



## A CASE CONTROL STUDY ON EXPRESSION OF CYCLIN D1, P16 AND RETINOBLASTOMA PROTEIN IN CARCINOMA OF THE GALL BLADDER

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### ABSTRACT

**Background:** Carcinoma of the gall bladder is a highly malignant tumor, with poor prognosis except when incidentally diagnosed at an early stage after cholecystectomy for illness other than carcinoma. The incidence of carcinoma of the gall bladder varies considerably in different parts of the world. A large number of risk factors have been proposed in the etiopathogenesis of carcinoma of the gallbladder. Cholelithiasis with or without chronic cholecystitis has been on the top among these causes. **Objective:** To study the expression of cyclin d1, p16 and retinoblastoma protein in carcinoma of the gall bladder. **Method:** This clinical study was conducted in the Department of Surgical Oncology, Institute of Medical Sciences, in collaboration with Department of Pathology, Banaras Hindu University, Varanasi. 60 patients of carcinoma gall bladder were included for the study who were further divided in three groups 25 in (study group), 25 patients of cholelithiasis and 10 patients of normal gallbladder (control group). **Result:** In the present study a mean age of  $47.4 \pm 8.11$  years (range: 25-70) was observed in patients of carcinoma of the gallbladder with 68% being in the 4<sup>th</sup> and 5<sup>th</sup> decade of life. Majority of patients were female 19 (76%) out of 25. The female to male ratio was 3.2:1 in present study. The common presenting symptoms in our study were pain in 88%, loss of appetite in 56.0%, loss of weight loss in 36%, abdominal lump 32.0% patients. The most common clinical sign in our study was tenderness in right hypochondrium (72.0 %), gallbladder lump in 36.0 % patients. Pallor was present in 28% cases, icterus in 12% cases and hepatomegaly in 12.0 %. We found strong positive relation between tumor metastasis and cyclinD1 expression ( $\chi^2 = 5.114$ ,  $P$  Value = **0.024**;  $r = 0.452$ ,  $P$  Value = **0.012**). In our study Rb expression was present in 6(24%), 8(32%) and 5(50%) in carcinoma, cholelithiasis and normal gallbladder respectively. This difference is statistically not significant. ( $\chi^2 = 2.234$ ,  $P$  Value = 0.327). **Conclusion:** There was a strong positive relation between metastatic tumors and cyclinD1 expression suggesting that aggressive malignancy of the gallbladder have higher cyclin D1 expression and its expression may act as a predictive biomarker for behaviour of the gallbladder malignancy.

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### INTRODUCTION

Carcinoma of the gall bladder is a highly malignant tumor, with poor prognosis except when incidentally diagnosed at an early stage after cholecystectomy for illness other than carcinoma. The incidence of carcinoma of the gall bladder varies considerably in different parts of the world. A large number of risk factors have been proposed in the etiopathogenesis of carcinoma of the gallbladder. Cholelithiasis with or without chronic cholecystitis has been on the top among these causes. And the risk seems to correlate well with the stone size and polyps that are more than 1 cm single sessile and echogenic. Anomalous junction of pancreaticobiliary ductal union (AJPBDU) especially without choledochal cyst and porcelain gallbladder is additional risk factor that predispose to gallbladder cancer.<sup>[1]</sup> Infrequent association includes chronic bacterial infection, typhoid carrier state, certain occupational and environmental carcinogens, hormonal changes in women and certain social, dietary and familial factors.<sup>[2]</sup> Gallbladder carcinoma can spread by direct invasion or by lymphatic or hematogenous routes. Ultrasound is useful for detecting the primary tumor as well as adjacent liver invasion. CT is more useful for

evaluating the extent of the disease, such as direct extension to the liver, periportal and peripancreatic lymphadenopathy, extension to the biliary tree (resulting in biliary obstruction), and hematogenous metastases.<sup>[3]</sup> Surgery is the only curative option for carcinoma of the gall bladder. However for patients coming with advance malignancy and jaundice or duodenal obstruction, only palliative procedures are performed.

**Objective:** To study the expression of cyclin d1, p16 and retinoblastoma protein in carcinoma of the gall bladder.

### MATERIAL AND METHODS

**Method:** This clinical study was conducted in the Department of Surgical Oncology, Institute of Medical Sciences, in collaboration with Department of Pathology, Banaras Hindu University, Varanasi.

This study includes 50 Patient undergoing surgery for gallbladder carcinoma (25 patients) and cholelithiasis (25 patients) and 10 patients undergoing cholecystectomy or cholecystojejunostomy for cancers other than gallbladder (normal control) during the period of Nov.2007 to July 2009 recruited after explanation of procedure and consent of patient

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for study. A detailed history, clinical examination and investigations, TNM staging and treatment details recorded for every recruited patient on a preset proforma.

Gallbladder specimen collected after surgery were cut into slices at approximately 0.5 cm and the specimen were kept in 10% buffered formalin for 18-24 hrs for proper fixation, then the specimen were grossed by trained pathologist to obtain representative tissue section which were processed routinely in the conventional way for embedding in paraffin wax. 4µm section were cut and placed on glass slide, one slide of each tissue was stained with haematoxyline and eosin (H & E).

