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# Primary Dysmenorrhea: pathophysiology.

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**Key words**: menstruation; menstrual cycle; dysmenorrhea; primary dysmenorrhea; pathophysiology; inflammation.

**Abstract.** The present study was conducted to investigate and analyze the recent and relevant studies about primary dysmenorrhea and its pathophysiology. Literature searches were performed electronically in PubMed, Medline, ISI, DOAJ, Springer, Embase. Web of Knowledge, DOAJ, Google Scholar and the Cochrane Library for original articles written in English and in Scielo, Lantidex, Imbiomed-L, Redalyc and Google Scholar for original articles written in Spanish. The searches included the key words (Mesh): menstruation, menstrual period, menstrual cycle, dysmenorrhea, primary dysmenorrhea, inflammatory substance and inflammatory markers. Publications from January 1980 to February 2021 were reviewed. Dysmenorrhea is the most common gynecologic condition experienced by menstruating women. It is characterized by crampy lower abdominal pain that can range widely in severity, and associated to others symptoms. Its overall impact often has significant medical and psychosocial implications. The hallmark of primary dysmenorrhea is painful menses in the absence of any associated macroscopic pathologic process, and it occurs in up to 50% of menstruating females and causes significant disruption in quality of life and absenteeism. An excessive or imbalanced amount of prostanoids and possibly eicosanoids released from the endometrium during menstruation have been mentioned as the main cause of primary dysmenorrhea. The uterus is induced to contract frequently and dysrhythmically, with increased basal tone and increased active pressure. Uterine hypercontractility, reduced uterine blood flow and increased peripheral nerve hypersensitivity induce pain. Diagnosis rests on a good history with negative pelvic evaluation findings. This narrative review investigated and analyzed the pathophysiology of primary dysmenorrhea and the implications of other chemical substances.

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## Dismenorrea Primaria: fisiopatología.

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**Palabras clave:** menstruación; ciclo menstrual; dismenorrea; dismenorrea primaria; patofisiología; inflamación.

**Resumen.** La presente revisión fue realizada con el objeto de investigar y analizar los estudios más recientes y relevantes sobre la dismenorrea primaria y su fisiopatología. La literatura revisada fue realizada electrónicamente en PubMed, Medline, ISI, DOAJ, Springer, Embase. Web of Knowledge, DOAJ, Google Scholar y la Librería Cochrane para los artículos escritos en inglés. Scielo, Lantidex, Imbiomed-L, Redalyc y Google Scholar fueron revisados en búsqueda de artículos escritos en español. La búsqueda incluyó las palabras claves (Mesh): menstruación, periodo menstrual, ciclo menstrual, dismenorrea, dismenorrea primaria, substancias inflamatorias y marcadores inflamatorios. Se revisaron publicaciones desde enero 1980 a febrero 2021. La dismenorrea es la condición ginecológica más común presente en la mujer menstruante. Se caracteriza por dolor en el abdomen bajo tipo cólico, su severidad es variable y está asociada con otros síntomas. Su impacto general tiene implicaciones médicas y psicosociales significativas. La característica de la dismenorrea primaria son las menstruaciones dolorosas en ausencia de un proceso patológico macroscópico, ocurre hasta en un 50% de las mujeres menstruantes y causa alteración de la calidad de vida y ausentismo. Un exceso o desbalance de la cantidad de prostanoides y posiblemente liberación de eicosanoides por el endometrio durante la menstruación han sido mencionados como la principal causa de la dismenorrea primaria. El útero es inducido a contraerse frecuente y disritmicamente con aumento del tono basal e incremento de presión activa. La hipercontractibilidad del útero reduce el flujo sanguíneo e incrementa la hipersensibilidad periférica lo cual induce dolor. Esta revisión narrativa investigó y analizó la patofisiología de la dismenorrea primaria y las implicaciones de otras sustancias químicas.

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### INTRODUCTION

The uterus is a dynamic organ. It not only responds and changes in a sensitive way to action of the classic hormonal signals (the endocrine events of the menstrual cycle), but it is also composed of complex tissues, with important autocrine and paracrine functions. The most dynamic tissue of the uterus is the endometrium. Every endometrial cycle has, as its only goal, the support of an early embryo (1). If the pregnancy does not occur, menstruation follows by the end of the menstrual cycle. As we know, menstruation, also known as menses, menstrual period or period, is the regular discharge of blood and mucosal tissue from the inner lining of the uterus through the vagina. Approximately 50% of the menstrual detritus is expelled in the first 24 hours of menstrual flow. The menstrual fluid is composed of the autolyzed endometrial tissue, inflammatory exudate,

red blood cells and proteolytic enzymes. The autolyzed endometrial tissue is composed of a variety of functional states including disarray and breakage of glands, fragmentation of vessels and stroma with persisting evidence of necrosis. The high fibrinolytic activity allows the emptying of the uterus by liquefaction of tissue and fibrin (1,2).

It is becoming increasingly accepted that many normal reproductive processes exhibit signs of inflammation. Such processes, include ovulation, menstruation, implantation and parturition (3). These events are associated with up-regulation in the expression of a host of inflammatory mediators, which include cytokines, growth factors and lipid mediators that influence the growth and function of the immune and vascular compartments (3-5). In addition, the female reproductive tract has the remarkable characteristic, which is the capacity to resolve these inflammatory events rapidly in order to re-establish the normal reproductive functions (5). The resolution of these inflammation events is the clearance of leukocytes and tissue debris as well as restoration of mucosal and vascular function in the affected tissue. Inflammation is an active process that involves the release of inflammatory cytokines, chemokines and peptide growth factors. This establishes a gradient for the recruitment of neutrophils and macrophages to the site of injury. Injury also promotes the activation of the coagulation and fibrinolysis system, which operates in tandem to control clotting and remodelling of the vasculature. This facilitates tissue regeneration and extravasation of neutrophils at the site of injury via dilatation and edema. Tissue remodeling also involves production of local inflammatory mediators such as kinins, histamine and eicosanoids such as prostanoids (prostaglandins, prostacyclins and thromboxanes) and leukotrienes (5).

The endometrium secretes many substances and regulating molecules. Besides, to produce a supportive and nutritive environment for the early embryo, the endometrium plays an important role in suppressing the immune response within the pregnant uterus. The endometrium secretes or produces different substances or regulating molecules involved in inflammation and immune responses such as prostaglandins, leukotrienes, tromboxanes, different cytokines: interleukin (IL)-1α, IL-1β, IL-6, Interferon-γ, colony stimulating factor-1, tumor necrosis factor-α, leukemia inhibiting factor (1).

There are three disorders related to the menstruation or menstrual period: premenstrual syndrome or premenstrual dysphoric disorder, premenstrual migraine and dysmenorrhea (6),

The objective of this narrative review article is to review and analyze the pathophysiology and the role of the different inflammatory markers have in the pathogenesis of the primary dysmenorrhea (PD).

## MATERIAL AND METHODS

### Study design

The present study was conducted to investigate and analyze recent and relevant studies about PD and inflammatory markers. Studies published in English and Spanish were included in the review.

In accordance with the PRISMA guidelines, we identified published studies through a systematic and electronical review of the literature searches of PubMed, Medline, ISI, DOAJ, Springer, Embase. Web of Knowledge, DOAJ, Google Scholar and the Cochrane Library for original articles written in English and in Scielo, Latindex, Imbiomed-L, Redalyc and Google Scholar for original articles written in Spanish. The searches included the key words (MESH): menstruation, menstrual period, menstrual cycle, dysmenorrhea, primary dysmenorrhea, inflammatory substance, inflammatory markers, and the following search terms: Dysmenorrhea" AND "menstrual cycle OR menstrual period OR inflammatory markers OR inflammatory substances OR pathophysiology". In addition, reference lists and citation histories were checked during the search.

**Inclusion Criteria**

Selection criteria included randomized clinical trials, observational trials, open-label non-randomized trials and case reports. The Cochrane Library was searched for reviews. Publications from January 1980 to February 2021 were reviewed.

**Information Extraction**

The electronic search and eligibility of the studies were evaluated by the author. The author reviewed, analyzed and discussed the PD and inflammatory markers (see Fig. 1).

