Role of leukotrienes in the pathogenesis of dysmenorrhea in adolescent girls

İlke Kılıç[1], Nuray Öksüz-Kanbur[1], Orhan Derman[1], Tarık Aksu[2], Özge Uysal-Soyer[3]
Ömer Kalaycı[3], Tezer Kutluk[1]
Units of [1]Adolescent Medicine and [3]Pediatric Allergy and Asthma, Department of Pediatrics and [2]Department of Gynecology and Obstetrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Kılıç İ, Öksüz-Kanbur N, Derman O, Aksu T, Uysal-Soyer Ö, Kalaycı Ö, Kutluk T. Role of leukotrienes in the pathogenesis of dysmenorrhea in adolescent girls. Turk J Pediatr 2008; 50: 521-525.

Although dysmenorrhea is a leading cause of gynecologic complaints among adolescents, its pathogenesis is incompletely understood. The purpose of this study was to determine the role of prostaglandins and leukotrienes in the pathogenesis of dysmenorrhea. Twenty patients with dysmenorrhea aged 16.2 ± 1.2 years and 20 healthy age-matched controls with eumenorrhea (absence of pain during menstruation) were included in the study. Serial measurements of serum $PGF_{2\alpha}$ and urinary LTE_4 levels during the menstrual cycle were obtained; serum progesterone was measured and ultrasonographic evaluations were made. LTE_4 and $PGF_{2\alpha}$ levels decreased on the third day and recovered on the 10th day of the menstrual cycle in both groups. Urinary LTE_4 levels were higher in the control group than in the patient group on the 1st, 3rd and 10th days of the cycle ($p < 0.05$ for each). This study suggests that there is a distinct pattern of leukotriene production during the menstrual cycle, but the changes in the systemic level are not responsible for their role in the pathogenesis of dysmenorrhea. Further studies at the local level in the target organ are necessary to elucidate the role of the lipid mediators in the pathogenesis of dysmenorrhea.

Key words: dysmenorrhea, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), leukotriene E_4 (LTE_4).

Dysmenorrhea is the feeling of pain during menstruation and may be severe enough to affect daily life in 15-60% of adolescents. Dysmenorrhea can be primary or secondary. Secondary dysmenorrhea is due to organic pelvic pathology[1-6]. In primary dysmenorrhea, there is no obvious underlying cause and its pathogenesis is incompletely understood.

In recent years, lipid mediators such as prostaglandins and leukotrienes have been implicated in the pathogenesis of dysmenorrhea by causing dysrhythmic uterine contractions and decreasing uterine blood flow[1-6]. Prostaglandins, especially $PGF_{2\alpha}$, cause uterine contractions by increasing the flow of calcium into the smooth muscle cells. PGE_2 and $F_{2\alpha}$ were found to be increased in the serum, menstrual fluid and endometrial tissue of patients with primary dysmenorrhea[7,8]. Similarly, the concentration of leukotrienes is increased in the uterine tissue and menstrual fluid samples of patients with dysmenorrhea[9,10]. In a pilot study, Harel et al.[11] demonstrated that urinary LTE_4 is increased in patients with primary dysmenorrhea. An interventional study by the same group, however, failed to show a beneficial effect of leukotriene receptor antagonism by montelukast on dysmenorrhea[12].

Even though both leukotrienes and prostaglandins have been implicated in the pathogenesis of dysmenorrhea, the combined effects of prostaglandins and leukotrienes in the biological fluids of the same patients have not been investigated. Therefore, we investigated the serial changes of $PGF_{2\alpha}$ in serum and LTE_4 in the urine during the course of a menstrual cycle in adolescent girls with and without dysmenorrhea.

Material and Methods

Adolescent girls who presented to the Adolescent Unit of Hacettepe Children's Hospital with dysmenorrhea between March-October 2006 were the subjects of the study. Twenty adolescents with primary dysmenorrhea and the first 20 adolescent girls with eumenorrhea who presented with minor problems and expressed their willingness to participate in this study formed the control group.

