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SERUM HOXA10 AND EMX2 EXPRESSION AND CHANGES IN ESTROGEN METABOLISM-RELATED INDICATORS IN ENDOMETRIOSIS AND ADENOMYOSIS

EKSPRESIJA SERUMSKIH HOXA10 I EMX2 I PROMENE U INDIKATORIMA POVEZANIM SA METABOLIZMOM ESTROGENA KOD ENDOMETRIOZE I ADENOMIOZE

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Summary

Background: Endometriosis (EMT) and adenomyosis (AM) are common estrogen-dependent gynecologic disorders with overlapping clinical manifestations yet distinct pathological features. This study was designed to characterize serum HOXA10 and EMX2 expression and to examine their association with estrogen-related biochemical alterations in EMT and AM.

Methods: A total of 168 women of reproductive age treated in our hospital between May 2024 and October 2025 were included, comprising 64 controls, 49 patients with EMT, and 55 patients with AM. Serum estradiol (E2) and estrone (E1) were measured by electrochemiluminescence immunoassay and enzyme-linked immunosorbent assay, respectively. Serum RNA was isolated and reverse-transcribed after quality assessment. The expression levels of HOXA10, EMX2, and six estrogen metabolism-related genes were then measured by real-time quantitative PCR. Correlations between the core genes and estrogen-related indicators were analyzed, and receiver operating characteristic (ROC) analysis was performed to evaluate the discriminatory efficacy of HOXA10 and EMX2 for identifying endometriosis (EMT) and adenomyosis (AM).

Results: Serum HOXA10 expression was decreased, whereas EMX2 expression was increased, in both the EMT and AM groups compared with controls. The HOXA10/EMX2 ratio was also reduced in both disease groups, with a significantly greater decline observed in the AM group compared with the EMT group ($P<0.05$). Serum E2 and

Kratak sadržaj

Uvod: Endometrioza (EMT) i adenomioza (AM) su česti estrogen-zavisni ginekološki poremećaji sa preklapajućim kliničkim manifestacijama, ali i različitim patološkim karakteristikama. Ova studija je osmišljena da okarakteriše ekspresiju serumskih HOXA10 i EMX2 i da ispita njihovu povezanost sa biohemijskim promenama povezanim sa estrogenom u EMT i AM.

Metode: Ukupno je uključeno 168 žena reproduktivnog doba lečenih u našoj bolnici između maja 2024. i oktobra 2025. godine, od čega 64 kontrolne grupe, 49 pacijentkinja sa endometriozom (EMT) i 55 pacijentkinja sa adenomiozom (AM). Serumski estradiol (E2) i estron (E1) mereni su elektrohemiluminiscentnim imunotestom i enzimski povezanim imunosorbentnim testom, respektivno. Serumska RNK je izolovana i reverzno transkribovana nakon procene kvaliteta. Nivoi ekspresije HOXA10, EMX2 i šest gena povezanih sa metabolizmom estrogena zatim su mereni kvantitativnom PCR u realnom vremenu. Analizirane su korelacije između osnovnih gena i indikatora povezanih sa estrogenom, a izvršena je i ROC analiza (receiver operating characteristic) kako bi se procenila diskriminatorna efikasnost HOXA10 i EMX2 za identifikaciju endometrioze (EMT) i adenomioze (AM).

Rezultati: Ekspresija serumskog HOXA10 je smanjena, dok je ekspresija EMX2 povećana, i u EMT i u AM grupi u poređenju sa kontrolnom grupom. Odnos HOXA10/EMX2 je takođe smanjen u obe grupe obolelih, sa značajno već im padom primećenim u AM grupi u poređenju sa EMT

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E1 levels were elevated in both disease groups. Genes involved in estrogen synthesis and activation were generally upregulated, while those related to estrogen inactivation were downregulated, indicating enhanced estrogenic activity, especially in AM. HOXA10 and EMX2 were correlated with several estrogen-related indicators, although the correlation patterns differed between EMT and AM.
Conclusion: EMT and AM are accompanied by coordinated changes in serum HOXA10, EMX2, and estrogen metabolism-related indices.

Keywords: endometriosis, adenomyosis, HOXA10, EMX2, biomarkers, serum, estrogen metabolism, estrogen-related indicators

grupom (P<0,05). Nivoi serumskog E2 i E1 bili su povišeni u obe grupe obolelih. Geni uključeni u sintezu i aktivaciju estrogena su generalno bili pojačani, dok su oni povezani sa inaktivacijom estrogena bili smanjeni, što ukazuje na pojačanu estrogenu aktivnost, posebno kod AM. HOXA10 i EMX2 su bili u korelaciji sa nekoliko indikatora povezanih sa estrogenom, iako su se obrasci korelacije razlikovali između EMT i AM.
Zaključak: EMT i AM su praćeni koordinisanim promenama serumskih HOXA10, EMX2 i indeksa povezanih sa metabolizmom estrogena.

