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Original Article

Pathology Section

Association of Epithelial Changes in Fallopian Tube and Epithelial Neoplasms of Ovary using p53 Tumour Marker- A Cross-sectional Study in a Tertiary Care Centre

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ABSTRACT

Introduction: Ovarian cancer is one of the major reasons of mortality in women due to gynaecological malignancies. The origin of ovarian carcinomas was considered to be de novo previously. But recent studies have shown that the type II ovarian carcinomas, majority constituting of High Grade Serous Carcinomas (HGSCs) originate from the fimbrial end of the fallopian tubes.

Aim: To establish an association of epithelial changes of fallopian tube with epithelial neoplasms of ovary in light of p53 expression.

Materials and Methods: An observational, descriptive, cross-sectional study was done in the Department of Pathology in a tertiary care centre of Eastern India for a period of 18 months from July 2019 to December 2020. Informed written consent was taken from all patients prior to the study. The epithelial ovarian neoplasms and fimbrial ends of the fallopian tubes using Sectioning and Extensively Examining the FIMbrial (SEE-FIM) end technique were submitted in 51 cases. The sections were stained with Haematoxylin and Eosin (H&E) and p53 tumour marker. The obtained results were tabulated and data analysis was done using Chi-square test and Fisher's-exact test with the help of statistical software International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 25.0. A p-value of <0.05 was considered statistically significant.

Results: On histopathological examination, 84.31% (43/51) cases were found to be serous tumours of the ovary, out of which 54.9% (28/51) cases showed histological features of HGSCs, 100% of these HGSCs were p53 positive. Thirteen cases (46.42%) of fimbrial ends of HGSCs showed nuclear atypia, pleomorphism and stratification and p53 positivity were categorised as Serous Tubal Intra Epithelial Carcinomas (STIC). Total 10 cases (10/28=35.71%) of fimbrial ends which showed p53 positive in minimum of 12 cells in the fimbrial ends were labelled as 'p53 signatures'. The remaining five cases did not show any significant finding.

Conclusion: Overall, the fimbrial end of the fallopian tube could be considered as the origin of these HGSCs and these lesions are precancerous in nature.

Keywords: Fimbria, Ovarian high grade serous carcinoma, Serous tubal intraepithelial carcinoma

INTRODUCTION

Ovarian cancer is one of the leading causes of death due to gynaecological malignancies. The World Health Organisation (WHO) Histological Classification classifies ovarian neoplasms depending on the most probable tissue of origin. Most of them arise ultimately from one of these three ovarian structures- Surface/fallopian tube epithelium and endometriosis; germ cells, which migrate to the ovary from the yolk sac; and stromal cells, which also involve the sex cords [1].

A new model of ovarian carcinogenesis based on clinicopathologic and molecular genetic data classifies surface epithelial tumours into two broad categories: Type I and Type II. They allude to tumourigenic pathways and are not histopathologic diagnostic terms [2]. Type I tumours include Low Grade Serous Carcinoma (LGSC), Endometrioid, Clear cell, Mucinous and Brenner carcinomas [3,4]. They evolve in a stepwise manner and the majority are associated with benign and borderline developmental stages. LGSC likely begins with Ovarian Epithelial Inclusions (OEI) forming Cortical Inclusion Cysts (CIC) which later leads to the serous cystadenoma progressing to serous borderline tumour finally leading to the formation of invasive carcinoma [5,6]. Genetically, they are characterised by several mutations such as Kirsten RAt Sarcoma virus gene (KRAS), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), ERBB2, Phosphatase and tensin homolog (PTEN), CTNNB1, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA), AT-Rich Interaction Domain 1A (ARID1A) and Protein phosphatase 2A, regulatory subunit A, alpha (PPP2R1A) [3,7]. KRAS and BRAF mutations being the most common are present in 65% of cases [8,9].

