Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pathology

Immunohistochemical Expression of Proliferative Marker (Ki-67) and Microvascular Density Marker (CD-34) in Cholecystectomy Specimens

Dr. Ronal Singh Rajkumar[1], Dr. Mutum Reeta Devi[2], Dr. Babina Sarangthem[3*]

[1]Senior Resident, Department of Pathology, JNIMS, Imphal
[2]Associate Professor, Department of Pathology, RIMS, Imphal
[3]Associate Professor, Department of Pathology, RIMS, Imphal

DOI: 10.36347/sjams.2021.v09i12.029 | **Received:** 11.11.2021 | **Accepted:** 19.12.2021 | **Published:** 30.12.2021

***Corresponding author:** Dr. Babina Sarangthem
Associate Professor, Department of Pathology, RIMS, Imphal

Abstract **Original Research Article**

Background: Cholelithiasis is the most common disease of the gall bladder afflicts 10% to 20% of adult populations in developed countries. It may develop into carcinoma by the dysplasia carcinoma sequence. Over 80% of invasive gallbladder cancers are present in areas adjacent to the carcinoma in situ and epithelial dysplasia. Ki67 expression is correlated with the aggression of various histopathological changes in the epithelium of the gallbladder and may act as prognostic marker in gallbladder carcinoma. CD34 expression is also increased in severe dysplasia and carcinoma. This study aims to assess the proliferative activity and microvascular density associated with lesions like hyperplasia, metaplasia, dysplasia and malignant lesions in cholecystectomy specimens. ***Materials and methods:*** It is a Hospital based cross sectional study conducted in department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur for two years from October 2017. Cholecystectomy specimens with features of metaplasia, dysplasia and malignancy are analysed. Immunostaining of the sections done with Ki 67 and CD34 and interpreted both qualitatively and quantitatively. Statistical Analysis done using SPSS IBM VERS.21. ***Results and observations:*** A total of 150 cholecystectomy specimens are studied with Male to female ratio is 1:9. Gallbladder carcinoma seen more commonly in females. The most common type is adenocarcinoma NOS with mean age of 41.9 years +/-14. ***Conclusion:*** Determination of immunohistochemical expression of Ki67 and CD34 may act as prognostic marker and help in assessing the aggressiveness of premalignant and malignant lesions of gall bladder.

Keywords: Gall bladder malignancies, Proliferative marker, Microvascular density marker.

INTRODUCTION

The gallbladder is among the most commonly surgically resected organ and the number of cholecystectomy has increased more than 50% in the past decade. Approximately 600,000 cholecystectomies are performed annually in the United States, mostly for Gallstone – related disease, which accounts for an estimated overall cost of $6 to $8 billion each year [1]. Cholelithiasis which is the most common disease of the gall bladder afflicts 10% to 20% of adult populations in developed countries. Most cases are silent and most individuals remain free of symptoms or other complications for decades. Other common diseases of the gallbladder include acute cholecystitis, chronic cholecystitis and neoplastic lesions [2]. Cholelithiasis is linked to carcinoma of the gall bladder by the dysplasia carcinoma sequence. Dysplasia in the gallbladder is graded as mild, moderate and severe. It can be classified as high or low grade. Generally high grade dysplasia which is equivalent to intraepithelial carcinoma in situ (CIS) is regarded risky [3,5]. Over 80% of invasive gallbladder cancers are present in areas adjacent to the CIS and epithelial dysplasia. Metaplasia, dysplasia and CIS are present in the mucosa adjacent to the cancer in 66%, 81.3% and 69% respectively [3-5]. Ki67 is one of the most important cell proliferation markers. Its expression is correlated with the aggression of various histopathological changes in the epithelium of the gallbladder [6]. Many studies have been done to assess Ki67 as prognostic marker in gallbladder carcinoma. It was found that the expression of Ki67 in chronic cholecystitis rose from less than 10% in metaplasia to 20% in severe dyplasia to 90% in carcinoma [6, 7]. CD34 is a microvascular density marker: Various studies have

shown the difference in expression of CD34 in non neoplastic and neoplastic diseases of gallbladder [8]. MVD is found to be 5-9 vessels /field in areas adjacent to pyloric metaplasia in chronic cholecystitis, 10-20 vessels/ field in severe dysplasia and 15-30 vessels / field in gall bladder carcinoma [1]. This study has been undertaken to assess the proliferative activity and microvascular density associated with lesions like hyperplasia, metaplasia, dysplasia and malignant lesions in cholecystectomy specimen received in our centre by immunohistochemical studies [9].

OBJECTIVES

To study the histomorphology of cholecystectomy specimens by assessing and comparing the proliferative activity (Ki67) and the microvascular density (CD34) in specimens with metaplasia, dysplasia and malignancy.

