Evaluation of the Expression of Estrogen Receptor, Progesterone Receptor and Her2 Neu in Ovarian Cancer Patients

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Summary

Steroid hormone receptors expression in epithelial ovarian cancers has been proposed to have therapeutic and prognostic relevance. Steroid hormones, primarily estrogen, progesterone and HER 2 Neu have been implicated in ovarian carcinogenesis. The prognostic characterisation of ovarian cancer patients, based on clinicopathological parameters such as age, menopausal status, stage, histology, grade, CA 125 level and treatment. This study mainly used to evaluate the expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and Her 2 Neu in ovarian cancer patients and correlate with clinicopathological parameters using immunohistochemistry technique. Nuclear ER expression was noted in tumor tissue of 60% (30/50) in ovarian cancer patients. Significatly higher ER expression was noted with pre-menopausal status. A trend of higher ER expression in Grade 2 tumors. Increased incidence of disease relapse and over death noted in ER positive patients than ER negative patients. Nuclear PR expression was found to be positive in 60% (31/50) cases. Significantly higher PR expression was noted in Grade 2 tumors. Similar incidence of disease relapse and death was noted in positive PR expression and negative PR expression. Membranous HER 2 Neu expression was found to be positive in 18% (09/50) cases. Significantly higher HER 2 Neu receptor expression was noted in CA 125 normal level and histological type of Mucinous Adenocarcinoma which was statistically significant. Higher incidence of disease relapse and death was noted in positive HER 2 Neu and negative HER 2 Neu patients.

Keywords: ER, PR, Her-2-neu, Ovarian Cancer

Introduction

Ovarian cancer is the second most common gynecologic malignancy, and in developed countries, in women it remains the fifth leading cause of cancer death.[1] In India, it is the third leading cancer amongst women, after cervix, and breast cancer. It is about 1 in every 70 women have a lifetime risk of developing ovarian cancer.[2] Age is considered as a significant risk of ovarian cancer. Ovulation, growth factors, cytokines, and environmental agents may play an important role in the initiation as well as progression of ovarian cancer.[3] The majority of cases are sporadic while about 5-10% cases of ovarian cancers are familial. However, the risk for developing ovarian cancer increases four fold in women with affected first degree relative. Lack of knowledge about the etiology and pathogenesis of the tumor leads to its late diagnosis at advanced stage which presents it with highest mortality rate. Therefore, new therapeutic strategies and reliable screening methods for diagnosis are urgently needed. Estrogen Receptor (ER) and Progesterone Receptor (PR) are main secreted hormones by ovary acting through specific receptors.[4] It is known fact that these two hormones and their specific receptors are involved in the process of tumor genesis in ovarian cancer. In addition, evaluation of ER and PR by immunohistochemistry would have advantage in the understanding of the difference in distribution of the expression of the protein between tumor tissues as well as surrounding normal tissue. As well, the determination of hormone receptor in malignant ovarian neoplasms may probably aid in selection of patients for endocrine therapy in a manner similar to that has been already established for certain hormone dependent cancers.[5] Human epidermal growth factor receptor type 2(Her 2 Neu) a proto-oncogene that encodes a transmembrane receptor protein involved in the development and progression of the majority of cancers. Studies have shown that Her2 Neu is overexpressed in approximately 15-30% of ovarian carcinomas.[6] It has also been tested as potential biomarkers of individualized clinical behaviour of cancers, however, findings regarding the overexpression and prognosis are still conflicting.[6] So, the present study aims at evaluation of the expression of ER, PR and Her 2 Neu Receptor in ovarian cancer patients. Furthermore to correlate their expression with various clinicopathological parameters.

Material and Method

Patients

This retrospective study was approved by institutional scientific and ethics committees, included 50 ovarian cancer patients diagnosed and treated at The Gujarat Cancer & Research Institute.

Detailed clinical history such as age, menopausal status, histopathological type, grade, CA125 levels, treatment offered and stage of the disease were recorded from the case files maintained at the Medical Record Department of the Institute. Disease staging was done according to AJCC classification. Disease status was assessed by clinical examination, radiological investigations and biochemical investigations.

Immunohistochemical Localization

Localization of markers Estrogen Receptor (ER), Progesterone Receptor (PR) and Her 2 Neu was performed on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Primary antibodies were procured commercially from Ventana, Roche Diagnostics. The primary antibodies and secondary antibody were incubated as follows: ER (SP1, RTU, Ventana) for 16 minutes, PR (1E2, RTU, Ventana) for 16 minutes, Her 2 Neu (4B5, RTU, Ventana) for 32 minutes, HRP multimer for 8 minutes.