Dysmenorrhea was scored by the patients on visual and numeric analog scales as previously described[13,14]. In the visual analog scale, the severity of dysmenorrhea is scored by the patient on a horizontal line on a scale from 0 to 10 with zero being "no feeling of pain" and 10 being the "most severe pain". In the numeric analog scale, on the other hand, subjects graded the severity of their dysmenorrhea on a scale labeled from 0 to 10 with each number between 0 and 10 displayed on the scale[13]. Patients who had both visual and numeric analog scale scores greater than seven were included in the study. In all adolescents, a pelvic ultrasonography was done on the third day of the menstrual cycle to confirm the absence of a pelvic pathology. A retrovert uterus was considered as a normal variant and not as a cause of dysmenorrhea. Adolescents with an acute or chronic illness; with a history of abdominal and pelvic surgery; irregular menses; and those who were using contraception or were sexually active were excluded from the study.

In order to search for a relationship between the mediator levels and the ovulation status, ovulatory activity was determined in each cycle. A serum progesterone level greater than 6 ng/ml on the 21st day of the ovulatory cycle was considered to indicate ovulation[15].

Mediator Measurements

Urine and plasma samples were obtained from each subject on the 1st, 3rd and 10th days of the menstrual cycle between 9-11 a.m. Quantification of $PGF_{2\alpha}$ in plasma and LTE_4 in urine was performed with commercial EIA (ELISA immunoassay) kits according to the manufacturer's instructions (Cayman Chemicals; Ann Arbor, MI, USA). LTE_4 levels were standardized against urinary creatinine levels and the results were expressed as pg/mg creatinine.

All study procedures were done in accordance with a protocol previously approved by the Ethics Committee of Hacettepe University. All subjects provided written informed consent for the study procedures.

Statistical Analyses

Statistical analyses were performed with SPSS 11.0 for Windows. $PGF_{2\alpha}$ and LTE_4 levels were natural log transformed to obtain normal distribution ($Ln.PGF_{2\alpha}$, $LnLTE_4$). Mediator levels were compared by Student's t test and repeated measures of ANOVA. A p-value <0.05 was considered statistically significant. Post-hoc analysis for pairwise comparisons was done using the Student-Newman-Keuls method.

Results

The study and control populations were well matched with respect to age, age of menarche, body mass index values and progesterone levels ($p>0.05$) (Table I). There was no difference between the two groups with regards to the ratio of individuals with retrovert uterus.

Comparisons of Lipid Mediator Concentrations at Days 1, 3 and 10 During the Course of the Menstrual Cycle.

Table I. Characteristics of the Study Population

	Study group (n=20)	Control group (n=20)	p
Age (years)	16.2 ± 1.2	16.0 ± 1.1	>0.05*
Years since menarche	2.6 ± 1.4	3.0 ± 1.5	>0.05*
Body mass index	21.3 ± 2.9	23.7 ± 4.9	>0.05*
Visual analog scale	8.7 ± 0.9	0.9 ± 1.1	<0.05*
Numeric analog scale	8.7 ± 0. 9	0.9 ± 1.0	<0.05*
Progesterone	6.1 ± 5.0	5.5 ± 5.8	>0.05*
Retrovert uterus	4	3	>0.05†

*: Student's t test.
†: Chi square.

Study group

Among the girls with dysmenorrhea, a significant difference was observed in the $PGF_{2\alpha}$ levels on days 1, 3 and 10 ($p=0.036$, Repeated Measures ANOVA) (Fig. 1). Post hoc analysis showed that there was a significant difference between days 1 and 3 ($p=0.037$) and days 3 and 10 ($p=0.04$), but not between days 1 and 10.

Even though the urinary LTE_4 levels followed a similar pattern (Fig. 2), there was no statistically significant change between different time points in the menstrual cycle.