MATERIALS AND METHODS

It is a Hospital based cross sectional study conducted in department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur for two years from October 2017 to September 2019. All Cholecystectomy specimens received showing features of metaplasia, dysplasia and malignancy are included in the study. Autolysed samples, treated cases of gallbladder carcinoma with history of radiotherapy or Chemotherapy are excluded from the study. The study variables also include age, sex and ethnicity. Representatives sections are taken from the formolsaline fixed specimen, processed and stained with haematoxylin and eosin as per standard protocol. Immunostaining of the sections done with Ki 67 and CD34 (monoclonal mouse antibody against Ki 67 and CD34, master diagnostic). Both positive and negative control taken and results are interpreted both qualitatively and quantitatively. Ki67: Positive control - mature lymphocytes.

Negative control - primary antibody omitted sample. Grading carried out according to the following criteria: Qualitative scoring: Score 0, 1+, 2+, 3+ as no staining, mild staining, moderate staining and intense staining respectively. Quantitative scoring: Score 0, 1+, 2+, 3+ as no staining, <10%, 10-50%, >50% respectively. CD34: Positive control - Capillary endothelial cells. Negative control -Primary antibody omitted sample. Grading will be carried out according to the following criteria: Qualitative scoring of CD34; 0, 1+, 2+ as negative, regionally positive and diffusely positive respectively. Quantitative scoring of CD34: 0, 1+, 2+ as <10%,10-50%, >50% respectively. Microvascular density (MVD) will be assessed as number of CD34 positive vessels / 5 representative fields will be taken as mean. Ethical clearance taken from institutional ethical committee. Statistical Analysis done using SPSS IBM VERS.21. Descriptive statistics, mean T-test and chi square test was used to find the association between relevant variables.

RESULTS AND OBSERVATIONS

A total of 150 cholecystectomy specimens with an age range of 8 to 60 years are included in the study. Male to female ratio is 1:9. The most common non malignant lesion is found to be chronic cholecystitis with gall stones sent together with specimen in 81 cases. Gallbladder carcinoma seen more commonly in females.

Table 1: Age and gender wise distribution of cases

Age range (years)	Male	Female	Percentage of total cases (n=150)
10 -20	0	3	2%
20 -30	2	18	13.4%
30 – 40	5	28	22%
40 – 50	6	39	30%
50 – 60	1	41	28%
60 – 70	1	6	4.6%

The gall bladder diseases are seen mainly in the 4th to 6th decade of life.

The following table shows distribution of cases according to diagnosis.

Table 2: Distribution of cases according to the diagnosis

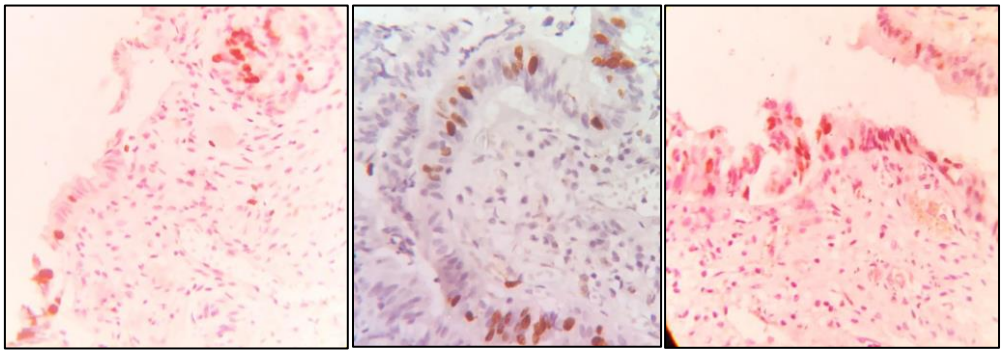
SN	TYPE	No of cases (n= 150)	%
1	Malignant cases		
	Adenocarcinoma(moderately differentiated)	02	1.3%
2	Premalignant and malignant like lesions:		
	Dysplasia	2	1.3%
	metaplasia	10	6.7%
3	Chronic cholecystitis without premalignant lesions	136	72.7%

The most common type is found to be adenocarcinoma NOS, moderately differentiated. and peak incidence is seen in 51 to 60 years of age with mean age of 41.9 years +/-14.9 years. The two cases of gall bladder carcinoma are seen both in female.

The following table shows Ki-67 quantitative expression levels in different groups. Maximum quantitative scoring of >50% is seen in the carcinoma cases.

