Original Article

DOI: 10.7860/JCDR/2021/45905.14562

Pathology Section

Immunohistochemical Expression of HER2/neu, Ki-67 and MUC1 in Benign and Malignant Gall Bladder Lesions and its Association with Clinicopathological Parameters

RANIA G ROSHDY[1], HEBA M RASHAD[2], ENAS IBRAHIM[3]

BY-NC-ND

ABSTRACT

Introduction: Gall Bladder Carcinoma (GBC) is a diagnostic and a therapeutic challenge. Although it is increasing, chronic cholecystitis remains the most worldwide gall bladder lesions, harboring many epithelial changes that may end in carcinoma.

Aim: To investigate the expression of HER2/neu (Human Epidermal Growth Factor Receptor 2), Ki-67 and MUC1 (Mucin 1) in malignant and non-malignant gall bladder lesions, and to evaluate its relation with clinicopathologic parameters of GBC.

Materials and Methods: This retrospective study included 40 cases of GBC, eight cases of gall bladder dysplasia, 10 cases of gall bladder metaplastic changes and 25 cases of chronic cholecystitis as a control group. The blocks were collected from the Department of Pathology of Benha University Hospital, from January 2012 to December 2019. Immunohistochemical staining results of HER2/neu, Ki-67 and MUC1 were analysed and correlated by Statistical Package for the Social Sciences (SPSS) version 16 and Chi-square test or Fisher's-exact tests.

Results: Positive HER2/neu expression (+2, +3) was detected in 47.5% (19/40) of malignant cases and 12.5% (1/8) of dyspastic group, in the same time it was completely absent in the metaplastic and cholecystitis cases (p<0.001). Similarly, Ki-67 Labeling Index (LI) (≥20%) expression was found in 55% (22/40) of malignant group, while it was completely absent in the other three studied groups. All cases of malignant group 100% (40/40), 50% (4/8) of dysplastic one, one case of metaplastic (1/10) showed cytoplasmic expression of MUC1 in the same time it was completely absent in control group (0/25) (p<0.001). High MUC1 expression was found in 75% of both malignant (30/40) and dysplastic (6/8) studied cases and only one case (10%) of metaplastic group (p<0.001). There was a significant correlation between MUC1 expression and studied parameters of GBC.

Conclusion: HER2/neu, and Ki-67 are overexpressed in GBC cases compared with control and dysplastic group. The study also highlights that MUC1 would be a better marker of malignant transformation of gallbladder epithelium and its depolarised expression would be reliable for detection of invasive carcinoma, so a new therapeutic agents can target these cell surface adhesion molecule (MUC1). HER2/neu can be considered as a candidate for targeted therapy in GBC treatment strategy.

Keywords: Cell surface associated, Gall bladder carcinoma, Human epidermal growth factor receptor, Ki labeling index

INTRODUCTION

GBC is considered as a relatively rare neoplasm worldwide with poor prognosis, rising incidence in the recent past, and unfortunately less treatment options. It occupies the sixth most common cancer of Gastro Intestinal Tract (GIT) and forms 80-95% of biliary tract carcinomas. It is predominantly disease of elderly female; it affects women with two to six times more than men [1].

GBC is usually diagnosed at an advanced state, therefore it is characterised by high mortality with an overall survival rate of six months [2]. It has a wide geographical variation that may be due to various risk factors, which include cholelithiasis (especially untreated chronic calculous cholecystitis), obesity, and reproductive factors. These suspected factors likely represent promoters of carcinogenesis [3]. The molecular biology of GBC is unclearly understood. Most of these studies have focused on the gene abnormalities and deletion of *R as, TP53, and p16Ink4/CDKN2 [4].*

Tyrosine Kinase (TK) has been recently implicated in pathogenesis of various neoplasms. Several oncogenes, which encode for growth factor receptors, have TK activity. The EGFR family includes the Epidermal Growth Factor Receptor (EGFR, HER-1) and c-erbB-2 (HER-2) are with TK activity [5]. HER-2 is a normal cellular gene, also called c-erbB2, located on chromosome 17q12q21.28. It encodes a TK that is strongly related to receptor for EGFR [6]. The overexpression of this receptor due to gene amplification and protein overexpression is widely detected in various solid tumours [7].

Mucins (known as high molecular weight glycoproteins) that plays vital roles in carcinogenesis or tumour invasion. There are about 20 MUC-encoding genes, with cell membrane localisation (MUC2, MUC5AC, MUC6) and transmembranous mucins (MUC1, MUC4) [8]. Normally, MUC1 expression is present in breast, pancreas, the respiratory tract, salivary gland, and, prostate, but sparse in GIT including the gallbladder. It is a high molecular weight transmembrane glycoprotein that is involved in lubrication and hydration of luminal surface. In the pathogenesis of various cancers, MUC1 has an important role in cell adhesions and cellular interactions [9].

In the recent times, these oncogenes were widely studied and were evaluated with the availability of specific chemotherapeutic targets against them in cancer treatment plan. Only few studies focusing on expression of HER2/neu and MUC1 in GBC exist. Ki-67 is an important proliferative marker, which is expressed in all phases of the cell cycle except G0. It corresponds to a nuclear non-histone protein which was described in 1983 [10]. It can be exclusively detected within the nucleus of proliferating tumour cells, so the fraction of Ki-67-positive tumour cells (the Ki-67 LI) is considered, as an excellent prognostic marker. It is associated with ribosomal RNA transcription. Ribosomal RNA synthesis is inhibited when antigen Ki-67 is inactivated [11].

