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Pathology

Study on P53 Expression in Association with Histopathological Grading of Ovarian Serous Carcinoma

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Abstract **Original Research Article**

Background*:** Ovarian cancer is a disease of significant morbidity and mortality. It is the most common cancer among women worldwide. Early and accurate detection and grading of ovarian serous carcinoma is of utmost significance for prolongation of patient survival. Panels of immunomarkers have been tested to overcome limitation of histopathology. Immunomarker p53 is commonly used among them. This study was undertaken to evaluate the significance of p53 immunomarker in ovarian serous carcinoma. ***Objective*:** Objective of this study to see p53 expression and its association with different histopathological grading of ovarian serous carcinoma. ***Method: This cross-sectional study was conducted in the Department of Pathology, Rajshahi Medical College, over a period of two years from July 2017 to June 2019. A total of 32 clinically suspected cases of ovarian carcinoma admitted in the RMCH and later on histologically confirmed as ovarian serous carcinoma were included in the study. Histories of the patient were obtained from the hospital records. Tissue biopsy from the site of lesion or operated specimen of tissue was fixed with 10% formalin and was processed stained with haematoxyline and eosin stain and was examined. ***Result:*** Immunohistochemistry was done for p53 from significant paraffin embedded block. Of the 32 cases 20 were histologically confirmed as high grade and the rest 12 were low grade ovarian serous carcinoma. In this present study, age distribution showed that, about 60% of the patients were within 2nd to 5thdecades.The mean age of the patients was 41.34 years. The sensitivity of p53 was found high (80.2%) in case of serous ovarian carcinoma and its different grade. ***Conclusion:*** The study concluded that p53 is a highly sensitive immunomarker for detecting the grade of ovarian carcinoma specially serous ovarian carcinoma.

Keywords: Ovarian cancer, histopathology, haematoxyline, eosin.

INTRODUCTION

Ovarian carcinoma is the seventh most common cancer among woman worldwide and eighth leading cause of death, with an estimation of 2,39,000 new cases and 1,52,000 deaths in a year throughout the world. Incidence of ovarian cancer is high in Europe and Northern America; and lowest incidence in Africa and Asia [1].

Ovarian cancer accounts for 6% of all cancers in the female and fifth most common form of cancer in women in the United States [2]. The annual mortality rate due to ovarian cancer per 1,00,000 population in Bangladesh has increased at 40.3% since 1990 and per yearly average rate is 1.8% [3].

Surface epithelial ovarian carcinoma is the most common type and consists of about 60%-70% among all type of ovarian carcinoma [4]. The fatality rate is high among ovarian carcinoma and most of which are due to high grade serous adenocarcinoma. Most of the cases with subtype of adenocarcinoma present at advanced stage with poor prognosis [5].

Ovarian carcinogenesis has been shown that the various different tumors are all derived from the ovarian surface epithelium (mesothelium) and that

subsequent metaplastic changes lead to the development of the different cell types (serous, endometrioid, clear cell, mucinous and transitional cell). The normal ovary, however, has no constituents that resemble these tumors [6].

The fallopian tube is the site of origin for "ovarian" high grade serous carcinoma and low grade serous carcinoma is originated from borderline serous carcinoma [7].

Fallopian tube, especially the fimbrial end is examined thoroughly in these days, because of recent developments pointing that fallopian tubes are not only the source of most of the ovarian high grade serous papillary carcinoma but also for low grade serous tumors [8].

Low grade ovarian serous carcinoma (LG-OSC) is thought to evolve in a step wise fashion from serous cyst adenomas to serous borderline tumors to invasive carcinoma. The majority of ovarian epithelial inclusions are derived from the fallopian tube rather than ovarian surface epithelium, and that the tubal secretory cell is the likely the cell origin of LG-OSC [8].

p53 is called the guardian of the genome. p53, a tumor suppressor gene is located in the short arm of chromosome 17 on the locus 17p13.1. It regulates cell cycle progression, DNA repair, cellular senescence and apoptosis [9]. Epithelial ovarian carcinoma have been found to harbor p53 mutation in over 90% of cases [10].

p53 gene encodes a nuclear phosphoprotein p53, which contain 393-aa (aa=amino acid). A point mutation of p53 leads to formation of an altered protein product that has prolonged half life, loss of suppressive capacity and uncontrolled cell proliferation which can be detected by immunohistochemistry [11]. Some study have shown that abnormality of p53 expression occur commonly in ovarian carcinoma and is associated with reduced survival rate. Expression of mutated form of this protein which protect neoplastic cells against apoptosis induced by chemotherapeutic agents and also become radio-resistant.

