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Original Article

Association of Gut Dysbiosis With Hormonal Imbalance in Perimenopausal Women

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ABSTRACT

Background: Perimenopause is a hormonally dynamic transitional stage often accompanied by menstrual irregularity, vasomotor symptoms, mood disturbance, sleep problems, and increasing metabolic vulnerability. Emerging evidence suggests that gut dysbiosis may influence estrogen metabolism, symptom burden, and cardiometabolic health through the estrobolome and related inflammatory-metabolic pathways. Objective: To determine the association of gut dysbiosis with hormonal imbalance, menopausal symptom severity, and metabolic health in perimenopausal women attending a tertiary care hospital in Multan, Pakistan. Methods: This cross-sectional analytical study included 180 perimenopausal women aged 40 to 55 years recruited through non-probability consecutive sampling. Data were collected using a structured proforma covering sociodemographic, menstrual, gastrointestinal, lifestyle, and medical variables. Menopausal symptoms were assessed using the Menopause Rating Scale, hormonal status was evaluated through serum estradiol, follicle-stimulating hormone, and luteinizing hormone, and metabolic assessment included body mass index, waist circumference, fasting blood sugar, HbA1c, lipid profile, and blood pressure. Participants were categorized into dysbiosis and non-dysbiosis groups according to protocol-defined clinical and laboratory criteria. Results: Gut dysbiosis was identified in 104 of 180 women (57.8%). Compared with women without dysbiosis, affected women had significantly higher mean total Menopause Rating Scale score (15.8 ± 5.9 vs 11.2 ± 4.8), lower serum estradiol (58.6 ± 21.4 pg/mL vs 74.9 ± 24.7 pg/mL), and higher FSH and LH levels. They also had higher BMI, waist circumference, fasting blood sugar, HbA1c, triglycerides, LDL cholesterol, and systolic blood pressure, with lower HDL cholesterol. On multivariable analysis, severe menopausal symptoms, low estradiol, high waist circumference, diabetes mellitus, and low physical activity remained independently associated with dysbiosis. Conclusion: Gut dysbiosis was significantly associated with hormonal imbalance, greater menopausal symptom burden, and adverse metabolic health in perimenopausal women. These findings support a more integrated clinical approach to perimenopausal care and justify further prospective studies to clarify temporality and mechanism. Keywords: Perimenopause; Gut dysbiosis; Hormonal imbalance; Estrogen metabolism; Estrobolome; Menopause Rating Scale; Metabolic health; Pakistan.

INTRODUCTION

Perimenopause is a biologically active transitional phase in the female reproductive lifespan, characterized by fluctuating ovarian function, menstrual irregularity, vasomotor complaints, sleep

disturbance, mood changes, and progressive metabolic vulnerability. Rather than representing a single event, it reflects a dynamic stage of endocrine instability preceding menopause, during which many clinically meaningful symptoms first emerge and begin to affect quality of life, cardiometabolic health, and daily functioning (9,10). Because estrogen variability during this period influences thermoregulation, mood, vascular tone, glucose handling, lipid metabolism, and body fat distribution, the perimenopausal transition has increasingly been recognized as a multidimensional clinical state rather than an isolated reproductive phenomenon (14-17).

Recent scientific interest has expanded beyond ovarian aging alone to include the contribution of the gut microbiome to women's hormonal and metabolic health. The gut microbial ecosystem performs important regulatory functions related to digestion, immune modulation, mucosal integrity, vitamin synthesis, and host metabolism. When this ecosystem becomes imbalanced, a state commonly described as gut dysbiosis may develop, characterized by reduced microbial diversity, loss of beneficial organisms, overrepresentation of potentially harmful species, and altered metabolic activity. Such dysregulation has been associated with systemic inflammation, insulin resistance, obesity, lipid abnormalities, and altered sex hormone metabolism, making it increasingly relevant to midlife female health (1-4,14).

A central mechanistic concept linking gut health with reproductive endocrinology is the estrobolome, the collection of gut microbial genes involved in estrogen metabolism. Through enzymes such as beta-glucuronidase, intestinal bacteria can deconjugate estrogen metabolites excreted in bile, thereby influencing whether estrogens are eliminated or reabsorbed into the circulation. Disturbance of this microbial activity may alter estrogen homeostasis and intensify endocrine instability during a phase when hormonal fluctuations are already pronounced (2,7). This relationship is biologically plausible in perimenopause, where even modest perturbations in estrogen availability may have clinically visible effects on menstrual regularity, vasomotor symptoms, mood, and metabolic adaptation.

