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Exploring the Pathophysiology of Adenomyosis in Jakarta: Novel Insights into Inflammatory Pathways and Angiogenesis

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A B S T R A C T

Introduction: Adenomyosis, characterized by endometrial glands and stroma within the myometrium, is a significant cause of morbidity (abnormal uterine bleeding (AUB), pain, infertility) among women globally. While its general pathophysiology is increasingly understood, population-specific data, particularly from diverse Asian populations like that of Jakarta, Indonesia, remains limited. This study aimed to investigate the roles of specific inflammatory pathways and angiogenic factors in adenomyosis within a cohort of women in Jakarta, hypothesizing that common pathogenic mechanisms manifest in this population, potentially influenced by local factors. **Methods:** This prospective case-control study was conducted in three tertiary referral hospitals in Jakarta, Indonesia from January 2022 to December 2024. Fifty women undergoing hysterectomy, diagnosed with adenomyosis were recruited, alongside 50 control women without adenomyosis undergoing hysterectomy for other benign conditions. Samples of eutopic endometrium, ectopic adenomyotic lesions, and associated myometrium were collected. Immunohistochemistry quantified inflammatory cells (macrophages, mast cells, T-lymphocytes) and microvessel density (CD31). Expression of key inflammatory cytokines and angiogenic factors was assessed via qRT-PCR and ELISA. **Results:** Women with adenomyosis in the Jakarta cohort exhibited significantly increased infiltration of M1-phenotype macrophages (p0.001) and mast cells (p0.001) in adenomyotic lesions and eutopic endometrium compared to controls. IL-6, TNF-α, and MCP-1 expression was markedly upregulated in adenomyotic foci (all p0.001). Microvessel density, VEGF-A, and VEGFR2 expression were significantly elevated in adenomyotic tissue (all p0.001). Strong positive correlations were observed between macrophage density, IL-6 levels, VEGF-A expression, and microvessel density within adenomyotic lesions, similar to findings in other populations. **Conclusions:** This study, conducted in Jakarta, provides strong evidence for a heightened pro-inflammatory and pro-angiogenic microenvironment in adenomyosis, consistent with general pathogenic theories. These findings in an Indonesian population underscore the universal importance of these pathways and suggest that novel therapies targeting inflammation and angiogenesis could be relevant for women in this region. Further research should explore potential local modulatory factors.

1. Introduction

Adenomyosis, a benign uterine pathology defined by the heterotopic presence of endometrial glands and stroma within the myometrium, often surrounded by hypertrophic and hyperplastic smooth muscle, poses a considerable health burden for women worldwide. Its prevalence, once thought to be primarily in parous women of advanced reproductive age, is now recognized as being higher and affecting a wider demographic, including younger and nulliparous women, largely due

to significant improvements in diagnostic imaging modalities such as transvaginal ultrasonography (TVUS) and magnetic resonance imaging (MRI). The clinical sequelae of adenomyosis are varied and often debilitating, commonly including abnormal uterine bleeding (AUB), particularly heavy menstrual bleeding (HMB), severe dysmenorrhea, chronic pelvic pain (CPP), and dyspareunia. Moreover, a growing corpus of evidence links adenomyosis to compromised reproductive outcomes, including subfertility, recurrent implantation failure following assisted reproductive technologies, and an increased risk of adverse obstetric events like miscarriage and preterm labor. Despite its prevalence and profound impact on quality of life, the intricate etiological and pathophysiological mechanisms driving adenomyosis are not fully elucidated, which consequently hampers the development of targeted, effective, and uterine-sparing medical therapies. Understanding these mechanisms in diverse populations is crucial for global women's health.[1-3]

The genesis of adenomyosis is likely multifactorial. The prevailing theory suggests that adenomyosis originates from the direct invagination of the endometrial basalis layer into the myometrium, facilitated by disruptions or weaknesses at the endometrial-myometrial junction (EMJ), also known as the J-zone. Such disruptions might be induced by various factors including uterine trauma from childbirth (especially vaginal delivery and cesarean sections), surgical procedures like curettage, chronic endometrial inflammation, or altered hormonal milieus leading to increased intrauterine pressure or tissue vulnerability. Alternative hypotheses include de novo metaplasia of displaced Müllerian remnants or differentiation of pluripotent stem cells residing within the myometrium into endometrial-like tissue. The potential role of adult stem/progenitor cells, possibly derived from bone marrow or local uterine niches, in initiating and promoting adenomyotic lesion growth through differentiation and paracrine signaling is also an area of active investigation.[4-6] These theories are not necessarily mutually exclusive, and it is conceivable that different pathogenetic pathways converge to result in the varied clinical and pathological presentations of adenomyosis.

While the initiating events are still debated, the establishment, persistence, and growth of adenomyotic lesions are critically dependent on the local uterine microenvironment, particularly hormonal influences, inflammatory processes, and aberrant angiogenesis. Estrogen is widely acknowledged as a primary driver, promoting the proliferation and survival of the ectopic endometrial cells. The role of progesterone is more complex, with evidence of progesterone resistance in adenomyotic lesions potentially contributing to their persistence and associated AUB, despite the systemic use of progestins in management. Beyond hormonal control, local chronic inflammation is increasingly recognized as a central player. Ectopic endometrial tissue within the myometrium is believed to provoke an inflammatory cascade, characterized by the recruitment and activation of various immune cells, including macrophages, mast cells, T-lymphocytes, and neutrophils. These cells, in turn, secrete a complex array of signaling molecules such as cytokines (Interleukin [IL]-1β, IL-6, IL-8, Tumor Necrosis Factor-alpha [TNF-α]), chemokines (Monocyte Chemoattractant Protein-1 [MCP-1]), and growth factors. This inflammatory soup can perpetuate inflammation, stimulate cell proliferation, induce tissue fibrosis and remodeling, and sensitize local nerve fibers, thus contributing to the pain symptoms characteristic of adenomyosis. Macrophages, particularly their polarization into pro-inflammatory M1 or anti-inflammatory/pro-repair M2 phenotypes, appear to be crucial in modulating the disease course. Mast cells, through the release of mediators like histamine and tryptase, are also implicated in neurogenic inflammation and adenomyosis-associated pain.[7-10]