Ključne reči: endometrioza, adenomioza, HOXA10, EMX2, biomarkeri, serum, metabolizam estrogena, indikatori povezani sa estrogenom

Introduction

Endometriosis (EMT) and adenomyosis (AM) are two common estrogen-dependent gynecologic diseases in women of reproductive age. The incidence of EMT in this population is estimated at 10%–15% (1), while the incidence of AM in this population is estimated at 20%–30% (2). Both conditions are characterized by abnormal proliferation and invasion of endometrial cells, and both are closely regulated by estrogen-related signaling pathways. Even so, they are not identical diseases. They differ in the mode of cellular invasion, the pattern of lesion expansion, and the molecular networks involved in disease progression (3, 4). Although substantial work has been devoted to their pathogenesis (5, 6), , which continues to limit a deeper understanding of their pathological heterogeneity and precise clinical, which continues to limit a deeper understanding of their pathological heterogeneity and precise clinical differentiation.

Homeobox genes encode key transcription factors that are essential for maintaining endometrial homeostasis and regulating pathological processes. Among them, HOXA10 and EMX2 have been implicated in several critical biological events closely related to endometrial physiology and pathology, including endometrial cell cycle regulation and epithelial-mesenchymal transition (7, 8), and their transcriptional activity can be directly modulated by the estrogen nuclear receptor pathway (9). Existing studies have confirmed that HOXA10 is downregulated while EMX2 is upregulated in the ectopic endometrial tissues of patients with EMT or AM, which is closely related to the abnormal invasive and proliferative capacity of endometrial cells; however, these findings are mostly limited to lesional tissue samples, and the expression pattern of the two genes in peripheral serum of patients with both diseases has not been systematically compared. However, most previous studies have concentrated on functional validation of a single gene in lesion tissues from only one disease type (10, 11). Comparative evidence regarding the expression patterns of HOXA10 and EMX2 in EMT and AM remains limited, and their relationship with estradiol, estrone, and other major components of the estrogen metabolic pathway is still not clear. As a result, an important gap remains in our understanding of estrogen-dependent transcriptional regulation in these two disorders.

In the present study, women of reproductive age were enrolled, fasting serum samples were collected, and real-time quantitative PCR was used to detect the mRNA expression levels of HOXA10, EMX2, and key genes in the estrogen metabolic pathway. By comparing patients with EMT, patients with AM, and healthy controls, we sought to define the differences in expression of these core target genes across the three groups. The findings may provide laboratory evidence for distinguishing the molecular characteristics of EMT and AM, enrich current data on estrogen-dependent endometrial disease, and offer a basis for further mechanistic investigation.

Materials and Methods

Study subjects

Women of reproductive age admitted to the Department of Gynecology of our hospital between May 2024 and October 2025 were included in this study. A total of 168 eligible participants were enrolled and divided into three groups: a control group (64 cases), an EMT group (49 cases), and an AM group (55 cases). All participants signed written informed consent, and the study protocol was reviewed and approved by the institutional ethics committee. No statistically significant differences were found among the three groups in age, gravidity, parity, or other baseline variables (P>0.05. *Table I*), indicating good comparability at baseline.

Table I Baseline characteristics of the study subjects.

Age	Control group	EMT group	AM group	F or χ^2	P
	36.95±3.59	36.33±5.34	35.82±4.38	0.987	0.375
Body mass index (kg/m^2)	22.16±2.79	22.18±2.70	21.31±2.59	1.854	0.160
Menstrual cycle length (days)	29.77±2.63	30.59±2.99	29.69±3.86	1.281	0.281
Gravidity	1.44±1.05	1.37±1.07	1.51±1.07	0.230	0.795
Parity	0.50±0.59	0.55±0.61	0.69±0.69	1.411	0.247
Past gynecologic history				1.761	0.415
Yes	12 (18.75)	12 (24.49)	16 (29.09)		
No	52 (81.25)	37 (75.51)	39 (70.91)		

Note: Past gynecologic history was defined as a previous history of benign gynecologic diseases, including uterine fibroids, ovarian cysts, cervical intraepithelial neoplasia, and pelvic inflammatory disease, excluding malignant gynecologic tumors.

Inclusion and exclusion criteria

Inclusion criteria

Control group: Women of reproductive age who received healthy physical examination in our hospital during the same period, with no organic gynecological diseases confirmed by pelvic ultrasound and gynecological examination, regular menstrual cycles (21–35 days), no history of hormone medication use in the past 3 months, and no abnormal findings in routine blood and biochemical tests.

EMT group: Patients with EMT confirmed by laparoscopic surgery and postoperative pathological examination, in line with the revised American Society for Reproductive Medicine (rASRM) diagnostic criteria for endometriosis.

AM group: Patients with AM confirmed by postoperative pathological examination, or diagnosed by standardized pelvic ultrasound criteria in line with the Morphological Uterus Sonographic Assessment (MUSA) consensus, with typical clinical manifestations.

All enrolled participants were aged 20–45 years, with regular menstrual cycles (21–35 days), and voluntarily signed the written informed consent.

