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THE COLLATION ONTOGENESIS BETWEEN P53 AND BCl-2 MARKERS IN THE PROGNOSIS OF BENIGN SURFACE EPITHELIAL TUMOURS OF OVARY

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ABSTRACT Ovarian cancer is the most colloquial cause of death among all gynecologic malignancies and 90 % are of epithelial origin that undergo multiple genetic alterations during malignant transformation of these epithelial cells. Bcl-2 and p53 play a crucial role in tumor formation.The relationship between Bcl-2 and p53 gene expression in benign ovarian surface epithelial tumors gives the progression of a benign lesion undergoing metamorphosis to a malignant Form .The Study Design for our research article November 2012 – December 2015. All benign surface epithelial tumors of the ovary , biopsies as well as resected specimens are included in this study. Statistical Methods like Sensitivity, Specificity And Chi Square Testwere used. Routine H & E stained slides were studied under light microscope and after the cases diagnosed as tumours they are classified according to WHO classification into benign, borderline and malignant .All the cases were subjected to Bcl-2 and p53 immunohistochemical staining using Novocastra Lyophilized Mouse Monoclonal Antibody Bcl-2 and p53 protein for their expression.Strong expression of Bcl-2 in epithelium and stroma was seen in all benign and borderline tumors except few case each of mucinous cystadenoma.p53 expression was negative both in the epithelium and stroma in all benign tumors except few cases of mucinous cystadenoma (mild staining in the epithelium alone). Positive p53 in epithelium can be taken as reliable criteria of malignancy and Bcl-2 positivity can be relied upon to differentiate between benign , borderline and malignant tumors.Both the markers tend to show an inverse correlation in benign surface epithelial tumours of ovary.

KEYWORDS : Benign Surface Epithelial Tumours p53 Bcl-2

INTRODUCTION:

Ovarian cancer is the most common cause of death among all gynecologic malignancies and 90 % are of epithelial origin that undergo multiple genetic alterations during malignant transformation of these epithelial cells. Bcl-2 and p53 play a crucial role in tumor formation.[1,2]Mutation of p53 gene 3,4and Bcl-2 gene [5] is associated with many cancers including Ovarian cancers which we have studied in the present research. The mechanism of action of the Bcl-2 protein has not been fully defined but may involve oxidative phosphorylation and/or mitochondrial electron and metabolite transport , and its main effect is to prolong cell survival by avoidance of apoptosis [6-8].Stem cells in epithelia, neurones, and memory B cells all expressBcl-2. In epithelial tissues, which are continually renewed, Bcl-2 expression is seen in the basal layers of the epithelium but is lost as cells approach the surface of the epithelium prior to undergoingapoptosis [9]. Bcl-2 is also expressed in glandular cells, such as those in the female breast, in which regulation of hyperplasia and involutionis controlled by hormones and growth factors [10]. The p53 protein is a transcriptional activator that binds to specificDNA sequences in the control regions of genes, influencing their expression[18]. This leads to expression of specific genes necessary for inhibition of cell growth or, alternatively, apoptosis [19]. Alterations of p53 activity, either as a result of point mutations or deletionsor due to protein stabilization in the absence of obvious genetic changes, are the most frequent abnormalities seen in human cancers[18,19] .These alterations lead to the loss of wild-type p53 function and may thus allow uncontrolled growth of damaged cells. Indeed, accumulation of the protein has been shown to be a prognostic marker of reduced survival in breast, gastric, and non-small cell lung cancer [19,20]. Key regulator of several crucial processes determining cell fate is the tumour suppressor protein p53 . Also, the p53-encoding gene,*p53*, is the gene most often found mutated in ovarian cancer (Cho & Shih 2009).However, mutations are only a part – albeit an important one – of the whole puzzle. Studies have shown that altered expression or regulation of other members of the p53 pathway can also lead to responses similar to those seen with mutations of *p53* that abolish p53 function. For molecular profiling to guide thechoice of chemotherapeutic drugs, the molecular responses and factors behind the activity of a specific drug must also be understood. The knowledge gained by some of the studies published in the literature does not confirm the correlation between p53 and Bcl-2 in diagnosing benign, borderline and malignant surface epithelial tumours of the ovary. The present study in addition to the importance of p53 and Bcl-2 in differentiating these tumours there is an attempt to know whether this knowledge can help in the treatment .Not many research papers are published in this regard. No Indian study has been done on this matter.

