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Gynecology

ROLE OF P65 AND P53 EXPRESSION IN DIFFERENT STAGE AND GRADE OF OVARIAN CANCER

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ABSTRACT

Introduction: Incidence of ovarian cancer is increasing in female population in urban India. Till now most of the treatments are guided by the tumor parameters and many occasions the results of treatment have been unpredictable. This study aims to various molecular factors and will try to link then with the tumor factors. This study was evaluated prognostic significance of like p65 (NF-κB) and p53 expression in ovarian cancer patients.

Material and Methods: In this hospital based study 106 ovarian cancer patients attend at OPD in our institute of Eastern India. NF-κB and p53 protein expression was measured from cancer tissue sample by both Western Blot and RT-PCR Technique. For statistical analysis data were entered into a Microsoft excel spreadsheet and then anlysed by SPSS 20.0.1 and GraphPad Prism version 5.

Results: NF-κB was overexpressed with 66(66.2%) patients and p53 was activated with 69(65.21%) patients in ovarian carcinoma in Indian population. Statistically significant associations of p65 (NF-κB) and p53 positive tumors were found in advance stage, grade, histopathology type and lymph node metastasis.

Conclusions: It was concluded NF-κB and p53 is an independent prognostic marker of Indian ovarian cancer. Thus the study can in a pioneering work in establishing new risk stratification system which will be an importance selecting appropriate adjuvant therapy following surgery. No such host-tumor integrating study has come out from this subcontinent and therefore is be of importance in establishing ovarian cancer patient's guideline for general Indian population.

KEYWORDS : .Ovarian cancer, p53, p65, Prognostic marker, Stage

Introduction:

Ovariancancer is one of the most common leading gynecological cancer in worldwide as well as India. (Age standardized incidence rate: 6.6/100000). Ovarian cancer has poor prognosis among all gynecological carcinoma [1]. The overall survival for five years is approximately 45% due to the advance stage at the time of diagnosis [2]. In Indian scenario ovarian cancer is third leading side of cancer among female next to cervical and breast cancer [1, 3]. Survival of ovarian cancer depends on various pathological and molecular factors [4, 5, 6, 7]. But nothing has been proved conclusive to be the best for overall survival of ovarian cancer. Prognosis of ovarian cancer can be depended on clinical factors, histopathological parameters and molecular based markers. Clinical factors include age, menopausal status and stage of the disease at diagnosis (FIGO). Histopathological parameters are type of carcinoma, tumor size, grade and lymph node metastasis. Several studies have reported that there are various prognostic markers in ovarian cancer [5,6,7].The nuclear factor-κB (NF-κB) family of genes are associated with cell proliferation, angiogenesis, metastasis, oncogenesis and survival of ovarian cancer [8, 9]. Some studies demonstrated that the association between NF-κB and tumor progression commonly involved the p65 subunit [11-13]. NF-κB regulates over 500 genes involved in cellular transformation, survival, proliferation, invasion, angiogenesis, metastasis, and inflammation. The NF-κB signaling pathway has become a potential therapeutic target [14]. p53 expression is regulated by NF-κB. Activation of NF-κB signaling pathway leads to the induction of target genes that can inhibit the apoptosis, interact with cell cycle regulation, cell invasion, contribute to tumourigenesis, inflammation and metastatic growth as well as chemo resistance and radio resistance in [15]. Activation of NF-κB interacts with P53 mediated by up regulating anti-apoptotic genes and down regulating p53 levels with damaging survival cell. Recent studies repotted that p53 and NF-κB are commonly mutated in variety of cancer [15]. The NF-κB/REL family of transcription factors is included of a RELA/p65, c-REL, RELB, p105/NF-κB1 and p100/NF-κB2 [10]. The members of this family are characterized by the presence of a REL homology domain (RHD) in the N-terminus, which is complex in sequence-specific DNA binding and translocation. The C-terminal regions of these proteins have domains responsible for either transcriptional activation (RELA, c-REL and RELB) or the inhibition of REL protein activity (p105 and p100). The p105 and p100 proteins can be processed by proteolytic cleavage into p50 and p52, respectively. NF-κB interferes with the transcriptional movement of p53, an important determinant of stress- or DNA damage-induced apoptosis. Common transcriptional antagonism between p53 and NF-κB includes competition for a regulating pool of their joint transcriptional coactivators, p300 and CREB-binding protein [23]. p53 also suppresses the expression of Bcl-3, a transcriptional coactivator of p52/ NF-κB, so switching p52/Bcl-3 activator centers to p52/HDAC1 repressor complexes [24]. Such transcriptional interference has also been attributed to a direct interaction between p53 and RelA and NF-κB -mediated phosphorylation of p53 [25]. Finally, IKK2-mediated activation of NF-κB has been shown to protect cells from DNA damage-induced apoptosis by increasing Mdm2 levels and limiting the stabilization of p53 [26]. Irrespective of the specific mechanism(s) elaborated, the mutual antagonism between NF-κB and p53 may regulate the balance between the expression of pro-apoptotic and survival genes. This study aims to validate the role of activation of NF-κB/p65 as a prognostic