**Dysmenorrhea**

Dysmenorrhea is one of the most common gynecologic problems in reproductive age women and is the most common gynecological symptom reported by women, irrespective of nationality and age (6-8). Dysmenorrhea is the pain that occurs with menstruation, usually cramping in nature and centered in the lower abdomen (6). The term dysmenorrhea is derived from the Greek words *dys* (difficult, painful, or abnormal), *meno* (month), and *rrhea* (flow). Dysmenorrhea has significant medical and

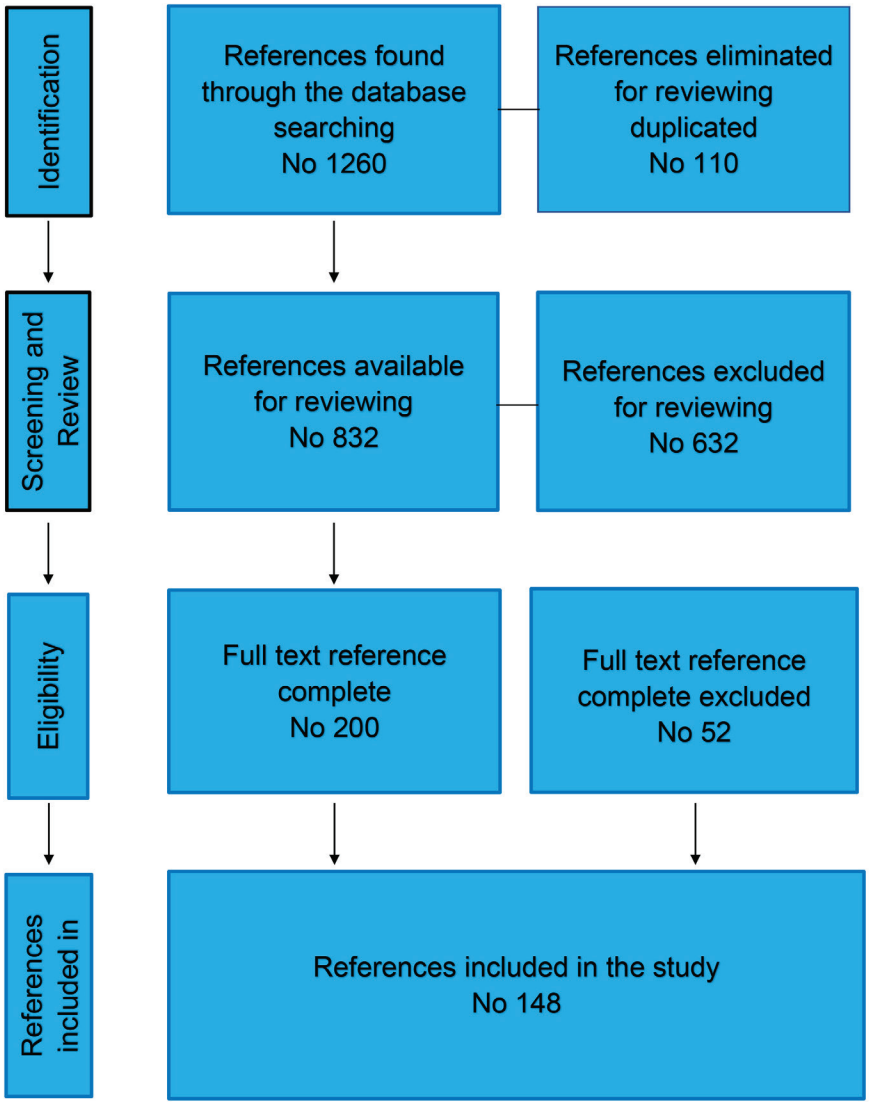


**Fig. 1.** Flow chart of study selection.

psychosocial implications. Dysmenorrhea is potentially the most underdiagnosed gynecologic condition because of common societal beliefs regarding a lack of effective treatments and expectations about the burden of menstruation (9). According to World Health Organization, the prevalence of dysmenorrhea ranges between 1.7% to 97% (10). Ninety percent of women experience some severity of menstrual pain, which is variable by geographical location and population. One third to one half of these women report moderate or severe symptoms (6-8,11-20). Symptoms are frequently associated with time lost from school, work or other activities (21). In spite of the frequency and severity of dysmenorrhea, most women do not seek medical treatment for this condition (22).

Dysmenorrhea is classified as primary and secondary (6). Table I shows the differences between primary and secondary dysmenorrhea. The age is an important and determinant factor of menstrual pain, being more pronounced in adolescents than in older women (22,23). Associated factors for more severe episodes of dysmenorrhea may include early menarche, heavy and increased duration of menstrual flow and family history (7,12, 24). There is some evidence that parous women have less severe dysmenorrhea (23-25)· The studies have shown that smoking and alcohol consumption worsens primary menstrual pain (7,19, 23, 25, 26)· There is some suggestion that frequent life changes, fewer social supports, and stressful close relationships may be associated with increase of dysmenorrhea (27). Different authors (28,29) mentioned that mood disorders are associated with primary dysmenorrhea (PD). There may be an increased prevalence of dysmenorrhea in lower socioeconomic groups (11). Also, there is controversy about the association of obesity (12,24), physical activity (24,26) and alcohol (12,24-26) with PD.

**Primary Dysmenorrhea**

Primary dysmenorrhea usually begins during adolescence, only after ovulatory cycles are established; usually, the symptoms start after the first 6-months and 24 months after the onset of menarche (6,30-32); 20–45% of teenage girls are ovulatory by 2 years and 80% by 4–5 years after menarche (6).

The prevalence of primary dysmenorrhea is highly underestimated, and is difficult to establish, because few affected women seek medical treatment, despite the substantial distress experienced, as many consider the pain to be a normal part of the menstrual cycle rather than a disorder (28). Many cases thus remain undocumented (33). Due to the lack of standard methods for assessing the severity of dysmenorrhea, the prevalence has been estimateted between 45 and 95% of menstruating women (34,35), with very severe primary dysmenorrhea estimated to affect 10–25% of women of reproductive age and this is not affected by height, weight, or regularity of the menstrual cycle (12,36). The overall prevalence of primary dysmenorrhea among adolescent girls is between 60 to 90% (6,7,20, 30, 37- 40), and an approximately 15% of adolescent girls seek medical attention (6). Between 2 and 29% will suffer from severe pain (6).

**Risk factors**

PD is positively associated with stress, family history of dysmenorrhea, a body mass index less than 20 or over 30 kg/m$^2$, depression, early menarche (before age 11), longer intermenstrual intervals (≥ 35 days) and duration of bleeding (≥7 days), heavy bleeding, premenstrual molimina, nulliparity, history of sexual assault, frequent consumption alcohol, smoking (Table II) (6, 7, 25, 32). About 50% of students reported a family history of dysmenorrhea (30). PD decreases with: age increases (prevalence of PD decreases to 67% by age 24), parit (but not in those who had a miscarriage or abortion), exercises, stable relationships, and the use of oral contraceptives (6, 30, 32, 41). Adolescents ranging from 7.7% to 57.8% miss

**TABLE I**
DYSMENORRHEA DIFFERENCES.

| Dysmenorrhea | Primary | Secondary |
|---|---|---|
| Onset | Within 3 years after menarche | More after 5 years after menarche |
| Age | 12-25 years old | Over 30 years old |
| Aging | Gradually improve | Become worse |
| Pathophysiology | No underlaying gynecological pathology | Underlying pathology |
| Time | Menstruation | Menstruation and/or other time |
| Duration | 4-72 hours | > 1 day |
| Nature | Spasmodic | Variable-achy, spasmodic |
| Accompanying symptoms | Heavy menses, nauseas, vomit, headache, backache, syncope, diarrhea | Dyspareunia, dyschezia, sinusorraghia, infertility, heavy menses, vaginal discharge, intermenstrual bleeding, chronic pelvic pain, bowel and urinary symptoms |
| Location | Central | Variable, often eccentric |
| Marriage | Improve | No change |
| Post-partum | Improve | No change |
| Finding | Normal | Different gynecological pathologies: endometriosis, adenomyosis, PID, etc. |
| Clinical investigation | Normal | Fixed retroverted uterus, thickened uterosacral ligaments, endometriotic nodules, on vaginal exam myomas, enlarged tender uterus, adnexal masses |
| Special investigation | Ultrasound: normal pelvic | Ultrasound: myomas, adenomyosis, endometriomas, |
| Relieving factors | NSAIDs, OCs, heat, childbirth, cervical dilation, others. | NSAIDs, heat, menstrual suppression |
| Response to NSAID/ COC | Yes | Yes, but may require further treatments |

NSAIDs: non-steroidal anti-inflammatory drugs; OCs: oral contraceptives.

school or work, 21.5% of them miss social activities and 15% have to limit their daily activities despite the use of medication (6, 30). As it was mentioned before, the prevalence of PD decreased to 67% by age 24, with 10% still reporting some limitations.

