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Prevalence of p53 Mutation in serous Carcinoma Ovary and its Predictive Value for Response to Platinum-Based Chemotherapy

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Abstract:- Objectives: To study the prevalence of p53 in serous ovarian carcinoma, and its association with grade of the tumor and response to platinum-based chemotherapy.

Methods: It was an analytical study with a quantitative correlational study design. Convenience non-probability sampling method was used to enroll 40 patients. Their tumor grades, p53 status and response to chemotherapy were measured. Then correlation of p53 with both these parameters was identified.

Results: Out of the 40 patients enrolled, 34 (85%) had high grade serous carcinoma while 6 (15%) patients had low grade disease. They presented mostly with Stage III i.e 67.5% followed by stage IV i.e 27.5%. Complete response to chemotherapy was seen in 4% of the patients, whereas 50% patients exhibited partial response. For correlation of p53 and grade, chi square test was used. The p value was 0.03 and therefore significant. For chemotherapy response chi square was used and turned out to be 0.05 and was also significant. The regression analysis was run and the "R" coefficient for clinical response was 0.17. The goodness of fit test for this regression model is 0.447. The estimated co-efficient of wild type p53 was 1.692 keeping the other category "mutant" as reference category shows that there is positive relation of wild type and worst response of chemotherapy and more chances to "no response/stable" in "wild type" as compared to "mutant" cases. The p-value was significant i.e (≤0.05) for all response elements.
Conclusions: p53 positive ovarian serous carcinomas are more prevalent in our patient population and there was a significant relation between p53 positivity and high tumor grade. Also, p53 positive tumors had aggressive disease pattern.

Keywords:- *p53; serous ovarian carcinoma; platinum chemotherapy; response.*

I. INTRODUCTION

By the end of 2019, around 22,000 new cases of ovarian cancer were diagnosed. Among these the most common histological type was serous epithelial ovarian carcinomas (52%).[1] Ovarian cancers also contribute to highest mortality in women with gynecological malignancies i.e. at 5% [1]. In Pakistani women, ovarian cancer is the 4th commonest malignancy and is the most common cancer out of gynecological cancers[2].

Serous ovarian carcinomas (SOCs) are broadly categorized into high and low grade[3]. The majority of epithelial ovarian carcinomas (89-90%) are high grade serous ovarian carcinomas (HGSOC). HGSOCs are extremely aggressive, have a tendency to present in advanced stages and have a 5-year survival of about 30%. In contrast, low-grade serous ovarian carcinomas (LGSOCs) have a relatively slower growth pattern than HGSOC, but their response to platinum containing chemotherapy is poor[4][5]. The survival of platinum sensitive ovarian cancer is 2 years, whereas platinum-resistant ovarian cancer has a median survival of 9–12 months and less than 15% respond to subsequent chemotherapy regimens [10].

Histopathology can guide us about diagnosis in most of these cases, however, in doubtful cases immune histo chemistry (IHC) is an important tool used to differentiate between high grade and low grade SOCs[4].

TP53 gene encodes the 53-KDa nuclear protein. The function of this protein is to maintain the DNA and is the guardian of the genome[6]. Mutations in the p53 gene leads to disturbance in the normal DNA synthesis, and can cause breast and ovarian cancers[7]. Presence of p53 mutation predicts earlier and better response to chemotherapy but it is a poor prognosticator[8]. Mutations in the p53 may be identified through immune histo chemistry (IHC).

Standard treatment of serous Ovarian Carcinomas (EOC) is taxane and platinum-based chemotherapy with cytoreductive surgery. Majority of EOCs relapse and eventually are chemo-resistant. HGSOC have poor prognosis, increased chances of developing resistance to standard chemotherapeutic regimes and thus have poor outcomes[5][9]. Resistance is mainly because of the switching of p53 DNA repair pathways by tumor[10].

There is a need to identify prognosticators of poor outcome in ovarian carcinoma so that further therapeutic strategies can be explored[11]. Several investigators have studied the association of p53 mutation with ovarian cancers with contradicting results[7] [12].

