

Relationship between 17β estradiol (17β E) levels and Catechol -O- methyltransferase (COMT) levels in ovarian cancers

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Objectives: Epidemiological data show that induction of ovarian cancer is related to estrogen exposure and metabolism. In addition catechol metabolites of estrogen also contribute to carcinogenesis. O methylation by Catechol -O- methyltransferase is a phase II metabolic inactivation pathway for catechol estrogens. The goal of this study was to evaluate a potential correlation between COMT and 17β estradiol levels and ovarian cancer. **Subjects and methods:** COMT and 17β E levels were measured in ovarian tissue and serum from 80 subjects: 30 with malignant ovarian tumors, 30 with benign ovarian tumors and 20 healthy controls. **Results:** Tissue and serum levels of Catechol -O- methyltransferase and 17β estradiol were determined using enzyme linked immunosorbant assay. According to our results Catechol -O- methyltransferase inhibition in the malignant group was associated with high levels of 17β estradiol, while in benign group high levels of COMT was associated with low levels of 17β estradiol in both serum and tissue homogenates. **Conclusions:** Low level of COMT and high tissue/serum levels of 17β estradiol may be contributory factors for the development of ovarian cancer. This supports the notion that targeting the metabolism of estrogen can be another way to reduce ovarian cancer risk.

Keywords

Ovarian cancer; Catechol -O- methyltransferase; 17β estradiol (E₂)

1. Introduction

During reproductive years, granulosa cells secrete both estradiol (E₂) and estrone (E₁) after stimulation of the sex steroid hormone synthesis in the ovary [1]. After menopause, estrogens are formed locally in various tissues. Estradiol is produced by circulating androgen and estrogen precursors and transported to ovarian epithelial cells [2]. Previous studies have demonstrated that 17β estradiol, its interconvertible metabolite estrone and their catechol metabolites are carcinogenic [2, 3]. Oxidative metabolism of estradiol/estrone to catechols involves reactive metabolites formation, generating mutagenic DNA adducts. Free radicals from estrogen metabolic activation cause mutations, and their accumulation lead to neoplastic transformation of proliferating cells [3–7].

O-methylation by Catechol -O- methyltransferase (COMT) has a major role in blocking the further oxidation to catechols [3]. The importance of regulating oxidative metabolism of estrogen to catechols was studied in MCF-10F cells, highlighting the importance of COMT enzyme [6].

In rats and humans, COMT has demonstrated a higher catalytic activity towards estrogen catechol metabolites (CEs) [8, 9]. In addition 2-methoxyestradiol (2MeOE₂) formed by COMT enzyme has been shown to increase apoptosis, inhibit growth and inhibit angiogenesis [10–14]. Thus COMT is an important protective enzyme against carcinogenesis and COMT activity determination is immensely significant as an oxidative metabolism regulator of estrogen in ovarian cancer with the contrasting effects of 17β estradiol levels.

2. Subjects and methods

Our study was conducted on 80 subjects divided into three groups: 30 patients with malignant epithelial ovarian tumors, 30 patients with benign ovarian tumors and 20 healthy age-matched individuals as a control group. Patients were recruited from El Shatby Maternity Hospital (Alexandria University) from 2018 to 2019. Patients with ovarian cancer were diagnosed according to the Ovarian Cancer International Federation of Gynecology and Obstetrics (FIGO). Exclusion criteria for the study were: patients with other related gynecological malignancies such as cervical and endometrial cancer.

This study was approved by the Ethics Committee of the Faculty of Medicine at Alexandria University.

Informed written consent for patients' participation in Clinical Research was obtained from all participants before enrollment into the study.

Table 1. Comparison between the three studied groups according to Catechol -O- methyl transferase.

	Control (n = 20)	Benign (n = 30)	Malignant (n = 30)	H	p
COMT tissue	529.55 ± 77.89	650.97 ± 130.32	386.50 ± 96.47	46.968*	<0.00*
Significance between groups: $p_1 = 0.039$, $p_2 < 0.001^*$, $p_3 < 0.001^*$					
COMT serum	323.40 ± 18.30	475.20 ± 214.34	367.70 ± 80.64	28.752*	<0.00*
Significance between groups: $p_1 < 0.001^*$, $p_2 = 0.022^*$, $p_3 = 0.001^*$					
Tissue vs. serum	<0.001*	<0.001*	0.028*		
Ratio tissue/serum	1.65 ± 0.32	1.50 ± 0.38	1.06 ± 0.18	35.420*	<0.00*
Significance between groups: $p_1 = 0.289$, $p_2 < 0.001^*$, $p_3 < 0.001^*$					

H, H for Kruskal Wallis test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test). p : p value for comparing between the studied groups. p_1 : p value for comparing between Control and Benign. p_2 : p value for comparing between Control and Malignant. p_3 : p value for comparing between Benign and Malignant.

