

Original Research Article

Ki 67 Expression in Various Diseases of Gallbladder

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Abstract

Cell proliferation is an important prerequisite for the development of a neoplasm. Ki-67 is one of the most important cell proliferation markers. Its expression is correlated with the aggression of various histopathologic changes in the epithelium of gallbladder.

This study aims to assess the expression of Ki-67 in various pathological processes that affects the epithelium of gallbladder.

Gallbladders from 146 patients were studied histopathologically. Then sections from the paraffin embedded tissues were evaluated for the expression of Ki-67 by using immunohistochemical method. The results of Ki-67 labeling index were estimated for each pathologic category and were analyzed for their significance using Mann-Whitney test.

The mean value of Ki-67 LI in the epithelium of normal gallbladder was (0.7%), in acute cholecystitis (1.14%), epithelial hyperplasia (8.38%), metaplasia (21.18%), dysplasia (38%) and (70.6%) in the carcinoma group. There was no statistically significant difference with regard to ki-67 between the epithelium of normal GB and those with acute inflammation ($p= 0.904$) and the difference was highly significant when the normal gallbladder epithelium was compared with carcinoma group. There was statistically highly significant difference when the comparison was performed between normal epithelium and metaplasia group ($p< 0.001$) and the group of dysplasia ($p= 0.001$).

From the present study a conclusion can be made is that Ki-67 expression increases in chronic inflammation and further increment occurs in premalignant and malignant lesions of the gall bladder epithelium and can be used as a marker of aggression of histopathologic lesions.

Key Words: Ki 67, gallbladder, cell proliferation

الخلاصة

تكاثر الخلايا هو أحد أهم متطلبات ظهور الأورام كما وأن Ki67 يعتبر من أهم معلمات التكاثر الخلوي و أضهاره يتماشى مع ضراوة التغيرات النسيجية في بطانة المرارة. تهدف هذه الدراسة إلى تقييم أظهار ال Ki 67 في الأمراض التي تصيب بطانة المرارة.

درست 146 مرارة من الناحية النسيجية ثم درس أظهار ال Ki 67 بطريقة الفحص المناعي النسيجي الكيمائي وتم تحديد ظهور هذا المعلم لكل مجموعة مرضية و عولمت النتائج بطريقة Mann-Whitney test لتقييم الأهمية المعنوية الإحصائية.

تبين أن معدل أظهار Ki 67 في بطانة المرارة الطبيعية هو 0.7% و في حالات الألتهاب الحاد 1.14% و في التكاثر البطني 8.38% و في الحؤول الخلوي 21.18% و في النمو ألتاذ 38% و بلغ المعدل 70.6% في الحالات ألسرطانية. و تشير نتائج هذه الدراسة إلى عدم وجود فرق معنوي من الناحية الإحصائية إذا قورنت معدلات ألتظهار بين ألبطانة ألتبيعية و حالات ألتلتهاب الحاد ($p= 0.904$) و لكن ألتفرق مهم جدا إذا تمت ألتقارنة مع ألتحالات ألسرطانية.

كما و أن ألتفرق ألتمعنوي مهم جدا إذا تمت ألتقارنة بين ألتنسيج ألتبيعي و حالات ألتحؤول ألتنسيجي ($P < 0.001$) و حالات ألتنمو ألتاذ ($p= 0.001$) تظهر هذه ألتدراسة أن ألتظهار ال Ki67 يزداد في حالة ألتلتهاب ألتزمن و في ألتأقات قبل ألسرطانية و ألتحالات ألسرطانية و يمكن أستعماله ألتلالاة على ضراوة ألتأقات ألتنسيجية.

Introduction

As a group, biliary diseases are extremely common medical problems affecting a significant proportion of the populations worldwide and the vast majority is represented by cholelithiasis or cholecystitis and the majority requires surgical intervention. About 10-20% of adults' population in developed countries suffered gall stones. Over 700,000 cholecystectomies were done every year in the United States [1].

Gall stones predispose to various pathologic lesions in the gallbladder, most importantly is inflammation and cancer[2].

Severe dysplasia/carcinoma in situ is currently reported. In the gallbladder epithelium adjacent to invasive carcinoma, dysplasia is noted in 40 - 60% of cases and in about 1% of all elective cholecystectomies done because of gallstones, an occult GB cancer was detected [2,3].

Cancer of the gallbladder is an aggressive malignancy and usually affects the elderly. It ranks fifth among the gastrointestinal malignancy. Women are affected 2-3 times more than men. Pathologically speaking, 90% of cases are adenocarcinoma and the rest are squamous cell carcinoma and others. In 70% of cases the growth is diffuse and may be difficult to distinguish it from chronic cholecystitis and in 30% it has a polypoid appearance [4].

