# Expression of Catechol O methyl transferase (COMT) with the contrasting effects of 17β estradiol (E2): Implications for the mechanism of estrogen-induced ovarian carcinogenesis.

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## Abstract

# Background

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 methyl transferase (COMT) is a phase II metabolic inactivation pathway for catechol estrogens. The goal of the present study was to investigate the role of COMT level in ovarian carcinogenesis with the contrasting effects of 17 β estradiol level.

# Subjects and methods

Our study was conducted on 80 subjects divided into 30 patients with malignant ovarian tumors ,30 patients with benign ovarian tumors and 20 healthy controls. Tissue and serum levels of COMT and 17 17 β estradiol were determined using ELISA

# Results

According to our results COMT inhibition in the malignant group was detected as high as 7.1 pmol/L E2 in serum and 15.6 pmol/L E2 in tissue homogenate. This inhibition was absent in the benign group as high as 7.53 pmol/L E2 in serum and as high as 14.9 pmol/L E2 in tissue homogenates.

# Conclusions

Our results provide evidence for the protective effect of COMT in benign ovaries against neoplastic transformation. This supports the notion that targeting the metabolism of estrogen can be an another way to reduce ovarian cancer risk.

## 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. There (E2) is produced by circulating androgen and estrogen precursors and taken up to ovarian epithelial cells by transporters.[2] Previous studies have demonstrated that 17β estradiol (E2), its interconvertible metabolite estrone (E1) and their catechol metabolites are carcinogenic.[2, 3] Oxidative metabolism of E2/E1 to catechols involves the formation of reactive metabolites leading to the generation of mutagenic DNA adducts. Free radicals from the metabolic activation of estrogen will cause mutation. Accumulation of mutations will lead to neoplastic transformation of proliferative cells.[3–7]

O methylation by COMT has a major role in blocking the further oxidation to catechols.[3] The importance of regulating oxidative metabolism of estrogen to catechols to quinone metabolites (phase II metabolic inactivation) was studied in MCF-10F cells, representing the importance of COMT.[4]

Both in rat and human COMT has been shown to have a higher catalytic activity towards catechol estrogen metabolites (CEs).[8, 9] 2-methoxyestradiol (2MeOE2) which is formed by COMT has been shown to increase apoptosis, inhibit growth and inhibit angiogenesis.[10–14] It will be important to determine COMT activity as a regulator of oxidative metabolism of estrogen in ovarian cancer with the contrasting effects of 17 β estradiol levels.

# Subjects And Methods

Our study was conducted on 80 subjects divided into 3 groups; 30 patients with malignant epithelial ovarian tumors, 30 patients with benign ovarian tumors and 20 healthy age-matched individuals as control group. Patients were recruited from El Shatby Maternity Hospital (Alexandria University), from November 2017 to February 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 Alexandria University.

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

### Methods

### Sample collection

Samples of normal ovaries and ovarian carcinomas were frozen in RPMI media and stored at -80°C. Before analysis, ovarian tissues were homogenized in the media to obtain tissue homogenate

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 both serum samples and tissue homogenates obtained from ovarian tissue using an ELISA from E bioseps (SNF Medical) The assays were performed in duplicate according to the manufacturer's protocols.

### Determination of catechol O methyl transferase concentration and 17 β estradiol levels by enzyme linked immunosorbant assay (ELISA)

In °brief, standards, test samples and control wells were set on pre-coated 96-well ELISA plates with captured antibodies (anti COMT antibodies for COMT and anti E2 antibodies for 17β estradiol). Duplicate aliquots (50 µl microliter per well) of diluted sera and different concentrations of standard protein 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 COMT and anti E2) 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 HRP 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 the samples were determined by comparison to the standard concentration curves.

### Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**).** The Kolmogorov- Smirnov test was used to verify the normality of distribution of variables, Comparisons between groups for categorical variables were assessed using **Chi-square test. Mann Whitney test** was used to compare two groups for abnormally distributed quantitative variables while **Kruskal Wallis test** was used to compare different groups for not normally distributed quantitative variables and followed by **Post Hoc test (Dunn's for multiple comparisons test)** for pairwise comparison. **ANOVA** was used for comparing between more than two groups. Significance of the obtained results was judged at the 5% level.[15-16]

## Results

### Subjects' demographic data

Age distributions and menstrual state among benign, malignant ovarian tumors and healthy control groups is illustrated in table (1). There were no statistically significant differences (p=0.053, 0.452) respectively

### Clinical data

Data are illustrated in table 2

### COMT concentrations

COMT concentrations were measured in both serum and ovarian tissues as illustrated in table 3. There was a significant increase in COMT level in tissue when compared to its level in serum in all the studied groups. Tissue /serum ratio was significantly lower in malignant group when compared to both control and benign groups ($p<0.001$). Both tissue and serum levels of COMT in patients with malignant tumors were significantly lower in comparison to control and benign groups ($p<0.001$) for tissue and ($p= 0.022$, $p=0.001$) for serum.

### 17β estradiol concentrations

17β estradiol concentrations were measured in both serum and ovarian tissues as illustrated in table 4. There was a significant increase of 17β estradiol level in tissue when compared to its level in serum. Tissue /serum ratio was higher in malignant group when compared to both control and benign groups

(p=0.007, p= 0.673). respectively. Both tissue and serum levels of 17β estradiol in patients with malignant tumors were significantly higher in comparison to control and benign groups (p<0.001) for tissue, (p<0.001, p<0.003) for serum. Also both tissue and serum levels of 17β estradiol were significantly decreased in postmenopausal females when compared to premenopausal females in patients with benign and malignant ovarian tumors as illustrated in table 5.

### Correlation between COMT and 17 β estradiol levels

A negative correlation was found between COMT and both tissue/serum 17 β estradiol levels in patients with benign and malignant ovarian tumors as illustrated in figures (1-4)

# Discussion

The goal of the present study was to explore the effects of alterations in COMT activity on cellular levels in ovarian cancer.

Our results demonstrated that ovarian tissue concentrations of E2 were more than 1.4 fold higher in tissue than in serum in normal ovaries. Also E2 was higher in benign tissue than in serum 1.5 fold and, 1.7 fold higher in neoplastic tissue than in serum. This may indicate an important role of ovarian tissues in tumor biology.

In vitro experiments have shown the potential of estrogens to stimulate the proliferation of OSE cells.[17] However, based on differences in concentrations between different ovarian tumor groups, postmenopausal women with malignant ovarian tumors presented lower median tissue hormone levels. Ovarian cancer is usually seen after the age of 50 years.[2] E2 levels in our postmenopausal women was 17.14 pmol/L (15.58–21.84) versus 19.85 pmol/L (17.32–34.15) in premenopausal group. In benign group E2 level in postmenopausal women was 13.2 pmol/L (12.4–16.2) while in premenopausal women was 15.6 pmol/L (13.9–18). Thus our results were not coherent with the previously suggested increased production of gonadal hormones in ovarian cancer tissues which agrees with Lendgren et al. 2002.[18]

Experimental evidences have suggested that catechol metabolites contribute to estrogen carcinogenicity. Methylation of catechol estrogen by COMT is a phase II inactivation pathway for CEs.[19] In the light of this, it is important to understand the effects of altered COMT activity in ovarian cancer.

Various chemo-preventive agents such as sulforaphane and resveratrol have been shown in cell culture to block oxidative metabolism of E2/E1 and thus prevent DNA damage.[3] This supports the notion that targeting the estrogen/estrone metabolism pathway may be another way to reduce cancer risk in neoplastic tissue.

According to our results COMT inhibition was detected in ovarian neoplasm as high as 7.1 pmol/L serum E2 levels and as high as 15.6 pmol/L in tissue. This inhibition in neoplastic tissues reflects the role of altered COMT activity in ovarian cancer.

Lavingie et al 2001 in a study on MCF-7 cells treated with E2 suggested that COMT inhibitor blocked 2MeOE2 formation.[20] This was associated with increased 2OH E2 and 8-oxo dG levels.[21] This provides evidence consistent with the hypothesis that COMT is an important enzyme protecting from CE metabolites carcinogenicity.

