past year. In addition, they were told that all information will remain confidential.

The amount of menstrual bleeding was measured and completed by the subjects during the menstrual periods of the past year with a Hygam Chart or Pictorial Blood Loss Assessment Chart (PBLAC) (Figure 1), as well as the number and amount of sanitary pads impregnated with blood. The researcher then calculated the amount of menstrual bleeding (by cc) based on the completed Hygam chart image. In this study, menstrual irregularities were measured using the Smith-Dijulio criteria in such a way that the interval between the longest and shortest lengths of the menstrual cycle is irregular if it is more than 6 days and regular if it is less than or equal to 6 days. The content validity method was used to determine the scientific validity of the personal / family profile questionnaire and the menstrual profile questionnaire. Thus, after studying valid scientific books and articles and fully recognizing the intervening variables, a questionnaire form was prepared and then approved by a number of faculty members. The Hyigam chart instrument is also valid for measuring the amount of menstrual bleeding and has been used in Zia study (2018) (17). The 10-point visual pain scale is a valid and reliable tool and its reliability was confirmed in the Alghadir study (2018) by the equivalent reliability method with a correlation coefficient of 91% (18).

In the second stage, students who had late menarche and girls who had normal menarche were selected, and after training before hormonal tests, the tests were performed at Hafez Educational and Medical Center. Tests included prolactin (to eliminate hyperprolactinemia), dehydroepiandrosterone ion sulfate (DHEA-S) to eliminate adrenal disease, thyroid-stimulating hormone (TSH) (to eliminate hypothyroidism), follicle-stimulating hormone (FSH), LH hormone (LH) and testosterone (TES).

46 people in each group were estimated according to the objectives, type of study and the opinion of a statistical expert in this field, taking into account the following assumptions and formula:

$$n=\frac{2(z_{1-\alpha/2}+z_{1-\beta})^2}{(\mu_1-\mu_2)^2}$$

1- β=90 α=0.05 SD=2.2

$\mu_{1-}\mu_{2=1.5}$

Considering a possible drop of 10%, finally the sample size of 50 people in each group was estimated. In the second stage, random sampling was performed on girls aged 15-18 years with the consent of parents in two groups of A (individuals with normal menarche) and B (individuals with late menarche). It should be noteded that the sample size in group A was 50, wheras it was 12 in group B, as out of 2000 students, only 12 ones had late menarche.

Figure 1. Measurement of menstrual bleeding based on the diagram of blood loss assessment (PBLAC)

Days of menstrual bleeding	1	2	3	4	5	6	7	8	9	10
The amount of hygiene pad impregnated										
A- Mild										
B- Medium										
C- Severe										
Small clot										
Big clot										

A 10-cc blood sample was taken from each person under optimal conditions (without anticoagulant). The samples were immediately placed in a 3000 rpm centrifuge for 10 minutes. CBC test was performed by High Cell **blood counter** and hormonal tests were performed by Genesys gamma counter device. After serum isolation, testosterone and DHEA-S tests were performed by radioimmunoassay and the rest of the hormones were based on sandwich immunoassay. The levels of LH, FSH, TSH and prolactin were measured using IRMA kit and testosterone and DHEA-S were measured using RIA CT kit. Besides, due to the high cost of hormonal tests, the sampling did not drop and the girls applied for complete hormonal tests.

Data were analyzed by SPSS 21 software from descriptive statistics to determine the mean, standard deviation and adjustment of absolute and relative frequency distribution tables and one-way analysis of variance to examine the significant relationship between hormone types and menstrual intervals.Significance level is considered $p < 0.05$.

The local Ethics Committee of Shiraz University of Medical Sciences approved the study protocol (number 7173). Permissions were also received through the authorities in the schools. Written informed consents were collected from all the participants.

Results

Out of 62 students, the highest number is related to the age of 16 years with 25 students (40.3%), 15 years old with 21 students (33.9%) and 18 years old with 11 people (17.7%) and the lowest number of ranges of the age was 17 years with 5 students (8.1%). In the distribution of sex hormone levels, 57 students (92.5%) had normal TSH, three students (4.8%) had low TSH (hyperthyroidism) and two students (2.6%) had high TSH (hypothyroidism). 60 students(96.8%) had normal prolactin and two students (3.2%) had high prolactin (hyperprolactinemia).

Table 1 shows the frequency distribution of sex hormones in each of the groups with and without menarche. Table 2 shows the results of one-way analysis of variance between hormone types and menstrual intervals. There is a significant relationship between TSH, Prolactin and menstrual intervals, but there is no significant relationship between LH, FSH, DHEAS, Testosterone and menstrual intervals.

Table 1. Frequency distribution of sex hormones in school girls

Hormone	Mean ± SD	Number	The lowest amount	The maximum amount	Standard value
Normal menarche					
TSH	2.25±1.94	50	0.03	12.76	0.25-5.85
FSH	5.36 ±1.74	50	0.74	10.42	2.3-9.2
LH	8.9 ±8.56	50	0.34	55.3	1.8-10
Prolactin	13.22 ±10.41	50	3.3	74.3	1.8-25
Testosterone	0.74 ±0.36	50	0.1	1.67	0.1-2.5
DHEAS	279 ±157.12	50	80	824	10-220
Late menarche					
TSH	8.6±23.12	12	0.02	81.96	0.25-5.85
FSH	5.45 ±1.8	12	1.16	7.44	2.3-9.2
LH	11.36 ±9.43	12	1.37	30.58	1.8-10
Prolactin	10.3 ±4.43	12	5.7	16.2	1.8-25
Testosterone	0.73 ±032	12	0.33	1.33	0.1-2.5
DHEAS	236.41 ±148.43	12	101	599	10-220