Antigen Ki-67 is a protein that corresponds to a nuclear non-histone protein expressed by cells in the proliferative phases G1, G2, M, and S. The original antibody against this marker worked only on fresh frozen sections, but monoclonal antibodies have been developed that detect formalin-resistant epitopes (MIB-1 and MIB-3). In general, there is a good correlation between Ki-67 staining and mitotic count [4,5].

Since the description of Ki-67, several antibodies, such as MM-1, Ki-S5, and SP6 have been assessed on paraffin sections after antigen retrieval. Mostly, Ki-67 is measured

on paraffin sections by an immunohistochemical method, using the MIB-1 antibody. The score of Ki-67 means the percentage of total number of tumor cells with nuclear staining[6].

Ki-67 is important for cell proliferation and has a relation to ribosomal RNA transcription, when there is inactivation of Ki-67, the synthesis of ribosomal RNA is inhibited. Because it is well characterized at the molecular level, Ki-67 protein is extensively used as a marker for cell proliferation [8].

There is a good correlation of Ki-67 labeling index and the morphologic aggression indicators of hyperplastic, dysplastic and malignant diseases of the gallbladder in addition to its prognostic significance[8-10].

The aim of this study is to assess the proliferative activity of the gallbladder epithelium utilizing Ki-67 score in various gall bladder pathologies.

Materials and Methods

This cross sectional study included 146 patients who underwent cholecystectomy for various reasons and was conducted in Duhok City-Iraq. Specimens collection started at January 2011 to end of September 2011.

Gallbladder specimens were taken immediately after cholecystectomy, gross examination including measurement, cut sectioning & inspection for any mass and stones were performed and also gallbladders were palpated for any mass. Three sections were taken from the fundus, body and neck of the gallbladder specimens if there was no grossly visible lesion, otherwise more sections were taken as justified [4].

All the specimens were immediately fixed in 10% formalin for 24 hours at room temperature to be ready for processing. The histological examination of all Hematoxyline and Eosin-stained slides included evaluation of mucosa for signs of inflammation, ulceration, hyperplasia,

metaplasia, dysplasia and malignant change. Muscularis layer is examined for features of inflammation, Rokitansky-Aschoff sinuses or malignant invasion. Finally, adventitia was looked for inflammation or invasion. Immunohistochemical evaluation for the expression of Ki-67 was done by the use of Novocastra (trade mark) Ready-to-Use Mouse Monoclonal Antibody (Ki67; code: RTU-Ki67-MM1) and evaluated under light microscopic observation at x 400 magnification. In the immunostaining analysis, positive brown nuclei were detected and discriminated from negative blue hematoxyline nuclei. Calculations were based on 5 fields in each section[11].

The results of ki-67 were expressed in mean and range and were analyzed for level of significance among normal and different pathological processes by using Mann-Whitney Test.

Results

Of the included 146, 30 (20.5%) were males and 116 (79.5%) are females. Ten (6.8%) of them had no gallstones and all the rest suffered cholelithiasis. The age of patients ranged from 19-92 years. The histologic examination revealed normal gallbladder tissue in 10 patients (6.85%) (Figure 1), acute cholecystitis in 7 patients (4.79%) (Figure 2), acute on chronic cholecystitis in 20 patients (13.7%), chronic cholecystitis in 90 patients(61.64%)(Figure 3) some of them

associated with epithelial hyperplasia, antral or intestinal metaplasia. Dysplasia was detected in 5 cases (3.42%) (Figure 4), chronic eosinophiliccholecystitis in 4 patients (2.74%) (Figure 5) and carcinoma of the gallbladder in 10 patients(6.85%) (Figure 6 &7).

The mean value of Ki-67 LI in the epithelium of normal gallbladder was (0.7%), in acute cholecystitis (1.14%) (Figure 8), epithelial hyperplasia (8.38%), metaplasia (21.18%) (Figure 9), dysplasia (38%) and a maximum value was recorded in carcinoma (70.6%)(Figure 10). Results of Ki-67 expression analysis of different histopathological categories are demonstrated in (Table 1).

There was no statistically significant difference with regard to ki-67 between the epithelium of normal GB and those with acute inflammation ($p= 0.904$), the difference was highly significant when the normal gallbladder epithelium was compared with carcinoma group. There was statistically highly significant difference when the comparison was performed between normal epithelium and metaplasia group ($p< 0.001$) and the group of dysplasia ($p= 0.001$). It was statistically significant difference when normal epithelium is compared with group of hyperplasia and chronic cholecystitis ($p= 0.056$) and (0.007) consequently (Table 1).

