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ORIGINAL ARTICLE

Tumour morphology after neoadjuvant chemotherapy as a predictor of survival in serous ovarian cancer: an experience from a tertiary care centre in India

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Abstract

Serous ovarian cancer is the most common malignant ovarian tumour. Traditional management consists of surgical resection with postoperative chemotherapy. Currently neoadjuvant chemotherapy is offered to patients with advanced stage disease. The present study aims to analyse the histomorphological alterations in serous ovarian cancer following neoadjuvant chemotherapy. Correlation of these morphological alterations with survival is also presented here. Serous ovarian cancers from 100 advanced stage cases were included; 50 were treated with pre-surgery chemotherapy. Semi-quantitative scoring was used to grade the alterations in tumour morphology. Survival data was correlated with the final morphological score. Tumour morphology was significantly different in cases treated with neoadjuvant chemotherapy (CT group) as compared to cases with upfront surgery. The CT group cases showed more fibrosis, calcification, and infiltration by lymphocytes, plasma cells, foamy and hemosiderin-laden macrophages. The residual tumour cells had degenerative cytoplasmic changes with nuclear atypia. Patients with significant morphological response had a longer median survival, although it did not attain statistical significance in the current study. With the increasing use of neoadjuvant chemotherapy in management, the pathologist needs to be aware of the altered morphological appearance of tumour. Further studies are required to establish a grading system to assess the tissue response which can be helpful in predicting the overall therapeutic outcome and the prognosis of patients.

Key words: Serous ovarian cancers, neoadjuvant chemotherapy, morphological response

INTRODUCTION

Ovarian cancer is the second most frequent cause of cancer in women.[1] Incidence of carcinoma ovary is lower in India compared to the West.[2] It usually occurs in females in the sixth and seventh decades of life.[3] The lifetime risk of developing sporadic ovarian cancer is 2 per cent. However, in patients with a positive family history the risk is 10 to 40 per cent.[4] The neoplasms of ovary are heterogeneous in origin. The serous subtype of the surface epithelium is the most frequent and lethal.[5-8] Almost 70 per cent of patients present at an advanced stage with a poor long-term prognosis. The traditional management consists of surgical resection of the entire grossly visible tumor (cytoreduction), followed by postoperative chemotherapy.[9] An inverse relationship exists between the volume of residual tumour after the initial cytoreduction and survival of the patient.[10] In recent years, there has been an increasing trend to treat patients having advanced stage ovarian cancer (FIGO stage, IIIc and IV) with neoadjuvant chemotherapy (NACT), followed by interval debulking surgery, and post-surgical chemotherapy. The aim of this protocol, commonly known as the 'sandwich therapy', is to reduce the tumour volume, optimise cytoreduction and improve

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TABLE 1: Grading of necrosis and fibrosis

Grade	Criteria
0/absent	Necrosis/fibrosis absent in the whole section
1+/minimally present	Necrosis/fibrosis present in up to 10% of the sections
2+/focally present	Necrosis/fibrosis present in up to >10 - 20% of the sections
3+/widespread occurrence	Necrosis/fibrosis present in up to >20% of the sections

the chances of complete remission. This was initially introduced for the patients unsuitable for a successful primary surgery, and is currently under evaluation for the primary management of advanced ovarian cancer.[8-9] There are clinical (symptomatic improvement and post-treatment quality of life), radiological (Computer Tomography scan) and biochemical (serum CA 125 levels) indicators to predict the response to treatment and prognosis of patients. However, despite all these parameters being favourable, therapeutic outcome after 'sandwich therapy' has not been found to be uniform in all these patients. Thus, it has become essential to document the chemotherapy-induced histomorphological alterations in tumour, as this might be helpful in prediction of tissue response to treatment. Currently, very few studies are available on a limited number of cases.[9,11] In the present study, with the largest number of cases till date, we analysed the histomorphological changes in serous ovarian cancer following NACT and correlated these findings with survival.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, with collaboration from the Department of Medical Oncology and the Department of Obstetrics and Gynaecology, AIIMS. It was a combined retrospective and prospective study, with tumour samples collected from January 2001 to December 2010. Retrospective tumour tissue samples were retrieved from the archives of Pathology Department, AIIMS.

