Original Article

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Expression of COX-2 in Carcinoma Breast

Pathology Section

ASHISH SHARMA[1], NISHA MARWAH[2], PADAM PARMAR[3], RAJEEV SEN[4]

ABSTRACT

Introduction: Early detection of breast cancer with chemoprevention is needed to decrease cancer related mortality at an early stage. The role of Cyclooxygenase-2 (COX-2) in carcinogenesis and tumour progression has been a subject of interest in breast cancer.

Aim: To study the spectrum of COX-2 expression in normal breast tissue, Ductal Carcinoma In Situ (DCIS) and invasive breast cancer.

Materials and Methods: Fifty cases of primary breast cancer undergoing radical or modified radical mastectomy constituted the study group. Histopathological diagnosis was established on routine Haematoxylin and Eosin (H&E) stain and various histologic prognostic parameters were assessed. Immunohistochemical profile of the tumour was assessed by subjecting one section each from a representative block of tumour to ER, PR, HER2/neu and COX-2. Immunohistochemical Score (IHS) of COX-2 was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score).

The results obtained were interpreted and correlated statistically. When the data was qualitative, a chi-square test was used to assess the association. Correlation of COX-2 IHS with clinicopathological parameters and different areas was calculated by Spearman rank correlation (rs). The significance of correlation was evaluated by using critical values table for Spearman's coefficient of correlation.

Results: COX-2 IHS was negative in (n=17, 34%) and moderately positive in (n=33, 66%) of the tumour cases examined. Among normal breast tissues, negative and moderate positivity was seen in (n=14, 28%) and (n=36, 72%) of the cases respectively. Amongst the 23 cases with DCIS component, (n=20, 86%) of the cases revealed moderately positive COX-2 IHS. COX-2 expression was correlated within normal breast tissue, DCIS component and invasive areas, as paired samples. Paired areas examined for COX-2 expression with group of normal-invasive, normal-DCIS and tumour-DCIS and all the three components together. Correlation of COX-2 expression among the paired areas examined was statistically significant.

Conclusion: Based on present results, COX-2 exerts autocrine and paracrine effects and is involved in early breast cancer carcinogenesis. Inhibition of COX-2 may represent a potential target for preventing breast cancer oncogenesis.

Keywords: Autocrine effect, Cancer carcinogenesis, COX-2 expression, Paracrine effect

INTRODUCTION

Breast carcinoma is the most common malignant tumour and a cause of cancer related mortality in women worldwide annually [1]. There has been a significant worldwide increase in mortality from breast cancer due to modern lifestyle (delayed childbearing, reduced breast-feeding, obesity due to richer diet and alcohol consumption) in the western world [2]. The incidence of breast cancer in India is also rising. Cancer of the breast and cervix are more common in the urban population with higher cancer mortality [3].

Various treatment modalities are available for breast carcinoma, therefore, it is important to provide accurate prognostic information on which the therapeutic decision would be based. The prognostic factors can be clinicopathological factors (tumour size, histologic subtype and grade, lymph node metastases and lymphovascular invasion) and biomarkers including hormonal profile [4,5].

The role of COX-2 in carcinogenesis and tumour progression has been a subject of interest in past few decades. Apart from the traditional immunomarkers {Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2/neu}, COX-2 is being studied extensively in breast cancer tissue [6,7].

Higher COX-2 expression have been observed in a number of human cancer: colon, lung, gastric and esophageal adenocarcinomas [8]. Based on colon cancer studies, it has been seen that COX-2 over expression increases production of Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), transforming growth factor-1, PDGF and endothelin-1 which contribute to the neovascularisation of tumours [9,10].

Increased level of COX-2 in breast carcinoma has been linked with an increased estradiol synthesis and development of breast cancer [10]. While a few studies demonstrated a positive correlation between COX-2 expression and histopathologic parameters associated with shorter disease free survival, a few others could not find such relation in literature [10-15]. However, there is a paucity of data on COX-2 expression in the normal breast tissue and on the changes in COX-2 expression from normal tissue via DCIS lesions to invasive cancer. This expression of COX-2 can bring a dramatic change in the treatment protocol. We conducted a study to observe the spectrum of COX-2 expression in the normal breast tissue, DCIS, wherever possible, for invasive cancer.

