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Original Article

Study of tumor transglutaminase 2 expression in gallbladder cancer – *Is it a novel predictor of survival*?

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Backgrounds/Aims: Transglutaminase 2 (TG2) is known to be an important mediator of inflammation induced carcinogenesis pathway. Chronic inflammation is the most important causative factor in Gallbladder cancer (GBC) carcinogenesis. We analyzed the expression of TG2 in GBC and its role as potential prognostic marker, first of its kind study. **Methods:** We analyzed TG2 expression in 100 cases of GBC and 28 cases of non-cancer gallbladder specimen (calculus cholecystitis). We studied TG2 expression in GBC in comparison to control group and evaluated its role as a potential prognostic marker. **Results:** TG2 score (1-9) was calculated by multiplying percentage cytoplasmic expression (P) with intensity of expression (I) in tumor cells. Positive TG-2 expression was observed in 62% of GBC patients compared to only 21% (n=6) in control group (p=0.001). In curative resection subgroup (n=54), TG2 positive patients showed shorter disease free survival rate (p=0.04) and higher rate of recurrence (p=0.03) compared to TG2 negative patients. TG2 positive expression was observed in 15/16 of patients with recurrent disease. In palliative treatment subgroup, patients with strong TG2 positive expression had poorer disease specific survival (p=0.01) as compared to weakly positive group. On multivariate analysis, lymph node status (p=0.03) and TG2 expression (p=0.037), were found to be significant predictor of recurrence and eventual survival. **Conclusions:** Positive TG2 expression was related to higher recurrence rates post curative surgery, shorter disease free and overall survival and ultimately portended poor prognosis. It may be helpful in better prognostication and tailoring therapeutic approach for better management of GBC. **(Ann Hepatobiliary Pancreat Surg 2020;24:460-468)**

Key Words: Transglutaminase 2; Gallbladder cancer; Inflammation; Prognostic factor

INTRODUCTION

Gallbladder cancer (GBC) is the most common malignancy of the biliary tract and the fifth most common gastrointestinal (GI) cancer.[1] The disease is characterized by late onset of symptoms, advanced stage at presentation and a rapidly progressive disease with a median survival of 6 months in advanced disease.[2] In early-stage disease, a 5-year survival rate up to 75% can be achieved if stage-adjusted treatment is given. Chronic calculous cholecystitis and gall stones are associated with GBC in 68 to 98% cases.[3,4] Chronic inflammation induced by gall stones is recognized as an important factor in gallbladder carcinogenesis and cancer progression. The gallbladder epithelium undergoes a recurrent cycle of gallstone induced damage and repair leading to an inflammatory environment that promotes carcinogenesis. Since no prognostic or predictive markers are known for GBC, we analyzed the expression of TG2 and its role as novel prognostic marker in GBC. It plays an important role in inflammation and is known to facilitate cancer growth and progression. Evaluation of TG2 expression in GBC patients and its correlation with survival characteristics in GBC has not been studied till date.

TG2 is a trans-peptidase with a wide distribution in various tissues. It is known to mediate cross-linking of proteins and participate in signal transduction via activating and hydrolyzing guanidine tri-phosphate (GTP) enzyme.

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It is also an active participant in promoting malignant cell mobility and invasion mainly through epithelial mesenchymal transition (EMT) induction, and in inducing chemo-resistance of cancer cells.[5,6]

Role of TG2 in various inflammatory processes like fibrosis, celiac disease, atherosclerosis and autoimmune disease are well known.[7] Many studies have demonstrated a close link between inflammation and tumor.[8] TG2 involved in chronic inflammation is known to play an important role in tumorigenesis and cancer progression.[9] Role of TG2 in cancer progression has been studied in various cancers including ovary,[8] colorectal,[10] breast,[11] renal[12] and lung.[13] These studies showed that high TG2 expression group had poorer overall survival rate than those in the low expression group. We aim to analyze the expression of TG2 in GBC and its role as potential prognostic marker, a first of its kind study.

Table 1. Calculation of TG2 score based on cytoplasmic expression and intensity of TG2 expression

Transglutaminase-2 (TG-2) score	
Cytoplasmic expression of TG2 (P)	＜30%=1 31-50%=2 ＞50%=3
Intensity of expression (I)-	Nil to mild=1 Moderate=2 Strong=3
TG2 score=P×I (range-1-9)	

MATERIALS AND METHODS

Patients

The present study evaluated TG2 expression in patients of GBC and the control group calculous cholecystitis (non-cancer Gallbladder) by immunohistochemistry (IHC). We studied the correlation of molecular markers expression of TG2 with clinco-pathological features of the tumor as histological type, grade of differentiation, lymph node status, level of invasion of the tumor (T stage), disease free and disease specific survival. Patients presenting in our Department from October 2013-October 2017 (4 years) with histological diagnosis of GBC and tissue blocks available for immunohistochemical analysis were considered. Both prospective and retrospective patients were included in the study. Non-cancer gallbladder specimens were obtained from patients operated in General Surgery department for gallstone disease. Informed consent was obtained from all individual participants included in the study in a prescribed format. This study was granted Ethical approval by Institutional Ethics Committee.