Type 2 tumours include HGSC, high grade endometrioid carcinomas, malignant mixed mesodermal tumours and undifferentiated carcinomas. They are characterised by a definite absence of KRAS and BRAF but very frequently contain Tumour protein 53 (Tp53) mutations and florid genetic instability [3]. TP53 mutations occur in upto 80-90% of cases and they also show a very high MIB-1 proliferation index of 50-75% [8,10]. In the past, they have been said to arise 'de novo'. Recent data, however, suggest that HGSC arise from intraepithelial carcinomas originating from the tubal fimbriae [11]. There are few cases of stage I HGSC that have been studied harbour TP53 mutations [12]. Therefore, it appears that conventional HGSCs even in its earliest stage of development resemble advanced stage serous carcinoma at both the morphologic and molecular level. Further TP53 mutations have been detected in STIC, and also in its recently described putative precursor lesion known as 'p53 signature' [13].

Just like the junctions between different types of epithelium (e.g., the gastroesophageal and anorectal junctions) are known to be hotspots for carcinogenesis, the presence of the Tubo-peritoneal junction in and around the fimbriae suggests that this junction could also be the source of serous carcinomas [14]. Tumour protein p53 (Tp53), also known as guardian of the genome is a tumour suppressor gene that regulates cell cycle progression, Deoxyribonucleic Acid (DNA) repair, cellular senescence and

apoptosis. It is the most frequently mutated gene in human cancers. With loss of p53 function, DNA damage goes unrepaired, driver mutations accumulate in oncogenes and other cancer genes, and the cell undergoes malignant transformation [15].

In this study, authors have evaluated the immunostaining of different ovarian epithelial neoplasms using p53 with special focus on the serous carcinomas. Authors have also compared the p53 immunopositivity of the respective fimbrial ends of the fallopian tube. Also compared the histological sections with the immunostaining of the fimbrial end to assign the precursors as STIC and 'p53 signatures'. This information can be further utilised in the treatment of the ovarian neoplasms. Therefore, the aim of the present study was to establish an association between epithelial changes of fallopian tube with the epithelial neoplasms of the ovary using p53.

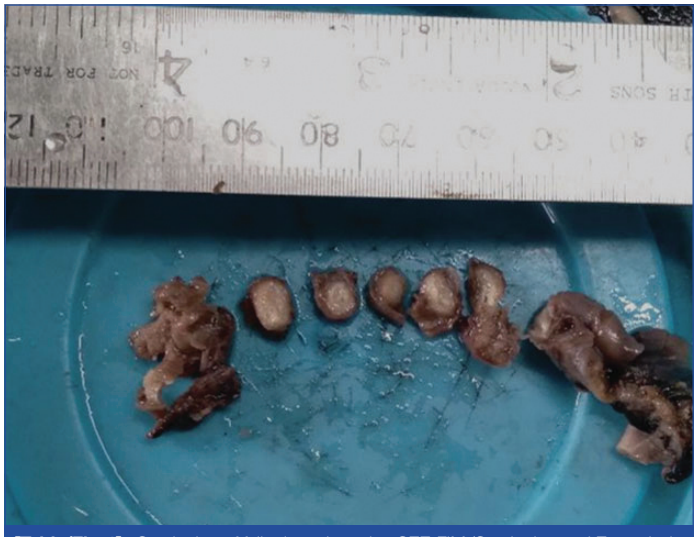
MATERIALS AND METHODS

The present study was an observational, descriptive, cross-sectional study done in the Department of Pathology in RG Kar Medical College and Hospital of Eastern India for a period of 18 months from July 2019 to December 2020. The study was approved by Institutional Ethical Committee (IEC No. ECR/322/Inst/WB/2013). Informed written consent was taken from all patients prior to the study. The total number of samples that were received in the Department of Pathology of the institution fulfilling the inclusion criteria during the study period constituted the sample size which was 51 cases.

Inclusion criteria: The inclusion criteria being the specimens of adnexal masses sent to the Department of Pathology, by unilateral or bilateral salphingo-oophorectomy with or without hysterectomy and histologically proven epithelial tumours of the ovary, with the attached fallopian tube.

