



# Immunohistochemical Expression of P63 in Ovarian Tumors among Sudanese Women

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Ovarian cancer is a deadly illness with little understanding of its biology. The tumor suppressor p63 is a homolog gene of p53. P63 has an important role in the development and differentiation of reproductive epithelium and interacts with p53 in human tumorigenesis.

**Objective:** The study was aimed at assessing the immunohistochemical expression of p63 in benign and malignant epithelial ovarian tumors.

**Methods:** In this hospital-based analytical retrospective cross-sectional study, we evaluated at p63 immunoexpression in (80) formalin-fixed paraffin-embedded blocks from patients with ovarian tumors; 40 (50%) were malignant, and 40 (50%) were benign. Malignant samples with histological subtypes include 32 (40%) epithelial ovarian cancer samples and 8 (10%) granulosa cell tumors. We used a mouse monoclonal antibody against the p63 antigen (Dako), which identifies all p63 variations, and tumors were designated p63 positive if 5% or more cells showed nuclear immunostaining.

**Results:** We discovered that 90% of the samples expressing the gene were from benign ovarian tumors and 10% were from malignant ovarian tumors, with a p-value of 0.000. In terms of histological subtypes of malignant tumors, p63 expression was found in 7.5% of epithelial ovarian tumors and 2.5% of granulosa cell tumors, with a p-value of 0.792.

**Conclusion:** Patients with ovarian tumors are often older than 50 years old. Epithelial ovarian cancer is the most histologically significant kind of ovarian cancer. p63 expression was low in ovarian cancer, and there was no significant relationship between p63 expression and ovarian tumor subtypes or tumor grade.

*Keywords: Ovarian tumor; immunohistochemical; p63; Sudan.*

## 1. INTRODUCTION

“Tumor protein 63 (p63) is a transcription factor of the p53 gene family, which is located at chromosome 3q28. P63 regulates the activity of many genes involved in the growth and development of the ectoderm and derived structures and tissues, such as basal layer keratins and cell cycle control genes” [1]. “p63 is expressed in basal cell layers of various organs, squamous epithelial cells of many organs, and urothelium” [1–4], “p63 is used routinely for tumor type determination and also differentiates squamous cell carcinoma from adenocarcinoma in lung biopsies, or urothelial carcinoma from renal cell carcinoma, as well as determining the tumor origin of metastases from unknown primary tumors, More than 2000 studies have evaluated p63 expression by IHC in various tumors, leading to quite discrepant p63 positivity rates in several tumor entities” [2,3–16]. For example, the proportion of p63 positive cases in small cell lung cancer ranged from 0 to 77% [6,17], 50 to 100% in squamous cell lung cancer [7,12], 0 to 84% in Merkel cell carcinoma [8,13], 0 to 82% in papillary thyroid carcinoma [9,14], 1.4 to 100% in colorectal adenocarcinoma [5,11], 0 to 100% in urothelial adenocarcinoma [10,15], “In 2016, it is estimated that there will be more than 22,200 new cases of ovarian cancer and more than 14,200 deaths from ovarian cancer in

the United States” [18]. “In the entire Sudan, the incidence rate of ovarian cancer has yet to be identified; however, in hospitals, the National Cancer Institute and the Radiation Isotopes Center in Khartoum, according to a collected, data set (2009–2010), ovarian cancer ranked sixth among all cancers diagnosed and fourth among female cancers” [19].

## 2. METHODOLOGY

From January 2016 to December 2019 (80) Sudanese women with ovarian malignancies who attended RICK were enrolled in this hospital-based analytical retrospective cross-sectional study in Khartoum state at the Radiation & Isotopes-Center of Khartoum (RICK) and Elrahama Medical Center. For the case group, forty (40) paraffin blocks were previously diagnosed as ovarian carcinomas, and forty (40) paraffin blocks were previously diagnosed as benign tumors. The histological subtypes of malignant samples were 32 (40%) epithelial ovarian cancer, 8 (10%) granulosa cell tumors, and the remaining samples were granulosa cell tumors.

