Histopathological study of gall bladder malignancies with special reference to p53 expression

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1. Introduction

Carcinoma of the gall bladder, overall, is the most common cancer of the biliary tract and one of the highly malignant tumors with poor prognosis.¹ It is the fifth most common gastrointestinal malignancy following colon, pancreas, stomach and oesophagus.² Incidence increases with age. Almost 90% patients are 50 years of age or older at the time of diagnosis. It is more common in females than males (3 to 4:1 ratio).³ Gall bladder cancer is very common in South American countries, the Mediterranean region, Japan and northern parts of India.⁴ Chile has the highest incidence rate of gallbladder cancer (7.5/100,000) especially among American females.⁵ According to the Indian Council of Medical Research cancer register, in the north the incidence of gallbladder cancer is 2.5-8.5/100,000 females and 1.6-3.7/100,000 in males whereas in southern India it is only 0.7-0.8/100,000 females and 0.5-0.6/100,000 males population.⁶

The etiology of gallbladder cancer has been a source of speculation. The incidence of GBC parallels the prevalence of gallstone disease; large and longstanding gallstones being associated with a higher risk of GBC. The risk of GBC in patients with gallstones has been reported to have increased four to seven times (Tyagi et al., 2008).⁷ The association between an abnormal pancreaticobiliary duct junction, a porcelain gallbladder, and other biliary disorders such as cholelithiasis, primary sclerosing cholangitis, Mirrizi’s syndrome and gallbladder cancer has also been recognized (Pandey et al., 2003).⁸

Primary carcinoma of the gallbladder is an unexpected histopathological finding in 1-3% specimens after elective cholecystectomy for benign gall bladder disease. The overall prognosis has remained dismal with the 5-year survival of 5-10% due to the late detection of the disease.⁹ Prior to the era of ultrasonography and CT scanning, the rate of the correct pre-operative diagnosis was only 8.6%, which has improved considerably to 75–88%, with the use of these newer imaging techniques. Still, a pre-operative diagnosis of early carcinoma of the gall bladder is
seldom made, where the 5-year survival is 91-100%. The most common clinical manifestations of gallbladder carcinoma are right upper quadrant abdominal pain and anorexia, and the most common abnormal laboratory finding is elevated alkaline phosphatase level.

Grossly, gallbladder carcinoma may present as diffusely growing (70%) or polypoidal (30%) mass. Most of the cancers originate in the fundus. As the tumor progresses gall bladder may fill with tumor or may contain pus, mucus or stones. Gallbladder carcinomas usually also contain calculi (80-90% cases) and exhibit marked fibrosis of the wall. The fact that some gall bladder carcinomas are not obvious on gross examination indicates the need for microscopic examination of every excised gallbladder.

Microscopically most carcinomas of the gallbladder are adenocarcinomas (80-95%), and can be papillary, tubular, mucinous or signet cell type and less common include: undifferentiated or anaplastic carcinoma (2-7%), squamous cell carcinoma (1-6%), and adenosquamous carcinoma (1-4%). Carcinoid tumors, small – cell carcinomas, malignant melanomas, lymphomas and sarcomas are particularly rare.

In the search for prevention, diagnosis and treatment of this cancer, genetic changes involved in the origin of gallbladder carcinoma should be determined. Most studies have focused on mutations of oncogenes K-ras or tumor suppresser genes p53, FHIT, p14 and p16, p63. Of these, alterations in the tumor suppressor gene, p53, is commonly observed in most human cancers.

The p53 gene, a tumor suppressor gene, located on short arm of chromosome 17, encodes a 53-kD nuclear phosphoprotein, which acts as an inhibitor of cell proliferation. Mutations in p 53 gene are the most common genetic lesions found which lead to gall bladder carcinoma. These mutations play a key role in the multistep process that leads to carcinogenesis. These mutations are generally thought to alter the functional capabilities of the cell. Most mutations described are missense mutations resulting in an abnormal protein that accumulates in cells by virtue of an increased half life. These intranuclear accumulations are easily detected by immunohistochemistry. Overexpression of p53 is an important early event in gallbladder tumor formation and progression and is reflected in the biological behavior and prognosis of the cancer.

This study aims to outline the histopathological spectrum of gall bladder malignancies with special reference to p53 expression.

2. Materials and Methods

A total of 80 histologically proven cases of gall bladder malignancies from January 2009 and December 2014 received in the Department of Pathology, SRMS IMS, Bareilly were included. The study was approved by the ethical committee of the institute.

All the histopathologically proven cases of gall bladder malignancy were examined for p53 expression immunohistochemically. Various sections were taken, fixed in 10% buffered formalin autoprocessed and blocks were embedded in paraffin. Sections were cut at 3-4 um thickness. These sections were stained with haematoxylin and eosin (H&E).

