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GSTM1, GSTP1, p53 as some probable predictors of prognosis in primary and metastatic epithelial ovarian cancer

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ABSTRACT

Objectives: Ovarian carcinomas are responsible for the death of more women than all other gynecologic malignancies in the Western world. Ovarian carcinomas are detected in an advanced stage of the disease in approximately 80% of the patients. Glutathione S-transferases (GSTs) are an important family involved in the detoxification of several xenobiotics. Thus, this mechanism protects tissues from the harmful effects of oxidative stress and chemical-induced damages. The expression of them may contribute to the characteristics of ovarian carcinoma as they can metabolise both exogenous and endogenous compounds, which are implicated in the development of ovarian cancer. Therefore, our aim was to determine the expressions of GST Mu 1 (GSTM1), GST Pi 1 (GSTP1), and also p53, which is a tumor suppressor gene, in benign and malign ovarian tumors and metastasis tissues.

Methods: A total of the 99 patients with ovarian tumor enrolled in the study. Thirty-one of the tissues was benign tumor, 17 was malign tumor and 51 was metastasis. The immunohistochemical GSTM1, GSTP1, and p53 staining characteristics of these tissues were investigated.

Results: The highest GSTM1, GSTP1, and p53 expression was noted in the malignant group followed by the metastasis group. GSTP1 expression was significantly higher in malignant tissues than benign ones ($p = 0.015$). No statistically significant difference was observed in the level of GSTM1 expression between groups ($p = 0.524$). p53 expression was significantly higher in the metastasis and malignant tissues than the benign ones ($p < 0.001$).

Conclusions: The higher expressions of GSTP1 and p53 in malignant and metastasis tissues than benign ones indicate that these expressions could be important biomarkers in ovarian cancer development and progression. Further studies with more cases are required to confirm the results of our present study.

Keywords: Ovary carcinoma, glutathione-S-transferase, p53, immunohistochemistry

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Ovarian cancer is a type of cancer that starts in the ovaries and generally spreads through out the body [1]. It is a heterogeneous disease with a low survival rate and rapid spread, and is the most important cause of death from gynecological cancer [2]. Different types of tumors can develop from each cell type. These tumors are epithelial tumors, germ cell tumors (originating from ovarian cell and follicle) and stromal tumors. Epithelial tumors arise from epithelial cells that cover the outer surface of the ovary. Germ cell tumors are derived from the ovary. Stromal tumors, on the other hand, consist of structural cells that hold the ovarian cells together and produce female hormones, progesterone and estrogen. Most of these tumors are benign and do not spread beyond the ovary [3]. Tumors originating from nonspecific connective tissue cells and tumors originating from another organ by metastasis [1].

The metabolism of xenobiotics is a two-phase process. Phase I reaction is mostly carried out in the liver by the microsomal enzyme system. Phase I reaction may also occur in the lung, kidney, intestine, skin, testis, placenta, and adrenal gland limitedly. Lipid-soluble xenobiotics become more polar by the phase I reactions [4, 5]. Phase II reactions are conjugation reactions carried out by many cytosolic enzymes. Polar metabolites, which are formed as a result of detoxification, combine with endogenous substances by conjugation reactions and are eliminated inactively [6]. Reactive species formed by the Phase I enzymes with glutathione enter into conjugation and eventually bind with cell macromolecules (DNA, RNA, protein), preventing cell damage [7].

Glutathione S-transferases (GSTs) is a family of Phase II detoxification enzymes responsible for the metabolism of chemotherapeutic agents, reactive oxygen molecules and xenobiotics including environmental carcinogens. GSTs catalyze the reactions between various electrophilic compounds and glutathione. GSTs protect DNA from alkylation by conjugation of active metabolites with glutathione. GSTs are dimeric enzymes that inactivate electrophilic xenobiotics and enable their conjugation for removal from the body. Glutathione protects the organism against reactive chemical compounds by binding to compounds with its nucleophilic sulfhydryl group [8]. GSTM1 isoenzymes are predominantly expressed in the liver and a lesser extent in the lung while GSTM3 is an important isoenzyme in lung tissue [9]. GSTP1-1 enzymes show resistance to chemotherapy and radiotherapy applied in many different cancers [10]. It is estimated that half of all cancer cases are associated with mutations in the p53 gene. This prediction suggests that p53 controls a key event in cell proliferation and that this regulation is not cell- or tissue-specific [11]. Since different mutations of p53 cause the structure of the protein to change, it cannot bind to DNA [12]. Mutation occurring in a single allele of the gene behaves as if there is no functional p53 protein in the cell. Homozygous loss of this gene results in non-repairing of DNA damage and the cell undergoes malignant transformation [13]. In our study, we aimed to investigate the differences in the expressions of GSTM1 and GSTP1, which play essential roles in xenobiotic metabolism, and p53, a tumor suppressor gene, in benign and malignant ovarian tumor tissues. Our other goal was to determine the GSTM1, GSTP1, and p53 expressions in metastatic tissues and assess whether these proteins have a role in the progression of the disease.Moreover, the relationship of these expressions with age was also aimed to investigate.