Uniform exclusion criteria

Participants who met any of the following criteria were excluded: ① Concurrent other gynecological organic diseases (except the target disease in the case group); ② Malignant tumors of any system; ③ Endocrine system diseases (such as polycystic ovary syndrome, hyperthyroidism, hypothyroidism, diabetes); ④ History of hormone therapy, oral contraceptives, or intrauterine device placement within the past 3 months; ⑤ History of pelvic surgery within the past 6 months; ⑥ Severe liver and kidney dysfunction, acute or chronic inflammatory diseases, or autoimmune diseases; ⑦ Visibly hemolyzed or lipemic serum samples that could not be used for detection.

Sample collection

All samples were collected under a unified standardized protocol: for each participant with regular menstrual cycles (21–35 days), 5 mL of fasting whole blood was collected during the mid-proliferative phase (days 5–14) of the menstrual cycle into an enzyme-free sterile procoagulant tube (BD). The tubes were kept upright at room temperature for 30 min to allow complete clotting, and were then centrifuged at 3500 r/min for 15 min at 4 °C using a benchtop high-speed refrigerated centrifuge (Eppendorf). Inside a clean bench sterilized under ultraviolet light for 30 min, the upper serum layer was carefully aspirated while avoiding the cellular layer and interface; visibly hemolyzed samples were discarded. Qualified serum specimens were aliquoted into enzyme-free, pyrogen-free cryovials (Axygen) with 200 μL in each vial, and three independent aliquots were prepared separately for estradiol testing, estrone testing, and RNA extraction to avoid repeated freeze-thaw cycles of the same sample. After labeling with unique blinded codes, all samples were immediately stored in a -80 °C ultra-low-temperature freezer (Thermo Fisher). Each specimen underwent only one freeze-thaw cycle to minimize loss of hormone activity and degradation of nucleic acids.

Serum estrogen measurement

Serum E2 was measured on a cobas e 601 electrochemiluminescence analyzer (Roche Diagnostics) with the corresponding reagents from the same manufacturer. The system was calibrated before use, and routine batch controls at low, medium, and high levels were run in parallel. The calibration range was 10–3000 pg/mL. Before analysis, serum samples were thawed on ice, kept at room temperature for 30 min, and centrifuged at 10000 r/min for 5 min at 4 °C. The supernatant was used directly, and each sample was measured in duplicate.

Serum E1 was determined with a high-sensitivity human estrone ELISA kit (R&D Systems) using an automated plate washer and a multifunctional microplate reader (Tecan). Sample handling before assay was the same as that for E2. After equilibration of the plate to room temperature, blank, standard, and sample wells were prepared according to the kit instructions. Seven standards were included. Samples were analyzed in duplicate, incubated at 37 °C for 90 min, washed five times, incubated with enzyme conjugate for 60 min at 37 °C, washed again, and then developed in the dark for 15 min at 37 °C. Absorbance was read at 450 nm within 10 min after stopping the reaction, and concentrations were derived from the standard curve. The calibration range was 10–3000 pg/mL. All serum E2 test values of the included subjects were within this calibration range; for any sample with a test value outside the range, we would perform sample dilution and retesting according to the standard operating procedure of the detection system. Before analysis, serum samples were thawed on ice, kept at room temperature for 30 min, and centrifuged at 10000 r/min for 5 min at 4 °C.

All runs were evaluated against predefined acceptance criteria. Blank OD values were kept at or below 0.05, duplicate CVs within 5%, and inter-assay variation within 8% by use of a retained control sample. Standard curves were accepted only when R^2 reached at least 0.995 for E2 and 0.99 for E1. Runs outside these limits were repeated after recalibration. All measurements were completed by the same operator under blinded coding. For the actual detection in this study, the intra-assay coefficient of variation (CV) was 2.18%–3.25% and the inter-assay CV was 3.42%–4.51% for serum E2 detection; the intra-assay CV was 3.05%–4.12% and the inter-assay CV was 4.36%–5.78% for serum E1 detection. All actual CV values were within the predefined acceptable range, confirming the good stability and reliability of the detection methods.

Total RNA extraction from serum

Total RNA was extracted from serum with Trizol LS reagent (TaKaRa). Briefly, 200 μL serum was mixed with 1 mL Trizol LS, incubated at room temperature for 10 min after vortexing, and then subjected to chloroform extraction. Following centrifugation at 12000 r/min for 15 min at 4 °C, the aqueous phase was collected, precipitated with an equal volume of precooled isopropanol at -20 °C for 30 min, washed twice with 75% ethanol, and dissolved in 20 μL enzyme-free DEPC water (Solarbio) containing 1 U RNase inhibitor (TaKaRa).