marker in patients with ovarian cancer in Indian subcontinent. This study aimed to find any association, if any, between the p53 and NF-κB/p65 among the parameters such as age, menstrual status, stage of the disease, histopathology type, tumor size, grade and lymph node metastasis in ovarian cancer and was conducted for the first time with the ovarian cancer patients of Eastern India. The study was undertaken in the Institute of Post Graduate Medical Education & Research (IPGME&R) and Seth Sukhlal Karnani Memorial Hospital (SSKM), Kolkata, India.

Materials and methods:

Patient selection:

The patients were divided into two groups, first group (Group-A) comprised of 106 female patients with ovarian carcinoma previous untreated by chemotherapy, radiotherapy, hormone therapy or a combination of any of the modalities who presented at outpatient door in our institute, Kolkata, West Bengal, India between 2014 to 2016 were included in this research work. 32 female patients who were histological and clinically benign ovarian disease treated as control group (Group-B). One part of the tissue ovarian carcinoma tumors were fixed in 10% neutral-buffered formalin for 24 h, measured the tumor size, nodal status, grade and embedded in paraffin and sectioned. All patients were followed up for a period of five years. Information of these patients was maintained in the department of G & O in this institute. All patients were treated with standard therapeutic protocol like surgery followed by chemotherapy /radiotherapy as appropriate.

Tissue Processing:

Another part of the ovarian cancer tissue, the specimens were washed with phosphate buffered saline (PBS), cut into small pieces and immersed in collagenase at 37°C for 4-6 hrs. Collagenase incubated tissue was minced and treated with 0.125% trypsin-EDTA for 10 min. Total protein was extracted by homogenizing cells in ripa : lysis buffer mixture (1:3) at 4°C and measured spectrophotometrically by Lowry's method.

Western Blot analysis:

For whole cell lysates, cells were resuspended and homogenized in buffer (100mM Tris-Cl, pH 7.4, 300mM NaCl, 1% NP-40, and 0.25% sodium-deoxycholate). All the buffers were supplemented with protease and phosphatase inhibitor mixtures. For direct Western blot analysis, the cell lysates or the particular fractions were separated by SDS-PAGE, transferred to nitrocellulose membrane (Amersham Hybond-P, GE Healthcare) and probed with specific antibodies, e.g., anti –p65(NF-κB) and anti –p53 produced from Santa Cruz thereafter the immunoblots were visualized by chemiluminescence or alkaline phosphatase method. Equal protein loading was confirmed with α-actin antibody (Santa Cruz).

Statistical Analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 20.0.1 and GraphPad Prism version 5. Without other qualification, 'chi-squared test' often was used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

Results:

In present study, NF-κB was overexpressed with 66(66.2%) patients and p53 was activated with 69(65.21%) patients in ovarian carcinoma but both NF-κB and p53 was not activated in benign ovarian tumor. It was found that 73(68.9%) patients were in age group >40 years whereas 33(31.1%) patients were in age group ≤40 years. 69 (65.1%) patients were in premenopausal women but 37(34.9%) patients were in postmenopausal women. In present study, it was showed that 11(10.4%) patients had in stage –I, 17(16.0%) patients had in stage-II, 61(57.5%) patients had in stage-III and 17(16.0%) patients had in stage-IV.