*, Statistically significant at $p \leq 0.05$.

Table 2. Comparison between the three studied groups according to 17 β estradiol.

	Control (n = 20)	Benign (n = 30)	Malignant (n = 30)	H	p
17 β estradiol tissue	11.35 ± 0.71	15.20 ± 1.44	21.18 ± 5.50	65.312*	<0.001*
Significance between groups: $p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 < 0.001^*$					
17 β estradiol serum	8.71 ± 1.12	9.90 ± 1.96	13.46 ± 4.90	23.450*	<0.001*
Significance between groups: $p_1 = 0.034^*$, $p_2 < 0.001^*$, $p_3 = 0.003^*$					
Tissue vs. serum	<0.001*	<0.001*	<0.001*		
Tissue/serum ratio	1.33 ± 0.24	1.57 ± 0.22	1.73 ± 0.62	7.995*	0.018*
Significance between groups: $p_1 = 0.020^*$, $p_2 = 0.007^*$, $p_3 = 0.673$					

H, H for Kruskal Wallis test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test). p : p value for comparing between the studied groups. p_1 : p value for comparing between Control and Benign. p_2 : p value for comparing between Control and Malignant. p_3 : p value for comparing between Benign and Malignant.

*, Statistically significant at $p \leq 0.05$.

2.1 Methods

2.1.1 Sample collection

Tissues of normal ovaries and ovarian carcinomas were frozen in Roswell Park Memorial Institute (RPMI) media and stored at -80°C . Before analysis, ovarian tissues were homogenized in the media to obtain tissue homogenates.

For serum samples, five mL of blood was obtained and centrifuged at 3000 rpm for 10 minutes.

Both COMT and 17 β estradiol levels were determined in serum samples and tissue homogenates using enzyme-linked immunosorbent assay (ELISA) from E bioseps (SNF Medical). The assays were performed in duplicate according to the manufacturer's protocols.

2.1.2 Determination of COMT and 17 β estradiol levels by ELISA

In brief, standards, test samples and control wells were set on pre-coated 96-well ELISA plates with captured antibodies (anti-Catechol -O- methyltransferase antibodies for COMT and anti 17 β estradiol antibodies for 17 β estradiol). Duplicate aliquots (50 μL per well) of diluted sera and different standard protein concentrations were loaded onto the ELISA plate. The plates were then incubated for 30 min at 37°C . Unbound materials were washed out, and biotinylated secondary antibodies (anti-Catechol -O- methyltransferase and anti 17 β estradiol) were added to each well. The plates

were incubated for 30 min at 37°C . After extensive washing, color development was performed by incubation with horseradish peroxidase substrate (substrate A and substrate B). After adding stop solution, the optical density (O.D.) at 450 nm was determined for each well using a microplate reader, and the concentrations of samples were determined by comparison to the standard concentration curves.

2.2 Statistical analysis

Data were fed to the computer and analyzed by IBM SPSS software package version 20.0. (IBM Corp, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to verify the normality of distribution of variables, and comparisons between groups for categorical variables were assessed by Chi-square test. Mann-Whitney test was utilized to compare two groups for abnormally distributed quantitative variables. In contrast, Kruskal-Wallis test was employed to compare different groups for abnormally distributed quantitative variables, followed by Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison. ANOVA was deployed to compare between more than two groups. Significance of the obtained results was judged at the 5% level [15, 16].

Table 3. Relation between menopausal status and different parameters.