The objective of this study was aimed to investigate the expression of HER2/ neu, MUC1 and Ki-67 in GBC, comparing with premalignant lesions and cholecystitis as control by immunohistochemistry, to elucidate their relation to the carcinoma development and to correlate them with different clinicopathological parameters of GBC as prognostic markers.

MATERIALS AND METHODS

This retrospective study was conducted in Department of Pathology of Banha University Hospital, January 2012 to December 2019. The study included 83 blocks and related clinical data. Cases were classified into four groups:

A Malignant group: GBC (40 cases)

B Premalignant group: dysplasia (8 cases)

C Metaplastic group: chronic cholecystitis with intestinal and pseudo pylori metaplasia (10 cases)

D Chronic cholecystitis without metaplastic and dysplastic changes, as a control group (25 cases)

Archived specimens were retrieved from archived records in hospital information system sections were prepared at 4 micron thickness from each tissue block, one of them was stained by Haematoxylin and Eosin (H&E) for histopathological re-evaluation. The sections were reviewed and graded according to World Health Organisation (WHO) classification and 8th edition of AJCC staging system (2019) [12].

Immunohistochemical staining: Sections were pretreated by sodium citrated buffer (pH6.0) as heat-antigen retrieval for 15 minutes in microwave. The sections were then cooled for five minutes and rinsed in tapwater. After blockage of biotin and peroxidase, immunohistochemical staining was performed and left for incubation period as shown in [Table/Fig-1]. Slides were subsequently stained by the universal immunoperoxidase method, according to the manufacturer's protocol. All study cases were approved by Ethical Committee in Benha Faculty of Medicine (Rc2.10.2020).

Antibody	Source	Dilution	Incubation period	Positive control
HER2/neu	LAB vision	Ready to use	60 min	Breast carcinoma
MUC1	Novocastra)	Ready to use	30 min	Lung tissue
KI-67	Thermo scientific	Ready to use	60 min	Breast carcinoma

[Table/Fig-1]: The studied markers.

HER2/neu expression interpretation: CAP/ASCO (College of American Pathologists/American Society of Clinical Oncology) guidelines are used to measure HER2/neu positivity as in [Table/Fig-2]. The immunohistochemical criteria for HER2/neu positivity in breast cancer is applied [13].

Score	Criteria
0 (negative)	No immunoreactivity or immunoreactivity in 10% of tumour cells but only a portion of the membrane is positive (Incomplete).
+1 (negative	Faint weak immunoreactivity in >10% of tumour cells but only a portion of the membrane is positive (Incomplete).
+2 (positive)	Weak to moderate complete membrane immunoreactivity in >10% of tumour cells.
+3 (positive)	Moderate to strong complete immune reactivity in >10% of tumour cells.

[Table/Fig-2]: HER2/neu scoring.

MUC1 interpretation: The presence of MUC1 expression was graded based on the sum score of expression rate and intensity of staining [Table/Fig-3] [14].

The positive intensity was divided into two groups: 1 (faint) and 2 (strong) for statistical analysis, sum scores (0-5) of the extent and intensity scales were divided into low expression (sum score 0-3) and high expression (sum score 4-5).

Score	Criteria
0 (negative)	Percentage of positive cells in <10%
+1	Percentage of positive cells in 10-25%
+2	Percentage of positive cells in 26-50%
+3	Percentage of positive cells in >50%

[Table/Fig-3]: MUC1 immunostaining score [14].

Ki-67 expression interpretation: Ki-67 LI was calculated as the percentage of positively stained tumour cell nuclei out of the total tumour cells counted (n=1000). A percentage of stained cells was considered positive regardless of the intensity of staining. A percentage ≥20% of stained cells was considered positive, regardless of the intensity of staining [15].

STATISTICAL ANALYSIS

The data collected were analysed using SPSS version 16.0. Determining the probability factor (p-value) assessed the significance of results. Chi-square test or Fisher's-exact tests were applied to evaluate the relation between variables. The p-value <0.05, the results were considered statistically significant.

RESULTS

This retrospective study included four groups, classifying into: 1-malignant group of 40 gall bladder adenocarcinoma cases with the median age of this group was 53.6±12.46 year (ranged from 39 to 79 years) and female predominance (80%). 2- Eight cases of dysplasia, 3- 10 cases of metaplastic changes (antral and intestinal) 4- finally 25 cases of cholecystitis taken as control group with the median age of studied 25 cholecystitis cases was 39.4±5.7 year (ranged from 28 to 70 years) and female predominance (90%).

Immunohistochemical results: Positive HER2/neu expression (+2, +3) was detected in 47.5% (19/40) of malignant cases and 12.5% (1/8) of dysplastic group was positive, in the same time it was completely absent in the metaplastic group and control cases (p<0.001). High MUC1 expression was found in 75% of both malignant (30/40), and dysplastic (6/8) studied cases beside 10% (1/10) of metaplastic group p<0.001. All cases of malignant group 100% (40/40), 50% (4/8) of dysplastic, 10% of metaplastic (1/10) and none of control group (0/25) revealed cytoplasmic MUC1 expression p<0.001. Ki-67 positive expression was registered in 55% (22/40) of malignant group while it was complete absent in the other three studied groups [Table/Fig-4].