Emerging evidence suggests that menopause is associated with measurable changes in gut microbial composition and function, with possible implications for cardiometabolic health and symptom burden. Studies comparing premenopausal and postmenopausal women have reported reduced microbial diversity, altered taxonomic abundance, and shifts in microbial metabolic pathways that resemble patterns seen in aging and adverse metabolic states (1,3,4). More recent literature has further highlighted the bidirectional nature of the sex hormone–gut microbiome axis, in which sex hormones shape microbial ecology while gut microbes influence hormone bioavailability and downstream physiological responses (8,19,20). These interactions may be especially relevant during perimenopause, when endocrine regulation is inherently unstable and susceptibility to symptom amplification may be heightened.

The clinical importance of this issue extends beyond reproductive symptoms alone. During the menopausal transition, women frequently experience progressive increases in central adiposity, dyslipidemia, elevated blood pressure, and impaired glucose regulation, changes that collectively contribute to rising long-term cardiovascular and metabolic risk (14-18). Gut dysbiosis may plausibly interact with these processes through inflammatory activation, altered intestinal permeability, metabolite signaling, and disruption of estrogen metabolism. Consequently, gut health may influence not only gastrointestinal well-being but also the broader metabolic and symptomatic profile of perimenopausal women (6,21-25).

Despite growing international interest in the menopause–microbiome relationship, important gaps remain. Much of the published literature has focused on postmenopausal women, experimental models, narrative synthesis, or microbiome characterization without parallel clinical assessment of symptom burden and metabolic parameters. Data from South Asian populations remain particularly limited, and hospital-based evidence from Pakistan examining gut dysbiosis in relation to hormonal profile, menopausal symptoms, and metabolic health in the same perimenopausal population is scarce. This is clinically relevant because women in local settings often present with overlapping complaints such as

bloating, constipation, poor sleep, mood fluctuation, irregular bleeding, and weight gain, yet these symptoms are usually evaluated in isolation rather than through an integrated hormonal-metabolic framework (21-25).

A clearer understanding of this association may have practical implications for earlier risk identification and more holistic care. If gut dysbiosis is linked with lower estradiol levels, greater symptom severity, and adverse metabolic markers during perimenopause, then accessible interventions such as dietary modification, physical activity optimization, weight control, and microbiome-supportive strategies may offer meaningful benefit in tertiary care settings where women commonly present with mixed symptom clusters (21,22,25).

Therefore, the present cross-sectional analytical study was designed to examine the association of gut dysbiosis with hormonal imbalance in perimenopausal women attending a tertiary care hospital in Multan, Pakistan, with particular focus on estrogen-related hormonal changes, menopausal symptom burden, and metabolic health indicators. The study further aimed to determine whether gut dysbiosis remained independently associated with clinically important hormonal and metabolic variables after adjustment for relevant confounders. It was hypothesized that women with gut dysbiosis would demonstrate lower estradiol levels, more severe menopausal symptoms, and a less favorable metabolic profile than women without dysbiosis.

MATERIALS AND METHODS

This hospital-based cross-sectional analytical study was conducted at a tertiary care hospital in Multan, Pakistan, to evaluate the association of gut dysbiosis with hormonal imbalance in perimenopausal women, with additional assessment of menopausal symptom burden and metabolic health. Recruitment was carried out through the gynecology outpatient department, medical outpatient department, and affiliated diagnostic units during the study period. The tertiary care setting was selected because it serves women from both urban and rural backgrounds and therefore provided a clinically relevant and heterogeneous perimenopausal population for analysis. The study was planned in accordance with accepted reporting principles for observational research, with prespecification of the study variables, comparison groups, and analytical approach.

Eligible participants were women aged 40 to 55 years who were in the perimenopausal stage and attending the study site during the recruitment period. Perimenopause was operationally defined on the basis of menstrual transition and clinical presentation as the phase preceding menopause in which spontaneous menstruation was still occurring but cycle regularity, menstrual flow pattern, or menopausal-transition symptoms had changed. Women were included if they were willing to participate and able to provide informed consent. Exclusion criteria were applied to reduce clinical and biochemical confounding and included surgical menopause, pregnancy, lactation, known malignancy, previously diagnosed inflammatory bowel disease or chronic gastrointestinal disease, chronic liver disease, renal failure, acute infection at the time of interview, severe psychiatric illness impairing reliable response, and recent exposure to factors likely to alter gut microbiota or hormonal profile, including antibiotics, probiotics, prebiotics, hormone replacement therapy, oral contraceptive pills, or corticosteroids within the protocol-defined exclusion window.

A non-probability consecutive sampling strategy was used, and every eligible woman presenting during the study period was approached for participation until the target sample size was achieved. The required sample was determined using a standard prevalence-based sample size approach for cross-sectional studies, incorporating an expected prevalence derived from related literature, a 95% confidence level, an acceptable margin of error, and an additional allowance for incomplete responses. Recruitment and enrollment were performed by trained study personnel. Written informed consent was obtained before interview, examination, and specimen collection. Participants were informed of the study purpose, the voluntary nature of participation, and the confidentiality of all collected information.