Blocks of the viable and tumour representative area were selected and immunohistochemistry (IHC) was done for p16, cyclin D1 and pRb

**RESULTS**

In group I majority (19; 76%) of patients were between the age group 41- 60 year and most of the patients were in 41-50year (52%). In group II most of the patients were between 41-50 years (48%).

**Table 1** Age distribution

	Group I (n=25)	Group II (n=25)	Group III (n=10)
Age group(years)	No. of patients (%)	No. of patients (%)	No. of patients (%)
≤20	-	-	-
21-30	01 (4%)	02(8%)	-
31-40	04(16%)	05(20%)	-
41-50	13(52%)	12(48%)	01(10%)
51-60	06(24%)	04(16%)	06 (60%)
≥60	01(4%)	02(8%)	03(30%)

**Table 2** Gender wise distribution

Sex	Group I (n=25)	Group II (n=25)	Group III (n=10)
Female	19 (76%)	21	04
Male	06 (24%)	04	06

Pearson Chi-Square - 7.227 (P Value - 0.027)

Pain abdomen was the most common symptom in study group (group I) which was present in all the patients followed by loss of appetite in 56.0%, lump abdomen in 32.0%, and fever in 16.0 % and jaundice in 8.0 % patients.

**Table 3** Clinical Symptoms

Symptoms	Group I (n=25)	Group II (n=25)	Group III (n=10)	Pearson Chi-Square (p value)
Pain Abdomen	22 (88%)	21	08	1.509 (0.825)
Vomiting	02	12	06	12.840 (0.002)
Jaundice	02 (8%)	01	04	9.542 (0.008)
Lump abdomen	08 (32%)	02	02	5.794 (0.055)
Fever	04	07	03	1.304 (0.521)
Anorexia	14 (56%)	07	06	5.051 (0.080)
Weight loss	09 (36%)	02	01	6.875 (0.032)

Simple cholecystectomy was performed in 01 patients (4.0%) followed by extended cholecystectomy in 17patients (68.0 %), palliative bypass in 04 patients (16%) and simply biopsy and closure was done in 03 patients (12%). All case (n=25) in cholelithiasis group (group-II) underwent simple choleystectomy and similarly all cases in normal gallbladder group (group-III) underwent pancreaticoduodenectomy for some other malignancy.

**Table 4** Surgical Treatment

Surgical Treatment	Group I(n=25)	Group II(n=25)	Group III(n=10)
Simple cholecystectomy	01 (4%)	25	00
Extended cholecystectomy	17 (68%)	00	00
Palliative bypass	04(16%)	00	00
Biopsy and closure	03(12%)	00	00
Pancreaticoduodenectomy	00	00	10

Positive staining for p16, cyclin D1 and retinoblastoma(Rb) protein expressed as brown granules , were mainly distributed in nuclei.

**Table 5** Expression of cyclinD1, Rb and p16 in the entire three groups

Variable	Carcinoma (n=25) Group I	Cholelithiasis (n=25) Group II	Normal (n=10) Group III	Pearson Chi-Square (p value)
CyclinD1				1.435(0.488)
(+)	10	10	2	
(-)	15	15	8	
Rb				2.234(0.327)
(+)	06 (24%)	08 (32%)	05(50%)	
(-)	19	17	05	
P16				4.704(0.095)
(+)	03	07	00	
(-)	22	18	10	

Table 6 shows distribution of patients according to cyclin D1 expression. Cyclin D1 expression was almost equivalent in both gallbladder carcinoma (10; 40%) and cholelithiasis(10; 40%) group. And it was 20% in group III (normal gallbladder)

**Table 6** Cyclin D1 expression

Cyclin D1 expression	Group I(n=25) No. of patients (%)	Group II(n=25) No. of patients (%)	Group III(n=10) No. of patients (%)
0	15(60%)	15(60%)	08(80%)
+1	06	07	01
+2	03	03	01
+3	01	0	0
Total (+)	10(40%)	10(40%)	02(20%)

Pearson Chi-Square - 2.955 (P Value - 0.814)

Table 7 shows distribution of patients according to Rb expression. Rb expression was 24% in group I (carcinoma gallbladder), 36% in group II (cholelithiasis) and it was 50% in group III (normal gallbladder).

**Table 7** Rb expression

Rb expression	Group I(n=25) No. of patients (%)	Group II(n=25) No. of patients (%)	Group III(n=10) No. of patients (%)
0	19(76%)	17(64%)	05(50%)
+1	05	05	02
+2	01	02	02
+3	0	01	01
Total(+)	06(24%)	09(36%)	05(50%)

Pearson Chi-Square - 5.170 (P Value - 0.522)

Table 8 shows distribution of patients according to p16 expression. P16 expression was 12% in group I (carcinoma gallbladder), 28% in group II(cholelithiasis) and there was no positivity at all in group III.