Angiogenesis, the formation of new blood vessels, is indispensable for the growth and survival of any tissue, and pathological angiogenesis is a known feature of conditions involving abnormal tissue proliferation, such as endometriosis and various cancers. In adenomyosis, ectopic endometrial implants must secure an adequate blood supply to establish, expand, and persist within the myometrial layer. Indeed, increased microvessel density (MVD) has been consistently observed in adenomyotic lesions and often in the eutopic endometrium of affected women. This heightened vascularization is believed to be orchestrated by an imbalance of pro-angiogenic and anti-angiogenic factors. Vascular Endothelial Growth Factor (VEGF), especially VEGF-A, is a potent stimulator of angiogenesis and its expression is frequently elevated in adenomyotic tissue, correlating with MVD and potentially contributing to HMB via increased vascular

permeability and fragility. Other angiogenic systems, including angiopoietins (Ang-1, Ang-2, and their receptor Tie-2), fibroblast growth factors (FGFs), and platelet-derived growth factors (PDGFs), are also implicated, although their specific roles and interactions in adenomyosis are still being defined.[11,12]

The interplay between these inflammatory and angiogenic processes is of particular interest. Inflammatory mediators can directly induce angiogenesis; cytokines like TNF-α and IL-6 can upregulate VEGF expression, and activated immune cells, notably macrophages, are rich sources of pro-angiogenic factors. Conversely, newly formed, often structurally abnormal, blood vessels can enhance the recruitment of inflammatory cells to the site of pathology, establishing a self-perpetuating cycle that fosters lesion development and symptom persistence. While these general mechanisms are being unraveled, there is a recognized need for studies in diverse global populations. Most research on adenomyosis pathophysiology originates from Western or East Asian (Japan, Korea, China) populations. Data from Southeast Asian countries, including Indonesia, is relatively scarce. Jakarta, the capital of Indonesia, is a large, multicultural metropolis with a diverse population. Studying adenomyosis in this specific cohort is important to determine if the fundamental pathophysiological mechanisms observed elsewhere are also prevalent here, and to explore any potential unique characteristics or influencing factors related to genetics, environment, or lifestyle that might be relevant in this population. Such data are crucial for tailoring diagnostic and therapeutic strategies effectively for Indonesian women.[13]

Therefore, this study was designed to provide novel insights into the pathophysiology of adenomyosis by comprehensively investigating key inflammatory pathways and angiogenic processes specifically within a cohort of women in Jakarta, Indonesia. The primary aims were: 1) To characterize and quantify specific inflammatory cell infiltrates (macrophages, mast cells, T-lymphocytes) in eutopic endometrium, ectopic adenomyotic lesions, and associated myometrium from women with adenomyosis in Jakarta compared to control uterine tissues from the same population. 2) To determine the expression profiles of selected pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α, MCP-1) in these tissues. 3) To evaluate the extent of angiogenesis by measuring MVD and the expression of key angiogenic factors (VEGF-A, VEGFR1, VEGFR2, Ang-1, Ang-2). 4) To explore potential correlations between these markers and clinical symptom severity. We hypothesized that, similar to findings in other populations, adenomyotic lesions in women in Jakarta are characterized by a distinct pro-inflammatory and pro-angiogenic signature, and that understanding these pathways will provide a basis for improved management strategies relevant to this specific demographic.

2. Methods

This prospective case-control study was conducted across three major tertiary referral hospitals in Jakarta, Indonesia. Patient recruitment and sample collection took place between January 2022 and December 2024. The study protocol received full ethical approval from the Ethics Committee of CMHC Research Center, Palembang, Indonesia, and conformed to the principles of the Declaration of Helsinki. All participants provided written informed consent in Bahasa Indonesia prior to their enrollment in the study.

A total of 128 women scheduled for hysterectomy for benign gynecological conditions were initially screened for eligibility. The adenomyosis group (AD group, n=50) comprised women pre-operatively diagnosed with adenomyosis based on standardized transvaginal ultrasound (TVUS) criteria outlined by the Morphological Uterus Sonographic Assessment (MUSA) group (globular uterus, myometrial heterogeneity, cysts, fan-shaped shadowing, indistinct EMJ) and/or MRI findings (junctional zone thickness ≥12 mm, high-intensity foci on T2-weighted images). The diagnosis was subsequently confirmed by post-operative histopathological examination in all cases.

The control group (CTRL group, n=50) consisted of women from Jakarta undergoing hysterectomy for benign conditions without evidence of adenomyosis, such as uterine prolapse with no endometrial pathology or small, asymptomatic leiomyomas not distorting the endometrial cavity or EMJ. These control participants had no ultrasonographic or MRI signs suggestive of adenomyosis. Post-operative histopathology confirmed the absence of adenomyosis, endometrial hyperplasia, or malignancy in all control subjects.

Exclusion criteria for participants in both groups were: age 18 years or >55 years; current or recent (within the preceding 3 months) use of hormonal medications (including GnRH analogues, oral contraceptives, progestins, danazol, or hormonal intrauterine systems) that could significantly alter endometrial or myometrial biology and inflammatory status; active pelvic inflammatory disease (PID); concurrent endometriosis diagnosed as the primary pathology or requiring extensive surgical management (minimal co-existing endometriosis, if incidentally found and not the main indication for surgery, was noted but these patients were limited to 10% of the cohort to minimize confounding inflammatory signals); prior uterine artery embolization or other uterine-sparing interventions for adenomyosis; known or suspected gynecological malignancies; pregnancy or lactation within the 6 months prior to surgery; and significant systemic inflammatory conditions (rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease) or known immunodeficiency disorders.

Comprehensive clinical data were collected for each participant. This included: demographic information (age, ethnicity – predominantly Indonesian with specific sub-ethnicities Javanese, Sundanese, Betawi, socioeconomic status based on education and occupation), parity, gravidity, body mass index (BMI, kg/m^2), detailed menstrual history, menstrual cycle phase at the time of surgery (determined by last menstrual period and subsequently confirmed by endometrial histology where feasible), primary symptoms (AUB, dysmenorrhea, CPP, dyspareunia), visual analog scale (VAS) scores for pain intensity (0-10 scale), and pictorial blood loss assessment chart (PBAC) scores for quantification of menstrual blood loss (35). Information on dietary habits and exposure to specific environmental factors prevalent in Jakarta was considered for collection via a supplementary questionnaire, though not a primary endpoint of this specific pathophysiological study.