The commonly used techniques that are used for ovarian carcinoma are bimanual pelvic examination, USG imaging of the ovaries and serum tumor markers. Most low grade ovarian carcinoma is incidentally detected in the early stage and five-year survival rates of over 90% can be achieved. Along with it a minimum improvements achieved in the past 20 years in mortality with aggressive treatment of advanced disease. So early detection and grading of tumor is necessary. A 5 years survival rate is 85% or more seen in the low grade well differentiated carcinoma. If patient can be diagnosed early then they may be cured with conventional surgery and chemotherapy. Detection of a greater fraction of ovarian cancers in early stage might significantly affect survival. For this reason early detection of grading is top priority [2].

The patients of ovarian cancer cannot be adequately monitored by computed tomography (CT scan) or ultrasound scans only. There has been a pressing need for immunohistochemical assay of biomarker in ovarian cancer. Immunomarker p53 has been described as a useful marker for detection of grading, treatment and monitoring of ovarian cancer. Oncogenic alterations are necessary for development of ovarian carcinoma. Mutation in tumor protein p53 is the main event in epithelial ovarian carcinoma, especially in the serous subtype (90 %) [12].

p53 staining pattern yielded a sensitivity of 87% and a specificity of 100% in detecting p53 missense mutations, demonstrating the utility of p53 immunostaining as a surrogate for p53 mutation in the histologic diagnosis of ovarian serous carcinoma [13].

Therefore, detection of p53 protein expression with immunomarker is an important measure for grading and detecting the prognosis of ovarian carcinoma.

OBJECTIVE

General Objective

- To see p53 expression and its association with different histopathological grading of ovarian serous carcinoma.

Specific Objectives

- To confirm the diagnosis and grading of the ovarian serous carcinoma by histopathology.
- To find the intensity and pattern of p53 expression in patients with ovarian serous carcinoma.
- To find out the association between p53 protein expression and histopathological grading of ovarian serous carcinoma.

MATERIAL AND METHODS

Study Design: The study was carried out with cross sectional type of descriptive study.

Place and period of study: The study was carried out for two years from July 2017 to June 2019 in the Department of Pathology, RMC.

Study population

Clinically suspected patients of ovarian carcinoma who were admitted in Gynae and Obstetric dept. of RMCH and later on histopathologically confirmed as ovarian serous carcinoma. Study population was further defined using eligibility criteria as follows :

Eligibility Criteria

Inclusion Criteria

All the cases of histopathologically confirmed ovarian serous carcinoma among clinically suspected patients with ovarian carcinoma who were admitted in Gynae and Obstetric dept. of RMCH.

Exclusion Criteria

Clinically suspected patients of ovarian carcinoma but not histopathologically confirmed as ovarian serous carcinoma.

Determination of sample size

Calculation of sample size:

Sample size was Calculated by using Cochran's formula.

$$n = \frac{Z^2 xpq}{d^2}$$

Here,

n= Estimated sample size

Z= Standard normal deviation. Usually assumed at 1.96 which corresponds to 95% confidence interval.

P= 3.3 %= 0.03 (Prevalence of ovarian carcinoma of all carcinoma is 3.3%)

(Ref: Comprehensive update on cancer scenario of Bangladesh, 2013).

q= (1-p)= (1-0.03) = 0.97

d= 0.5 (Marginal error considered as 5%).

$$n = \frac{(1.96)^2 x0.03x0.97}{(0.05)^2}$$

= 44.7 = 45 (for infinite population)

(Average 3-4 ovarian cancer specimens are obtained per months in Pathology department of RMCH. Therefore theoretically total specimen during study period 1.5 years x 12 months = 18 months is 4x18 =72)

$$n_c = \frac{n}{1+\frac{n}{N}}$$

(Here n_c=corrected sample size, n= 45, N=72)

$$= \frac{45}{1+\frac{45}{72}}$$

=27.69= 28

(10% dropout/design defect 3 more cases to be added).