PD is characterized or described as a sharp ache or dull pain, intermittent spams or cramps usually located in the midline suprapubic area that begins between a few hours before and a few hours after the onset of the menstrual bleeding (9, 22, 31,). The pain can irradiate to the back or inner of the legs or/and the lower back. Nausea, vomiting, diarrhea, fever, fatigue, malaise, and lightheadedness are systemic symptoms very common. Symptoms or pain peak with the maximum blood flow and may persist up to 2 to 3 days (6, 9, 31).

**TABLE II**
RISK FACTORS.

| |
|---|
| Adolescence |
| Anxiety or stress |
| Body mass index<20 or >30 kg/m$^2$ |
| Depression |
| Disrupted social networks |
| Family history, especially in a first-degree relative |
| Early Menarche (before 12 years old) |
| Longer intermenstrual periods |
| Heavy and longer bleeding |
| Nulliparity |
| Pre-menstrual molimina |
| History of sexual assault |
| Smoking |
| Alcohol |

**Etiology**

Zhou *et al*. (30) mentioned four possible etiological aspects of PD:

1.- Brain abnormality: in recent years, there have been several scientific reports on brain abnormal structure changes and connectivity in dysmenorrheic females using fMRI. Tu *et al*. (42) in 2010 among women with PD found that they had smaller gray matter volume in brain regions in pain transmission and higher levels sensory processing, and larger gray matter volume in regions in pain modulation and endocrine function regulation compared with healthy controls. In 2016, Liu *et al*. (43) reported that PD women had significantly increased cortical thickness in the orbitofrontal cortex (OFC), insula (INS), primary/secondary sensory area (SI/SII), superior temporal cortex (STC), precuneus, and posterior cingulate cortex (PCC) but PD women also have decreased subcortical volumes of the caudate, thalamus, and amygdala. Dun *et al*. (44) found lower gray matter density in the left anterior insula (aINS) in PD patients. In addition, it has been observed changed in neuro-connectivity in patients with PD. Different reports have revealed abnormal white matter integrity involved in pain transmission and modulation systems at periovulation in these patients. Dun *et al.* (45) and Liu *et al.* (46) have mentioned that these white matter microstructure alterations could be closely related with the intensity of menstrual pain. In addition, an abnormal anterior cingulate cortex (ACC) connectivity has been related to the PD pain sensation and regulation (47). PD patients have a dynamic regional spontaneous activity which changes during the whole period cycle and the altered brain regions are involved in decreasing pain modulations, the default mode network (DMN), and the sensory modulation (48). Wu *et al*. (49) reported that in PD women had a regional reduction in homogeneity in the ventromedial prefrontal cortex part of the DMN, during the periovulatory phase; hypoconnectivity of the DMNsalience network and hyperconnectivity of DMNexecutive control network across the menstrual cycle. Also, young women with PD have increased theta oscillations in different brain regions: in the right side parahippocampal gyrus, right posterior insula, and left anterior/middle cingulate gyrus during the menstrual phase. In the left side: the bilateral anterior insula and the left middle/ inferior temporal gyrus during the periovulatory phase.

2.- Gene variation: Zhou *et al.* (30) suggest that there are several genotype and allele frequencies in PD women compared with the healthy controls, including TNF α308, macrophage migration inhibitory factor gene 173, estrogen receptor-1 gene brain derived neurotrophic factor gene Val66Met, cytochrome P450 2D6 gene, glutathione S transferase Mu gene, Familial Mediterranean fever (MEFV) gene, and others. The genotype and allele frequencies of TNF α308 genetic polymorphisms differ significantly between dysmenorrhea patients and controls. Dogru *et al*. (51) proved that TNF α308 GG genotype might be a useful tool to predict the susceptibility to the dysmenorrhea. Mac-

rophage migration inhibitory factor gene 173G>C polymorphism is also significantly associated with age at menarche and a history of back pain among dysmenorrhea patients (51). Lee *et al.* (52) mentioned that the brain derived neurotrophic factor gene Met/Met homozygosity, may be associated with an increased risk of PD and a possible regulator of menstrual pain and pain related emotions in PD. Erten *et al.* (53) reported that patients with dysmenorrhea showed a significant increase in the frequency of the Familial Mediterranean fever (MEFV) gene mutations compared with the control group. Also, Liedman *et al.* (54) reported that the gene expression for the oxytocin receptor was significantly lower in women with dysmenorrhea than in healthy women.

3.- Metabolism: some studies have shown that serum nitric acid (NO) levels are higher and serum homocysteine levels are lower among PD patients, than in the healthy controls. Lee *et al.* (52) hypothesized that NO affects the homocysteine metabolic pathway, and contribute to dysmenorrheal symptoms. Yeh *et al.* (55) and Turhan *et al.* (56) reported higher plasma Malondialdehyde (MDA) levels, an oxidative stress marker in patients with dysmenorrhea compared to those in the healthy controls. MDA is generated during lipid peroxidation, and serves as a marker of tissue injury (57). Dikensoy *et al.* (58) further revealed that the serum levels of MDA, NO, and adrenomedullin were significantly higher in PD patients compared to that of the control group on the first and the 21st day of the menstrual cycles, suggesting that the possibility that lipid peroxidation and oxidative stress might play a significant role in the etiopathogenesis of PD. The plasmatic concentration of oxytocin is significantly higher during the menstrual period and vasopressin plasmatic concentrations are lower during the ovulation in PD patients compared with healthy women (30). Liedman *et al.* (54) found that the gene expression for the oxytocin receptor was significantly lower in women with dysmenorrhea than in healthy women. AbdulRazzak *et al.* (59, 60) reported a relationship among low levels of Vitamin D, low calcium intake and dysmenorrhea in adolescents and young women. The same authors mentioned that women, who have dysmenorrhea, have a high prevalence of Vitamin D-deficient, secondary hyperparathyroidism and low dietary calcium intake. Different authors (61-63) have shown that the Vitamin D supplementation intake among PD patients had a significant reduction of pain compared with the placebo group. Orimadegun *et al.* (64) found the level of alpha tocopherol (vitamin E) was considerably lower in women who experience PD than controls suggesting relative deficiency of this antioxidant.

4.- Pain threshold difference: recent studies indicated that women with PD are hypersensitive to experimental pain compared with controls. Iacovides *et al.* (28) published a detailed review about the hyperalgesia in PD patients. They pointed out that two features of this hyperalgesia in women with PD: 1) an increased sensitivity to experimental pain during the menstruation as well as during painfree follicular phase of the menstrual cycle; 2) the hyperalgesia occurred in muscles within and outside the area of menstrual pain.

**Endometrial Menstrual Cycle**

The menstrual cycle and its physiology, is characterized by the addition of a series of anatomic, functional and hormonal changes within the glandular component of the endometrium, as well as its vascular and stromal constituents, which are mediated by feedback mechanisms that respond to endocrine, autocrine and paracrine factors. It is defined as the interval from the first day of menstruation to the beginning of the next one (65,66). The changes can be divided into five phases: 1) the preparation of the menstrual endometrium, 2) the proliferative phase, 3) secretory phase, 4) the endometrial preparation for the implantation, and

5) the endometrial breakdown phase. The entire process is an integrated evolutionary cycle of endometrial growth and regression, which is repeated some 400 times during the adult life of the human female (1).

The endometrium is divided into two distinctive layers. Its upper 2/3 constitutes the *functionalis* layer, which has the function of preparing the uterus for implantation of the blastocyst; thus, it has a pivotal importance in the processes of proliferation, secretion and, particularly, endometrial degeneration during menstruation. Its regeneration is provided by the *basalis* layer, which is located on the lower 1/3 of the endometrium (1).

The first episode of menstrual bleeding is known as menarche, which occurs approximately two years after telarche, between the ages of 8.5 to 13 years old, marking the beginning of women's reproductive life, which ceases when they achieve menopause. Menarche is followed by approximately 5–7 years of increasing regularity as cycles shorten to reach the usual reproductive-age pattern. In the 40s, cycles begin to lengthen again. The usual duration of menstrual flow is 4–6 days, but many women have flow for as little as 2 days and as much as 8 days. The normal volume of menstrual blood loss is 30 mL; greater than 80 mL is abnormal. Approximately 50% of the menstrual detritus is expelled in the first 24 hours of menstrual flow. The menstrual fluid is composed of the autolyzed *functionalis* layer, inflammatory exudate, red blood cells, and proteolytic enzymes, especially, plasmin which lyses fibrin clots as they form. The high fibrinolytic activity advances emptying of the uterus by liquefaction of tissue and fibrin. If the rate of flow is great, clotting can and does occur. Most women (90%) have menstrual cycles with an interval of 24–35 days (1,6).