All uterine tissue samples were obtained by pathologists immediately following hysterectomy in the operating theatres of the participating Jakarta hospitals. Specifically, for the AD group, samples were meticulously dissected, these included: Eutopic Endometrium (AD-EuE), which was endometrial tissue carefully scraped or excised from an area of the uterine cavity macroscopically distant from any visible adenomyotic lesions, typically the fundus or anterior/posterior wall; Ectopic Adenomyotic Lesions (AD-EcL), consisting of discrete adenomyotic foci (identified as poorly demarcated, often firm, whitish, or trabeculated areas within the myometrium, sometimes containing small, blood-filled or clear fluid-filled cysts) that were carefully excised from the surrounding myometrium, with efforts made to obtain lesions from the inner myometrium; Adjacent Myometrium (AD-AdjM), comprising myometrial tissue immediately enveloping (within approximately 0.5 cm) an excised adenomyotic lesion; and Distant Myometrium (AD-DistM), which was myometrial tissue sampled from an area of the uterine corpus apparently free of macroscopic adenomyosis, usually from the outer myometrial layer, at least 2 cm away from any visible lesion. For the CTRL group, corresponding samples were collected, including Control Endometrium (CTRL-EuE), which was endometrial tissue from a standardized location in the uterine fundus or upper uterine body, and Control Myometrium (CTRL-M), consisting of myometrial tissue from the inner third of the myometrial layer.

Each tissue sample was meticulously divided. One portion was immediately fixed in 10% neutral buffered formalin for 24-48 hours for routine histopathological processing and subsequent immunohistochemical (IHC) analysis. A second portion was snap-frozen in liquid nitrogen vapor within sterile cryovials and then stored at -80°C for later RNA and protein extraction. A third small portion was placed in sterile transport medium (DMEM/F-12; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) with an antibiotic-antimycotic solution for potential future cell culture studies.

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were processed according to standard histological protocols. Serial sections of 4 µm thickness were cut using a microtome and stained with hematoxylin and eosin (H&E). Experienced gynecologic pathologists, blinded to the initial grouping, reviewed all H&E slides to confirm the diagnosis of adenomyosis using the established criterion of endometrial glands and stroma situated at least 2.5 mm deep from the endometrial-myometrial junction within the myometrium, or one low-power field. The specific type (diffuse, focal, adenomyoma) and an estimate of the extent/depth of myometrial infiltration by adenomyosis were recorded.

Control tissues were meticulously examined to confirm the absence of adenomyosis, endometrial hyperplasia, malignancy, or other significant confounding pathologies.

Immunohistochemistry was performed on 4 µm thick FFPE sections obtained from the tissue blocks. The panel of primary antibodies included: Mouse anti-human CD68 (Clone PG-M1, Dako, Glostrup, Denmark; 1:100 dilution) for identifying pan-macrophages; Rabbit anti-human CD163 (Clone EPR19518, Abcam, Cambridge, UK; 1:250 dilution) as a marker for M2-phenotype macrophages. M1-like macrophages were inferred by CD68+CD163- staining profile or identified using specific M1 markers (iNOS); Mouse anti-human Mast Cell Tryptase (Clone AA1, Dako; 1:500 dilution) for detecting mast cells; Rabbit anti-human CD3 (Clone SP7, Abcam; 1:200 dilution) for pan T-lymphocytes; Mouse anti-human CD31 (Clone JC70A, Dako; 1:75 dilution) for endothelial cell staining to determine microvessel density (MVD); Rabbit anti-human VEGF-A (Clone EP1176Y, Abcam; 1:150 dilution); Rabbit anti-human VEGFR2 (KDR/Flk-1) (Clone 55B11, Cell Signaling Technology, Danvers, MA, USA; 1:100 dilution).

Standard IHC protocols were followed. Briefly, sections were deparaffinized in xylene and rehydrated through graded ethanol series. Antigen retrieval was performed using heat-induced epitope retrieval (HIER) with either citrate buffer (pH 6.0) or Tris-EDTA buffer (pH 9.0) in a decloaking chamber or water bath, optimized for each antibody. Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen peroxide in methanol for 20 minutes. Non-specific antibody binding was blocked using an appropriate blocking serum (5% normal goat serum or 5% bovine serum albumin in TBS) for 30-60 minutes at room temperature. Sections were then incubated with the diluted primary antibodies overnight at 4°C in a humidified chamber. Following washes with Tris-buffered saline with Tween-20 (TBST), sections were incubated with an HRP (horseradish peroxidase)-conjugated secondary antibody system (EnVision+ Dual Link System-HRP, Dako) for 60 minutes at room temperature. The immunoreaction was visualized using 3,3'-diaminobenzidine (DAB) as the chromogen, followed by light counterstaining with Mayer's hematoxylin. Negative controls involved omission of the primary antibody or substitution with an isotype-matched control immunoglobulin. Known positive control tissues (human tonsil for immune cells, placental tissue for VEGF/VEGFR, tissues with known high vascularity for CD31) were included in each IHC run to validate the staining procedure.

All IHC-stained slides were scanned using a digital slide scanner (Aperio CS2, Leica Biosystems, Wetzlar, Germany) for archiving and to facilitate quantitative analysis. Quantification was performed by two independent observers (a pathologist and a trained researcher) blinded to the patient group and clinical data, using digital image analysis software (ImageJ, NIH, Bethesda, MD, USA).

For inflammatory cell counts (CD68+, CD163+, Mast Cell Tryptase+, CD3+), five to ten non-overlapping representative high-power fields (HPFs; 400x magnification, area approx. 0.0625 mm^2 per field) were selected from areas of highest cellular density ("hotspots") within the endometrial stroma, adenomyotic glandular/stromal areas, or myometrial stroma. The number of positively stained cells was counted in each field, and the average count per HPF (or cells/mm^2) was calculated.

For MVD (CD31+), individual endothelial cells or clusters clearly separated from adjacent structures were counted as single microvessels. Vessels with a distinct lumen, or elongated structures without a lumen but clearly identifiable as vessels, were included. The five areas with the highest vascular density were selected at low magnification (100x), and vessels were counted at higher magnification (200x or 400x). MVD was expressed as the mean number of vessels per HPF or per mm^2.

For VEGF-A and VEGFR2 expression, a semi-quantitative H-score was calculated. This score incorporates both the intensity of staining (graded as 0 = no staining, 1 = weak, 2 = moderate, 3 = strong) and the percentage of cells staining at each intensity level (P0, P1, P2, P3). The H-score = $(0 \times P0) + (1 \times P1) + (2 \times P2) + (3 \times P3)$, resulting in a score from 0 to 300. Staining in glandular epithelium and stromal cells was assessed separately where appropriate.

Inter-observer reliability for quantitative IHC was assessed using the intraclass correlation coefficient (ICC); an ICC > 0.80 was considered good agreement. Discrepancies were resolved by consensus review at a

multi-headed microscope or via joint digital pathology review.

Total RNA was extracted from snap-frozen tissue samples (approx. 20-30 mg) using the RNeasy Mini Kit (Qiagen, Hilden, Germany) or TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) following the manufacturer's instructions, including an on-column DNase digestion step (Qiagen) to eliminate genomic DNA contamination. RNA concentration and purity (A260/A280 and A260/A230 ratios) were determined using a NanoDrop One spectrophotometer (Thermo Fisher Scientific). RNA integrity was assessed using the Agilent 4200 TapeStation System (Agilent Technologies, Santa Clara, CA, USA), and samples with an RNA Integrity Number (RINe) ≥ 7.0 were used for subsequent analyses.

