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Original Research Article

A Clinicopathological Study and Immunohistochemical Expression of p53 in Ovarian Tumors

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Abstract

Background: Ovarian tumours are heterogenous neoplasms with a varied clinical, morphological and histological features. p53 overexpression has been associated with poor prognosis, poor overall survival and altered sensitivity to chemotherapy in patients with ovarian cancer.

Aim and Objectives: To study the frequency, age distribution and the diverse histomorphological spectrum of ovarian tumors and to evaluate the frequency of immunohistochemical expression of p53 in different types of ovarian tumors.

Materials and Methods: This is a retrospective study of 100 ovarian tumours diagnosed in the Department of Pathology, Kilpauk Medical college, Chennai, Tamilnadu, India. After gross examination of specimens, representative bits were taken, routinely processed and stained with H and E.Tumors were classified as per WHO classification. p53 expression was assessed immunohistochemically.

Results: There were 14 malignant cases among 100 ovarian tumours of which commonest was Surface Epithelial tumours. p53 was found to be overexpressed in 85.7% of malignant tumours.

Keywords: Ovarian Tumours; p53; Immunohistochemistry.

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Introduction

Ovary is one of the common sites for neoplasm in female genital tract. Ovarian cancer is fifth most common cause of the death from gynaecological malignancy [1]. Ovarian tumours account for about 30% of female genital tract neoplasms and is the fifth leading cause of death among cancer death in female [2]. Among all the ovarian tumours about 80% are benign, and remaining 20% of these tumours are malignant [3]. In most of the population based cancer registries in India, ovarian cancer is the third leading site of cancer among women, behind cervix and breast cancer [2,4]. p53 genome maps to chromosome 17 p13.1, which regulates the expression of various genes involved in apoptosis, growth arrest, inhibition of cell cycle progression, cell differentiation and DNA repair or senescence in response to genotoxic or cellular stress. Inactivation of p53 by direct mutation of the gene is one of the most frequent genetic lesions in human tumours [5]. In Ovarian Carcinoma, p53 is altered in 30–70% of cases.

Molecular and genetic studies have further confirmed the relevance of p53 in the development, progression of Ovarian Carcinoma. p53 is one of the most studied genes in relation to prognosis and prediction of response to adjuvant chemotherapy of variant cancer [6,7,8]. This study is undertaken in order to evaluate the incidence of ovarian neoplasms in our institution with reference to age, histopathological and immunohistochemical features.

Aim and Objectives

1. To study the various clinico pathological factors of ovarian neoplasm including age of incidence, location of tumour, clinical features, gross appearance and histologic grade.
2. To determine the immunohistochemical expression of p53 in ovarian neoplasm.
3. To study the association of expression of p53 in ovarian neoplasm with known prognostic factors like age, histological grade of tumors and other variables like gross appearance and type of specimen received.
4. To study the prognostic significance of expression of p53 in ovarian neoplasm and its association with survival.

Materials and Methods

This is a retrospective study conducted in the Department of Pathology, Government Kilpauk Medical College, Chennai. A total of 100 resected specimens (Cystectomy, Oophorectomy, Total Abdominal Hysterectomy with Bilateral Salpingo-Oophorectomy) from the Department of Obstetrics and Gynaecology, Govt. Kilpauk Medical college Hospital, which were received in Department of Pathology, Govt. Kilpauk Medical College and reported as Benign, Borderline, Malignant neoplasms were included for the study. This study was done over two years in histologically proven tumours and non-neoplastic lesions were not studied.

Data was collected from the registers and case records in a standard format. Retrospectively patients' tissue blocks were analysed by immunohistochemical study for the expression of p53 and graded appropriately. Age, clinical features, duration, gross appearance, histopathological diagnosis and grade of tumour were correlated with p53 expression.

Immuno Histochemical Evaluation

Immuno histochemical analysis was done in paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology. 4 micron thick sections from formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with rabbit polyclonal antibody p53 AIP1 proteins and then detected by the addition of secondary antibody conjugated with horse radish peroxidase polymer and diamino benzidine substrate.

The immunohistochemically stained slides were analysed for the presence of reaction, cellular localization, percentage of cells stained and intensity of nuclear staining for p53 positivity.

Results

In this study ovarian neoplasms were in the age group ranging from 15 to 71 years. The highest incidence of ovarian neoplasms is seen in the age group between 31-40 years (30% of patients). There were 14 patients with malignant ovarian tumours and of them 7 patients were in their 4th decade.

Most of the cases presented with multiple clinical features. Abdominal pain was the most common clinical presentation (46. 1%). A majority of tumours presented as cystic masses. (40%). (Table 1).