IHC analysis was performed at Molecular Quest Healthcare Pvt. Ltd. (MolQ) Gurgaon, Haryana. Tumor stage was determined according to the pTNM staging

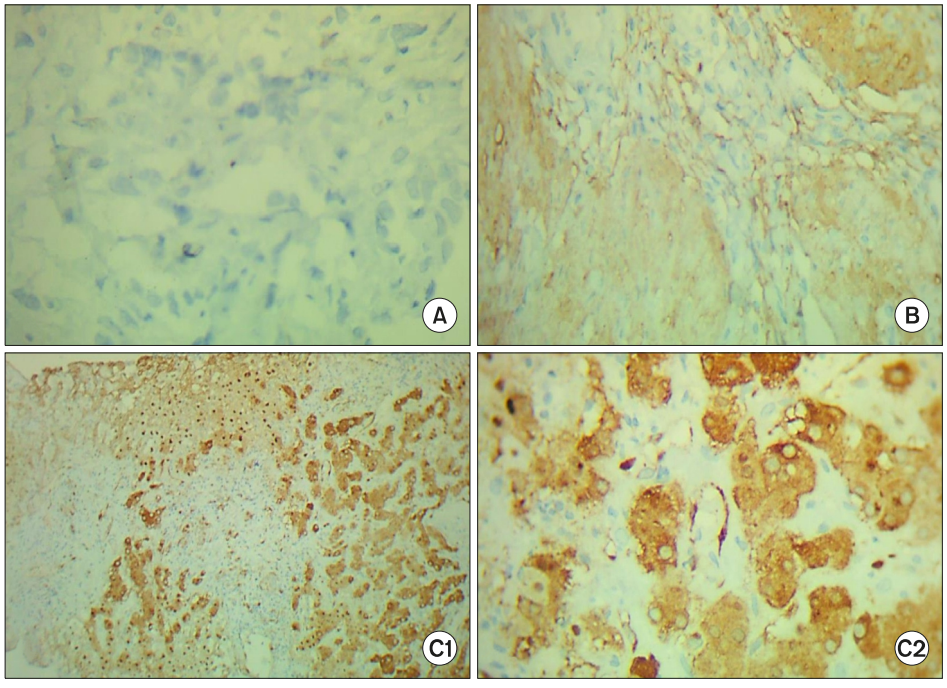

Fig. 1. Immunohistochemical TG2 expression in gallbladder cancer specimen (20×/40× magnification). (A) Negative expression (TG2 score ＜3), (B) weakly positive expression (TG2 score 3-6), (C1) strongly positive TG2 expression by tumor cells (TG2 score ＞6), (C2) strongly positive TG2 expression shown by tumor cells with minimal cytoplasmic staining seen in 40× magnification (TG2 score ＞6).

guideline published by the 2017 American Joint Committee on Cancer (8th Edition AJCC). Three monthly follow up was done by clinical examination and USG abdomen in operated patient group while symptomatic improvement (like pain, appetite, jaundice)was assessed in palliative patient group for response assessment.

Immunohistochemical staining

Following a review of the tumor slides, a representative area from each tumor block was selected and sectioned. Immunohistochemical staining was performed on 5-μm-thick sections. Polyclonal rabbit anti-TG2 antibody (*Neomarkers*, *CUB7401*) was used for IHC staining.

Evaluation and scoring

Cytoplasmic expression of TG2 intensity was studied and evaluated. Percentage of TG2 expression (P) was categorized in three categories ＜30%=1, 31-50%=2, ＞50%=3 while intensity of expression (I) were nil to mild (1), moderate (2) and strong (3) expression. TG2 score was calculated by multiplying percentage expression category (P) with intensity (I) [TG2 score=P×I] (Table 1, Fig. 1). Scores in GBC and non-cancer gallbladder group (control group) were compared to generate cut-off value for positive score in GBC group by plotting Receiver Operator Characteristic (ROC) curve. Maximum sensitivity (69%) and specificity (79%) were obtained with cut-off value of 2.7 (AUC=0.72, 95% confidence interval: 0.63-0.82, $p<0.001$) (Fig. 2). Thus, we considered score ≥3 (Range-3-9) as positive and ＜3 as negative. Positive TG2 expression was classified as strongly positive if score is ＞6 and weakly positive if score is between 3 to 6 (Range of TG-2 expression score-0-9) (Fig. 1).