### 2.1 Histopathological Tissue Preparation

From each tissue block, two paraffin sections were cut into 3µm thicknesses using a rotary

microtome (Leica), sections were taken on glass slide and covered with diluted alcohol (30%) then the sections floated into a preheated floating water bath at 40°C then sections were placed in coated glass slide for Immunohistochemistry, and other section was placed in the clean microscopic slide for Hematoxylin and Eosin, then incubated in the oven at 58°C for overnight for partial deparaffinization,

## 2.2 Haematoxylin and Eosin

Mounted samples were deparaffinized in xylene; slides were rehydrated through a graded series of alcohol and placed in distilled water for two minutes each. Then samples were stained with Mayer's hematoxylin (RAL-France) for 10 minutes, blued in running tap water for 10 minutes, and then stained with Eosin for 3 minutes, dehydrated through ascending ethanol, cleared in xylene, and mounted in DPX. The samples were confirmed for histological grade by an MD clinical pathologist.

## 2.3 Immunohistochemical Staining

"Immunohistochemical staining was carried out as follows: Tissue sections (3µm) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized slides (Thermo – USA). Following deparaffinization in xylene, slides were rehydrated through a graded series of ethyl alcohol: 100% for 4 min, 90% for 2 min, 70% for 2 min, and water for 2 min. The antigens were retrieved using high PH (9) by water bath (histone-TEC 2601-Italy) at 95°C for 40 minutes. After washing with PBS for 3 min Endogenous peroxidase activity was blocked by a 3% peroxidase blocker (hydrogen peroxide and methanol) for 10 minutes (Dako, USA), and after washing with PBS for 3 minutes, the slides were then incubated with (100µL) of mouse monoclonal antibody (p63, Dako), against p63 antigen for 30 minutes at room temperature in a moisture chamber, then washed in phosphate buffer saline for 3 minutes. The binding of antibodies will be detected by incubating for 20 min with dextran labeled polymer (Dako - USA), then slides washed in three changes of phosphate buffer saline, then incubated in 3,3-diaminobenzidine tetrahydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antigen-antibody complex for up to 5 minutes. After that washed in distilled water for 3 minutes, slides were counterstained in Mayer's hematoxylin (RAL-France) for one minute, then slides were washed

in running tap water for several minutes (7–10) (bluing). After that, it was dehydrated, cleared, and mounted in DPX" [19].

## 3. RESULTS

All quality control procedures were followed. During immunohistochemical staining, positive and negative control slides were employed. Positive p63 staining should be seen as dark brown nuclear staining. If 5% or more of the tumor cells showed nuclear immunostaining, the tumor was identified p63 positive.

### 3.1 Statistics

After examination of the sections, the results of the laboratory investigation as well as the demographic data from the patient's records were processed using Statistical Packages for Social Sciences (SPSS) computer program. Frequency, mean, and chi-square test values were calculated value <0.05 and considered statically significant.

The study includes eighty samples, of which 40 (50%) were malignant tumors and 40 (50%) were benign tumors. The age of the study population ranges between 30 and 79 years, with a mean age of 54 years. Most patients were older than 50 years, representing 48 patients (60%), and the remaining 32 (40%) were younger than 50 years) (Table 1). The histopathological diagnosis of the study population includes 32 (40%) epithelial ovarian cancer, 8 (10%) granulosa cell tumors, and 40 (50%) benign ovarian tumors (Table 2). The tumor grade of study samples revealed 14 (35%) samples were grade I, 8 (20%) samples were Grade, II, and 18(45%) samples were grade III (Table 3). P63 positive expression was found in (4/40) samples (10%) of malignant tumors and (36/40) samples (90%) showed negative expression, while in benign ovarian tumors, positive expression was found in (36/40) samples (90%) and (4/40) samples (10%) showed negative expression for P63. This result revealed showed significant association (*P. value* = 0.000) (Table 4). The relation between p63 expression and types of the malignant tumor showed positive expression of p63 in 3 (7.5%) samples of epithelial ovarian tumors, only one (2.5%) sample of granulosa cell tumor, with no association between p63 expression and type of malignant tumor (*P. value* =0.792) (Table 5). p63 positive expression was found in 4 (10%) samples of grade I, while negative expression revealed 10 (25%) samples of grade I, 8 (20%)