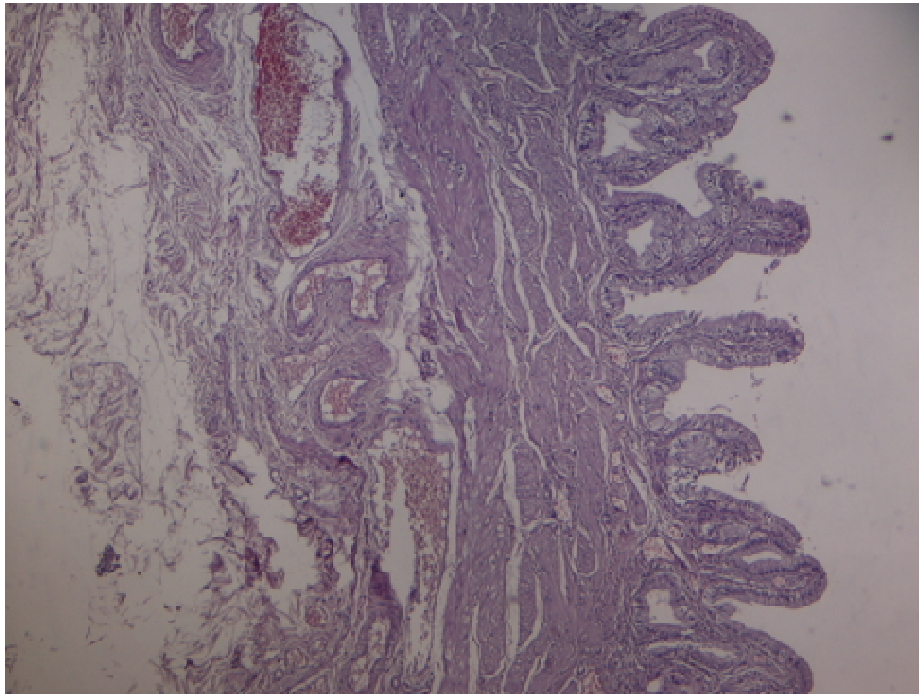


Figure 1: Normal histology of the gallbladder. (H & E stain) (X20)

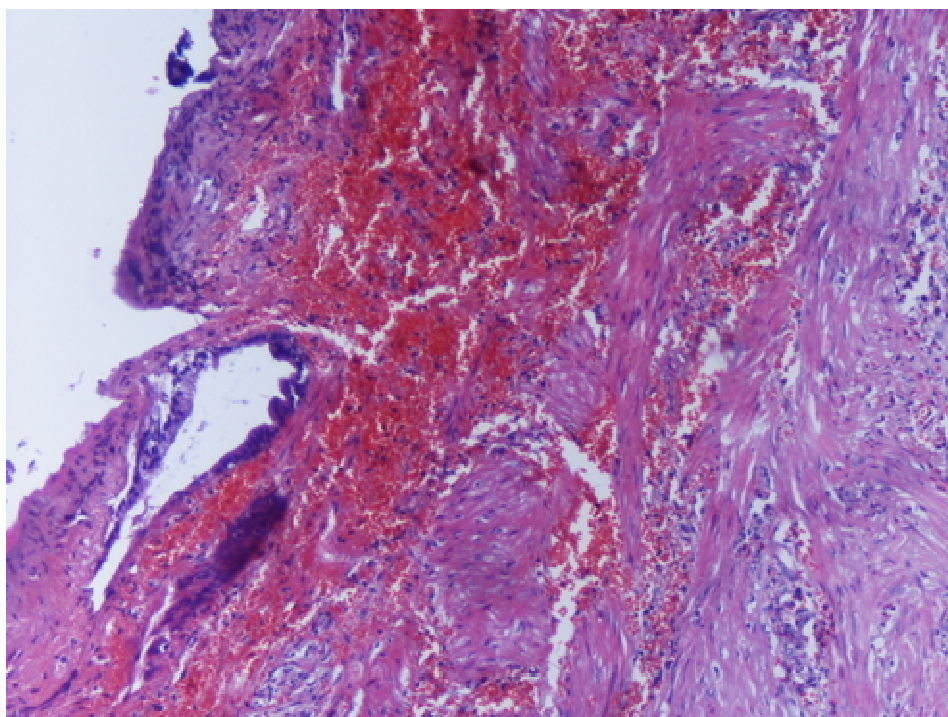


Figure 2: Gallbladder Acute (H & E stain) (X20) Acute haemorrhagic cholecystitis.