As per table-1, 20(60.6%) NF-κB positive patients were in ≤40 years age group and 46(63.0%) NF-κB positive patients were in >40 years age group but this was not statistically significant (p=0.8128). It was showed that 44(66.7%) premenopausal patients and 20(54.1%) postmenopausal patients were in NF-κB positive tumor but this was not statistically significant (p=0.2016). According to clinical staging, NF-κB positive tumors were found in 2 (18.2%) tumors with stage I, 3(17.6%) tumors with stage II, 46(75.4%) tumors with stage III and 15(88.2%) tumors with stage IV. This association was statistically significant (p<0.0001).

In table-2, According to histopathological grading, 2(18.2%) in grade-I, 9(34.6%) in grade-II and 55(79.7%) in grade-III tumors were in NF-κB Positive. Over expression of NF-κB was increased with higher grade and which was statistically significant (p<0.0001). NF-κB was activated 10(90.9%) tumor with Clear Cell Carcinoma, 14(53.8%) tumor with Mucinous Carcinoma and 42(71.2%) tumor with Serous Carcinoma. This association was statistically significant (p<0.0001). 2(50.0%) patients with T1 tumors, 30(52.6%) patients with T2 tumors and 34(75.6%) with T3 tumors were NF-κB positive but this was not statistically significant (p=0.0526). Patients with lymph node negative tumors 5(19.2%) and lymph node positive tumors 61(76.3%) were found with NF-κB positive tumors and this association was statistically significant (p<0.001).

As per table-3, 22(66.7%) p53 positive patients had in age group ≤40 years and 47(64.4%) p53 positive patients had in age group >40 years but this was not statistically significant (p=0.8193). 47(68.1%) premenopausal patients and 22(59.5%) postmenopausal patients were in p53 positive tumors but this was not statistically significant (p=0.3728). In the p53 positive tumors, 3(27.3%) had stage I, 5(29.4%) had stage II, 47(77.0%) had stage III and 14(82.4%) had stage IV disease. This association was statistically significant (p < 0.0001).

In table-4: In grade I, 3(27.3%) out of 11 patients, in grade II 11 (42.3%) out of 26 patients, in grade III 55(79.7%) out of 69 patients were p53 positive which was statistically significant (p=0.0001). According to histopathology type of ovarian carcinoma, 1(9.1%) patient was Clear Cell Carcinoma, 1(10.0%) patient was Endometrioid Carcinoma, 16(61.5%) patients were Mucinous Carcinoma and 51(86.4%) were Serous Carcinoma with p53 positive and this association was statistically significant (p<0.0001). p53 was activated in 3(75.0%), 34(59.6%) and 32(71.1%) patients with T1, T2 and T3 tumor size respectively but this was not statistically significant (p=0.4419). Patients with lymph node negative tumors 9(34.6%) and lymph node positive tumors 60(75.0%) were found with p53 positive tumors and this association was statistically significant (p<0.0001).

Discussion & Conclusion:

In this study, activation of NF-κB and p53 was higher proportion (66.2% and 65.21%) in ovarian carcinoma but both NF-κB and p53 was not activated in benign ovarian tumor. Various study reported that there are various prognostic factors in survival for ovarian cancer [4, 5, 6, and 7]. In present study, high grade tumor was associated with poor prognosis and a large proportion of these tumors were observed to be NF-κB positive. There was strong association between lymph node metastasis among p53 positive tumors and NF-κB activation. NF-κB positive tumors showed higher number in T3 tumor. NF-κB positivity was observed in advanced stage of disease like stage-III and stage-IV. Activation of p53 was directly related with NF-κB positive tumors. This implies that NF-κB is associated with aggressive tumor biology such as large T-Size, high grade and poor differentiation. D. Jana et al suggested that overexpression NF-κB was poor prognostic marker and also associated with higher grade, advance stage, large tumor size and more lymph node metastasis in breast cancer [16]. It was found that activation of NF-κB was significantly associated with advanced stage, (stage-III and stage-IV) in ovarian cancer. Christina M at al demonstrates that role of NF-κB progression was correlated with advanced ovarian cancer [17]. Various study reported that NF-κB was a poor prognostic marker in ovarian cancer [18, 19, 20]. No correlation was found between NF-κB expression vs age at diagnosis. Use of several compounds for inhibition of NF-κB activity was an important role in treatment and prevention of various cancers [21]. Activation of p53 was associated with high grade and advance stage in epithelial ovarian carcinoma. Lynn C at al found that p53 expression was significantly correlated with decrease overall survival by using univarate analysis [22]. So abnormality of p53 was found commonly in patients with ovarian carcinoma in our scenario. NF-κB promotes tumor genesis, cell cycle progression, angiogenesis, proliferation and survival of ovarian cancer [16]. NF-κB expression was directly correlated with advance stage, higher grade, large tumor size, and more limb node metastasis, which implies a poor prognosis. High level of p53 and NF-κB were associated with poor overall survival in ovarian cancer patients. The key role of NF-κB signaling is regulating the differentiation of the ovarian cancer cell: coupled with the fact that dysregulation of this pathway is associated with adverse clinico pathological parameters, making it a potential target for anti-cancer therapeutic application.

