Original Article

Profile of p53 Expression in Epithelial Ovarian Carcinomas: A Multicenter Study from South-East Nigeria

Abstract

Background: Therapeutic targeting of mutated protein 53 (p53) will require the knowledge of mutated p53 expression. Currently, there is paucity of data on the expression of p53 in epithelial ovarian cancer (EOC) in Nigeria. **Objective:** This study therefore aims to carry out an immunohistochemical study of histologically diagnosed EOCs in Nnewi, South-East Nigeria. **Materials and Methods:** Hematoxylin and eosin slides and paraffin blocks of all histologically diagnosed cases of epithelial ovarian carcinomas in the two histopathology laboratories in Nnewi, Anambra State, over a 7-year period, were retrieved from the archives. Archival paraffin blocks of histologically normal ovaries in these laboratories were also retrieved to serve as controls. Sections were made from the tissue blocks and stained with p53 immunostain. **Results:** Fifty EOC specimen and twenty histologically normal ovaries were enrolled in this study. While 58% of EOC showed p53 positivity, none of the histologically normal ovaries showed p53 positivity. p53 expression was more common in those above 50 years of age, yet no association was found between p53 expression and age of patient. However, there was a statistically significant association between p53 positivity and tumor grade ($P < 0.01$), histologic subtype ($P = 0.009$) and molecular subtype ($P < 0.01$), with p53 positivity being more common in high-grade EOC, serous tumors, and Type 2 EOC. **Conclusion:** Overall, these data support the dualistic model of ovarian carcinogenesis in Nigerian patients and therefore, recommend intensified research into p53-targeted therapy.

Keywords: *Carcinoma, immunohistochemistry, Nigeria, ovary, p53*

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Introduction

Epithelial ovarian cancer (EOC) is second only to cervical cancer among the most common gynecological cancer and accounts for more deaths than all combined gynecological cancers.[1] Despite extensive research, progress has been slow in understanding the pathobiology. EOC is identified as a heterogeneous malignancy with various histological subtypes. It is now well known that these different histological subtypes show differences in terms of presentation, response to treatment, immunohistochemical (IHC) reactivity, and molecular profiling.[2] EOC is often diagnosed in the advanced stages of the disease, usually after distant metastasis has occurred.[3] This is probably due to lack of effective screening methods to detect the disease at an early stage. It responds poorly to conventional chemotherapy (paclitaxel/carboplatin) and presently, the 5-year survival rate of such patients is <20%.[4] EOCs have also been associated with mutations in protein 53 (p53).

p53 (*Tp53*, tumor protein p53) is one of the most relevant human oncosuppressor genes. Accordingly, inactivation of p53 by direct mutation of the gene is one of the most frequent genetic lesions in human tumors.[5] Molecular and genetic studies have further confirmed the relevance of p53 in the development and progression of EOC. p53 IHC can be used as a surrogate marker of *TP53* mutation. Therapeutic targeting of mutated p53 will require the knowledge of mutated p53 expression. Similarly, p53 expression has been suggested as markers to predict aggressive behavior in EOC.[6]

This study aims to determine the rate of expression of p53 in EOC. Unfortunately, these data are rare in Nigeria. It may also shed more light on the relationship between p53 and aggressive behavior of EOC in Nigerian patients.

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Materials and Methods

Ethical approval was obtained from the Ethical Review Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, before commencement of this research.

This is a cross-sectional IHC study of EOCs diagnosed in the two histopathology laboratories in Nnewi, Anambra State, namely Histopathology Department of Nnamdi Azikiwe University Teaching Hospital, Nnewi, and Pathocon Specialist Clinic and Research Institute, Nnewi. Hematoxylin and eosin (H and E) slides and paraffin blocks of all histologically diagnosed cases of epithelial ovarian carcinomas in these facilities from July 2009 to June 2016 were retrieved from the archives.

Inclusion criteria included well-preserved tissue blocks with adequate data and tissue left for sectioning. Exclusion criteria included tissue blocks with inadequate tissue left for sectioning, cases with missing tissue blocks, and cases with incomplete data such as age.

