Original Article

Three models of primary dysmenorrhea via estradiolbenzoate and oxytocin induction: an experimental study

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Objective: To evaluate the PGF2 profile and its association with symptoms in several models of primary dysmenorrhea.

Methodology: Forty Sprague Dawley rats were placed into four groups, each with ten rats: control, primary dysmenorrhea 1 (PD1), primary dysmenorrhea 2 (PD2), and primary dysmenorrhea 3 (PD3). The pain as main dysmenorrhea symptom was assessed by observing writhin glatency and frequency. The enzyme linked immunosorbent assay method was used to evaluate PGF2 levels in serum. Using hematoxyline eosin staining, the uterus was examined histopathologically.

Results: The PD3 model had writhing characteristics, PGF2 levels, and degree of inflammation that are comparable to the PD2 model.

Conclusion: Our proposed PD model has clinical and biomolecular and histopathological characteristics comparable to existing models. Thus, this new model can be an alternative for modeling primary dysmenorrhea.

Keywords: Primaryd ysmenorrhea, inflammation, uterus, prostaglandins, experimental animal models.

INTRODUCTION

Dysmenorrhea is a Greek term meaning trouble with menstrual flow.[1] Dysmenorrhea is described as pain experienced throughout the menstrual cycle that is either associated with pelvic disease (secondary dysmenorrhea) or absent (primary dysmenorrhea/PD). Severe pain can interfere with everyday activities, education, or employment.[2] According to a study spanning the last 60 years, 45–95% of women worldwide who are menstruation suffer from this illness.[3] Several Asian nations have greater rates of dysmenorrhea than European nations.[4,5]

The basic mechanism of PD is still unknown, although one idea holds that this pathology is based on increased prostanoid production due to cyclooxygenase pathway activation, which includes prostaglandins, thromboxanes, and prostacyclin.[6-8] PGF2 and PGE are two prostanoids that are frequently researched in Parkinson's disease. When the endometrium creates and releases too much PGF2, it causes uterine smooth muscle spasms and contractions, resulting in an increase in PGE. The PGF/PGE ratio will accurately reflect PG's effect on the uterus. However, the PGF/PGE ratio remains a source of contention. Raising the PGF/PGE ratio has a detrimental influence on the endometrium.[9] Complementary therapies cause uterine relaxation by increasing or decreasing the PGF/PGE ratio.[10,11]

To study pathomechanisms and therapy, experimental animal models are used. To the knowledge of researchers, there are two models of PD through the induction of estradiol benzoate and oxytocin.[12,13] In both models, prostaglandin measurements are carried out directly in the uterus. This makes this model irrelevant for studying PD progression through measuring PG dynamics. Therefore, this study aims to compare the dynamics of circulating PG in the two existing PD models. In addition, we also investigated new PD models by modifying the dose of PD induction drugs.

METHODOLOGY

The study was approved by the research ethics committee of the Faculty of Medicine, Sebelas Maret University, Surakarta, Centralof Java, Indonesia (Approval number: 162/UN27.06.11/KEP/EC/2022; Date: November 22, 2022).

Subjects: This study had 40 female Sprague Dawley

rats. All were healthy and active. Estrous cycle screening was performed after 7 days of acclimatization. The rats wererandomizedforthetrialifthey had normal estrouscycles. The group was divided into four groups with 10 in each group: the control group, the primary dysmenorrhea model 1 group (PD1), the primary dysmenorrhea model 2 group (PD2), and the primary dysmenorrhea model 3 group (PD3).

Modeling of primary dysmenorrhea: Subcutaneous injections of estradiolbenzoate and intraperitoneal injections of oxytocin were used to induce primary dysmenorrhea. We used the prior method for the first group (PD1).[14] On days 1 and 10 of thee strous cycle, rats were given 0.5 mg of estradiolbenzoate and 0.2 mg on days 2 to 9. After 1 hour of the previous day's treatment, rats were given 2 U of oxytocin.