METHODS

We investigated the immunohistochemical staining characteristics of GSTM1, GSTP1, and p53 in malignant (n = 17), benign (n = 31), and metastasis (n = 51) ovarian tumor tissues from 99 patientsin Ankara Keçiören Training and Research Hospital. The expression patterns of the tissues were compared based on immunohistochemical staining intensity.Ethics committee approval was provided by the decision of the Ankara Keçiören Training and Research Hospital Clinical Research Ethics Committee, with the decision number of 2012-KAEK-15/2215 (09.02.2021).

Immunohistochemical Staining

The GSTM1, GSTP1, and p53 were studied by immunohistochemical staining in the tumor tissues of the patients. For immunohistochemistry, the formalin-fixed tissue sections dewaxed in xylene and rehydrated in ethanol sections were washed with distilled water for 3 min. The sections were peroxidase-incubated for 10 minutes using 3% hydrogen peroxide in methanol (v/v). Subsequently, the sections were washed with

distilled water for 3 min and antigen retrieval was performed for 3 min using a 0.01M citrate buffer, pH 6.0 in a domestic pressure cooker. Sections were placed in Tris-buffered saline (TBS) containing 0.15M sodium chloride and 0.05 M Tris-HCL pH 7.6. Sections were incubated at room temperature for 10 min with superblock (SHP125; Scy Tek laboratories, west logan, UT). The primary antibody was diluted through a diluting solution, based on the manufacturer company instructions. After sections were incubated with the primary antibody for anti-GSTP1 (Sc-28,494; Santa Cruz Biotechnology, Inc) diluted 1:500, anti-p53 (M00001-4, Boster Biological Technology) diluted 1:300, anti-GSTM1 (Sc-517262; Santa Cruz Biotechnology.,Inc) diluted 1:100. The sections were incubated at room temperature with a biotinylated link antibody (SHP125; ScyTek Laboratories) followed by streptavidin/HRP complex (SHP125; ScyTek laboratories). After washing with TBS for 15 min, the sections were incubated at room temperature with biotinylated link antibody (SHP125; ScyTek Laboratories) then diaminobenzidine was used to visualize peroxidase activity in tissues. Nuclei were lightly counterstained with hematoxylin, and then the sections were dehydrated and mounted. Light microscopy and scoring of immunohistochemically stained sections were performed by a pathologist, who was unaware of the patients' clinical information scoring for each enzyme was: -, negative (no staining);1, weak staining; 2, moderate staining; 3, strong staining.

Statistical Analysis

Statistical analyses were performed with the IBM SPSS software (Statistical Package for the Social Sciences, Version 25.0). Data were presented as mean ± standard error of the mean (SEM) and minimum-maximum staining intensity. Homogenity of variances was tested by Levene test. Normality of distribution was assessed via Shapiro-Wilk and Kolmogorov-Smirnov tests. The data were not normally distributed. Thus, the Kruskal-Wallis test was performed to compare differences between groups followed by the post hoc bonferonni correction. The point biserial correlation analysis was used to evaluate the correlation between data. The statistical significance level was defined as $p < 0.05$.

RESULTS

The study covers 99 female subjects with benign ovarian tumor (n = 31), malignant ovarian tumor (n = 17), and metastasis (n = 51). The mean age was 47.29 ± 2.87 years in benign ovarian tumor group, 60.56 ± 2.35 years in malignant ovarian tumor group and 61.02 ± 1.37 years in metastasis group (Fig. 1).