samples of grade II, and 18 (45%) samples of grade III. This result showed no significant association between p63 expression and tumor grade (*P. value* = 0.06) (Table 6).

#### 4. DISCUSSION

The current investigation included 80 ovarian tumors stained for p63 by immunohistochemistry, 40 of which were benign and the remaining 40 were malignant. “Concerning the age group of the study population, the study showed that the majority of patients were older than 50 years, indicating that women older than 50 are more affected by ovarian cancer. This result is

compatible with DO Abuidris *et al.*, who reported that the incidence rate of ovarian cancer increased greatly in women aged 55 years or older. I also agree with Jermaine *et al.*, who reported that the risk of developing ovarian cancer increases with age” [20]. and disagreeing with Dafalla, *et al.*, who reported that “ovarian malignancies tend to occur at younger ages in Sudan” [19]. The histopathological diagnosis of the study population showed that epithelial ovarian cancer was the more frequent type of ovarian cancer. This result is compatible with Poli Neto *et al.*, who reported that “epithelial tumors represent more than 85% of all ovarian malignancies” [3]. “In this study, we observed

**Table 1. Distribution of age among the study population**

Age group	Frequency	Percentage
50 and more than 50 years	48	60%
Less than 50 years	32	40%
Total	80	100%

**Table 2. Distribution of histopathological diagnosis among the study population**

Histopathological diagnosis	Type	Frequency	Percentage
Benign	Benign ovarian tumor	40	50%
Malignant	Epithelial ovarian cancer	32	40%
	Granulosa cell tumor	8	10%
Total		80	100%

**Table 3. Distribution of malignant tumor grade**

Grade	Frequency	Percentage
Grade I	14	35%
Grade II	8	20%
Grade III	18	45%
Total	40	100%

**Table 4. Relation between histopathological diagnosis and p63 expression**

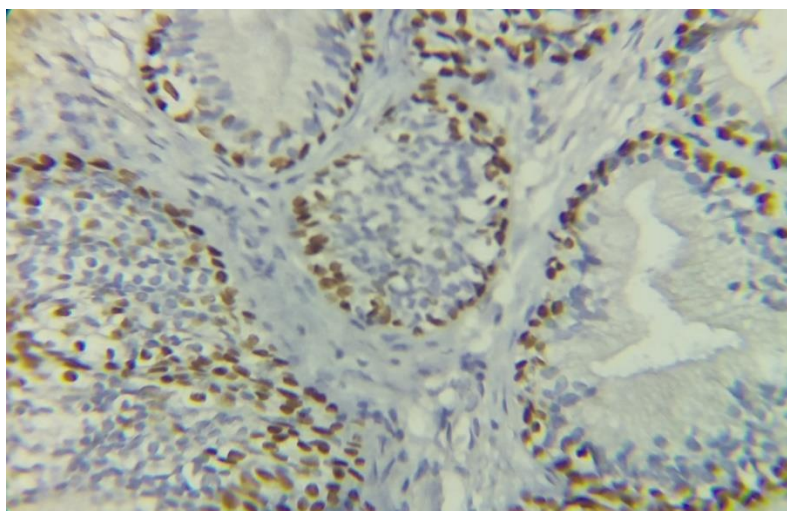
Variables	P63 expression		P. value
Histopathological diagnosis	Positive N (%)	Negative N (%)	
Benign	36 (90%)	4 (10%)	0.000
Malignant	4 (10%)	36 (90%)	
Total	40(100%)	40 (100%)	

