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Can Caspase-3 act as a potential prognostic biomarker in breast cancer? - A retrospective pilot study in central India

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Abstract

Background: Caspase-3 is involved in the apoptosis induced by many agents, including antineoplastic drugs in cancers. Loss of caspase-3 expression and activity has been reported in primary human breast cancers. It is suggested that it might promote tumour development by preventing and reducing senescence. It was a retrospective observational pilot study aimed to evaluate the prognostic potential role of caspase-3 in breast cancer patients.

Method: It was a retrospective observational pilot study in which fifty breast cancer patients were enrolled. Haematoxylin and eosin-stained slides of the cases were screened to obtain the best paraffin block for IHC, on which immunohistochemical expression of caspase-3 marker was done and studied. It was correlated with the known prognostic markers of breast cancer.

Results: Out of 21 cases with moderate caspase-3 staining, 85.7% cases were grade III and 14.2% were grade II. From 16 cases with strong caspase-3 staining, 75% cases were grading III followed by 25% cases of grade II. There was no significant correlation between caspase-3 staining with histological grade, size of tumour, lymph node involvement, ER, PR and Her2/neu status (p=0.68, 0.24, 0.20, 0.68, 0.45 and 0.11 respectively). Correlation of survival was significantly associated with lymph node involvement (p=0.01) while it was insignificant for tumour size and histological grade (p=0.36 and 0.42 respectively).

Conclusion: The survival of breast cancer patients was correlated only with lymph node metastasis. It was better with smaller number of lymph node involvement. While, Caspase-3 did not have any direct association with prognostic markers of breast carcinoma like tumour size, grade of tumour, lymph node and ER, PR and HER-2/neu status. It can be probably used as a prognostic biomarker in future if bigger study is able to establish the significance in breast cancer.

Keywords: Breast cancer, caspase-3, immunohistochemistry, prognosis, survival

Introduction

"Apoptosis" is defined as "a programmed cell death". It is a biochemically and morphologically defined form of cell death [1]. It is usually characterized by specific morphological characteristics and energy-dependent biochemical mechanisms. It is considered an important part of various processes including normal cell turnover, functioning of the immune system, embryonic development and chemical induced cell death. There are many conditions were inappropriate apoptosis (either too little or too much) is found which includes autoimmune disorders, neurodegenerative diseases, ischemic damage and many types of cancer [1, 2]. "Caspases", the family of aspartate-directed cysteine proteases, plays an essential role not only in the transduction of the apoptotic signals but also in the execution of apoptosis in mammalian cells [3].

Impaired apoptosis is a telltale sign of human cancers. Two major pathways initiate apoptosis; intrinsic and extrinsic pathway [4]. The extrinsic pathway, which is also known as [death receptor (DR)] pathway is activated in response to ligand binding of Dr. Superfamily members which results in activation of caspase-3. The intrinsic pathway, which is also known as mitochondrial pathway is triggered by the release of cytochrome-c from mitochondria, leading to the formation of Apoptotic protease activating factor-1 (Apaf-1). Subsequently, cytochrome-c complex with the help of ATP activate caspase-9 followed by caspase-3 [5].

Caspases are cysteine aspartyl proteases and 14 family members have been identified till date [6]. Based on their function, structural characteristics and locations, caspases are generally classified as apoptotic caspases or proinflammatory caspases. Typical apoptotic caspases are classified into two groups: the initiator caspases (caspase-2, 8, 9 and 10) and the effector caspases (caspase-3, 6 and 7). All caspases exist as inactive zymogens [7].

An effector caspase is activated by an initiator caspase by cleavage at the internal Asp residue, causing disassembly of the large and small subunits. However, the inhibitor caspaseis activated by dimerization via the signal obtained from death receptors [8]. Caspase-3 is an executor caspase and once activated, rapidly leads to the cellular changes observed in apoptosis. Caspase-3 is involved in the apoptosis induced by many agents, including antineoplastic drugs which are a part of routine cancer management [9].

