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Original Research Article

Grading ovarian serous carcinoma using a two tier system: Does it have prognostic significance?

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Abstract

Background: A uniformly accepted, clinically useful grading system for ovarian serous carcinoma has not been defined.

Objective: To evaluate a two tier system for grading of ovarian serous carcinoma as compared to the three tier system and to determine whether a predictive relationship exists between grade and survival.

Methods: A retrospective collection of all cases of ovarian serous carcinomas diagnosed during five years in a tertiary care centre were chosen. The histopathological features were analysed and cases were categorised into two tier grading system as low grade and high grade, based primarily on the assessment of nuclear atypia with mitotic figures used as a secondary feature. For comparison, tumours were also graded using the system proposed by Shimizu/Silverberg and categorized as well, moderately and poorly differentiated. Median survival was calculated using the Kaplan-Meier method and the curves were compared using the log rank tests. Multivariate analysis was performed using Cox proportional hazard method. Contingency tables were used to compare the two grading systems.

Results: Forty five cases of ovarian serous adenocarcinomas were studied and categorized into high grade (60%) and low grade (40%). For comparison, the cases were also graded using the Shimizu-Silverberg system and redistributed into grade 1, grade 2 and grade 3. When survival was modelled using a proportional hazard model with the two grading system as predictors, the p values for the two tier grading and Shimizu/Silverberg grading were p=0.62 and p=0.69 respectively.

Conclusion: Significant difference was not noted in survival between low grade and high grade of two tier grading system and the three grades of Shimizu/Silverberg system. Majority of high grade carcinoma cases were placed in grade two of Shimizu/Silverberg grading system. Similarly, majority of cases of low grade carcinomas were placed in grade one of Shimizu/Silverberg grading system.

Keywords: Grading system, High grade, Low grade, Ovary, Serous carcinoma, Three tier, Two tier

1. Introduction

The histological grade of the primary tumour is an important prognostic factor as it helps to determine the likelihood of response to specific therapeutic modalities and the probability of survival in ovarian serous carcinomas. A uniformly accepted, clinically useful grading system for prognosis has not yet been defined and there is no agreement regarding the designation of different categories or number of strata.[1,2,3]

The current existing universal grading system for ovarian epithelial cancer proposed by Silverberg and colleagues is a three-tier grading

system; it analyses three parameters of: architectural patterns, nuclear pleomorphism and mitotic activity, however no criteria exist for defining the precise threshold for the grades leading to inter observer variability.[4,5,6] Recently, it has been proposed that this three tier (well, moderately, and poorly differentiated) grading system for ovarian serous carcinomas could be replaced by a two-tier grading system (low grade and high grade).[7,8] Malpica *et al*, evaluated a two tier system based on well-defined morphologic features of nuclear atypia with the mitotic rate used as a secondary feature.[9,10] The

two tier grading system was shown to be useful in predicting outcome and is also in line with current thinking on serous ovarian tumour pathogenesis. [8,11,12]

This study was done to evaluate a two tier system for grading of ovarian serous carcinoma, as compared to the three tier system and to determine whether a predictive relationship exists between grade and survival.

2. Materials and Methods

A retrospective collection of all cases of ovarian serous carcinomas diagnosed between June 2005 to July 2010, for a period of five years in the department of pathology, Kasturba Medical College, Manipal, a tertiary care centre, were chosen for the study. The institutional ethic clearance was obtained (IEC127/2010 dated 8/09/2010). The diagnosis of ovarian carcinoma showing an exclusive serous morphology was the criterion of search to retrieve these cases. Cases displaying other histologic types such as endometrioid, transitional and undifferentiated as defined by the WHO classification were excluded.

The histopathological features were analysed systematically and cases were categorised into two tier grading system as low grade and high grade, based primarily on the assessment of nuclear atypia with mitotic figures used as a secondary feature.[Table 1] The nuclear features were recorded in epithelial areas showing maximum atypia. The mitotic index (number of mitoses per 10 HPFs) was evaluated in the most mitotically active area of epithelial component of the tumour.[9]For comparison, tumours were also graded using the system proposed by Shimizu/Silverberg and categorized as well, moderately and poorly differentiated. [Table 2][Figure 1,2]

Clinical information was obtained in all cases from the Medical Records Department. The clinical and pathological parameters were evaluated. Surgical procedures, details of chemotherapy and follow up duration were noted for all the patients. The survival duration was measured from the date of diagnosis to the date of last contact or death.