Correlation between HER2/neu and clinicopathological factors in carcinoma group.

HER2/neu over expression was more evident in older patients, 50 years of age (p=0.001 (r=0.696), site of the tumor (p=0.001) (r=0.441). HER2/neu overexpression did not correlate significantly with sex, level of tumour infiltration (T), presence of lymph node or distant metastasis. There was no significant difference in expression between early and advanced stages [Table/Fig-5-7].

Correlation between Ki-67 and clinicopathological factors in malignant group.

Ki-67 positive expression was found in 55% (22/40) of studied malignant cases. Ki-67LI significantly correlated with age (p=0.001) (r=0.493), and stage of the tumor (p=0.012) (r=0.433), while other variables showed no significant difference, despite its expression was more in positive LN metastatic cases than in cases with no LN metastasis, as shown in [Table/Fig-8, 9a-b].

Correlation between MUC1 extension rate and clinicopathological factors in carcinoma

There was a significant difference in MUC1 expression among age group (p=0.0001) (r=0.591), sex of the patient (p=0.001) (r=0.498),

			Malignant (1) (n=40)	Premalignant (2) (n=8)	Metaplastic (3) (n=10)	Cholecystitis (4) (n=25)	p-value
HER2/neu		Negative	21 (52.5%)	7 (87.5%)	10 (100%)	25 (100%)	<0.001**
		Positive	19 (47.5%)	1 (12.5%)	0 (0%)	0 (0%)	
MUC1	Scoring	Low	10 (25%)	2 (25%)	9 (90 %)	25	<0.001**
		High	30 (75%)	6 (75%)	1 (10%)	0	
	Pattern	Apical	0	4 (50%)	9 (90 %)	25	<0.001**
		Cytoplasmic	40	4 (50%)	1 (10%)	0	
Ki-67		<20%	18 (45%)	8 (100%)	10 (100%)	25 (100%)	<0.001**
		≥20%	22 (55%)	0 (0%)	0 (0%)	0 (0%)	

[Table/Fig-4]: HER2/neu, Ki-67 and MUC1 expression in studied four groups.
**significant

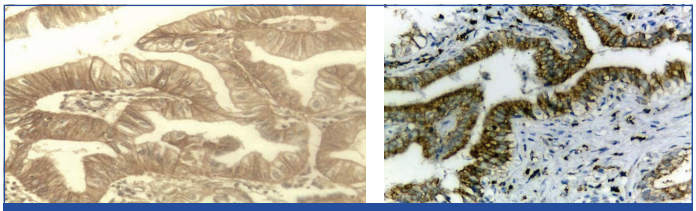
Variable	Total	HER2 /neu				p-value
		Negative No (%)	1 No (%)	2 No (%)	3 No (%)	
Age (years)						
<50	17	12 (70.6%)	3 (17.6%)	2 (11.8%)	0	0.001**
≥50	23	2 (8.7%)	4 (17.4%)	6 (26.1%)	11 (47.8%)	
Mean±SD	53.6±12.46					
Sex						
female	26	7 (26.9%)	4 (15.4%)	6 (23.1%)	9 (34.6%)	0.076
Male	14	7 (50%)	3 (21.4%)	2 (14.3%)	2 (14.3%)	
Site						
Fundus	16	12 (75%)	1 (6.2%)	1 (6.2%)	2 (12.5%)	0.001**
Body	13	1 (7.7%)	4 (30.8%)	4 (30.8%)	4 (30.8%)	
Neck	11	1 (9.1%)	2 (18.2%)	3 (27.3%)	5 (45.5%)	
Grade						
(WDC)	9	2 (22.2%)	1 (11.1%)	1 (11.1%)	5 (55.6%)	0.532
(MDC)	20	10 (50%)	4 (20%)	4 (20%)	2 (10%)	
(PDC)	11	2 (18.2%)	2 (18.2%)	3 (27.3%)	4 (36.3%)	
Infiltration level						
T1	10	6 (60%)	2 (20%)	1 (10%)	1 (10%)	0.293
T2	17	6 (35.5%)	1 (5.9%)	4 (23.5%)	6 (35.3%)	
T3	13	2 (15.4%)	4 (30.8%)	3 (23.1%)	4 (30.8%)	
LN						
Negative	19	6 (31.6%)	2 (10.5%)	5 (26.3%)	6 (31.6%)	0.391
Positive	21	8 (38.1%)	5 (23.8%)	3 (14.3%)	5 (23.8%)	
DM						
Absent	24	11 (45.8%)	4 (16.7%)	5 (20.8%)	4 (16.7%)	0.720
Present	16	3 (18.7%)	3 (18.8%)	3 (18.8%)	7 (43.7%)	
Stage						
I	10	6 (60%)	2 (20%)	1 (10%)	1 (10%)	0.775
II	8	1 (12.5%	2 (25%)	3 (37.5	2 (25%)	
III	6	2 (33.3%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	
IV	16	5 (31.2%)	1 (6.2%)	3 (18.7%)	7 (43.9%)	
Total	40	14 (35%)	7 (17.5%)	8 (20%)	11 (27.5%)	

[Table/Fig-5]: HER2/neu scoring according to clinico-pathological variables of malignant group.
WDC: Well differentiated carcinoma; MDC: Moderately Differentiated Carcinoma; PDC: Poorly differentiated carcinoma; LN: Lymph node; DM: Distant metastasis; **significant

grade groups (p=0.001) (r=0.503), tumour site (p=0.002) (r=0.485), level of infiltration (p<0.001) (r=0.493), the presence of lymph node p=0.001) (r=0.516), distant metastasis (p=0.027) (r=0.349), and tumour stage (p=0.001) (r=0.526), as detailed in [Table/Fig-10,11a-b,12].