Loss of caspase-3 expression has been reported in primary human breast cancers. It has been suggested that it might promote tumour development by preventing and reducing senescence [9]. Indeed, several pieces of evidence suggest that loss of caspase-3 correlates with resistance to drug-induced apoptosis in a variety of human cancer cell lines, including breast cancer cells [10]. One of the study on this aspect has demonstrated that restoration of caspase-3 expression in caspase-3 deficient MCF-7 breast cancer cells, can sensitize to doxorubicin and etoposide-induced apoptosis, suggesting caspase-3 deficiency may be a possible mechanism for chemo resistance [11, 12].

Breast cancer is the most common cause of cancer related mortality in Indian women with an incidence of 30-33 per 100000 women in urban India. It is the second commonest cancer in rural women as well [13, 14]. The prognosis of breast cancer depends on several clinical and pathological factors including immunohistochemical markers. Very few studies have been conducted to decide role of caspase-3 on prognosis in India as per our knowledge. So, the present study was carried out to evaluate the prognostic potential of caspase-3 in breast cancer patients. We decided to conduct this study as a pilot study to get initial results to plan for a bigger study.

Materials and Methods

Study design and study setting: It was a retrospective observational pilot study which was conducted in the Department of Pathology at Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram, Wardha, Maharashtra, a rural tertiary care hospital in central India.

Case selection: We selected 50 consecutive cases of infiltrating duct carcinoma diagnosed on histopathology of surgical specimen in between years 2015 and 2016 from records of Department of Pathology. These cases were followed up for 5 years. The relevant clinical information like age, side of breast involvement and clinical data like grade of tumour, dimensions of the tumour and the presence of lymph node metastases was collected from the clinical documents and pathological documents like grossing records and histopathological reports of the patients. Follow up data of these patients was also obtained from hospital information system, department of radiotherapy and the records of the cancer registry of the region as well as on patients follow up period.

Inclusion criteria: The breast cancer cases diagnosed as infiltrating duct carcinoma on histopathological specimen were included in the study.

Exclusion criteria: The cases where adequate representative tissue was not available for immunohistochemistry (IHC) or complete clinical details were not available were excluded from the study.

Immunohistochemistry: We screened haematoxylin and eosin-stained slides of the cases to obtain the best paraffin block for IHC. Then we retrieved formalin-fixed paraffin embedded tissue blocks from archives of our department. A tissue section from these blocks was used in the study. IHC staining for Caspase-3, Estrogen Receptor (ER), Progesterone Receptor (PR) and Her2/neu was done by standard technique according to IHC system manual. The positive control tissue (appendix for Caspase-3 and normal breast tissue for ER, PR and Her2/neu antibodies) sections were usedto establish homogenous accurate and reproducible results. We obtained sections of 3-5 µ in thickness and this tissue was fixed in formalin for 12 hours. The unmasking of antigen (Antigen retrieval) was done with the help of microwave oven (operated at 5 minutes at 750 watts and then 15 minutes at 200 watts). 3% hydrogen peroxidase was used for inactivation of endogenous peroxidase. Then, removal of non-specific antibodies to highly charged sites (power blocking) was achieved. The cases with appropriate positive and negative controls were stained with prediluted primary Anti-Caspase-3 protein antibody [Monoclonal, Immunogen: Recombinant full length human recombinant Caspase-3 protein, Clone: monoclonal 3CSP03, Species: Mouse, Ig class: IgG2a, Protein concentration: 200 ug/ml thermoscientific UK, MBL; Code: M058-3]. Staining with ER, PR and Her2/neu was done in order to find out the hormonal status of the study cases. In case of ER staining, a primary antibody (1:100 dilution of mouse monoclonal anti-human ER; DAKO M7047) was applied overnight at 4 °C. We used a biotinylated rabbit anti-mouse immunoglobulin (DAKO E413) as the secondary antibody (1:350 dilutions) for 1 hour. In case of PR staining, a primary antibody [1:4 dilution of mouse monoclonal anti-human PR; DAKO M7047) was applied overnight at 4 °C. We used a biotinylated rabbit anti-mouse immunoglobulin (DAKO E413)] as the secondary antibody (1:350 dilutions) for 1 hour. For Her2/neu staining, the tissue section was treated with 100 µl prediluted primary antibody HER-2/neu [Monoclonal, Immunogen: A synthetic peptide corresponding to residues near the C-terminus of human HER2, Clone: EP1045Y, Species: Rabbit, Ig class: IgG, Protein Conc: 50mg/ml, Catalog no: AN7260717, BioGenix, USA] following blocking of the non-specific binding and endogenous peroxidase activity. Following rinsing, the tissue sections were incubated with the biotinylated rabbit anti-rat antibody (1:100 dilutions) for 30 minutes at room temperature. In all cases of ER, PR and Her2/neu staining, Streptavidin (DAKO) was applied for 30 minutes and sections were visualised with DAB (3,3'-Diaminobenzidine) solution.