Table 1: Gross Appearance of the Ovarian Tumours in the Study

Gross Appearance	No. of cases	Percentage
Pure Solid	10	10.0
Pure cyst	40	40.0
Mixed solid with cyst	12	12.0
Solid with variegated appearance	4	4.0
Cyst with papillary excrescences	8	8.0
Cyst with hair, sebaceous material	26	26.0
Total	100	100.0

Of the total 100 neoplasms, surface epithelial tumours predominates with 65 cases (65%), followed by germ cell tumours with 27 cases (27%) and sex cord – stromal tumours 6 cases (6%) and metastasis 2 cases (2%). (Table 2).

Table 2: Classification of Ovarian Tumors in the Study Population

Types	No. of cases	Percentage
Surface epithelium	65	65.0
Sex-cord	6	6.0
Germ cell	27	27.0
Metastasis	2	2.0
Total	100	100.0

Among 80 Benign neoplasms, Surface Epithelial Tumours constitute highest number of cases (62. 5%) (Table 3).

Table 3: Different Morphological Subtype of Benign Ovarian Neoplasm

Types	No.of cases	Percentage
Serous Cystadenoma	21	26.25
Serous Cystadenofibroma	4	5
Mucinous Cystadenoma	24	30
Brenner Tumor	1	1.25
Fibroma	1	1.25
Fibroma-Thecoma	3	3.75
Mature Cystic Teratoma	26	32.5
Total	80	100

Malignant serous tumours are graded according to recent 2-tier system of classification. Table 5 shows that, 2/5 cases were low grade carcinomas and 3/5 cases were high grade. High grade serous carcinomas are common among all serous carcinomas. All 4 cases of mucinous carcinomas showed expansile type of invasion. Sex-cord stromal tumours-In this granulosa cell tumour were 2/14 (14. 28%), Germ cell tumours-In this Mixed germ cell tumour 1/14 (7. 14%) and Krukenberg tumour 2/14 (14.28%).

Regarding p53 expression, 15 cases of 100 were positive, of which 12 were malignant and 3 were borderline. Two malignant cases were negative for p53 expression. Highest percentage of p53 over expression was seen among patient with Serous cystadencarcinoma (5/5 cases), among which high grade (3 cases), and low grade (2 cases) followed by Mucinous cystadenocarcinoma (4/4 cases), Krukenberg tumor (2/2 cases) and Mixed germ cell tumor (1/1case).

Table 4: Different Morphological Subtype of Malignant Ovarian Neoplasm in Study Population

Types	No.of cases	Percentage
Serous CystadenoCarcinoma	5	35.71
A) Low Grade	2	
B) High Grade	3	
Mucinous CystadenoCarcinoma	4	28.57
Types Of Invasion-A)Expansile	4	
B) Infiltrating	0	
Granulosa Cell Tumor	2	14.28
Mixed Germ Cell Tumor	1	7.14
Krukenberg Tumor	2	14.28
Total	14	100

Table 5: p53 Expression with BorderlineOvarian Neoplasm in Study Population

Types	Positive	Negative	No.of cases
Borderline Serous Cystadenoma	2	1	3
Borderline Mucinous Cystadenoma	1	2	3
Total	3	3	6

Table 6: p53 Expression with Malignant Ovarian Neoplasm

Types	Positive	Negative	No.of Cases
Serous cystadenocarcinoma	5	0	5
a)low grade	2	0	2
b)high grade	3	0	3
Mucinous cystadeno Carcinoma	4	0	4
Granulosa cell tumor	0	2	2
Germ cell tumor	1	0	1
Krukenberg tumor	2	0	2
Total	12	2	14

Highest percentage of p53 over expression was seen among patients with Malignant ovarian neoplasm (12/14 cases, 85.70%), followed by Borderline Ovarian Neoplasm (3/6 cases, 50%),

Benign ovarian neoplasm (0/80 cases, 0%) and the association was statistically significant p=0.0005 (p <0.05).

Table 8: p53 Expression with Ovarian Neoplasms

Types	Negative	Positive
Benign	80	0
Borderline	3	3
Malignant	2	12
	p53 Expression	
	Negative	Positive
Benign	100%	0%
Borderline	50%	50%
Malignant	14.30%	85.70%

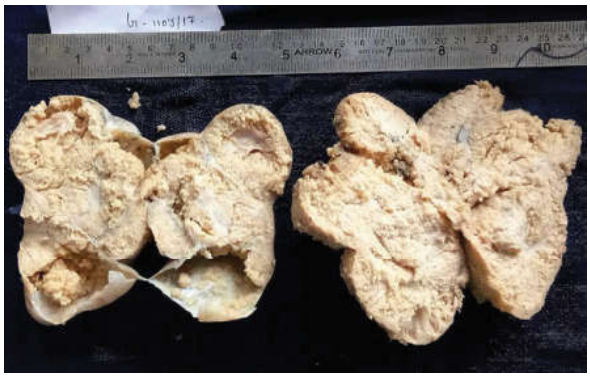

Fig. 1: Bilateral Papillary Serous Cystadenocarcinoma with Solid and Cystic Areas