Correlation between HER2/neu expression. MUC1 and Ki-67

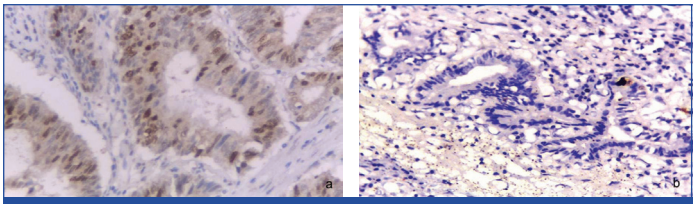
There was an insignificant correlation between HER2/neu and Ki-67 expression within carcinoma cases (p=0.28) (r=0.667). Among 22 cases that were positive for Ki-67, 10 cases (45.5%) registered positive HER2/neu expression. Although most of HER2/neu positive cases (55.5%) (15/27) showed high MUC1 expression but with no statistical correlation (p<0.501) as shown in [Table/Fig-13].



[Table/Fig-6]: HER2/neu expression in GBC showing moderate to strong complete membranous immuno reactivity in >10% of tumour cells score 3 (Avidin Biotin Complex ABC x400). **[Table/Fig-7]:** HER2/neu expression in dysplastic gland showing weak to moderate complete membrane immuno reactivity in >10% of tumour cells score 2 (Avidin Biotin Complex ABCx 400). (Images from left to right)

Variable	Total	Ki -67 labeling index (LI)		p-value
		LI <20% No (%)	LI ≥20% No (%)	
Age (years)				
<50	17	1 (5.9%)	16 (94.1%)	0.001**
≥50	23	17 (73.9%)	6 (26.1%)	
Sex				
Female	26	10 (38.5%)	16 (61.5%)	0.603
Male	14	8 (57.2%)	6 (42.8%)	
Site				
Fundus	16	6 (37.5%)	10 (62.5%)	0.231
Body	13	7 (53.8%)	6 (46.2%)	
Neck	11	5 (45.5%)	6 (54.5%)	
Grade				
(WDC)	9	5 (55.6%)	4 (44.4%)	0.105
(MDC)	20	8 (40%)	12 (60%)	
(PDC)	11	5 (45.5%)	6 (54.5%)	
Infiltration level				
T1	10	7 (70%)	3 (30%)	0.640
T2	17	7 (41.2%)	10 (58.8%)	
T3	13	4 (30.8%)	9 (69.2%)	
LN				
Negative	19	9 (47.7%)	10 (52.3%)	0.241
Positive	21	9 (42.9%)	12 (57.1%)	
Metastasis				
Absent	24	11 (45.8%)	13 (54.2%)	0.756
Present	16	7 (43.7%)	9 (56.3%)	
Stage				
I	10	8 (80%)	2 (20%)	0.012*
II	8	4 (50%)	4 (50%)	
III	6	2 (33.3%)	4 (66.7%)	
IV	16	4 (25%)	12 (75%)	
Total	40	18 (45%)	22 (55%)	

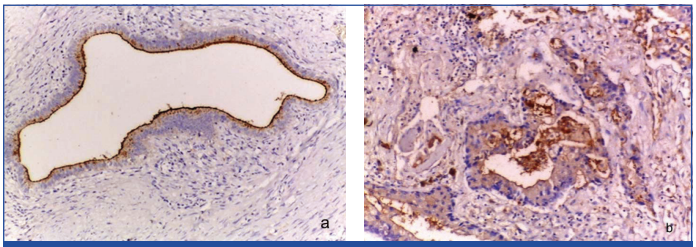
[Table/Fig-8]: Correlation between Ki-67 and clinicopathological factors in carcinoma.
WDC: Well differentiated carcinoma; PDC: Poorly differentiated carcinoma; LN: Lymph node; DM: Distant metastasis



[Table/Fig-9]: a) High nuclear Ki- 67expression in GBC (Avidin Biotin Complex (ABC) 400 X; b) Negative Ki-67 expression in dysplastic glands (Avidin Biotin Complex (ABC) x 200.