This COMT inhibition is absent in benign group even as high as 7.53 pmol/L E2 in serum and as high as 14.9 pmol/L E2 in tissue homogenates. This may reflect a protective effect of COMT in benign tissue against DNA damage and neoplastic transformation. This agrees with findings from different studies.[10–14] Whereas, the levels of specific metabolites were not determined in our work, this is similar to Han and Liehr study [19] who demonstrated that in the microsome system COMT catalyzed the inactivation of catechols.[22–27]

COMT inhibition in neoplastic tissue while this inhibition is absent in benign group may reflect that this defect could be a primary impairment or genetic disorder in COMT gene in neoplastic group, which could result in decreased CEs detoxification. Polymorphism in COMT gene could result in 3–4 fold less COMT activity.[28–31]

We can conclude that low COMT activity and high tissue/serum level of 17β estradiol may be a contributary factor for the development of ovarian cancer while the absence of COMT inhibition in benign group is protective against imbalance in estrogen homeostasis and neoplastic transformation. This supports the notion that targeting the metabolism of estrogen can be an another way to reduce ovarian cancer risk. We recommend additional mechanistic studies and perhaps the development of 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 methyl transferase, CEs :catechol estrogen metabolites (MeOE2) methoxyestradiol, dG deoxyguanine

# Declarations

- **Ethics approval and consent to participate:** This study was approved by the Ethics Committee of the Faculty of Medicine Alexandria University. Informed written consent for patient's participation in a Clinical Research was obtained from all participants before enrollment into the study
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data used and analyzed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
- **Funding:** There are not any financial ties to include.

- **Authors' contributions:** HN conceived and designed the study; NME collected the samples; EO carried out EO, NME and NAE analyzed the data; HN and EO shared in writing the paper.
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# Tables

**Table (1): Comparison between the three studied groups according to demographic data**

| | Control (n = 20) | Benign (n = 30) | Malignant (n = 30) | Test of Sig | p |
|---|---|---|---|---|---|
| **Age** | | | | | |
| < 40 | 1 (5%) | 10 (33.3%) | 3 (10%) | $c^2$= | 0.053 |
| 40 – 50 | 5 (25%) | 8 (26.7%) | 9 (30%) | 9.351 | |
| > 50 | 14 (70%) | 12 (40%) | 18 (60%) | | |
| Min. – Max. | 38 – 62 | 11 – 75 | 17 – 75 | F= | 0.058 |
| Mean ± SD. | 53.3 ± 6.3 | 44.7 ± 16.3 | 51.1 ± 13.4 | 2.954 | |
| **Menopausal** | | | | $c^2$= | 0.452 |
| Premenopausal | 12 (60%) | 23 (76.7%) | 21 (70 %) | 1.587 | |
| Postmenopausal | 8 (40%) | 7 (23.3%) | 9 (30%) | | |

$c^2$: **Chi square test** **F: F for ANOVA test**
p: p value for comparing between the studied groups
*: Statistically significant at p ≤ 0.05

**Table (2): Distribution of the studied cases according to diagnosis, staging and grading (n = 60)**

| | No. (%) |
|---|---|
| **Diagnosis of Benign group** | |
| Benign ovarian cystadenoma | 15 (50.0%) |
| Endometrioma | 6 (20.0%) |
| Ovarian fibrothecoma | 4(13.3%) |
| Dermoid cyst | 3(10.0%) |
| Ovarian inflammatory mass | 2 (6.7%) |
| **Diagnosis of Malignant group** | |
| Serous adenocarcinoma | 18(60.0%) |
| Endometrioid adenocarcinoma | 8(26.7%) |
| Mucinous adenocarcinoma | 4(13.3%) |
| **Staging of Malignant group** | |
| I | 13(43.3%) |
| II | 5(16.7%) |
| III | 12(40.0%) |
| **Grading of Malignant group** | |
| 1 | 4 (13.3%) |
| 2 | 14 (46.7%) |
| 3 | 12 (40%) |