Scoring

Two individual observers scored the sections. Nuclear staining pattern was observed for ER and PR, while Her 2 Neu showed membranous staining pattern. The sections were scored positive and negative for statistical analysis.

Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc., USA). Univariate survival analysis was carried out by Kaplan Meier and Log Rank statistics was used to assess the prognostic significance of disease free survival (DFS) and overall survival (OS). P values $\leq$ 0.05 were considered to be significant.

Results

Patient's Characteristics and Outcome

This retrospective study included 50 patients, 30% had age $\leq$ 53 years, whereas 70% patients had >53 years. Majority of the patients i.e. 80% had postmenopausal status. In relation to pathological characteristics more than 50% were of late stage, having grade 3 tumor with serous papillary adenocarcinoma and higher CA125 levels. (Table 1)The primary treatment offered to the patients was surgery followed by adjuvant chemotherapy (Paclitaxel + Carboplatin). The maximum follow-up period was 68 months with a median follow-up of 12 months.

ER Expression

Nuclear expression of ER was noted in 60% of the tumors. A significant higher incidence of ER expression was noted with premenopausal women as compared to postmenopausal women (p=0.03) whereas similar incidence of ER expression was observed with age group (Table 1; Figure 1). A trend towards higher incidence of ER expression was observed in patients with Grade II (p=0.09) as compared with their counterparts. No significant correlation was observed with other clinical and pathological parameters. (Table 1)

ER expression in relation to survival

According to Kaplan Meier univariate survival analysis, with respect to DFS, higher incidence of disease relapse was noted in ER positive (20%, 4/30) than ER negative patients (5%, 1/20). (Table 2; Figure 2a) While with respect to OS, higher incidence of death was noted in ER positive patients (10%, 3/30) than ER negative patients (0%, 0/20). (Table 3; Figure 2b)

PR Expression

Nuclear expression of PR was noted in 60% of the ovarian cancer cases. No significant correlation of PR expression was observed with clinical parameters. (Table 1; Figure 3) A significant higher incidence of PR expression was observed with Grade II patients (p=0.02) as compared to their counter parts. While no other pathological parameters were found to be significantly associated with PR expression. (Table 1)

PR expression in relation to survival

According to Kaplan Meier univariate survival analysis, with respect to DFS, a trend higher incidence of disease relapse was noted in PR positive (23%, 7/30) than PR negative patients (0%, 0/20). (Table 2; Figure 4a) While with respect to OS, higher incidence of death was noted in PR positive patients (10%, 3/30) than PR negative patients (0%, 0/20). (Table 3; Figure 4b)

Her 2 Neu Expression

Membranous Her 2 Neu expression was observed in 18% of the patients. No significant correlation of clinical parameters with Her2 Neu expression was observed. (Table 1, Figure 5)

With pathological correlations, a significant higher incidence of Her 2 Neu expression was observed with mucinous adenocarcinoma as compared to other histologic type. Also, a significant higher incidence of Her 2 Neu expression was observed with normal CA125 level than higher CA125 level. (Table 1)

Table 1: Correlation of ER, PR and Her2Neu expression with clinicopathological parameters

	N	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
	50 (100)	20 (40)	30 (60)	20 (40)	30 (60)	41 (82)	09 (08)
Age (Years)							
<53	15 (30)	04 (27)	11 (73)	06 (40)	09 (60)	13 (87)	02 (13)
≥53	35 (70)	16 (46)	19 (54)	14 (40)	21 (60)	28 (80)	07 (20)
Menopausal Status							
Premenopausal	10 (20)	01 (10)	09 (90)	03 (30)	07 (70)	09 (90)	01 (10)
Postmenopausal	40 (80)	19 (47)	21 (53)	17 (42)	23 (58)	32 (80)	08 (20)
Histological Type							
Surface Epithelial Adenocarcinoma	06 (12)	01 (17)	05 (83)	02 (33)	04 (67)	06 (100)	00 (00)
Serous Papillary Adenocarcinoma	28 (56)	11 (39)	17 (61)	10 (36)	18 (64)	24 (86)	04 (14)
Mucinous Adenocarcinoma	09 (18)	06 (67)	03 (33)	06 (67)	03 (33)	04 (44)	05 (56)
Clear Cell Carcinoma	01 (02)	01 (100)	00 (00)	01 (100)	00 (00)	01 (100)	00 (00)
Stromal Tumor	06 (12)	01 (17)	05 (83)	01 (17)	05 (83)	06 (100)	00 (00)
Histological Grade(HG)							
Grade I	07 (14)	04 (57)	03 (43)	04 (57)	03 (43)	04 (57)	03 (43)
Grade II	13 (26)	02 (15)	11 (85)	01 (08)	12 (92)	12 (92)	01 (08)
Grade III	30 (60)	14 (47)	16 (53)	15 (50)	15 (50)	25 (83)	05 (17)
Stage							
Early Stage	13 (26)	05 (39)	08 (61)	06 (46)	07 (54)	10 (77)	03 (23)
Advanced Stage	37 (74)	15 (41)	22 (59)	14 (38)	23 (62)	31 (84)	06 (16)
CA125 level							
Normal	06 (12)	03 (50)	03 (50)	03 (50)	03 (50)	03 (50)	03 (50)
High	44 (88)	17 (39)	27 (61)	17 (39)	27 (61)	38 (86)	06 (14)