MATERIALS AND METHODS

This prospective study was done in the Department of Pathology, Pandit Bhagwat Dayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India (2013 to 2015). This study protocol was approved by the Institutional Board. Fifty cases of primary breast cancer undergoing radical or modified radical mastectomy constituted the study group. Patients with breast cancer other than primary adenocarcinoma such as lymphoma, sarcoma, stromal tumour and metastasis were excluded. In the present study, 23 cases had both DCIS and a tumour area. From each specimen, normal breast tissue piece was also taken.

In the department, specimens were examined grossly for tumour size, along with axillary lymph node status. Specimens were fixed and processed by routine histological technique for paraffin embedding. Representative blocks were prepared from tumour, normal tissue, area adjacent to tumour, tumour margins, overlying skin, deepest resection margin and axillary lymph nodes. Histopathological diagnosis was established on routine H&E stain and various histologic prognostic parameters including histologic type, histologic grade and lymph node metastasis were assessed [16]. Histological grading was done by Modified Bloom-Richardson system (MBR) taking into account the scores for tubule formation, nuclear pleomorphism and mitotic count [17]. Using size, MBR histologic grade and lymph node stage, Nottingham Prognostic Index (NPI) were calculated [17].

Immunohistochemical (IHC) profile of the tumour was assessed by subjecting one section each from a representative block of tumour to ER, PR, HER2/neu and COX-2. Immunoquantification was performed using light microscopy at 4X magnification. Positive and negative controls were done for each batch of IHC stain. Positive control for ER, PR and HER2/neu was from a positive case of carcinoma breast while positive control for COX-2 was from a positive case of carcinoma colon. For negative control, primary antibody was substituted with an antibody of irrelevant specificity.

The Interpretation of Immunohistochemical Stains

ER, PR and HER2/neu staining: Brown diffuse or grainy nuclear staining was taken as positive for ER/PR and assessed by Quick scoring based on assessment of proportion and intensity [18]. Patients with tumours scoring 2 or less were regarded as ER/PR negative. Uniform, intense brown membrane staining of >30% of the tumour cells was taken as positive for HER2/neu [19].

COX-2 Staining: Positive cases showed brown cytoplasmic stain. The IHS was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score), as follows [20]:

Quantity Score was rated on a scale of 0 to 4, with

Score 0: 0-5% of cells stained

Score 1: 6-25% of cells stained

Score 2: 26-50% of cells stained

Score 3: 51-75% of cells stained

Score 4: 76-100% of cells stained

Staining intensity was rated on a scale of 0 to 3, with

0: Negative

1: Weak

2: Moderate

3: Strong

When there was multifocal immunoreactivity and significant differences in staining intensities between foci, the average of the least intense and most intense staining was recorded. The raw data was converted to the IHS by multiplying the quantity and staining intensity scores. The scores theoretically ranged from 0 to 12.

Interpretation of IHS scoring:

0 to 3: Negative

4 to 8: Moderate

9 to 12: Strong

The COX-2 score was correlated with clinicopathological parameters including age, tumour size, tumour type, histological tumour grade, axillary lymph node status and NPI along with ER, PR and HER2/neu status.

STATISTICAL ANALYSIS

The results obtained were interpreted and correlated statistically using all the data obtained, analysed statistically using IBM SPSS statistics for windows, version 20.0. (IBM Corp., Armonk, NY). Mean and standard deviations were calculated. When the data was qualitative, a chi-square test was used to assess the association between these parameters. A p-value <0.05 was taken as significant (S) and p-value <0.01 was taken as highly significant (HS) whereas the p-value of more than 0.05 was taken as non-significant. Correlation of COX-2 IHS with clinicopathological parameters and different areas (normal breast, DCIS and tumour) was calculated by Spearman rank correlation (rs). It gave a value of 'rs' between (-1 to +1). The significance of correlation was evaluated by using critical values table for Spearman's coefficient of correlation (statistically significant with a p-value ≤0.05).