We evaluated the intensity of the immunostaining for caspase-3 by dividing the staining reaction in four groups[15] as cytoplasmic staining intensity as 1 for weak, 2 for moderate, 3 for strong and 4 for very strong intensity. The quantity of the immunostaining was evaluated with respect to percentage of tumour cells showing cytoplasmic

positivity as; 0 forno positive immunostaining, 1 for < 25%, 2 for 25-50%, 3 for 50-75% and 4 for > 75% of tumor cells. A combined score for the immunostaining, was calculated by adding both the qualitative and quantitative score which were then be divided into three groups as +: No or weak immunostaining (score 0-2), ++: Moderate immunostaining (score 3-5) and +++: Strong immunostaining (score 6-8) (Figure 1A, B and C).

We also scored immunostaining results semi-quantitatively for ER and PR on the basis of the microscopically estimated percentage of positively stained tumour cell nuclei.[16] The nuclear staining intensity was scored for tumour cell nuclei as (0) for negative/no staining, (1+) for weak, (2+) for intermediate and (3+) for strong staining (Figure 1D and E). We scored a minimum of 100 tumour cells and the percentage of tumour cell nuclei in each category were recorded. The overall percentage of positively stained tumour cell nuclei was calculated from sum of three staining categories. ER/PR H-score was then calculated as sum of the intensity of IHC tumour cell nuclei as follows:

H-score = (% of positively stained tumour cell nuclei at weak intensity category × 1) + (% of positively stained tumour cell nuclei at intermediate intensity category × 2) + (% of positively stained tumour cell nuclei at strong intensity category × 3).

The intensity for HER-2/neu staining was done as follows[17]; Negative (0) for the cases with either no staining or membrane staining in < 10% of the tumour cells, negative (1+) for cases where faint/barely perceptible membrane staining is detected in > 10% of tumour cells. Weakly positive (2+) for the cases which showed weak to moderate complete membrane staining is observed in > 10% of tumour cells, strongly positive (3+) for the cases where a strong complete membrane staining is observed in > 10% of tumour cells (Figure 1F).

Ethical approval: The approval was taken from Institutional ethics committee before beginning the study vide letter number MGIMS/IEC/PATH/84/2017, dated 09-10-2017. The patient confidentiality was maintained during all research procedures. After taking informed consent, the patients were only contacted in the form of interview at the time of their follow up visits.

Statistical analysis: The statistical analysis was performed by using inferential statistics using Pearson correlation coefficient and descriptive (such as mean, frequency, standard deviation). The software used in the analysis was SPSS22.0 (IBM Corp. Released 2015. IBM Statistics for Windows, Version 20.0: Armonk, New York, United States) and graph pad PRISM 6.0 version. The p value < 0.05 was considered as level of significance.

Results

Demography and clinical information of study population (untabulated data):

In present study, the age ranged from 28-79 years, 53.5 years being the mean age. 64% of the patients presented with the tumour size between 2 to 5 cm. 34% patients had tumour size > 5 cm, the mean tumour size being 4.6 cm. Right and left breasts involvement was 58% and 42% respectively. 80% and 20% of the patients had grade III and grade II tumour respectively. Amongst all cases, 28% had 1-3 positive nodes, 20% had 4-9 positive nodes and 4% had > 10 positive lymph nodes. In case of caspase-3 staining, maximum cases have exhibited moderate staining (42%), followed by strong staining (32%) and weak/negative staining (26%). ER and PR positivity was 64% and 74% respectively. In cases of Her2/neu, 2+ and 3+ staining was observed in 14% cells each while 0 and 1+ staining was observed in 72% cases.