**Table (3): Comparison between the three studied groups according to COMT**

| | Control (n = 20) | Benign (n = 30) | Malignant (n = 30) | H | p |
|---|---|---|---|---|---|
| **COMT tissue** | | | | | |
| Min. – Max. | 489.9 – 812.1 | 435.5 – 803.2 | 281.1 – 678.7 | 46.968* | <0.001* |
| Mean ± SD | 529.55±77.89 | 650.97±130.32 | 386.50±96.47 | | |
| Median (IQR) | 493.9 (489.9 – 544.1) | 654.1 (558.8 – 773.9) | 376.3 (323.6 – 424.1) | | |
| **Sig. bet. grps.** | $p_1$=0.039*,$p_2$<0.001*,$p_3$<0.001* | | | | |
| **COMT serum** | | | | | |
| Min. – Max. | 298.9 – 340.7 | 302.7 – 938.5 | 281.1 – 674.8 | 28.752* | <0.001* |
| Mean ± SD | 323.40±18.30 | 475.20±214.34 | 367.70±80.64 | | |
| Median (IQR) | 334.7 (298.9 – 336.7) | 397.3(348.9 – 449.1) | 344.3(315.4-400) | | |
| **Sig. bet. grps.** | $p_1$<0.001*,$p_2$=0.022*,$p_3$=0.001* | | | | |
| **Tissue vs. serum** | <0.001* | <0.001* | 0.028* | | |
| **Ratio tissue / serum** | | | | | |
| Min. – Max. | 1.44 – 2.72 | 0.9 - 2.0 | 0.6 – 1.4 | 35.420* | <0.001* |
| Mean ± SD | 1.65±0.32 | 1.50±0.38 | 1.06±0.18 | | |
| Median (IQR) | 1.48(1.46-1.73) | 1.7 (1.2-1.8) | 1.1 (1.01-1.2) | | |
| **Sig. bet. grps.** | $p_1$= 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 p1: p value for comparing between Control and Benign p2: p value for comparing between Control and Malignant p3: p value for comparing between Benign and Malignant

*: Statistically significant at p ≤ 0.05

**Table (4): Comparison between the three studied groups according to 17 β estradiol**

| | Control (n = 20) | Benign (n = 30) | Malignant (n = 30) | H | p |
|---|---|---|---|---|---|
| **Beta 17 β tissue** | | | | | |
| Min. – Max. | 10.7 – 12.4 | 12.4 – 18.0 | 15.6 – 34.2 | 65.312* | <0.001* |
| Mean ± SD | 11.35±0.71 | 15.20±1.44 | 21.18±5.50 | | |
| Median (IQR) | 11.1 (10.7 – 12.0) | 15.6 (13.9 – 16.2) | 19.1 (17.3 – 21.8) | | |
| **Sig. bet. grps.** | $p_1<0.001^*$,$p_2<0.001^*$,$p_3<0.001^*$ | | | | |
| **Beta Serum** | | | | | |
| Min. – Max. | 7.5 – 9. 9 | 7.5 – 14.6 | 7.1 – 26.3 | 23.450* | <0.001* |
| Mean ± SD | 8.71±1.12 | 9.90±1.96 | 13.46±4.90 | | |
| Median (IQR) | 8.2 (7.5 – 9.9) | 9.3 (8.4 – 10.8) | 13.9 (10.2 – 15.1) | | |
| **Sig. bet. grps.** | $p_1= 0.034^*$, $p_2$,$<0.001^*$,$p_3= 0.003^*$ | | | | |
| **Tissue vs. serum** | $<0.001^*$ | $<0.001^*$ | $<0.001^*$ | | |
| **Ratio tissue / serum** | | | | | |
| Min. – Max. | 1.0 - 1.6 | 1.1 - 1.9 | 0.7 – 3.3 | 7.995* | 0.018* |
| Mean ± SD | 1.33±0.24 | 1.57±0.22 | 1.73±0.62 | | |
| Median (IQR) | 1.4 (1.1-1.6) | 1.5 (1.5-1.8) | 1.7 (1.3 – 2.1) | | |
| **Sig. bet. grps.** | $p_1= 0.020^*$,$p_2=0.007^*$ ,$p_3= 0.673$ | | | | |