Data were collected using a structured proforma developed in line with the study objectives and pretested before final implementation to improve clarity, sequencing, and completeness. Sociodemographic information included age, residence, education, marital status, occupation, and socioeconomic background. Clinical history covered menstrual and reproductive characteristics, including age at menarche, parity, cycle pattern, menstrual irregularity, cycle-length variation, bleeding characteristics, and prior gynecological problems. Relevant medical and lifestyle factors were also recorded, including diabetes mellitus, hypertension, thyroid disease, medication history, dietary pattern, bowel habits, sleep status, and physical activity. To improve data consistency, interviews were conducted in a private setting by trained investigators using the same data collection format for all participants, and completed forms were reviewed on the day of collection for internal completeness and recording accuracy.

Menopausal symptom burden was assessed using the Menopause Rating Scale or an equivalent validated symptom-based instrument administered in interview format. Participants were asked about vasomotor, psychological, somatic, and urogenital symptoms, including hot flushes, sweating, palpitations, sleep disturbance, irritability, anxiety, low mood, musculoskeletal discomfort, sexual complaints, and bladder-related symptoms. Individual symptom scores were summed to generate a total symptom burden score, and symptom severity was categorized according to the scale's standard interpretive framework. This variable was used both descriptively and analytically to examine the relationship between symptom burden and dysbiosis status.

Gut dysbiosis was assessed using a protocol-defined composite classification based on gastrointestinal symptom burden supported by laboratory correlation where available. A structured gut health assessment captured bloating, constipation, diarrhea or loose stools, altered stool frequency, excessive gas, abdominal discomfort, food intolerance, and incomplete evacuation. These features were used to derive a dysbiosis-related symptom score. Stool sampling and routine microbiological or laboratory evaluation were performed under standardized aseptic guidance in the subset of participants for whom such testing was feasible within routine hospital workflow. Available laboratory indicators included stool pH, microscopic findings, culture findings where relevant, and clinical correlation. To reduce misclassification, final categorization into dysbiosis and non-dysbiosis groups was based on the prespecified study criteria rather than on any single symptom or isolated laboratory parameter.

Hormonal assessment was performed using venous blood samples collected under standard aseptic conditions. Serum estradiol, follicle-stimulating hormone, and luteinizing hormone were measured because these variables were central to the study objective and represent key endocrine markers during the menopausal transition. Given the irregularity of menstrual timing in perimenopausal women, blood collection was aligned with menstrual timing where practically possible; in women with irregular cycles, samples were obtained at presentation and interpreted in conjunction with menstrual history and clinical context. Hormonal imbalance was evaluated using observed laboratory values in relation to expected reproductive-aging ranges and accompanying clinical features.

Metabolic assessment included fasting blood sugar, glycated hemoglobin where available, and lipid profile parameters including total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Blood pressure was measured after adequate seated rest using standard sphygmomanometric technique. Anthropometric measurements were obtained using calibrated instruments and standardized procedures. Body weight and height were measured with participants in light clothing and without shoes, body mass index was calculated as kilograms per meter squared, and waist circumference was recorded as an index of central adiposity. These variables were selected because of their established clinical relevance during the menopausal transition and their plausible biological relationship with gut dysbiosis.

Several steps were taken to minimize bias and strengthen internal validity. Uniform eligibility criteria were applied throughout recruitment, the questionnaire was pretested before formal use, measuring

instruments were checked regularly, and laboratory analyses were conducted under routine institutional protocols. Collection of sociodemographic, clinical, hormonal, and metabolic variables in the same participants allowed assessment of important confounders. Particular attention was given to age, body mass index, diabetes mellitus, waist circumference, physical activity, and related lifestyle factors because these variables could influence both gut health and hormonal or metabolic outcomes. Exclusion of participants with major gastrointestinal disease, acute infection, recent microbiota-altering medications, and exogenous hormonal exposure further reduced the likelihood of substantial measurement distortion.

Data were entered and analyzed using SPSS. Continuous variables were summarized as mean and standard deviation when normally distributed and as median with interquartile range when distributional assumptions were not satisfied. Categorical variables were presented as frequencies and percentages. Participants were divided into dysbiosis and non-dysbiosis groups according to the predefined study classification. Between-group comparisons for continuous variables were performed using the independent-samples t test when parametric assumptions were satisfied; otherwise, appropriate non-parametric methods were used. Associations between categorical variables were examined using the chi-square test. Correlation between dysbiosis score and key hormonal, symptomatic, and metabolic variables was assessed using Pearson or Spearman coefficients according to data distribution and measurement properties. To evaluate independent associations and address confounding, multivariable logistic regression and, where relevant, linear regression modeling were planned using clinically relevant covariates selected a priori from the literature and univariable assessment. Adjusted effect estimates were reported with 95% confidence intervals, and a p value of 0.05 or less was considered statistically significant.

