ORIGINAL RESEARCH

Expression of P53 In Ovarian Tumors' And Its Correlation To The Morphological Differentiation: Report From A Tertiary Care Hospital Of Bihar

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Abstract: Ovarian tumors represent 3% of female malignancies, with over 140,000 worldwide annual associated deaths.Epithelial tumors constitute over 90% of all the ovarian cancers.This study aims to evaluate the expression of p53 by immunohistochemistry (IHC) in the different histological types and grades of epithelial ovarian tumors (EOT) and attempts to compare it with various Clinicopathological prognostic factors, namely, age, clinical presentation, stage, gross morphology, histopathology, grade, serum CA-125, etc.**Materials and Methods:** EOT specimens from 40 patients received in the Department of Pathology, Indira Gandhi Institute of Medical Science, Patna, Bihar, India from January 2021 to December 2021 were studied. Ethics committee permission was obtained, and ethical practices were followed. All specimens obtained were subjected to detailed gross and histopathological examinations.**Results:** Among the 40 cases of EOT, 24 (60%) were benign, 6 (15%) were borderline, and rest 10 (25%) were malignant. Serous malignancies were the largest group with followed by mucinous and clear cell carcinomas.**Conclusion:** Understanding of p53 staining patterns is mandatory to use it along with a panel of other antibodies for the correct classification and further research of morphologically confusing EOT.

Key Words: P53 in Ovarian Tumors, Morphological Differentiation.

Introduction

Ovarian tumors represent 3% of female malignancies, with over 140,000 worldwide annual associated deaths.[1, 2] Epithelial tumors constitute over 90% of all the ovarian cancers. [3] The incidence is either steady or slowly increasing in the western nations and rapidly increasing in the Asian subcontinent. [4]

Ovarian tumors are a heterogeneous group of tumors with multiple, poorly understood etiopathogenesis and dismal prognosis which is partly due to the lacunae in the understanding of their pathogenesis. [5, 6] Screening for ovarian cancer has been based on strategies using serum tumor markers or ultrasound imaging of the ovaries. However, serum CA125 is elevated in only about 50% of patients with clinically detectable early-stage ovarian carcinomas. [7] Insight into their pathogenesis requires an understanding of the genetic mutations, tumor

suppressor/oncogenes, and cell cycle regulators of ovarian cancers to develop new technologies to identify other biomarkers that can be used for early detection.

TP53 is the most frequently altered gene in human cancers and loss of functional p53 protein occurs in most epithelial ovarian cancers.[8] Association between p53 IHC positivity and histological subtype in the literature has been controversial. Hence, the need to study p53 IHC in an Indian cohort considering all the technical factors that could potentially affect the staining (the antibody clone, IHC technique, interpretation of staining, etc.).

This study aims to evaluate the expression of p53 by immunohistochemistry (IHC) in the different histological types and grades of epithelial ovarian tumors (EOT) and attempts to compare it with various Clinicopathological prognostic factors, namely, age, clinical presentation, stage, gross morphology, histopathology, grade, serum CA-125, etc.

Materials and Methods

EOT specimens from 40 patients received in the Department of Pathology, Indira Gandhi Institute of Medical Science, Patna, Bihar, India from January 2021 to December 2021 were studied. Ethics committee permission was obtained, and ethical practices were followed. All specimens obtained were subjected to detailed gross and histopathological examinations. Relevant clinical details were collected by reviewing the medical records using a structured pro forma. After examining the hematoxylin and eosin stained slides, the tumors were classified according to WHO 2014 classification. IHC was performed according to heat-induced epitope retrieval method and IHC of the marker p53 (DAKO- DO 7, ready to use mouse monoclonal antibody against p53-wild and mutant staining) was done in all cases. Bloom Richardson grade-II breast carcinoma and colon carcinoma were used as positive controls as recommended in the product datasheet of DAKO DO-7 mouse monoclonal antibody against p53 in the initial run. In subsequent runs, cases that were found to be positive were also kept as positive controls. Negative controls (sections in which p53 antibody omitted and IHC done) were also kept.

P53 expression of all tumors was studied. Clinical and histomorphological parameters like age, laterality of tumor, ascites, capsule rupture, tumor size, stage at presentation, metastasis, histological differentiation of tumor, tumor grade were studied. SerumCA-125 levels and clinical details were collected from the case records. Ovarian tumors treated with neo-adjuvant chemotherapy or radiotherapy was excluded as it could potentially interfere with IHC staining.