**Table 5. Relation between p63 expression and types of malignant tumor**

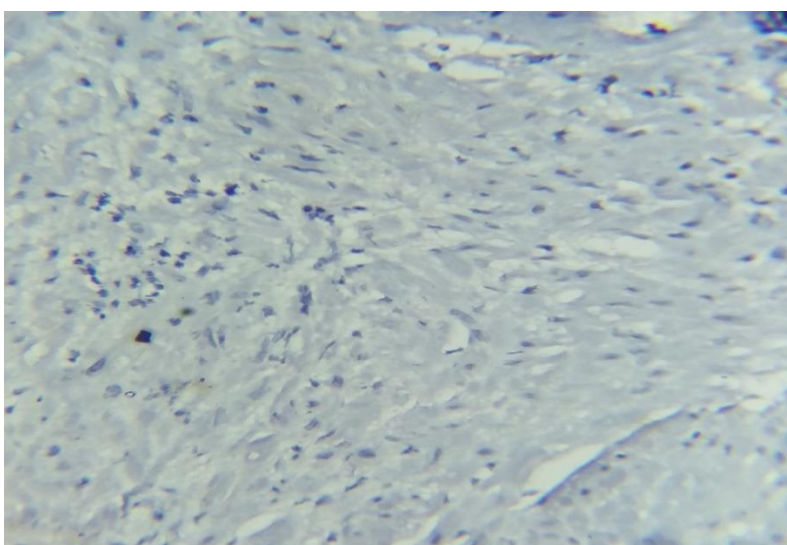
Variables	Type of malignant tumor			Total	P. value
P63 expression	Epithelial cancer	ovarian	Granulosa cell tumor		
Positive	3		1	4(10%)	0.792
Negative	29		7	36(90%)	
Total	32		8	40(100%)	

**Table 6. Relation between p63 expression and ovarian cancer grade**

P63 expression	Tumor grade			Total	P. value
	Grade I	Grade II	Grade III		
Positive	4 (10%)	0 (0.0%)	0 (0.0%)	4 (10%)	0.06
Negative	10 (25%)	8 (20%)	18 (45%)	36 (90%)	
Total	14 (35%)	8 (20%)	18 (45%)	40 (100%)	



**Fig. 1. serous cystadenoma showed positive expression of p63 (40X)**



**Fig. 2. Serous cystadenocarcinoma showed negative expression of p63 (40X)**

strong p63 nuclear staining in 4 of malignant conditions compared to 36 of 40 benign conditions ( $P = 0.000$ ), which suggests that p63 expression is more frequently expressed in benign conditions. This result agrees with that of Poli Neto, *et al.*, who reported that 85.7% of positivity was in benign tumors, 50% in borderline tumors, and 8.7% in invasive ovarian cancer ( $P.0001$ )” [2]. A similar pattern of immunostaining has been reported by

several groups [2,21], whose results showed that “most of the ovarian carcinomas were p63 negative”. The present study revealed that there was no significant association between p63 expression and the type of malignant tumor. This finding was in agreement with Reis-Filho, *et al.* [22-28] who reported that “no association was found between the histological type and the expression of p63 in ovarian neoplasm” [21].

## 5. CONCLUSION

Patients with ovarian tumors are often older than 50 years old. Epithelial ovarian cancer is the most histologically significant kind of ovarian cancer. p63 expression was low in ovarian cancer, and there was no significant relationship between p63 expression and ovarian tumor subtypes or tumor grade.

## CONSENT AND ETHICAL APPROVAL

Ethical approval for the study was obtained from the Board of the Faculty of medical laboratories sciences, at University of Al-Neelain. The written informed consent form was obtained from each guardian of the participant as well as from the subject himself before recruitment into the study. All protocols in this study were done according to the Declaration of Helsinki (1964).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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