RNA concentration and purity were assessed with a NanoDrop 2000 microspectrophotometer (Thermo Fisher). Integrity was further examined by 1% agarose gel electrophoresis and confirmed on an Agilent 2100 Bioanalyzer. All extracted RNA samples were subjected to full quality control assessment including RIN value detection, and only samples meeting all the preset quality criteria (OD260/OD280 1.8–2.1, OD260/OD230 ≥1.8, 28S/18S ≥1.8, and RIN ≥7) were used for subsequent reverse transcription and qPCR analysis.

cDNA synthesis by reverse transcription

Reverse transcription was carried out with the PrimeScript RT reagent Kit (TaKaRa) using qualified total RNA. Each 20-μL reaction contained 4 μL 5×PrimeScript Buffer, 1 μL PrimeScript RT Enzyme Mix I, 1 μL Oligo dT Primer, 1 μL Random 6 mers, and 1 μg RNA template, with enzyme-free DEPC water added to volume. The reactions were assembled on ice and run on a Veriti 96-well PCR instrument (Applied Biosystems) at 37 °C for 15 min and 85°C for 5 s, followed by holding at 4 °C.

A portion of each cDNA product was diluted 10-fold and checked by GAPDH pre-amplification. Ct values between 18 and 25 were considered acceptable. Qualified cDNA was aliquoted and stored at -20°C in the dark, and each aliquot was thawed only once before use.

Real-time quantitative PCR (qPCR)

Primers for HOXA10, EMX2, ESR1, ESR2, CYP19A1, HSD17B1, HSD17B2, CYP1A1, and GAPDH were designed with Primer Premier 6.0 based on human reference sequences from the NCBI GenBank database. Amplicon lengths were limited to 100–200 bp, primer Tm values were set at 60°C, and specificity was checked by NCBI BLAST. Primer performance was verified with a 5-fold serial dilution of pooled cDNA; only primer pairs with amplification efficiencies of 90%–110%, $R^2 \geq 0.99$, and a single melting peak were retained (*Table II*).

qPCR was performed with SYBR Premix Ex Taq II (TaKaRa) in a 25 μL reaction containing 12.5 μL premix, 1 μL each of forward and reverse primers, 2 μL cDNA template, and enzyme-free DEPC water.

Table II Primer sequences.

	F (5′-3′)	R (5′-3′)	bp
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG	101
HOXA10	CCCTGGACGAGTACCTGAAG	GCTGCTGCTGCTGCTTATC	142
EMX2	ACCCAGTCCAAGATCGAGAAG	GGTAGTTGATGGTGATGTTGGC	137
ESR1	TGCCTGGCTACAGTGTGAC	GATGATGAAGGTGGCGATGTT	128
ESR2	CCAGCACATCGACATCAAGA	GGTGATGTAGCGGAAGATGAG	156
CYP19A1	GGAAATGCTGAACCCGATACA	GGTGATGTTGCCAAGATCTGG	119
HSD17B1	GCTGTCATCGCCAAGGAGAT	GGTGATGGTGATGAAGCGG	133
HSD17B2	CCATCGCCATCCTGATCAAG	GATGAAGCCGATGGTGATGAG	147
CYP1A1	ACTTCATCCGAGACACTGAGG	GATGATGCCGTTGATGTTGG	125

Amplification was run on an ABI 7500 real-time PCR system (Applied Biosystems) at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s, with melting-curve analysis performed afterward. Each sample was tested in triplicate, and relative mRNA expression was calculated by the $2^{-\Delta\Delta}$Ct method using GAPDH as the internal reference. No-template and no-reverse-transcription controls were included on each plate, and runs with evidence of contamination were excluded.

Statistical analysis

All data were analyzed using SPSS 26.0 statistical software (IBM). Outliers in gene expression data and estrogen level data were identified by the two-sided Grubbs test ($\alpha=0.05$), and outlier values were excluded to improve data stability. No sample was fully excluded from the study due to outlier values, and the sample size of each group remained unchanged after outlier processing. Normality was assessed using the Shapiro-Wilk test. Estrogen level data conformed to a normal distribution after logarithmic transformation. Measurement data with a normal distribution are presented as mean ± standard deviation ($\bar{x}\pm s$). Comparisons among multiple groups were performed using one-way analysis of variance. For pairwise comparisons, the LSD-t test was used when variance was homogeneous, whereas Dunnett's T3 test was applied when variance was heterogeneous. Pearson correlation analysis was used to evaluate linear relationships between two variables. Receiver operating characteristic (ROC) curves were used to assess the discriminatory value of single indicators by calculating the area under the curve (AUC), 95% confidence interval, sensitivity, and specificity. All tests were two-sided, and $P<0.05$ was considered statistically significant.

Results

Differential expression of HOXA10 and EMX2 mRNA

After outlier exclusion via the Grubbs test as described in the statistical methods, the sample size of each group remained consistent with the baseline enrollment, with 64 cases in the control group, 49 cases in the EMT group, and 55 cases in the AM group. The mRNA expression levels of these two genes were measured in serum samples from the three groups, and a significant overall difference was observed ($F=429.182$, $P<0.05$). Pairwise comparisons showed that HOXA10 mRNA expression was decreased in both the EMT and AM groups relative to the control group, and the reduction was greater in the AM group ($P<0.05$). By contrast, EMX2 mRNA expression was increased in both disease groups, with a more marked elevation in the AM group than in the EMT group ($P<0.05$). In line with these findings, the HOXA10/EMX2 expression ratio was significantly reduced in both the EMT and AM groups compared with the control group, and the ratio was significantly lower in the AM group than in the EMT group ($P<0.05$). These results suggest that dysregulation of the two core genes is closely related not only to disease occurrence but also to the pathological differences between the two disorders (*Figure 1*).