Median survival was calculated using the Kaplan- Meier method and the curves were compared using the log rank tests. Multivariate analysis was performed using Cox proportional hazard method. Contingency tables were used to compare the two grading systems.

3. Results

Forty five cases of ovarian serous adenocarcinomas were studied. Among which, 27 cases (60%) showed high grade nuclear features and 18 cases (40%) showed low grade nuclear features according to the criteria considered for grading in the present study. Fourteen cases with high grade nuclear features showed ≥ 12 mitotic figures per 10 HPFs while remaining 13 cases showed mitotic count of $< 12/10$ HPFs. Among the 18 cases which showed low grade nuclear features, 12 cases showed < 12 mitosis/10 HPFs whereas only six cases showed ≥ 12 mitosis /10 HPFs. For comparison, the cases were also graded using the Shimizu-Silverberg system and redistributed into grade 1, grade 2 and grade 3.(Table 3)

The age ranged from 22 years to 73 years (mean: 49.6 years). The tumour size ranged from 2 to 20.5 cms in the low grade category (mean size: 7.7cms) and from 1.1 to 22.4 cms (mean 9.1 cms) in the high grade group.

The patients in both the groups were treated with total abdominal hysterectomy and bilateral salphingoophorectomy. Six out of eighteen patients (33.3%) with low grade serous carcinomas had received neoadjuvant chemotherapy which involved a platinum based regimen and 15 (83.3%) received subsequent adjuvant chemotherapy. In the high grade group, seventeen out of 27 patients (62.96 %), received neoadjuvant chemotherapy while 23 (85.18%) received adjuvant chemotherapy. It was seen that the rate of receiving neoadjuvant chemotherapy was comparatively higher in high grade carcinomas as compared to the low grade carcinomas whereas the incidence of adjuvant chemotherapy, was almost the same in both the groups.

In the low grade carcinoma group, the follow up period ranged from 2 months to 4.5 years with mean of 19.6 months. One of the patients died due to cause unrelated to the disease. In the high grade serous carcinoma group, the follow up period ranged from 2 months to 4.1 years with mean of 19.9 months. Three out of 27 patients died, two patients died of disease while one patient died of cause unrelated to disease. (Table 4)

Chi-square test was run between two variables: Grade and Survival. Correlation between two tier tumour grade and survival was analysed, p value obtained was 0.96(>0.05). Relationship between Shimizu/Silverberg grading system and survival was also correlated; p value was 0.35.

When survival was modelled using a proportional hazard model with the two grading system as predictors, the p values for the two tier grading and Shimizu/Silverberg grading were $p = 0.62$ and $p = 0.69$ respectively, none of the grading systems had any significant predictive ability. Thus the relationships between two tier grade and survival as well as Shimizu /Silverberg grade and survival were not statistically supported in this study. Further, survival curves were plotted using Kaplan Meier survival analysis with Log rank test to test the significant difference between the curves. A p value of <0.05 was set to be statistically significant. Mean survival time was plotted with death/alive as the

events; p values in both the groups were insignificant. (Figure 3) Grade three of Shimizu/Silverberg grading system had a visibly poorer survival as compared to grade one and grade two. (Figure 3)

Therefore this study did not demonstrate a statistically significant difference in survival between low grade and high grade of two tier grading system and the three grades of Shimizu/Silverberg system, though majority of high grade carcinoma cases had marked cytological atypia with a mitotic count of $\geq 12/10$ HPFs and were placed in grade two of Shimizu/Silverberg grading system and majority of cases of low grade carcinomas were placed in grade one of Shimizu/Silverberg grading system.

Table 1: Two Tier Grading System

Grade	Nuclear features	Mitotic figure/ 10 HPF
Low Grade	Mild to moderate atypia, uniform round/oval nuclei, conspicuous to inconspicuous nucleoli. (Figure 3A)	<12
High Grade	Severe atypia, pleomorphism $\geq 3:1$, irregular chromatin, macronucleoli. (Figure 3B, 3C, 3D, 3E)	≥ 12

Table 2: Universal Grading System: Shimizu/Silverberg System [9,10,11,16]