Exclusion criteria: The specimens diagnosed microscopically as non epithelial tumours of the ovary were excluded from the study.

After receiving the specimens in the Department of Pathology, histopathological sectioning was done from the ovarian tumour and fallopian tube according to the Sectioning and Extensively Examining the FIMbrial (SEE-FIM) technique [Table/Fig-1]. The fimbriated end of the fallopian tube was amputated from the rest of the tube and serially sectioned at 2 mm intervals along the long axis. The entire length of the remaining tube was cut perpendicular to the long axis ("bread loafed") at 2 mm intervals [16].



[Table/Fig-1]: Sectioning of fallopian tube using SEE-FIM (Sectioning and Extensively Examining the FIMbrial end) technique.

The sections were submitted for histological examination. Paraffin blocks were prepared following routine histopathological techniques. Sections of 4 µ thickness were stained with H&E stains. Light microscopic examination was done and results were noted in the histopathological data record form. Immunohistochemical analysis was done for p53 expression in paraffin embedded sections. Ultrathin sections were obtained using microtomy from the formalin fixed paraffin embedded blocks. They were picked on poly-L-lysine coated slides, dried, deparaffinised and rehydrated in the descending grades of alcohol. Heat Induced Epitope Retrieval (HIER) procedure was performed by microwave method using TRIS Buffer. Endogenous peroxidise activity was blocked with PolyExcel Peroxidase Block, (PathnSitu). Incubation with primary antibody was done at 37°C for 60 minutes. (Primary antibody: p53-Mouse Monoclonal Antibody, PathnSitu, Lot No.- R06101NA). Serial incubation for 30 minutes each was carried out with PolyExcel Target Binder (PathnSitu); Poly HRP (PolyExcel HRP DAB Detection System PathnSitu) and chromogen (Polyexcel Stunn DAB Buffer and Polyexcel Stunn DAB Chromogen, PathnSitu). Lastly, the sections were counterstained with Harris Haematoxylin and mounted.

Cases of invasive breast carcinoma, known positive for p53 were taken as positive control and negative control was supplied by vendor. Brown coloured strong nuclear staining in atleast 80% of tumour cells was considered as positive with p53 expression [17].

The fimbrial ends of the fallopian tube were histologically corroborated with the immunohistochemical study for identifying STIC and 'p53 signature' lesion. An algorithmic approach by Vang R et al., was followed for this [18]. STIC lesions showed morphological stratification and associated nuclear pleomorphism and atypia with p53 positive. While the morphologically normal looking tubal epithelium with p53 immunoreactivity in atleast 12 secretory cells were designated as 'p53 signatures'.

STATISTICAL ANALYSIS

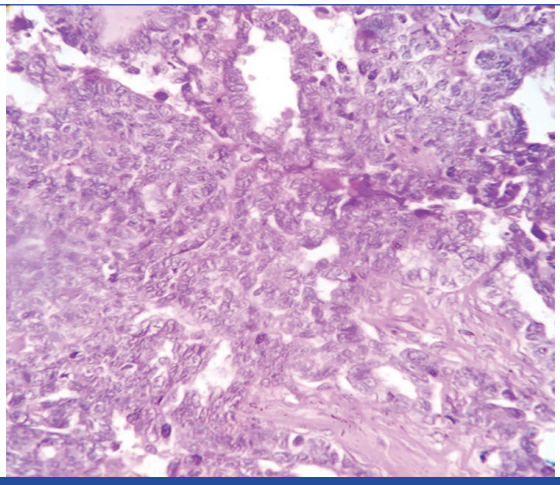
Results were compiled and data analysis was done. Chi-square tests and Fisher's-exact tests were performed, wherever applicable, for association of ordinal qualitative data using appropriate statistical software IBM SPSS 25.0. A p-value of <0.05 was considered statistically significant.