Immunohistochemical expression of GSTM1, GSTP1, and p53 was determined in benign ovarian tumor, malign ovarian tumor and metastasis tissues and the resuls were shown in Table 1. Some of the general images obtained for pathological microscopy ex-

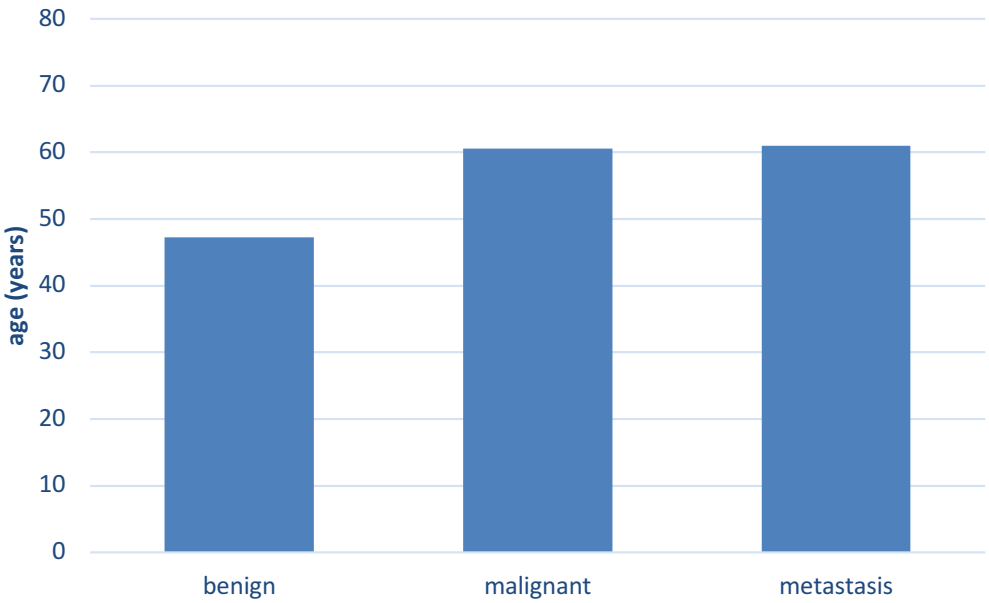

Fig. 1. Age distribution of patients according to benign, malignant and metastasis groups

Table 1. Expression levels of GSTM1, GSTP1, and p53 proteins in benign and malignant ovarian tumor samples and metastasis tissues

	Benigngroup (n = 31)	Malignant group (n = 17)	Metastasis group (n = 51)	*p* value
GSTM1				
Positive (1)	11/31 (35.48%)	7/17 (41.18%)	14/51 (27.45%)	0.524
Negative (0)	20/31 (64.52%)	10/17 (58.82%)	37/51 (72.55%)	
Mean	0.35 ± 0.09 (0-1)	0.41 ± 0.12 (0-1)	0.27 ± 0.06 (0-1)	
GSTP1				
Positive (1)	15/31 (48.39%)	15/17 (88.24%)	37/51 (72.55%)	**0.010**
Negative (0)	16/31 (51.61%)	2/17 (11.76%)	14/51 (27.45%)	
Mean	0.48 ± 0.09 (0-1)	0.88 ± 0.08 (0-1)	0.73 ± 0.06 (0-1)	
p53				
Positive (1)	0/31 (0%)	11/17 (64.71%)	26/51 (50.98%)	**< 0.001**
Negative (0)	31/31 (100%)	6/17 (35.29%)	25/51 (49.02%)	
Mean	0	0.65 ± 0.12 (0-1)	0.51 ± 0.07 (0-1)	

Data were presented as mean ± SEM and minimum-maximum staining intensity. The Kruskal-Wallis test was used for the statistical analysis. The statistical significance level was defined as $p < 0.05$.