On correlation of caspase-3 staining with histological grade of the tumour, it was found that out of 21 cases with moderate caspase-3 staining, 85.7% cases were grade III tumours and 14.2% were grade II tumours. While, out of 16 cases with strong caspase-3 staining, 75% cases were grade III tumours followed by 25% cases which were grade II tumours. The difference was not statistically significant (p=0.68) [Table 1A]. On correlating caspase-3 staining with size of tumour, we found that out of 13 cases with negative/weak caspase-3 staining, 61.5% cases had tumour size between 2 to 5 cm in size, followed by 38.4% cases with tumour size more than 5 cm in size. Those with moderate caspase-3 staining (N=21), 76.1% cases had tumour size between 2 to 5 cm in size, while 23.8% cases had size more than 5 cm [Table 1B].

When we looked for lymph node involvement, we found that in case of moderate caspase-3 staining (N=21), maximum cases (47.6%) had no lymph node involvement, 42.8% had 1-3 positive nodes, and 4.8% had 4 to 9 and more than 10 positive nodes each. In cases of strong caspase-3 staining (N=16), maximum cases (50%) had no lymph node involvement, 25% had 4-9 positive nodes, and 18.7% had 1 to 3 positive nodes and 6.25% had > 10 positive nodes [Table 1C]. Thus, the difference was statistically insignificant in histological grade, size of tumour and lymph node involvement (p=0.68, 0.24 and 0.20 respectively).

When caspase-3 was correlated with ER, 32 cases were ER positive while 18 cases were ER negative. Amongst 21 cases with moderate caspase-3 staining, 71.4% were ER positive and 28.5% were ER negative. On correlating strong caspase-3 staining (N=16), 68.6% were ER positive and 31.2% were ER negative. The findings were not statistically significant (p=0.68) [Table 2A]. The correlation with PR staining showed that out of 13 cases with negative/weak caspase-3 staining, 61.5% were PR positive while 38.4% were PR negative. Whereas, in case of 21 cases with moderate caspase-3 staining, 81% and 19% had shown PR positivity and negativity respectively. Out of 16 cases with strong caspase-3 staining, 75% cases had positive PR status and 25% had negative PR status. The findings were not statistically significant (p=0.45) [Table 2B]. Similarly, statistically insignificant (p=0.11) difference was found in case of Her2/neu staining [Table 2C].

Out of 50 cases in our study, the survival status was available in 21 cases. 7 cases each showed negative/weak, moderate and strong caspase-3 staining. Correlation of caspase-3 staining with survival of patients showed that out of 7 cases with negative/weak caspase-3 staining, 57% cases were alive while 43% cases expired. Out of 7 cases with moderate caspase-3 staining, 71.43% cases expired whereas 28.57% were alive and out of 7 cases with strong caspase-3 staining, 57% cases expired and 43% cases were alive. The findings were not statistically significant (p=0.55) [Table 3].

Correlation of survival of patients with clinicopathological parameters showed significant results with lymph node involvement (p=0.01) with maximum patients in the group of 4-9 lymph node involvement) while there were insignificant findings with histological grade and size of tumour (p=0.36 and 0.42 respectively) [Table 4].

Correlation of survival of patients with ER status showed that out of 13 patients with negative ER status, 69.23% patients expired whereas 30.77% patients were alive. Out of 8 patients with positive ER status, 37.5% patients expired whereas 62.5% patients were alive. The difference was not statistically significant (p=0.15) [Table 5A]. When correlated with PR status, out of 16 patients with negative PR status, 62.5% patients expired whereas 30.7% patients were alive. Out of 5 patients with positive PR status, 37.5% patients expired whereas 62.5% patients were alive. The difference was not statistically significant (p=0.15) [Table 5B].

In case of HER-2/neu status, out of 14 patients with weak HER-2/neu status, 50% patients were either alive or dead. Out of 4 patients with moderate HER-2/neu staining, 75% patients expired whereas 25% patients were alive. Out of 3 patients with strong HER-2/neu staining, 66.67% patients expired whereas 33.33% patients were alive. The difference was not statistically significant (p=0.60) [Table 5C].