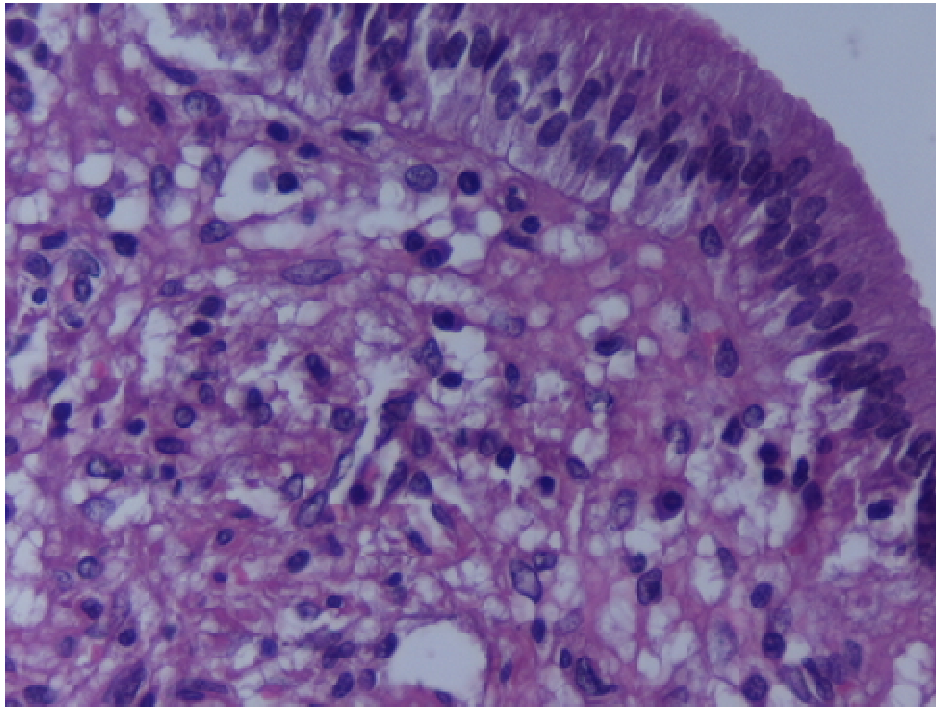


Figure 3: Gallbladder (H & E stain) (X40): Plasma cell infiltration in chronic cholecystitis

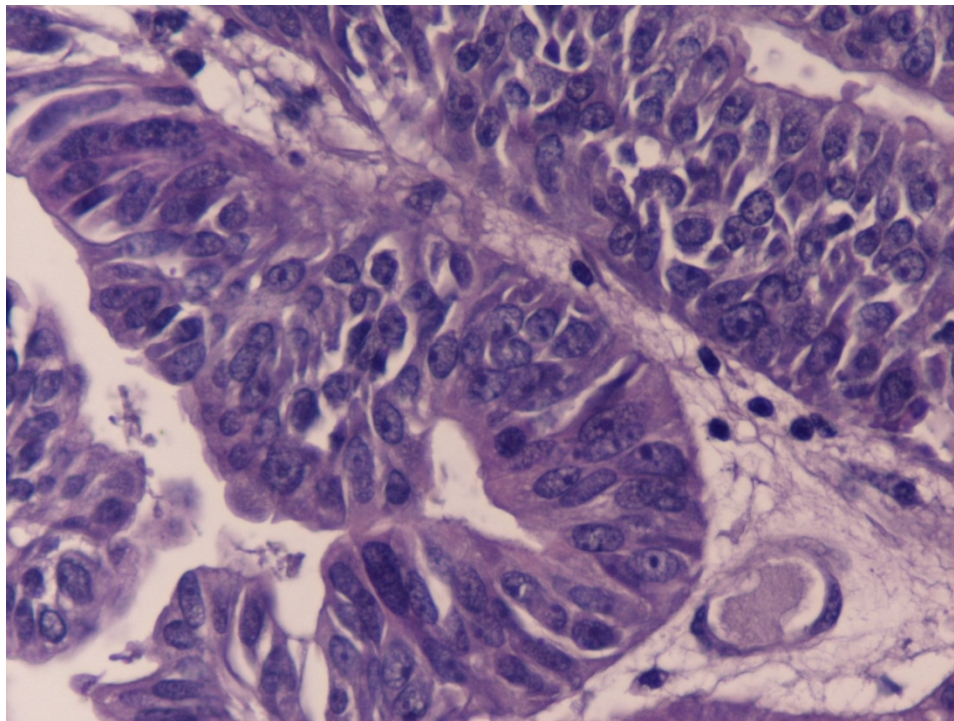


Figure 4: Gallbladder (H & E stain) (X40): Dysplasia of gallbladder epithelium. There are pleomorphic cells with intact basement membrane