NF-κB and p53 overexpression was associated with large tumor size, high grade, positive lymph node metastasis and Serous Carcinoma which is poor prognostic outcome of the disease. We concluded that p65 and p53expression was significantly correlated with advance stage that means poor overall survival. Inhibition NF-κB overexpression may reduction tumor advancement in patients and may block carcinogenesis, reducing the incidence of ovarian carcinoma in patients at high risk. In conclusion, Activation of NF-κB implies aggressive tumor biology with p53 mediated in ovarian carcinoma & it can predict tumors likely to have poor prognosis. Patients with NF-κB and p53 positive tumors need to be treated aggressively in clinical practice.

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Conflict of Interest:
The authors have stated that they have no conflict of interest.

Table-1: Clinical details according to NF-κB status

Table:1		NF-κB Negative		NF-κB Positive		p-Value
		Number	Percentage	Number	Percentage	
Age (Years)	≤40	13	39.4	20	60.6	0.8128
	>40	27	37.0	46	63.0	
Menopausal Status	Pre	23	33.3	46	66.7	0.2016
	Post	17	45.9	20	54.1	
Stage	I	9	81.8	2	18.2	<0.0001
	II	14	82.4	3	17.6	
	III	15	24.6	46	75.4	
	IV	2	11.8	15	88.2	

Table-2: Association between Clinical details according to NF-κB status

Table:2		NF-κB Negative		NF-κB Positive		p-Value
		Number	Percentage	Number	Percentage	
Grade	I	9	81.8	2	18.2	<0.0001
	II	17	65.4	9	34.6	
	III	14	20.3	55	79.7	
Histopathology Type	Clear Cell Carcinoma	1	9.1	10	90.9	<0.0001
	Endometrioid Carcinoma	10	100.0	0	0.0	
	Mucinous Carcinoma	12	46.2	14	53.8	
	Serous Carcinoma	17	28.8	42	71.2	
Tumor Size	T1	2	50.0	2	50.0	0.0526
	T2	27	47.4	30	52.6	
	T3	11	24.4	34	75.6	
Nodal Status	Negative	21	80.8	5	19.2	<0.0001
	Positive	19	23.8	61	76.3	

Table-3: Relationship between pathological details and p53 status

Table:3		p53 Negative		p53 Positive		p-Value
		Number	Percentage	Number	Percentage	
Age (Years)	≤40	11	33.3	22	66.7	0.8193
	>40	26	35.6	47	64.4	
Menopausal Status	Pre	22	31.9	47	68.1	0.3728
	Post	15	40.5	22	59.5	
Stage	I	8	72.7	3	27.3	0.0001
	II	12	70.6	5	29.4	
	III	14	23.0	47	77.0	
	IV	3	17.6	14	82.4	

Table-4: Relationship between pathological parameters and p53 status

Table:4		p53 Negative		p53 Positive		p-Value
		Number	Percentage	Number	Percentage	
Grade	I	8	72.7	3	27.3	0.0001
	II	15	57.7	11	42.3	
	III	14	20.3	55	79.7	
Histopathology Type	Clear Cell Carcinoma	10	90.9	1	9.1	<0.0001
	Endometrioid Carcinoma	9	90.0	1	10.0	
	Mucinous Carcinoma	10	38.5	16	61.5	
	Serous Carcinoma	8	13.6	51	86.4	
Tumor Size	T1	1	25.0	3	75.0	0.4419
	T2	23	40.4	34	59.6	
	T3	13	28.9	32	71.1	
Nodal Status	Negative	17	65.4	9	34.6	<0.0001
	Positive	20	25.0	60	75.0	

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