Data integrity was supported through pretesting of the data collection instrument, daily review of forms, standardized measurement procedures, and cross-checking of entered data against source documents. Analyses were conducted on complete available observations for each variable, and only verified values were entered into the final database. Ethical approval was obtained from the relevant Institutional Review Board or Ethical Committee of the participating hospital before initiation of the study, and all participants were enrolled only after written informed consent. The study was conducted in a manner consistent with accepted ethical standards for human-subject research, with confidentiality maintained throughout data collection, analysis, and reporting.

RESULTS

A total of 180 perimenopausal women were included in the final analysis. Based on the prespecified dysbiosis assessment criteria, 104 women were classified into the gut dysbiosis group and 76 into the non-dysbiosis group, yielding an observed dysbiosis prevalence of 57.8%. Across the dataset, the dysbiosis group demonstrated a consistently less favorable profile with respect to menopausal symptom burden, hormonal status, and metabolic indicators. The most pronounced between-group contrasts were observed for total menopausal symptom burden, serum estradiol, triglycerides, waist circumference, and gastrointestinal symptom frequency.

Table 1. Baseline Demographic and Clinical Characteristics of the Study Participants

Variable	Total (n=180)	Dysbiosis (n=104)	Non-Dysbiosis (n=76)	Effect Estimate	95% CI	p-value
Age (years), mean ± SD	46.9 ± 3.8	47.3 ± 3.7	46.4 ± 3.9	0.90	-0.23 to 2.03	0.118
Urban residence, n (%)	98 (54.4)	59 (56.7)	39 (51.3)	OR = 1.24	0.69 to 2.25	0.471
Married, n (%)	168 (93.3)	98 (94.2)	70 (92.1)	OR = 1.40	0.43 to 4.52	0.587
BMI (kg/m²), mean ± SD	28.1 ± 4.5	29.2 ± 4.3	26.6 ± 4.4	2.60	1.31 to 3.89	0.001
Waist circumference (cm), mean ± SD	92.8 ± 9.6	95.4 ± 8.8	89.2 ± 9.8	6.20	3.41 to 8.99	<0.001
Hypertension, n (%)	51 (28.3)	36 (34.6)	15 (19.7)	OR = 2.15	1.08 to 4.31	0.031
Diabetes mellitus, n (%)	42 (23.3)	31 (29.8)	11 (14.5)	OR = 2.51	1.17 to 5.39	0.019
Low physical activity, n (%)	101 (56.1)	66 (63.5)	35 (46.1)	OR = 2.04	1.11 to 3.72	0.022

Women in the dysbiosis group were comparable in age to women without dysbiosis, with a non-significant mean difference of 0.9 years. In contrast, metabolic and lifestyle contrasts were clinically relevant. Mean BMI was 2.6 kg/m² higher in the dysbiosis group, and waist circumference was higher by 6.2 cm, indicating a materially greater burden of overall and central adiposity. Hypertension and diabetes were approximately twice as common among women with dysbiosis, while low physical activity was also more frequent, supporting the presence of a broader unfavorable metabolic phenotype rather than an isolated gastrointestinal presentation.

Table 2. Menstrual and Reproductive Characteristics of the Participants

Variable	Dysbiosis (n=104)	Non-Dysbiosis (n=76)	Effect Estimate	95% CI	p-value
Irregular menstrual cycles, n (%)	81 (77.9)	43 (56.6)	OR = 2.70	1.41 to 5.17	0.002
Heavy menstrual flow, n (%)	39 (37.5)	18 (23.7)	OR = 1.93	1.00 to 3.75	0.049
Cycle length variation >7 days, n (%)	69 (66.3)	34 (44.7)	OR = 2.44	1.33 to 4.47	0.005
Multiparity, n (%)	73 (70.2)	50 (65.8)	OR = 1.23	0.65 to 2.31	0.536
History of dysmenorrhea, n (%)	35 (33.7)	19 (25.0)	OR = 1.52	0.79 to 2.94	0.217

Menstrual disturbance was more prominent among women with dysbiosis. Irregular cycles were 2.7-fold more likely in the dysbiosis group, while cycle-length variation greater than 7 days showed a 2.4-fold higher odds. Heavy menstrual flow was also more frequent, although the confidence interval approached the null, indicating a weaker degree of precision than that seen for cycle irregularity. By contrast, parity and previous dysmenorrhea did not differ significantly between groups, suggesting that the principal reproductive signal related to dysbiosis lay in current menstrual instability rather than reproductive history.