Differences in the levels of core estrogen indicators

Serum E2 and E1 levels were measured simultaneously, and both disease groups showed evidence of abnormal estrogen synthesis and activation. Compared with the control group, serum E2 and E1 levels were elevated in both the EMT and AM groups ($P<0.05$). The E2 level in the AM group

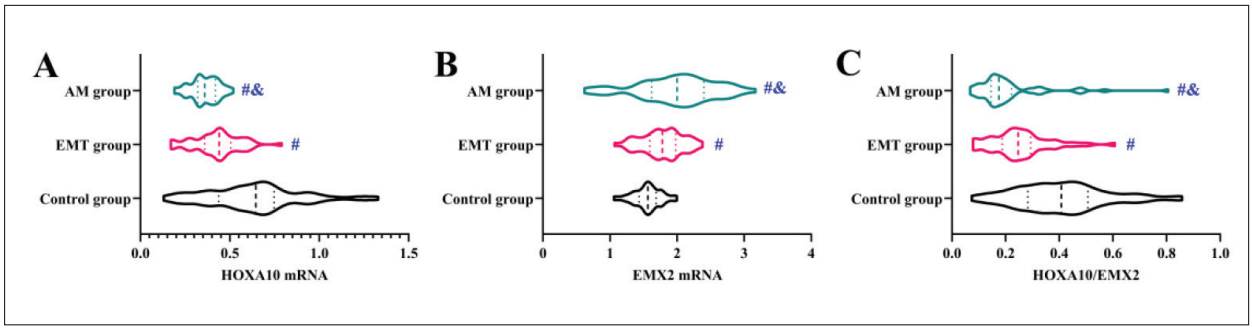

Figure 1 Differential serum expression of HOXA10, EMX2, and the HOXA10/EMX2 ratio among the control, EMT, and AM groups.

(A) Serum HOXA10 mRNA expression in the three groups. (B) Serum EMX2 mRNA expression in the three groups. (C) Serum HOXA10/EMX2 expression ratio in the three groups. # indicates $P<0.05$ compared with the control group; & indicates $P<0.05$ for the comparison between the EMT group and the AM group.

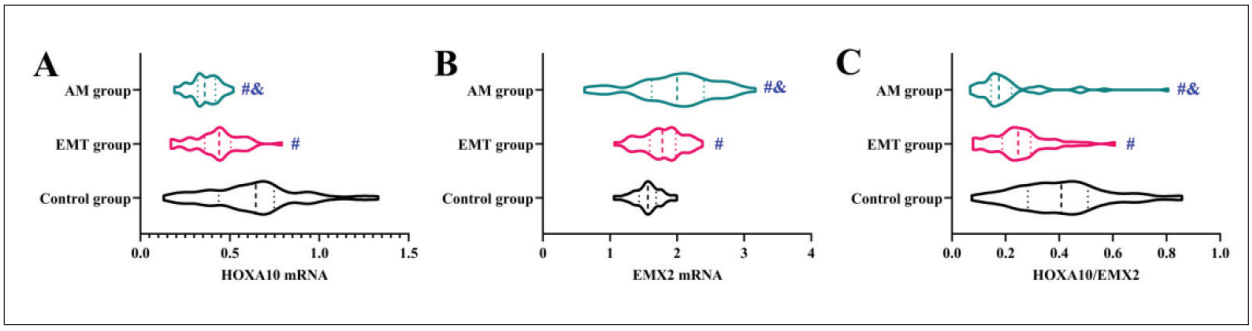

Figure 2 Differences in serum estrogen levels among the control, EMT, and AM groups.

(A) Serum estradiol (E2) levels in the three groups. (B) Serum estrone (E1) levels in the three groups. # indicates $P<0.05$ compared with the control group, & indicates $P<0.05$ compared with the AM group.