All cases included had a pre-treatment diagnosis of ovarian serous carcinoma either by Computed tomography guided fine needle aspiration cytology (FNAC) or by biopsy. We enrolled a total of 100 cases of advanced stage (FIGO stage IIIc or IV) serous ovarian cancer, comprising of two groups, each with 50 patients. Patients receiving 'sandwich therapy' were included in the NACT group (CT group, n=50). Other group underwent upfront surgery followed by six cycles of chemotherapy (US-CT group, n=50).

Pathology evaluation

Haematoxylin and eosin stained tissue sections representing the entire specimen were analysed for tumour morphology. The sections were evaluated for the following histopathological parameters: tumour architecture, fibrosis, necrosis, psammoma bodies, inflammatory cells (lymphocyte and/ plasma cells) and presence of foamy and hemosiderin-laden macrophages. In addition, nuclear features including anisonucleosis with bizarre nuclei (large nuclei with >1 lobe with/without chromatin smudging) along with cytoplasmic degenerations and vacuoles were also noted.

Each of the morphological parameter was graded semi-quantitatively. Initially, all the slides were reviewed individually by two pathologists (BK, SM); thereafter the slides were reviewed together using a multi-headed microscope and a consensus on grading was attained. Necrosis and fibrosis was calculated morphometrically using image pro-plus software and a scoring of 0 to 3+ was done (Table 1). Anisonucleosis was graded as mild, moderate and severe. Other features apart from necrosis, fibrosis, and anisonucleosis were graded from 0 to 3+ using the criteria described in Table 2.

TABLE 2: Morphological grading of other parameters

Grade	Criteria
0/absent	Absent in the entire section
1+/minimally present	Present in up to 4/10 HPF
2+/focally present	Present in up to 5-7/10 HPF
3+/widespread occurrence	Present in up to >7/10 HPF

Survival data

Follow-up data was obtained from the records of the Department of Medical Oncology, AIIMS. Duration of survival and outcome at the end of the follow-up period was noted.

Statistical analysis was done using non-parametric tests (Pearson chi square and Fisher exact test) and Kaplan-Meier survival analysis with log-rank test. The Stata 11.0 software was used.

Ethics

The study was started after approval by the Institute Ethical Committee and was carried out according to the recommendations and guidelines of the Institutional (AIIMS) Ethical Committee.

RESULTS

Age and stage distribution

The patients were between 30 to 70 years of age with a mean age of 50 years. 93 patients were in stage IIIC. The rest were in stage IV.

Morphological analysis

The architecture of most of the tumours was papillary with solid areas. Fibrosis and calcification was found significantly more in the CT group ($p = 0.005$ and 0.002 respectively, Table 3). On the other hand, necrosis was more in the US-CT group ($p > 0.05$, Table 3). None of the tumours in the US-CT group had cholesterol clefts (Table 3). Foamy and haemosiderin-laden macrophages and inflammatory cells were significantly more in the CT group (Figure 1d-f). Similarly, cytoplasmic degenerations and a high degree of anisonucleosis with presence of many bizarre tumour cell nuclei were significantly more common in the CT group (Table 3, Figure 2).

Survival analysis

Survival analysis was done for 29 patients of the CT group with available follow-up data (Table 4). The range of follow-up period was 1 to 68 months, with a mean follow-up duration of 31 months. 12 patients died during follow up. The median overall survival was 32 months.

For analysing correlation of survival outcome with histological parameters, a combined score of numerical grades of all the nine significant histopathological parameters (Table 3) was taken. Significant cut-off score was 10, after adding all parameters. Cases with a score of equal to or more than 10 were labelled as 'morphological responders'. Cases with a score below 10 were labelled as 'morphological non-responders'. Kaplan-Meier survival analysis for overall survival was done for the morphological responders (solid line) versus non-responders (dashed line) (Figure 3). Median survival was longer in 'morphological responder'; however it did not attain statistical significance.