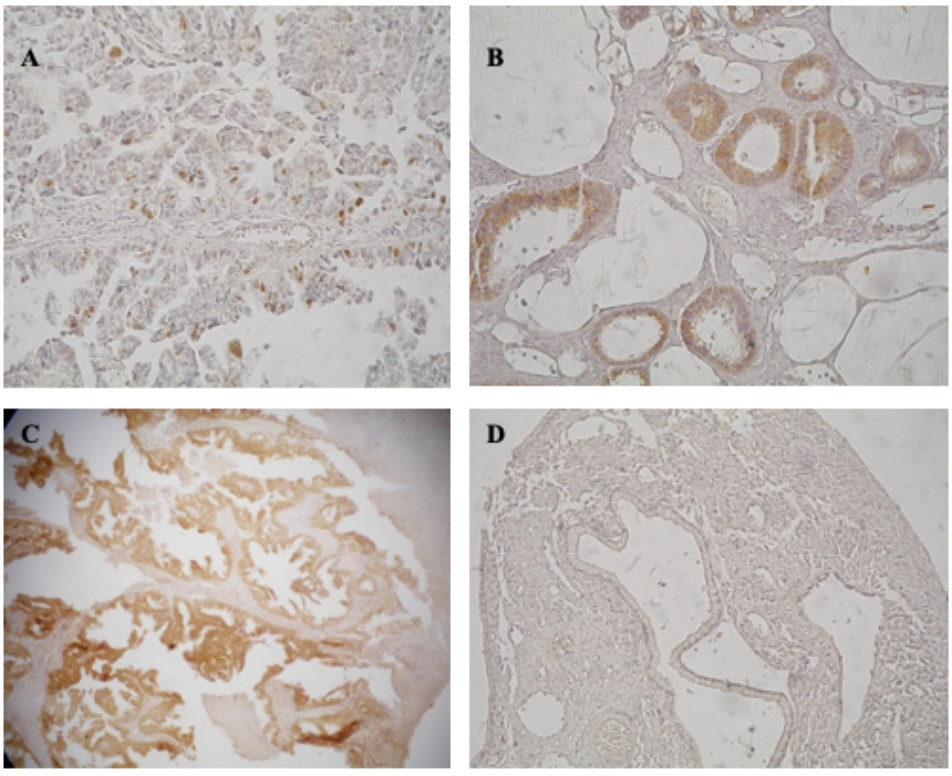

Fig. 2. Immunohistochemical expression of GSTM1 and GSTP1 isoenzymes, and p53 protein in ovarian benign and tumor tissues, (A) p53 protein expression on tumor tissue; (B) GSTP1 protein expression on tumor tissue; (C) GSTM1 protein expression on tumor tissue; and (D) GSTP1 protein expression on benign tissue.

amination of preparative tissues obtained as a result of immunohistochemical applications are given in Fig. 2.

The results showed that the highest GSTM1 expression was observed in the malignant group. GSTM1 was positively expressed in 35.48% of benign tissues, while 41.18% of malignant ones. Positive GSTM1 expression was found in 27.45% of metastasis tissues. However, there were no statistically significant differences between groups in terms of GSTM1 expression ($p = 0.524$).

There was a statistically significant GSTP1 expressions between groups ($p = 0.010$). The highest GSTP1 expression was noted in the malignant group followed by the metastasis group. Positive GSTP1 expression was observed in 88.24% of malignant, 72.55% of metastasis, and 48.39% of benign tissues. GSTP1 expression of malignant tissues was 1.83-times higher than that of benign tissues ($p = 0.015$). Metastasis tissues were exhibited 1.5-fold greater GSTP1 expression than that of benign tissues. There was no significant difference in GSTM1 expressions between malignant and metastatic tissues ($p = 0.700$). The highest p53 expression was noted in the malignant group followed by the metastasis group. 64.71% of malign tissues displayed positive p53 expression, while 50.98% of metastasis ones. None of the samples had positive p53 expression in the benign group. Both malignant and metastasis tissues exhibited statistically significantly higher p53 expression than benign tissues ($p < 0.001$). Malignant and metastasis tissues exhibited similar p53 expression patterns. No significant difference in p53 expressions was found between the malignant and the metastasis groups ($p = 0.941$).

The point biserial correlation analysis was performed. The relationships between the patients's age and the expression levels were examined. Hex binned scatter plots of expressions versus age were shown in Fig. 4. The increase in expressions of p53 was observed to be positively correlated with the age in a ratio of 32.70% ($p = 0.001$). No significant correlation was observed between age and GSTM1 and GSTP1 expressions ($p = 0.422$ and $p = 0.427$, respectively).

DISCUSSION

It is now known that genes and proteins produced by the cell for different functions are also involved in the mechanism of this disease, rather than studies on target genes and proteins at the molecular level, in studies aimed at elucidating the mechanisms of cancer. Genes and proteins and their metabolism, which are not directly involved in cancer formation such as detoxification and drug metabolism, and intracellular immunity, but cause cancer as a result of structural deterioration, are also shown among the causes of cancer formation. Detoxification mechanisms are of great importance in protecting cells from carcinogenic effects. Detoxification (biotransformation) is the mechanisms of making harmful substances such as xenobiotics (toxic substances, metabolites, epoxides) harmless

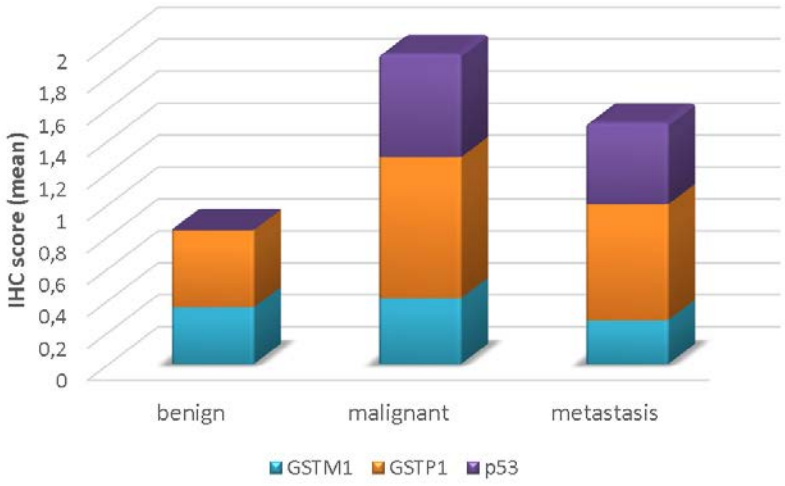

Fig. 3. Immunohistochemical staining scores of subjects in benign, malignant and metastasis groups. IHC = Immunohistochemical.