Materials and Methods:

The study period was from November 2012 – May 2014.The Inclusion Critireon was , all benign surface epithelial tumors of the ovary , biopsies as well as resected specimens are included in this study. The sample size was 50 cases .Statistical Methods Like Sensitivity, Specificity And Chi Square test were done. All tissues were collected in 10 %formalin .Paraffin embeded blocks were used to prepare the sections of 4 micron thickness. Routine H & E stained slides were studied under light microscope and after the cases diagnosed as tumours they are classified according to WHO classification into benign, borderline and malignant .All the cases were subjected to Bcl-2 and p53 immunohistochemical staining using Novocastra Lyophilized Mouse Monoclonal Antibody . Bcl-2 and p53 protein for their optimum expression were subjected to High temperature antigen unmasking technique for immunohistochemical demonstration on Paraffin sections.Unmasking solution used was made by boiling 0.01 M citrate buffer pH 6.0 (pressure cooker method) after blocking endogenous peroxidase with 0.5% hydrogen peroxide/ methanol. This was followed by protein block and incubation with primary antibody , post-primary block , treatment with biotinylated secondary antibody, DAB & staining with Mayer's haematoxylin in that order. Following antigen retrieval, sectionswere rinsed in TBS.Novolink Polymer Detection Tecnique was followed Draining of excess TBS & block endogenous peroxidase activity for 5 mins (using Peroxidase Block RE7101)was done followed by Washing in TBS(Tris -buffered saline (abbreviated TBS) for 5 mins followed by Incubation in protein block (RE 7102) (. ... Before using antibodies to detect proteins by immunohistochemistry) for 5 mins,further on Washed in TBS.Application of optimally diluted primary antibody for 60 mins is done,followed by Washing slides in TBS. Incubation

with post primary block for 30 mins and Washing slides in TBS was done.Incubation with NOVOLINK polymer for 30 minutesand than slides were washed in TBS.Incubation in freshly prepared DAB (DAB peroxidase substrate kit, 3,3'-diaminobenzidine) solution for 10 mins was done followed by Rinsing in TBS & transfer to running water. Counterstaining in hematoxylin , dehydrating , clearing and mounting was followed.Written consent of patients and ethical clearance was taken before starting the research ,as it was a part of thesis studies.

RESULTS

Strong expression of Bcl-2 in epithelium and stroma was seen in all(45) benign tumors except five cases of mucinous cystadenoma .p53 expression was negative both in the epithelium and stroma in all benign tumors except five cases of mucinous cystadenoma (miild to moderate staining in the epithelium alone).(FIG 1)

EXPRESSION OF Bcl-2 IN BENIGN TUMOURS: Strong expression of Bcl-2 in epithelium and stroma was seen in 32(92%) benign cases except 8 cases of serous cystadenoma 8(4%) which was were mildle staining/ negative for Bcl-2 and (4%) cases of mucinous cystadenoma where there was decreased intensity.(FIG 3)

EXPRESSION OF p53 IN BENIGN TUMOURS:p53 expression was negative both in the epithelium and stroma in all benign tumours except five case of mucinous cystadenoma where there was weak staining in the epithelium alone.**(FIG2)**

FIGURE 1

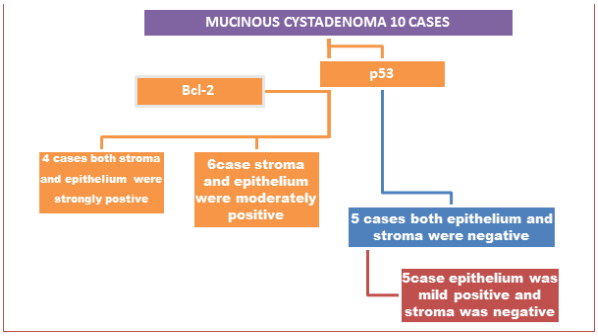

Figure 2:P53 NEGATIVE IN BOTH STROMA AND EPITHELIUM

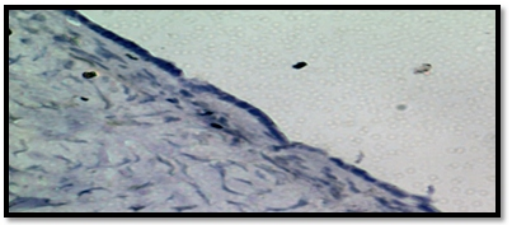

Figure 3:BCL-2 POSITIVE IN BOTH STROMA AND EPITHELIUM

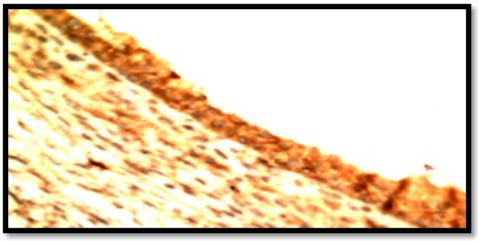

Table no. 2 :Location of benign surface

Sl.no.	Side of involvement of ovary	Benign
1	BILATERAL	02(4%)
2	LEFT	20(40%)
3	RIGHT	28(56%)
4	Total	50(100%)

Table no.3 -Histological types of benign tumours:

Histological types	No. of cases	Percentage
Serous cystadenoma	28/50	56%
Papillary cystadenoma	12/50	24%
Mucinous cystadenoma	10/50	20%
Total	50	100%

STATISTICS: In this study we calculated sensitivity and specificity for both the markers that is Bcl-2 and p53 by using following formulas:

Sensitivity = True Positives(TP)

True Positives (TP) + False Negatives (FN)

Specificity = True Negatives (TN)

True Negatives (TN) + False Positives (FP)

Table no.4: Sensitivity and Specificity of Bcl-2 in detecting benign cases:

MARKER	BENIGN	BORDERLINE+ MALIGNANT(control)
Bcl-2+	**48(TP)**	**16(FP)**
Bcl-2-	**02(FN)**	**01(TN)**

Sensitivity= (TP/TP +FN) X 100= (48/48+2) X100
= 96 %
Specificity= (TN/TN+FP) X100= (2/1+16) X100
=5.89%

Table no 5: Sensitivity and Specificity of p53 in detecting benign surface epithelial tumours:

MARKER	Benign	Borderline +Malignant
p53+	**5 (TP)**	**15 (FP)**
p53-	**45 (FN)**	**02 (TN)**

Sensitivity= (TP/TP +FN) X100 = (5/45+5) X 100
= 10%
Specificity = (TN / TN+ FP) X 100= (2 / 2+15) X100
= 11.76 %

The sensitivity of p53 was very low as well as specificity in benign surface epithelial tumours.

Table no. 6 - Sensitivity and Specificity of Bcl-2 and p53 to detect Benign tumours:

Marker	Sensitivity	Specificity
Bcl-2	**96%**	**5.89%**
p53	**10%**	**11.76%**

Bcl-2 is a highly sensitive marker in benign surface epithelial tumours.p53 as a marker for benign surface epithelial tumours does not hold well.Chi square = 19.4% with p value of less than 0.0005 which is significant and shows a reciprocal or indirect /inverse correlation between Bcl-2 and p53 staining in benign surface epithelial tumours of ovary.(TABLE6)

Statically the conclusion favours the significant use of Bcl-2 in the diagnosis of benign ovarian tumours .In total there is an increase in expression of p53 in more aggressive and malignant tumours wheras bcl-2 expression is seen in the benign counterparts.

Discussion: The efficacy of cancer chemotherapy is restricted by the ability of the tumours to resist or develop resistance to treatment. Ovarian cancers show high response rates to first line chemothe rapy but are characterized by recurrence and the development of resistance to chemotherapy. Therefore, prognosis is poor, with only a minority of patients surviving 5 years.

Resistance to chemotherapy has been associated with decreased susceptibility to apoptosis[16,17] raising the possibility that cell death

determinants may influence the outcome of treatment. The Bcl-2 gene, the first negative regulator of cell death to be identified, was discovered through the t (14:18) translocation, which frequently occurs in B cell Lymphomas.[17]

P53 protein is a transcriptional activator that binds to specific DNA sequences in the control region of genes, influencing their expression.[18] This leads to expression of specific genes necessary for inhibition of cell growth or, alternatively apoptosis.

Alternations of p53 activity, either as a result of point mutations or deletions or due to protein stabilization in the absence of obvious genetic changes, are the most frequent abnormalities seen in human cancers.[19,20]

p53 protein is overexpressed in 50-60% of ovarian cancers.[21-25] Restoration of the function of p53 in tumor cells is one of the therapeutic approach. Important progress has been made recently in this field, using viral and non viral vectors[12, 13], or p53 activating peptides[12,27] seems an attractive target for cancer immunother apy. Henriksen R et al 2 studied Bcl-2 expression in 12 cases of benign epithelial tumours found 83.3 %(10/12) cases with positive expression ,In comparison, our study showed 96% Bcl-2 positivity in benign tumours which is almost correlating with Henriksen R et al study result .Chan WY et al studied Bcl-2 in 11 cases of benign surface epithelial tumours where he found 100 % Bcl-2 positivity,So there is correlation between all 3 studies in benign tumours. Our study showed p53 expression in 10 % cases of benign. Chan WY et al 7 found 18% positive benign cases . Torre FJ et al 8 found low nuclear immunoreactivity (less than 25% tumour cells) in 71% of benign tumours.No cases of benign showed high nuclear immunoreactivity (75% of cells) in all three studies mentioned above. Thus immunohistochemically and statistically p53 appears to be a promising marker with better specificity and sensitivity and significance in distinguishing benign, borderline and malignant tumours in comparison to Bcl-2 protein which does not stand as a reliable marker for distinguishing benign, borderline and malignant surface epithelial tumours. The results of this study can be utilized especially p53 over expression in ovarian carcinomas for the immunotherapy and an inverse correlation between the two can be used for the same.

Conclusion:Positive p53 in epithelium can be taken as reliable criteria of malignancy and Bcl-2 positivity can be relied upon to differentiate between benign , borderline and malignant tumors.Mild staining intensity for p53 in the epithelium in mucinous cystadenoma may suggest possibility of its progressing into malignant tumor .The above information can be utilized especially p53 overexpression in ovarian carcinomas for the immunotherapy and the inverse relation between the two markers in benign surface epithelial tumours can be taken in consideration .

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