The normal menstrual cycle, constituted by follicular and luteal phases, has a duration of 28 ± 7 days, lasting around 2-7 days. Nonetheless, this length is completely variable between women, changing from menarche to menopause, and constantly showing high variability during each particular phase of the cycle. The follicular phase lasts around 10 to 23 days, a 14.6-days mean duration; while the luteal phase has a duration of 7 to 19 days (mean:13.6 days). Menstruation is the external sign of women's cyclicity and it takes place at the beginning of the follicular phase and at the end of the luteal phase (66,67). Thus, the follicular phase is constituted by menstruation and the proliferative phase, and the luteal phase includes the secretory phase (1). The uterus blood is supplied by the two uterine arteries, which are branches of the internal iliac arteries; at the lower part of the uterus, the uterine artery separates into the vaginal artery and an ascending branch that divides into the arcuate arteries. The arcuate arteries go parallel to the uterine cavity and anastomose with each other, forming a vascular ring around the cavity. Small centrifugal branches (the radial arteries) leave the arcuate vessels, perpendicular to the endometrial cavity, to supply the myometrium. Once these arteries enter to the endometrium, small branches (the basal arteries) extend laterally to supply the basalis layer. These basal arteries do not demonstrate a response to hormonal changes. The radial arteries continue in the direction of the endometrial surface, now assuming a corkscrew appearance and change the name for spiral arteries so that they supply blood to the *functionalis* layer of the endometrium. This spiral artery segment is very sensitive to hormonal changes. One reason that the *functionalis* layer is more vulnerable to vascular ischemia is that there are no anastomoses among the spiral arteries. The endometrial glands and the stromal tissue are supplied by capillaries that emerge from the spiral arteries, at all levels of the endometrium. The capillaries drain into a venous plexus and eventually, into the myometrial arcuate veins and into the uterine veins. This unique vascular architecture is important to allow the repeated sequence of endometrial growth and desquamation (1) (see Fig. 2).

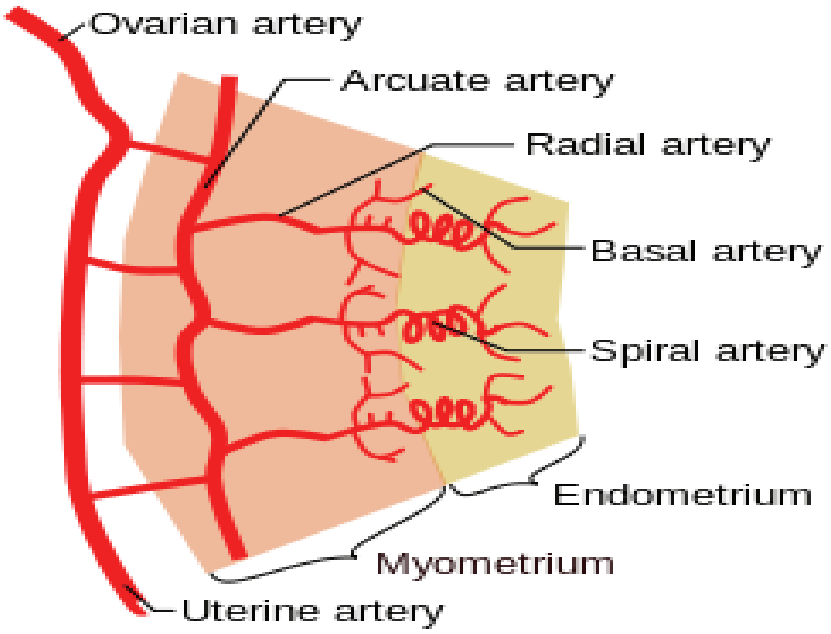


**Fig. 2.** The Uterine Vasculature.
Adapted from Uterine blood flow during pregnancy. Apaza-Valencia J, Guerrero MH. Rev Peru Ginecol Obstet. 61(2); abr./jun. 2015. Available from: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S2304-51322015000200006. Reviewed on March 29, 2021.

The menstrual endometrium is a relatively thin but dense tissue. As we mentioned before, the endometrium is composed by a stable, *basalis* component and a variable, but small, amount of residual stratum *spongiosum*. During the menstruation, this latter tissue displays a variety of functional states including disarray and breakage of glands, fragmentation of vessels and stroma with persisting evidence of necrosis, white cell infiltration, and red cell interstitial diapedesis. When the remnants of menstrual shedding dominate the overall appearance of this tissue, evidence of repair in all tissue components can be detected. Endometrial regeneration begins and originates in epithelial and stromal stem cells. Endometrial epithelial stem cells have been found in glands within the basalis layer and are thought to be responsible for the re-epithelialization of the exposed surface of the endometrium and subsequent glandular proliferation to regenerate the *functionalis* layer under the influence of increasing estrogen levels following menses. Endometrial mesenchymal stem/progenitor cells are found around blood vessels in the *basalis* layer. These progenitor cells are thought to contribute to regeneration and growth of the endometrial *functionalis* stroma (68).

Once menstruation starts, during the follicular phase, estradiol (E2) level starts progressively rising, as well, leading to the endometrial proliferation by the mid-follicular phase. The hypothalamus-pituitary-gonadal (HPG) axis is activated, inducing the secretion of follicle-stimulating hormone (FSH), via gonadotropin-releasing hormone (GnRH), through a positive feedback of the axis. During the follicular phase, the dominant ovarian follicle secretes most of E2 and growths at a rate 1-4 mm/day (65,67,69). The follicle growth and increased E2 secretion, as well as the increase in E2 receptors

throughout the endometrium, are characteristics of the proliferative phase, with proliferation of the endometrial glands, stromal and endothelial cells, and the restoration of the endometrium provided by the *basalis* layer (1). Considering that ovarian hormones constitute the main mediators of the HPG axis in females (65), the high concentration of E2 decreases FSH levels via a negative feedback of the HPG axis, but a release of luteinizing hormone (LH) takes place, marking the beginning of the luteal phase. Thirty-six hours later, the oocyte is released from the ovarian follicle, a process known as ovulation, ceasing epithelial proliferation. The high LH levels induce the transformation of the dominant follicle into the corpus luteum, which secretes progesterone (P); however, P production also takes place during the follicular phase, although it is quantitatively lower (1,65,67). The expression of P receptors throughout the endometrium is induced by the high levels of E2, during the follicular phase, via E2 receptor-α (ER-α), which are then inhibited by P. Progesterone prepares the endometrium for the embryo implantation, during the days 21 or 22 of the cycle, and also, for decidualization, which plays a pivotal role in menstruation, implantation and placentation, defined as endometrial hemostasis. If fertilization and implantation do not take place, the corpus luteum will regress and the secretion of P ceases, with vasoconstriction of spiral arterioles that produces an epithelial lesion, induced by hypoxia, characterized by vasomotor reactions, apoptosis and tissue loss, causing the consequent degeneration and desquamation of the *functionalis* layer of the endometrium and, thus, marking the beginning of the next follicular phase (1,67).

**Inflammatory responses during menstruation**

As it has been mentioned before, the endometrium is constituted by a simple columnar epithelium lying over a multicellular stroma, comprised by connective tissue components, fibroblast-like stromal cells, tubular glands, spiral arteries and recruited innate immune cells. Endometrial stromal cells are originated form the differentiation of endometrial progenitor stem cells, located in its basal layer, and, through a process known as decidualization, are transformed into decidual cells, which induce a propitious environment for the embryo implantation and consequent placental development. This process begins with the intracellular production of cAMP in the perivascular endometrial stromal cells, which eventually spread through the entire stroma, and induces the expression of progesterone-dependent proteins (70).

The female reproductive system presents molecular and cellular mediators associated to inflammation, which are expressed fundamentally during ovulation and menstruation, particularly, during the early follicular phase (71,72). P withdrawal produces endometrial tissue edema, increased vascularization, and vessel permeability and fragility (71). Changes in the endometrium are examples of cyclic inflammatory activity, not only characterized by cellular inflammation, but by an outer tissular destruction as well, and whose repair mechanisms prepare the endometrium for the onset of the next menstrual cycle (71,72). Concerning steroid hormones (estrogens, androgens and glucocorticoids), the androgen receptor is downregulated in the endometrial functional layer's stromal cells during the secretory phase and upregulated in the endometrial epithelial cells during the last stages of the proliferative phase. In addition, once locally generated cortisol binds to the nuclear glucocorticoid receptor in the endometrium, it limits inflammation at other tissue sites, mainly inhibiting angiogenesis. On the other hand, the concentrations of the estrogen receptor, located in the nuclei of both, epithelial and stromal cells of the endometrium, decline in the luteal phase, while the proges-

terone receptor's concentrations tend to decline only in the glandular epithelium, augmenting during the proliferative phase (71).