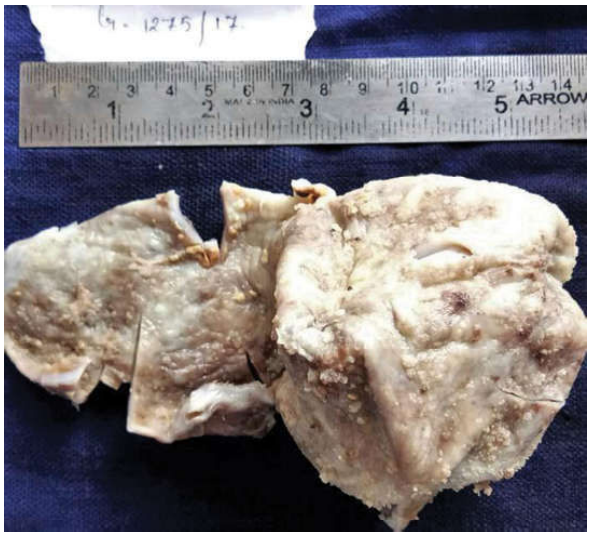

Fig. 2: Bilateral Borderline Serous Cystadenoma. Cut Surface- Multiloculated Cysts with Numerous Tiny Papillary Excrescences

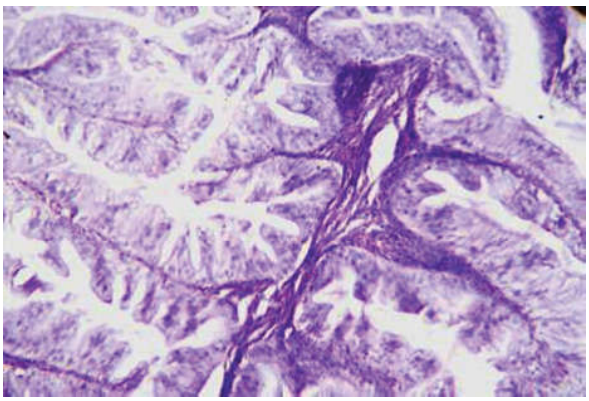

Fig. 3: Mucinous Carcinoma Showing Expansile Invasion – Closely Packed (Back to back) Glands with Little/No Stromal Support

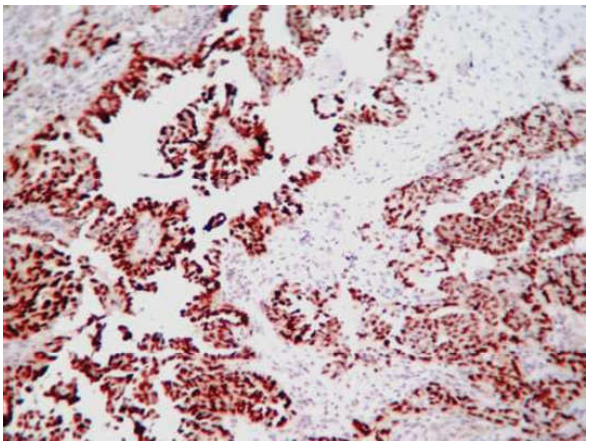

Fig. 4: High Grade Serous Carcinoma -Diffuse p53 Nuclear Staining

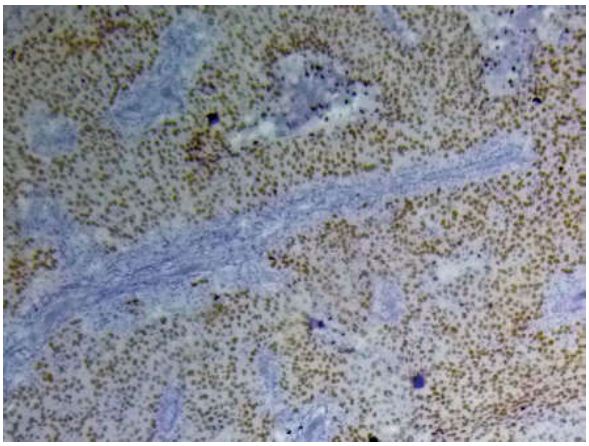

Fig. 5: Mixed Germ Cell Tumour-Diffuse p53 Nuclear Staining

Discussion

Two thirds of ovarian tumours occur in women of reproductive age Group [9]. Majority of

malignant neoplasms occurred in age group 31-40 yrs (7/14 cases, 50%). In this study most of the patients with ovarian tumors were in the 2nd to 5th decade which is similar to the Shreya Hegde. Dravid N. V et al. 2014 who studied 145 cases out of which most common lesion was surface epithelial tumors (serous cystadenoma) followed by germ cell tumors (mature cystic teratoma) [10]. In this study also total benign cases were 80%, among which commonest benign tumor was Surface Epithelial Tumors with 50 cases (62. 5%), followed by Germ Cell Tumors with 26 cases (32.5%) and Sex cord – Stromal tumors (4 cases 5%). Deeba et al. 2015 who reported Serous Cystadenocarcinoma (57.1%) was common, followed by Mucinous Cystadeno carcinoma (17. 9%) [11].