Score	Architectural Predominant Pattern	Nuclear Pleomorphism	Mitotic Figures/ 10HPFs
1	Glandular (Figure 2A)	SLIGHT [Relatively uniform vesicular nuclei (varying $\leq 2:1$ in diameter), a low nuclear: cytoplasmic ratio, with no chromatin clumping or prominent nucleoli.]	0 - 9
2	Papillary (Figure 2B)	MODERATE [Intermediate variation in nuclear size (between $2:1$ and $4:1$) and shape, nucleoli recognizable but small, some chromatin clumping with no bizarre cells]	10 - 24
3	Solid (Figure 2C)	MARKED [Marked variation in nuclear size ($>4:1$) and shape, a high nuclear: cytoplasmic ratio, prominent chromatin clumping, thick nuclear membrane and large eosinophilic nucleoli, possible presence of bizarre cells.]	>25

To determine the overall grade, the scores from each of the three parameters outlined in columns 2, 3 and 4 were added:

Final grade 1 (well differentiated) = total score of 3, 4 or 5

Final grade 2 (moderately differentiated) = total score of 6 or 7

Final grade 3 (poorly differentiated) = total score of 8 or 9

Table 3: Shimizu Silverberg Grading System

	Grade 1	Grade 2	Grade 3
Low grade (n = 18)	14 (77.7 %)	3 (16.6%)	1 (5.5%)
High grade (n = 27)	5 (18.5)	17 (62.69%)	5 (18.5)

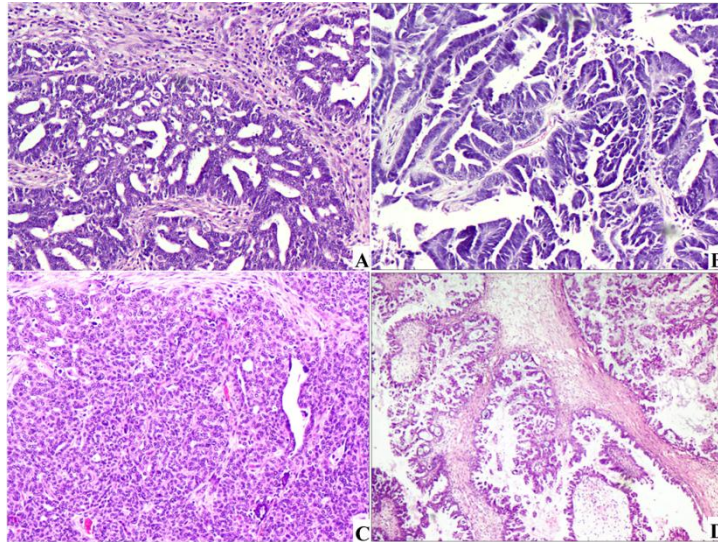
Table 4: Means and Medians for Survival Time

Grade	Mean ^a				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Low grade	19.671	3.045	13.702	25.639	18.000	1.235	15.580	20.420
High grade	19.943	2.605	14.837	25.048	18.000	0.786	16.460	19.540
Overall	19.840	1.966	15.987	23.693	18.000	0.571	16.880	19.120

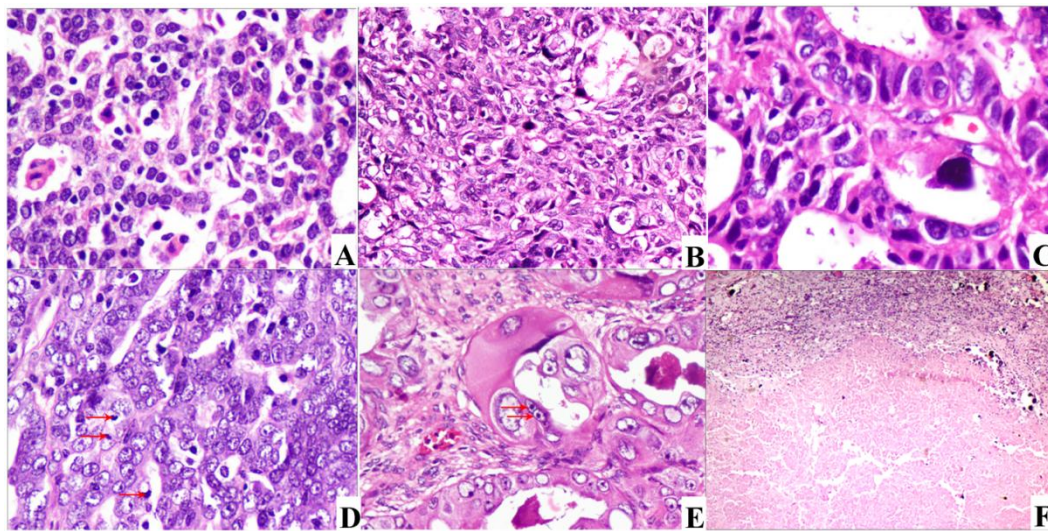
a. Estimation is limited to the largest survival time if it is censored.