The decidualized stroma, in response to the reduction of steroid hormones, stimulates the release of cytokines (interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α), chemokines and their ligands (CCL11, CCL2, CXCL10 and CXCL8), adhesion molecules, and granulocyte-macrophage colony-stimulating factor (GM-CSF), followed by the release of leukocyte's matrix metalloproteinase causing the endometrial glandular layer's disruption, as a product of P withdrawal, processes that are regulated by cyclo-oxygenase 2 (COX-2) and NF-kappa B (NF-kB) (70,72). The intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), whose expression elevates during the early and mid-follicular phases, including ovulation, are also involved in the inflammatory process of leukocyte trafficking (71). The tissue's disruption is exacerbated by the entrance of endometrial neutrophils that also contain high levels of metalloproteinases (70,72).

Quantitatively, the endometrial leukocyte population varies between 8.2% to 15% of the endometrial stromal cell compartment, during the proliferative phase of the menstrual cycle, while immune cells vary between 20-25% to 40-45% of the cell compartment, during the same phase. The resolution of menstrual-related inflammation also depends on the phagocytosis of apoptotic cells by macrophages, which can either proliferate in the endometrium or be derived from monocytes that were recruited into the endometrial tissue, under the action of IL-4 in Th2 inflammatory responses, as well as other chemotactic stimuli, such as CCL2 or monocyte chemoattractant protein-1 (MCP-1), highly expressed in the endometrium during the late secretory phase (71).

During the onset of menstruation, prostaglandin F2α (PGF2α) and endothelin-1 induce the vasoconstriction of the spiral arteries of the uterus; while excessive PGE2 production, at the expense of PGF2α, allows their vasorelaxation, causing the proliferation of the vascular smooth muscle cells in endometrial blood vessels, allowing an increase in blood flow, due to a regulation of the vascular tone and contractility, by smoothelin and calponin, and thus inducing arteriogenesis (70,72). However, decreased vasoconstriction during menstruation does not only depend on the decreased production of the former vasoactive substances, but also on the aberrant maturation of the spiral arterioles of the uterus, throughout the preceding menstrual cycle. Vasoconstriction may also induce hypoxia in the menstrual endometrium, which studies have shown that is necessary for the initiation of menstruation. The cellular response to hypoxia is regulated by hypoxia inducible factor (HIF), which has been detected in the endometrium, but only during the perimenstrual phase, and has proven to have roles in inflammation, metabolism, angiogenesis, mitogenesis and apoptosis. HIF-1 also promotes the increase of the vascular endothelial growth factor (VEGF) in the endometrial tissue during menstruation, characterized by its angiogenic effects, contributing to an efficient endometrial repair (70, 73).

The levels of C-reactive protein (CRP), as a hepatic pro-inflammatory marker, reach their peak during the early follicular phase, considering that their increase is associated with endogenous progesterone's levels. Cathepsins B, L, and S are also endometrial proteinases that allow its normal development and functionality during the entire menstrual cycle, in relation to proteolytic processes. Regarding the peak for both selectins, -P and E-selectins-, it tends to vary. Chiareti *et al*. (71) observed a slight increase of P-selectin during the luteal phase, while E-selectin remained unaltered during both phases of the cycle. IL-6 also elevates during the follicular phase of the menstrual cycle, inducing the synthesis of, both, CRP and pentraxin-3 (PTX-3), which is upregulated during ovulation; it also constitutes

an early marker for endothelial dysfunction, and has proven to play a role in innate immunity, female fertility and pro-coagulation activity, being largely responsible for the inflammatory activity during the early follicular phase (71).

Due to an increase in erythrocytes' catabolism, reticulocyte counts, and nitric oxide (NO) and progesterone expression and activation of heme oxygenase 1 (HO-1), particularly in the myometrial, vascular endothelial and smooth muscle cells, the production of endogenous carbon monoxide (CO) is elevated during the luteal phase of the menstrual cycle, providing protection against the endothelial oxidative stress. NO levels augment in the midcycle (74).

**Pathophysiology of dysmenorrhea**

It is becoming increasingly accepted that many normal reproductive processes display signs of inflammation. Such processes include ovulation, menstruation, implantation and parturition (3, 5). All of these events are associated with up-regulation in the expression of a host of inflammatory mediators, which include cytokines, growth factors and lipid mediators that influence the growth and function of the immune and vascular compartments (3,75,76).

Another remarkable feature of the female reproductive tract is its capacity to resolve these inflammatory events rapidly to re-establish normal reproductive function. The resolution of inflammation involves the clearance of leukocytes and tissue debris as well as restoration of mucosal and vascular function in the affected tissue. Until recently, resolution of inflammation was considered a passive process that came about as a result of dissipation in the expression of local inflammatory mediators. In response to the tissue injury there are specific anti-inflammatory and pro-resolution biochemical pathways that are activated, which facilitate the reestablishment of homeostasis in the affected tissues (5,76). Furthermore, it is well recognized that inflammation mediated alterations in immune cell and vascular function are important components of many pathologies which include cancer, chronic inflammatory diseases, allergy, asthma, atherosclerosis, autoimmunity, transplant rejection and metabolic and degenerative diseases. Moreover, alterations or disruption in the onset of the pro-resolution pathways may lead to uncontrolled inflammation and the onset of disease and there is evidence in animal models that the administration of pro-resolution mediators can help control and resolve inflammation (76).

The female reproductive system presents molecular and cellular mediators associated to inflammation, which are expressed fundamentally during ovulation and menstruation, particularly during the early follicular phase (71,72). Progesterone withdrawal produces endometrial tissue edema, increased vascularization, and vessel permeability and fragility (70). Changes in the endometrium are examples of cyclic inflammatory activity (71), not only characterized by cellular inflammation, but by an outer tissular destruction, as well, whose repair mechanisms prepare the endometrium for the onset of the next menstrual cycle (72). Concerning steroid hormones (estrogens, androgens and glucocorticoids), the androgen receptor is downregulated in the endometrial functional layer's stromal cells during the secretory phase and upregulated in the endometrial epithelial cells during the last stages of the proliferative phase. In addition, once locally generated cortisol binds to the nuclear glucocorticoid receptor in the endometrium, it limits inflammation at other tissue sites, mainly inhibiting angiogenesis. On the other hand, the concentrations of the estrogen receptor, located in the nuclei of both, epithelial and stromal cells of the endometrium, decline in the luteal phase, while the progesterone receptor's concentrations tend to decline only in the glandular epithelium, augmenting during the proliferative phase (70).

In spite of the huge amounts of studies, the mechanism of PD is not fully understood. Previous studies have shown that dysmenorrhea is a complex process that may depend on many factors (77-79). It is well known that the menstrual cycle is dependent on cyclic changes in ovarian hormone concentrations secretions and therefore also on cyclic changes in prostaglandin level and uterine contractile activity (89-82). In 1965, Pickles *et al.* (82) noted that one of the factors contributing to dysmenorrhea might be an increase in prostaglandin concentrations before menstruation. This suggestion was confirmed years later by other authors who showed that prostaglandins production increases in dysmenorrhea (83). Although the specific contributions of these various mechanisms and substances are not yet perfectly understood, the pathophysiology of dysmenorrhea has solidly moved beyond a psychosomatic cause (9,30).

In normal eumenorrheic women, the uterus has well defined contraction patterns that are influenced by sex steroids, prostaglandins, and other uterotonic substances throughout the menstrual cycle and menstrual period (84). During the menstruation, the uterine basal tone is minimal, less than 10 mm Hg, there are 3-4 contractions in 10 minutes period with active pressures at the peak of a contraction reaching up to 120 mm Hg and the contractions are synchronous and rhythmical (84). Women with primary dysmenorrhea, uterine contractions during menses begin from an elevated level of basal tone (>10 mm Hg), generate higher intrauterine pressures that frequently reach 150–180 mm Hg and can exceed 400 mm Hg, occur more frequently (>4–5/10 minutes), and are not rhythmic or coordinated. When intrauterine pressure exceeds arterial pressure for a sustained period of time, ischemia results in the production of anaerobic metabolites that stimulate small type C pain neurons, which contributes to the pain of PD. When more than one abnormal contraction occurs, they synergize with each other so that the pain threshold is exceeded with smaller changes than if only one abnormal contraction is present. Classically, as it has been mentioned before, PD begins just before or coincident with the onset of is exceeded with menses and declines gradually over the subsequent 72 hours. The menstrual cramps are intermittent, vary in intensity, and usually are centered in the suprapubic region, although some women also experience pain in their thighs and lower back (6, 84).