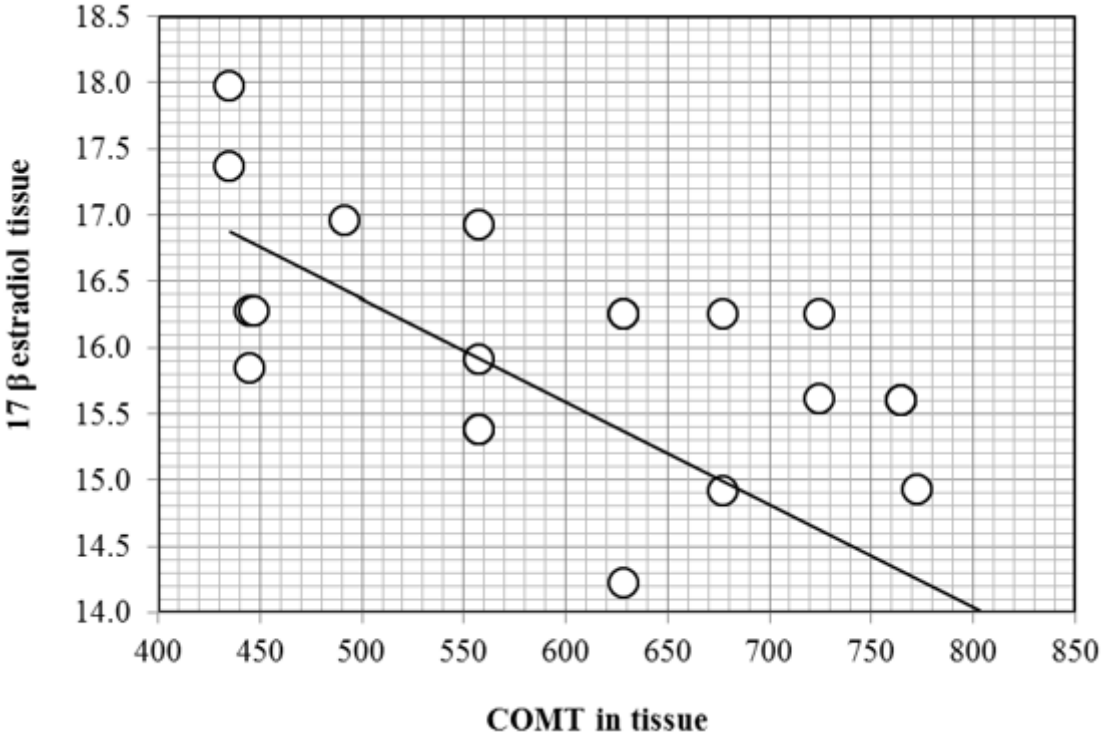
H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
p: p value for comparing between the studied groupsp1: p value for comparing between Control and Benign p2: p value for comparing between Control and Malignantp3: p value for comparing between Benign and Malignant
*: Statistically significant at $p \leq 0.05$

**Table (5): Relation between menopausal status and in 17β estradiol in different groups (n=80)**

| | Menopausal | | U | p |
|---|---|---|---|---|
| | Premenopausal | Postmenopausal | | |
| **Beta 17 β tissue** | | | | |
| **Control group** | **(n = 12)** | **(n = 8)** | | |
| Min. – Max. | 10.71 - 12.35 | 10.71 - 12.35 | 33.0 | 0.270 |
| Mean ± SD | 11.54 ± 0.71 | 11.23 ± 0.71 | | |
| Median | 11.55 | 10.71 | | |
| **Benign group** | **(n = 23)** | **(n = 7)** | | |
| Min. – Max. | 13.9 – 18 | 12.4 – 16.2 | 16.500* | 0.001* |
| Mean ± SD | 15.69±1.09 | 13.58±1.32 | | |
| Median | 15.6 | 13.2 | | |
| **Malignant group** | **(n = 21)** | **(n = 9)** | | |
| Min. – Max. | 17.32 - 34.15 | 15.58 - 21.84 | 25.0* | 0.001* |
| Mean ± SD | 22.72 ± 5.85 | 17.57 ± 1.87 | | |
| Median | 19.85 | 17.14 | | |
| **Beta 17 β serum** | | | | |
| **Control group** | **(n = 12)** | **(n = 8)** | | |
| Min. – Max. | 7.53 - 9.89 | 7.53 - 9.89 | 27.0 | 0.115 |
| Mean ± SD | 9.04 ± 1.07 | 8.21 ± 1.05 | | |
| Median | 9.89 | 7.71 | | |
| **Benign group** | **(n = 23)** | **(n = 7)** | | |
| Min. – Max. | 7.5 – 14.6 | 7.5 – 8.6 | 25.500* | 0.005* |
| Mean ± SD | 10.42±1.96 | 8.20±0.45 | | |
| Median | 10.1 | 8.4 | | |
| **Malignant group** | **(n = 21)** | **(n = 9)** | | |
| Min. – Max. | 8.49 - 26.29 | 7.10 - 19.07 | 36.50* | 0.007* |
| Mean ± SD | 14.75 ± 4.73 | 10.44 ± 4.05 | | |
| Median | 13.85 | 8.35 | | |