Table 3: Ki-67 quantitative expression levels in different groups

SN	Group	Ki-67 expression		
		<10%	10-50%	>50%
1	Carcinoma	0	0	2
2	Metaplasia	4	6	0
3	Dysplasia	0	2	0
4	Without premalignant lesions	134	2	0



A **B** **C**

Figure 1: proliferative activity as shown by Ki 67 staining in metaplastic, dysplastic and carcinoma (a, b, c)

The following table shows Quantitative scoring of CD34 for the different groups.

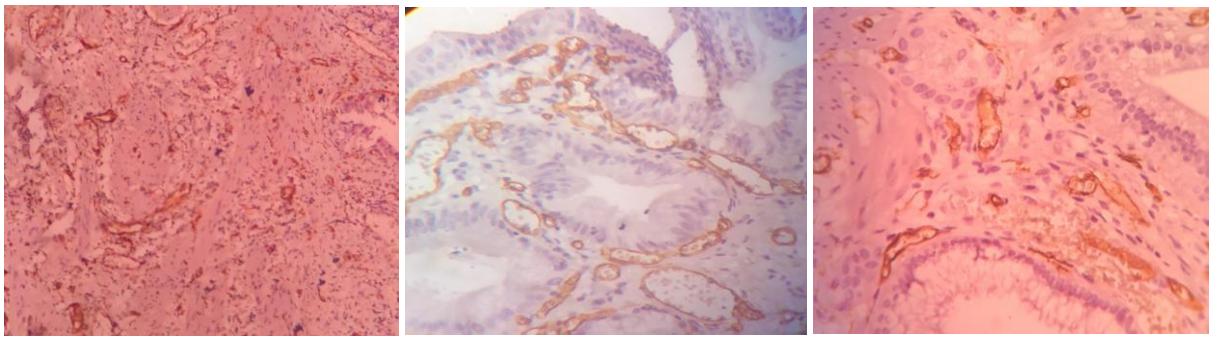
Table 4: Quantitative scoring of CD34 for the different groups

SN	Group	Total no of cases n=150	CD34 quantitative scoring		
			<10%	10 -50%	>50%
1	Carcinoma	2	0	0	2
2	Metaplasia	10	0	0	10
3	Dysplasia	2	0	0	2
4	Without premalignant lesions	136	0	110	26

The following table shows MVD calculated as a mean of the number of CD34 positive vessels /5 representative fields.

Table 5: MVD in various categories

SN	Group	2-9 vessels / field	10 – 15 vessels / field	16 – 20 vessels / field	21-25 vessels/ field	26-30	>31
1	Carcinoma	0	0	0	1	0	1
2	Metaplasia	1	5	4	0	0	0
3	Dysplasia	0	0	0	2	0	0
4	Without premalignant changes	107	24				



A **B** **C**

Figure 2: MVD as shown by CD34 staining in metaplasia, dysplasia & carcinoma (a, b, c)

DISCUSSION

Ki-67 is a proliferative marker which is a nuclear protein expressed in all cell cycle phase except G0 and early G1 phase. Ki-67 has a prognostic value in different tumour types and is reliable and accurate in assessing the growth fraction of neoplasms [10,11]. CD34 is used in this study to assess the microvascular density proliferation. In our study the most common age range of dysplasia and carcinoma was found to be between 51 and 60 years of age [10]. There is female preponderance as seen in other studies [11]. The mean age was found to be 41.9 +/- 14.9 years in our study. Male to female ratio was found to be 1: 9.

In this study, the Ki67 score increased from less than 10% in simple cholecystitis cases to between 10 and 50% in metaplastic and dysplastic cases. In carcinoma cases, the score increased to more than 50%. These findings are comparable to other studies. Stancu M *et al.*, [10] found that the Ki67 score increased from 10% in simple chronic cholecystitis to 20% in severe dysplastic associated lesions up until 90% in carcinoma. They also found that the MVD (microvascular density) was 5-9 vessels in areas adjacent to pyloric metaplasia and 10 -20 vessels in severe dysplastic areas and 15 -30 vessels in carcinoma cases. In our study the MVD was found to be between 10 -20 in metaplastic cases and 15 to 25 vessels in dysplastic cases and in carcinoma cases it was found that one case had MVD 20-25 and another 30 to40 vessels /field.

Pin our study increased Ki67 positivity was seen in the carcinoma cases compared to the other metaplastic and dysplastic cases. The dysplastic cases also showed increased Ki67 positivity in comparision to the metaplastic cases but still lesser than the carcinoma cases. Thus Ki67 score in our study suggest that it gradually increased with increase in the metaplasia dysplasia carcinoma sequence. Also in our study with increase in the age, the Ki67 scoring also increased with metaplastic and dysplastic changes and more so in carcinoma cases. This suggest that the age is also a factor towards progression to gallbladder carcinoma [11-13].