Variable	Total	MUC1		p-value
		Low	High	
Age				
<50	17	11 (64.7%)	6 (35.3%)	0.0001**
≥50	23	2 (8.7%)	21 (91.3%)	
Sex				
Female	26	4 (15.4%)	22 (84.6%)	0. 001**
Male	14	9 (64.3%)	5 (35.7%)	
Site				
Fundus	16	9 (65.2%)	7 (43.8%)	0. 002*
Body	13	4 (30.8%)	9 (69.2%)	
Neck	11	0	11 (100%)	
Grade				
(WDC)	9	7 (77.8%)	2 (22.2%)	0. 001**
(MDC)	20	5 (25%)	15 (75%)	
(PDC)	11	1 (9.1%)	10 (90.9%)	
Infiltration level				
T1	10	8 (80%)	2 (20%)	0.001**
T2	17	3 (16.7%)	14 (82.4%)	
T3	13	2 (15.4%)	11 (84.6%)	
LN				
Negative	19	11 (57.9%)	8 (42.1%)	0.001**
Positive	21	2 (9.5%)	19 (90.5%)	
Metastasis				
Absent	24	11 (45.8%)	13 (54.2%)	0. 027*
Present	16	2 (12.5%)	14 (87.5%)	
Stage				
I	10	8 (80%)	2 (20%)	0.01**
II	8	2 (25%)	6 (75%)	
III	6	1 (16.7%)	5 (83.3%)	
IV	16	2 (12.5%)	14 (67.5%)	
Total	40	13 (32.5%)	27 (67.5%)	

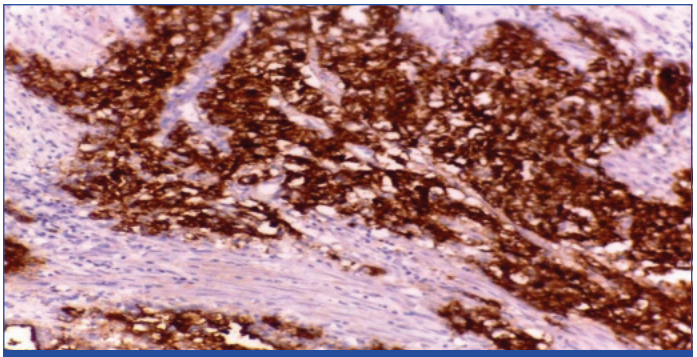
[Table/Fig-10]: Correlation between MUC1 expression and clinicopathological factors in carcinoma.
WDC: Well differentiated carcinoma; MDC: Moderately differentiated carcinoma; PDC: Poorly differentiated carcinoma; LN: Lymph node; DM: Distant metastasis; **Significant



[Table/Fig-11]: a) Apical membranous and low MUC1 expression in cholecystitis (Avidin Biotin Complex (ABC)x 100; b) Apical membranous and cytoplasmic MUC1 expression in dysplasia (Avidin Biotin Complex (ABC)x 200.

DISCUSSION

Carcinoma of gallbladder, an aggressive disease with poor prognosis, is the most common malignancy of the biliary tract and the sixth most common malignancy of the GIT worldwide. The poor prognosis, increasing incidence besides ineffective therapy of GBC makes challenging in its management. Therefore, there is a need of effective therapeutic agents for proper targeted therapy [16].



[Table/Fig-12]: High cytoplasmic expression of MUC1 expression in GBC (Avidin Biotin Complex ABC x400).

		Total	HER2/neu negative	HER2/neu positive	p-value
Ki-67	<20%	18	9 (50%)	9 (50%)	0.28
	≥20%	22	12 (54.5%)	10 (45.5%)	
MUC1	Low	13	9 (69.2%)	4 (30.8%)	0.501
	High	27	12 (44.5%)	15 (55.5%)	

[Table/Fig-13]: Relation between HER2/neu, Ki-67 and MUC1 expression within malignant group.

The present study was planned to investigate the expression of HER2/neu, MUC1and Ki-67 in GBC, comparing with dysplastic and metaplastic lesions, beside the correlation with clinico pathological parameters of GBC by immunohistochemical staining technique. Subsequently, these markers were studied to evaluate their value as targeted biomarkers in the therapy of GBC treatment.

The mean age of patients in the malignant group was 53.6±12.46 years, which was higher than that in control group 39.4±5.7 years. Female predominance was noted. This observation is comparable to that of Chaube A et al., [17]. HER-2/neu, a member of the EGFR family, acts as oncogene. It plays pivotal role in tumourigenesis of breast cancer. Its overexpression, as a result of gene amplification, is reported in other solid tumours [18].

In this current study, 47.5% (19/40) of GBC cases, and 12.5% (1/8) of dysplastic group respectively were HER2/neu positive compared by the control group (cholecystitis) and metaplastic group in which HER2/neu expression was completely absent.

Positive expression of HER2/neu in GBC ranges from 2-46.5% across many studies [19-21]. This wide range can be due to variable methodology and different HER2/neu scorning system in these different studies. Some authors [17,22] considered +2 and +3 score as positive, as done in present study while Roa I et al., considered only +3 to be HER2 /neu positive [23]. Both of Doval DC et al., [24] and Pujani M et al., [25] used breast cancer scoring system for HER2/neu. This wide controversy highlights the need for a uniform consensus for HER2/neu scoring in GBC like that in breast carcinoma.

Close to present study results, HER2/neu was registered in 24% of GBC cases in study done by Pujani M et al., and absence of its expression in control group [25]. The study done by Yoshida H et al., supports present study findings, as HER2/neu positive expression was in 23% of GBC cases [26]. In the same study, all non-neoplastic gallbladder lesions were HER2/neu negative. The results of Roa I et al., who registered HER2/neu over-expression in in GBC with negative immune-staining in the control group that is matching the present study [23].