Then, n_c= 28+3 = 31.

Sampling Technique: The study was carried out with purposive sampling method.

Sample Collection

Microscopic examination of H&E sections of tissue blocks from each ovarian mass suspected of malignancy was done. Only the cases of ovarian tumour that were confirmed microscopically as ovarian serous carcinoma were enrolled for the final analysis. Ovarian tumours other than ovarian serous carcinoma were excluded.

Routine histopathological examination:

In the laboratory, tissue processing, paraffin embedding, sectioning of paraffin blocks, H & E staining were done according to the standard protocol. Later H&E sections of all the submitted blocks extensively examined, and the findings were recorded in the data sheet.

Statistical analysis and result

The data was analyzed by statistical package for social science (SPSS)-25 software program. The analytical tests were performed using test statistics.

RESULTS

The findings of the study obtained from data analysis were documented below:

Age of the study patients

Table 1: Distribution of study subject according to age group (n=32)

Age group	Frequency (n)	Percentage (%)
≤ 20	03	09
21-30	05	16
31-40	06	19
41-50	12	37
51-60	06	19
Total	32	100.0
Min=14; Max=60; Mean=41.34 (± 11.4)		

Distribution of study subject according to age group (n=32).In this study, mean age of the patients were 41.34±11.4 with age range from 14 to 60 years. Most (78%) of the patients belonged to 3rd to 5th decade. There was no case below 14 years or above 60 years. The least number of samples was below 20 years of age (3 case, 09 %).

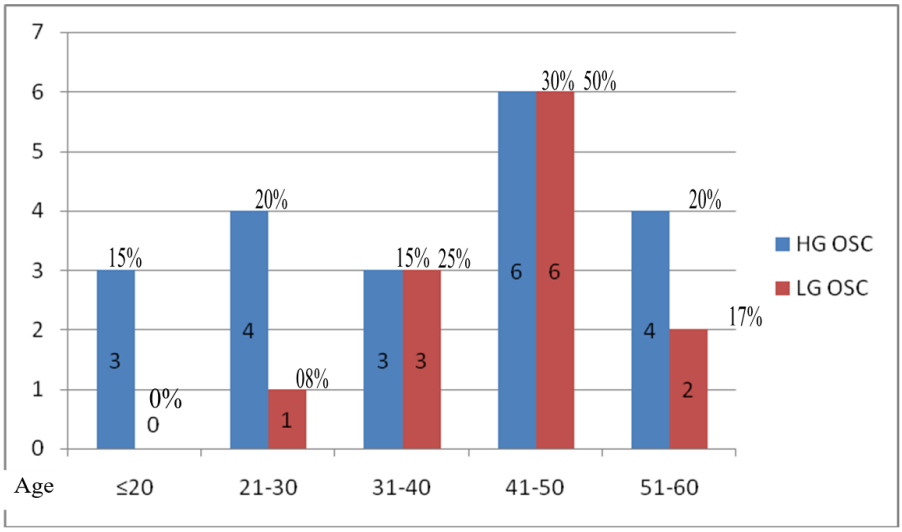

Figure 1: Bar diagram of distribution of age group between LG & HG

From the above diagram, it was observed that in HG-OSC, lowest age was 14 years which were found in 03(15%) cases and highest age range was 51-60 years which were found in 04(20%) cases. In LG-OSC, lowest age range was 21-30 years which were found in 01(08%) cases and highest age range was 51-60 years which were found in 02(17%) cases. HG-OSC was commonly occurred in 3rd-5th decades. On the other hand, LG-OSC was commonly occurred between 2rd-4thdecades**.**

Marietal status of study patients

Table 2: Distribution of study subject according to marietal status (n=32)

Marietal status	LG-OSC		HG-OSC	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Unmarried	02	17	04	20
Married	10	83	16	80

This study showed that, most of the women were married. 02(17%) patients of LG-OCS were unmarried and 10(83%) patients were married. Only 4(20%) patients of HG-OSC were unmarried and 16(80%) were married.