A prior study[15] was used for the second group (PD2). During the estrous cycle, rats were given 5 mg of estradiolbenzoate on days 1 and 10 and 3 mg on days 2 to 9. After 1 hour of the previous day's treatment, rats were given 3 U of oxytocin. We offered a new technique for the third group (PD3). During the estrous cycle, rats were given 1 mg estradiolbenzoate on days 1 and 10 and 0.5 mg estradiolbenzoate on days 2 to 9. After 1 hour of the previous day's treatment, rats were given 1.5 U ofoxytocin.

Detection of writhing latency and frequency: The main dysmenorrhea model was evaluated by examining each rat's movement for a duration of 20 minutes. A twisting reaction in rat models (abdominal contraction, concave, trunk, and hind limb extension, rotation of one limb, uterine contraction) suggested the presence of a pain in primary dysmenorrhea model.[18]

Measurement of PGF2α levels: We used enzyme linked immunosorbent assay technique to measure PGF2 levels in serum. We use the Rat PGF2 ELISA Kit brand FINE TEST catalog number ER1257 (Wuhan Fine Biotech Co., Ltd. China). We carried out detailed measurement steps according to manufacturer guidelines.

Histopathological analysis: After oxytocin induction, euthanasia and organ isolation will be carried out. Euthanasia was performed by cervical dislocation. The uterus was removed and washed with physiological saline and then soaked in a 4% formaldehyde solution for 72 hours.[20] Next, the uterine tissue was dehydrated in stages, then transparent, waxed immersion, embedded, cut to a thickness of 3-5 µm and then stained with hematoxylin eosin.[21] The preparations were then observed to calculate endometrial thickness, number of vacuolated cells, and inflammation in the endometrium using an optical microscope (CX23, Olympus, Japan).

Measurement of the thickness of the uterus from the edge of the cavity to the border of the endometrium was carried out in cross section and viewed from eight zones, then the results are taken as an average of the eight zones. Counting the vacuole cells from eight zones then taking the average of the results from the eight zones with 400x magnification. Inflammation assessment was done using scoring techniques. Score 1 if plasma cells and lymphocytes were found in small numbers. Score 2 if plasma cells, eosinophils and neutrophils were found in large numbers. Score 3 if there were many inflammatory cells and microabscesses.

Statistical analysis: All data were tabulated and analyzed using non parametric test. $p<0.05$ was stated to be significantly different. The statistical analysis were performed using SPSS version 16.

RESULTS

The three model groups showed a greater frequency of writhing than the control group ($p<0.05$), but there were no significant differences between the three groups ($p>0.05$). Meanwhile, writhing latency was also found to increase in the three model groups compared to controls ($p<0.05$). There were no significant differences in writhing latency between the three research groups (Table 1).

On the first day of observation, no significant differences were found in the PGF2α levels of all study groups. The same results were also found for the tenth day. For the fifth day, PGF2α levels in the PD1 group were significantly smaller than those in the control group ($p<0.05$). Mean while, PGF2α levels increased significantly in PD2 and PD3 compared to the control group ($p<0.05$), but the values were comparable for the two groups ($p>0.05$) (Fig. 1).

Endometrial thickness and cell vacuolization did not differ significantly between all groups ($p>0.05$) (Fig. 2). The degree of inflammation in the three model groups was significantly higher than the control group ($p>0.05$). This increase was

Table 1: Writhing frequency and latency in allgroups.