a:χ^2 =4.688;r=-0.306;p=0.03; **b**:χ^2 =4.695;r=-0.045;p=0.09; **c**:χ^2 =7.761;r=-0.101;p=0.02;
d:χ^2 =11.71;r=0.04;p=0.02; **e**:χ^2 =4.73;r=-0.308; p=0.03

Table 2: Univariate survival analysis for disease free survival

Marker Expression N(%)	**Remission N(%)**	**Relapse N(%)**
ERExpression		
Negative 20(40)	19 (95)	01(05)
Positive 30(60)	26 (80)	04 (20)
	Log Rank=1.04, df=1, p=0.308	
PR Expression		
Negative 20 (40)	20 (100)	00 (00)
Positive 30 (60)	23 (77)	07 (23)
	Log Rank=3.55, df=1, p=0.06	
HER2 Neu Expression		
Negative 41 (82)	35 (85)	06 (15)
Positive 09 (18)	08 (89)	01 (11)
	Log Rank=0.073, df=1, p=0.780	

Table 3: Univariate survival analysis for overall survival

Marker Expression N(%)	**Alive N(%)**	**Dead N(%)**
ER Expression		
Negative 20 (40)	20 (100)	00 (00)
Positive 30 (60)	27 (90)	03 (10)
	Log Rank=1.60, df=1, p=0.205	
PR Expression		
Negative 20 (40)	20 (100)	00 (00)
Positive 30 (60)	27 (90)	03 (10)
	Log Rank=1.410, df=1, p=0.235	
HER2 Neu Expression		
Negative 41 (82)	39 (95)	02 (05)
Positive 09 (08)	08 (89)	01 (11)
	Log Rank=0.395, df=1, p=0.530	

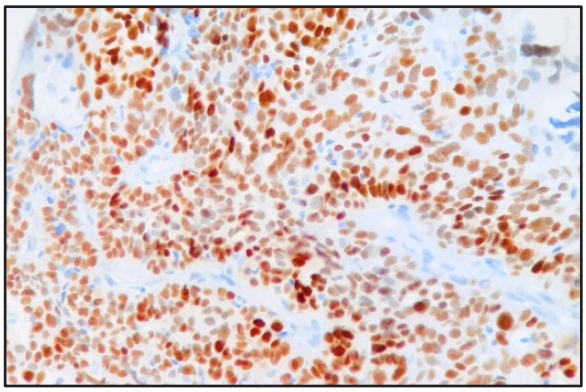

Figure 1: Nuclear staining of ER expression in ovarian tumor cells

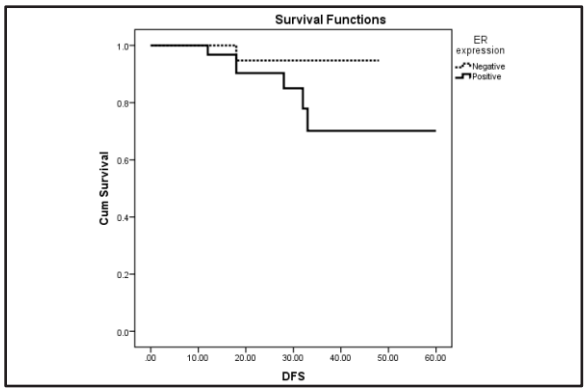

Figure 2a: ER expression in Kaplan Meier univariate survival analysis with respect to DFS