RESULTS

The number of cases in each histopathological type encountered in this study, is shown in [Table/Fig-2]. Out of 51 cases, 84.31% of the

Histopathological tumour	Frequency	%
Serous cystadenoma	5	9.8
Low Grade Serous Carcinoma (LGSC)	10	19.6
High Grade Serous Carcinoma (HGSC)	28	54.9
Mucinous cystadenoma	2	3.9
Mucinous borderline tumour	1	2.0
Mucinous carcinoma	2	3.9
Brenner tumour	3	5.9
Total	**51**	**100**

[Table/Fig-2]: Frequencies of histopathological types (n=51).



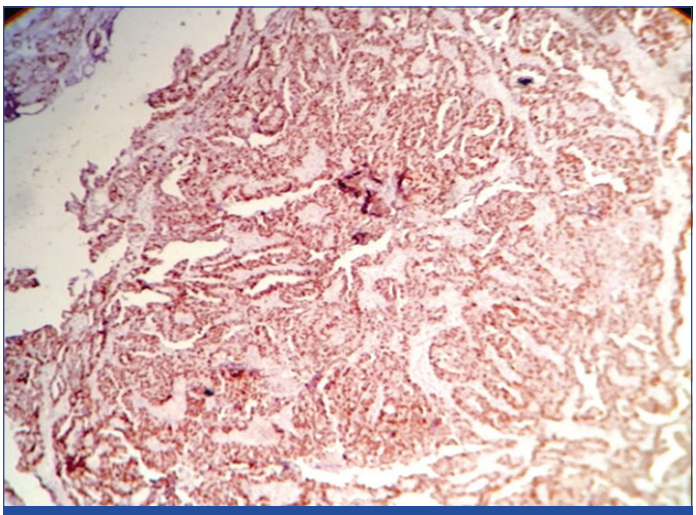
[Table/Fig-3]: Photomicrograph high grade serous carcinoma, ovary (H&E; X400).

cases (43/51) belonged to the serous tumours of the ovary while 54.9% (28/51) constituted HGSC [Table/Fig-2]. [Table/Fig-3] shows a photomicrograph of H&E stained ovarian HGSC.

A 69.76% cases (30/43) of serous tumours were positive for p53 and all the non serous tumours were p53 negative [Table/Fig-4]. [Table/Fig-5] shows a photomicrograph of HGSC p53 positive.

Tumours	p53 positive	p53 negative	% of positivity
Serous tumours	30	13	69.76
Non serous tumours	0	8	0

[Table/Fig-4]: Frequencies of p53 staining in the histological sections of the ovarian tumours (n=51).
p-value was <0.001 (by Fisher's-exact test) [significant]



[Table/Fig-5]: Photomicrograph High Grade Serous Carcinoma (HGSC) (p53, Mag: X40).

When the p53 positive serous tumours of the ovary were analysed, it was seen that 100% HGSC were p53 positive and only 20% LGSC were p53 positive [Table/Fig-6]. The fimbrial end was p53 positive in 80% of the p53 positive serous tumours [Table/Fig-7]. Remaining 20% cases had p53 negative fimbrial ends.

Serous carcinoma	p53 positive in ovary	p53 negative in ovary	% of positivity
LGSC	2	8	20
HGSC	28	0	100

[Table/Fig-6]: p53 status in the serous carcinomas of ovary (n=38).
p-value was <0.001[**1] (by Fisher's-exact test) (significant); LGSC: Low grade serous carcinoma; HGSC: High grade serous carcinoma