Table 5: Redistribution of Cases According To Shimizu/Silverberg Grading

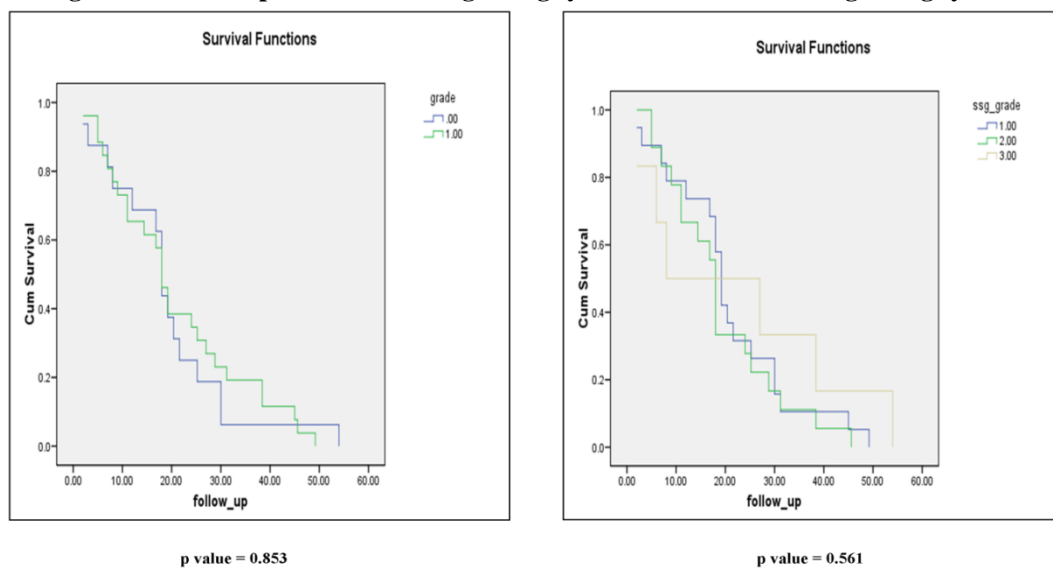
Shimizu/Silverberg grading	Malpica <i>et al</i> [22]		Present study	
	High Grade	Low Grade	High Grade	Low Grade
Grade 1	-	47 (94%)	5 (18.51%)	14 (77.77%)
Grade 2	14 (28%)	3 (6%)	17 (62.96%)	3 (16.66%)
Grade 3	36 (72%)	-	5 (18.51%)	1 (5.55%)

Figure 1: Architectural patterns of ovarian serous carcinoma

A: Glandular Pattern, B: Papillary Pattern, C: Solid Pattern, D: Micropapillary Pattern. [H&E, X100]

Figure 2: Nuclear features and mitosis in ovarian serous carcinoma

A: Low grade nuclear features [H&E, X400], B: High grade nuclear features [H&E, X400], C: High grade nuclear features [H&E, X400], D: Mitotic figures [H&E, X400], E: Macronucleoli & bizarre nuclei [H&E, X400] F: Coagulative necrosis [H&E, X400].

Figure 3: Survival plot with two tier grading system and the three tier grading system

4. Discussion

The importance of grading of ovarian cancers for determining its prognosis is a well-established. While the World Health Organization (WHO) advocates the use of a three tier system such as the Shimizu/Silverberg system, the Royal College of Pathologists, UK does not advocate the use of any particular grading system on grounds of low reproducibility and uncertain prognostic value. However, wherever appropriate, the inclusion of a comment on the differentiation/grade is recommended in the ovarian cancer minimum dataset.[11]

The earliest grading scheme for ovarian carcinomas was based on the Broders classification and the degree of differentiation and the number of mitotic figures were the parameters used in the assignment of a specific grade to a tumour.[13] No criteria exist for defining precise thresholds between grades; which could lead to interobserver variability in the assignment of grade. This would clearly impact on management of an individual patient.[14]