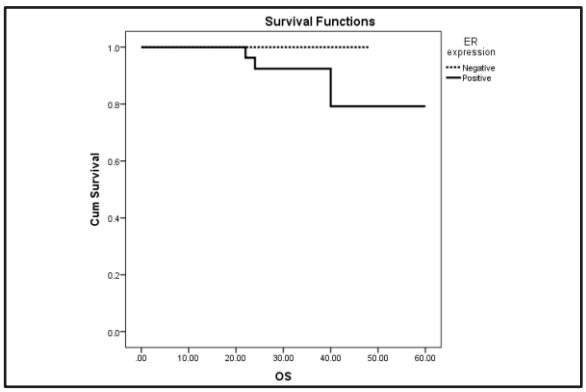

Figure 2b: ER expression in Kaplan Meier univariate survival analysis with respect to OS

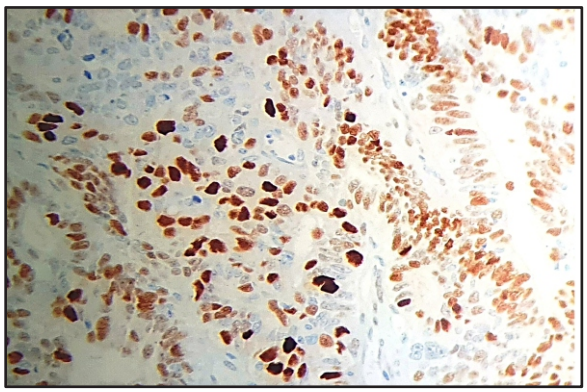

Figure 3: Nuclear staining of PR expression in ovarian tumor cells

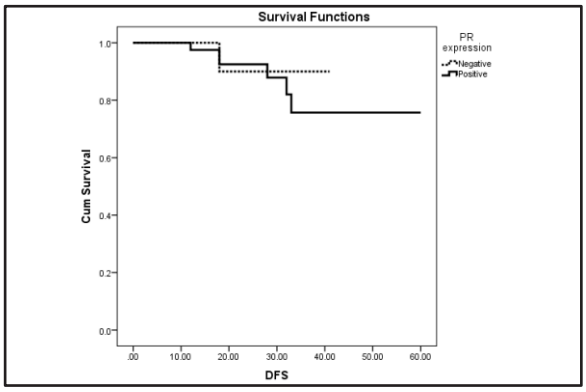

Figure 4a: PR expression in Kaplan Meier univariate survival analysis with respect to DFS

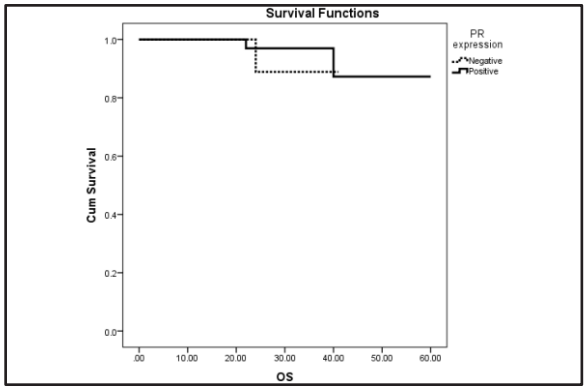

Figure 4b: PR expression in Kaplan Meier univariate survival analysis with respect to OS

Her 2 Neu expression in relation to survival

With respect to DFS, Kaplan Meier univariate survival analysis, revealed a similar incidence of disease relapse between Her 2 Neu positive (15%, 6/41) and Her 2 Neu negative patients (11%, 1/09) (Table 2; Figure 6a). While with respect to OS, the incidence of death was the same between Her 2 Neu positive patients (05%, 2/41) and Her 2 Neu negative patients (1%, 11/09). (Table 3; Figure 6b)

Discussion

Ovary is one of the most dynamic organ which undergoes intensive age-dependent and ovarian cycle dependent remodelling. In proliferation and apoptosis of ovarian cells an equilibrium needs to be maintained which helps with the remodelling process. In western countries, ovarian cancer is the fourth common cause of death in women.[7] Estrogen receptor signalling is less important in the development and progression of ovarian cancer than for breast or endometrial cancers. However clinical data, animal experiments, and receptor studies have shown that malignant as well as normal ovaries can be considered as endocrine related and hormone dependent.[8]