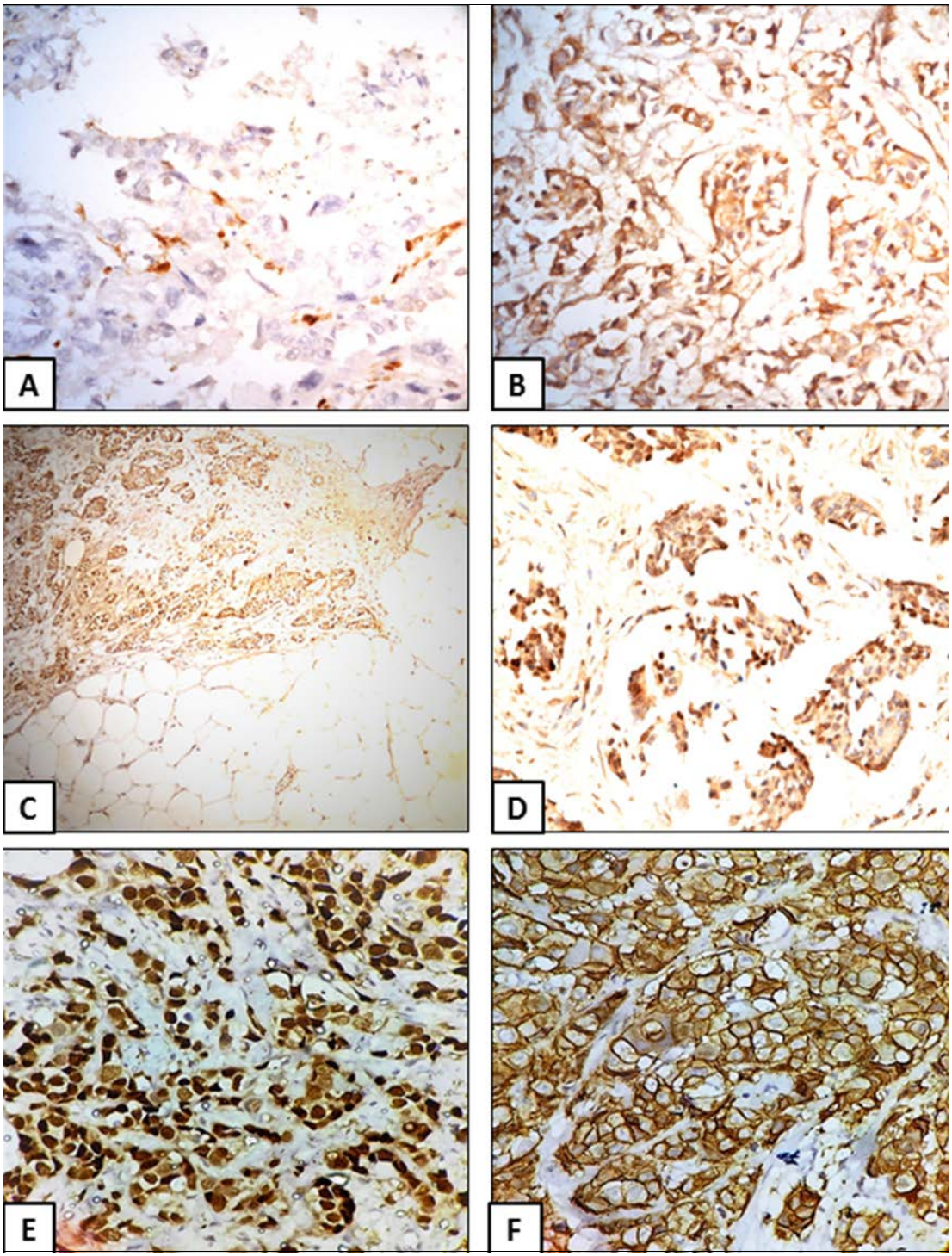

Fig 1: (A): Section showing caspase-3 expression with weak immunostaining, score 2 (1+1), (IHC, 400x) (B): Section showing caspase-3 expression with moderate immunostaining, score 3 (2+1), (IHC, 400x) (C): Section showing caspase-3 expression with strong immunostaining, score 7 (3+4), (IHC, 100x) (D): Section showing expression of estrogen receptor with positive staining (IHC, 100x) (E): Section showing expression of progesterone receptor with positive staining (IHC, 400x) (F): Section showing expression of HER2/neu receptor with positive staining (IHC, 100x)

