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Original Research Article

Evaluation of Over-Expression of C-MET /HGF Pathway in Pre-Neoplastic and Neoplastic Lesions of Gall Bladder

Khanam Bushra[1], Jaiswal Pooja[2], Mustafa Asif [3], Tandon Pradeep[4]

[1]Senior Resident, Department of Pathology, GMC Kannauj
[2]Associate Professor, Department of Pathology, Integral Institute of Medical Sciences and Research Lucknow
[3]Assistant Professor, Department of Biochemistry, GMC Kannauj
[4]Professor and HOD, Department of Pathology, Hind Institute of Medical Sciences Lucknow

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Corresponding Author: Dr. Tandon Pradeep
Conflict of interest: Nil

Abstract:

Background: Mesenchymal epithelial transition (c-MET) is a tyrosine kinase receptor that is activated by its ligand Hepatocyte Growth Factor (HGF). c-MET over-expression is implicated in initiation, progression, inhibition of apoptosis and tumor metastasis in many different organs of the body.

Objectives: The present study was done to investigate over-expression of c-MET in pre-neoplastic and neoplastic lesions of the gallbladder. The stage at which c-MET/HGF pathway alterations begin to appear during tumorigenesis was also investigated.

Material and Methods: The study was conducted in department of Pathology, Integral Institute of Medical Sciences and Research on 60 specimens of Gall bladder. Gall bladder showing chronic cholecystitis, metaplasia, dysplasia, and carcinoma were included in the study. Immunohistochemical expression of c-MET was evaluated in all specimens.

Statistical Analysis: Chi square test and Fisher's exact probability test was used in analysis of c-MET over-expression in all lesions of gall bladder.

Results: Over expression of c-MET was seen in 50% cases of gall bladder carcinoma. It was seen in 16.7% of cases showing dysplasia only. c-MET over-expression was not observed in any case of chronic cholecystitis and metaplasia.

Conclusion: We conclude that c-MET over-expression in gall bladder lesions appear with advent of dysplasia and increases as the lesion progresses from dysplasia to neoplasia. This suggests that over-expression of MET/HGF signaling may be responsible in pathogenesis of GBC. Elucidation of c-MET expression in GBC may act as a prognostic marker and could be a potential therapeutic target in future for treatment by c-MET pathway antagonists.

Keywords: c-MET/HGF Immunohistochemistry, Carcinoma Gall Bladder.

Introduction

Gall bladder cancer (GBC) – although a relatively rare tumor in other parts of the world - is a common malignancy in Indian subcontinent, particularly in northern and eastern parts of the country [1]. Many of the risk factors implicated in causation of GBC are known to promote inflammation that lead to sequential development of metaplasia, dysplasia and carcinoma [2].

A variety of genetic and epigenetic alterations are also seen in association with this malignancy [3]. GBC is an aggressive tumor. Even following surgery and cytotoxic chemotherapy, the outcome is disappointing. More recently, target-oriented agents have entered clinical trials [4,5]. However, these too provide no or marginal clinical benefit. Hence there is a need for identification of new therapeutic approaches. Mesenchymal epithelial transition receptor (c-MET) stimulation activates tyrosine kinase pathway. Abnormal c-MET activity plays critical role in tumor formation, progression, angiogenesis and metastasis. c-MET is stimulated by its ligand - hepatocyte growth factor (HGF).

Dysregulated c-MET/HGF pathway activity is reported in many pre-malignant and malignant disorders. However, very few studies have explored over-expression/inappropriate activation of MET-HGF pathway in pre-malignant and malignant lesions of gall bladder. We could not find any study from India that has explored this avenue of gall

bladder carcinogenesis. Therefore, the present study was planned to investigate over-expression of c-MET in pre-neoplastic and neoplastic lesions of GB.

Materials and Methods

This study was conducted in department of Pathology at Integral Institute of Medical Sciences and Research (IIMS&R), Lucknow in between the period June 2021 to February 2023. Sixty surgical specimens of gall bladder were analysed for c-MET expression after obtaining clearance from the Ethical and Research Committee of the Institute.