One microgram of total RNA was reverse transcribed into complementary DNA (cDNA) using the iScript cDNA Synthesis2 Kit (Bio-Rad Laboratories, Hercules, CA, USA) or the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific) with a mix of oligo (dT) and random primers, according to the manufacturer's protocol.

qRT-PCR was performed using TaqMan Fast Advanced Master Mix (Applied Biosystems) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The target genes analyzed included: IL-1β, IL-6, IL-8, TNF-α, MCP-1 (CCL2), VEGF-A, VEGFR1 (FLT1), VEGFR2 (KDR), Angiopoietin-1 (ANGPT1), and Angiopoietin-2 (ANGPT2). Validated primers for SYBR Green assays were either designed using Primer-BLAST (NCBI) spanning exon-exon junctions or sourced from published literature. For TaqMan assays, pre-designed validated Gene Expression Assays (Applied Biosystems) were used. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Beta-2-microglobulin (B2M) were evaluated and used as endogenous reference genes for normalization after confirming their stable expression across sample types using geNorm algorithms.

Thermal cycling conditions for SYBR Green assays were typically: 95°C for 3 minutes (initial denaturation), followed by 40 cycles of 95°C for 10 seconds and 60°C (or optimized annealing temperature) for 30 seconds. A melt curve analysis (65°C to 95°C with 0.5°C increments) was performed post-amplification to verify product specificity. For TaqMan assays, standard manufacturer-recommended cycling conditions were employed. All reactions were run in triplicate. Data were expressed as fold change relative to the control group.

Protein concentrations of selected key cytokines (IL-6, TNF-α, MCP-1) and VEGF-A in tissue homogenates were determined using commercially available sandwich ELISA kits (Quantikine ELISA Kits, R&D Systems, Minneapolis, MN, USA) as per manufacturers' detailed protocols.

Approximately 50-100 mg of snap-frozen tissue was homogenized in ice-cold lysis buffer (CelLytic MT, Sigma-Aldrich, St. Louis, MO, USA, or RIPA buffer) supplemented with a protease inhibitor cocktail (cOmplete Mini, Roche, Basel, Switzerland) and phosphatase inhibitors (PhosSTOP, Roche). Homogenates were centrifuged at 12,000-14,000 x g for 20 minutes at 4°C, and the clear supernatants were collected. Total protein concentration in the lysates was quantified using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Thermo Fisher Scientific).

For ELISA, tissue lysates were diluted as appropriate and incubated in antibody pre-coated microplates. Optical density was measured at 450 nm (with wavelength correction at 540 nm or 570 nm if recommended) using a microplate reader (Tecan Spark, Männedorf, Switzerland). Standard curves were generated using the provided recombinant human protein standards. Cytokine and growth factor concentrations were calculated based on the standard curve, normalized to the total protein content of the sample lysate, and expressed as pg/mg total protein or ng/mg total protein. All samples and standards were assayed in duplicate.

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous data was assessed for normality using the Shapiro-Wilk test normality test. Demographic and clinical characteristics were compared between the AD and CTRL groups. Normally distributed continuous data were presented as mean ± standard deviation (SD) and compared using an independent samples Student's t-test. Non-normally distributed data were presented as median and interquartile range (IQR) and compared using the Mann-Whitney U test. Categorical variables were expressed as frequencies and percentages (n, %) and4 compared using the Chi-square (x^2) test or Fisher's exact test, as appropriate.

For comparisons of IHC, qRT-PCR, and ELISA data across multiple tissue types (AD-EcL, AD-EuE, AD-AdjM, AD-DistM, CTRL-EuE, CTRL-M), one-way analysis of variance (ANOVA) followed by Tukey's or Bonferroni's post-hoc test was used for normally distributed data. For non-normally distributed data, the Kruskal-Wallis H test followed by Dunn's multiple comparison test was applied.

Correlations between different inflammatory markers, angiogenic factors, and between these biological markers and clinical parameters (VAS scores for pain, PBAC scores for HMB) within the adenomyosis group were analyzed using Pearson's correlation coefficient (r) for normally distributed data or Spearman's rank correlation coefficient (ρ) for non-normally distributed or ordinal data.

A two-tailed p-value 0.05 was considered statistically significant. Adjustments for multiple comparisons were applied where appropriate (Bonferroni correction for post-hoc tests). A pre-study power calculation indicated that a sample size of n=50 per group would provide >80% power to detect a medium effect size (Cohen's d = 0.5-0.6) for key variables like macrophage density or VEGF expression, at an alpha of 0.05.

3. Results

A total of 100 women from Jakarta were successfully recruited and completed the study: 50 in the adenomyosis (AD) group and 50 in the control (CTRL) group. The baseline demographic and clinical characteristics of these participants are detailed in Table 1.

The mean age of women in the AD group was 44.1 ± 5.8 years, comparable to the CTRL group at 45.7 ± 5.1 years (p=0.13). The majority of participants in both groups were of Indonesian ethnicity, with specific distributions (Javanese: AD 42%, CTRL 40%; Sundanese: AD 30%, CTRL 34%; Betawi: AD 18%, CTRL 16%; Other Indonesian: AD 10%, CTRL 10%) showing no significant difference in ethnic makeup between groups (p=0.85). BMI was similar between the AD group (26.2 ± 4.1 kg/m²) and the CTRL group (27.0 ± 3.8 kg/m²; p=0.29). Parity (median [IQR]: AD: 2 [1-3] vs. CTRL: 2 [1-3]; p=0.45) and socioeconomic status distribution were also comparable between the groups (p=0.38). The proportion of women in the proliferative versus secretory phase of the menstrual cycle at the time of hysterectomy did not differ significantly (p=0.52).

As anticipated, women in the AD group from Jakarta reported significantly higher rates of HMB (AD: 44/50 [88%] vs. CTRL: 7/50 [14%]; p0.001), dysmenorrhea (AD: 46/50 [92%] vs. CTRL: 9/50 [18%]; p0.001), and chronic pelvic pain (AD: 33/50 [66%] vs. CTRL: 6/50 [12%]; p0.001). Median VAS scores for dysmenorrhea (AD: 8 [7-9] vs. CTRL: 2 [0-4]; p0.001) and median PBAC scores (AD: 280 [200-400] vs. CTRL: 55 [35-80]; p0.001) were significantly higher in the AD group. These data confirmed a substantial symptom burden in the adenomyosis cohort from Jakarta, consistent with general clinical profiles of the disease. All cases in the AD group had adenomyosis confirmed by postoperative histopathology.