Archival paraffin blocks of histologically normal ovaries in these laboratories were also retrieved to serve as controls. Known p53-positive breast cancer tumors were used as the positive control. As the negative control, tumor specimens were immunostained under the same conditions without the primary antibody.

First, H and E-stained slides of these were reviewed for morphological consistency, and tumor grading was done according to the scoring system recommended by Shimizu *et al*.[7] According to this scoring system, nuclear atypia (mild = 1, moderate = 2, and severe = 3), mitotic activity (0–9 = 1, 10–24 = 2, and >25 = 3), and architecture (glandular = 1, papillary = 2, and solid = 3) were described, and total score was counted as follows: score 3–5 = Grade 1, score 6–7 = Grade 2, and score 8–9 = Grade 3. Grade 1 tumors were considered low-grade tumors, whereas Grade 2 and 3 tumors were both considered high-grade tumors in keeping with the WHO two-tier grading system.[1] Then, sections were made from the tissue blocks and stained with p53 immunostains.

IHC studies were done by the streptavidin–biotin immunoperoxidase method on formalin-fixed, paraffin-embedded tissue sections (4 μm) using p53 monoclonal mouse antibody (Santa Cruz p53; clone Pab 1801) at a dilution of 1:75 and biotinylated secondary anti-mouse labeled streptavidin biotin.

Interpretation

The antibody staining was reported as the percentage of cells with positive nuclear staining. The percentage of cells immunoreactive to p53 was estimated to the nearest 10%. Those with focal p53 expression (≤10%–50% positivity) were considered as p53 negative, whereas those that either showed diffuse p53 expression (≥60% positivity) or complete negativity (<5%) were considered p53 positive.

Data were analyzed using simple descriptive statistics such as Chi-square test which was used to measure associations with the level of significance ($P < 0.05$). The above analysis was done using the Statistical Package for the Social Sciences version 20.0; IBM Inc., Chicago, IL, USA.

Results

Fifty-two EOC specimens were retrieved from the archives within the study period. However, antigen could not be retrieved from two blocks during IHC staining due to poor block condition. Therefore, fifty EOC specimens were enrolled in this study. Also included in this study were twenty histologically normal ovaries.

The ages of the patients with EOC ranged from 23 to 90 years, with a mean of 52.1 ± 13.6 years and modal age range of 51–60 years. In addition, 56% of patients were aged above 50 years, whereas 44% were 50 years and below [Figure 1].

The EOC cases consisted of 31 (62%) serous, 14 (28%) mucinous, and 5 (10%) endometrioid carcinomas. Seventeen (34%) tumors were low-grade neoplasms and 33 cases (66%) were high-grade neoplasms [Table 1].

p53 expression in normal ovaries and epithelial ovarian cancer

p53 positivity was seen in 58% of EOC. While 15 (30%) EOC specimens showed diffuse p53 expression, none of the histologically normal ovaries showed diffuse p53 expression. Rather, focal p53 expression was present in only four (20.0%) of these normal ovaries, while the rest were completely negative. This is statistically significant ($\chi^2 = 20.319$; $P = 0.001$) [Table 2].