	Menopausal			
	Catechol -O- methyl transferase		17 β estradiol	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
Tissue				
Control group	(n = 12)	(n = 8)	(n = 12)	(n = 8)
Mean \pm SD	528.3 \pm 93.7	531.5 \pm 51.5	11.23 \pm 0.71	11.54 \pm 0.71
U (p)	40.50 (0.571)		33.0 (0.270)	
Benign group	(n = 23)	(n = 7)	(n = 23)	(n = 7)
Mean \pm SD	610.59 \pm 121.85	783.66 \pm 29.27	15.69 \pm 1.09	13.58 \pm 1.32
U (p)	10.500* (<0.001*)		16.500* (0.001*)	
Malignant group	(n = 21)	(n = 9)	(n = 21)	(n = 9)
Mean \pm SD	350.1 \pm 47.6	471.5 \pm 128.2	22.72 \pm 5.85	17.57 \pm 1.87
U (p)	28.0* (0.002*)		25.0* (0.001*)	
Serum				
Control group	(n = 12)	(n = 8)	(n = 12)	(n = 8)
Mean \pm SD	326.9 \pm 17.0	318.1 \pm 20.0	9.04 \pm 1.07	8.21 \pm 1.05
U (p)	38.50 (0.473)		27.0 (0.115)	
Benign group	(n = 23)	(n = 7)	(n = 23)	(n = 7)
Mean \pm SD	402.07 \pm 123.49	715.49 \pm 279.79	10.42 \pm 1.96	8.20 \pm 0.45
U (p)	16.500* (0.001*)		25.500* (0.005*)	
Malignant group	(n = 21)	(n = 9)	(n = 21)	(n = 9)
Mean \pm SD	337.6 \pm 38.6	437.9 \pm 109.2	14.75 \pm 4.73	10.44 \pm 4.05
U (p)	33.0* (0.004*)		36.50* (0.007*)	

U, Mann Whitney test.

*, Statistically significant at $p \leq 0.05$.

3. Results

3.1 Subjects' demographic data

Age distributions and menstrual state among benign, malignant ovarian tumors and healthy control groups were studied. No statistically significant differences existed ($p = 0.053$, 0.452), respectively.

3.2 Pathological data

Most patients with benign tumors were benign ovarian cystadenoma (50%), while others were endometrioma (40%) and ovarian fibroma (10%). Patients with malignant tumors were serous adenocarcinoma (60%), endometroid adenocarcinoma (30%) and mucinous adenocarcinoma (10%). All patients with serous adenocarcinoma were of high grade.

3.3 Catechol -O- methyltransferase concentrations

COMT concentrations were measured in serum and ovarian tissues of all studied groups, as illustrated in Table 1. A significant increase in COMT level existed in tissue than in serum in all groups. Both tissue and serum levels of COMT in patients with malignant tumors were significantly lower than in control and benign groups ($p < 0.001$) for tissue and ($p = 0.022$, $p = 0.001$) for serum.

3.4 17 β estradiol concentrations

17 β estradiol concentrations were measured in serum and ovarian tissues of all studied groups, as illustrated in Table 2. A significant increase of 17 β estradiol level existed in tissue than in serum. Both tissue and serum levels of 17 β estradiol

in patients with malignant tumors were significantly higher than in control and benign groups ($p < 0.001$) for tissue ($p < 0.001$, $p < 0.003$) for serum. Also, both tissue and serum levels of 17 β estradiol were significantly decreased in postmenopausal females than in premenopausal females in patients with benign and malignant ovarian tumors, as illustrated in Table 3.

3.5 Correlation between Catechol -O- methyltransferase and 17 β estradiol levels

A negative correlation was found between COMT and 17 β estradiol levels in patients with benign and malignant ovarian tumors, as illustrated in Fig. 1.

4. Discussion

Ovarian cancer can be initiated by unbalanced estrogen metabolism leading to estrogen–DNA adducts that cause mutations in critical genes in the ovarian epithelial cells. In addition estrogen metabolism is strongly implicated in developing ovarian and other hormonal cancers [17]. Many risk factors associated with ovarian cancer development are related to estrogen exposure [18].

COMT enzyme catalyzes formation of reactive estrogen metabolites and DNA adducts. Moreover, it has been associated with ovarian cancer when combined with other polymorphisms in the catechol estrogen pathway [19].

This study aimed to evaluate a potential correlation between COMT and 17 β estradiol levels and ovarian cancer.