RESULTS

In the present study, a total of 50 cases of invasive breast carcinoma constituted the study group with the age ranging from 21-70 years. Mean age (±SD) at presentation was 48.22±12.04 years. Premenopausal and postmenopausal cases were (n=19, 38%) and (n=31, 62%) cases respectively in present study. All the cases were divided into three groups depending on size i.e. <2 cm, 2-5 cm and >5 cm. Thirty nine (78%) cases belonged to 2-5 cm size group. Histologically, all the cases were infiltrating duct carcinoma (NOS type). The cases were graded using MBR grading system. Grade II constituted (n=27, 54%) of the cases followed by Grade I (n=16, 32%) and grade III (n=7, 14%) cases [Table/Fig-1].

Clinicopathologic parameters		COX-2 expression			p-value (rs)=T/N/DCIS
		Tumour	Normal	DCIS	
		%	%	%	
Tumour size	<2 cm	12	11	1	0.098 (0.237)/0.048 (0.281)/0.157 (0.305)
	2-5 cm	80	83	85	
	>5 cm	28	6	14	
Histologic grade	Grade I	39	38	45	0.098 (0.237)/0.048 (0.281)/0.011 (0.962)
	Grade II	51	48	40	
	Grade III	10	14	15	
Lymph node status	Stage I	45	48	45	0.251 (0.166)/0.142 (0.211)/2.42 (0.182)
	Stage II	33	30	25	
	Stage III	22	22	30	
NPI	Good	18	20	20	0.045 (0.285)/0.0245 (0.319)/0.239 (0.290)
	Moderate	62	60	55	
	Poor	20	20	25	
ER	Positive	63	58	50	0.332 (-0.140)/0.971 (0.005)/0.610 (0.112)
	Negative	37	42	50	
PR	Positive	60	55	50	0.051 (-0.278)/0.258 (-0.163)/0.610 (0.112)
	Negative	40	45	50	
HER2/neu	Positive	21	22	25	0.53 (0.091)/0.067 (0.645)/0.305 (0.157)
	Negative	79	78	75	

[Table/Fig-1]: Correlation of COX-2 IHS with various clinicopathological paramteres. Test-Spearman's rank correlation
DCIS-Ductal carcinoma in situ, N-Normal breast tissue, T-Tumour tissue, NPI-Nottingham prognostic index, ER-Estrogen receptor, PR-Progesterone receptor.
Statistically significant with a p-value ≤0.05)

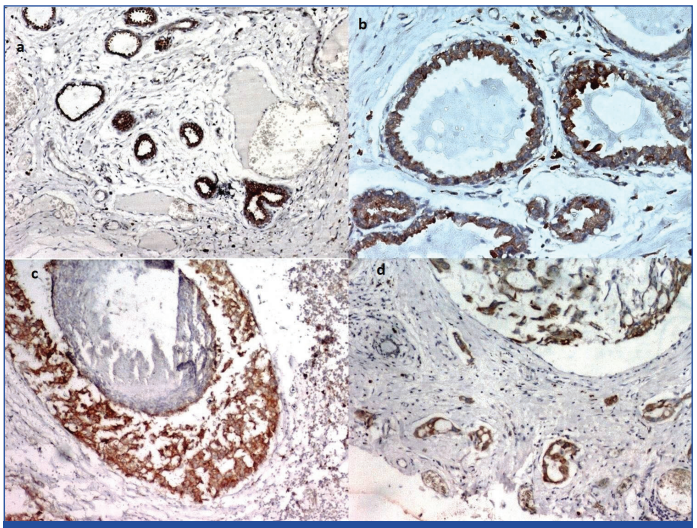
Lymph node involvement being an important prognostic variable was assessed in all cases and staging was done based on number of lymph nodes involved. In (n=21, 42%) of the cases, lymph node involvement was not seen (stage I), (n=15, 30%) of the cases were in stage II and (n=14, 28%) of the patients had four or more lymph nodes involvement falling in stage III. Based on tumour size, histologic grade and lymph node status, tumours were categorised into different prognostic groups. NPI was calculated using following formula:

0.2 × tumour size (cm) + grade (1-3) + lymph node stage (1-3)