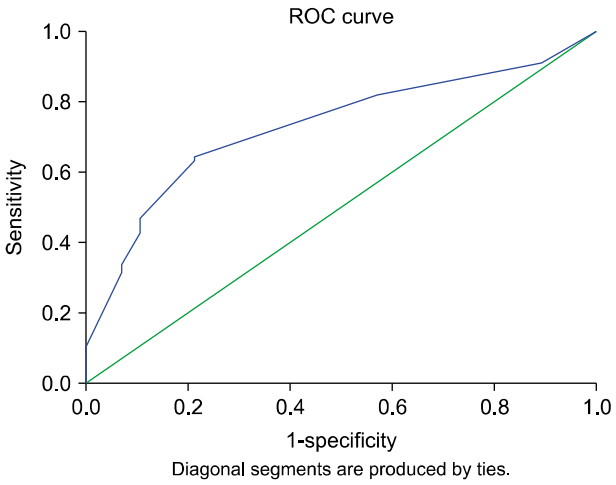

Fig. 2. Receiver operator characteristic (ROC) curve for determining cut off value of TG2 in GBC patients (maximum sensitivity (69%) and specificity (79%) were obtained with cut-off value of 2.7 (AUC=0.72, 95% confidence interval: 0.63-0.82, $p<0.001$).

Statistical analysis

SPSS version 23 was used for statistical analysis. The association between clinic-pathologic findings and TG expression was analyzed using the linear association. Disease free survival (DFS) and disease specific survival (DSS) were analyzed using the Kaplan-Meier method supported by the log rank test. All statistical analyses were 2-tailed, and a $p<0.05$ was regarded as statistically significant.

RESULTS

Clinico-pathologic characteristics

Total of 100 patients of GBC were included in the study. All GBC cases were divided in two groups-curative resection group (n=54) and palliative treatment group (n=46) (Table 2). Curative group comprised of patients who underwent curative resection (Radical cholecystectomy with or without CBD or adjacent organ resection), while palliative group comprised of patients with advanced stage (metastatic or locally advanced/inoperable disease) and were kept on palliative chemotherapy (Gemcitabine and Cisplatin Day 1 and 8, 3 weekly regimen). Curative resection group was further divided into two subgroup-Early stage including stage I and II (n=17) and locally

Table 2. General characteristic of GBC patients (n=100)

General characteristics (GBC group)	Distribution
M:F	1:3
Mean age (years)	50 years
Rural:urban distribution	73:27
Dietary habits	
Vegeterian	75
Non-vegeterian	25
Cholelithiasis	
Present	70
Absent	30
Curative surgery subgroup	54 (stage I-IVA)
Palliative treatment subgroup	46 (stage IVB)
Median follow up (months)	21 months (13-52)

advanced (stage III and IVA) group (n=37) (Table 3). Females (n=73, 73%) outnumbered males (n=27, 27%). The age of patients ranged from 20 to 80 years with peak incidence in fourth and fifth decade of life with mean age at diagnosis of 50 years. 70% of GBC patients in our study population had coexistent gallstones (Table 2).

Control group - A total of 28 patients of cholelithiasis who underwent Laparoscopic/open Cholecystectomy for symptomatic gallstone disease were recruited as control group. Out of 28 patients, 7 were male (25%) and 21 female (75%). Majority (71%) 20/28 of patients were in the age range of 31-50 years with median age of patients being 42 years.

TG2 expression

In the control group, TG2 expression was seen only in 21% (n=6) and none of them showed strong TG2 expression. In GBC group, TG2 expression could not be assessed in two patients due to insufficient tissue block, thus excluded from study. In stark comparison to only 6 patients showing positive TG2 expression in the control group (none in strongly positive group), positive TG2 expression was demonstrated in 62 GBC patients (63%), this differential expression of TG-2 being statistically significant (p=0.001). In 62 GBC patients with TG2 positive expression, 56 patients (90%) showed weakly positive expression (score 3-6) while rest 6 patients (10%) were strongly positive (score 7-9).

Of the 62 GBC patients with positive TG2 expression, 40/54 were in curative resection group while 22/46 were in palliative treatment group (p=.31). In curative resection group (n=54), more patients (28/37; 76%) with locally advanced disease showed positive TG2 expression as compared to early stage disease (12/17; 70%), though the difference was not statistically significant (p=.69).