**Table 8** p16 expression

p 16 expression	Group I(n=25) No. of patients (%)	Group II(n=25) No. of patients (%)	Group III(n=10) No. of patients (%)
0	22	18	10
+1	03	07	0
+2	0	0	0
+3	0	0	0
Total (+)	03(12%)	07(28%)	0(0%)

Pearson Chi-Square = 4.704 (P Value = 0.095)

## DISCUSSION

In the present study a mean age of  $47.4 \pm 8.11$  years (range: 25-70) was observed in patients of carcinoma of the gallbladder with 68% being in the 4<sup>th</sup> and 5<sup>th</sup> decade of life. Majority of patients were female 19 (76%) out of 25. This shows that elderly females are the main victim of carcinoma of the gallbladder. Carcinoma of the gallbladder is usually associated with elderly females with a peak incidence in 6<sup>th</sup> and 7<sup>th</sup> decade of life and a mean age of 65 years. [4] [5] the female to male ratio was 3: 1 in present study. Men: women ratio was 0.36:1.00 in a study. [6] The female to male ratio was 3.2:1 in present study. The common presenting symptoms in our study were pain in 88%, loss of appetite in 56.0%, loss of weight loss in 36%, abdominal lump 32.0% patients. The most common clinical sign in our study was tenderness in right hypochondrium (72.0 %), gallbladder lump in 36.0 % patients. Pallor was present in 28% cases, icterus in 12% cases and hepatomegaly in 12.0 % compared to 60.3%. [7] Majority of the patients in our study were of stage II (60.0 %). There were stage 16.0 % patients in stage III, 12% patients in stage IV and just one patient in stage I.

Standard treatment for carcinoma of the gallbladder is surgery, but surgical resection is possible only in 10-30% of the patients. [8] In our study we performed extended cholecystectomy in 68.0% patients. Palliative bypass was performed in four patients (16%) in which curative resection were not possible and bypass was the only left option to ameliorate the symptom. All the patients had adenocarcinoma gallbladder as histological type in our present study. As reported in various series include adenocarcinoma (82%), squamous cell carcinoma (3.3%), undifferentiated carcinoma (6.9%), adenoacanthoma (1.4%), carcinoma in situ (0.7%) and mixed malignant lesion of epithelial and mesothelial origin (1%). [9] It has been accepted that there is restriction point in cell-cycle progression, called the cell-cycle check points. The check point at G1-S is important. Rb, p16 and cyclin D1 are major restriction factors at this cell-cycle check point regulating the cell-cycle. Cell-cycle progression is normally regulated by cyclins and cyclin inhibiting proteins. [10] Cyclin D1 / p16 / Rb pathway has been shown to play critical role in tumorigenesis. [11] The overexpression of cyclin D1 has been reported in a wide range of human cancer. [12] In our study there was no difference at all between the carcinoma and cholelithiasis group, means in both these group cyclin D1 expression was present in 10 (40%) out of 25 patients. On contrary, only 2 out of 10 patients in normal gallbladder group showed the positive staining for cyclin D1 but this difference in cyclinD1 expression between normal Vs carcinoma/cholelithiasis was statistically not significant (P Value - 0.488). Out of three metastatic cases in our study cyclinD1 expression was positive in all the three cases so There is a strong positive relation between tumor metastasis and cyclinD1 expression ( $\chi^2 = 5.114$ , P Value = 0.024;  $r = 0.452$ , P Value = 0.012).

Rb gene is the first tumor suppressor gene located at chromosome 13q14 identified by the location cloning method. The product of Rb is a nuclear phosphoprotein. Loss of pRb has been demonstrated in a variety of cancers, including gastric, pancreatic and bladder cancers, small cell lung and colorectal carcinomas. [13] In our study Rb expression was present in 6(24%),8(32%) and 5(50%) in carcinoma, cholelithiasis and normal gallbladder respectively. This

difference is statistically not significant. ( $\chi^2 = 2.234$ , P Value = 0.327). There is no significant correlation between Rb and tumor size, nodal status, metastasis, staging and grading of the tumor. P16 gene located on chromosome 9p21, is a new tumor suppressor gene, which was identified by an American molecular geneticist in 1995 and is also called multiple tumor suppressor 1 (MTS1) for its suppressing function on multiple tumors. [14] In our study P16 expression was 12% in group I (carcinoma gallbladder), 28% in group II (cholelithiasis) and there was no positivity at all in group III. This difference was not statistically significant ( $\chi^2 = 4.704$ , P Value = 0.095). There is a positive correlation between p16 expression and nodal involvement in the study in carcinoma group ( $r = 0.369$ , P Value = **0.035**). There is no significant correlation between p16 and tumor size, metastasis, staging and grading of the tumor.

## CONCLUSION

There was a strong positive relation between metastatic tumors and cyclinD1 expression suggesting that aggressive malignancy of the gallbladder have higher cyclin D1 expression and its expression may act as a predictive biomarker for behaviour of the gallbladder malignancy. Also there is a positive correlation between p16 expression and nodal involvement in the study in carcinoma group and may have similar implications.

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