Table 3. Frequency of Gastrointestinal Symptoms Among Participants

Symptom	Dysbiosis Group (n=104)	Non-Dysbiosis Group (n=76)	Effect Estimate	95% CI	p-value
Bloating, n (%)	82 (78.8)	24 (31.6)	OR = 8.08	4.11 to 15.86	<0.001
Constipation, n (%)	57 (54.8)	19 (25.0)	OR = 3.64	1.91 to 6.95	<0.001
Diarrhea/loose stools, n (%)	28 (26.9)	9 (11.8)	OR = 2.74	1.21 to 6.23	0.015
Excessive gas, n (%)	76 (73.1)	21 (27.6)	OR = 7.11	3.66 to 13.81	<0.001
Abdominal discomfort, n (%)	64 (61.5)	17 (22.4)	OR = 5.55	2.85 to 10.84	<0.001
Food intolerance, n (%)	40 (38.5)	11 (14.5)	OR = 3.69	1.74 to 7.83	<0.001

The gastrointestinal symptom pattern strongly supported the dysbiosis classification. The largest between-group contrasts were seen for bloating and excessive gas, with odds ratios of 8.08 and 7.11, respectively. Abdominal discomfort and food intolerance were also substantially more frequent in the dysbiosis group, reinforcing that these women had a markedly greater everyday gastrointestinal burden rather than only a laboratory-defined classification.

Table 4. Menopausal Symptom Severity According to Menopause Rating Scale (MRS)

Symptom Category / Score	Dysbiosis (n=104)	Non-Dysbiosis (n=76)	Effect Estimate	95% CI	p-value
Mild symptoms, n (%)	18 (17.3)	31 (40.8)	OR = 0.30	0.15 to 0.60	<0.001
Moderate symptoms, n (%)	49 (47.1)	32 (42.1)	OR = 1.23	0.68 to 2.22	0.510
Severe symptoms, n (%)	37 (35.6)	13 (17.1)	OR = 2.68	1.30 to 5.50	0.007
Total MRS score, mean ± SD	15.8 ± 5.9	11.2 ± 4.8	4.60	3.01 to 6.19	<0.001
Hot flushes score, mean ± SD	2.8 ± 1.1	1.9 ± 1.0	0.90	0.59 to 1.21	<0.001
Sleep problems score, mean ± SD	2.9 ± 1.0	2.1 ± 0.9	0.80	0.52 to 1.08	<0.001
Anxiety/irritability score, mean ± SD	3.0 ± 1.2	2.2 ± 1.1	0.80	0.46 to 1.14	<0.001

Menopausal symptom burden was substantially higher in women with dysbiosis. Severe symptoms were present in 35.6% of the dysbiosis group compared with 17.1% of the non-dysbiosis group, corresponding to 2.68-fold higher odds. The mean total MRS score was higher by 4.6 points, while hot flushes, sleep disturbance, and anxiety/irritability were each elevated by 0.8 to 0.9 points. These findings indicate that

the relationship between dysbiosis and symptom burden was not limited to a single symptom domain but extended across vasomotor, psychological, and sleep-related complaints.

Table 5. Hormonal Profile of the Participants

Hormonal Parameter	Dysbiosis (n=104)	Non-Dysbiosis (n=76)	Effect Estimate	95% CI	p-value
Serum estradiol (pg/mL), mean ± SD	58.6 ± 21.4	74.9 ± 24.7	-16.30	-23.11 to -9.49	<0.001
Serum FSH (mIU/mL), mean ± SD	24.8 ± 8.9	19.3 ± 7.6	5.50	3.03 to 7.97	<0.001
Serum LH (mIU/mL), mean ± SD	16.7 ± 6.1	13.8 ± 5.4	2.90	1.20 to 4.60	0.002
Estradiol below reference range, n (%)	46 (44.2)	17 (22.4)	OR = 2.75	1.42 to 5.35	0.003

Hormonal differences between groups were both statistically and clinically meaningful. Women with dysbiosis had mean serum estradiol levels 16.3 pg/mL lower than those without dysbiosis, while mean FSH and LH were higher by 5.5 mIU/mL and 2.9 mIU/mL, respectively. In categorical terms, low estradiol was associated with a 2.75-fold higher odds of dysbiosis. This pattern is consistent with a more advanced or more disturbed menopausal transition among women in the dysbiosis group.