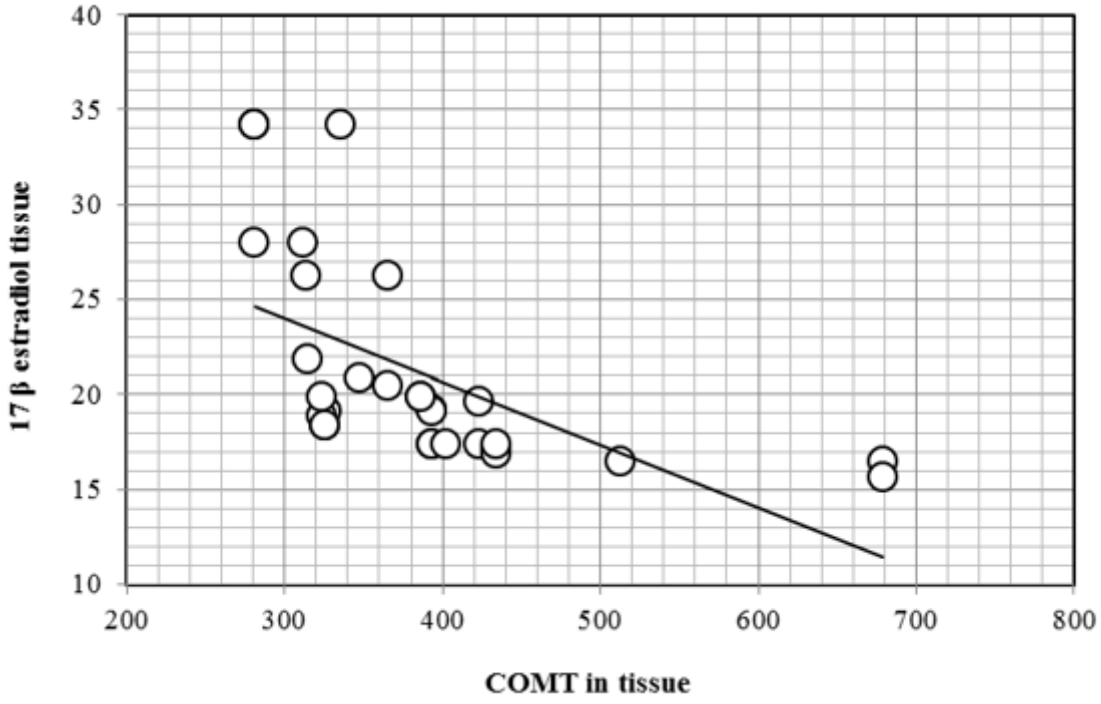
**U: Mann Whitney test**
*: Statistically significant at $p \leq 0.05$