The aim of the present study was to evaluate the association of p53 mutation with grade of EOC and its importance as a marker of response to chemotherapy. We studied the clinical response of the tumor to standard platinum-based chemotherapy.

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II. OBJECTIVES

- To determine the prevalence of p53 mutations among serous ovarian carcinoma patients.
- To study the association of p53 mutation with the grade in patients with serous ovarian carcinoma.
- To determine the predictive value of p53 as a marker of clinical response to platinum-based chemotherapy in serous ovarian carcinoma patients.

III. OPERATIONAL DEFINITIONS

A. Prevalence[13]

Prevalence, referred to as the proportion of persons in a population who have a particular disease or attribute at a specified point in time or over a specified period of time. It is calculated by diving all cases with the total population at a given point in time.

Grade	***Nuclear features***	***Mitotic figures/ 10 HPF***
low	Mild to moderate atypia, uniform round/oval nuclei, conspicuous to inconspicuous nucleoli.	<12
high	Severe atypia, pleomorphism 3:1, irregular chromatin, macro nucleoli	>12

Table 1 : Grades of serous ovarian carcinoma[3]

mutation	pattern	IHC interpretation
TP53 mutation absent	Wild type	normal
TP53 mutation present	Overexpression/complete absence/ cytoplasmic	mutant

Table 2 : P53 MUTATION[14]

B. *Response:* Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[15]

- Complete response: Disappearance of all target lesions and reduction in the short axis measurement of all pathologic lesions to ≤10 mm.
- Partial response: ≥30% decrease in the sum of the longest diameter of the target lesions compared with baseline.
- Progressive disease: ≥20% increase of at least 5 mm in the sum of the longest diameter of the target lesions compared with the smallest sum of the longest diameter recorded, OR, the appearance of new lesions, including those detected by FDG-PET.
- Stable disease: neither PR or PD

C. Chemotherapy Settings[16]

- **Neo-adjuvant Chemotherapy**
  Chemotherapy given before the definitive treatment (surgery or radiation). In our case the definitive treatment was surgery.
- **Adjuvant Chemotherapy**
  Chemotherapy given after the primary treatment to lower the risk of relapse. Other adjuvant therapies could be radiation therapy, hormonal therapy, targeted therapy or biological therapy.
- **Palliative Chemotherapy:**
  Treatment given to relieve the symptoms and reduce the suffering caused by cancer.

D. CA125 High/low[17]

The reference range of serum CA-125 is 0-35 units/mL (0-35 U/mL). Values above 35 are regarded as high and below 35 are considered low.

IV. MATERIALS AND METHODS

A. Setting:

The study was conducted at Medical Oncology Department, Fauji Foundation Hospital, Rawalpindi, after getting approval from the hospital ethical review committee.

B. Duration of study:

Study duration was 12-18 months.

C. Sample size:

Total 40 patients were enrolled in the study. Sample size was calculated by WHO sample size calculator 1.1.

D. Sampling Techniques:

Patients were enrolled using convenience non-probability sampling method.

E. Sample Selection:

- Inclusion Criteria:
  - Patient aged ≥ 12 years.
  - Newly diagnosed cases.
  - Both operated and non-operated cases were considered.
  - Histologically proven cases of SCO requiring platinum-based combination chemotherapy.
  - Stage I-IV
- Exclusion Criteria:
  - Ovarian cancer patients with other histologies than serous carcinoma or mixed histologies.
  - Those who were not candidates for future chemotherapy
  - Pregnancy
  - Other concomitant malignancy

F. Study Design:

It was an analytical study, with a quantitative correlational study design.