Lesions studied were chronic cholecystitis, chronic cholecystitis with metaplasia and dysplasia and adenocarcinoma of gall bladder. In our study, for purpose of analysis, gall bladder lesions have been grouped into benign, preneoplastic lesions and neoplastic lesions. Patients with gall bladder malignancy, who had received adjuvant chemo or radiotherapy prior to surgery, were excluded from our study. Clinical data of patients was obtained from their hospital case files. Gall bladder specimens were collected in 10% neutral buffered formalin. Hematoxylin and eosin-stained histological slides were prepared as per standard protocol. Representative sections from specimens were further studied for c-MET expression by PAP immunohistochemistry using mouse antic-MET monoclonal antibody (clone 3D4, Diagnostic Biosystems) as the primary reagent [6]. Positive c-MET staining was seen as membranous/cytoplasmic or nuclear staining.

Positive staining of at least 10% of cells (metaplastic, dysplastic or malignant cells) was taken as positive staining which was further graded subjectively as weak, moderate or strongly positive. Control used was colorectal carcinoma. Statistical analysis of the data obtained was done using Chi-square test and Fisher's exact probability test. A p-value of less than 0.05 was considered significant.

Results

The study group comprised of 10 cases of chronic cholecystitis (16.7% of total), 10 cases of chronic cholecystitis showing metaplasia (16.7% of total), 24 cases of chronic cholecystitis with dysplasia (39.9% of total) and 16 cases of adenocarcinoma (26.7% of total). The mean age of patients with chronic cholecystitis, metaplasia, dysplasia and GBC was 35.4, 44.5, 38.3 and 51.1years respectively. In the present study the male/female ratio was 1: 4.47. In our study, the most common site of involvement of GBC was fundus (7 cases 63.7%) followed by body (3 cases 27.3%) and neck of the gall bladder (1 case 9%). Pattern of tumor growth was polypoid in 9 cases (82%), followed by infiltrative and nodular growth pattern 1 case each (9%). In the present study, 10 cases of GBC were well differentiated adenocarcinoma (62.5%). 3 cases (18.75%) of tumors were moderately differentiated adenocarcinoma and 1 case (6.25%) was poorly differentiated adenocarcinoma. Carcinoma in-situ was seen in 2 cases (12.5%).

In our study, all cases of chronic cholecystitis and chronic cholecystitis with metaplasia were negative for c-MET expression. (Figure 1 and Figure 2). 4 out of 24 cases of chronic cholecystitis with dysplasia (16.7%) showed weakly positive staining for c-MET (Figure 3). c-MET expression was seen in 1 case (50%) of carcinoma in-situ. Amongst 16 cases of GBC, c-MET expression of varying grades (Table 1) was seen in 8 cases (50% of cases) (Figure 4 and Figure 5). As there was no expression of c-MET in benign, p value could only be calculated amongst premalignant (metaplasia and dysplasia) and malignant cases (GBC) and was found to be 0.0242 on chi-square test (Table 1). In the present study, c-MET expression was seen in 40% cases of well differentiated adenocarcinoma and in all cases of moderately differentiated adenocarcinoma. c-MET expression was not seen in any case of poorly differentiated adenocarcinoma. On correlating c-MET expression with histological grade, p value of 0.2559 on Fisher's exact probability test was seen.