Table 6. Metabolic Profile of the Participants

Metabolic Parameter	Dysbiosis (n=104)	Non-Dysbiosis (n=76)	Effect Estimate	95% CI	p-value
Fasting blood sugar (mg/dL), mean ± SD	109.6 ± 22.8	96.4 ± 18.7	13.20	7.02 to 19.38	<0.001
HbA1c (%), mean ± SD	6.1 ± 0.9	5.6 ± 0.7	0.50	0.26 to 0.74	<0.001
Total cholesterol (mg/dL), mean ± SD	208.7 ± 34.1	191.9 ± 29.6	16.80	7.31 to 26.29	0.001
Triglycerides (mg/dL), mean ± SD	179.5 ± 41.3	151.6 ± 35.7	27.90	16.49 to 39.31	<0.001
LDL cholesterol (mg/dL), mean ± SD	131.2 ± 26.5	118.4 ± 22.9	12.80	5.34 to 20.26	0.001
HDL cholesterol (mg/dL), mean ± SD	40.8 ± 7.1	45.3 ± 7.8	-4.50	-6.71 to -2.29	<0.001
Systolic BP (mmHg), mean ± SD	131.7 ± 14.8	124.3 ± 12.9	7.40	3.29 to 11.51	0.001

Table 7. Correlation of Dysbiosis Score With Hormonal, Symptom, and Metabolic Variables

Variable	Correlation coefficient (r)	Strength and Direction	p-value
Total MRS score	0.49	Moderate positive	<0.001
Serum estradiol	-0.41	Moderate negative	<0.001
Serum FSH	0.38	Weak-to-moderate positive	<0.001
BMI	0.29	Weak positive	<0.001
Waist circumference	0.35	Weak-to-moderate positive	<0.001
Fasting blood sugar	0.32	Weak positive	<0.001
Triglycerides	0.30	Weak positive	<0.001

The dysbiosis group also demonstrated a consistently more adverse metabolic profile. Mean fasting blood sugar was higher by 13.2 mg/dL and HbA1c by 0.5%, while triglycerides were elevated by 27.9 mg/dL and HDL cholesterol was reduced by 4.5 mg/dL. Systolic blood pressure was 7.4 mmHg higher among women with dysbiosis. Taken together, these differences indicate that gut dysbiosis clustered with both glycemic dysregulation and a more atherogenic lipid profile. Correlation analysis showed a graded relationship between dysbiosis severity and the key study variables. The strongest association was observed with total menopausal symptom burden, followed by serum estradiol in the opposite direction. Higher dysbiosis scores were also associated with higher FSH, greater adiposity, poorer glycemic status, and higher triglycerides. This pattern supports the interpretation that dysbiosis severity tracked simultaneously with symptomatic, hormonal, and metabolic worsening rather than with any single isolated outcome.

Table 8. Multivariable Logistic Regression for Factors Associated With Gut Dysbiosis

Variable	Odds Ratio (AOR)	95% CI	p-value
Severe menopausal symptoms	2.41	1.28 to 4.54	0.006
Low estradiol level	2.76	1.43 to 5.31	0.002

Variable	Odds Ratio (AOR)	95% CI	p-value
High waist circumference	2.19	1.16 to 4.14	0.015
Diabetes mellitus	1.88	1.01 to 3.76	0.047
Low physical activity	1.73	1.04 to 3.11	0.039

After multivariable adjustment, low estradiol remained one of the strongest independent correlates of dysbiosis, with an adjusted odds ratio of 2.76. Severe menopausal symptoms and high waist circumference also retained significant associations, while diabetes mellitus and low physical activity remained smaller but still independent predictors. These findings indicate that the observed relationship between dysbiosis and symptom burden was not explained solely by metabolic disease or lifestyle status.