Table 1. Baseline demographic and clinical characteristics of study participants.

Characteristic	Adenomyosis (AD) Group (n=50)	Control (CTRL) Group (n=50)	p-value
Demographics			
Age (years), mean ± SD	44.1 ± 5.8	45.7 ± 5.1	0.13
Ethnicity, n (%)			0.85
Javanese	21 (42%)	20 (40%)	
Sundanese	15 (30%)	17 (34%)	
Betawi	9 (18%)	8 (16%)	
Other Indonesian	5 (10%)	5 (10%)	
Body Mass Index (BMI, kg/m²), mean ± SD	26.2 ± 4.1	27.0 ± 3.8	0.29
Parity, median [IQR]	2 [1-3]	2 [1-3]	0.45
Socioeconomic Status, n (%)			0.38
Low	10 (20%)	12 (24%)	
Medium	25 (50%)	23 (46%)	
High	15 (30%)	15 (30%)	
Menstrual Cycle Phase, n (%)			0.52
Proliferative	27 (54%)	24 (48%)	
Secretory	23 (46%)	26 (52%)	
Clinical Symptoms & Scores			
Heavy Menstrual Bleeding (HMB), n (%)	44 (88%)	7 (14%)	<0.001
Dysmenorrhea, n (%)	46 (92%)	9 (18%)	<0.001
Chronic Pelvic Pain (CPP), n (%)	33 (66%)	6 (12%)	<0.001
VAS for Dysmenorrhea (0-10), median [IQR]	8 [7-9]	2 [0-4]	<0.001
VAS for Chronic Pelvic Pain (0-10), median [IQR]	7 [5-8]	1 [0-2]	<0.001
PBAC Score, median [IQR]	280 [200-400]	55 [35-80]	<0.001

Table 2 presents immunohistochemical staining and reveals marked differences in inflammatory cell populations across the tissue types. The density of total macrophages (CD68+) was profoundly elevated in ectopic adenomyotic lesions (AD-EcL) (mean ± SD: 132.5 ± 30.1 cells/HPF) and significantly increased in the eutopic endometrium of adenomyosis patients (AD-EuE) (103.7 ± 22.8 cells/HPF) compared to the endometrium from control women (CTRL-EuE) (38.1 ± 11.5 cells/HPF; p0.001 for both comparisons) and control myometrium (CTRL-M) (22.4 ± 9.0 cells/HPF; p0.001 for both). Within the AD group, AD-EcL harbored significantly more CD68+ cells than AD-EuE (p0.001), AD-AdjM (70.2 ± 16.9 cells/HPF; p0.001), and AD-DistM (33.5 ± 10.8 cells/HPF; p0.001). The AD-AdjM also exhibited higher macrophage density than AD-DistM (p0.001) and CTRL-M (p0.001).

Regarding macrophage phenotype, the density of M2-phenotype macrophages (CD163+) was increased in AD-EcL (45.3 ± 12.0 cells/HPF) and AD-EuE (35.8 ± 10.1 cells/HPF) compared to CTRL-EuE (18.2 ± 6.0 cells/HPF; p0.001 for both). However, the number of CD68+CD163- (M1-like) macrophages was disproportionately higher in AD-EcL and AD-EuE, indicating a shift towards a pro-inflammatory M1-dominant macrophage profile in adenomyotic tissues from this Jakarta cohort. Mast cell density was significantly greater in AD-EcL (60.3 ± 13.5 cells/HPF) and AD-EuE (44.1 ± 11.0 cells/HPF) compared to CTRL-EuE (9.2 ± 3.8 cells/HPF; p0.001 for both) and CTRL-M (6.0 ± 2.5 cells/HPF; p0.001 for both). AD-EcL contained significantly more mast cells than AD-EuE (p0.001), AD-AdjM (23.7 ± 7.1 cells/HPF; p0.001), and AD-DistM (10.5 ± 4.2 cells/HPF; p0.001). Mast cells were frequently observed near blood vessels and nerve fibers within the adenomyotic lesions. CD3+ T-lymphocyte infiltration was also significantly elevated in AD-EcL (75.9 ± 20.1 cells/HPF) and AD-EuE (60.2 ± 16.8 cells/HPF) relative to CTRL-EuE (25.3 ± 8.5 cells/HPF; p0.001 for both) and CTRL-M (17.0 ± 6.9 cells/HPF; p0.001 for both). These findings are consistent with an active adaptive immune response within the adenomyotic tissues of the Jakarta patient group.

Table 2. inflammatory cell infiltrates (cells/HPF, mean ± SD) in uterine.

Tissue Compartment	**CD68+ Macrophages**	**CD163+ (M2) Macrophages**	**Mast Cells (Tryptase+)**	**CD3+ T-Lymphocytes**
Adenomyosis Group (AD)				
Ectopic Lesions (AD-EcL)	132.5 ± 30.1	45.3 ± 12.0	60.3 ± 13.5	75.9 ± 20.1
Eutopic Endometrium (AD-EuE)	103.7 ± 22.8	35.8 ± 10.1	44.1 ± 11.0	60.2 ± 16.8
Adjacent Myometrium (AD-AdjM)	70.2 ± 16.9	N/A	23.7 ± 7.1	N/A
Distant Myometrium (AD-DistM)	33.5 ± 10.8	N/A	10.5 ± 4.2	N/A
Control Group (CTRL)				
Eutopic Endometrium (CTRL-EuE)	38.1 ± 11.5	18.2 ± 6.0 h	9.2 ± 3.8	25.3 ± 8.5
Myometrium (CTRL-M)	22.4 ± 9.0	N/A	6.0 ± 2.5	17.0 ± 6.9

Table 3 presents compelling molecular evidence from the Jakarta cohort, illustrating a significantly heightened pro-inflammatory microenvironment in adenomyotic tissues. It details the expression of key inflammatory cytokines and chemokines, revealing a consistent and marked upregulation of IL-6, TNF-α, IL-8 (mRNA), MCP-1, and IL-1β (mRNA) at both mRNA and, where measured (IL-6, TNF-α, MCP-1), protein levels in ectopic adenomyotic lesions (AD-EcL) and the eutopic endometrium of adenomyosis patients (AD-EuE) compared to control endometrium (CTRL-EuE) (all $p \leq 0.01$ or $p \leq 0.001$ for these comparisons). Notably, the pro-inflammatory signature was generally most pronounced within the ectopic lesions (AD-EcL), which consistently exhibited higher expression levels than the affected eutopic endometrium (AD-EuE), although AD-EuE itself was significantly more inflamed than control tissue. For instance, IL-6 and TNF-α mRNA showed approximately 18.5-fold and 15.2-fold increases in AD-EcL respectively, with corresponding protein elevations confirming active synthesis. This pronounced upregulation of multiple inflammatory mediators underscores a complex, localized inflammatory cascade crucial to adenomyosis pathophysiology in this population.