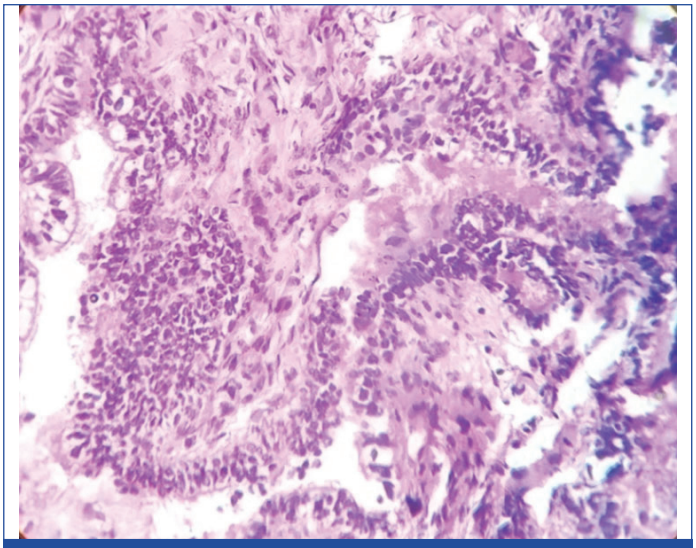
p53	Fimbrial end p53 positive	Fimbrial end p53 negative	% of positivity
p53 positive serous tumours	24	6	80
p53 negative serous tumours	0	13	0

[Table/Fig-7]: p53 immunopositivity of serous tumours and fimbrial end of the fallopian tube (n=43).
p-value was <0.001; (by Fisher's-exact test) [significant]

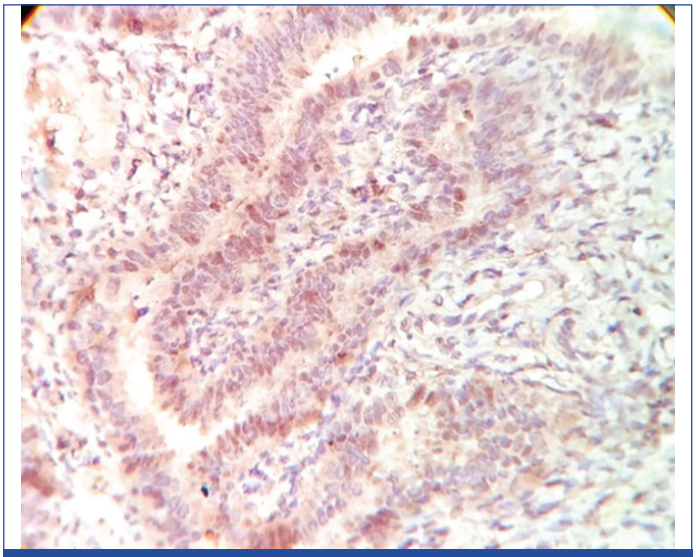
A 46.42% of the p53 positive HGSC had STIC [Table/Fig-8-10] while 35.71% exhibited p53 signature lesion in their corresponding fimbrial ends of the fallopian tubes [Table/Fig-11,12]. Both the lesions were found only in the fimbrial end of the fallopian tubes of the high grade serous ovarian tumours. They were absent in

Serous carcinoma	STIC present	STIC absent	% of cases with STIC
LGSC[1]	0	10	0
HGSC[2]	13	15	46.42

[Table/Fig-8]: Serous carcinomas and STIC (n=38).
p-value was 0.008 (by Fisher's-exact test), (Significant); STIC: Serous tubal intraepithelial lesion



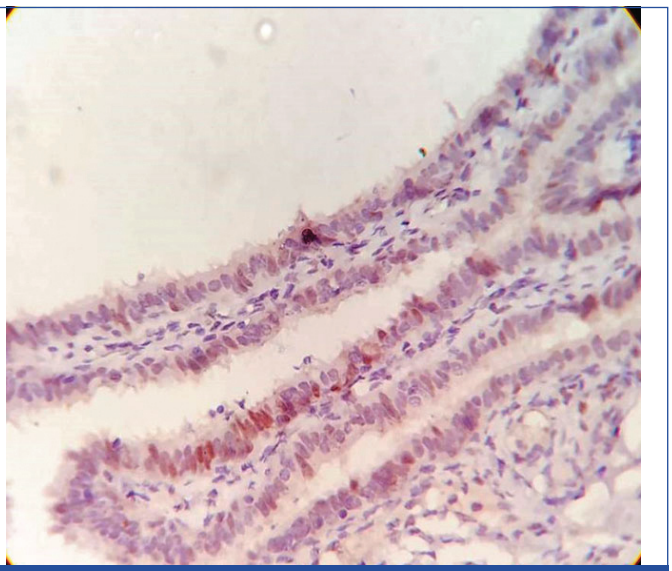
[Table/Fig-9]: Photomicrograph serous tubal intraepithelial carcinoma (H&E, Mag: X400).