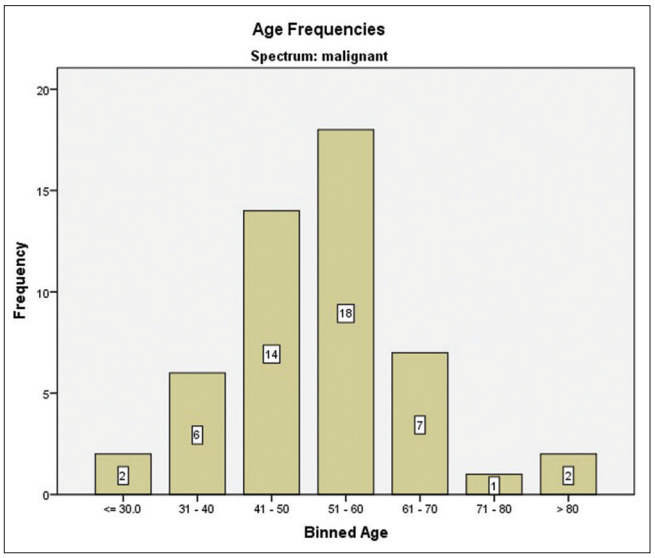

Figure 1: Distribution of epithelial ovarian carcinoma across age groups

p53 expression and patients' age

p53 immunopositivity was seen in 64.3% of those aged above 50 years but in only 50% of those aged 50 years and less, i.e., p53 mutation was more common among those aged above 50 years than those aged 50 years and below. However, no association was found between p53 expression and age of patient (χ^2 = 1.032; P = 0.310) [Table 3]. Complete negativity was more common (63.6%) in those aged 50 years and below, whereas diffuse positivity was the predominant pattern (61.1%) in those above 50 years of age [Table 4]. However, this was not statistically significant.

Table 1: Distribution of tumors across histological grades and subtypes

Tumor grade	Serous	Mucinous	Endometrioid	Total (%)
Low grade	4	10	3	17 (34)
High grade	27	4	2	33 (66)
Total (%)	31 (62)	14 (28)	5 (10)	50

Table 2: Protein 53 expression in normal and malignant ovaries

	Spectrum		Total	χ^2	*P*
	Malignant	Normal			
p53 expression					
Diffuse					
Count	15	0	15	20.319	0.001
Percentage	30.0	0.0	21.4		
Focal					
Count	21	4	25		
Percentage	42.0	20.0	35.7		
Negative					
Count	14	16	30		
Percentage	28.0	80.0	42.9		
Total					
Count	50	20	70		
Percentage	100.0	100.0	100.0		

p53: Protein 53

p53 expression and tumor grades

Comparison of p53 expression across the different tumor grades showed that 78.8% of high-grade carcinomas and 17.6% of low-grade carcinomas showed p53 positivity. This showed statistically significant association (χ^2 = 17.218; P = 0.000) [Table 3]. Though there was no association between tumor grade and pattern of p53 positivity, high-grade lesions showed mainly diffuse pattern of positivity, whereas low-grade lesions showed mainly complete negativity [Table 4].

p53 expression and epithelial ovarian cancer histologic subtypes

p53 positivity was seen in 74.2% of serous carcinomas, 35.7% of mucinous carcinomas, and 20% of endometrioid carcinomas. This was statistically significant (χ^2 = 9.373; P = 0.009) [Table 3 and Figures 2, 3]. Positivity in serous carcinomas was predominantly of the diffuse type, whereas mucinous carcinoma and endometrioid carcinoma showed mainly complete negativity. Nevertheless, this was not statistically significant [Table 4].

p53 expression and epithelial ovarian cancer molecular subtypes

Comparing p53 expression in high-grade serous carcinomas, i.e., Type 2 EOC (85.2% positivity) with others, i.e., Type 1 EOC (26.1% positivity), showed statistically significant difference (χ^2 = 17.807; P = 0.000) [Table 3]. While 60.9% of p53-positive Type 2 EOC showed diffuse type of p53 positivity, only 16.7% of p53-positive Type 1 EOC showed diffuse p53 positivity. This difference was not statistically significant [Table 4].

Table 3: Protein 53 expression across age, tumor grades, histologic subtypes, and molecular subtypes of epithelial ovarian cancer

	p53 status		Total (%)	χ^2	*P*
	Positive (%)	Negative (%)			
Age (years)					
≤50	11 (50.0)	11 (50.0)	22 (44.0)	1.032	0.310
>50	18 (64.3)	10 (35.7)	28 (56.0)		
Tumor grades					
Low grade	3 (17.6)	14 (82.4)	17 (34.0)	17.218	0.000
High grades	26 (78.8)	7 (21.2)	33 (66.0)		
Histologic subtypes					
Serous	23 (74.2)	8 (25.8)	31 (62.0)	9.373	0.009
Mucinous	5 (35.7)	9 (64.3)	14 (28.0)		
Endometrioid	1 (20.0)	4 (80.0)	5 (10.0)		
Molecular subtypes					
Type 1	6 (26.1)	17 (73.9)	23 (46.0)	17.807	0.000
Type 2	23 (85.2)	4 (14.8)	27 (54.0)		