	Control	PD1	PD2	PD3
Writhing frequency (unit)	0.0000 ± 0.0000	15.6666 ± 4.9328[a]	24.000 ± 6.2450[a]	18.0000 ± 2.6547[a]
Writhing latency (min)	0.0000 ± 0.0000	6.6667 ± 1.5275[a]	6.000 ± 1.7321[a]	6.6666 ± 1.5275[a]

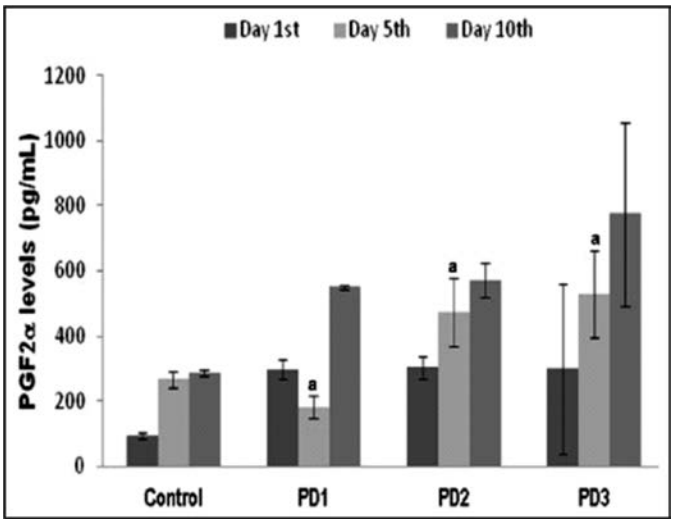

Fig. 1: Levelsof PGF2α serum in all groups of experiment.

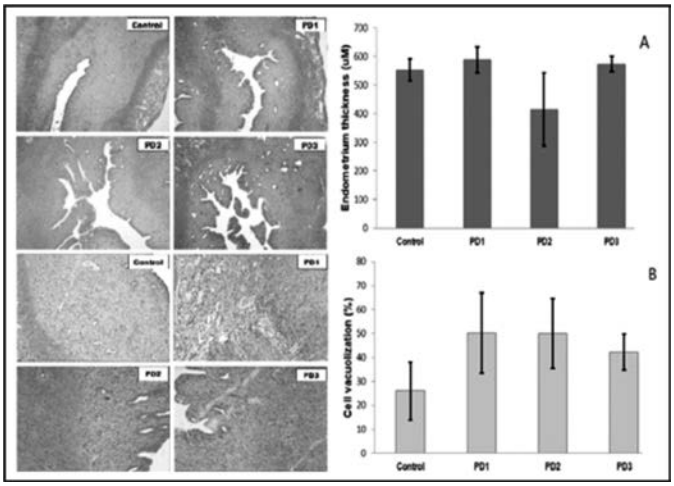

Fig. 2: Thickness of endometrium (A) and cell vacuolization (B) in variousgroups.

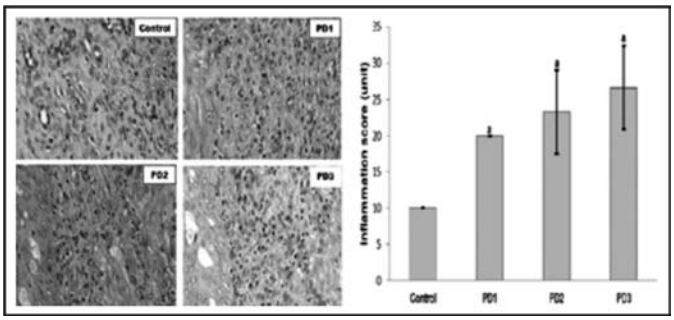

Fig. 3: Degreeofinflammation in the endometrium in all experimentalgroups.

comparable between the three model groups (p>0.05) (Fig. 3).

DISCUSSION

Animals chosen for study in order to elicit particular illnesses or symptoms have to fulfill a number of requirements. The animal must, first and foremost, exhibit a phenotype that is humanoid. Second, there must be no differences in the way diseases arise in people and animals. Third, in order to cure human diseases, experimental animals need to be sensitive to both pharmacological and non-pharmacological therapies. Fourth, animal models' biological mechanisms ought to resemble human biological systems in most cases. Fifth, creating models is less invasive and more reasonably priced.[22,23] Our primary discovery is that the PD animal model phenotype of our induction model is similar to that of earlier models.