CD34 quantitative score gradually increase with increase in the metaplasia dysplasia carcinoma sequence in our study, this finding is similar with other study Stancu M *et al.*, [10,14]. However the quantitave score was increased in all the categories: metaplastic, dysplastic and carcinoma cases. So CD34 quantitative score alone as a measure in the metaplasia dysplasia carcinoma sequence is not satisfactory Artico M *et al.*, [15]. However microvascular density (MVD) calculated as the number of vessels per high power field stained with CD34 as an average of five fields in representative areas such as areas adjacent to metaplasia or dysplasia or carcinoma definitely increased with increase in the grade in the sequence from metaplasia, dysplasia and carcinoma [10,14]. The MVD in carcinoma cases was more than 30 vessels in both carcinoma cases and 20 to 25 vessels /field in dysplastic cases comparable to the study by Stancu M *et al.*, [10].

CONCLUSION

Immunohistochemical expression of Ki67 and CD34 measured as quantitative score and microvascular density (MVD) correlate with the grade of the lesion and increases in expression with the maximum expression seen in carcinoma cases. This study strongly indicates the metaplasia, dysplasia carcinoma sequence in the pathogenesis of gallbladder carcinoma as the most probable sequence of events in the pathogenesis. Ki67 and CD34 (as a marker for MVD) has a potential role in the early detection of gallbladder carcinoma and together can help in confirming cases of gallbladder carcinoma and premalignant lesions along with the histomorphological study.

REFERENCES

1. Adsay NV. Gallbladder, Extrahepatic Biliary Tree and Ampulla. In:Mills S E, editor. Sternberg's Diagnostic Surgical Pathology. 5th ed, Vol.2. Philadelphia: Wolters Kluwer; 2010. p.1600-5.
2. Rosai J. Gallbladder and extra hepatic bile ducts. In Rosai and Ackerman's Surgical pathology. 9th ed. Vol 1; New Delhi, Elseviers; 2004.p1035-1060.
3. Jessurun J, Albores-Saavedra J. Gallbladder and Extrahepatic Biliary Ducts. in: Damjanov l, Linder J,editors. Anderson's Pathology. 10th Edition. St. Louis: Mosby; 1996. p. 1859-86.
4. Theise N.D. Liver and Gallbladder.In: Kumar V, Abbas A K, Aster J C, editors. Robbins & Cotran Pathologic Basis of Disease. South Asia Edition. Vol II. India:RELX; 2016. p. 821-80.
5. Yamagiwa H. Dysplasia of gallbladder, its pathological significance. Acta pathol Jpn 1987 ;37(5):747-54.
6. Bullwinkel J, Baron LB, Wedermann A, Wohlesberg C, Gerdes J, Scholzen T. Ki 67 Protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. J Cell Physio 2005 ; 206(3): 624-35.
7. Freirson HF. Gallbladder and extrahepatic biliary system,Histology for Pathologist.2nd ed. New York:Raven Press Newyork;1992.
8. Simmons DL, Sattrthwaite AB, Tenen DG, Seed B. Molecular cloning of a cDNA encoding CD34, a Sailomucin of human hematopoietic stem cells. J Immun 1992 ;148(1): 267-71.
9. Ivan R, Xabier D A, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer J Surg Oncol. 2006 ; 93(8):615-23.
10. Stancu M, Caruntu ID, Sajin M, Giusca S, Badescu A, Dobrescu G. Immunohistochemical markers in the study of gallbladder premalignant lesions and cancers. Rev Med Chir Soc Med Nat Lasi.2007; 111(3):734-43.
11. Ojha A, Agarwal T, Gupta S, Singh P, Agarwal A. immunohistochemical expression of Ki 67 in gall

bladder carcinoma. IJPO, April-june,2018;5(2):173-77

12. Grau LAH, Badia JM, Salvador CA , Monso TS, Canaleta JF, Nogues JMG. Gallbladder carcinoma: the role of p53 protein overexpression and Ki-67 antigen expression as prognostic markers. HPB 2004 ; 6(3):174-80.
13. Lee CS. Differences in cell proliferation and prognostic significance of proliferating cell nuclear antigen and Ki-67 antigen immunoreactivity in in-situ and invasive carcinomas of the extrahepatic biliary tract. Cancer 1996 ; 78(9): 1881-87.
14. Chen Y, Yan J, Yu S, Wang X, Zheng Q. Overexpression of Beclin-1 in gallbladder carcinoma and its relationship with prognosis. Contemp Oncol 2014 ; 18(3): 171-6.
15. Artico M, Bronzetti E, Alcino V, Ionta B, Bosco S, Grande C, et al. Human gallbladder carcinoma : Role of neurotrophins, MIB-1,CD34 and CA15-3. Eur J Histochem 2010 ; 54(10): 50-5.