DISCUSSION

Ovarian cancer is one of the leading causes of morbidity and mortality in females. In India it is the third leading cause of cancer among women.[2] In recent years, 'sandwich therapy' is being preferred in advanced stages with the aim of an improved survival.[8-12]

Morphological features of carcinoma ovary following NACT differ markedly from those of the native neoplasm. McCluggage *et al* compared nuclear and cytological alterations in serous ovarian carcinoma in the pre- and the post-chemotherapy samples. They classified the cases as 'good responders' (n = 14) and 'little or no responders' (n = 4). They also analysed the serum CA 125 levels to document serological response to treatment. However they did not find any correlation between morphological responses and serological response.[13]

In other tumours, such as non-small cell lung carcinoma,[14] breast carcinoma,[15-16] carcinoma of the stomach[17] and malignant bone tumours,[18] the histological alterations following chemotherapy have been well described in the literature. With the increasing use of NACT in the management of ovarian cancers, it is important to establish morphological criteria for adequate treatment response. Sassen *et al* were the first to describe the terms of 'morphological responders' and 'non-responders' in carcinoma of ovary. Their study showed that features like presence of solitarily scattered tumour cells, fibrosis, foamy macrophages, and foreign-body giant cells were present more significantly in cases receiving NACT. A few other features including presence of inflammatory cell infiltrates, psammoma bodies, and haemosiderin were also associated with NACT.[11]

In the current study, haemosiderin-laden macrophages, psammoma bodies, foamy macrophages, and inflammatory cells were more significantly present in cases of CT group (Figure 1), similar to the findings described in literature.[11] Fibrosis was significantly more in the CT group (Table 3). Necrosis was more in the

TABLE 3: Comparison of histopathological parameters between US-CT and CT groups

Histopathological Parameters	US-CT group (n=50)	CT group (n=50)	P value
Tumor Architecture			
Papillary (n=35)	13 (26%)	22 (44%)	
Papillary & solid (n=39)	20 (40%)	19 (38%)	
Solid (n=26)	17 (34%)	9 (18%)	0.101
Fibrosis			
0	25 (50%)	12 (24%)	
1+	13 (26%)	17 (34%)	
2+	12 (24%)	16 (32%)	
3+	0	5 (10%)	0.005
Necrosis			
0	25 (50%)	29 (58%)	
1+	14 (28%)	9 (18%)	
2+	9 (18%)	4 (8%)	
3+	2 (4%)	8 (16%)	0.081
Cholesterol clefts			
0	50 (100%)	43 (96%)	
1+	0	6 (12%)	
2+	0	1 (2%)	0.001
Calcification			
0	30 (60%)	19 (38%)	
1+	16 (32%)	13 (26%)	
2+	3 (6%)	15 (30%)	
3+	1 (2%)	3 (6%)	0.002
Haemosiderin-laden macrophages			
0	41(82%)	19 (38%)	
1+	9 (18%)	12 (24%)	
2+	0	17 (34%)	
3+	0	2 (4%)	<0.001
Foam cells			
0	40 (80%)	17 (34%)	
1+	9 (18%)	10 (20%)	
2+	1 (2%)	22 (44%)	
3+	0	1 (2%)	<0.001
Inflammatory cells			
0	22 (44%)	8 (16%)	
1+	22 (44%)	31 (62%)	
2+	6 (12%)	11 (22%)	0.004
Cytoplasmic degeneration			
0	48 (96%)	29 (58%)	
1+	2 (4%)	17 (34%)	
2+	0	4 (8%)	<0.001
Anisonucleosis			
Mild (n=16)	15 (30%)	1 (2%)	
Moderate (n=74)	34 (68%)	40 (80%)	
Severe (n=10)	1 (2%)	9 (18%)	<0.001
Bizarre nuclei			
0	41 (82%)	2(4%)	
1+	9 (18%)	5(10%)	
2+	0	23 (46%)	
3+	0	20 (40%)	<0.001