Table 4 presents a comprehensive assessment of angiogenic activity within uterine tissues from the Jakarta cohort, revealing a significantly enhanced and dysregulated pro-angiogenic microenvironment in women with adenomyosis. The data demonstrate a marked increase in microvessel density (MVD), as evidenced by CD31+ staining, in both ectopic adenomyotic lesions (AD-EcL, 49.5 ± 11.0 vessels/HPF) and the eutopic endometrium of adenomyosis patients (AD-EuE, 38.0 ± 9.2 vessels/HPF) compared to control endometrium (CTRL-EuE, 17.2 ± 5.8 vessels/HPF) ($p \leq 0.01$ to $p \leq 0.001$ for these comparisons). This heightened vascularity was strongly associated with the significant upregulation of Vascular Endothelial Growth Factor-A (VEGF-A) and its key receptor VEGFR2 at both protein (IHC H-score and ELISA) and mRNA levels in AD-EcL and AD-EuE relative to controls (all $p \leq 0.001$). Furthermore, the study highlights a critical imbalance in the angiopoietin system, with significantly decreased Angiopoietin-1 (ANGPT1, a vessel stabilizer) and increased Angiopoietin-2 (ANGPT2, a vessel destabilizer) mRNA expression in adenomyotic tissues, culminating in a markedly elevated ANGPT2/ANGPT1 ratio (median ratio of 14.86 in AD-EcL vs. 1.00 in controls, $p \leq 0.001$). These findings collectively indicate robust neovascularization and vascular instability, pivotal for lesion sustenance and likely contributing to symptoms such as heavy menstrual bleeding.

Table 3. Expression of inflammatory cytokines and chemokines in uterine tissues.

Analyte & Measurement Type	Adenomyosis - Ectopic Lesions (AD-EcL)	Adenomyosis - Eutopic Endometrium (AD-EuE)	Control - Eutopic Endometrium (CTRL-EuE)
Interleukin-6 (IL-6)			
mRNA (fold change), median [IQR]	18.5 [12.2-25.9]	9.8 [6.1-14.5]	1.0 [0.7-1.3]
Protein (pg/mg total protein), mean ± SD	285.3 ± 80.1	199.5 ± 60.7	49.0 ± 17.2
Tumor Necrosis Factor-α (TNF-α)			
mRNA (fold change), median [IQR]	15.2 [10.1-20.7]	7.9 [5.0-10.8]	1.0 [0.6-1.4]
Protein (pg/mg total protein), mean ± SD	170.2 ± 45.5	105.8 ± 28.0	33.1 ± 11.5
Interleukin-8 (IL-8)			
mRNA (fold change), median [IQR]	24.0 [17.5-33.1]	12.5 [8.8-17.9]	1.0 [0.5-1.5]
Monocyte Chemoattractant Protein-1 (MCP-1/CCL2)			
mRNA (fold change), median [IQR]	21.7 [14.0-29.8]	11.3 [7.2-16.1]	1.0 [0.8-1.2]
Protein (pg/mg total protein), mean ± SD	390.8 ± 100.3	230.1 ± 65.9	65.5 ± 22.0
Interleukin-1β (IL-1β)			
mRNA (fold change), median [IQR]	6.5 [3.9-9.2]	3.8 [2.5-5.9]	1.0 [0.6-1.1]

Table 4. Angiogenesis assessment in key uterine tissues.

Parameter & Measurement Type	Adenomyosis - Ectopic Lesions (AD-EcL)	Adenomyosis - Eutopic Endometrium (AD-EuE)	Control - Eutopic Endometrium (CTRL-EuE)
Microvessel Density (MVD)			
CD31+ (vessels/HPF), mean ± SD	49.5 ± 11.0	38.0 ± 9.2	17.2 ± 5.8
VEGF-A Expression			
Protein (IHC H-score), mean ± SD	235.8 ± 48.0	190.5 ± 40.1	90.3 ± 27.5
Protein (ELISA, pg/mg total protein), mean ± SD	490.1 ± 120.5	345.0 ± 90.2	105.3 ± 33.7
mRNA (fold change), median [IQR]	12.3 [8.0-17.5]	7.9 [5.1-10.8]	1.0 [0.6-1.3]
VEGFR1 (FLT1) Expression			
mRNA (fold change), median [IQR]	2.9 [2.0-4.0]	2.0 [1.4-2.8]	1.0
VEGFR2 (KDR) Expression			
Protein (IHC H-score), mean ± SD	210.0 ± 42.8	175.3 ± 37.2 b	77.8 ± 22.1
mRNA (fold change), median [IQR]	7.0 [4.9-10.1]	4.9 [3.2-7.0]	1.0 [0.7-1.4]
Angiopoietin System (mRNA Expression)			
Angiopoietin-1 (ANGPT1) (fold change), median [IQR]	0.35 [0.2-0.5]	0.55 [0.3-0.7]	1.0 [0.8-1.3]
Angiopoietin-2 (ANGPT2) (fold change), median [IQR]	5.2 [3.5-7.5]	3.6 [2.5-5.0]	1.0 [0.6-1.4]
Calculated ANGPT2/ANGPT1 Ratio (median)	14.86	6.55	1.00

Table 5 presents crucial insights into the interplay of pathological mechanisms and their clinical relevance in the Jakarta adenomyosis cohort by detailing significant correlations. Part A reveals strong positive associations within ectopic adenomyotic lesions (AD-EcL), indicating a synergistic relationship between inflammation and

angiogenesis; for instance, CD68+ macrophage density strongly correlated with both IL-6 mRNA (ρ=0.72, p0.001) and microvessel density (MVD) (ρ=0.78, p0.001). Similarly, VEGF-A mRNA levels showed a robust positive correlation with MVD (ρ=0.80, p0.001) and the ANGPT2/ANGPT1 ratio (ρ=0.53, p0.001). Part B further links these biological alterations to patient-reported symptoms. Notably, the severity of heavy menstrual bleeding (PBAC scores) correlated positively with MVD (ρ=0.48, p0.001) and VEGF-A mRNA levels in the eutopic endometrium of adenomyosis patients (AD-EuE) (ρ=0.45, p0.01). Dysmenorrhea severity (VAS scores) also showed significant positive correlations with inflammatory markers within ectopic lesions, including mast cell density (ρ=0.42, p0.01), IL-6 protein levels (ρ=0.39, p0.01), and CD68+ macrophage density (ρ=0.44, p0.01). Collectively, these findings underscore how interconnected inflammatory and angiogenic processes within adenomyotic tissue contribute to the disease's establishment and the severity of its clinical manifestations.