with the help of various enzymes or molecules and excreting them out of the body. Enzymes or molecules involved in these mechanisms also support this vital phenomenon.The GST enzyme family constitutes an enzyme system that creates Phase II reactions in detoxification metabolism. At the same time, GST enzymes have crucial roles in drug metabolism, elimination of intracellular oxidative damage, provide the detoxification of reactive intermediates and protect the cells from harmful effects such as cancer, necrosis, tissue and DNA damage. In light of this information, the roles of the members of this family have been explained in the literature in many studies such as normal intracellular antioxidant activity in cancer formation, drug resistance in drug metabolism, and detoxification metabolism. On the other hand, studies on the roles of GSTM1 and GSTP1 isozymes in cancer formation are very limited [8, 14, 15].

Similar to the results of our study, Marks *et al*.[16] found high levels of nuclear p53 protein expression in the malignant epithelium in 54 (50%) of 107 epithelial ovarian cancers in their study. Green *et al*.[17] reported that GSTP1 could not make a difference in 109 ovarian cancers (86 cancer and 23 normal) in normal and malignant tissues, but stained more intensely in the malignant epithelium. In patients resistant to chemotherapy, GSTP1 stained at higher intensity. GSTA1 and GSTM1 did not make a difference in malignant and benign cases, but stained with higher intensity in malignant [17].

In a study conducted in the southeast of England in 2001, GSTM1 mutation and null allele frequency were investigated in 293 ovarian cancer patients and 219 control group. The "null" allele frequency in the patient group (59%) compared to the "null" allele frequency in the control group (48.9%) and it was found to be significantly increased (p=0.025). With these results, it was observed that the GSTM1 “null” allele was not associated with endometriosis. Despite this, it has been reported to be a factor in endometriotic malignant transformation and clear-cell ovarian cancer [18].

In another study, similar to our study, while trying to determine the "null" allele frequency in the GSTM and GSTT gene regions with samples taken from 81 individuals with invasive ovarian tumors, it was determined whether p53 protein expression accompanies the null allele frequency in phase II enzymes in this patient group. The situation where it did not work was compared with the data obtained after the immunohistochemical method. A significant relationship could only be established in the group of patients who received chemotherapy with the group of patients with GSTM/GSTT “null” allele frequency ($p = 0.007$). No relationship could be established between p53 and all other parameters and conditions such as survival [19]. The relationship between polymorphisms in the GSTT1, GSTM1, and GSTP1 gene regions in the formation and course of the disease was investigated in 132 patients with epithelial ovarian cancer and 132 control. Considering the polymorphic situation in these gene regions and compared to the control group, the risk of encountering epithelial ovarian cancer was found 1.8-fold, 2.38-fold, and 11.28-fold higher in GSTP1 Ile/Ile, GSTM1 null plus GSTP1 Ile/Ile, and GSTM1 null plus GSTT1 null plus GSTP1 Ile/Ile than control [20].

CONCLUSION

The higher expressions of GSTP1 and p53 in malignant and metastasis tissues than benign ones indicate that these expressions could be important biomarkers in ovarian cancer development and progression. However, our study have some limitations. The tissues used in this study belong to patients who have not received chemotherapy and clinical data of the patients are not sufficient. Since GSTs are involved in drug metabolism, their expression in patients with ovarian tumor receiving chemotherapy needs to be investigated. Therefore, there is a need for new studies in which a larger number of patients and clinical data will be evaluated together in order to confirm our results.

Authors' Contribution

Study Conception: GÖ,SO; Study Design: GÖ,PK,SYS,SO; Supervision: SO; Funding: SO; Materials: GÖ,PK,SYS,SO; Data Collection and/or Processing: PK,OD,SYS,GGŞ,SO; Statistical Analysis and/or Data Interpretation: SYS,OD,SO; Literature Review: OD,MK; Manuscript Preparation: OD and Critical Review: AE,TÇ.

Conflict of interest

The authors disclosed no conflict of interest during

the preparation or publication of this manuscript.

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