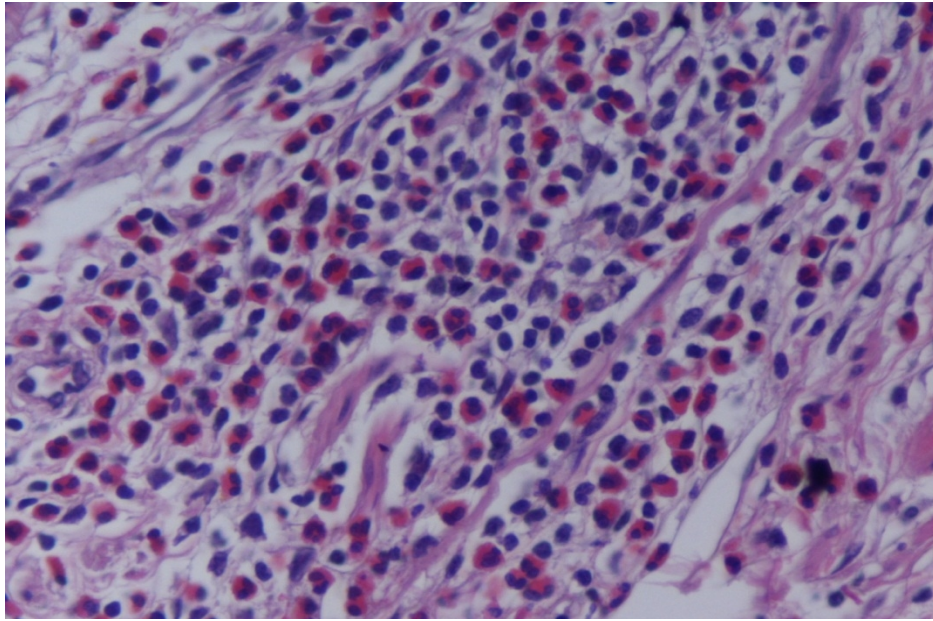


Figure 5: Eosinophilic infiltration in chronic cholecystitis. (H & E stain) (X40)

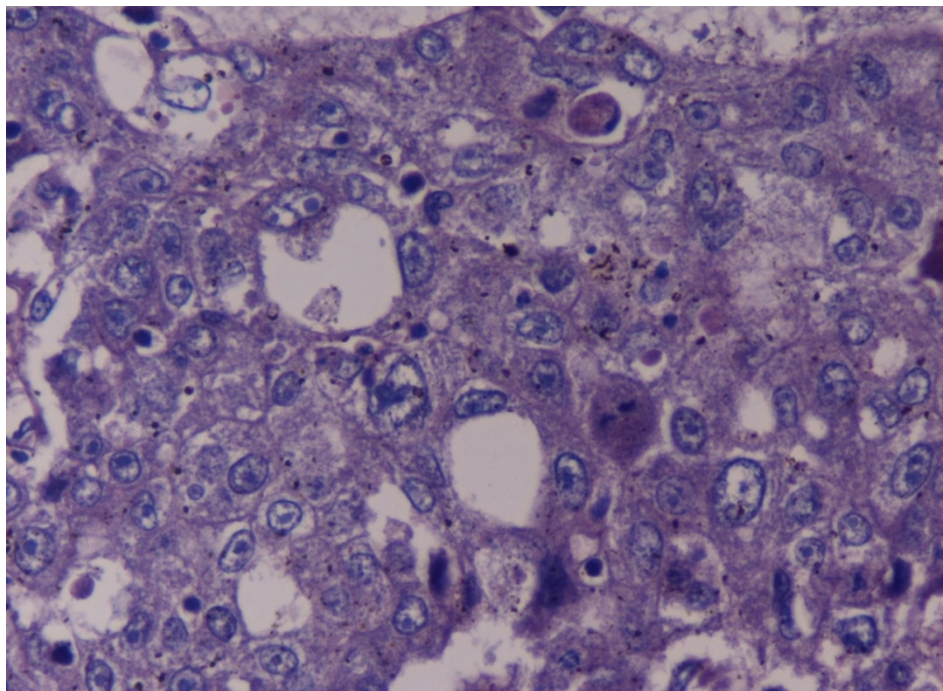


Figure 6: Gallbladder (H & E stain) (X40): Poorly differentiated adenocarcinoma of the gallbladder. There are large pleomorphic cells, increased nuclear cytoplasmic ratio, prominent nucleoli arranged in solid sheets and few glandular elements