This is in contrast with findings, reported by Zhou YM et al., who registered 70.7% of GBC was positive expression of HER2/neu and it was higher in dysplasia lesions (85.5%) [27].

Although HER2/neu score (+2) and (+3) are higher in stage IV and also in cases having distant metastasis within the current work, there is no statistical correlation with clinicopathological parameters of studied GBC except with the age and the site of the tumor. HER2/neu positive were detected in 73.9% (17/23) of GBC cases aged over 50 years while only 11.8% (2/17) of cases under 50-year-old showed positive HER2/neu expression.

This is in agreement with Roa I et al., and Doval DC et al., Pujani M et al., and Chow NH et al., [23-25,28]. It is also similar to Matsuyama S et al., who fail to find correlation with GBC grade [29].

Contradictory to present study results, correlation with advanced tumour stage and metastatic lymph node status was observed in studies done by Nakazawa K et al., and Puhalla H et al., [19,22], respectively. Kumari N et al., observed more HER2 /neu expression in well differentiated GBC and advanced staged ones that disagrees the current study [30].

The discrepancy between these findings and present study results may be explained by the use of different immunostaining procedures (e.g., monoclonal/vs polyclonal antibodies and sensitivity of the technique), time of fixation, preservation of antigenic sites and number of samples analysed.

Mucins are high molecular weight glycoproteins that are produced by various epithelial cells. They are essential for epithelial cell protection and homeostasis, acting as an anti-adhesion molecule preventing tumour cell invasion [31]. The current work found there was significant difference of MUC1 expression concerning the pattern and intensity between the studied groups as low MUC1 expression within all cases of cholecytitis cases (25/25) and 90% (9/10) of metaplastic group, with apical surface polarisation while it was significantly increased in dysplastic lesion and GBC (high expression in 75% of both) with cytoplasmic localisation.

Concerning the relation with clinicopathological parameters, 90.9% (10/11) of poorly differentiated GBC cases showed high MUC1 expression compared with 22.2% (2/9) of WD ones ($p<0.001$), high MUC1 expression was in 84.6% (11/13) of pT3 and in only 20% (2/10) of pT1 with significant difference. The current study registered that 90.5% (19/21) of positive nodal metastatic cases and 87.5% (14/16) of distant metastatic GBC cases were of high MUC1 expression. Parallel to present study Maurya KS et al., who found weak and apically located MUC1 expression in normal epithelial cells of control group in contrast to higher and extensive expression in malignant cells [32]. The loss of apical expression within malignant transformation leads to disturbance of cell-cell adhesion and facilitating the spread of carcinoma [32].

The results of Xiong L et al., Wang HH et al., Takagawa M et al., and Ghosh M et al., are similar to index findings concerning MUC1 overexpression in GBC [33-36]. Yamato T et al., study was in accordance with present study, as they reported rare expression of MUC1 in normal epithelial cells, while it was significantly higher in dysplastic and malignant tumour with depolarisation of its expression than in cholecystitis cases [37].

These findings are close to that of Bhoge A [38] who registered positive expression rate of MUC1 was significantly higher in GBC (85%) and dysplastic lesion with cytoplasmic pattern (83%) while was not found in chronic cholecystitis [38]. In the same study, also there was significant correlation between MUC1 and advanced stage of GBC.

Based on that, present study emphasises some evidence about the pathogenesis of GBC which progresses from cholecystitis, metaplaia, dysplasia, and invasive cancer as seen in literature. MUC1 transcripts/protein are occurred with the sequel of GBC development from cholecystitis up to carcinoma passing by dysplasia, with significant variance in its intensity and distribution patterns [33,38].

Concerning Ki-67, 55% (22/40) of malignant group showed positive Ki-67 expression, with complete absence in submitted dysplastic and control group.

The current study recorded increasing Ki-67 expression with advancing stage of GBC with statistically significant correlation ($p=0.012$).

The current work found a significant correlation between the Ki-67 expression and the age of patients ($p<0.001$). Doval DC et al., and Lee CS et al., agree with present study concerning this observation [24,39] . Similarly, Singh KR et al., did not find significant correlation with grade and metastatic status of GBC cases [40]. On the other side, the same study registered positive Ki-67 expression in 55% of GBC cases and complete absence in cholecystitis group that also support present study results. Pujani M et al., found 60% malignant cases with Ki-67 positive staining that is in agreement with present study [25], but against us concerning the benign group that showed Ki-67 positive in 4% of benign gall bladder lesions.

No significant correlation between the grade of differentiation and the wall infiltrate in relation to Ki-67 LI within the study done by Grau LH et al., similar to present study findings [41]. A noteworthy finding was that, Ki-67 expression (LI ≥20%) was observed in 56.3% (9/16) of cases with distant metastasis 57.1% (12/22) of positive lymph node metastatic cases compared, that justifies the more proliferation the more prevalent invasion and spread. Despite Lee CS approved the concerning significant correlation with the age and the stage of GBC patients but he found correlation with the grade that was not detected in the present study [39].

The continuous advance in targeted therapeutic approaches, that were directed against various cell adhesion molecules and cell cycle regulatory molecules, simultaneously with over expression of HER2/neu and MUC1 in GBC put more light on the validation of both markers as a candidate in GBC targeted therapy, especially in advanced stage tumours as that of breast cancer. In the same time, applied markers were significantly different between tested groups as diagnostic markers declaring their role in GBC development.