Control group

A significant difference was observed in the plasma $PGF_{2\alpha}$ levels on days 1, 3 and 10 ($p=0.008$, Repeated Measures ANOVA) (Fig. 1). Post hoc analysis showed that there was a significant difference between days 1 and day 3 ($p=0.006$, Student-Newman-Keuls), but not between days 1 and day 10 ($p=0.147$). The difference between days 3 and 10 approached significance ($p=0.07$).

The urinary LTE_4 levels followed a pattern similar to $PGF_{2\alpha}$ (Fig. 2). A significant difference was observed in the urinary LTE_4
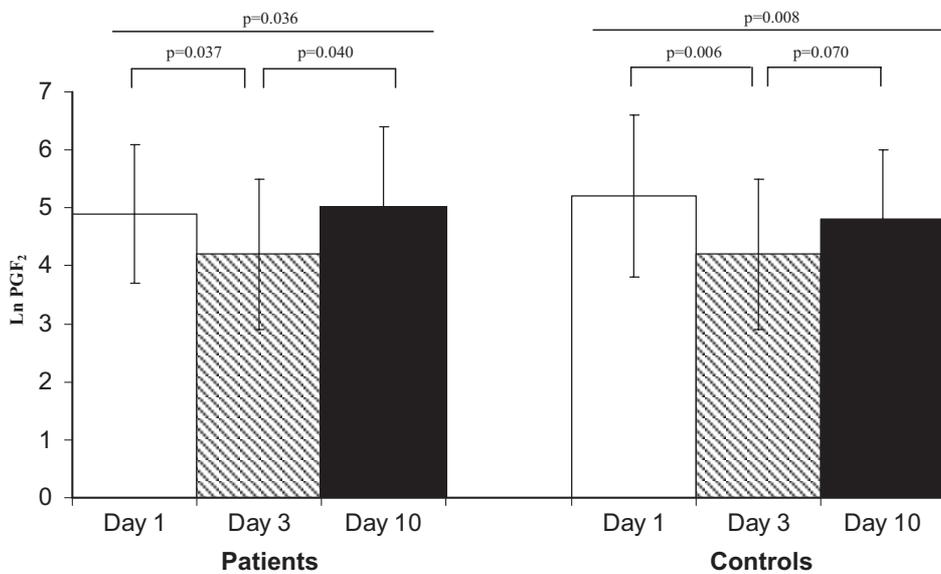

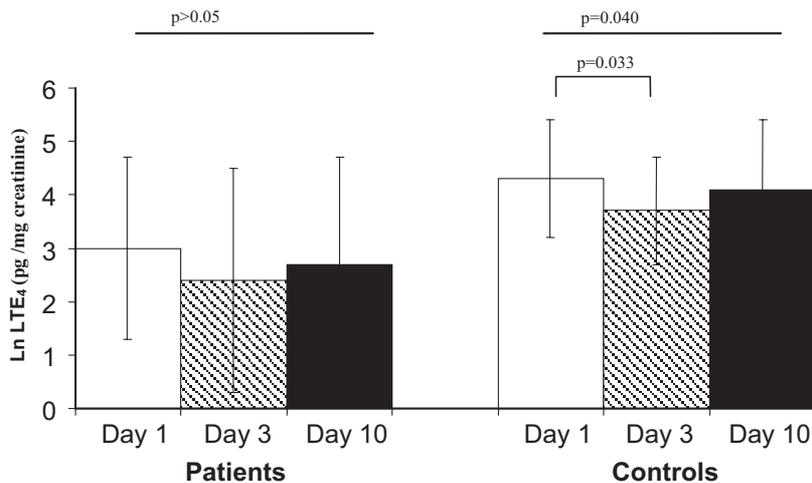**Fig. 1.** Plasma levels of $LnPGF_{2\alpha}$ in patients with dysmenorrhea and in controls.

Fig. 2. Uniray LnLTE4 levels in patients with dysmenorrhea and in controls.

levels on days 1, 3 and 10 (p=0.040, Repeated Measures ANOVA). Post hoc analysis showed that there was a significant difference between days 1 and 3 (p=0.033, Student-Newman-Keuls), but not between days 1 and 10 (p=0.3) or between days 3 and 10 (p=0.1).