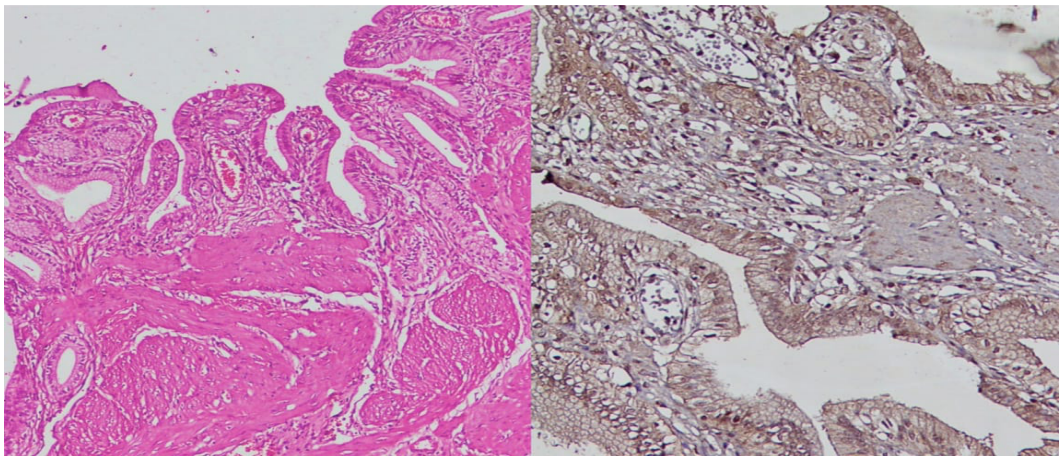

Figure 1: (a) H&E section of Chronic cholecystitis (100X)(b) Negative staining for c-Met (200X)

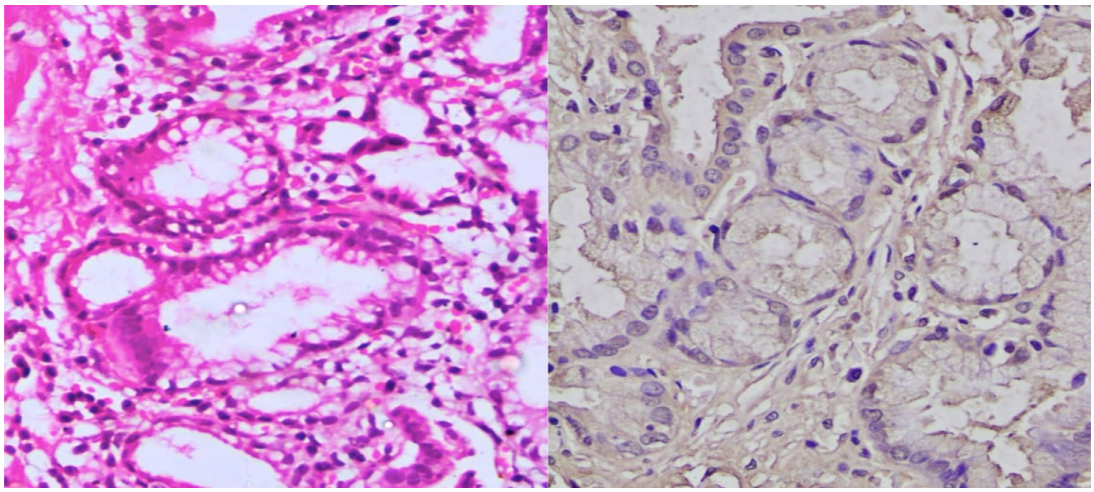

Figure 2: (a) H&E section of a case of chronic cholecystitis showing metaplasia (400X) (b) Negative staining for c-Met (400X)