Limitation(s)

The current study had potential limitations including the small size of dysplastic and malignant groups due to their rarity beside financial limitation.

CONCLUSION(S)

HER2/neu, and Ki-67 are overexpressed in GBS cases compared with control and dysplastic group with no significant correlation with the parameters except for age despite greater expression of both are more prevalent in advanced stage and cases with distant metastasis. Based on that, HER2/neu can be considered as a candidate for targeted therapy of GBC. Also, MUC1 core protein expression rate and pattern are significantly different within studied groups with statistical correlation with clinicopathological parameters of GBC suggesting that MUC1 core protein would be a marker of malignant transformation of gallbladder epithelium and its depolarised expression would also be a marker of GBC invasion. Subsequently, a new therapeutic agent can target these cell surface adhesion molecules (MUC1) in GBC treatment strategy.

REFERENCES

[1] Rawla P, Sunkara T, Thandra K. Epidemiology of gallbladder cancer. Clin Exp Hepatol. 2019;5(2):93-102.
[2] Lazcano-Ponce EC, Miquel JF, Munoz N. Epidemiology and molecular pathology of gall bladder cancer. Cancer J Clin. 2001;51(6):349-64.
[3] Ghosh Y, Thakurdas B. Carcinoma gall bladder. A review of literature. Int J Sci Study. 2015;2:98-103.
[4] Hanada K, Tsuchida A, Iwao T. Gene mutations of K-ras in gallbladder mucosa and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. Am J Gastroenterol. 1999;94:1638-42.
[5] Meric-Bernstam F, Hung MC. Advances in targeting human epidermal growth factor receptor-2 signaling for cancer therapy. Clin Cancer Res. 2006;12:6326-30.
[6] Ali HM, Yahya AQ, Mohammed HL. Chromogenic in situ hybridization technique versus immunohistochemistry in assessment of HER2/neu status in 448 Iraqi patients with invasive breast carcinoma. Open Access Maced J Med Sci. 2019;7(12):1917-25.