Table 1: Correlation of caspase-3 staining with clinical findings

A. Correlation of caspase-3 staining with histological grade of tumour					
Histological grade	**Caspase-3 staining**			**Total**	**χ^2 value***
	Negative/ Weak	**Moderate**	**Strong**		
Grade II	3 (23.09)	3 (14.29%)	4 (25%)	10 (20%)	0.75 p=0.68
Grade III	10 (76.92%)	18 (85.71%)	12 (75%)	40 (80%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	
B. Correlation of caspase-3 staining with size of tumour					
Maximum dimension of tumour	Caspase-3 staining			Total	χ^2 value*
	Negative/ Weak	Moderate	Strong		
< 2 cm	0 (0%)	0 (0%)	1 (6.25%)	1 (2%)	5.42 p=0.24
2-5 cm	8 (61.54%)	16 (76.19%)	7 (43.75%)	31 (62%)	
> 5 cm	5 (38.46%)	5 (23.81%)	8 (50%)	18 (36%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	
C. Correlation of caspase-3 staining with lymph node involvement					
Lymph node involvement	Caspase-3 staining			Total	χ^2 value*
	Negative/ Weak	Moderate	Strong		
Not involved	6 (46.15%)	10 (47.62%)	8 (50%)	24 (48%)	8.51 p=0.20
1 to 3 positive nodes	2 (15.38%)	9 (42.86%)	3 (18.75%)	14 (28%)	
4 to 9 positive nodes	5 (38.46%)	1 (4.76%)	4 (25%)	10 (20%)	
> 10 positive nodes	0 (0%)	1 (4.76%)	1 (6.25%)	2 (4%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	

*Pearson correlation coefficient

Table 2: Correlation of caspase-3 staining with hormonal status

A. Correlation of caspase-3 staining with ER staining					
ER staining	Caspase-3 staining			Total	χ^2 value*
	Negative/ Weak	Moderate	Strong		
Positive	6 (46.15%)	15 (71.43%)	11 (68.75%)	32 (64%)	2.45 p=0.68
Negative	7 (53.85%)	6 (28.57%)	5 (31.25%)	18 (36%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	
B. Correlation of caspase-3 staining with PR staining					
PR staining	Caspase-3 staining			Total	χ^2 value*
	Negative/ Weak	Moderate	Strong		
Positive	8 (61.54%)	17 (80.95%)	12 (75%)	37 (74%)	1.58 p=0.45
Negative	5 (38.46%)	4 (19.05%)	4 (25%)	13 (26%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	
C. Correlation of caspase-3 staining with Her2/neu staining					
Her2/neu	Caspase-3 staining			Total	χ^2 value*
	Negative/ Weak	Moderate	Strong		
0 and 1+	10 (76.92%)	17 (80.85%)	9 (56.25%)	36 (72%)	7.4 p=0.11
2+	3 (23.08%)	2 (9.52%)	2 (12.50%)	7 (14%)	
3+	0 (0%)	2 (9.52%)	5 (31.25%)	7 (14%)	
Total	13 (100%)	21 (100%)	16 (100%)	50 (100%)	

*Pearson correlation coefficient

Table 3: Correlation of caspase-3 staining with survival of patients

Patients' status	**Caspase-3 staining**			**Total**	**χ^2 value***
	Negative/ Weak	**Moderate**	**Strong**		
Expired	3 (42.86%)	5 (71.43%)	4 (57.14%)	12 (57.14%)	1.16 p=0.55
Alive	4 (57.14%)	2 (28.57%)	3 (42.86%)	9 (42.86%)	
Total	7 (100%)	7 (100%)	7 (100%)	21 (100%)	

*Pearson correlation coefficient

Table 4: Correlation of clinicopathological parameters with survival of patients

A. Correlation of survival of patients with histological grade				
Patient status	**Histological grade**		**Total**	**χ^2 value***
	II	**III**		
Expired	1 (33.33%)	11 (61.11%)	12 (57.14%)	0.81 p=0.36
Alive	2 (66.67%)	7 (38.89%)	9 (42.86%)	
Total	3 (100%)	18 (100%)	21 (100%)	
B. Correlation of survival of patients with size of tumour				
Patient status	Size of tumour		Total	χ^2 value*
	< 2 cm	2-5 cm	> 5 cm	
Expired	0 (0%)	9 (64.29%)	3 (50%)	12 (57.14%)	1.75 p=0.42
Alive	1 (100%)	5 (35.71%)	3 (50%)	9 (42.86%)	