[Table/Fig-10]: Photomicrograph serous tubal intraepithelial carcinoma (p53, Mag: X100).

Serous carcinoma	p53 Signature present	p53 Signature absent	% of positivity
LGSC	0	10	0
HGSC	10	18	35.71

[Table/Fig-11]: Serous carcinoma and p53 signatures (n=38).
p-value was 0.0281 (by Fisher's-exact test), (significant); LGSC: Low grade serous carcinoma; HGSC: High grade serous carcinoma



[Table/Fig-12]: Photomicrograph p53 signatures (p53, Mag: X400).

the low grade serous ovarian carcinoma as well as in the benign serous cystadenoma. These were not seen in the proximal end of the tubes.

DISCUSSION

The spectrum of epithelial neoplasms of the ovary is very wide. Clinical presentation is vague and non specific in most of the cases. Data related to clinical history and histopathological characteristics of these masses are limited in Indian scenario. Present study results have been compared to different published studies over different parameters. The above results were similar to the study by Hiroharu K and Michiyasu K, where they stated that p53 immunopositivity is diffuse and strong in HGSC [19]. In present study, 100% of the HGSC were p53 immunopositive. These findings were similar to the study conducted by Royal College of Obstetricians and Gynaecologists [20] where they also found that benign ovarian tumours do not stain positive for p53 immunostain. Tone A et al., stated that STICs and 'p53 signatures' were predominantly found in the fimbria, which was concordant to present study [21].

In the study by Vang R and Shih I, it was stated that the precursors of the ovarian HGSCs were not found in the random parts of the fallopian tube [22]. Present study findings were similar to the study conducted by Mittal N et al., which stated that the 'p53 signatures' were present in 65% cases of serous carcinomas of the ovary and were absent in the benign serous lesions and other tumours of the ovary [23]. Their study was highly significant as p-value was 0.003. However, they even found 2 out of 32 cases of serous carcinomas with serous intraepithelial lesion of the tube, but may be due to the small sample size, present study did not have any case with STILs. The study by Gao FF et al., reported that 92.2% of the STICs were found in ovarian HGSCs which was similar to the finding in present study [24]. Present study was disconcordant with the study conducted by Kindelberger D et al., where they found 93% of STICs involved the fimbriae 'p53 signatures' in the fimbrial end of 100% cases and in the proximal ends in 22% cases [25]. They also found 'p53 signatures' in the non neoplastic tubes, which suggested ovulation related oxidative stress. This non concordance could be due to restricted sample collection in this study.

The comment on the presence of Secretory Cell Outgrowth (SCOUTs) could not be made as BCL-2 was not done. Study by Chen EY et al., defined SCOUT as atleast 30 distinctive secretory epithelial cells with BCL-2 expression and without p53 expression [26].

Limitation(s)

The sample was small, difficulty in follow-up and minimal infrastructural facilities like BRCA mutation study were few limitations of this study. Study about SCOUT could not be done as BCL-2 was not done.

CONCLUSION(S)

Preventive strategies such as salpingectomies rather than therapeutics like immunotherapy can be considered, if the fimbrial end of the fallopian tube is proved to be the site of origin for serous carcinomas of the ovary. There have not been any known physiological benefits in sparing the fallopian tubes during hysterectomy for benign uterine indications. Also, the hormone profile of the patient remains unaltered after salpingectomy. Thus, whenever a hysterectomy needs to be performed for benign indications, simultaneous salpingectomies could be considered. p53 can also be used as targeted therapy in cases of ovarian HGSCs.

Long term prospective trials may be required which shall compare the occurence of adnexal serous carcinomas in women who underwent salpingectomy and the women who did not.

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- Was informed consent obtained from the subjects involved in the study? Yes
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