p53: Protein 53

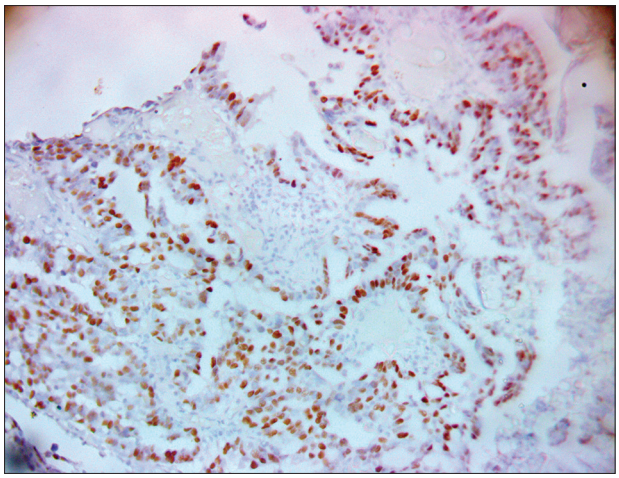

Figure 2: Diffuse (>60%) p53 positivity in high-grade serous carcinoma (×100)

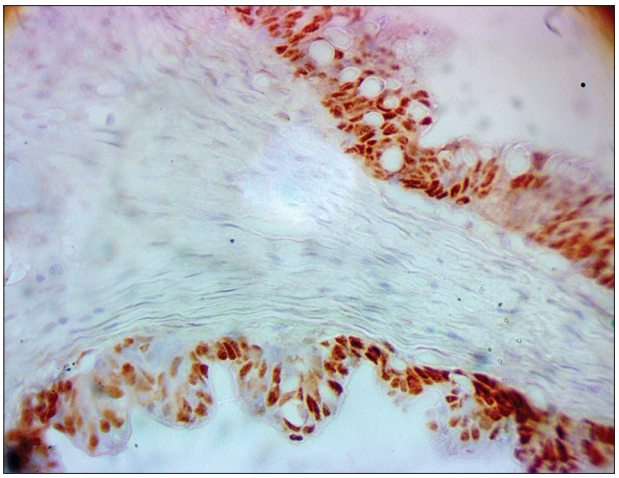

Figure 3: Diffuse (>60%) p53 positivity in mucinous cystadenocarcinoma (×400)

Table 4: Patterns of protein 53 positivity across age, tumor grades, and histologic and molecular subtypes

	Pattern of p53 positivity (%)			χ^2	*P*
	<5%	≥60%	Total		
Age (years)					
≤50	7 (63.6)	4 (36.4)	11 (37.9)	1.675	0.196
>50	7 (38.9)	11 (61.1)	18 (62.1)		
Tumor grades					
Low grade	3 (100.0)	0 (0.0)	3 (10.3)	3.585	0.058
High grade	11 (42.3)	15 (57.7)	26 (89.7)		
Histologic subtypes					
Serous	9 (39.1)	14 (60.9)	23 (79.3)	4.375	0.112
Mucinous	4 (80.0)	1 (20.0)	5 (17.2)		
Endometrioid	1 (100.0)	0 (0.0)	1 (3.4)		
Molecular subtypes					
Type 1	5 (83.3)	1 (16.7)	6 (20.7)	3.724	0.058
Type 2	9 (39.1)	14 (60.9)	23 (79.3)		

p53: Protein 53

Discussion

Malignant surface epithelial tumors of the ovary are generally more common in older women, between the ages of 45 and 65 years.[1] This is evident in the present study. The age range of patients with EOC in this study was 23–90 years, with a mean of 52.1 ± 13.6 years and a modal age range of 51–60 years. This is comparable with findings from other studies within and outside Nigeria, such as age range of 16–82 years, with a mean age of 52.2 ± 12.6 years reported in Ibadan, Nigeria,[4] and age range of 24–78 years, with a mean age of 51.26 ± 14.75 years reported in China.[8] The higher prevalence of EOC in the older age group may be because the role of *TP53* as a guardian of the genome diminishes with age, as the probability of mutation increases. *TP53* mutations account for approximately one-quarter of the aging-related rise in the worldwide incidence of all cancers.[9] These significant associations between *TP53* mutations and the rapid rise in cancer incidence with aging support a causal role for *TP53*. However, questions remain concerning the contribution of *TP53* mutations to neoplastic development and the role of factors such as genetic instability, obesity, and gene deficiencies other than *TP53* that reduce p53 activity.