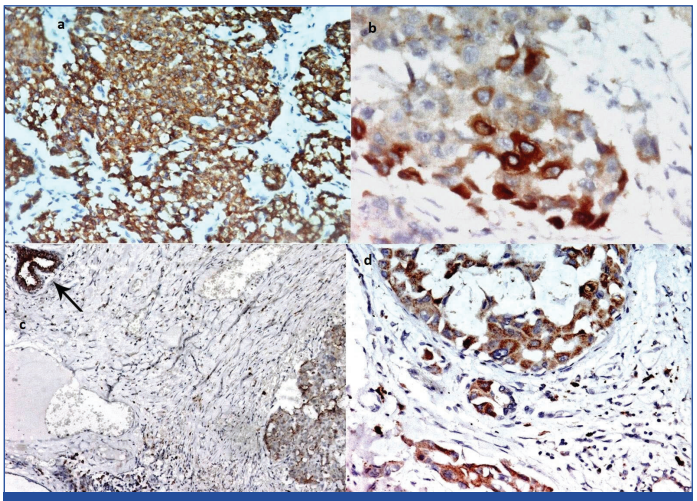
Twenty eight (56%) patients were in moderate prognostic group, (n=14, 28%) and (n=8, 16%) in poor and good prognostic group respectively.

The ER, PR and HER2/neu status was assessed. Thirty (60%) cases were ER positive and (n=26, 52%) were PR positive. Fourty percent cases were both ER/PR negative. Only 24% cases had HER2/neu positivity [Table/Fig-1].

The COX-2 expression was assessed as COX-2 IHS, defined as product of staining intensity and percentage of positive tumour cells. COX-2 IHS was separately calculated for normal breast epithelium (near to the tumour), DCIS (where ever possible, n=17) and tumour tissue. COX-2 was moderately positive in (n=33, 66%) of the cases of tumour, (n=36, 72%) of adjacent normal breast epithelial tissue and (n=20, 86%) of DCIS component. There was no significant difference in COX-2 expression in these groups [Table/Fig-2,3].



[Table/Fig-2]: COX-2 expression in normal breast tissue (score-4, intensity-2, IHS-moderate); a) (10X) and; b) (20X); c) COX-2 expression in DCIS. (Score-4, intensity-3, IHS-strong) (10X); d) COX-2 expression in normal breast tissue (score-4, intensity-2, IHS-moderate) and DCIS. (Score-4, intensity-3, IHS-strong) (10X).



[Table/Fig-3]: COX-2 expression in invasive breast carcinoma; a) score-4, intensity-1, IHS-moderate (10X) and; b) score-3, intensity-2, IHS-moderate (40X); c) COX-2 expression in invasive breast carcinoma (score-3, intensity-2, IHS-moderate) and normal breast tissue (arrow). (Score-4, intensity-3, IHS-strong) (10X); d) COX-2 expression in invasive breast carcinoma (score-2, intensity-3, IHS-moderate) and DCIS (Score-4, intensity-3, IHS-strong) (20X).

Majority of cases i.e., (n=47, 94%) cases showed same COX-2 expression level in between normal breast epithelium and corresponding tumour areas and this correlation was statistically significant (p-value <0.001, rs= 0.869).

In the present study, 23 cases had both DCIS and tumour area. Out of these 23 cases, three cases of paired DCIS and invasive tumour area were negative for COX-2 expresssion. Conversely, 90% (18/20) of DCIS lesion with moderate COX-2 expression were matched by a similar expression level in paired invasive cancer samples. Only two cases with moderate COX-2 expression in DCIS showed negative expression in corresponding tumour area. The correlation of level of COX-2 expression between tumour and DCIS was highly significant. (rs =0.735, p-value <0.001). In all of 23 cases, with DCIS component, COX-2 IHS between normal tissue and DCIS was similar and this correlation was highly significant (p-value <0.01, rs=1.0).

Statistically, the COX-2 IHS scoring was not significantly related with the age group, menopausal group and tumour size in the present study. COX-2 expression was statistically significant in DCIS areas in relation to histopathological grades, while no significant relation was seen in tumour tissue. COX-2 expression was compared in tumour and DCIS in relation to lymph node status and this was not found to be statistically significant. COX-2 expression in tumour was statistically significant in various prognostic groups while it was insignificant in DCIS areas. On statistical analysis, COX-2 expression was not significant in relation to ER, PR and HER2/neu status within tumour and DCIS area [Table/Fig-1].