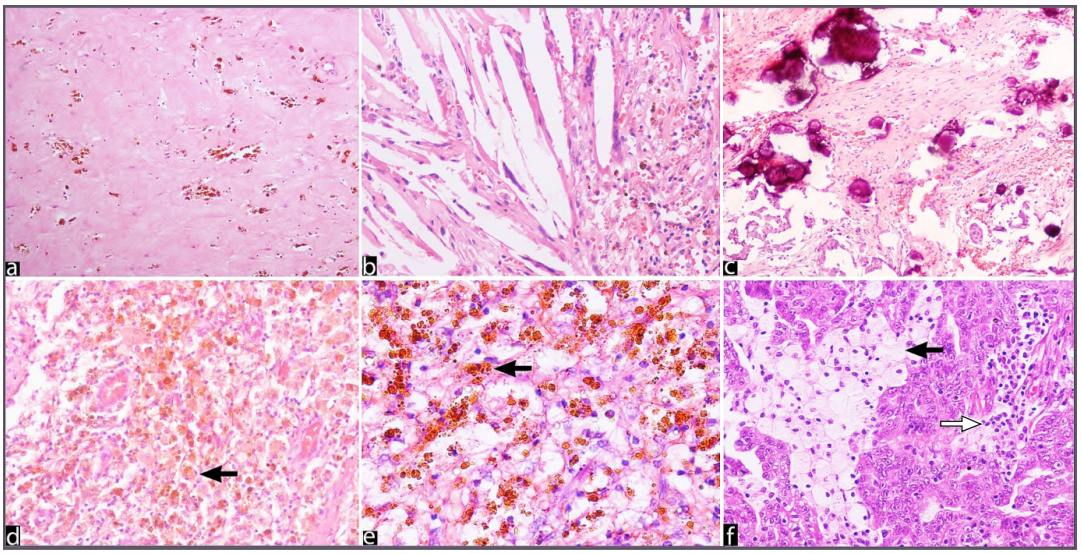

FIG 1: Post-neoadjuvant chemotherapy serous ovarian carcinoma (H&E stain) showing marked stromal hyalinisation (a, X100), cholesterol clefts (b, X200), psammomatous calcification (c, X100), haemosiderin-laden macrophages (black arrow; d, X100; e, X200), infiltration of foam cells (black arrow; f, X400) and plasma cells (white arrow; f, X400).

US-CT group (p>0.05, Table 3). This finding was different from the previous study done by Sassen and his colleagues.[11] Both macroscopical and microscopical measurements of the area showing necrosis may be helpful for determining the exact extent of tumour necrosis. Apart from necrosis and fibrosis, elastosis, myxoid degeneration and hyalinization of the stroma (Figure 1a) with widespread infiltration by inflammatory cells (lymphocytes and plasma cells) in the tumour were also noted in the cases receiving NACT.

The tumour cell morphology was significantly different in the CT group. Cytoplasmic degeneration and vacuoles were prominent in 29 cases (Table 3, Figure 2c). These changes can sometimes be so extensive that it may mimic either a primary clear cell carcinoma of the ovary or a metastatic renal cell carcinoma.[19] Supplementation of morphological diagnosis with immunohistochemistry may be deployed