In this study, clinically based on symptoms, it was seen that the three models displayed increased frequency and decreased writhing latency. Additionally, all three models showed increased inflammation. This indicates that various doses of estradiol benzoate and oxytocin will show the same clinical picture.

The interesting thing refers to PGF2α levels, in the form of an increase for PD2 and PD3, whereas for PD1 a significant decrease was found compared to controls. These indicate that PD2 and PD3 display better oxytocin sensitivity preparations than PD2. Estradiol benzoate functions to expand oxytocin sensitivity and trigger contractions of smooth muscle cells in the uterus. Oxytocin sensitivity is likely due to an increase in the number of oxytocin receptors in the cytoplasm of uterine smooth muscle cells. If this interaction occurs, prostaglandins will be produced and subsequent uterine spasms will occur.[26]

Prostaglandins are associated with pain and inflammation during menstruation. The amount of prostaglandin produced is positively correlated with pain severity. In this inflammatory process, PGF2α and PGE eachplay a specific role. The arcuate blood vessels will constrict due to PGF2α activation, resulting in localhypoxia in the endometrium. Specific to smooth muscle cells, contraction will occur. On the other hand, the action of PGE is determined by its receptor, including relaxing endometrial blood vessels or triggering swelling and recruitment of leukotrienes.

Furthermore, prostaglandins also trigger the recruitment of chemokines, growth factors, migration of neutrophils and leukocytes to the endometrium.[3] Our findings were an increase in serum PGF2α levels for PD2 and PD3 models, while a decrease for PD1. This increase in levels is consistent with previous studies.[12] If this is connected to the emergence of writhing then all models experience an increase in writhing. We hypothesize that this phenomenon is caused by the action of PGE determined by its receptor.[12]

In this study, we found inflammatory involvement in

all three PD models. This is consistent with previous studies that acute inflammation accompanies the development of dysmenorrhea.[22] This inflammation is the end result of an imbalance between pro-inflammatory and anti-inflammatory factors. Proinflammatory factors include PGF2α, TNFα, IL-6, CRP, and VEGF. Anti-inflammatory factors for example biliverdin.[6,23,24]

CONCLUSION

We conclude that our proposed PD model has clinical and biomolecular and histopathological characteristics comparable to existing models. Thus, this new model can be an alternative for modeling primary dysmenorrhea.

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SL: Conceptualization; project admiinistration; methodology; resources, data curation, investigation, formal analysis, software, writing – original draft.
DGT: Conceptualization; project administration; methodology; resources; writing – review and editing.
SS: Conceptualization; methodology; formal analysis, writing – review and editing.
DI: Conceptualization; project administration; methodology; resources; writing – review and editing.
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REFERENCES