The proportion of tumors showing p53 positivity is expressed as percentage. Statistical analysis is done using SPSS version 16. Chi-square test and Fisher's exact test were used to find if there is any statistically significant association between p53 expression and the parameters studied. The kept *P* value for level of significance is $P < 0.05$

Results

Among the 40 cases of EOT, 24 (60%) were benign, 6 (15%) were borderline, and rest 10 (25%) were malignant. Serous malignancies were the largest group with followed by mucinous and clear cell carcinomas[Figure 1]. Proportions of malignancies increased with the age of the patients. In the younger age group (those less than 50 years), the proportions of malignant tumors were less as compared to the patients above 50 years of age.

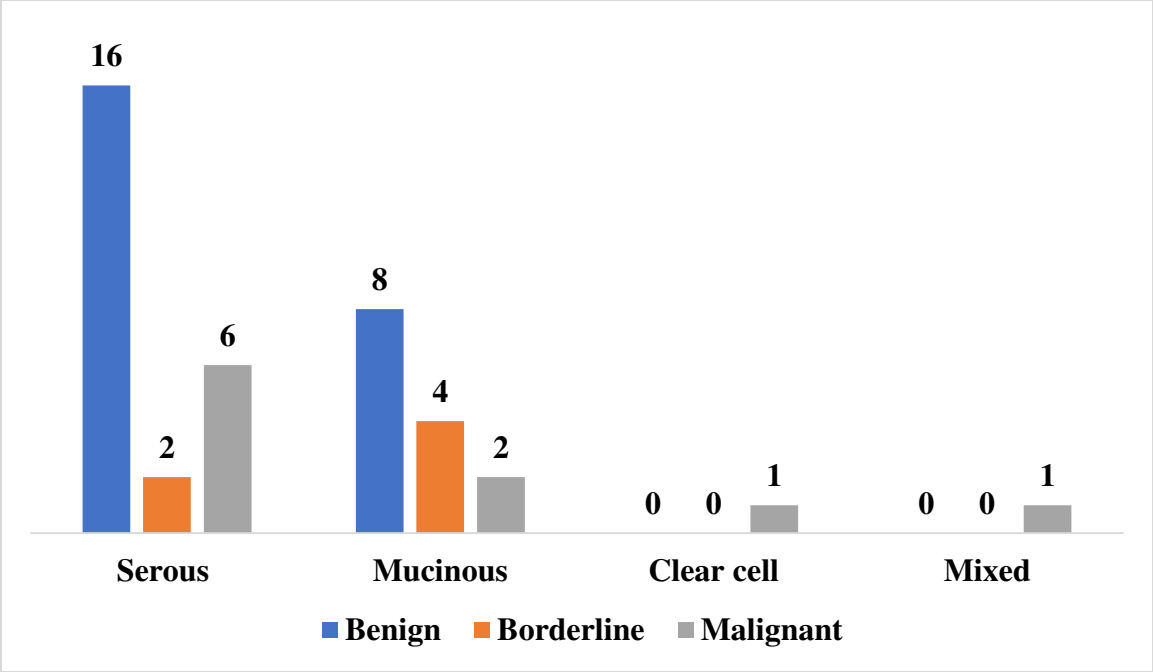

Figure 1: Distribution of tumors based on the relative proportion of benign, borderline, and malignant tumors among the various histological types

All benign and borderline EOT were p53 negative. Among the 10 malignant tumors, 6 (60%) were p53 positive and all of them were serous malignancies. That implies all the serous carcinomas were p53 positive while all the mucinous and clear cell carcinomas were p53 negative. Out of 6 p53 positive cases, 4 (66.7%) were High Grade Serous Carcinoma while rest 2 were Low Grade Serous Carcinoma. Out of 6 p53 positive cases, 1 was a case of stage 1 tumor, another was stage 2 tumor and rest 4 were in stage 3 and above. This result was statistically significant by Chi-square test (*P* value < 0.05). Hence, p53 positivity increased with higher stage. Among the 6 cases with p53 positive serous tumors, 4 cases (66.7%) had capsule rupture, while rest 2 caseshad an intact capsule. This was found to be statistically significant (p value < 0.05). P53 was found to be positive in 2 out of the 3 tumors with bilateral ovarian masses. Among all tumors with ascites, 50% were p53 positive and 50% were p53 negative. Fifty percentages of the tumors with ascites were p53 positive. All the p53 positive cases had ascites. The association of p53 positivity with ascites and bilateral ovarian masses were found to be statistically significant. The mean serum CA 125 was 427.9 u/ml for p53 positive cases with aberrantdiffuse staining, 1731.2 u/ml for p53 positive with null staining, and 49 u/ml for p53 negative tumors. This was found to be statistically significant. 93.5% of the tumors with metastasis were p53 positive. This was also found to be statistically significant.