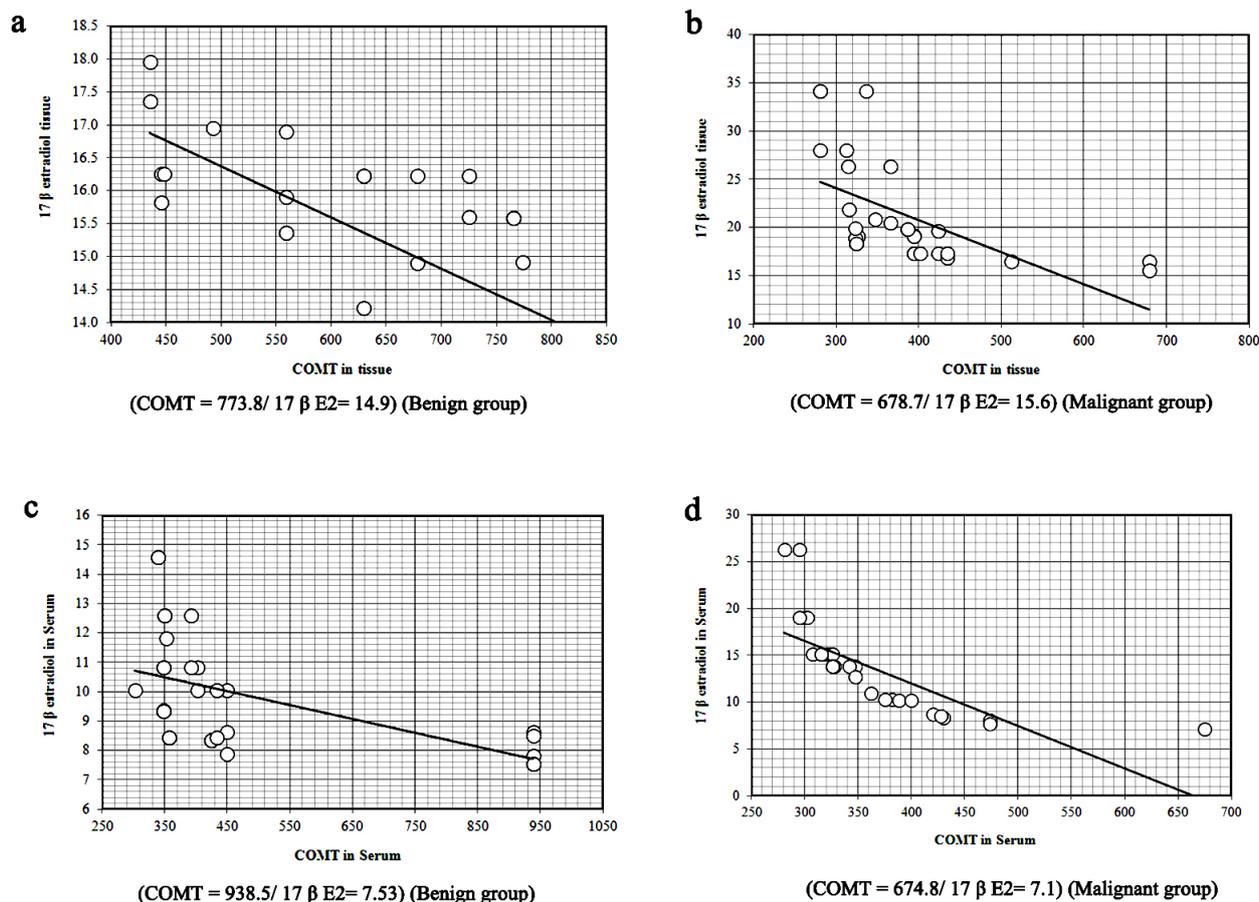


Fig. 1. Correlation between COMT and 17β estradiol in serum and tissue in each group. (a) Correlation between Catechol -O- methyltransferase (pg/mL) and 17β estradiol (pmol/L) in tissue in Benign group (negative correlation). (b) Correlation between Catechol -O- methyltransferase (pg/mL) and 17β estradiol (pmol/L) in tissue in Malignant group (negative correlation). (c) Correlation between Catechol -O- methyltransferase (pg/mL) and 17β estradiol (pmol/L) in serum in Benign group (negative correlation). (d) Correlation between Catechol -O- methyltransferase (pg/mL) and 17β estradiol (pmol/L) in serum in Malignant group (negative correlation).