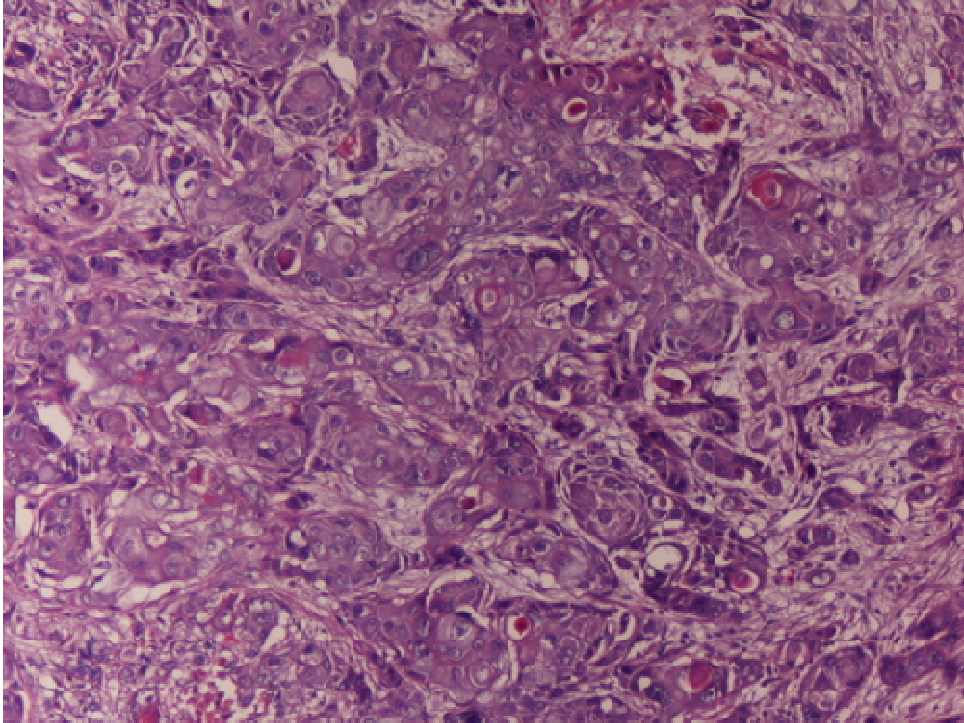


Figure 7: Squamous cell carcinoma of the gallbladder. There are sheets of malignant squamous cells with focal keratin formation. (H & E stain) (X40)

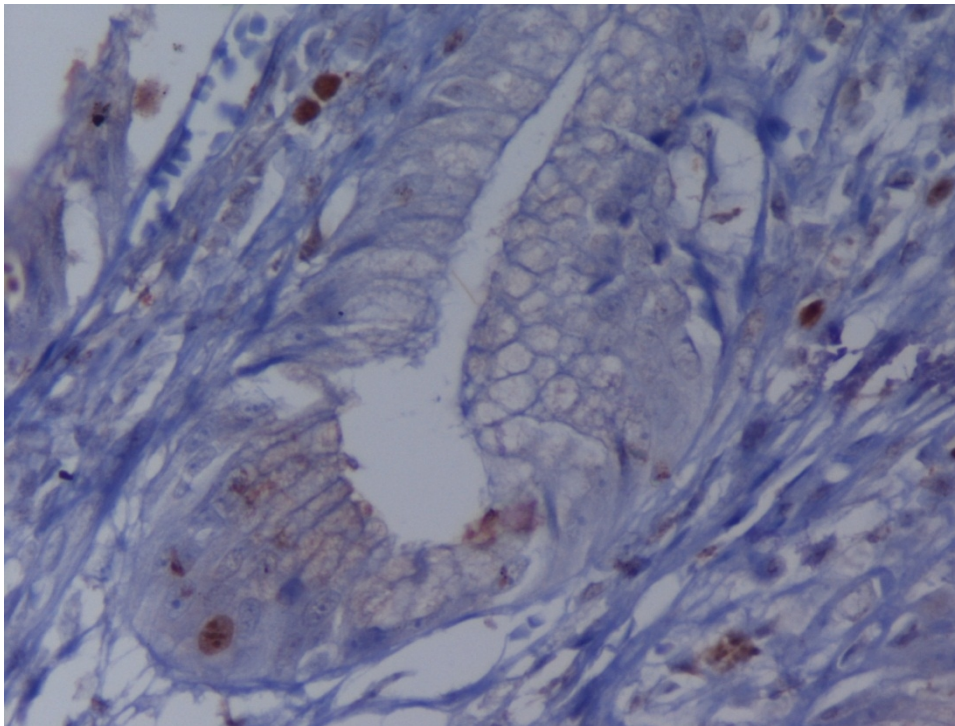


Figure 8: Low Ki-67 expression in acute cholecystitis. (X40)