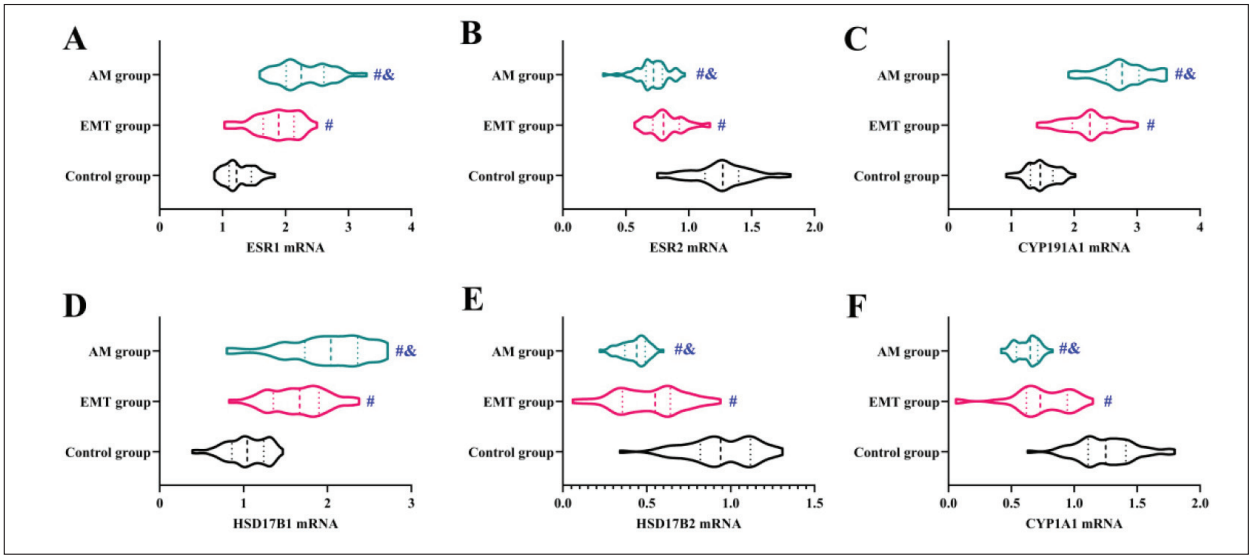

Figure 3 Differential expression of key estrogen metabolism-related genes in the control, EMT, and AM groups.

(A) ESR1 mRNA expression in the three groups. (B) ESR2 mRNA expression in the three groups. (C) CYP19A1 mRNA expression in the three groups. (D) HSD17B1 mRNA expression in the three groups. (E) HSD17B2 mRNA expression in the three groups. (F) CYP1A1 mRNA expression in the three groups. # indicates $P<0.05$ compared with the control group, & indicates $P<0.05$ compared with the AM group.

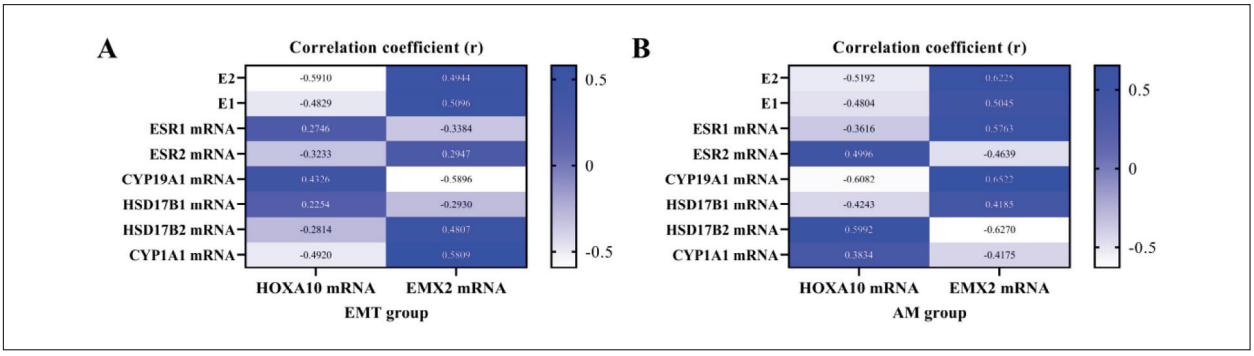

Figure 4 Correlation of serum HOXA10 and EMX2 with estrogen-related indicators in EMT and AM.

(A) Correlation heatmap of HOXA10 and EMX2 with estrogen-related indicators in the EMT group. (B) Correlation heatmap of HOXA10 and EMX2 with estrogen-related indicators in the AM group. # indicates P<0.05 compared with the control group, & indicates P<0.05 compared with the AM group.

was higher than that in the EMT group (P<0.01), whereas the difference in E1 between the two disease groups was not statistically significant (P>0.05). These findings indicate that both EMT and AM are accompanied by enhanced estrogen synthesis and activation, although the activation profile appears to be more pronounced in AM (*Figure 2*).

Differential expression of key genes in the estrogen metabolic pathway

Abnormal circulating estrogen levels reflect disturbances in estrogen synthesis, metabolism, and receptor-mediated signaling. We therefore further examined the mRNA expression levels of six key genes in this pathway and found significant overall differences among the three groups for all genes tested (P<0.05). Regarding receptor signaling, ESR1 (which mediates the proliferative effects of estrogen) was significantly upregulated in both the EMT and AM groups relative to the control group, whereas ESR2 (which exerts inhibitory regulatory effects on estrogen signaling) was significantly downregulated in both disease groups; the upregulation of ESR1 and downregulation of ESR2 were both significantly more pronounced in the AM group than in the EMT group (all P<0.01), suggesting that the pattern of estrogen responsiveness differs between the two diseases. As for estrogen-metabolizing enzymes, CYP19A1 and HSD17B1 (key genes involved in estrogen synthesis and activation) were significantly upregulated in both disease groups, while HSD17B2 and CYP1A1 (key genes responsible for estrogen inactivation and detoxification) were significantly downregulated in both the EMT and AM groups; the magnitude of the above expression changes in all four enzyme genes was significantly greater in the AM group compared with the EMT group (P<0.05). Overall, both diseases showed a pathway pattern characterized by enhanced estrogen synthesis and weakened inactivation, and this pattern was more obvious in AM, consistent with the circulating estrogen results (*Figure 3*).