Total	1 (0%)	14 (100%)		6 (100%)	21 (100%)	
C. Correlation of survival of patients with lymph node involvement						
Patient status	Lymph node involvement				Total	χ^2 value*
	Not involved	1-3 positive nodes	4-9 positive nodes	> 10 positive nodes		
Expired	1 (14.29%)	3 (50%)	7 (100%)	1 (100%)	12 (57.14%)	11.37 p=0.01
Alive	6 (85.71%)	3 (50%)	0 (0%)	0 (0%)	9 (42.86%)	
Total	7 (100%)	6 (100%)	7 (100%)	1 (100%)	21 (100%)	

*Pearson correlation coefficient

Table 5: Correlation of survival of patients with hormonal status

A. Correlation of survival of patients with ER status				
Patient status	ER status		Total	**χ^2 value***
	Negative	Positive		
Expired	9 (69.23%)	3 (37.5%)	12 (57.14%)	2.03 p=0.15
Alive	4 (30.77%)	5 (62.5%)	9 (42.86%)	
Total	13 (100%)	8 (100%)	21 (100%)	
B. Correlation of survival of patients with PR status				
Patient status	PR status		Total	χ^2 value*
	Negative	Positive		
Expired	10 (62.5%)	2 (37.5%)	12 (57.14%)	2.03 p=0.15
Alive	6 (30.77%)	3 (62.5%)	9 (42.86%)	
Total	16 (100%)	5 (100%)	21 (100%)	
C. Correlation of survival of patients with HER-2/neu status				
Patient status	HER-2/neu status			Total	χ^2 value*
	Weak	Moderate	Strong		
Expired	7 (50%)	3 (75%)	2 (66.67%)	12 (57.14%)	0.92 p=0.60
Alive	7 (50%)	1 (25%)	1 (33.33%)	9 (42.86%)	
Total	14 (100%)	4 (100%)	3 (100%)	21 (100%)	

*Pearson correlation coefficient

Discussion

We tried to study the correlation of caspase-3 staining in the breast cancer in rural population in central India. We did not find any statistically significant correlation of caspase-3 staining with different parameters like histological grade of the tumour, size of the tumour and lymph node involvement (p=0.68, 0.24 and 0.20 respectively) [Table 1]. Hadjiloucas I *et al.* [10] found strong caspase-3 immunoreactivity in grade 3 tumours as compared to grade 1 or 2 tumours as well as in node positive groups in their study. While, Yang XH *et al.* [11] found neither any significant correlation between strong caspase-3 staining with high grade tumours nor with lymph node metastases. Vakkala M *et al.* [15] and Jha K *et al.* [18] *et al* did not find association between strong caspase-3 staining and high-grade tumours in invasive breast carcinoma (p=0.69 and p=0.18 respectively) while; Jha K *et al.* [18] found significant association with positive lymph node status and caspase-3 staining (p=0.03). Nassar A *et al.* [19] found significant correlation with histological grade (p=0.041) but no association for lymph node metastases. While, Nakopoulou L *et al.* [20] found significant correlation between overexpression of caspase-3 and low nuclear grade. Therefore, the findings of various studies in the literature as well as present study shows that there is no consistent observation about correlation of Caspase-3 staining with histological grade of tumour, size of the tumour and lymph node status [10, 11, 15, 18, 19]. Some studies have shown association with grade [10, 19, 20] as well as with lymph node status [10, 18]. The findings were not consistent by these as well as present study. However, as per findings in table 1(C), the weak caspase-3 staining was associated with no lymph node or lesser number of lymph node involvements (less than 10). The cases with more than 3 lymph node involvement had moderate or strong caspase-3 staining. Although the findings were not statistically significant, it is possible that a bigger study may show a significant correlation with survival as well as lymph node involvement.