Serous carcinomas are the most common EOC.[1] This is also portrayed in the present study as serous EOC was the most common, while endometrioid EOC was the least. The histologic subtypes of the EOC specimen within the period under study consisted of 31 (62%) serous, 14 (28%) mucinous, and 5 (10%) endometrioid carcinomas. This picture is similar to published data from Ibadan, Nigeria, in which 70% were serous carcinoma, 26.7% were mucinous carcinoma, and 2.2% were endometrioid carcinoma.[4] Other studies from other countries made similar observation.[10,11] In the same vein, most carcinomas in this series were high-grade lesions. This might be explained by the preponderance of serous tumors, which were mostly high-grade tumors. This also agrees with many of the published works.[4,10]

In this study, p53 expression was scored as the percentage of positive tumor cells. This simple method was easy to apply compared to complex scoring schemes that take into account the combination of staining distribution (percentage of positive cells) and staining intensity.[12] In addition, evaluating the intensity of staining is problematic as it is difficult to reproduce and can vary with different protocols.[12]

Focal p53 expression was present in only four (20.0%) of the histologically normal ovaries but none showed diffuse p53 expression, and the rest were completely negative. These findings are consistent with other studies. Hutson *et al*. and Chan *et al*. observed that p53 immunoreactivity was seen in only 7.7% and 43% of normal ovaries, respectively, and their staining was weak.[13,14] Furthermore, Kuhn *et al*. also noted that, in general, normal epithelium

either showed complete negativity or contained scattered nuclei that were weakly positive for p53, a finding consistent with functional p53 protein and wild-type *TP53*.[15] Hence, evaluation of the complete absence of immunoexpression is made exclusively in tumor cells because lack of immunoexpression can be observed in normal nonneoplastic epithelium and it does not indicate *TP53* mutation.[12] It has been suggested that cellular stress may result in delayed degradation of wild-type p53, making it detectable by immunohistochemistry.[12]

IHC overexpression of p53 (≥60% positive cells) closely correlates with a *TP53* mutation. Even more importantly, it has been found that complete absence of immunolabeling is also indicative of a mutation. Missense mutations in the *TP53* gene lead to the formation of a stable protein resulting in IHC overexpression, whereas nucleotide deletions and nonsense mutations result in protein truncation and complete lack of immunolabeling.[12]

The finding of p53 positivity in 58% of EOC and diffuse p53 positivity in only 30% of EOC in this study is similar to that of other published works. In a meta-analysis by Kmet *et al*., it was also observed that, while 60% of EOC expressed p53, only 29% demonstrated diffuse p53 expression.[16] Similarly, with respect to diffuse p53 positivity, other authors have reported figures such as 25% in Sweden[17] and 40.7% in Iran.[18]

TP53's role as a guardian of the genome diminishes with age, as the probability of mutation increases.[9] It has been shown that, worldwide, about a quarter of the aging-related exponential rise in the diagnosed tumors at all sites, excluding prostate cancer, could be assigned to *TP53* mutations.[9] Accordingly, in the present study, p53 positivity was found in 64.3% of those above 50 years of age but in only 50.0% of those aged 50 years and below, i.e., p53 expression was more common among those above 50 years of age than those aged 50 years and below. However, no association was found between p53 expression and age of patient. Werness *et al*. also reported that cases with p53 expression are older than those without, and that these tumors are of the serous histological subtype and high-grade tumors.[19]