During the menstrual period, when exfoliation of the endometrium occurs, metalloproteinases (MMPs) play an important role. They are enzymes produced by endometrial cells and leukocytes. The secretion of MMPs is probably inhibited by the P, so when the P decreases, increase the MMPs secretion (80). Before menstruation, endometrium tissue gets the characteristics of inflammation process, it becomes red and edematous. Endometrial edema is consequence of the local increased production of chemokines including interleukin-8 (IL-8), proinflammatory cytokines such as IL-1, Il-6 and tumor necrosis factor (TNF) and leukocytes inflow: uterine NK cells, neutrophils, eosinophils, macrophages and activated mast cells (5,79). After the ovulation both hormones: P and E2 affect the endometrium. During the secretory phase, P level increases and has anti-inflammatory action, inhibiting the release and activation of MMPs. Three days before the onset of the menstrual period, P and E2 levels decrease which initiate the endometrial transformation or change: vasomotor reactions, apoptosis, tissue atrophy and bleeding or menstruation (85). After ovulation, fatty acids are accumulated in phospholipids in the cell membrane. Omega-6 fatty acid and arachidonic acid are released only when the level of progesterone begins to fall (86).

**Prostaglandins and prostanoids**

An excess or imbalance of prostaglandins (PGs), vasopressin and other chemical substances derived from phospholipids has been proposed to cause dysmenorrhea and is no longer heavily disputed (9). Evidence for

this theory includes measurements of the prostaglandins PGF2α, PGE2, and vasopressin in menstrual fluid that correlate with adverse symptoms of dysmenorrhea. In addition, these chemicals are known to cause symptoms of increased uterine contractility and cramping, nausea, vomiting, and diarrhea in other clinical situations (9). PGs produce vasoconstriction of the blood vessels, decreasing the blood supply to the uterus, and produce abnormal contractile activity of the uterus, which leads to ischemia, hypoxia of the uterus and increased sensitivity of the nerve endings (31, 79, 81). Evidences indicate that PD is caused by myometrial ischemia due to frequent and prolonged uterine contractions. Studies of uterine blood flow using Doppler ultrasonography have revealed that uterine and arcuate artery resistance on the first day of menses is significantly higher in women with PD than in women without dysmenorrhea, suggesting that constriction of uterine vessels is one of the causes of pain, which induces abnormal and intense uterine contractions. The uterine contractions decrease or reduce the uterine blood flow, leading to uterine hypoxia and ischemia and more painful uterine contractions (6, 9, 84). The association of PD with ovulation makes sense on a physiologic level because of the normal sequence of cyclical endometrial growth. The increase in serum P following ovulation in the secretory endometrium causes and increases substantial stores or reserves of arachidonic acid, which is a precursor to PGs: PGF2α and PGE2, prostacyclin, and leukotrienes: thromboxane $A_2$ (Tx $A_2$); all promote uterine contractions and act as potent vasoconstrictors during the menses (3, 9). Higher concentrations of PGF2-α and PGE2 have been proved to be present in the endometrium and menstrual fluid of women who have primary dysmenorrhea (9). PGF2α always stimulates uterine contractions and is the primary mediator of PD. Endometrial concentrations of PGF2-α and PGE2 correlate with the severity of dysmenorrhea. Uterine smooth muscle contractions are the cause of the crampy, spasmodic lower abdominal and low back pain typical of PD (see Fig. 3).

The increased secretion of vasoactive prostanoids is considered as one of the most important cause responsible for the etiology of PD and it is supported by: 1) the similarity between the clinical symptoms of PD and the uterine contractions and side and adverse effects that can be observed in prostaglandin induced abortion or labor; 2) it has been demonstrated an increase in prostanoids concentrations during the menstrual period in women with PD compared with eumenorrheic women; 3) numerous clinical trials have demonstrated the efficacy of cyclooxygenase inhibitors in relieving the pain of PD and in decreasing or suppressing the amount prostanoids in the menstrual fluid (32, 84).

The role of other protanoids such as Tx-A2, prostacyclin and leukotrienes in the pathogenesis of PD is neither fully understood, nor adequately studied. Prostacyclin, a potent vasodilator and uterine relaxant, appears to be reduced in primary dysmenorrhea (32). The increase of leukotriene production, by the 5-lipoxygenase pathway, may contribute to some forms of PD that are not responsive to nonsteroidal anti-inflammatory drugs (NSAID) (87). Human endometrium and myometrium can synthesize leukotrienes (87); this confirms that the functional activity of the 5-lipoxygenase pathway and leukotrienes are involved in myometrial contractions (88). In women with PD there are significantly higher concentrations of menstrual leukotrienes (89, 90), especially leukotriene C4 and leukotriene D4, than in women without dysmenorrhea (91). Because specific binding sites for leukotriene C4 are demonstrable in myometrial cells (92), it is likely that leukotrienes contribute to the uterine hypercontractility seen in PD (see Fig. 4).

PGs and prostanoids are biosynthesized from arachidonic acid through the COX pathway after production of arachidonic acid from hydrolysis of phospholipids by the lysosomal enzyme phospholipase A2. The stability of

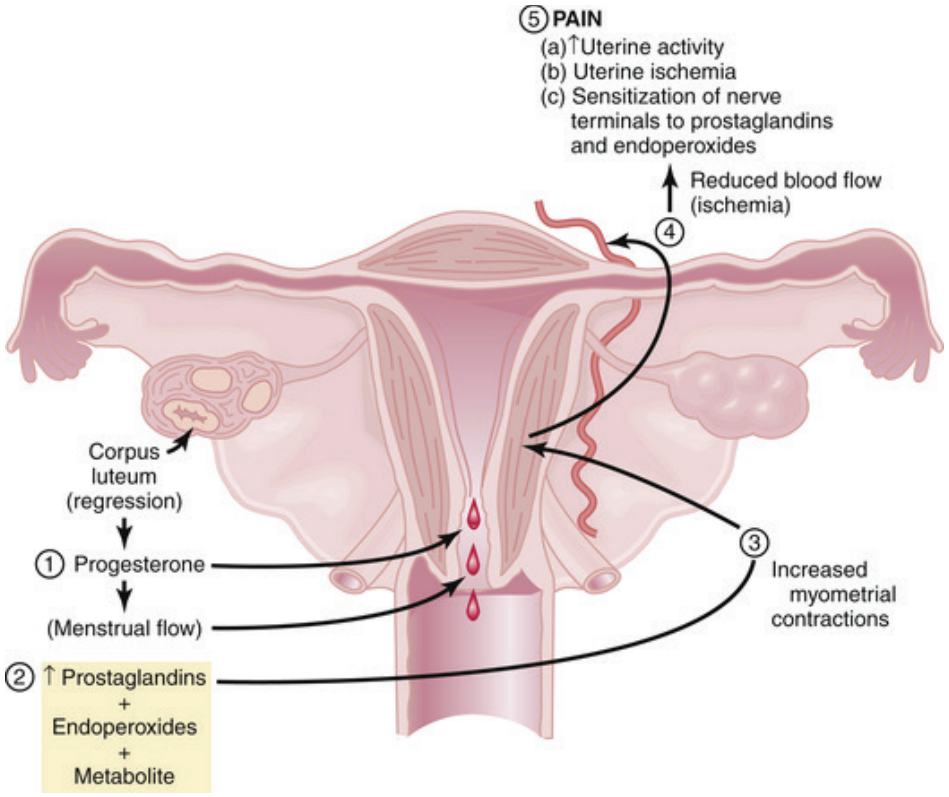


**Fig. 3.** Possible mechanism of primary dysmenorrhea.
*Adapted from* Dawood MY: Hormones, prostaglandins and dysmenorrhea. *In* Dawood MY [ed]: Dysmenorrhea. Baltimore, Williams & Wilkins, 1981).
Available from https://clinicalgate.com/pelvic-pain-2/. Reviewed on March 28, 2021.

lysosomal activity is regulated by several factors such as P levels. High levels of P produce the stability of lysosome activity (32, 36). When pregnancy does not occur, P levels decline during the late luteal phase. This stabilizing effect on endometrial lysosomes disappear and the phospholipase A2 is released causing the lability of lysosomes and release of their phospholipase enzyme, which then, hydrolyzes the cell membrane phospholipids to generate arachidonic acid as well as icosatetraenoic acid. These compounds serve as the precursors for the COX and lipoxygenase pathways (28). This is regulated by cyclic adenosine phosphate (cAMP). Via cAMP, PGs production can be stimulated by substances such as adrenaline, peptide hormones and steroid hormones, but also by mechanical stimuli and tissue trauma (28, 93,94).