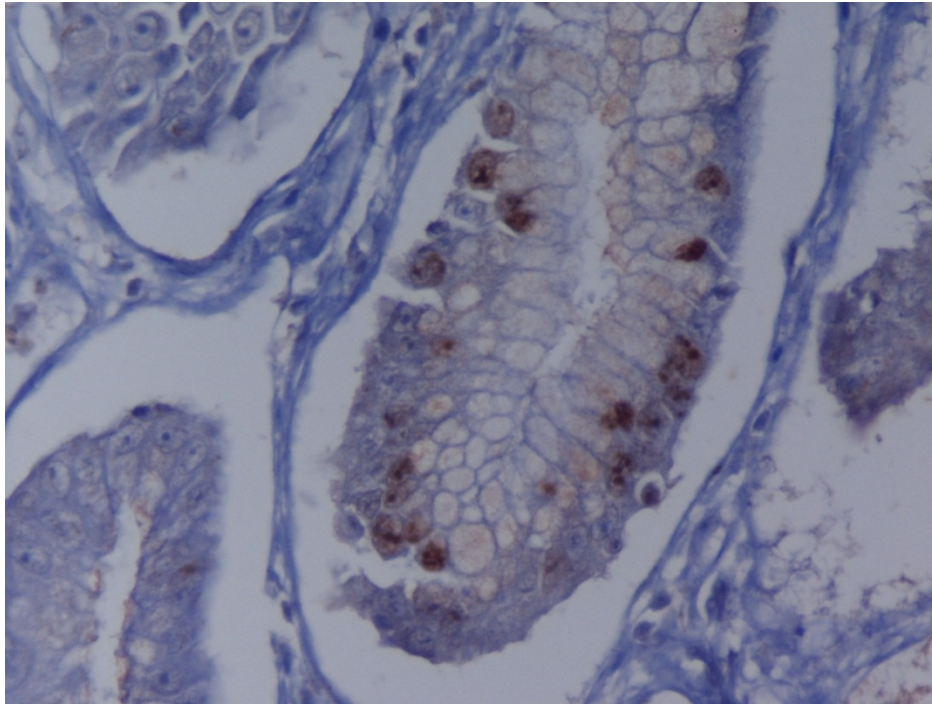


Figure 9: Low Ki-67 expression in metaplasia of GB epithelium and there is nuclear atypia (low grade dysplasia). (X40)

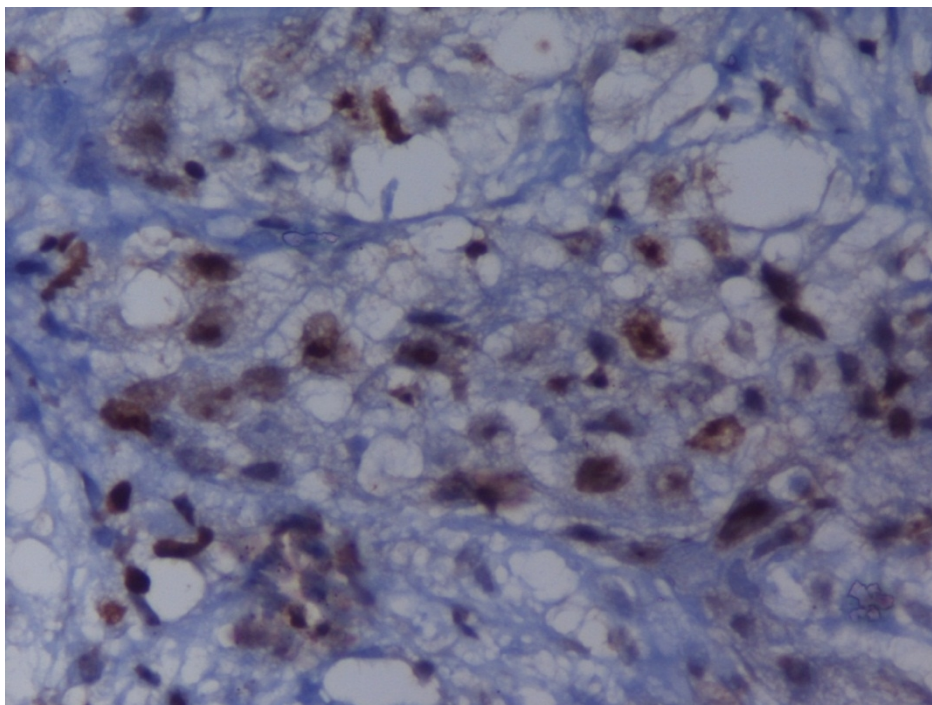


Figure 10: High Ki-67 expression in poorly differentiated adenocarcinoma. (X40)