According to Glen mc Cugage et al. & other studies, it is well established that there are two distinct types of serous carcinoma, namely low grade and high grade, low grade being much less common and arising from a serous borderline tumour [12]. In this study there are about 5 cases of serous carcinoma, of which 3 cases (60%) were high grade and 2 cases (40%) were of low grade was observed. Ovarian serous carcinomas are classified into low grade and high grade according to recent 2-tier grading system, which is based primarily on the assessment of nuclear atypia with mitotic rate as a secondary feature. Recent 2 tier grading system is based on the evidence that low and high grade tumors arise from different genetic pathways [13].

According to Masaki Mandai et al., Krukenberg tumor is the most common form of ovarian metastatic carcinoma, often found in the 4th decade [14]. It is applied to a clinicopathological entity characterised by presence of mucin filled signet ring tumor cells within cellular stroma. In this study, 2 cases of Krukenberg tumor was observed.

Serous Borderline Ovarian tumors are the most common histological subtype of borderline tumors accounting for 65% of all borderline ovarian tumours [15]. In contrast, serous and mucinous borderline tumors were same percentage in this study. According to Neeraj lalwani et al., approximately 1/3rd of serous borderline tumors are unilateral [16]. In this study also 1/3rd (1case, 33%) were unilateral. About 20-30% of serous borderline ovarian tumors are associated with peritoneal implants. In contrast, in this study none of the serous borderline tumours had associated peritoneal implants at the time of presentation.

In this study, p53 immunohistochemical staining of 100 cases of ovarian neoplasm (beingn, borderline, malignant) was done after classifying serous carcinoma as low grade and high grade based on recent 2 tier grading system. Of the 2 low grade serous tumors and 3 high grade serous tumors, all of them showed positivity for p53. Of the 4 cases of mucinous carcinoma, all showed positive for p53. The 2 Krukenberg tumors were also positive for p53. The single case of mixed germ cell tumor also showed positivity for p53. The reported 2 cases of sex-cord stromal tumors (granulosa cell tumor) was negative for p53. Total borderline tumor were 6 among which 3 were positive and 3 were negative for p53 expression. All the 80 benign neoplasm was negative for p53 expression, This finding in our study is well correlated with the studies conducted by Russell Vang et al. and Gad Singer et al., Monisha chowdary's study [17,18,19]. In this study p value of <0.05 was calculated which is highly significant association obtained between benign, borderline, malignant types of ovarian tumor and Eltabbakh GH studied show similar result [20].

There was no significant correlation between p53 overexpression and age of the patients. This is consistent with the results of other studies with the exception of a single one with a reported significant correlation [21,22,23]. p53 deficiency alone is not sufficient for ovarian epithelial tumorigenesis [24]. Other genetic lesions are likely to be required to develop ovarian cancer [24]. However, the leading role of alterations in p53 gene in the development of ovarian carcinoma was further substantiated by results of a study, which revealed a striking association between the number of life time ovulatory cycles and overexpression of mutant p53 protein in ovarian carcinoma tissue [25]. There was no significant correlation between p53 expression and histological type of tumours.

The degree of p53 expression increased with the increasing grade of ovarian tumors, as seen by the presence of strong p53 expression in grade III [15]. Apparently, cancers with p53 mutation demonstrated a trend toward more aggressive tumor behavior such as distant metastasis and poor cellular differentiation [26]. Epithelial ovarian malignancies showing p53 aberrations are significantly less sensitive to platinum based chemotheraphy and more aggressive than those with functional p53 and hence overexpression of p53 is a poor prognostic factor [27].

Conclusion

Surface epithelial tumors are the most common neoplasm of which Serous Cystadenoma is the commonest. Detecting overexpression of p53 is a

poor prognostic factor found to be overexpressed in 85.70% of malignant tumors. This study is an institution based one and has small sample size of 100 cases. So the results obtained may or may not reflect the actual histological pattern and age distribution of ovarian cancer in Indian women. The epidemiological pattern of cancers in developing countries differs in many aspects from developed nations. The age specific incidence of ovarian cancer and its subtypes presented in this study will serve as a useful point of reference for future studies and would help to specify their trend in future and encompass the community health programs to solve health problems.

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