1. Burnett M, Lemyre M. No. 345-primary dysmenorrhea consensus guideline. J Obstet. Gynaecol Can 2017;39(7):585–95.
2. Durand H, Monahan K, McGuire BE. Prevalence and impact of dysmenorrhea among university students in Ireland. Pain Med 2021;22(12):2835-45.
3. Iacovides S, Avidon I, Baker FC. What we know about primary dysmenorrhea today: a critical review. Hum Reprod Update 2015;21(6):762–78.
4. Abreu-Sánchez A, Parra-Fernández ML, Onieva-Zafra MD, Ramos-Pichardo JD, Fernández-Martínez E. Type of dysmenorrhea, menstrual characteristics and symptoms in nursing students in Southern Spain. Healthcare (Basel) 2020;8(3):302-6.
5. Karout S, Soubra L, Rahme D, Karout L, Khojah HM, Itani R. Prevalence, risk factors, and management practices of primary dysmenorrhea among young females. BMC Womens Health 2021;21(1):392.
6. Ferries-Rowe E, Corey E, Archer JS. Primary dysmenorrhea: diagnosis andtherapy. Obstet Gynecol 2020;136(5):1047–58.
7. Gan J, Zhu S, Ma X, Ding X, Deng Y, Wang Y, et al. The effect of Ding-kun-dan comparing with Marvelon on primary dysmenorrhea: A prospective, double-blind, multicenter, randomized controlled trial. J Ethnopharmacol 2024;318(Pt B):116975.
8. Harel Z. Cyclooxygenase-2 specific inhibitors in the treatment of dysmenorrhea. J Pediatr Adolesc Gynecol 2004;17(2):75–79.
9. Dawood MY, Khan-Dawood FS. Clinical efficacy and differential inhibition of menstrual fluid prostaglandin F2alpha in a randomized, double-blind, cross over treatment with placebo, acetaminophen, andibuprofen in primary dysmenorrhea. Am J Obstet Gynecol 2007;196(1):e1–e35.
10. Chen YX, Ma K. Evaluation of clinical application of Dingkun Dan. Zhongguo Zhong Yai Za Zhi 2015;40(20):3916–9.
11. Macedo CAF, de Paiva GO, Menezes PMN, Ribeiro TF, Brito MC, Vilela DAD, et al. *Lippiaoriganoides* essential oil induces tocolytic effect in virginrat uterus and inhibits writhing in a dysmenorrhea mouse model. J Ethnopharmacol 2022;290(23):115099.
12. Li X, Guo S, Chen Z, Ren K, Zhang H, Yu S, et al. Regulation of mild moxibustion on uterine vascular and prostaglandin contents in primary dysmenorrhea rat model. Evid Based Complement Alternat Med 2021;2021:9949642.
13. Bi W, Zhou, J, Zhao L, Wang C, Wu W, Zhang L, et al. Preventive effect of different citrus essential oil son primary dysmenorrhea: *in vivo*and*in vitro*study. Food Biosci 2021;42:101135.
14. An G, Chen X, Li C, Zhang L, Wei M, Chen J, et al. Pathophysiological changes in female rats with estrousсycle disorder induced by long-term heat stress. Biomed Res Int 2020; 2020:4701563.
15. Wang D, Cheng X, Fang H, Ren Y, Li X, Ren W, et al. Effect of cold stress on ovarian & uterine microcirculation in rats and the role of endothelin system. Reprod Biol Endocrinol 2020;18:29-34.
16. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci 2010;13(10):1161–9.
17. Poznyak AV, Silaeva YY, Orekhov AN, Deykin AV. Animal models of human atherosclerosis: current progress. Braz J Med Biol Res 2020;53(6):e9557.
18. Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. Biol Mood Anxiety Disord 2011;1 (1):9-14.
19. Peng Y, Qing X, Shu H, Tian S, Yang W, Chen S, et al. Proper animal experimental designs for preclinical

research of biomaterials for intervertebral disc regeneration. BiomaterTransl 2021;2(2):91-142.
20. Li J, Liu X, Jian M, Xu Y, Wang C, Hu Y, et al. Wenjing Zhitong recipe exhibits potential anti-primary dysmenorrhea properties by inhibiting COX2 and PKC signaling pathway in rats induced by estradiolbenzoate and oxytocin. J Trad Chinese Med Sci 2023;10(3):296-309.
21. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 2012;13(4):277–88.
22. Fajrin I, Alam G, Usman AN. Prostaglandin level of primary dysmenorrhea pain sufferers. EnfermeriaClinica 2020;30(Suppl 2):5-9.
23. Chen LX, Hu DJ, Xu WF, Li SP, Zhao J. Identification andd etermination of fructooligosaccharides in snowchrysanthemum (*Coreopsistinctorianutt.*). World J Tradit Chin Med 2021;7(1):78–85.
24. Zhang F, Liu XL, Wang W, Dong HL, Xia YF, Ruan LP, et al. Expression of MMIF, HIF-1α and VEGF in serum and endometrial tissues of patients with endometriosis. Curr Med Sci 2018;38(3):499–504.