G. Data Collection:

In this study, patients with histologically proven diagnosis of serous carcinoma of ovary were recruited from Medical Oncology Department of Fauji Foundation Hospital, Rawalpindi. After taking an informed consent, enrolment of patients was carried out on non-probability convenience sampling technique. Forty patients were enrolled in the study. Detailed history, examination, blood CP, renal and liver function tests and staging tests were performed. The presence or absence of p53 mutation was assessed on the biopsy

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specimen and correlated with the histological grade of the tumor. Patients were given platinum-based combination chemotherapy (in neo-adjuvant, adjuvant or palliative setting) and response was assessed at the end of treatment by RECIST criteria on CT scan and serum CA-125 levels.

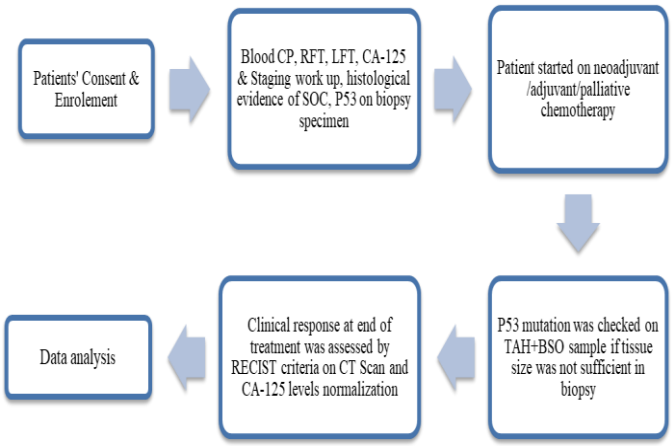

Fig 1 : Conceptual framework of the study

H. Data Analysis:

SPSS 23 was used to analyze the data. In descriptive statistics, means and standard deviations were calculated for age. Qualitative variables like chemo-administration settings, serum CA-125 levels, clinical stages, p53 and clinical responses were shown as frequency and percentages.

In analytical statistics, chi-square test was used to predict the correlation of p53 with histological grade. The correlation of P53 with the clinical response of serous ovarian carcinoma was also evaluated by applying chi square test. The predictability of cause effect relation of independent and dependent variables was checked by ordinal logistic regression.

V. RESULTS

In this study 40 patients were enrolled. Mean age was 56.58±9.9 yrs. Table 1 gives details of descriptive statistics of the study.

As per our first objective the prevalence of p53 positivity was 70% whereas 30% patients were negative for p53 in our study population

Clinicopathological features	*n (%)*
Age(years)	
Mean ± SD	56.58±9.9years
Range	36-74 years
Histological grade	
Low	6 (15%)
High	34 (85%)
Clinical stage at diagnosis	1 (2.5%)
I	1 (2.5%)
II	27 (67.5%)
III	11 (27.5%)
IV	
Pretreatment CA-125	
Normal	6 (15%)
Raised	34 (85%)
Post-treatment CA-125	
Normal	19 (47.5%)
Raised	21 (52.5%)
Chemo setting	
Neo-adjuvant	23 (57.5%)
Adjuvant	5 (12.5%)
Palliative	12 (30%)
P53 Mutation	
Positive	28 (70%)
Negative	12 (30%)

Response to chemotherapy	**Mutant**	**Wild type**
Complete	3(100%)	0(0%)
Partial	17(85%)	3(15%)
Stable	4(44%)	5(55%)
Progressive	4(50%)	4 (50%)

Table 3 : Baseline Characteristics of Patientd

The association of p53 and tumor grade was checked using chi square test as both variables were categorical. The statistical significance was shown by a p-value for co-relation to be 0.034 and hence signifying a positive correlation among the two parameters.