In these cases, p53 expression was observed by immunohistochemical method. Immunohistochemistry was performed using mouse monoclonal primary antibodies for p53 (clone DO-7, ready to use solution) using labeled Streptavidin – Biotin Peroxidase method.3-4 um thick sections after deparaffinisation in xylene were rehydrated in graded alcohol. Antigen retrieval was done using microwave pretreatment in citrate buffer pH(6.0). The sections were incubated with primary antibody for 1 hr at room temperature. After washing, the sections were treated with biotinylated secondary antibody for 30 min. A streptavidin HRP (horse – radish peroxidase) conjugate was applied for 15 min. The reaction was visualized by using DAB (diaminobenzidine) as chromogen. Counterstaining was done by Mayer’s hamatoxylin. A positive control was used simultaneously.

2.1. Evaluation of p53 immunohistochemical staining

We adopted a semi – quantitative scoring method as described by Wistuba et al. The intensity was graded as absent(0), mild (1), moderate (2), or intense(3). The Percentage of nuclei stained was categorised as / 3 was considered as a positive overexpression.

The results obtained will be correlated with grade and the stage of the malignancy.

3. Results

The present study included 80 cases of histopathologically proven cases of gall bladder malignancy. It was observed that out of 80 cases of gall bladder malignancy, 30(37.5%) cases were p53 positive and 50(62.5%) were p53 negative. (Table 1). It was observed that in p53 positive patients 6(20%) cases were well differentiated carcinoma, 10(33.3%) were moderately differentiated carcinoma and 14(46.7%) were poorly differentiated carcinoma. (Table 2).

It was observed that in p53 positive patients 2(6.7%) were in stage pT1, 6(20%) were in stage pT2, 10(33.3%) were in stage pT3 and 12(40%) were in stage pT4. Whereas in p53 negative patients 3(6%) were in stage pT1, 11(22%) were in stage pT2, 16(32%) were in stage pT3 and 20(40%) were in stage pT4. (Table 3).
Table 1: p53 over expression in gall bladder malignancy

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<thead>
<tr>
<th>p53</th>
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<th>Percentage</th>
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<tr>
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<td>37.5%</td>
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<tr>
<td>Negative</td>
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Table 2: Grading of malignancy in adenocarcinoma

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<td></td>
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<tr>
<td>MD</td>
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Table 3: Staging of malignancy

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<th>Stage</th>
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<tbody>
<tr>
<td></td>
<td>Cases</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>pT4</td>
<td>12</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
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Fig. 1: Microphotograph showing p53 score 3 (IHC,4X)

Fig. 2: Microphotograph showing p53 score 5 (IHC,10X)

4. Discussion

In our study we planned to determine the immunohistochemical expression of p53 in gall bladder malignant cases. Total 80 cases of gall bladder carcinoma were included in the study, 30 (37.5%) cases were p53 positive and 50(62.5%) cases were p53 negative. The reported incidence of p53 expression in gall bladder malignancy is variable(20-92%).

In a study conducted by Grau et al on 41 patients of histologically proven cases of GBC, 29 showed p53 positivity.

Roa et al in a study of 157 primary gall bladder carcinoma, found p53 expression in 45% cases.

In an Indian study conducted on 78 patients of GBC by Chaube et al p53 positivity was found in only 20% of patients.

p53 negative cases showed 20(47.6%) well differentiated cases, 18(42.9%) moderately differentiated cases and 4(9.5%) poorly differentiated cases. This difference was found to be significant (p=.003). This suggests an inverse relationship between differentiation and p53 expression. Assuming that poorly differentiated tumors behave more aggressively, p53 expression may correlate with aggressiveness of disease. In a similar study by Roa et al of 191 cases of gall bladder malignancy, they observed increased p53 expression with grade of the tumor. A study by Ghosh et al on 80 cases of GBC found that low grade tumor(well differentiated) shows low
expression of p53 (42.8%) in comparison to that in the moderately differentiated (62%) and poorly differentiated tumors (66.7%).

In a study by Chauhe et al on 20 cases of GBC none of the grade I case showed p53 expression, while 20% and 37% of grade II and grade III revealed overexpression respectively. In a study conducted by Kim et al on 71 cases of GBC, 48 cases showed p53 expression and no association with the grade of the tumor.

Among p53 negative patients also more number of patients were in stage pT3/pT 4 i.e 26(72%) as compared to stage pT1/ pT 2 i.e 4(28%). p53 scores showed no significant difference with the stage of the tumor. According to a study conducted by Ghosh et al on 80 patients of GBC 36 had stage I disease, 40 cases had stage II and 4 cases had stage IV disease.

In a study conducted by Wistuba et al on 52 cases of GBC thirty four showed p53 expression and no association was observed with correlation between p53 over expression in GBC and stage of the tumor. However, p53 expression didn’t correlate with the depth of the tumor invasion or the stage of the tumor in our cases. These results may indicate that p53 has a role in gall bladder carcinogenesis and its progression of the cancer from low grade to high grade but not in tumor invasiveness.

5. Conclusion

Out of 80 patients of gall bladder carcinoma evaluated for p53, p53 overexpression was detected in 37.5% cases. There was statistically significant difference between the histological grade of p53 positive and p53 negative adenocarcinomas with significantly higher number of patients of p53 overexpression presenting with poorly differentiated adenocarcinomas (P=.003). p53 overexpression showed inverse relationship with the grade of the tumor.

6. Source of funding

None.

7. Conflict of interest

None.

References

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