DISCUSSION

There has been an inconsistency in the literature regarding significance of COX-2 expression in the normal breast tissue and on the changes in level of COX-2 expression during progression of invasive cancer. Some studies have found no clinicopathological relevance at all, while others have concluded that COX-2 expression is an important biomarker in invasive breast cancer and pre-cancerous lesions, correlating with poor prognostic features [10,15,20-22].

The COX-2 was moderately positive in (66%) of the cases of tumour, (72%) of adjacent normal breast epithelial tissue and (86%) of DCIS component. There was no significant difference in COX-2 expression in these groups. COX-2 expressions in the present study were comparable to other studies in literature [Table/Fig-4] [13,15,20,23,24].

Studies (year)	No. of cases N/DCIS/T	COX-2 expression		
		Normal tissue (%)	DCIS (%)	Tumour (%)
Half E et al., [13] (2002)	48/16/42	81	63	43
Shim JY et al., [23] (2003)	0/42/64	-	76	72
Boland GP et al., [24] (2004)	120/187/65	23	67	63
Ranger GS et al., [15] (2004)	30/22/30	-	55	56
Leo C et al., [20] (2006)	39/29/39	54	55	59
Present study (2015)	50/23/50	72	86	66

[Table/Fig-4]: Distribution of COX-2 expression in various studies.
DCIS-Ductal carcinoma in situ, N-Normal breast tissue, T-Tumour tissue

About 94% of cases investigated, showed similar COX-2 expression level in between normal breast epithelium and corresponding tumour area in the same patient. The extent of COX-2 expression in normal breast epithelium correlated significantly to that in invasive breast cancer of the same patient (rs=0.869, p-value<0.001). Published data regarding COX-2 expression in normal breast tissue are conflicting [13,15,20]. In accordance to present study, Leo C et al., found (83%) of cases with a negative COX-2 expression in normal breast epithelium and the paired invasive breast cancer lesions were also negative [20]. On the contrary, in (95%) of cases with a moderate or strong COX-2 expression in normal breast epithelium, this was accorded by a moderate or strong COX-2 expression in the invasive breast cancer of the same patient.

However, some studies had different results. Half E et al., found COX-2 expression in 81% of benign adjacent tissue and described it to be of similar or reduced intensity relative to the malignant tissue within the same tissue sections [13]. They used reverse transcriptase polymerase chain reaction for the detection of COX-2 messenger RNA (mRNA). Ranger GS et al., did not find any COX-2 immunoreactivity in normal breast and Adjacent Non-Cancerous

Tissue (ANCT) [15]. This discrepancy can be partly explained by the paucity of ductal units in normal breast tissue as compared with malignant breast tissue or due to different methods in evaluation of immunohistochemistry results in the different studies.

Conversely, (90%) of DCIS lesions with moderate COX-2 expression were matched by a similar expression level in paired invasive cancer samples. We had a significant association between tumour and DCIS area. Similar observations were made by few available studies in literature who have examined DCIS areas in matched samples. In a study by Leo C et al., there was a statistically significant correlation between the COX-2 expression in DCIS and invasive breast cancer [20]. In (85%) of the cases with a negative COX-2 expression in DCIS, the paired invasive cancer lesions were also negative. Conversely, (94%) of DCIS lesions with moderate or strong COX-2 expression were matched by a similar expression level in the paired invasive breast cancer samples. Half E et al., showed that within the same tissue sections, COX-2 expression in invasive breast tumours and adjacent DCIS were highly correlated (p-value=0.019) [13]. Ranger GS et al., studied 30 patients of invasive breast cancer and a significant statistical association was observed between invasive carcinoma and concomitant DCIS lesions (p-value=0.007) [15].

Shim JY et al., studied 64 cases of breast cancer of which four cases were of pure DCIS, whereas 38 cases of invasive duct carcinoma with areas of DCIS [23]. Thirty-two out of 42 cases of total DCIS (76%) demonstrated COX-2 positivity. Out of 38 cases in which DCIS and invasive carcinoma coexisted, 31 cases showed COX-2 overexpression. Keeping in view high frequency of COX-2 in DCIS area, it can be suggested that COX-2 overexpression is involved in the progression to invasive cancer and may be an early event in breast carcinogenesis.