Table 1: Ki-67 immunohistochemical expression and statistical analysis of different gallbladder diseases compared to normal

Category	Subcategory	No. of cases	Range %	Mean %	Median	P-value*	SD
Normal		10	0-5	0.7	0.0		1.567
Acute Cholecystitis	Non-specific	7	0-7	1.14	0.0	0.904	2.609
Acute on chronic cholecystitis		20	0-12.8	3.1	0.0	0.269	4.351
Mild Chronic cholecystitis	Non-specific	28	0-28	4.5	1.5	0.095	7.315
Moderate And severe chronic cholecystitis	Non-specific	38	0-51	9.69	5.0	0.007	13.128
	Hyperplasia	13	0-30	8.38	2.0	0.056	11.478
	Metaplasia	11	0-31	21.18	22.0	<0.001	9.217
Dysplasia		5	29-42	38	41.0	0.001	5.612
Chronic eosinophilic cholecystitis		4	0-1	0.5	0.5	0.616	0.577
Carcinoma	SCC	1	60	60	Total mean 70.6	<0.001	13.874
	Well differentiated adenocarcinoma	6	55-80	66			
	Poorly differentiated adenocarcinoma.	3	82-89	86			

* From Mann-Whitney test.

SD: Standard Deviation. SCC: Squamous Cell Carcinoma.

Discussion

The investigation of Ki-67 brings important data regarding the carcinogenesis process in the GB, initiated on the background of a chronic cholecystitis. Ki-67 labeling index values in GB pathology sustain the hypothesis of the metaplasia-dysplasia-carcinoma sequence. It is found that Ki-67 was reported to be positive in all chronic cholecystitis cases (100%) and in all carcinoma cases (100%), which is explainable by the selection manner of chronic cholecystitis associating pre-malignant lesions and by the invasive pattern of the carcinomas[9].

In this study, the least value in the results of Ki-67 was a mean of (0.7%) in normal GB, while in acute cholecystitis (1.14%), epithelial hyperplasia (8.38%), metaplasia

(21.18%), dysplasia (38%) and a maximum value was recorded in carcinoma (70.6%). A conclusion can be made that Ki-67 expression increases from normal tissue to carcinoma passing through various hyperplastic, metaplastic and dysplastic processes. A similar conclusion was made by Stancu *et al* [9], who reported rising Ki-67 index rises from 10% in simple dysplastic lesions associated with chronic cholecystitis to 20% in severe dysplastic lesions associated with chronic cholecystitis reaching up to 90% in carcinoma.

Badescu *et al*[10] used Ki67 to evaluate the abnormal proliferation abilities found in the GBs with cholelithiasis and they establish a good correlation between the high activity of Ki67 and the morphological aggression

indicators in hyperplastic and dysplastic GB epithelium.

In the present study, there was an increase in Ki-67 expression to a mean of 65.83% for well differentiated adenocarcinoma and 85% for poorly differentiated adenocarcinoma. This finding demonstrates a correlation between Ki-67 expression and the grade of the tumor, obviously because high grade tumors have a higher proliferation index. Other investigators demonstrated a significant difference between well and poorly differentiated adenocarcinomas with regard to MIB (Ki-67 antibody)[11].

In another study, a staining index of Ki-67 expression with a mean of 25% in samples of non tumoral mucosa and 46% in GB cancer was reported. Seventy five percent of GB cancer samples had a staining index of more than 20%. Non tumoral GB mucosa samples have relatively a high proliferation index - in comparison with normal mucosa- which indicates cellular hyper-proliferation that may be related to the pathogenesis of GB cancer[12].

Gallbladder carcinomas have significantly higher MIB-1 indices than CIS (carcinoma in situ) and non-neoplastic lesions and they usually associated with poor prognosis. The poorly differentiated adenocarcinomas of the GB had higher mean MIB-1 index and reduced patient survival when compared with the moderately differentiated carcinomas[13].

The conclusion which can be made from the current work is that Ki-67 labeling index is a marker of aggression of histopathologic lesions.

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