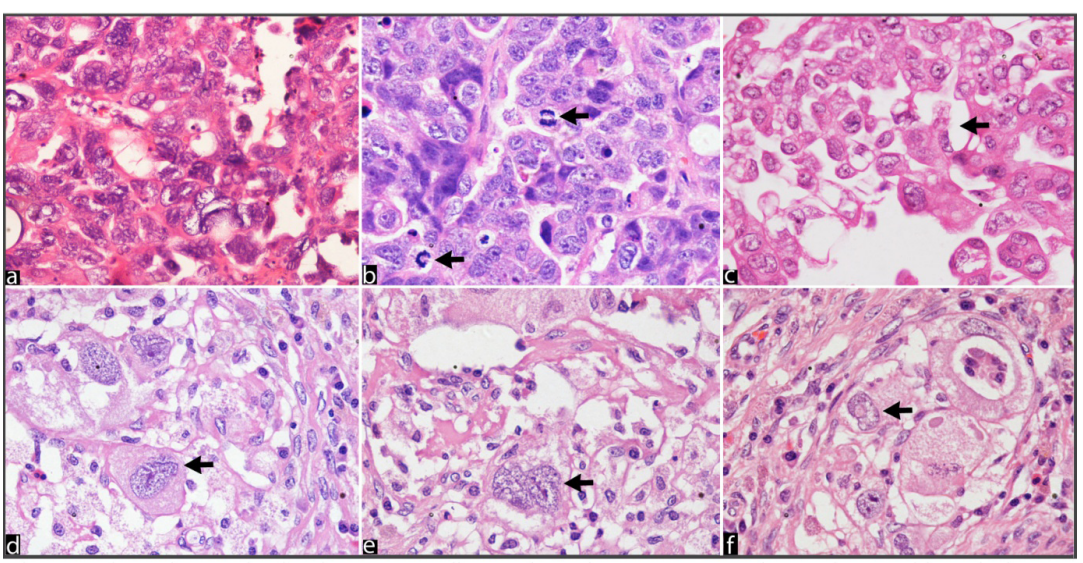

FIG. 2: Photomicrographs (H&E stain) showing post-neoadjuvant chemotherapy serous ovarian carcinoma with marked anisonucleosis (a, X400), brisk mitosis (arrow; b, X200), cytoplasmic degeneration and vacuolation (arrow; c, X200), and presence of bizzare tumor cell nuclei (arrow; d, e, X400; f, X200).

TABLE 4: Survival analysis

Cases (n = 29)	Death (n = 12)	Median Survival (Months)	P value
Non-responders (n = 17)	5	44	0.0897
Responders (n = 12)	7	52	

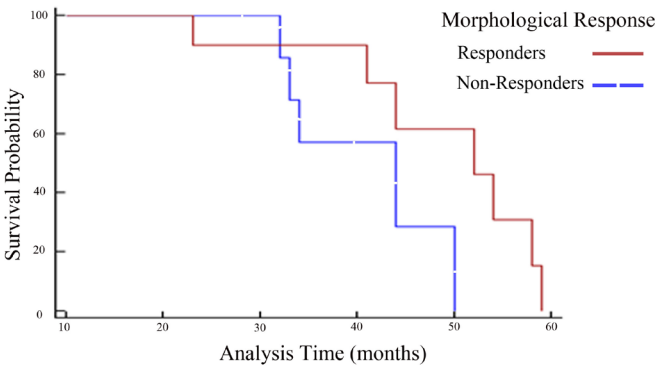

FIG. 3: Kaplan-Meier survival analysis curves depicting difference in survival outcome of morphological responders versus non-responders.

in such cases.

Marked anisonucleosis with presence of many bizarre tumour cell nuclei were found significantly more in the CT group (Figure 2a, 2d-f). 'Bizarre chemotherapy nuclei' has been described previously by other authors.[11,13,19] Grade of the tumours were not taken into comparison, as previous studies have shown a change in the grade in post-chemotherapy samples.[13] Following chemotherapy, the grade may be erroneously higher, as anisonucleosis, bizarre nuclei and necrosis are more in these tumours.

The numerical grades of fibrosis, calcification, cholesterol clefts, foamy and haemosiderin-laden macrophages, inflammatory cells, cytoplasmic degeneration, bizarre nuclei and anisonucleosis were added for the final score of morphological response (Table 3). The median survival was longer in cases showing a significant morphological difference, score ≥10 (52 months in the responders, in comparison to 44 months in the non-responders, Table 4); however the difference was not statistically significant ($p > 0.05$), which may be due to the smaller sample size. The findings were similar to the study done by Sassen *et al.*[11] With the increasing use of NACT the pathologist should be aware of the various morphological changes that occur following treatment. All the chemotherapy induced morphological features should to be carefully searched by sampling the entire tumour area and graded. Further studies on a larger scale are needed to formulate a gradation system for histomorphological response in post-chemotherapy ovarian cancer, which will help in determining the tissue response to treatment and in predicting the survival of the patients.

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