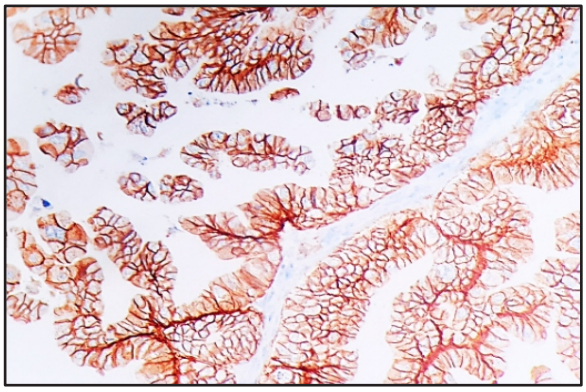

Figure 5: Membranous staining of Her 2 Neu expression in ovarian tumor cells

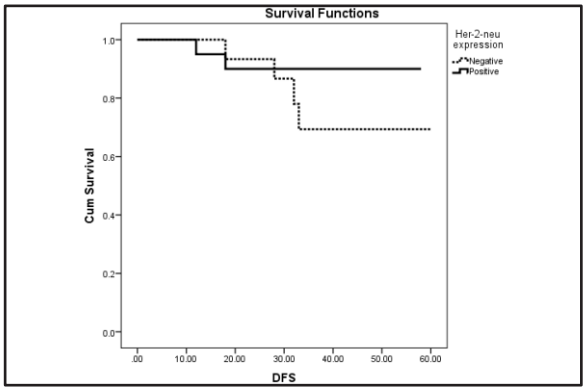

Figure 6a: Her 2 Neu expression in Kaplan Meier univariate survival analysis with respect to DFS

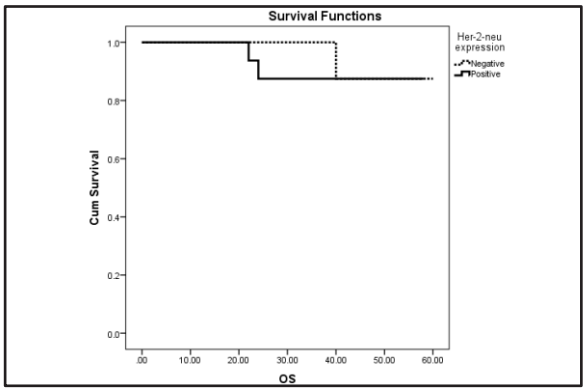

Figure 6b: Her 2 Neu expression in Kaplan Meier univariate survival analysis with respect to OS

In this study ER and PR expression was noted in 60% of ovarian tumor cells. A variable range of ER and PR expression have been demonstrated by various groups in the range of 33% to 90% in ovarian tumor.[9,10,11,12,13] In present study ER expression was higher in older age group. Studies by Sylvia et al, 2011 and Verma et al, 2018 showed higher ER expression in older age group.[11,12] Whereas, PR expression was similar in both age group in present study. A study by Jin et al, 2016 was found to have similar PR expression in younger and older age.[14] With respect to menopausal status, ER expression was significantly higher in premenopausal women as compared to postmenopausal women while PR expression was higher in postmenopausal women. Sylvia et al 2011 study showed higher positive ER and PR expression in postmenopausal women.[11] Whereas, Garg et al 2014 study showed high ER and PR expression in premenopausal women.[15] In present study ER and PR expression was significantly higher in grade II patients as compared to their counterparts. While other studies showed high ER and PR expression in grade III patients.[11,16] This study could not find any significant association between ER and PR expression with histological type as well histological grade. With respect to survival, present study showed higher incidence of disease relapse in ER and PR positive patients. Similarly higher incidence of death was noted in ER and PR positive patients. Other studies showed similar results with DFS and OS.[17,18] In present study Her 2 Neu expression was noted 18% of ovarian tumor cells. Overexpression of HER2 is seen in 20–30% patients with ovarian cancer. Berchuck et al was first to establish a close link between HER2 overexpression with poor survival in advanced epithelial ovarian cancer was first established by.[19] In this study Her 2 Neu expression was significantly correlated with mucinous carcinoma. Similar results were observed in study by Sarkar et al 2015.[20] Present study also showed significant higher incidence of Her 2 Neu expression with normal CA125 level than higher CA125 level. Whereas, in a study by Zorn et al, 2009, noted that higher HER 2 Neu expression was associated with increased CA 125 level.[21] With respect to survival, similar HER 2 Neu expression was noted with DFS and OS in the present study which was in accordance with a study by Shandiz et al 2016.[22]

Conclusion

In this study we found inconsistent findings of ER, PR and Her 2 Neu expression with clinical parameters with various other reports so we need to study these markers in larger cohort. While, ER and PR status may help to select the women with ovarian malignancy for hormonal therapy which is more likely to improve the response rate as well as prognosis. Her 2 Neu may be used as a potential marker to predict the poor prognosis of ovarian cancer patients, especially for patients with unclassified ovarian cancer

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