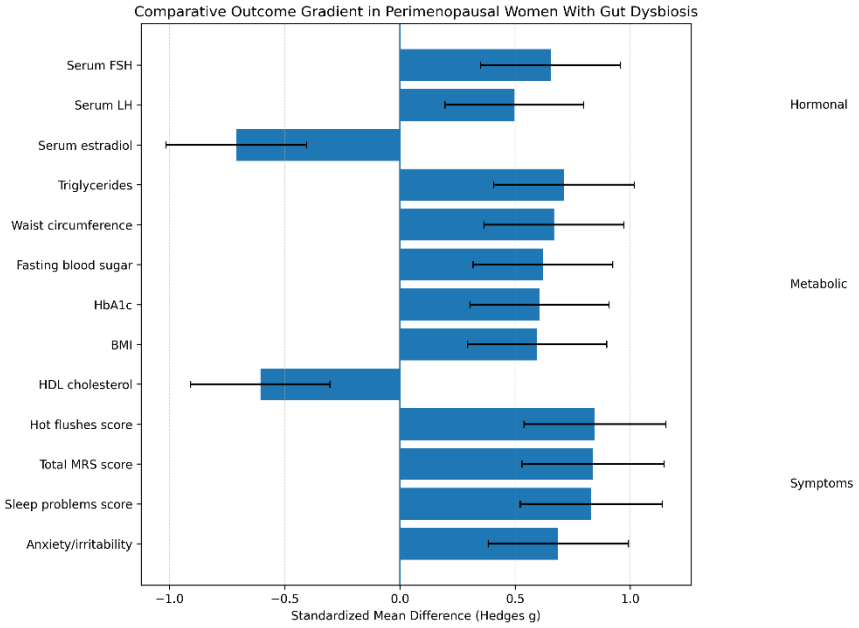

Figure 1 Comparative Outcome Gradient in Perimenopausal Women With Gut Dysbiosis

The integrated effect-gradient figure demonstrates that the strongest dysbiosis-associated shifts occurred in menopausal symptom burden and estrogen-related hormonal change. Standardized mean differences were large for hot flushes score (Hedges g = 0.85), total MRS score (g = 0.84), and sleep problems score (g = 0.83), while serum estradiol showed a moderate-to-large inverse shift (g = -0.71), indicating substantially lower estrogen levels in the dysbiosis group. Among metabolic variables, triglycerides (g = 0.71), waist circumference (g = 0.67), fasting blood sugar (g = 0.62), HbA1c (g = 0.61), and BMI (g = 0.60) all showed moderate adverse gradients, whereas HDL cholesterol moved in the opposite protective direction (g = -0.61). The figure therefore indicates that gut dysbiosis was associated not with a single isolated abnormality but with a coordinated pattern of symptomatic worsening, lower estradiol status, and clinically relevant metabolic deterioration across multiple domains.

DISCUSSION

The present study examined the association of gut dysbiosis with hormonal imbalance in perimenopausal women attending a tertiary care hospital in Multan and found a consistent pattern linking dysbiosis with greater menopausal symptom burden, lower estradiol levels, higher gonadotropins, more frequent menstrual irregularity, and a less favorable metabolic profile. These findings support the growing view that the menopausal transition is not solely an ovarian event but a broader biological state in which endocrine fluctuation, metabolic vulnerability, and gut-related mechanisms may intersect in clinically relevant ways (1-4,14,16). More than half of the participants were classified in the dysbiosis group, suggesting that altered gut health may be common among symptomatic perimenopausal women presenting to hospital care. This observation aligns with prior reports that menopause is accompanied by measurable changes in the gut microbiome and estrobolome, with downstream relevance for inflammation, metabolism, and sex hormone handling (1,3,4,6).

Although much of the existing literature has emphasized postmenopausal women, the present findings indicate that these relationships may already be clinically meaningful during perimenopause, when hormonal variability is still active and symptoms are emerging or intensifying.

The gastrointestinal symptom pattern in this study strengthens the internal credibility of the dysbiosis classification. Women placed in the dysbiosis group reported substantially higher frequencies of bloating, constipation, excessive gas, abdominal discomfort, and food intolerance, indicating that the observed differences were not restricted to laboratory or surrogate classification alone. This is consistent with contemporary menopause–microbiome literature, which increasingly links microbial imbalance with intestinal barrier changes, altered motility, inflammatory signaling, and symptom burden in midlife women (6,21,23,25). The coexistence of gut complaints with vasomotor, psychological, and menstrual symptoms in the same women is clinically important because these symptom clusters are often managed separately in routine practice. The present data suggest that such separation may overlook a potentially integrated pathophysiological pattern.

A notable finding of the study was the greater frequency of menstrual irregularity, heavy menstrual flow, and cycle-length variation among women with dysbiosis. Because menstrual instability is one of the defining clinical features of the perimenopausal transition, the stronger expression of these disturbances in the dysbiosis group raises the possibility that gut-related mechanisms may be interacting with endocrine instability. A biologically plausible explanation lies in the estrobolome, through which intestinal microbes participate in estrogen deconjugation and recirculation. Altered microbial composition or function may therefore influence the availability of active estrogen and, in turn, contribute to less stable cycle regulation during a hormonally labile stage of life (2,7,8). This interpretation is consistent with the present hormonal results, in which women with dysbiosis had lower estradiol concentrations and higher FSH and LH values, a profile compatible with a more disturbed menopausal transition.