Table 5. Correlations between inflammatory markers, angiogenic markers, and clinical symptom severity.
Part A: Correlations between Inflammatory and Angiogenic Markers within Ectopic Adenomyotic Lesions (AD-EcL)

Marker 1	**Marker 2**	**Spearman's Correlation Coefficient (ρ)**	**p-value**
CD68+ Macrophage Density	IL-6 mRNA Levels	0.72	<0.001
CD68+ Macrophage Density	TNF-α mRNA Levels	0.65	<0.001
CD68+ Macrophage Density	MCP-1 mRNA Levels	0.75	<0.001
Mast Cell Density	IL-6 mRNA Levels	0.58	<0.001
Mast Cell Density	TNF-α mRNA Levels	0.50	<0.001
Inflammatory Markers vs. Angiogenic Markers			
CD68+ Macrophage Density	Microvessel Density (MVD)	0.78	<0.001
CD68+ Macrophage Density	VEGF-A mRNA Levels	0.73	<0.001
IL-6 mRNA Levels	Microvessel Density (MVD)	0.68	<0.001
IL-6 mRNA Levels	VEGF-A mRNA Levels	0.62	<0.001
TNF-α mRNA Levels	Microvessel Density (MVD)	0.60	<0.001
TNF-α mRNA Levels	VEGF-A mRNA Levels	0.55	<0.001
Angiogenic Markers			
VEGF-A mRNA Levels	Microvessel Density (MVD)	0.80	<0.001
VEGF-A mRNA Levels	ANGPT2/ANGPT1 Ratio	0.53	<0.001

Part B: Correlations between biological markers and clinical symptom severity in adenomyosis patients.

Clinical Symptom/Score	**Biological Marker (Tissue specified if not AD-EcL)**	**Spearman's Correlation Coefficient (ρ)**	**p-value**
Heavy Menstrual Bleeding (HMB)			
PBAC Score	Microvessel Density (MVD) in AD-Eutopic Endometrium	0.48	<0.001
PBAC Score	VEGF-A mRNA Levels in AD-Eutopic Endometrium	0.45	<0.01
Dysmenorrhea			
VAS Score for Dysmenorrhea	Mast Cell Density in AD-Ectopic Lesions	0.42	<0.01
VAS Score for Dysmenorrhea	IL-6 Protein Levels in AD-Ectopic Lesions	0.39	<0.01
VAS Score for Dysmenorrhea	CD68+ Macrophage Density in AD-Ectopic Lesions	0.44	<0.01

4. Discussion

This study, conducted within a cohort of women in Jakarta, Indonesia, provides a comprehensive investigation into the roles of inflammatory pathways and angiogenesis in the pathophysiology of adenomyosis. Our findings clearly demonstrate a significantly heightened pro-inflammatory microenvironment and enhanced, dysregulated angiogenic activity within both ectopic adenomyotic lesions and the eutopic endometrium of these women,

when compared to uterine tissues from control individuals from the same population. Furthermore, the strong positive correlations observed between key inflammatory mediators and angiogenic factors underscore a probable synergistic interplay, which likely contributes to the development, progression, and symptomatology of adenomyosis in this specific Southeast Asian population.

The current investigation corroborates and extends the growing body of international literature implicating chronic inflammation as a cornerstone of adenomyosis pathophysiology, now providing specific evidence from an Indonesian cohort. We documented a marked increase in the infiltration of total macrophages (CD68+), with a notable skewing towards an M1 pro-inflammatory phenotype, alongside increased numbers of mast cells and T-lymphocytes, in both adenomyotic lesions (AD-EcL) and the eutopic endometrium (AD-EuE) of women from Jakarta. This cellular influx was paralleled by significantly elevated local expression of potent pro-inflammatory cytokines IL-6 and TNF-α, and the crucial chemokine MCP-1. These findings are largely consistent with studies from other global regions, suggesting that these core inflammatory mechanisms are fundamental to adenomyosis regardless of geographic or ethnic background. The high levels of MCP-1 likely drive the continuous recruitment of macrophages, sustaining the inflammatory milieu within the adenomyotic foci.[13,14]

The observation that the eutopic endometrium (AD-EuE) in women with adenomyosis from Jakarta also exhibits a significant pro-inflammatory state is particularly noteworthy. This supports the concept of a "global uterine dysfunction" or an altered "field effect" in adenomyosis, where the entire uterus may be affected, not just the areas with visible lesions. This generalized endometrial inflammation could contribute to symptoms like HMB and may also play a role in adenomyosis-associated infertility or recurrent implantation failure by creating an unreceptive endometrial environment for embryo attachment and development. This aspect warrants further investigation, especially in populations like Jakarta where fertility rates and family planning practices might differ from Western contexts. The increased inflammation in the myometrium adjacent to lesions (AD-AdjM) also points to a local spread of these pathological processes.[15-16]

The functional consequences of this intense inflammatory response within the uterine tissues of women in Jakarta are likely profound. Pro-inflammatory cytokines such as TNF-α and IL-6 are known to stimulate endometrial cell proliferation, inhibit apoptosis, promote tissue remodeling and fibrosis (common features of adenomyosis), and sensitize nociceptive pathways, thus contributing directly to pain. The increased mast cell density, and its correlation with dysmenorrhea scores in our Jakarta cohort, aligns with findings elsewhere highlighting the role of mast cell degranulation products (histamine, tryptase, prostaglandins, NGF) in neurogenic inflammation, hyperalgesia, and visceral pain. Addressing this inflammatory component is a key therapeutic avenue for managing adenomyosis-related symptoms in Indonesian women.[17,18]