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4.519	1	
Likelihood Ratio	4.131	1	0.034
Linear-by-Linear Association	4.406	1	0.042
N of Valid Cases	40		0.036

Table 4 : Chi-Square Tests for p53 and Histological Grade

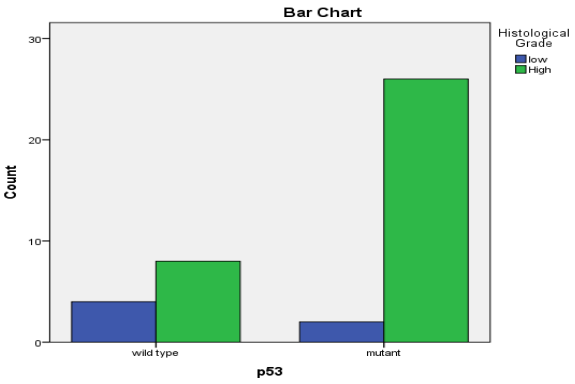

Fig. 2 : Bar chart showing relationship between p53 status and grade

Then the correlation of p53 with the clinical response of the tumor to platinum-based chemotherapy was analyzed by using chi-square test with large contingency table. Here the

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independent variable (IV) i.e p53 was nominal while the dependent variable (DV) of clinical response was measured as ordinal variable. The p-value for co-relation was 0.051 and hence significant, confirming that p53 correlated well with the response to chemotherapy.

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7.751	1	
Likelihood Ratio	8.505	1	0.051
Linear-by-Linear Association	5.961	1	0.037
N of Valid Cases	40		0.015

Table 5 : Chi-Square Tests for p53 and response to chemotherapy

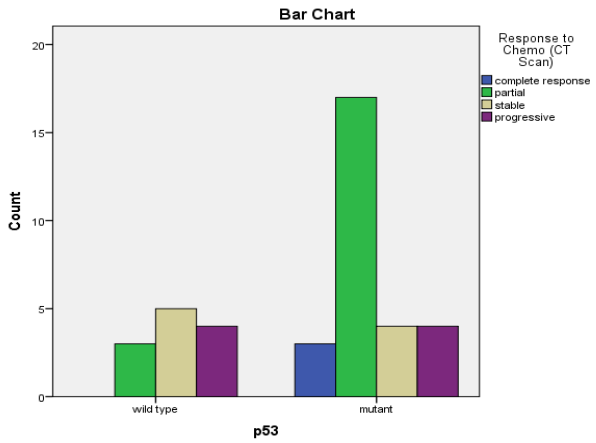

Fig 3 : Bar chart showing relationship between clinical response and p53

To find the predictability of cause effect relation of IVs with the DVs ordinal regression analysis was used. Here the output of analysis was "R^2" which is the "coefficient of determination". It signifies the predictable change that will be expected in the DV by any change in the IV, in other words R^2 predicts the cumulative effect of IV in any change in DV. The regression analysis was run and the "R^2" coefficient for clinical response was 0.17. The goodness of fit test for this regression model is 0.447 which shows that model is good fit and it implies that p53 mutation status has 17% contribution to response of chemotherapy. The estimated co-efficient of wild type p53 was 1.692 keeping the other category "mutant" as reference category shows that there is positive relation of wild type and worst response of chemotherapy and more chances to "no response/stable" in "wild type" as compared to "mutant" cases. The p-value was significant i.e (<0.05) for all response elements

R^2	Standard error of estimate	significance
0.17	1.692	.014

Table 6 : Regression analysis for response

VI. DISCUSSION

Ovarian carcinoma is the second commonest gynecological malignancy in women. It has a tendency to present in late stages and hence associated with aggressive disease patterns and frequent relapses[7]. Hence, by identifying certain prognostic mutations, response to chemotherapy can be predicted, and we can identify cases where we need to be more aggressive to prevent these relapses. One of the frequently studied markers is p53, which is an important prognosticator of disease pattern, response to chemotherapy and predicts an increased tendency towards relapse[7][8]. Other cancers with established role of p53 mutation include squamous cell carcinoma of head and neck (30% to 70%), hematopoietic malignancies (10%), lung cancers (70%) and colorectal carcinomas (60%)[19].

The mean age was 56.58±9.9 years which is close to other similar studies such as study done by Brachova and McDonald, in which the mean age of the patients was (40-56yr) and (57-60yr) respectively[9][12]. In other studies, the patient characteristics were quite similar to our study but the sample sizes were larger, such as in the study done by Luis Felipe Sallum the sample size was 106 and 264 in study done by Pavla Brachova[4][12]. Despite the smaller sample size in our study, our results are still comparable to these studies. This shows the similarity in presentation of ovarian cancer in most parts of the world.