Parity pattern of study patients

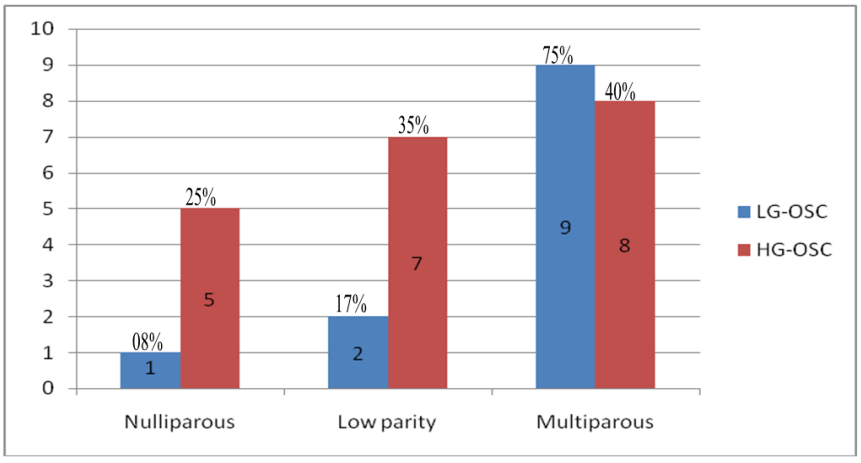

Figure 2: Bar diagram of distribution of study subject according to parity.

Among the cases 06 cases (19%) were nulliparous, 9 cases (28%) had low parity, 17 cases (53%) were multiparous. In this study most of the malignant epithelial cancers occurred in multipatity.

Menopausal status of study patients

Table 3: Distribution of study subject according to menopausal status

Menopausal status	LG-OSC		HG-OSC	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Premenopausal	03	25	08	40
Postmenopausal	09	75	12	60
Total	12	100	20	100

In this study among 12 LG-OSC, 03 cases (25%) occurred in premenopausal and 9 cases (75%) in postmenopausal. Among the 20 HG-OSC, 08 cases (40%) occurred in premenopausal and 12 cases (60%) in postmenopausal. Most HG-OSC occurred in post menopausal women.

Table 4: Intensity of immunostaining of p53 in OSC

Intensity of p53	HG	LG
0	02	02
1+	01	04
2+	01	04
3+	16	02
Total	20	12

In the present study, staining intensity of p53 in HG-OSC showed strong and diffuse positivity in nucleus of cells i.e. 3+ staining in 16 cases, out of 20 cases.Rest 2 cases were focally positive for p53 immunostain. 2 cases were negative.

In LG-OSC showed focally and weakly positivity in nucleus of cells i.e. 1+ or 2+ staining in 08 cases, out of 12 cases. Rest only 2 case were strongly positive for p53 . 2 cases were negative.

Immunostaining of p53 in different grade of ovarian serous carcinoma

Table 5: Intensity of immunostaining of p53 in different grade of ovarian serous carcinoma

Histopathological grade	Intensity of p53		Total
	≥ 75% of cell [3+]	<75% of cell [0,1+,2+]	
HG-OSC (n = 20)	16	04	20
LG-OSC (n = 12)	02	10	12
Total	18	14	32
$X^2 = 12.20$, df = 1, p< .05			

The Intensity of immunostaining of p53 in different grade of ovarian serous carcinomais shown in table 06. The sensitivity of the p53 in differentiating the grade was 80.2% and specificity of the test was 83.4%. The positive and negative predictive values of the test were 80.2% and 80.9% respectively, while the percentages of false positive and false negatives were 10% and 33.3% respectively. The overall accuracy of the test was 100 = 87.5%.

Chi square test at 5% level of significance against df =1, test statistics was greater than critical value (3.84), which showed statistically significant p value of <0.05 from the above variables.