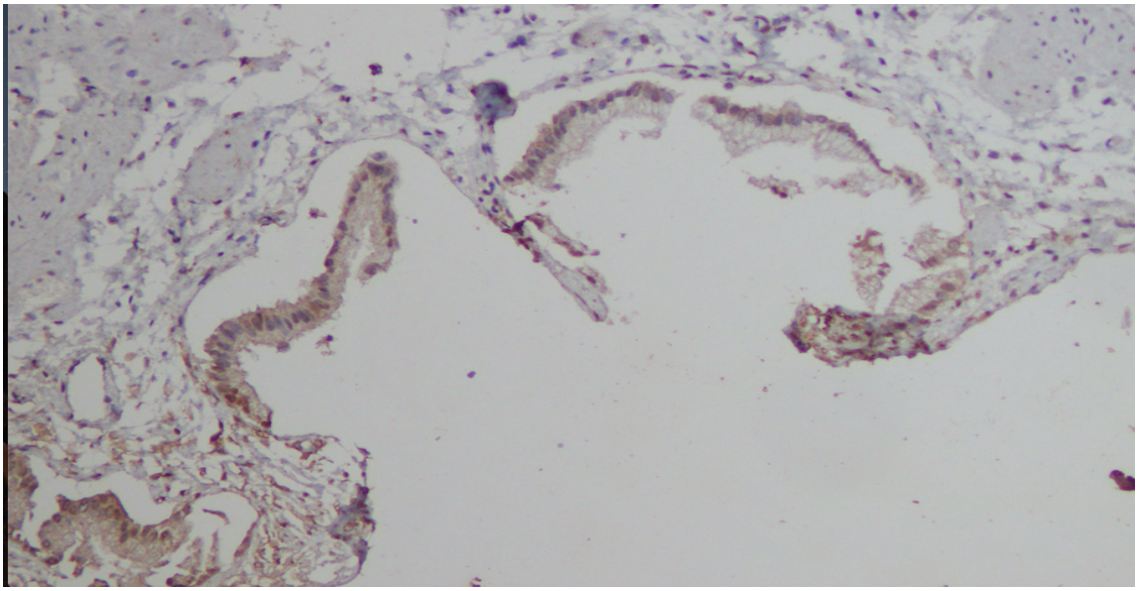

Figure 3: Case of Chronic cholecystitis with low grade dysplasia showing positive staining for c-Met (100X, 100X)

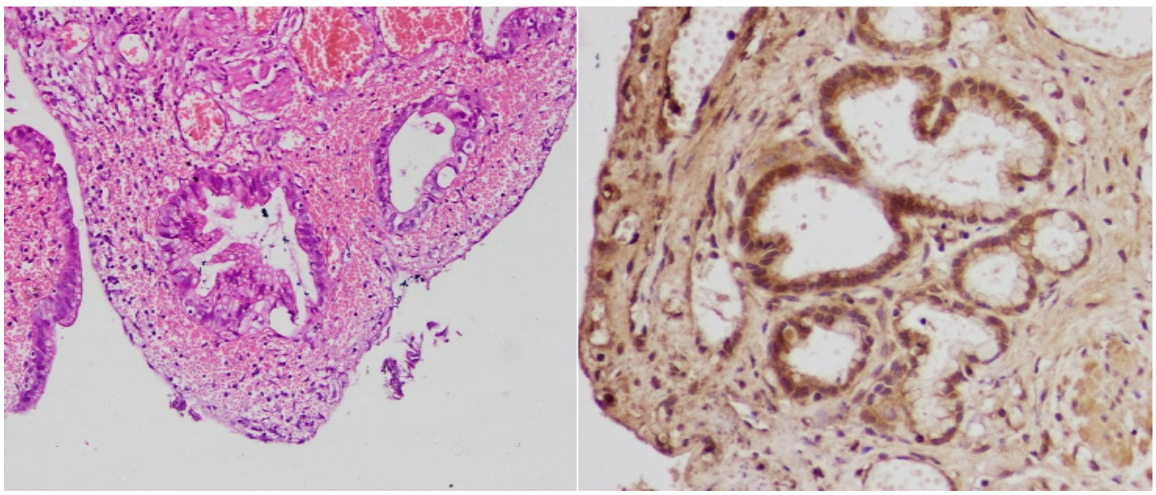

Figure 4: (a) H&E section of a case of Well differentiated adenocarcinoma (200X) (b) Positive staining for c-Met (400X)