Comparison of Lipid Mediator Concentrations Between the Study and Control Groups

There was no difference in the plasma $PGF_{2\alpha}$ levels on the matching days between the patient and control groups (p>0.05 for each of days 1, 3 and 10) (Table II). However, urinary LTE_4 levels were significantly higher in the control group compared to the study group on day 1 (p=0.006) and on day 10 (p=0.014). The difference on day 3 just failed to reach statistical significance (p=0.054) (Table II).

The Effect of Ovulation on Lipid Mediators

Nine subjects in the patient group and eight subjects in the control group had ovulatory cycles. There was no significant difference in the levels of LTE_4 and $PGF_{2\alpha}$ between the adolescents having ovulatory and anovulatory cycles within and between the groups.

Discussion

Our study shows that the lipid mediators $PGF_{2\alpha}$ and LTE_4 display a similar pattern in biological fluids during the course of the menstrual cycle. This pattern is represented by a significant fall from day 1 to day 3 followed by recovery to day 10. This pattern is observed in adolescents with and without dysmenorrhea and seems to be independent of the ovulation status. Interestingly, and in sharp contrast to

previous reports[11], our study shows that in this population, urinary LTE_4 levels are higher in adolescents without dysmenorrhea compared to those with dysmenorrhea on the matching days of the menstrual cycle.

The role of leukotrienes in the pathogenesis of dysmenorrhea is consistent with the observation that human uterine tissue synthesizes leukotrienes and possesses leukotriene receptors[9,10]. Increased leukotriene levels have been reported both in the urine, uterine tissue and in the menstrual fluid in patients with dysmenorrhea[10]. A recent study, upon sequential measurements on the 1st, 5th and 10th days of the menstrual cycle, found significantly elevated urinary LTE_4 levels in adolescents with dysmenorrhea on the 5th day of the cycle[11]. The results of leukotriene measurements in our study, however, do not support these observations but are consistent with a recent report that showed that treatment with a leukotriene receptor antagonist is ineffective in controlling symptoms of dysmenorrhea. Our finding of even higher leukotriene levels in patients who do not have dysmenorrhea suggests that there may be differences in receptor expression in the target organ level that render dysmenorrheic patients more susceptible to the effects of leukotrienes. In fact, since the patterns of urinary LTE_4 and systemic $PGF_{2\alpha}$ levels are identical in adolescents with and without dysmenorrhea, there is reason to believe that the pathogenetic role of the lipid mediators may actually be underlined by the receptor expression at the target level. Alternatively, considering the finding that the leukotrienes are lower in the study group, it can also be speculated that a negative feedback loop may be operative and result in lower leukotriene levels in patients with

Table II. LnPGF$_{2\alpha}$ and LnLTE4 Levels of the Groups

	Study group (n=20)	Control group (n=20)	p
LnPGF$_{2\alpha}$*			
1st day	4.9 ± 1.2 (2.9-6.9)	5.2 ± 1.4 (2.5-7.1)	>0.05
3rd day	4.2 ± 1.3 (2.3-7.9)	4.2 ± 1.3 (2.6-6.8)	>0.05
10th day	5.0 ± 1.4 (1.5-6.5)	4.8 ± 1.2 (2.4-6.2)	>0.05
LnLTE4*			
1st day	3.0 ± 1.7 (0.0-5.0)	4.3 ± 1.1 (2.4-7.0)	<0.05
3rd day	2.4 ± 2.1 (0.0-5.1)	3.7 ± 1.0 (0.0-4.9)	<0.05
10th day	2.7 ± 2.0 (0.0-4.7)	4.1 ± 1.3 (0.0-6.2)	<0.05

Mean ± standard error (interval)*
LnPGF$_{2\alpha}$: Natural log transformation of $PGF_{2\alpha}$.
LnLTE4: Natural log transformation of LTE4.

dysmenorrhea. Apparently, racial and unidentified genetic differences may be important factors; however, the factors underlying the discrepant results remain to be elucidated.