Comparison of p53 positivity across the different tumor grades showed statistically significant difference. While 78.8% of high-grade carcinomas showed p53 positivity, only 17.6% of low-grade carcinomas showed p53 positivity. Hence, p53 positivity was dependent on tumor grade. This is similar to observations made by other authors.[14,16] The importance of p53 accumulation as a marker of adverse outcome in ovarian carcinoma has been demonstrated in several studies. Expression of p53 is associated with unfavorable prognostic factors such as advanced International Federation of Gynecology and Obstetrics stage, suboptimal cytoreduction, serous histologic subtype, and increasing tumor grade. Nevertheless, its independent prognostic value remains controversial. Some investigators have demonstrated that p53 mutation or overexpression is a significant prognostic factor.[20] Other studies were unable to confirm such results.[17,21]

In this study, p53 positivity was present in 74.2% of serous carcinomas, 35.7% of mucinous carcinomas, and 20% of endometrioid carcinomas. This is comparable to findings by other authors. According to Arik *et al*., 60.7% of serous EOCs were p53 positive.[21] p53 positivity in mucinous EOC varies widely in literature from 25% to 80% of these tumors.[18,20,22,23]

In this study, however, diffuse p53 immunopositivity was most prevalent among serous carcinomas. While 60.9% of serous carcinomas showed diffuse p53 positivity, only 20% of mucinous carcinomas and none of the endometrioid carcinomas showed diffuse p53 positivity. Correspondingly, Yemelyanova *et al*. found that 57.7% of serous EOC showed diffuse p53 expression.[12] Similarly, Sreeja *et al*. reported diffuse p53 positivity in 5% of mucinous EOC.[22]

However, association between p53 immunopositivity and histologic subtype in literature has been controversial. While some investigators have noted a statistically significant correlation between the p53 expression and the serous histological type,[20] others reported that p53 status was not related to histological subtype and observed no difference between serous and nonserous tumors.[17]

It has been noted that, although p53 mutations have been detected in all histological types of EOC, they are more strongly associated with high-grade serous carcinomas.[24] Similarly, 85.2% of Type 2 EOC showed diffuse p53 positivity, whereas only 26.1% of Type 1 EOC showed p53 positivity. These observations are similar to those by Cole *et al*. who observed diffuse p53 positivity in 95.8% of Type 2 EOC.[25]

Conclusion

p53 immunopositivity was found in 58% of EOC, but not in normal ovaries. While p53 immunopositivity was significantly associated with high-grade tumors in general and high-grade serous carcinomas in particular, no association was found between p53 immnoexpression and age of patient.

Overall, these data suggest that the tumor suppressor gene/cell cycle regulator, p53, is mutated in EOC among Nigerian patients. It also supports the dualistic model of ovarian carcinogenesis, in which p53 mutation is important in high-grade serous carcinomas (Type 2 EOC) but not in Type 1 EOC. Hence, ovarian carcinogenesis in Nigerian patients is not different from that reported in literature on their counterparts in other parts of the world.