The hormonal findings are among the most important results of the present study. Women with gut dysbiosis had significantly lower serum estradiol and significantly higher gonadotropin levels, while low estradiol remained independently associated with dysbiosis in the multivariable model. These observations are compatible with the bidirectional model of the sex hormone–gut microbiome axis described in recent literature, in which sex hormones influence microbial ecology and the gut microbiome, in turn, may alter sex hormone metabolism and bioavailability (8,19,20). However, the present study design does not allow determination of directionality. It is possible that dysbiosis contributes to altered estrogen metabolism, that hormonal transition itself reshapes the gut environment, or that both processes occur simultaneously within the same biological context. This distinction is important and should be emphasized to avoid causal overstatement.

The association between dysbiosis and menopausal symptoms was similarly robust. Women with dysbiosis had significantly higher total Menopause Rating Scale scores and greater frequencies of severe symptoms, with especially marked differences in hot flushes, sleep disturbance, and anxiety or irritability. These findings are clinically relevant because they indicate that dysbiosis was associated not only with altered laboratory values but also with the lived symptom experience of perimenopausal women. Prior studies and reviews have suggested that menopause-related microbial changes may influence symptom severity through effects on inflammation, estrogen handling, gut-brain signaling, and metabolic regulation (1,6,21-23). The present data are consistent with that framework, although the observed associations should still be interpreted as clinical correlations rather than proof of mechanism.

The metabolic findings also merit attention. Women with dysbiosis had higher BMI, larger waist circumference, higher fasting blood sugar, higher HbA1c, more adverse lipid values, and higher systolic blood pressure. This pattern is aligned with literature describing the menopausal transition as a cardiometabolic turning point marked by increasing central adiposity, dyslipidemia, insulin resistance, and vascular risk (14-18). In the present analysis, waist circumference and diabetes mellitus remained

independently associated with dysbiosis, suggesting that dysbiosis clustered with a broader adverse metabolic phenotype. This may reflect shared biological pathways involving inflammation, gut permeability, microbial metabolites, sedentary behavior, and altered hormonal regulation rather than a purely gastrointestinal disturbance.

The correlation analysis further reinforced the overall pattern by demonstrating that higher dysbiosis scores were associated with greater menopausal symptom burden, higher FSH, higher BMI, larger waist circumference, higher fasting blood sugar, and higher triglycerides, while estradiol showed a negative correlation. The graded nature of these associations is important because it suggests that dysbiosis severity tracked with clinical worsening across multiple domains rather than simply distinguishing one binary group from another. Even so, these correlations were weak to moderate in magnitude and should be interpreted accordingly. They indicate meaningful clinical relationships but not deterministic effects.

The study has several strengths. It focused specifically on perimenopausal women, a stage that is often underrepresented compared with postmenopausal populations despite being the phase in which physiological transition is actively unfolding. It also evaluated symptom burden, hormonal measures, menstrual characteristics, and metabolic markers within the same analytic framework, allowing a more integrated interpretation of midlife female health. At the same time, several limitations should be recognized. The cross-sectional design precludes causal inference and temporal sequencing. Dysbiosis was assessed using a pragmatic composite approach based on symptom inquiry with available laboratory support rather than universal advanced molecular microbiome profiling, which introduces the possibility of classification imprecision. The hospital-based sampling frame may also limit generalizability to the wider community, particularly to women with milder or unreported symptoms. In addition, residual confounding from diet, psychosocial stress, and other behavioral or environmental exposures cannot be fully excluded despite adjustment for important metabolic and lifestyle variables.

Taken together, the findings suggest that gut dysbiosis may represent an important and underrecognized correlate of hormonal disturbance, menopausal symptom severity, and metabolic vulnerability during perimenopause. The results are directionally consistent with the evolving literature on the estrobolome, menopause-related microbiome shifts, and cardiometabolic transition in midlife women (1-4,7,8,14,16,21-25). Rather than implying a unidirectional causal pathway, the present study supports a more integrated clinical model in which gut health, endocrine change, and metabolic risk may interact during the menopausal transition. Larger prospective studies using standardized dysbiosis definitions and detailed microbiome analysis are needed to clarify temporality, mechanism, and potential intervention targets in Pakistani and other South Asian populations.

CONCLUSION

Gut dysbiosis was significantly associated with lower serum estradiol levels, higher gonadotropin levels, greater menstrual irregularity, increased menopausal symptom burden, and a more adverse metabolic profile among perimenopausal women attending a tertiary care hospital in Multan, Pakistan. These findings suggest that gut health may be an important clinical correlate of the hormonal and metabolic changes observed during the menopausal transition. Although the cross-sectional design does not permit causal inference, the observed associations support a more integrated view of perimenopausal care in which gastrointestinal symptoms, endocrine changes, and metabolic risk are considered together rather than in isolation. Further longitudinal and mechanistic studies are warranted to determine temporal direction, refine dysbiosis assessment, and evaluate whether gut-focused interventions can improve symptom burden and metabolic outcomes in this population.

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