The utility of two tier grading system has been confirmed by the study of Hsu *et al.*[8] The two tier grading system is easier to learn and shows excellent agreement among both gynaecologic and general surgical pathologists in different institutes.[15] Clinically, high-grade serous carcinomas are aggressive neoplasms frequently affecting women in the perimenopausal or postmenopausal age-group, whereas low-grade serous carcinomas are relatively indolent and affect younger women. Low-grade serous carcinomas are more refractory to platinum based chemotherapy as compared with high-grade serous carcinomas, probably because of their low proliferative rate. They develop slowly in a stepwise fashion from noninvasive, micropapillary serous carcinomas that in turn arise from atypical proliferative serous (borderline) tumours with which they also show genetic similarities, specifically the presence of BRAF or KRAS mutations and absence of p53 mutation. High grade carcinomas have not been shown to have a morphological precursor and appear to arise from dysplastic changes in the surface or inclusion cyst epithelium, with p53 mutation typically occurring as an early event. The two groups also differ in allelic imbalance and chromosomal instability, while this is progressive and occurs in a step-wise fashion in low-grade serous ovarian carcinomas, high-grade lesions are characterized by very high instability at the earliest stages.[11,16-18]

In the present study, 60% were graded as high grade and 40% as low grade based on nuclear

features. This finding is consistent with some studies which state that low grade serous carcinomas are relatively uncommon as compared to high grade carcinomas.[12,19] The nuclear characteristics were the principal criteria for distinguishing between low grade carcinomas and high grade carcinomas.¹² The utility of two tier grading system has also been confirmed by yet another study, in which the authors demonstrated by computerized morphometry that nuclear size as measured by mean nuclear area and volume percentage of epithelium is an excellent adjunctive tool for distinguishing low grade from high grade serous tumours.[8] The present study had the limitation of unavailability of computerized morphometry and the molecular genetic profiles of low grade and high grade ovarian serous carcinomas were not assessed.

Ovarian serous carcinomas with features that were intermediate (nuclear grade two) between low grade and high grade were studied and it was concluded that the molecular genetic profile and behaviour of grade two tumours were virtually the same as those of grade three tumours, supporting the use of two tier grading system.[11]

In the study by Malpica, a good correlation was seen between the two tier grading system and the Shimizu/Silverberg system and it was emphasised that two tier grading system was easier to follow. They found a correlation between histologic grade and survival (4.2 years vs 1.7 years median survival for low grade and high grade categories, respectively). In the present study, 77.77% cases of low grade carcinomas were graded into grade 1 as compared to 94% cases of low grade carcinomas in the study done by Malpica. Similarly, majority of the high grade tumours (62.9%) were graded into grade 2 in the present study but in the study done by Malpica, majority of the tumours (72%) were graded as grade 3. Also, all cases of high grade carcinomas (100%) were subdivided into combined grade 2 and grade 3. This observation is in concordance with the findings in the present study where 81.47% of all high grade cases were subdivided into combined grade 2 and grade 3 (Table 5). The numerical values in the present study are in concordance with the study done by Malpica with majority of high grade tumours being redistributed in grade 2 and grade 3 of Shimizu/Silverberg grading system and majority of the high grade cases showing a mitotic count of $\geq 12/10\text{HPFs}$. [9]

When survival was modelled using a proportional hazard model with the two grading system as predictors the p values for the two tier grading and Shimizu/Silverberg grading were $p =$

0.62 and $p = 0.69$ respectively, with neither grade having a significant predictive ability. This was not in concordance with the study by Malpiciain which, the pvalues for the two tier grading system and the Shimizu/Silverberg classification system were $p = 0.02$ and 0.24 respectively.⁹ The two tier grade had a significant predictive ability. The predictive ability of two tier grades over survival was found to be insignificant in our study with p value of 0.62 but was found to be significant in the study of Malpiciawith a p value of 0.040 . [9] Further the predictive ability of Shimizu/Silverberg grade in the present study and in the study by Shimizu/Silverberg grading system was found to be insignificant with p value of 0.69 and 0.24 respectively.

5. Conclusion

The prognostic utility of the two tier grading system was not statistically supported in the present study, however, good overall correlation was observed between the two tier system and the Shimizu/Silverberg grading system. The present study follows several recent reports utilizing a highly comparable scoring strategy to determine tumour grade but was limited by the small sample size and multiple exclusion criteria. Utilising a large tumour bank with a longer follow up period might serve to fortify the results obtained in this study and to reduce confounding factors.

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