In our study, we did not find any significant association between caspase-3 staining and ER, PR and HER-2/neu status (p=0.68, 0.45 and 0.11 respectively) [Table 2]. The relationship between caspase-3 and ER, PR and HER-2/neu status in patients have not been demonstrated in literature till date. Many of the studies did not observe any correlation [18-19] or some observed correlation with all the three parameters [6] or few observed correlations with some of the parameters [11] whereas few observed negative correlations [10]. The reason could be attributed to number of study samples, different methods of staining and analysis pattern of caspase-3 in tumour cells.

Follow up was available only 21 patients in our study. We did not find significant correlation of caspase-3 with survival of patients (p=0.55) [Table 3]. Vakkala *et al.* [15] and Nassar A *et al.* [19] found no association between overall and disease-free survival and caspase-3 immunoreactivity. Jha K *et al.* [18] found that overall, 3-year survival rate for patients with caspase-3-positive breast carcinomas (88.6%) was less than that for patients with caspase-3 negative tumours (91.3%), but the difference was statistically not significant (p=0.46). Yang XH *et al.* [12] found that an increased expression of caspase-3 had a negative influence on the overall survival of the patient with breast cancer and poor prognosis. Devarajan E *et al.* [12] found that lack of caspase-3 expression in breast cancer patients renders breast cancer cells resistant to apoptosis in response to certain apoptotic stimuli including chemotherapeutic drugs and thus may affect the outcome and prognosis of the disease. Nakopoulou L *et al.* [20] found a significant negative influence of caspase-3 expression on patients' overall survival (p=0.029). The reason behind different findings in

each study could be associated with a smaller number of samples in our study and it is possible that a bigger study may show a significant correlation for the same.

We did not find any significant correlation of caspase-3 expression with survival of patients with respect to histological grade and tumour size (p=0.36, 0.42 respectively). While, it was significantly associated with lymph node involvement (p=0.01) [Table 4]. We could find only single study in literature which tried to find out association between clinicopathological parameters with survival of patients till date. Pu X *et al.* [6] found that high caspase-3/high calpain-1, high caspase-3/high calpain-2 and high caspase-3/low calpastatin expression was significantly associated with adverse breast cancer-specific survival in the total patient cohort group. They also found that combinational caspase-3/calpain-1 has important prognostic value in basal-like patients (p=0.047). Only caspase-3/calpastatin expression was identified as an independent factor for breast cancer specific survival in their study. Thus, they concluded that high caspase-3 expression was significantly associated with adverse breast cancer specific survival. We also tried to find association of hormonal status with survival of patients in study cases. There was no significant correlation of caspase-3 expression with survival of patients with respect to ER, PR and HER-2/neu status (p=0.15, 0.15 and p=0.60 respectively) [Table 5]. We could not find any study in the literature for the same.

Findings of the present studies have not been able to find any associations between survival, tumour size, tumour grade, ER, PR status and HER-2/neu expression with caspase-3 expression. The only correlation which was observed was with lymph node involvement. We observed that survival was better when there was weak or negative Caspase-3 staining, when the tumour size was less than 2 cm as well as positive ER and PR status. However, a greater number of patients died when there was moderate to strong HER-2/neu receptor expression. Though these findings were not statistically significant, it does show that these factors which have been found so important for patient prognosis in literature are also important in present study though not in statistically significant numbers.

The major limitation of present study was that the follow up was available in less than 50% of patients. The studies in literature for survival analysis had shown a significant number of patients. Also, the stage of the disease is an important parameter in survival studies. However, as this was a laboratory-based study, we correlated caspase-3 expression with no prognostic parameters which were easily available from patient data in laboratories. Therefore, the stage was not correlated individually with survival analysis. We could not find any Indian study which tried to explore the expression and prognosis of caspase-3 expression in breast cancer patients with clinicopathological parameters till date. So, this is first pilot study in Indian literature on this aspect. We recommend a larger study with a large follow up period and disease-free survival which will help to warrant the role of caspase-3 as a prognostic marker in breast cancer.

Conclusion

Caspase-3 might play an important role in human tumorigenesis. It did not have any direct association with other prognostic marker of breast carcinoma like tumour size, grade of tumour, lymph node status and ER, PR and HER-2/neu status from the present study. The survival of patients was correlated only with lymph node involvement. It was better with small number of lymph node involvement. Caspase-3 can be used probably as a prognostic biomarker in future if the bigger study is able to establish the significance in breast cancer.

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Conflict of Interest

Not available

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Not available

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