Our study robustly demonstrates that adenomyosis in the Jakarta cohort is characterized by significantly enhanced and structurally abnormal angiogenesis. We observed markedly increased MVD in both AD-EcL and AD-EuE, a finding that mirrors results from studies in other populations (25,26). This neovascularization is crucial for supplying nutrients and oxygen to the ectopic endometrial implants, thereby supporting their survival, growth, and infiltration within the myometrium. The often dilated and irregular appearance of these new vessels could contribute to their fragility and propensity for bleeding, providing a plausible explanation for the HMB frequently experienced by women with adenomyosis.[19-20] The positive correlation we found between MVD in the eutopic endometrium and PBAC scores in our Jakarta participants supports this link.
This pathological angiogenesis was closely associated with the overexpression of the potent pro-angiogenic factor VEGF-A and its primary receptor, VEGFR2, at both mRNA and protein levels in adenomyotic tissues. This is consistent with numerous international studies that have identified VEGF-A as a central player in driving adenomyotic vascularity.[17-19] The concurrent upregulation of VEGFR2 suggests an amplified responsiveness of the local endothelial cells to VEGF-A signaling. Furthermore, our investigation of the angiopoietin system revealed a significant decrease in

the vessel-stabilizing factor Ang-1 and a concomitant increase in the vessel-destabilizing factor Ang-2 in adenomyotic tissues. This resulted in a markedly elevated Ang-2/Ang-1 ratio, a condition known to promote vascular leakage, destabilization, and active angiogenic sprouting in the presence of VEGF. These findings indicate that the angiogenic process in adenomyosis among women in Jakarta is not merely an increase in vessel quantity but also involves a significant qualitative dysregulation leading to an abnormal and inefficient vasculature.

A particularly important finding from our study in the Jakarta population is the strong evidence supporting an intricate and likely synergistic interplay between local inflammatory processes and angiogenesis. We demonstrated significant positive correlations between the density of pro-inflammatory immune cells (especially M1-like macrophages and mast cells), the local concentrations of pro-inflammatory cytokines (IL-6, TNF-α), and various markers of heightened angiogenesis (MVD, VEGF-A expression). This suggests that inflammation is a potent driver of angiogenesis in adenomyosis within this cohort. Activated macrophages are well-established sources of numerous pro-angiogenic molecules, including VEGF-A, FGFs, and even TNF-α itself, which can directly stimulate endothelial cell proliferation, migration, and tube formation. Furthermore, inflammatory cytokines like IL-6 and TNF-α, produced by both immune cells and stromal cells, can directly upregulate VEGF expression in endometrial and myometrial cells.

Conversely, the newly formed, often leaky and immature, blood vessels characteristic of pathological angiogenesis can facilitate the extravasation and continued recruitment of inflammatory cells from the bloodstream into the uterine tissue, thereby amplifying and perpetuating the local inflammatory response. The increased Ang-2/Ang-1 ratio, by promoting vascular permeability, likely contributes to this enhanced inflammatory cell trafficking. This establishes a vicious positive feedback loop where inflammation begets more angiogenesis, and the resulting abnormal neovasculature further fuels inflammation. This cycle likely drives lesion growth, persistence, and the generation of symptoms in women with adenomyosis in Jakarta, as it does elsewhere.

Conducting this study in Jakarta provides valuable data from a Southeast Asian population, which has been underrepresented in adenomyosis research. While our core findings on inflammation and angiogenesis align broadly with international studies, suggesting these are conserved fundamental mechanisms, the specific baseline characteristics of the Jakarta cohort (ethnicity, potential dietary influences, environmental exposures unique to a large tropical urban center like Jakarta) are now documented. It is plausible that such local factors could subtly modulate the intensity or specific molecular players within these general pathways, though our current study was not designed to isolate such influences. Future research could investigate whether specific dietary patterns prevalent in Indonesia known to have anti-inflammatory or pro-inflammatory effects might interact with adenomyosis risk or severity. Genetic predisposition within the Indonesian population also warrants further exploration.

The findings have direct implications for women's health in Jakarta and Indonesia. The high prevalence of debilitating symptoms like HMB and dysmenorrhea observed in our Jakarta cohort underscores the need for effective local management strategies. The molecular pathways elucidated here (IL-6, TNF-α, MCP-1, VEGF pathways) offer potential targets for novel, non-surgical therapies that could be particularly beneficial in a setting where access to advanced surgical interventions might vary. However, the translation of these findings into clinical practice requires careful consideration of cost-effectiveness, accessibility, and potential side effects in the local healthcare context.

The strengths of this study include its prospective case-control design, the recruitment from multiple tertiary centers in Jakarta ensuring a degree of representativeness for urban Indonesia, the comprehensive analysis of multiple inflammatory and angiogenic markers using robust methodologies, and the careful histopathological confirmation. Nevertheless, certain limitations are acknowledged. As with many studies on adenomyosis, tissue samples were primarily obtained from women undergoing

hysterectomy, which may represent a more severe spectrum of the disease. Findings may not be fully generalizable to women with milder forms of adenomyosis or those who do not opt for surgery in Jakarta. The cross-sectional nature of the study identifies associations but cannot definitively establish causality. While we documented ethnicity, a deeper exploration of genetic or specific environmental factors was beyond the scope of this particular pathophysiological investigation. Further research, including longitudinal studies to track disease progression and response to therapies in Indonesian women, is crucial. Functional studies using *in vitro* models derived from Jakarta patient tissues or relevant animal models could further delineate the specific mechanisms and test therapeutic interventions. Collaborative efforts within Indonesia and Southeast Asia could also help build a larger, more detailed picture of adenomyosis in the region. The potential influence of co-morbidities like endometriosis, although minimized by exclusion criteria, should always be considered.

Future research in Jakarta could also focus on identifying non-invasive biomarkers (in blood or menstrual fluid) for early diagnosis and monitoring of adenomyosis, which would be particularly valuable in resource-limited settings. Investigating the impact of traditional Indonesian medicines or lifestyle factors on adenomyosis symptoms and pathophysiology could also yield interesting and locally relevant insights.

5. Conclusions

This study, conducted specifically within a cohort of women in Jakarta, Indonesia, provides robust evidence that the pathophysiology of adenomyosis is intrinsically linked to a uterine microenvironment characterized by intense chronic inflammation and aberrant, dysregulated angiogenesis. We have demonstrated significantly increased infiltration of pro-inflammatory immune cells, elevated local concentrations of key inflammatory cytokines and chemokines, and enhanced expression of pro-angiogenic factors coupled with increased microvessel density in both ectopic adenomyotic lesions and the eutopic endometrium of these women. Critically, our findings reveal a strong synergistic relationship between these inflammatory and angiogenic processes, suggesting they fuel each other in a vicious cycle that likely promotes lesion establishment, perpetuates growth, and contributes significantly to clinical symptoms like heavy menstrual bleeding and pelvic pain in this population. These insights, while confirming universal mechanisms, provide a crucial evidence base from an underrepresented Southeast Asian population. They highlight the potential for novel therapeutic strategies targeting these pathways to be relevant and beneficial for women suffering from adenomyosis in Jakarta and broader Indonesia. Further research should focus on translating these findings into locally appropriate diagnostic and therapeutic innovations.

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