## Others chemical substances, cytokines and others pro-inflammatory factors in primary dysmenorrhea

### Vasopressin

It has also been shown that vasopressin plays a role in the pathophysiology of dysmenorrhea (95), although, the role of vasopressin in the pathogenesis of PD is controversial (32, 96, 97). Vasopressin is a hormone secreted by the pituitary gland, its secretion is stimulated by cyclic changes in estradiol levels. This hormone may contribute to uterine contraction activity (86). Its concentration is lower during the follicular phase and increases during the ovulation. Also, the increased levels of circulating vasopressin during the menstrual period that have been reported in women with PD, can

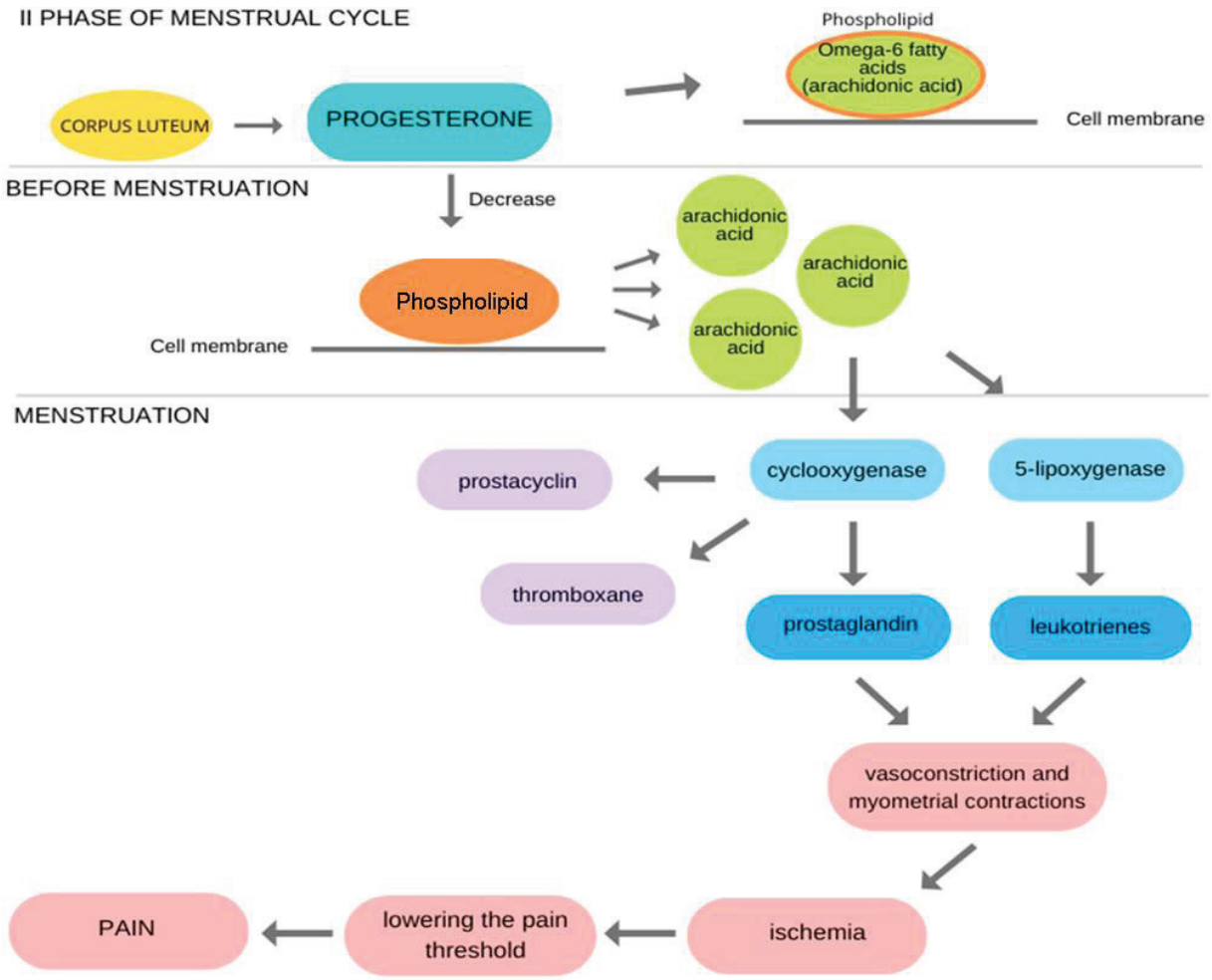


**Fig. 4.** Role of prostaglandins and prostanoids in primary dysmenorrhea.
Adapted and modified from Barcikowska Z, Rajkowska-Labon E, Grzybowska ME, Hansdorfer-Korzon R, Zorena K. Inflammatory Markers in Dysmenorrhea and Therapeutic Options. Int J Environ Res Public Health. 2020 Feb 13;17(4):1191. *doi: 10.3390/ijerph17041191.*

produce dysrhythmic uterine contractions that reduce uterine blood flow and cause uterine hipoxia (98). Vasopressin exerts its uterine effects mainly via the vasopressin V1a receptor (99,100). The etiological importance of vasopressin in dysmenorrhea has been postulated by the therapeutic effect of the peptide analogue 1-deamino-2-DTyr (Oet)-4-Thr-8-Orn-oxytocin, which competitively blocks the vasopressin V1a and oxytocin receptors of the uterus (101). An orally active vasopressin V1a and the oxytocin receptor blocking agent, SR 49059, was also shown to inhibit vasopressin effects on uterine contractility and to be therapeutically active in dysmenorrhea when given prophylactically before the onset of symptoms (102,103). However, other investigators could not confirm elevated plasma vasopressin in women with PD and have found that the vasopressin antagonist atosiban had no effect on menstrual pain, intrauterine pressure, or uterine artery pulsatility index in PD women (97).

**Tumor necrosis factors alpha**

Activated macrophages produce proinflammatory cytokines: TNF-α, interleukin-1 (IL-1), IL-6, etc. responsible for upregulating inflammatory responses (104,105). It has also been reported that these mediators stimulate the synthesis or release of prostaglandins (105,106), producing excessive contraction of the uterine muscle, which leads to ischemic pain of primary dysmenorrhea. Plasma IL-6 and TNF-α levels has been found higher in women with dysmenorrhea compared to women without menstrual disorders (104). Also, TNF-α is a cytokine that is responsible for inhibiting endometrial proliferation and induces apoptosis. Previous studies have shown that endometrial cells produce increased concentration of TNF-α during menstruation (105). Moreover, Dogru *et al.* (107) showed that the TN -308G > A gene polymorphismis strongly associated with susceptibility to dysmenorrhea in the Turkish female population. The authors (107) demonstrated that the presence of the -308A TNF-α allele can protect against dysmenorrhea and suggested that the TNF-α-308, GG genotype may be a useful tool for predicting susceptibility to dysmenorrhea. Recent studies have shown that intensive aerobic exercises not only cause a reduction in the levels of the metabolite of prostaglandins:13,14-dihydro-15-ketoprostaglandin F2 αlfa (KDPGF2α), but also reduce the level of TNF-α, as well as reduce the intensity of pain associated with dysmenorrhea (105).

**Interleukin 6**

IL-6 is a pleiotropic cytokine with multi-directional effects on the cells of the innate and acquired immunity system. The main role of IL-6 is to initiate and regulate the acute inflammatory response and to facilitate the development and targeting of the acquired response. IL-6 exhibits pro and anti-inflammatory properties and is now considered an important target for clinical intervention (108). In a study of women with a normal menstrual cycle, significant variability in plasma cytokine levels, including IL-1β, IL-6, IL-8, and IL-10 were observed (109). Levels of several factors increased during ovulation and then achieved their peak during menstruation, which is considered by some scientists to be a proinflammatory event (85). Levels of IL-1β, IL-6, and IL-8 were inversely correlated with estradiol and progesterone levels. However, Angstwurm *et al.* (110) demonstrated an increase in IL6 concentration in the follicular phase when the level of E2 increased. After ovulation, in the luteal phase, when there was a 10-fold increase in P, the level of IL-6 in plasma decreased 1.5–4.4 times. Others authors have shown (104, 111, 112) an increased IL-6 concentration in women with dysmenorrhea compared to women without dismenorrhea. Konecna *et al.* (112) have demonstrated that the level of IL6 was statistically significantly higher during the luteal phase compared to the follicular phase.