# Figures



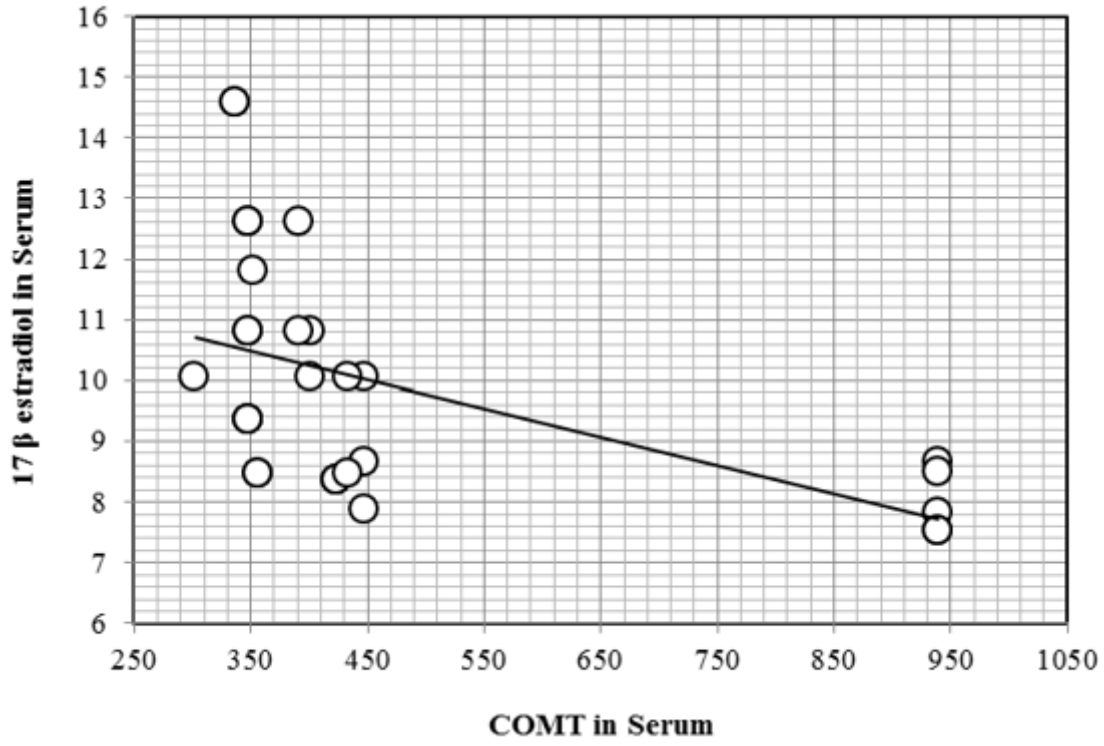

## Figure 1

Correlation between COMT and 17 β estradiol in tissue in Benign group



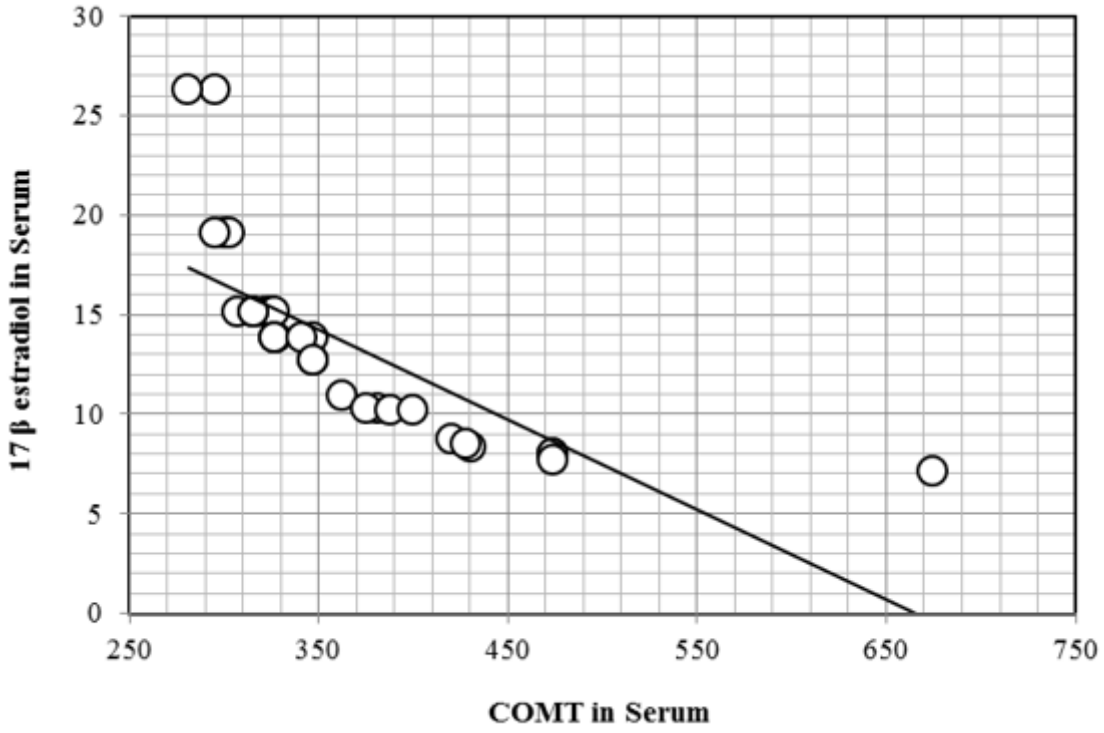

## Figure 2

Correlation between COMT and 17 β estradiol in tissue in Malignant group




## Figure 3

Correlation between COMT and 17 β estradiol in serum in Benign group




## Figure 4

Correlation between COMT and 17 β estradiol in serum in Malignant group