[7] Rakha EA, Pinder SE, Bartlett JM. Updated UK Recommendations for HER2 assessment in breast cancer. Journal of Clinical Pathology. 2015;68(2):93-99.
[8] Yamada N, Kitamoto S, Yokoyama S Hamada T, Goto M, Tsutsumida H, et al. Epigenetic regulation of mucin genes in human cancers. Clin Epigenet. 2011;2:85-96.
[9] Onckheere Nand VanSeuningen I. The membrane-bound mucins from cell signalling to transcriptional regulation and expression in epithelial cancers. Biochimie. 2010;92(1):01-11.
[10] Yerushalmi R, Woods R, Ravdin PM. Ki-67 in breast cancer: Prognostic and predictive potential. Lancet Oncol. 2010;11:174-80.
[11] Ustymowicz KG, Pryczynicz A, Kemona A. Correlation between proliferation markers: PCNA, Ki-67 MCM-2 and anti-apoptotic protein bcl2 in colorectal cancer. Anticancer Research. 2009;29(8):3049-52.
[12] Kim N, Park DJ, Ryu YJ. Validation of AJCC 8th edition stage for gall bladder cancer. European Journal of Surgical Oncology. 2019;145(2):69.
[13] Summary of ASCO/CAP HER2 Guideline Recommendations. 2012. http://www.cap.org/apps/docs/committees/immunohistochemistry/summary_of_recommendations.pdf.
[14] Kim SM, OH SJ, Bang H. Expression of MUC1 and MUC4 in gallbladder adenocarcinoma. Korean J Pathol. 2012;46(5):429-35.
[15] Grau LAH, Badia JM, Salvador CA. Gallbladder carcinoma: The role of p53 protein overexpression and Ki-67 antigen as prognostic markers. HPB (Oxford. 2004;6:174-80.
[16] Javle M, Zhao H, Abou-Alfa GK. Systemic therapy for gallbladder cancer. Chin Clin Oncol. 2019;8(4):44.
[17] Chaube A, Tewari M, Garbyal RS. Preliminary study of p53 and c-erbB-2 expression in gallbladder cancer in Indian patients. BMC Cancer. 2006;6:126.
[18] Mitri Z, Constantine T, O'Regan. The HER2 receptor in breast cancer: pathophysiology: Clinical use, and new advances in therapy. Chemother Res Pract. 2012;2012:743193.
[19] Nakazawa K, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. J Pathol. 2005;206:356-65.
[20] Kawamoto T, Krishnamurthy S, Tarco E, Trivedi S, Wistuba II. HER receptor family: novel candidate for targeted therapy for gallbladder and extrahepatic bile duct cancer. Gastrointest Cancer Res. 2007;1:221-27.
[21] Shafizadeh N, Grenert JP, Sahai V, Kakar S. Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence in situ hybridization in adenocarcinomas of the biliary tree and gallbladder. Hum Pathol. 2010;41:485-92.
[22] Puhalla H, Wrba F, Kandioler D. Expression of p21(Waf1/Cip1), p57(Kip2) and HER2/neu in patients with gallbladder cancer. Anticancer Res. 2007;27:1679-84.
[23] Roa I, de Toro G, Schalper K. Overexpression of the HER2/neu gene: A new therapeutic possibility for patients with advanced gallbladder cancer. Gastrointest Cancer Res. 2014;7:42-48.
[24] Doval DC, Azam S, Sinha R. Expression of epidermal growth factor receptor, p53, Bcl2, vascular endothelial growth factor, cyclooxygenase-2, cyclin D1, human epidermal receptor-2 and Ki-67: Association with clinicopathological profiles and outcomes in gallbladder carcinoma. J Carcinog. 2014;13:10.
[25] Pujani M, Makker I, Makker A. Expression of Human Epidermal Growth Factor Receptor (Her 2/neu) and proliferative marker Ki-67: Association with clinicopathological parameters in gallbladder carcinoma. APJCP. 2016;17(8):3903.
[26] Yoshida H, Shimada K, Kosuge T. A significant subgroup of resectable gallbladder cancer patients has an HER2 positive status. Virchow Archif. 2016;468(4):431-39.
[27] Zhou YM, Li YM, Cao N, Feng Y, Zeng F. Significance of expression of Epidermal Growth Factor (EGF) and its receptor (EGFR) in chronic cholecystitis and gallbladder carcinoma. Ai Zheng. 2003;22:262-65.
[28] Chow NH, Huang SM, Chan SH, Mo LR, Hwang MH, Su WC. Significance of cerbB-2 expression in normal and neoplastic epithelium of biliary tract. Anticancer Res. 1995;15:1055-59.
[29] Matsuyama S, Kitajima Y, Sumi K, Mori D, Satoh T, Miyazaki K. Gallbladder cancers rarely overexpress HER-2/neu, demonstrated by Hercep test. Oncol Rep. 2004;11:815-19.
[30] Kumari N, Kapoor VK, Krishnani N. Role of C-erbB2 expression in gallbladder cancer. Indian J Pathol Microbiol. 2012;55:75-79.
[31] Yonezawa S, Higashi M, Yamada N. Mucins in human neoplasms: Clinical pathology, gene expression and diagnostic application. Pathol Int. 2011;61:697-716.
[32] Maurya KS, Tewari M, Mahendra KT. Stage dependent expression of MUC1 glycoprotein in gallbladder carcinoma. Journal of Cancer Research and Experimental Oncology. 2009;1(1):01-07.
[33] Xiong L, Yang Z, Yang L, Liu J, Miao X. Expressive levels of MUC1 and MUC5AC and their clinicopathologic significances in the benign and malignant lesions of gallbladder. J Surg Oncol. 2012;105:97-103.
[34] Wang HH, Afdhal NH, Gendler SJ, Wang DQ. Evidence that gallbladder epithelial mucin enhances cholesterol cholelithogenesis in MUC1 transgenic mice. Gastroenterology. 2006;131(1):210-22.
[35] Takagawa M, Muguruma N, Oguri K, Imoto Y, Okamoto K, Ii K, et al. Prediction of prognosis in gallbladder carcinoma by mucin and p53 immunohistochemistry. Dig Dis Sci. 2005;50(8):1410-13.
[36] Ghosh M, Kawamoto T, Kamma H, Koike N. Different expression of MUC1 in the gallbladder disease. Internet J. Surg. 2003;2(4):45.
[37] Yamato T, Sasaki M, Watanabe Y, Nakanuma Y. Expression of MUC1 and MUC2 mucin core proteins and their messenger RNA in gall bladder carcinoma: an immunohistochemical and in situ hybridization study. J Pathol. 1999;188:30-37.
[38] Bhoge A, Sinai Khandeparkar SG, Joshi AR. Immunohistochemical study of MUC1 and MUC5AC expression in gall bladder lesions. J Clin Diagn Res. 2017;11(7):EC12-16.
[39] Lee CS. Differences in cell proliferation and prognostic significance of proliferating cell nuclear antigen and Ki67 antigen immunoreactivity in in-situ and invasive carcinomas of the extrahepatic biliary tract. Cancer. 1996;78(9):1881-87.
[40] Singh KR, Choudhary V, Goel MM, Gupta V, Agarwal P, Makkar A, et al. Ki-67 expression in premalignant and malignant lesions of gallbladder. Journal of Medical Science and Clinical Research. 2017;5(5):1260.
[41] Xuan YH, Choi YL, Shin YK, Kook MC, Chae SW, Park SM, et al. An immunohistochemical study of the expression of cell-cycle-regulated proteins p53, cyclin D1, RB, p27, Ki67 and MSH2 in gallbladder carcinoma and its precursor lesions. Histol Histopathol. 2005;20(1):59-66.

PARTICULARS OF CONTRIBUTORS:
1. Lecturer, Department of Pathology, Banha University, Banha, Egypt.
2. Lecturer, Department of Pathology, Banha University, Banha, Egypt.
3. Lecturer, Department of Pathology, Banha University, Banha, Egypt.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Heba M Rashad,
Lecturer, Department of Pathology, Banha University, Banha, Egypt.
E-mail : heba_massoud@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 01, 2020
- Manual Googling: Nov 17, 2020
- iThenticate Software: Dec 19, 2020 (7%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 27, 2020**
Date of Peer Review: **Sep 18, 2020**
Date of Acceptance: **Nov 19, 2020**
Date of Publishing: **Feb 01, 2021**