Rees et al.[10] reported increased amounts of leukotrienes in the uterine tissues obtained during hysterectomy in adult women with dysmenorrhea. Nigam et al.[16] reported increased amounts of LTC_4 and LTD_4 in the menstrual fluid samples of women with dysmenorrhea with close relation to the severity of dysmenorrhea. Therefore, the differences in the lipid mediator concentrations can be detected in the menstrual fluid even though they can not be detected at the systemic level. Adolescent girls in Turkey have a low rate of coitus and culturally it is not possible to offer the use of tampons for collection of menstrual fluid to allow local measurement of lipid mediators.

In conclusion, our study suggests that there is a distinct pattern of leukotriene production during the menstrual cycle, but the changes in the systemic level are not responsible for their role in the pathogenesis of dysmenorrhea. Further studies at the local level in the target organ are necessary to elucidate the role of the lipid mediators in the pathogenesis of dysmenorrhea.

REFERENCES

1. Dawood MY. Primary dysmenorrhea. Advances in pathogenesis and management. Obstet Gynecol 2006; 108: 428-441.

2. Kennedy S. Primary dysmenorrhea. Lancet 1997; 349: 1116.

3. Coco AS. Primary dysmenorrhea. Am Fam Physician 1999; 60: 489-496.

4. Harel Z. A contemporary approach to dysmenorrhea in adolescents. Paediatr Drugs 2002; 4: 797-805.

5. Schroeder B, Sanfilippo JS. Dysmenorrhea and pelvic pain in adolescents. Pediatr Clin North Am 1999; 46: 555-571.

6. Durain D. Primary dysmenorrhea: assessment and management update. J Midwifery Womens Health 2004; 49: 520-528.

7. Lumsden MA, Kelly RW, Baird DT. Is $PGF_{2\alpha}$ involved in the increased myometrial contractility of primary dysmenorrhea? Prostaglandins 1983; 25: 683-692.

8. Lumsden MA, Kelly RW, Baird DT. Primary dysmenorrhea: the importance of both prostaglandins E_2 and $F_{2\alpha}$. Br J Obstet Gynaecol 1983; 90: 1135-1140.

9. Abu JI, Konje JC. Leukotrienes in gynaecology: the hypothetical value of anti-leukotriene therapy in dysmenorrhea and endometriosis. Hum Reprod Update 2000; 6: 200-205.

10. Rees MC, Dimarzo V, Lippins JR, Turnbull AC. Leukotriene release by endometrium and myometrium throughout the menstrual cycle in dysmenorrhea and menorrhagia. J Endocrinol 1987; 113: 291-295.

11. Harel Z, Lilly C, Riggs S, Vaz R, Drazen J. Urinary LTE_4 in adolescents with dysmenorrhea: a pilot study. J Adolesc Health 2000; 27: 151-154.

12. Harel Z, Riggs S, Vaz R, Flanagan P, Harel D. The use of leukotriene receptor antagonist montelukast (singulair®) in the management of dysmenorrhea in adolescents. J Pediatr Adolesc Gynecol 2004; 17: 183-186.

13. Larroy C. Comparing visual-analog and numeric scales for assessing menstrual pain. Behav Med 2002; 27: 179-181.

14. Derman O, Kanbur NÖ, Baltacı G, Akbayrak T, Tokur T. The pain intensity level in adolescents with primary dysmenorrhea. The Pain Clinic 2004; 16: 349-352.

15. De Leo V, Musacchio MC, Morgante G, Piomboni P, Petraglia F. Metformin treatment is effective in obese teenage girls with PCOS. Hum Reprod 2006; 21: 2252-2256.

16. Nigam S, Benedetto C. Increased concentrations of eicosanoids and platelet-activating factor in menstrual blood from women with primary dysmenorrhea. Eicosanoids 1991; 4: 137-141.