DISCUSSION

Ovarian cancer is the seventh most common cancer and the eighth cause of death from cancer in women. Worldwide, approximately 239,000 women are diagnosed with ovarian cancer annually, with an estimated 152,000 associated deaths[1]. Although ovarian cancer accounts for only 3% of all cancers in women, it has one of the highest death-to-incidence ratios, which has been primarily attributed to the lack of effective screening tools, the absence of early phase symptomatology in many patients, and common presentation at advanced stages when prognosis is poor [7].

To validate this concept, this cross sectional study was done with the aim to detect high and low grade ovarian serous carcinoma, and further characterize the lesions by immunohistochemistry with p53 antibody. The study included 32 cases ovarian serous carcinoma. Of them 20 cases were high grade and 12 were low grade.

The study showed higher mean age of 46 years with an agerange of 33-78 years. In this study, it was observed that HG-OSC is commonly occurred in 3rd -5th decades. On the other hand, LG-OSC was commonly occurring between 2rd-4^{h} decades**.**

Familial predisposition has been described in 5–10% of a younger subset of women who develop ovarian cancer, and most of these cases are associated with mutations in the BRCA1 and BRCA2 genes [14]. Inherited germ line mutation in both BRCA1or BRCA2

gene increases susceptibility to breast and ovarian cancers. Mutations in BRCA1 markedly increase the risk of developing ovarian carcinoma, which occurs in as many as 20% to 40% of carriers. BRCA2 confers a smaller risk for ovarian carcinoma (10% to 20%) (Lester, 2014) [15]. In this study, family history of ovarian carcinoma was found in 8(40%) cases of high grade ovarian serous carcinoma. In low grade, only four patients were reported to had a family history.

This study showed that most of the women were married. Among LG-OCS 02(17%) patients were unmarried and 10(83%) patients were married. Only 4(20%) patients of HG-OSC were unmarried and 16(80%) married. Among the married women 06 cases (19%) were nulliparous, 9 cases (28%) had low parity, 17 cases (53%) were multiparous. In this study most of the epithelial cacinoma occurred in multiparous woman, which disagreed with the study of Hennessy *et al*., 2009.

In this study among 12 LG-OSC, 03 cases (25%) occurred in premenopausal and 9 cases (75%) in postmenopausal. Among the 20 HG-OSC, 08cases (40%) occurred in premenopausal and 12 cases (60%) in postmenopausal. Most HG-OSC occurred in postmenopausal women which agreed with the study of [16].

This p53 staining pattern yielded a sensitivity of 87% and specificity of 100%. In the present study, staining intensity of p53 in HG-OSC showed strong and diffuse positivity in nucleus of cells i.e. 3+ staining in 16 cases, out of 20 cases. Rest 2 cases were focally positive for p53 immunostain and 2 cases were negative.

In LG-OSC showed focally and weakly positivity in nucleus of cells i.e. 1+ or 2+ staining in 08 cases, out of 12 cases. Rest only 2 caseswere strongly positive for p53 and 2 cases were negative.

In this study, immunohistochemistry with p53 antibody was done in all 32 cases. The sensitivity of the p53was 80.2% and specificity of the test was 83.4%. The positive and negative predictive values of the test were 80.2% and 80.9% respectively, while the percentages of false positive and false negatives were 10% and 33.3% respectively. The overall accuracy of the test is 87.5%.

With few difficulties in detection of OSC along with histopathology, the characterization of immunomarkers for the early detection and grading of OSC had become a high priority. The results of this study were in conformity with the previously conducted studies and thus immunomarker p53 played a pivotal role in grading of OSC.

Summarizing the findings of the present study and those of other investigators compared and contrasted, it is evident that p53 is highly sensitive marker in case of grading and detection of prognosis of OSC.

CONCLUSION

This study was undertaken with the aim to see the association of p53 expression and histopathological grading of ovarian serous carcinoma. According to the recent concept, a good number of high grade ovarian serous carcinoma showed strong and diffuse positivity in p53 immunostaining. In low grade showed focally and weakly positivity. The result obtained from analysis of data of this study have some similarities and dissimilarities with those of other studies done at home and abroad. Further large scale long term population based studies are needed to ascertain the actual status prevailing in the field of ovarian serous carcinoma in our country.

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