Table 2. The relationship between hormones and menstrual intervals in the student

Hormone	N	Mean ± SD	Standard error	F	Significance level
TSH					
<21	7	4.07 ±3.98	1.5		
21–35	33	1.93 ±1.3	0.22		
36–45	5	2.62 ±1.003	0.44	3.034	0.039
>45	5	1.44 ±0.41	0.18		
Total	50	2.25±1.94	0.27		
FSH					
<21	7	5.6 ±2.13	0.807		
21–35	33	5.26 ±1.56	0.27		
36–45	5	3.93 ±2.08	0.93	2.395	0.080
>45	5	6.004 ±1.24	0.55		
Total	50	5.36 ±1.74	0.24		
LH					
<21	7	6.44 ±2.69	1.01		
21–35	33	9.91 ±10.08	1.75		
36–45	5	5.65 ±5.13	2.29	0.570	0.637
>45	5	8.87 ±3.87	1.73		
Total	50	8.9 ±8.56	1.21		
Prolactin					
<21	7	26.18 ±22.83	8.63		
21–35	33	10.58 ±4.16	0.72		
36–45	5	12.14 ±4.09	1.82	5.544	0.002
>45	5	13.62 ±6.17	2.76		
Total	50	13.22 ±10.41	1.47		
Testosterone					
<21	7	0.75 ±0.42	0.16		
21–35	33	0.72 ±0.34	0.06		
36–45	5	0.69 ±0.54	0.24	0.473	0.703
>45	5	0.93 ±0.25	0.11		
Total	50	0.74 ±0.36	0.05		
DHEAS					
<21	7	272 ±146.15	55.24		
21–35	33	290.39 ±171.63	29.87		
36–45	5	217.4 ±113.03	50.55	0.306	0.821
>45	5	257.2 ±126.63	56.63		
Total	50	279 ±157.12	22.22		

Discussion

The present study is the first study to investigate the relationship between age between menarche, menstrual intervals and sex hormones in a large sample of adolescent girls in Shiraz. As a result, our study showed that there was a significant relationship between TSH, Prolactin and menstrual intervals, but not with other hormones. According to the study of hormones, the rate of hyperprolactinemia among the cases we studied was high similar to other studies. The prevalence varies from 0.4% in the normal adult population to 9-17% in women with menstrual disorders (19). In the Rajiwade study, hyperprolactinemia was seen in 7.7% of cases of secondary amenorrhea and 1.9% of oligomenorrhea (20). Another study (21) showed a 5.5% prevalence of hyperprolactinemia of in cases with secondary amenorrhea and 2.6% in abnormal uterine bleeding in adolescents. The Bieniasz study was performed on 117 adolescent girls aged 14-18 years; 86 patients had menstrual disorders, 9.2% of which was due to hyperprolactinemia (22). This result is consistent with the present study. 33 adolescents with menarche had a

bleeding interval of 21-35 days similar to other studies (23). According to the American College of Obstetricians and Gynecologists, using the menstrual cycle calendar as an additional vital sign is a powerful tool for assessing the normal process and eliminating pathological conditions. Identifying abnormal menstrual patterns during adolescence may allow early identification of potential health concerns for adulthood. In a study of 325 female patients, Borna et al. identified the prevalence and pattern of menstrual disorders. 55.8% of hyperthyroid patients and 51.3% of hypothyroid patients had a normal menstrual pattern. 44.2% of hyperthyroid patients had menstrual disorders, the pattern of which was more in the form of oligomenorrhea, and 48.7% of hypothyroid patients had menstrual disorders, the pattern of which was more in the form of polymenorrhea, oligomenorrhea and menorrhagia. The researchers concluded that menstrual disorders are one of the most common symptoms of thyroid disease and screening for thyroid disease is an easy and inexpensive solution to help these people. It was recommended that thyroid hormones and TSH be measured in the first step in dealing with patients with menstrual disorders (24), which, like the present study, the majority of patients had hypothyroidism. According to evaluated articles, there is a lack of studies on the prevalence of thyroid disorders in adolescents with menstrual disorders. Available data describe menstrual disorders in patients with thyroid dysfunction of women in the reproductive age group. Evidence suggests that the prevalence of menstrual disorders in thyroid dysfunction is not different from that of healthy individuals or is less associated with menstrual irregularities (19). In our study, we tried to investigate the prevalence of thyroid disorders in adolescent girls with and without menstrual disorders. The prevalence of hypothyroidism and hyperthyroidism was higher in girls with menstrual disorders than in menarche, which is consistent with other studies (21).

One of the limitations of this study is limiting the age of study inclusion criteria of the girls of 9-18 years old, which indicates the prevalence of adolescent girls. The distribution of symptoms and causes may not represent all adolescent girls with menstrual problems because the study population was selected from schools. In this regard, the data obtained in our study can be a guide for government centers to implement an appropriate health policy for the reproductive health of adolescent girls in Shiraz while the prognosis of future fertility depends on preventive assessments made during adolescence.

Conclusion

The results showed that TSH and prolactin disorders are the most common hormonal disorders in girls with menstrual disorders. It is recommended to check the serum level of these hormones in girls with various menstrual disorders after eliminating structural disorders and before starting different treatments for menstrual disorders.

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Conflicts of interest

Authors declared no conflicts of interest.

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