In all the 23 cases of DCIS, we found a significant correlation between COX-2 expression in DCIS and normal breast epithelium. (rs= 1.0, p-value <0.01) There is significant correlation between the COX-2 expression levels in normal breast tissue and DCIS lesion of the same patient. This was in concordance with studies done by Leo C et al., Shim V et al., and Boland GP et al., [20,21,24]. Present observation that COX-2 is upregulated in the surrounding epithelial tissue raises the strong possibility that the adjacent normal epithelium was part of the disease process in DCIS, was further supported by the study of Shim V et al., which stated that COX-2 intensity in the normal adjacent epithelium is stronger than in the lesion itself and correlated with DCIS nuclear grade [21].

In the present study, correlation of COX-2 expression with patient's age was statistically insignificant and present observation is in accordance to various other studies in literature [10,13,20,22,25]. COX-2 expression in tumour when compared to different tumour sizes was not found statistically significant in the present study. Our findings were in accordance to studies by Ranger GS et al., and Leo C et al., [15,20]. While Ristimaki A et al., had a statistically significant association of COX-2 expression with respect to tumour size [10].

In the present study, we did not observe a statistically significant correlation between COX-2 expression and MBR grade in tumour areas, (chi-square test, p-value= 0.098), but it was significant in DCIS areas (chi-square test, p-value=0.011). Ranger GS et al., Leo C et al., and Shim V et al., also did not find any significant association between COX-2 expression and tumour grade [15,20,21]; on the contrary, studies by Ristimaki A et al., and Takeshita E et al., found statistically significant correlation between COX-2 expression levels and tumour grades [10,22]. The discrepancy in the observation can be partly explained by more number of cases with higher grade (grade III) in both the studies whereas in the present study grade III cases constituted the smallest group. The other factors which might have influenced the results could be the number of cases studied and histological type.

Number of cases with positive COX-2 expression was higher in good and moderate prognostic groups; however, in poor prognostic group COX-2 expression was less. COX-2 expression in the tumour was statistically significant with prognostic groups (p-value= 0.045). None of the studies had NPI as a parameter for studying its correlation with COX-2 expression.

In the present study, positive COX-2 expression was seen in both ER/PR positive/negative group and HER2/neu positive/negative groups; it was not found dependent on hormonal receptor status. On statistical analysis, COX-2 expression was not found significant in relation to hormonal receptor status. Most of the literature [13,15,20-23] pertaining to correlation of COX-2 expression among the tumour areas and hormonal status had unanimity that there is no correlation except for Ristimaki A et al., Boland GP et al., and Perrone G et al., who found a significant correlation. This discrepancy could be explained partly by selection of high grade cases and with different histological types [10,24,25].

These observations support the possibility that the adjacent normal epithelium is the part of disease process in DCIS and this could be an early event preceding the changes in DCIS and tumour areas.

LIMITATION

The histological types in present study solely comprised of infiltrating duct carcinoma (NOS) as per WHO classification whereas, other studies had different histological types as their study group.

Failure to follow up many of present patients compounded by unavailability of significant clinical details in some cases adversely affected our ability to provide correlative data regarding clinical behaviour and survival information.

CONCLUSION

Based on present results, we conclude that a statistically significant correlation exists between tumour, adjacent normal epithelium and DCIS: therefore, suggesting that COX-2 exerts paracrine effect and is involved in early breast cancer carcinogenesis. Since, most infiltrating breast carcinomas are believed to originate from DCIS, available data suggests that inhibition of COX-2 may represent a potential target for preventing breast cancer oncogenesis and as an adjuvant treatment following surgery to reduce local recurrence. Although, present results are also consistent with this hypothesis, further studies are needed in setting of large clinical DCIS trials to explore COX-2 expression in adjacent tissue as a marker for recurrence and also a potential therapeutic agent.

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PARTICULARS OF CONTRIBUTORS:
1. Resident, Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India.
2. Professor, Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India.
3. Senior Resident, Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India.
4. Senior Professor and Head, Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Padam Parmar,
Senior Resident, Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak-124514, Haryana, India.
E-mail: drpadamparmar@gmail.com

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