Correlation analysis of HOXA10, EMX2, and estrogen system indicators

To clarify the internal relationship between HOXA10, EMX2, and the estrogen system, Pearson correlation analysis was performed separately in the two disease cohorts. Both core genes were correlated with the estrogen pathway, but the correlation profiles were clearly different between EMT and AM. In the EMT group, HOXA10 expression was negatively correlated with serum E2, E1, ESR2, and HSD17B2, whereas positive correlations were observed with ESR1 and HSD17B1; CYP19A1 was also associated with HOXA10 expression. EMX2 showed an overall opposite pattern. These findings suggest that, in EMT, the two genes are mainly involved through estrogen synthesis and receptor-related pathways. In the AM group, however, the associations between the core genes and the estrogen system were stronger and covered a broader regulatory range, implying that in AM they may participate in both estrogen synthesis and estrogen inactivation. This broader bidirectional involvement may represent one of the major molecular differences between the two disorders (*Figure 4*).

Discriminative performance of the core target genes

The results showed that HOXA10 and EMX2, when analyzed individually, could effectively distinguish patients with EMT from healthy controls, with AUC values of 0.758 and 0.724, respectively (P<0.05). In AM, both HOXA10 and EMX2 also showed good discriminatory performance, with AUC values of 0.835 and 0.775, respectively. Notably, the discriminatory performance of both markers

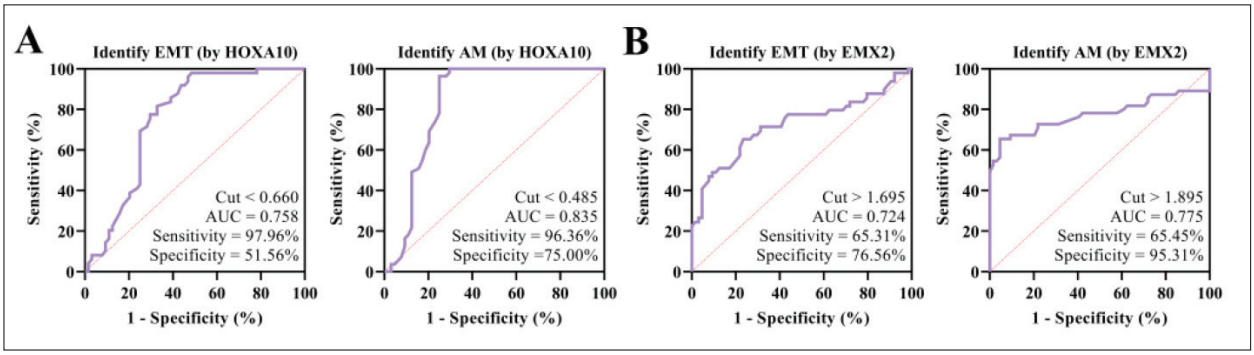

Figure 5 ROC analysis of serum HOXA10 and EMX2 for discriminating EMT and AM.

(A) ROC curves of HOXA10 for identifying EMT and AM. (B) ROC curves of EMX2 for identifying EMT and AM. # indicates $P<0.05$ compared with the control group, & indicates $P<0.05$ compared with the AM group.

was significantly better in AM than in EMT: the Z value for the AUC comparison of HOXA10 between AM and EMT was 3.462 ($P<0.001$), and the Z value for the AUC comparison of EMX2 between AM and EMT was 2.641 ($P=0.008$, *Figure 5*).

Discussion

By analyzing serum samples from patients with EMT, patients with AM, and healthy controls, the present study identified distinct expression patterns of the homeobox genes HOXA10 and EMX2 and further revealed their disease-specific associations with estrogen synthesis and metabolism.