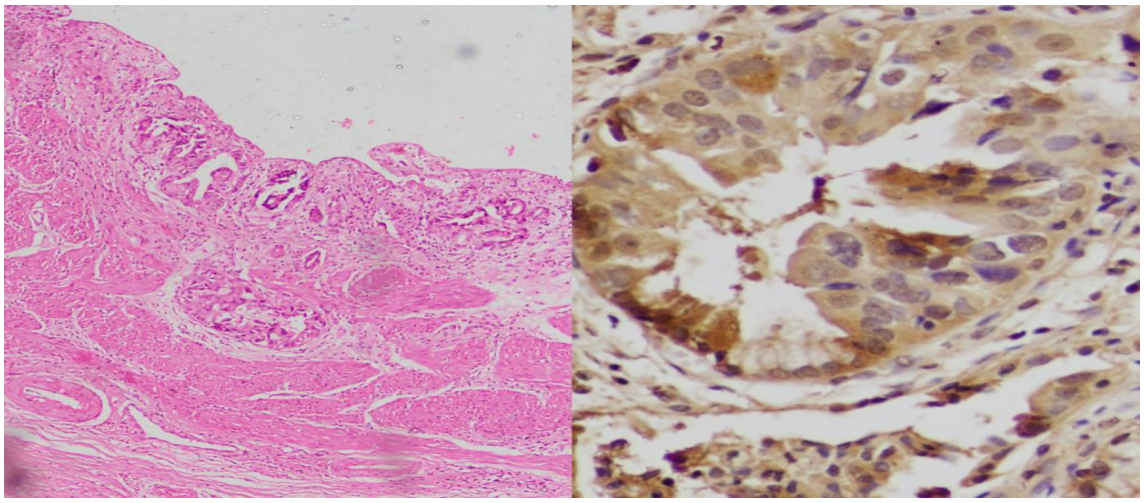

Figure 5: (a) H&E section of a case of moderately differentiated adenocarcinoma (100X) (b) Positive staining for c-Met (400X)

Table 1: c-MET expression in different lesions (p value was calculated only amongst premalignant and malignant)

c-MET expression	Chronic cholecystitis	Metaplasia (n)	Dysplasia (n)	Carcinoma (n)	P value
	Benign	Premalignant		Malignant	
Negative	10/10	10/10	20/24	8/16	0.0242
Weakly positive	0/10	0/10	4/24	2/16	
Moderately positive	0/10	0/10	0/24	2/16	
Strongly positive	0/10	0/10	0/24	4/16	

Table 2: Comparison of c-MET expression in GBC in different studies

Study	No of cases of GBC	Criteria of c-MET over-expression	c-MET overexpression (n)	c-MET overexpression (%age)
Present study	16	Staining in >10% of TC	8/16	50%
Moon et al.[13]	IC 35 CIS 08 Total 43	Staining in >10% of TC	IC-26/35 CIS – 5/8	IC 74 % CIS 62%
Heo et al.[14]	12	Staining >10% of TC	5/12	41.7%
Sanada et al.[15]	IC 14 CIS 13 Total 27	Staining in >30 % of TC	17/27	IC 28.6 % CIS 100%
Yang et al.[16]	108	Staining in >25% TC	60/108	55.6%
Kim et al.[3]	93	Moderate to strong staining in > 50% TC	37/93	39.8%
Nakazawa et al.[17]	89	2+ or 3+ staining in any %age of cells	5/89	5.6%
IC – Invasive carcinoma; CIS – carcinoma in-situ; TC – Tumour cells				

Table 3: Comparison of c-MET expression in pre-malignant lesions of different organs of Gastrointestinal Tract

Study	Organ	No of cases (n)	Criteria of over-expression	Cases showing over-expression (n)	Percentage showing over-expression
Present study	Gall bladder	Dys-24 Meta-10	Staining >10% cells	4/24 0/10	Dys 16.7% Met 0 %
Ashima et al.[18]	Bile ducts	Dys - 23	Staining >10% cells	8/23	35%
Ecker et al.[19]	Esophagus	Dys -42	Moderate to strong staining in > 50% cells	18/42	42.9%
Wang et al.[20]	Esophagus	Dys - 54	2+ or 3+ staining in any percentage of cells	9/54	16.7%
Zhoa et al.[21]	Stomach	Dys-62 Meta-55	Weak to strong in any percentage of cells	38/62 35/55	Dys - 61.9% Meta - 63.4%
Dys – dysplasia, Meta - metaplasia					