**C-Reactive Protein**

C-Reactive Protein (CRP) is a clinically recognized as an acute phase protein. It is assumed that normal CRP concentrations in healthy people should not exceed 3 mg/L. During the acute phase reaction, which is a defense response to inflammation, infection or injury, the concentration of serum CRP can increase up to 1000-fold, reaching its maximum concentration after 24–48 h (113,114). CRP is an important marker of the ongoing inflammatory response, with a relatively short half-life (~48 h), its concentration returns to its baseline in 7 to 12 days (114). CRP also supports the process of phagocytosis, affecting monocytes, macrophages, and neutrophils, as well as acting in a chemotactic and opsonizing way (115). In addition, it induces monocytes/macrophages to synthesize pro-inflammatory cytokines and inhibits the synthesis of anti-inflammatory cytokines. Studies conducted in adult women have shown that increased levels of CRP varied significantly across

the menstrual cycle. CRP was highest during menses, decreased during the follicular phase, was lowest on the expected day of ovulation, and increased in the luteal phase (116). Another study, showed that a ten-fold increase in progesterone was associated with a 23% increase in CRP (P = 0.01), a ten-fold increase in estrogen was associated with a 29% decrease in CRP (P = 0.05) (1`17). In a study, with the participation of healthy women, CRP levels were positively correlated with the severity of menstrual symptoms, the strongest being mood and pain symptoms (118).

**Vascular Endothelial Growth Factor**

Few studies indicate the involvement of vascular endothelial growth factor (VEGF) in the process of dysmenorrhea among women with endometriosis (119, 120). It is known that VEGF is the strongest factor involved in the embryonic development, menstrual cycle, and in ovarian endometriomas (121). Although VEGF is produced by cells and tissues of the reproductive tract, such as the endometrium, ovary and placenta; VEGF receptors are found only in the endothelial cells. VEGF has been shown to stimulate endothelial cell proliferation, migration and increase vascular permeability (122). Recent reports have shown a relationship between the production of VEGF, macrophage migration inhibitory factor (MMIF), hypoxia-inducible factor-1α (HIF-1α) and the stage of endometriosis, as well as the severity of dysmenorrhea (119). The expression of all three proteins in endometrial tissues and in serum increased significantly with the severity of pain (P <0.05). Thus, the authors conclude that MMIF, HIF-1α and VEGF expression in serum can be used to assess the stage of endometriosis, as well as the severity of dysmenorrhea (119).

**Asymmetric dimethylarginine**

Increase in the concentrations of asymmetric dimethylarginine (ADMA) is accepted as a marker of endothelial dysfunction (123). By inhibiting nitric oxide (NO), ADMA level increases, and has been shown to be a risk factor for endothelial dysfunction and cardiovascular disease in end-stage renal disease (124). It is responsible for increased cardiovascular morbidity and mortality in patients with renal disease (125), cause worse outcome in idiopathic pulmonary hypertension patients (126), and probably, play a role in the pathogenesis of cerebrovascular disorders by increasing vascular stiffness and decreasing cerebral perfusion (127). ADMA is an endogenous inhibitor of NO synthase, and its accumulation has been associated with reducing NO bioavailability and increasing superoxide generation (128). NO is a potent inhibitor of platelet aggregation and a powerful vasodilator; it is responsible for uterine quiescence during pregnancy and also appears to relax the nonpregnant myometrium (129, 130). Inhibition of NO synthesis cause vasoconstriction and increase in oxidative stress, both of which would be reflected in increased ADMA concentrations, as it has been demonstrated by Alkdemir *et al.* (131). Unfortunately, the results of use of NO donors to treat PD have been controversial. Some authors (132, 133) have reported an effective relief of pain in 90% of the patients and statistically superior results, compared to placebo. However, Facchinetti *et al*. (134) reported a reduced efficacy compared to diclofenac. Alkdemir *et al*. (131) mentioned that it should be kept in mind in a patient with PD, since she could have a endothelial dysfunction because of elevated ADMA levels. Knowing that dysmenorrhea affects only a part of the female population, the imbalance between vasodilatation and vasoconstriction is present only in some women. Alkdemir *et al.* (131) have showed that ADMA concentrations are increased in women with PD compared to healthy controls. This might provide further insight into the pathophysiology of this common and devastating disorder.

**Nitric oxide**

NO is a vasodilatory substance produced by endothelial or other cells that play a role in the physiological control of blood pressure (135). It is involved in many different physiological processes, including blood pressure regulation, platelet aggregation, and cytotoxicity (136). NO is an important source of free radical production in tissue damage and NO levels decrease in the case of endothelial dysfunction (137). There are results showing that serum NO levels are higher and serum homocysteine levels are lower among PD patients than in the healthy controls. It is hypothesized that NO affects the homocysteine metabolic pathway, and contribute to dysmenorrheal symptom (138).

**Malondialdehyde**

Malondialdehyde (MDA) is generated during lipid peroxidation, serving as a marker or indicator of lipid peroxidation, an oxidative stress marker, and tissue injury (64, 139). Turhan *et al*. (56), Orimadegun *et al.* (64), and Yeh *et al.* (140), have reported higher plasma MDA levels in patients with dysmenorrhea compared to those in the healthy controls. Also, Dikensoy *et al.* (141) found that the serum levels of MDA, NO, and adrenomedullin were significantly higher in PD patients compared to that of the control group on the first and the 21st day of the menstrual cycles, suggesting that the possibility that lipid peroxidation and oxidative stress might play a significant role in the etiopathogenesis of PD.

**Heme oxygenase 1**

Heme oxygenase-1 (HO-1) is a ratelimiting enzyme which has an important role in the oxidative catabolism of heme to generate carbon monoxide, iron, and biliverdin. HO-1 has been reported to have cytoprotective effects in oxidative stress conditions (142). Different authors (143-147) have shown that HO-1 has extensive tissue protection via anti-oxidative, anti-inflammatory, anti-apoptotic and immunoregulatory activities. Akoy *et al*. (143) found in fasting blood samples taken from 28 nulliparous women with PD, that they had higher HO-1, MDA, and NO levels compared with controls. HO-1 levels are increased by compensatory mechanisms to protect the balance between oxidant and antioxidant system in patients with dysmenorrhea (143).

**Nitrotyrosine**

3-Nitrotyrosine(3-NT) is a product of tyrosine nitration mediated by reactive nitrogen species such as peroxynitrite anion and nitrogen dioxide. Nitrotyrosine is identified as an indicator or marker of cell damage, inflammation as well as NO production. 3-NT is formed in the presence of the active metabolite NO. Generally, in many disease states, oxidative stress increases the production of superoxide (O2−) and NO forming peroxynitrite (ONOO−) a destructive free radical oxidant. The production of ONOO− is capable of oxidizing several lipoproteins and of nitrating tyrosine residues in many proteins. It is difficult to determine the production of ONOO−, usually nitrotyrosine in proteins are the detectable marker for indirectly detecting ONOO−. It is detected in large number of pathological conditions and is considered a marker of NO-dependent, reactive nitrogen species-induced nitrative stress. Orimadegun *et al*. (64) found higher levels of 3-HT in patients with PD compared to control patients, however, Turhan *et al*. (56) and Yeo *et al*. (148) have reported no significant difference in the plasma level of 3-NT of women with dysmenorrhea and healthy control.

## CONCLUSIONS

Today, it is well accepted that reproductive processes in women are regulated by inflammatory events. The control of the onset and resolution of these inflammatory events ensures a normal reproductive function. Exacerbated or premature activation of inflammation can contribute to disease. Un-

derstanding the molecular control of inflammation and its resolution in the reproductive tract may give us insight into how these may be corrected therapeutically in disease

PD is commonly a simple diagnosis that can be made accurately with an attentive history and, in young women who have classic symptoms and no specific indication, a pelvic examination is often unnecessary in the initial evaluation. The opportunity for primary care practitioners to support women is the best approach to this chronic recurrent discomfort and to minimize an adverse life impact is significant and valuable. Identification of patients who are incapacitated by their symptoms, or have symptoms that represent underlying pathology, is a critical component of a careful history. The wide range of treatments available for PD ensures that all females who have the symptoms can find relief with relatively safe and inexpensive treatments with limiting negative side effects. The opportunity to counsel and support healthy lifestyle choices contribute positively to the general health and provide symptom relief of this condition. As so many disorders encountered in primary care medicine, dysmenorrhea give to clinicians the opportunity to teach, counsel, and support patients toward not only the relief of symptoms, but also, to get an optimal health.

As it has been mentioned before, the pathophysiology of PD is primarily linked to elevated levels of PGs. Low P levels in the late luteal phase of the menstrual cycle is reported to increase the synthesis of PGs, pro-inflammatory cytokines (IL-6 and TNF-α) and other, possible, pro-inflammatory factors are also implicated in the pathogenesis of primary dysmenorrhea, but a better understanding of the pathophysiology of PD may lead to better treatments.

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