Following or preceding complete surgery, platinum-based chemotherapy is the mainstay of treatment in serous ovarian carcinoma. Chemotherapy can also be given in palliative setting in case of stage IV disease. Since our study had majority of patients with advanced stage disease i.e stage III (67.5%) followed by stage IV (27.5%) we gave chemotherapy in adjuvant, neo-adjuvant as well as palliative setting. This is similar to the study done by Luis Felipe Sallum in which 68% of high-grade ovarian carcinoma patients had stage III & IV disease[4]. Similarly in studies done by Pavla Brachova and Megan E. McDonald the percentage of study population with advanced stage was approximately 90%[9] [12].

SOCs are broadly categorized into high and low grade. [3]. Studies have shown that higher the grade of ovarian carcinoma, better is the response to platinum-based chemotherapy, though the overall prognosis is still poor[18]. When we checked the correlation of p53 with histological grade in our study, we found a positive correlation of mutant p53 with grade of ovarian carcinoma. These finding were similar to the patients' characteristics in the studies conducted by Oaknin[8]. In this study the vast majority of patients with higher grade had mutated p53 i.e 92% whereas 99% of low-grade SOCs were wild type p53. In study done by Mariana Rezende Alves, p53 positivity was seen in 72.9% in which >90% were classified as high grade[23]. However, the percentage of p53 positivity in high grade carcinomas in our study was only 70%. Similar percentage was also identified in previous studies done on ovarian carcinoma in the population of Karachi[2]. Globally, there is more focus on the tumor genetics and molecular characters of the tumors across a population and similarity of our patients tumor profile to other

Pakistani studies is an evidence of this[9]. Interestingly, in another study conducted in sudanese population almost similar results were obtained, hence we can say that regardless of the geographical distribution, patients of ovarian carcinoma with similar population characteristics have comparable tumor biology and disease patterns[21].

The clinical response to chemotherapy as assessed by RECIST criteria in our study showed that the majority of our patients had partial response i.e 75% and only 4% patients had complete response in the p53 mutant subgroup. In contrast to this p53 wild type subgroups showed no complete responses and only 15% had a partial response. Despite the aggressive nature of the disease as predicted by p53 mutation status the response seen to the initial chemotherapy was better in p53-mutated patients, as expected. However, in this subset, the anticipated incidence of relapses and disease complication is also much higher[9]. The association of p53 with clinical response is varied in different studies done previously. In studies done by Brachova P and Lavarino C p53 mutated patients had better response to chemotherapy. The similarity of results seen in our study with them can be due to similar tumor characteristics among these patients hence leading to similar response[12][22]. In contrast, in studies done by Luis Felipe Sallum, Megan E. McDonald and Kim Yong Man no association was established between p53 and clinical response[4][9][24]. The reason for this inconsistent correlational pattern of p53 and tumor response can be explained by the varied expression of p53 mutation subtypes[24]. These subtypes have an important role in determining the tumor response to treatment. Three major subtypes defined are loss of function mutations, unclassified missense mutations and oncomorphic mutation. Among these the oncomorphic subtype of p53 mutations have the highest resistance whereas the unclassified p53 mutations have the best response to chemotherapy[12] [22]. Thus, we can presume that may be different subtypes across different study population may have contributed to these contrasting results, though we need further elaborative studies on p53 subtypes to explain this phenomenon.

VII. CONCLUSION

Thus, we conclude that P53 is an important prognostic marker in ovarian carcinoma. Its positivity implies a more aggressive disease pattern requiring frequent monitoring and aggressive disease management to combat its lethal nature.

VIII. LIMITATIONS

This study had small sample size and was single institution based. For future, larger multi-centric randomized controlled trials are needed to determine the exact association of p53 mutation subtypes with the chemotherapy response.

IX. ACKNOWLEDGEMENTS

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