Several studies have indicated that 17β -estradiol (E2) can increase the risk of epithelial ovarian cancer development by directly acting on OSE cells. When treated with E2, these cells are more susceptible to neoplastic transformation due to E2 ability to increase proliferation and production of reactive oxygen species in these cells [20–22].

According to our results, both tissue and serum levels of COMT were significantly decreased in patients with malignant ovarian tumors compared to benign and control groups. This inhibition in neoplastic tissues reflects the role of altered COMT activity in ovarian cancer development. In accordance with our results, Zahid *et al.* [17] concluded that estrogen metabolism was unbalanced and estrogen DNA adducts were significantly higher in women with ovarian cancer compared to control women without cancer ($p < 0.0001$). These results suggest that formation of estrogen–DNA adducts by COMT plays a critical role in ovarian cancer initiation [17].

In addition Lavingie *et al.* [23] in a study on MCF-7 cells treated with E2 stated direct relationship between carcinogenic estrogen metabolites and oxidative DNA damage in the

absence of COMT activity and O-methylated metabolites.

COMT inhibition in neoplastic tissue may reflect that this defect could be a primary impairment of COMT gene in patients with malignant ovarian tumors. In contrast, the absence of this inhibition in patients with benign ovarian tumors may reveal a protective effect of COMT gene against DNA damage and neoplastic transformation. This was in agreement with findings from different studies [10–14].

In accordance with our results, other studies concluded that polymorphism in COMT gene, which codes for a low activity variant of COMT enzyme, is associated with increased risk of breast cancer development [24–28].

In addition, our results demonstrated higher concentrations of 17β estradiol in ovarian tissues compared to serum samples in all studied groups with elevated tissue/serum ratio of 17β estradiol. This may indicate an essential role of ovarian tissues concentrations of estradiol in tumor biology. Besides, both tissue and serum levels of 17β estradiol in patients with malignant tumors were significantly higher than in control and benign groups. Consistent with our findings, Lavi-

ollette *et al.* [29] stated that exogenous 17 β -estradiol (E2) accelerates ovarian cancer progression in the transgenic mouse model of disease.

However, based on differences in concentrations of gonadal hormones between different ovarian tumor groups, postmenopausal women with ovarian tumors have decreased E2 tissue levels. In the malignant group, E2 levels were 17.14 pmol/L (15.58–21.84) and 19.85 pmol/L (17.32–34.15) in postmenopausal women and premenopausal women, respectively. In the benign group, E2 levels were 13.2 pmol/L (12.4–16.2) and 15.6 pmol/L (13.9–18) in postmenopausal women and premenopausal women, respectively. Therefore, our results were incoherent with Lindgren *et al.* [30], who suggested an increased production of gonadal hormones in ovarian cancer tissues of postmenopausal females.

Correlation analysis showed a negative correlation between COMT and 17 β estradiol in tissue and serum of patients with benign and malignant ovarian tumors. This confirms that COMT enzyme holds a vital function in the decreased production of carcinogenic estrogen metabolites, and low COMT is associated with developing malignant ovarian tumors.

5. Conclusions

We can conclude that low COMT activity and high tissue/serum level of 17 β estradiol may be contributory factors for ovarian cancer development, while the absence of COMT inhibition in benign group is protective against imbalance in estrogen homeostasis and neoplastic transformation. This corroborates the notion that targeting estrogen metabolism can be an alternative way to reduce ovarian cancer risk. We recommend additional mechanistic studies and perhaps developing an appropriate mouse model to provide more insights into the role of COMT activity and polymorphisms affecting their levels in ovarian tissue and ovarian cancer.

Abbreviations

FIGO, International federation of Obstetrics and Gynecology; COMT, Catechol -O- methyltransferase; CEs, catechol estrogen metabolites; MeOE2, methoxyestradiol dGdeoxyguanine; OSE, ovarian surface epithelium.

Author contributions

HN conceived and designed the study; NME collected the samples; EO carried out ELISA. EO, NME and NAE analyzed the data; HN and EO shared in writing the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculty of Medicine at Alexandria University. Informed written consent for patients' participation in Clinical Research 2018 (serial number 0304867) was obtained from all participants before enrollment into the study.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

The raw data used and analyzed during the current study are available from the corresponding author on reasonable request.

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