Recommendation

This study recommends further studies including multicenter studies from other parts of Nigeria in order to determine the true extent of these mutations nationwide as well as intensified research into p53-targeted therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kurman RJ, Carcangiu M, Herrington C, Young R. Tumours of the ovary. In: WHO Classification of Tumours of Female Reproductive Organs. Lyon: IARC Press; 2014. p. 12-3.
2. Madhuri TK, Tailor A, Haagsma B, Coley H, Butler-Manuel S. Relevance of immunohistochemical expression of p57kip2 in epithelial ovarian carcinoma - A systematic literature review. J Ovarian Res 2012;5:46.
3. Verri E, Guglielmini P, Puntoni M, Perdelli L, Papadia A, Lorenzi P, *et al.* HER2/neu oncoprotein overexpression in epithelial ovarian cancer: Evaluation of its prevalence and prognostic significance. Clinical study. Oncology 2005;68:154-61.
4. Ajani M, Salami A, Awolude O, Oluwasola A, Akang E. The expression status of human epidermal growth factor receptor 2 in epithelial ovarian cancer in Ibadan, Nigeria. South Afr J Gynaecol Oncol 2016;8:9-13.
5. Moretti F, Mancini F. p53 Network in Ovarian Cancer. In: Farghaly SA, editor. Ovarian Cancer - Basic Science Perspective. Shanghai: InTech; 2012. p. 271-86.
6. Min K, Park M. The expression of c-erbB-2, EGFR, p53 and Ki-67 in ovarian borderline tumors and carcinomas of the ovary. Korean J Pathol 2007;41:296-306.
7. Shimizu Y, Kamoi S, Amada S, Akiyama F, Silverberg SG. Toward the development of a universal grading system for ovarian epithelial carcinoma: Testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. Cancer 1998;82:893-901.
8. Wang D, Zhu H, Ye Q, Wang C, Xu Y. Prognostic value of KIF2A and HER2-neu overexpression in patients with epithelial ovarian cancer. Medicine (Baltimore) 2016;95:e2803.
9. Richardson RB. P53 mutations associated with aging-related rise in cancer incidence rates. Cell Cycle 2013;12:2468-78.
10. Cai Y, Wang J, Zhang L, Wu D, Yu D, Tian X, *et al.* Expressions of fatty acid synthase and HER2 are correlated with poor prognosis of ovarian cancer. Med Oncol 2015;32:391.
11. Mohammed RA, Makboul R, Elsers DA, Elsaba TM, Thalab AM, Shaaban OM, *et al.* Pattern of HER-2 gene amplification and protein expression in benign, borderline, and malignant ovarian serous and mucinous neoplasms. Int J Gynecol Pathol 2017;36:50-7.
12. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IeM, *et al.* Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: An immunohistochemical and nucleotide sequencing analysis. Mod Pathol 2011;24:1248-53.
13. Hutson R, Ramsdale J, Wells M. P53 protein expression in putative precursor lesions of epithelial ovarian cancer. Histopathology 1995;27:367-71.
14. Chan WY, Cheung KK, Schorge JO, Huang LW, Welch WR, Bell DA, *et al.* Bcl-2 and p53 protein expression, apoptosis, and p53 mutation in human epithelial ovarian cancers. Am J Pathol 2000;156:409-17.
15. Kuhn E, Kurman RJ, Vang R, Sehdev AS, Han G, Soslow R, *et al.* TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma – Evidence supporting the clonal relationship of the two lesions. J Pathol 2012;226:421-6.
16. Kmet LM, Cook LS, Magliocco AM. A review of p53 expression and mutation in human benign, low malignant potential, and invasive epithelial ovarian tumors. Cancer 2003;97:389-404.
17. Skirnisdottir I, Seidal T. Association of p21, p21 p27 and p21 p53 status to histological subtypes and prognosis in low-stage epithelial ovarian cancer. Cancer Genomics Proteomics 2013;10:27-34.
18. Abbasi F, Esmaili A, Yekta Z, Saffarifard A. Expresion of P53 in ovarian epithelial tumours and its correlation with histopathological parameters. J Ayub Med Coll Abbottabad 2016;28:3-6.
19. Werness BA, Freedman AN, Piver MS, Romero-Gutierrez M, Petrow E. Prognostic significance of p53 and p21(waf1/cip1) immunoreactivity in epithelial cancers of the ovary. Gynecol Oncol 1999;75:413-8.
20. Elnashar AT, Hafez AS. Immunohistochemical expression of CD44V6, P53 and BCL-2 in epithelial ovarian tumors. J Am Sci 2012;8:258-64.
21. Arik D, Kulaçoğlu S. P53, bcl-2, and nm23 expressions in serous ovarian tumors: Correlation with the clinical and histopathological parameters. Turk Patoloji Derg 2011;27:38-45.
22. Sreeja TT, Chandrasekhar S, Magar SL, Durga K. 2 years study on p53 expression on serous and mucinous tumors of ovary. Int Arch Integr Med 2016;3:150-8.
23. Kamal CK, Simionescu CE, Mărgăritescu C, Stepan A. P53 and ki67 immunoexpression in mucinous malignant ovarian tumors. Rom J Morphol Embryol 2012;53:799-803.
24. Kupryjańczyk J, Thor AD, Beauchamp R, Merritt V, Edgerton SM, Bell DA, *et al.* P53 gene mutations and protein accumulation in human ovarian cancer. Proc Natl Acad Sci U S A 1993;90:4961-5.
25. Cole AJ, Dwight T, Gill AJ, Dickson KA, Zhu Y, Clarkson A, *et al.* Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. Sci Rep 2016;6:26191.