Discussion

Worldwide, GBC is the commonest malignancy of the biliary tract. There is marked geographical and ethnic variation in incidence and demography of GBC. High incidence countries are Chile, Columbia, Korea, Japan, parts of India and Pakistan. The incidence of this tumor in north India is 10 -22/100,000 population, which is amongst the highest in the world [1]. Even within India, marked regional variation in incidence is seen. Indian Council of Medical Research (ICMR) has reported age-adjusted incidence rate of 8.9 per 100,000 populations in Delhi compared to age-adjusted incidence rate of 0.8 per 100,000 female populations in Chennai, South India [7]. As incidence of GBC shows wide geographical and regional variation, it is possible that apart from environmental and other factors like diet, food and water contamination, higher incidence of bacterial infection and variable molecular profile of the tumor in different population groups may be responsible for the dissimilarity.

GBC is an aggressive tumor with a 5-year survival rate of 5% [8]. Less than 10% of patients have tumors that are resectable at the time of surgery, while nearly 50% have lymph node metastasis [9]. Similar to colonic carcinoma, a step wise progression from metaplasia, dysplasia to carcinoma has been proposed for GBC [2]. Multiple genetic and epigenetic alterations are seen in association with GBC. Most commonly affected genes belong to ErbB signaling pathway - affecting nearly 37% of cases [3]. Following discovery of aberrant ErbB signaling pathway in GBC, targeted therapy against EGFR and vascular endothelial growth factor involving tyrosine kinase inhibitors and monoclonal antibodies was used in clinical trials [4]. However, no significant benefits were achieved.

Mesenchymal-epithelial transition (MET) gene located on chromosome 7 encodes for a cell membrane receptor c-MET. It is activated following binding to its ligand - hepatocyte growth factor (HGF). c-MET is found on surface of epithelial cells of several organ and its stimulation leads to activation of multiple downstream signaling pathways, including mitogen activated protein kinases (MAPK), phosphoinositide 3 kinase (PI3K), signal transducer/activator of transcription (STAT), NF-kB and mammalian target of rapamycin (mTOR) [10]. Apart from stimulating cell proliferation, MET gene is also involved in cell cycle progression, tissue repair and wound healing. It stimulates angiogenesis through induction of vascular endothelial growth factor (VEGF). c-MET/HGF pathway also induces epithelial to mesenchymal transition (EMT). As use of target-oriented agents, involving monoclonal antibodies and tyrosine kinase inhibitors against EGFR and vascular endothelial growth factor has not provide desirable clinical benefit in patients of GBC, search for newer molecular targets is needed.

Targeting MET gene is now being evaluated in clinical trials for different tumors [3]. Several drugs have been developed that target MET or HGF. These agents include MET inhibitors (crizotinib, cabozantinib, capmatinibtepotinib); anti-MET monocloncal antibodies (onartuzumab and emibetuzumab); and anti-HGF antibodies (ficlatuzumab, rilotumumab). However, overex-pression of c-MET in GBC has not been evaluated adequately. We did not find any study from India exploring the role and over-expression of c-MET in premalignant and malignant lesions of gall bladder. Absence of data concerning molecular profile of GBC in different population groups will pose problems in selecting appropriate targeted therapy. Hence the present study was planned to detect aberrant c-MET expression in cases of GBC in Indian population. Song X et al. in their study have reported about the ongoing trials of c-Met inhibitors on GBC [11].

In the present study,60 cases with different pathologies of gall bladder were investigated for c-MET expression by IHC. Previous studies have demonstrated that MET gene expression level closely correlate with protein overexpression as assessed by IHC. Thus, MET expression by IHC used in the present study can be considered as a reasonable assay for c-MET gene overexpression.