We found that serum HOXA10 expression was significantly reduced in both EMT and AM, whereas EMX2 showed the opposite upregulation trend, and this expression imbalance was more marked in AM. This observation is consistent with earlier findings in eutopic and ectopic endometrial lesion tissues from patients with EMT or AM (12, 13). The consistent expression pattern of HOXA10 and EMX2 between peripheral serum and local lesion tissues suggests that the dysregulation of the two genes in the systemic circulation is synchronized with the molecular changes in the focal lesions, which provides a pathophysiological basis for using serum HOXA10 and EMX2 as noninvasive surrogate biomarkers to reflect the pathological state of the lesions (14, 15). Reduced HOXA10 expression weakens its inhibitory effect on epithelial-mesenchymal transition and thereby facilitates the invasive and proliferative behavior of endometrial cells, creating favorable conditions for ectopic implantation (16). EMX2, by contrast, has a clear antagonistic relationship with HOXA10 and can directly counteract HOXA10-mediated regulation of endometrial cell behavior; once EMX2 is upregulated, the abnormal invasive phenotype of endometrial cells may be further amplified (17). Unlike many previous studies, which focused mainly on eutopic or ectopic endometrial tissue (18), our data demonstrated the same expression pattern in circulating serum. Notably, mRNA in peripheral serum is mainly encapsulated in extracellular vesicles such as exosomes and apoptotic bodies, which can avoid degradation by RNases and maintain stable expression in the circulation. This suggests that peripheral blood HOXA10 and EMX2 mRNA may accurately reflect the local molecular transcriptional state of the lesions and may therefore have important value in noninvasive detection of EMT and AM. The more pronounced imbalance observed in AM is also consistent with the pathological nature of this disease. Compared with the relatively localized lesions of ovarian EMT, AM is characterized by diffuse infiltration of endometrial tissue into the myometrium, which places greater demands on invasive and proliferative capacity. It is therefore not surprising that dysregulation of these core regulatory genes is more evident in AM.

This characteristic gene-expression imbalance was closely related to abnormalities in the estrogen metabolic pathway, which is one of the main pathological drivers shared by both diseases. Our results showed that both disorders were accompanied by enhanced estrogen synthesis and activation together with reduced inactivation, and HOXA10 and EMX2 were correlated with key indicators of the estrogen system. However, the pattern of association was not the same in EMT and AM. Previous in vitro studies have confirmed the presence of estrogen response elements in the promoter regions of both HOXA10 and EMX2 (15, 19). After estrogen binds to the nuclear receptor ERα, it can directly suppress HOXA10 transcription while promoting EMX2 expression. This mechanism also helps explain the correlations observed here with serum estradiol and ESR1 expression. Compared with EMT, AM showed more pronounced upregulation of ERα and a higher estrogen activation index, which may have strengthened estrogen-dependent transcriptional regulation of these two homeobox genes (20). This

is consistent with the stronger correlations observed in the AM group. In addition, EMX2 in AM was negatively correlated with the estrogen-inactivating enzyme HSD17B2, suggesting that in AM, EMX2 may not simply be a downstream target of estrogen signaling but may also participate in feedback regulation of estrogen metabolism. This feature may be relevant to the molecular heterogeneity between the two diseases, although it has received limited attention in previous studies.

From a clinical perspective, HOXA10 and EMX2 each showed reasonable discriminatory value for identifying disease groups, especially AM. Most previous biomarker studies in this field have focused on individual molecules, yet the pathological overlap between EMT and AM often limits the performance of single indicators (21, 22). In this context, the present findings suggest that core transcriptional regulators linked to the estrogen pathway may offer useful information for molecular evaluation of these two disorders.

From a laboratory perspective, the present study is clinically relevant because the target molecules were assessed in serum rather than in lesion tissue, which makes them more suitable for noninvasive testing. At the same time, several limitations of this study need to be acknowledged. First, this was a single-center case-control study with a limited sample size, and only ovarian EMT was included, which may restrict the generalizability of the findings. Second, although pre-analytical and analytical quality-control measures were applied throughout the study, the serum qPCR assay was not subjected to a full analytical validation for routine laboratory use, and no external validation cohort was available. Third, we did not compare the discriminatory performance of HOXA10 and EMX2 with established clinical diagnostic tools such as pelvic ultrasound imaging or conventional serum markers (e.g., CA125). This means that the incremental diagnostic value of the studied markers over existing clinical tools cannot be determined in the current study. Fourth, although we strictly controlled the pre-analytical process of serum samples and standardized all experimental operations, serum mRNA detection still has potential limitations as a clinical biomarker: the stability of serum mRNA is susceptible to pre-analytical factors such as sample processing time, freeze-thaw cycles, and hemolysis, which may affect the consistency of test results among different laboratories; in addition, the qPCR method used in this study has high requirements for laboratory operation and quality control standards, which may limit its wide application in primary medical institutions. For that reason, the current results should be interpreted as evidence of diagnostic potential rather than proof of immediate clinical applicability.

Conclusion

In summary, serum HOXA10 and EMX2 expression was altered in both endometriosis and adenomyosis and was accompanied by broader changes in estrogen metabolism-related indices. The overall pattern was more pronounced in adenomyosis, suggesting that these circulating molecular and biochemical alterations may reflect differences in disease-associated endometrial regulation. From a laboratory perspective, the present findings suggest the potential value of integrating serum mRNA assessment with conventional biochemical measurements in the study of estrogen-dependent gynecologic disorders. Further work is still needed to strengthen assay validation, confirm reproducibility in independent cohorts, and clarify how these serum findings relate to established clinical laboratory parameters.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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