Discussion

Ovarian tumors are a heterogeneous group of tumors with poorly defined etiopathogenesis. p53 mutation is one of the most frequent mutations among ovarian tumors. Recent WHO grouping of ovarian tumors into type 1 and type 2 based on studies done by Kurman *et al*. highlights the importance of p53 mutations in ovarian tumors.[9, 10] Earlier studies had proved that immune-histochemical staining patterns of p53 (>60%, <5%) can serve as a surrogate marker for TP53 mutations in ovarian carcinoma. [11, 12] The more recent concept of classification of ovarian

carcinomas into 5 main histological types is: high-grade serous carcinoma (HGSC), clear-cell carcinoma, endometrioid carcinoma, mucinous carcinoma, and low-grade serous carcinoma (LGSC), which differs with respect to their biology, clinical presentation, and response to chemotherapy. [13, 14] Hence, IHC can be used as a robust adjunct tool for the sub-classification of ovarian carcinomas.

Among the 10 malignant tumors, 6 (60%) were p53 positive. All benign and borderline EOT were p53 negative. This is in concordance with the previous studies which showed mutation or inactivation of p53 in average 50% (range 13.7–82%) of invasive ovarian tumors, rarely in borderline tumors and virtually nonexistent in benign tumors or normal ovarian epithelium. [15-17] In the present study, all the p53 positive tumors were serous malignancies. Malignant mucinous tumors and clear cell tumors were p53 negative. These results correlated well with older studies. [17] Conversely, p53 positivity was seen in 100% of serous carcinomas. The results were comparable to studies by Havrilesky *et al*., Leitao *et al*., and Chiesa *et al*. but relatively lower positivity rates were seen in Lassus *et al*. and Sylvia *et al*. [17-21] This may be due to (1) small sample size, (2) the method of counting p53 positivity, and (3) the inter-observer variability in interpretation of slides.

When serous carcinomas are graded by the two-tier system, 66.7% were high-grade serous carcinomas (HGSC), while rest were low-grade serous carcinoma. It was also observed that 4 out of 4 HGSC were p53 positive. There was only one LGSC in the study and it was also p53 negative, which reflects the current IHC expression profile by WHO. Studies by Chiesa-Vottero *et al*. in 2007 and Bilyk *et al*. in 2011 indicate p53 positivity in 80% of serous ovarian carcinomas and protein expression differences depending on the degree of differentiation, high-grade tumors being diffusely p53 positive. [21, 22] Even though the pathogenic ways in the ovarian carcinogenesis are thought to be independent for type 1 (low grade) and type 2 (high grade) tumors, studies have proven the occurrence of high-grade serous ovarian carcinomas from low-grade lesions.[23-25] There are also reports of borderline synchronous tumors or recurrent borderline tumors recurring as high-grade carcinomas. [23]

Among the p53 positive serous tumors, 4 had capsule rupture, while 4rest 2 had an intact capsule. Studies correlating the hypothesis of serous tubal *in situ* carcinoma with capsule rupture in HGSC show p53 positivity. [24, 25] P53 was found to be positive in majority of cases with bilateral ovarian masses. This association was statistically significant and is comparable to studies done by Skirnisdottir *et al*.[26] All the p53 positive cases had ascites. Study by Sylvia *et al*. showed 84.21% positivity in tumors with ascites. [17]

The mean CA 125 level was significantly higher for positive staining as compared to normal values for benign tumors. This was in concordance with the results obtained by Sylvia *et al*.[17] and Angelopoulou *et al* [27] Study by Hafner *et al*. reports that p53 could be more sensitive than CA 125 in detecting residual disease. [28] P53 positivity was associated with higher grade and stage. Higher stage and advanced disease have been found to be associated with p53 positivity by Sylvia *et al*. [17] This is in contrary to a study by Kadkhodayan *et al*. in 2004 which reported no correlation between p53 and tumor type, grade, and stage. [29]

Conclusion

P53 IHC is a surrogate marker for p53 gene mutation in clinical practice which is seen in serous EOT, the expression being higher in high-grade serous carcinomas and in the advanced stage. It helps to differentiate between borderline and malignant tumors and high-grade from low-grade serous carcinomas. It can also assist in differentiating the endometrioid carcinomas from the

serous types. Understanding of p53 staining patterns is mandatory to use it along with a panel of other antibodies for the correct classification and further research of morphologically confusing EOT.

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