In our study, the age range of GBC was 30-70 years and the peak age range for this lesion was between 4th and 7th decade. These findings are similar to that reported by other workers from India [12]. Table 2 shows c-MET expression in GBC in our study with findings reported by other workers as is evident from the data shown in table 2, the criteria for determining c-MET over- expression varies amongst different workers [13-17]. In the present study, staining of more than 10% of tumor cells is regarded as positive. Similar criterion has been employed by Moon et al. and Heo et al. [13,14]. In our study, c-MET expression in GBC is less than the figures reported by Moon et al. and more than the figures reported by Heo et al. [13,14] In our study, analysis of expression of c-MET in different histological grades of adenocarcinoma reveals that c -MET overexpression was seen in 50% cases of carcinoma in-situ, in 40% cases of well differentiated adenocarcinoma and in all cases of moderately differentiated adenocarcinoma. However, c-MET overexpression was not seen in any case with poorly differentiated adenocarcinoma. Thus, a p- value of 0.2559 is seen on correlating c-MET expression and histological grade of GBC using Fisher's exact probability test. Sanada et al. have mentioned that immunostaining of c-MET in neoplastic biliary epithelium relates to

the degree of pathologic differentiation - being highest in well-differentiated tumors and relatively low in poorly differentiated tumors [15].

In contrast, Heo et al. in their study, encompassing cancers of whole of biliary tract, observed c-MET expression in 16.7% cases of well differentiated carcinoma, 34.7% cases of moderately differentiated carcinoma and 55.6% cases of poorly differentiated carcinoma [14]. It is possible that c-MET expression differs at different sites in malignancies of the biliary tract. The difference in c-MET expression in relation to histological grade in different studies can be due to racial and geographical variation in population group. Sanada et al. have also indicated that immuno-staining of c-Met is highest in well-differentiated tumours and relatively low in poorly differentiated tumours [15] . They suggest that c-MET overexpression might be related to early events of carcinogenesis rather than tumor progression.

In our study, 4 out of 24 cases of dysplasia (16.7%) showed weak positive staining for c-MET. No case of chronic cholecystitis (10 cases) or metaplasia (10 cases) showed positive staining for c-MET. We could not find any report in literature that explores expression of c-MET in dysplastic or metaplastic lesions of the gall bladder. Hence, we have compared c-MET expression in metaplastic and dysplastic lesions of gall bladder of our study with c-MET expression in similar lesions of other organs of gastrointestinal tract as reported by other workers (Table 3) [18-21].

In our study, 16.7% cases of dysplasia showed weak c-MET expression. With criteria similar to those used in the present study, Ashima et al. observed c-MET positivity in 35% cases of dysplasia of bile ducts [18]. Comparison with other studies is not tenable as criteria for c-MET positivity differs in different studies. Thus, results of our study indicate that c-MET expression is seen more often in invasive stage of gall bladder malignancy compared to early dysplastic lesion. Moon et al. propose that c-MET plays an important role in tumor invasion [13]. Our findings concur with this proposal. In contrast, Sanada et al. and Yang et al. found that c-MET overexpression correlates with adverse clinic-pathological indicators like frequency of lymph node metastasis, invasion, and decreased overall survival [15,16].

Conclusion

We conclude that c-MET expression in gall bladder increases as the lesion progresses from pre-neoplastic to neoplastic state, suggesting that over-expression of MET/HGF signaling may be responsible in pathogenesis of GBC. Elucidation of c-MET expression in GBC may act as a prognostic indicator and in future could be a potential therapeutic target for treatment by c-MET pathway antagonists.

Limitations

Correlation between c-MET expression and known adverse histological features (like histological grade and disease stage etc) could not be done because of small sample size, heterogenous study population and absence of established universal criteria for grading c-MET over-expression. Also due to Lack of data correlation of c-MET expression with clinical progression of the disease could not be made.

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