Role of leukotrienes in the pathogenesis of dysmenorrhea in adolescent girls

İlke Kılıç1, Nuray Öksüz-Kanbur1, Orhan Derman1, Tarık Aksu2, Özge Uysal-Soyer3 Ömer Kalaycı3, Tezer Kutluk1

Units of 1Adolescent Medicine and 3Pediatric Allergy and Asthma, Department of Pediatrics and 2Department of Gynecology and Obstetrics, Hacettepe University Faculty of Medicine, Ankara, Turkey


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 PGF2α and urinary LTE4 levels during the menstrual cycle were obtained; serum progesterone was measured and ultrasonographic evaluations were made. LTE4 and PGF2α levels decreased on the third day and recovered on the 10th day of the menstrual cycle in both groups. Urinary LTE4 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 F2α (PGF2α), leukotriene E4 (LTE4).

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 pathology1-6. 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 flow1-6. Prostaglandins, especially PGF2α, cause uterine contractions by increasing the flow of calcium into the smooth muscle cells. PGE2 and F2α were found to be increased in the serum, menstrual fluid and endometrial tissue of patients with primary dysmenorrhea7,8. Similarly, the concentration of leukotrienes is increased in the uterine tissue and menstrual fluid samples of patients with dysmenorrhea9,10. In a pilot study, Harel et al.11 demonstrated that urinary LTE4 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 dysmenorrhea12.

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 PGF2α in serum and LTE4 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\textsuperscript{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\textsuperscript{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 21\textsuperscript{st} day of the ovulatory cycle was considered to indicate ovulation\textsuperscript{15}.

Mediator Measurements

Urine and plasma samples were obtained from each subject on the 1\textsuperscript{st}, 3\textsuperscript{rd} and 10\textsuperscript{th} days of the menstrual cycle between 9-11 a.m. Quantification of PGF\textsubscript{2α} in plasma and LTE\textsubscript{4} in urine was performed with commercial EIA (ELISA immunoassay) kits according to the manufacturer’s instructions (Cayman Chemicals; Ann Arbor, MI, USA). LTE\textsubscript{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\textsubscript{2α} and LTE\textsubscript{4} levels were natural log transformed to obtain normal distribution (Ln.PGF\textsubscript{2α}, LnLTE\textsubscript{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

<table>
<thead>
<tr>
<th></th>
<th>Study group (n=20)</th>
<th>Control group (n=20)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.2 ± 1.2</td>
<td>16.0 ± 1.1</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Years since menarche</td>
<td>2.6 ± 1.4</td>
<td>3.0 ± 1.5</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Body mass index</td>
<td>21.3 ± 2.9</td>
<td>23.7 ± 4.9</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Visual analog scale</td>
<td>8.7 ± 0.9</td>
<td>0.9 ± 1.1</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Numeric analog scale</td>
<td>8.7 ± 0.9</td>
<td>0.9 ± 1.0</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Progesterone</td>
<td>6.1 ± 5.0</td>
<td>5.5 ± 5.8</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Retrovert uterus</td>
<td>4</td>
<td>3</td>
<td>&gt;0.05†</td>
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*: 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$_4$.
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 1$^{st}$, 5$^{th}$ and 10$^{th}$ days of the menstrual cycle, found significantly elevated urinary LTE$_4$ levels in adolescents with dysmenorrhea on the 5$^{th}$ 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>
<thead>
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<th>Table II. LnPGF$_{2\alpha}$ and LnLTE4 Levels of the Groups</th>
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<tr>
<td>LnPGF$_{2\alpha}^*$</td>
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<tr>
<td>Study group (n=20) Control group (n=20) p</td>
</tr>
<tr>
<td>1$^{st}$ day 4.9 ± 1.2 (2.9-6.9) 5.2 ± 1.4 (2.5-7.1)  &gt;0.05</td>
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<tr>
<td>3$^{rd}$ day 4.2 ± 1.3 (2.3-7.9) 4.2 ± 1.3 (2.6-6.8)  &gt;0.05</td>
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<tr>
<td>10$^{th}$ day 5.0 ± 1.4 (1.5-6.5) 4.8 ± 1.2 (2.4-6.2) &gt;0.05</td>
</tr>
<tr>
<td>LnLTE4$^*$</td>
</tr>
<tr>
<td>1$^{st}$ day 3.0 ± 1.7 (0.0-5.0) 4.3 ± 1.1 (2.4-7.0) &lt;0.05</td>
</tr>
<tr>
<td>3$^{rd}$ day 2.4 ± 2.1 (0.0-5.1) 3.7 ± 1.0 (0.0-4.9) &lt;0.05</td>
</tr>
<tr>
<td>10$^{th}$ day 2.7 ± 2.0 (0.0-4.7) 4.1 ± 1.3 (0.0-6.2) &lt;0.05</td>
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</tbody>
</table>

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.\textsuperscript{10} reported increased amounts of leukotrienes in the uterine tissues obtained during hysterectomy in adult women with dysmenorrhea. Nigam et al.\textsuperscript{16} reported increased amounts of LTC\textsubscript{4} and LTD\textsubscript{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 cannot 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.

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