In curative resection group, median follow up was 21 months (13-52 months). Following curative surgery, local or metastatic recurrence was reported in 29.6% (16/54) of patients. TG2 positive expression was present in 93.8% (15/16) of patients with recurrent disease (local or metastatic) indicating that TG2 expression may be a marker of inherent aggressive biological behavior and thus associated with higher likelihood of disease recurrence (p=0.03). TG2 positive patients had mean DFS of 21.3 months (95% confidence interval 17.02-25.60) as compared to 33.3 months (95% confidence interval 26.7-39.8) in TG2 negative patients with a difference of about 12 months which was statistically significant (p=0.04). Correlation of recurrence with TG2 expression demonstrated TG2 negative patients had better survival as compared to TG2 positive patients, difference being statistically significant (log rank test, p=0.04) (Fig. 3A). Weakly positive patients (21.7 months; 95% confidence interval 17.1-26.3) had slightly better DFS as compared to strongly TG2 positive patients (20 months; 95% confidence interval 10.9-29) though this difference was not statistically significant (p=0.84) (Table 4, Fig. 3B).

Univariate analysis was performed for TG2 expression and its correlation with seven clinic-pathological factors like patient age, sex, T stage, N stage, tumor histological grade, AJCC stage, association with cholelithiasis was

Table 3. Clinical characteristics of GBC patients in curative surgery group (n=54)

Clinico-pathological profile (GBC group)	Number (%)
Type of surgery	
Radical cholecystectomy	48 (89%)
Completion cholecystectomy	6 (11%)
T stage	
T1	5 (9%)
T2	23 (43%)
T3	21 (39%)
T4	5 (9%)
N stage	
N0	28 (52%)
N1	21 (39%)
N2	05 (9%)
Histological type	
Adenocarcinoma	45 (83%)
Papillary adenocarcinoma	8 (15%)
Adenosquamous	1 (2%)
AJCC Stage	
I	4 (7%)
II	13 (24%)
IIIA	11 (20%)
IIIB	17 (31%)
IVA	4 (7%)
IVB	5 (9%)
Incidental GBC	
Post open cholecystectomy	4 (9.3%)
Post lap cholecystectomy	2 (3.7%)
Total	6 (12%)
Recurrence	
Local	10 (62.5%)
Metastatic	6 (37.5%)
Total	16

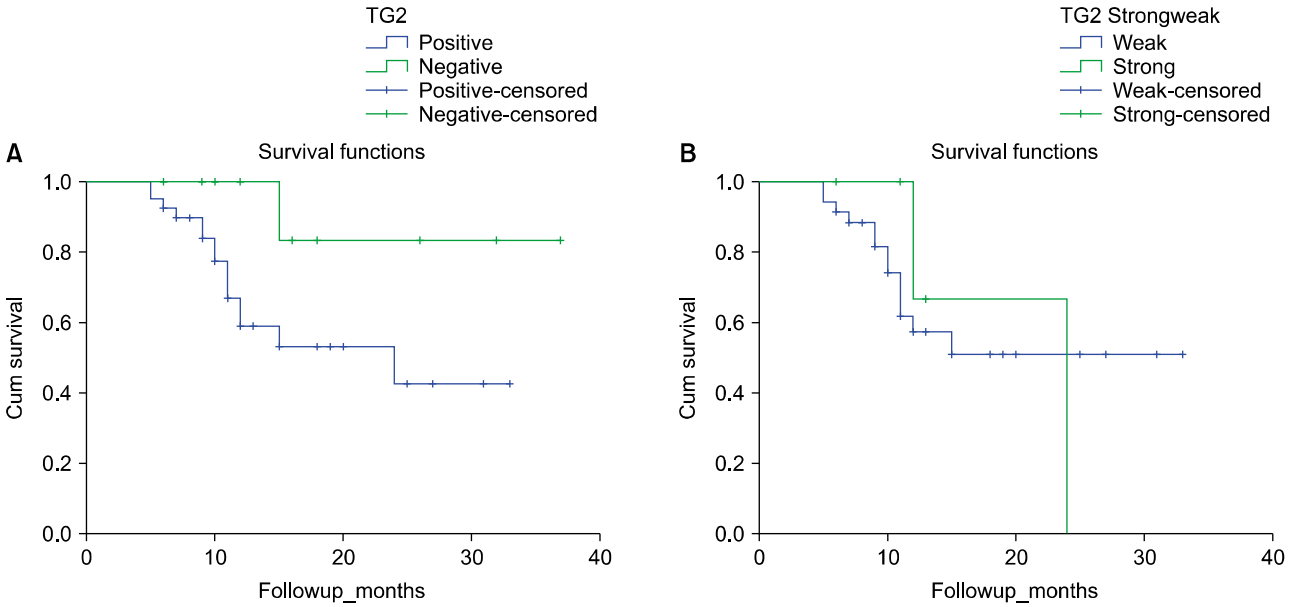

Fig. 3. Kaplan Meier curves showing TG2 expression and its correlation with recurrence affecting survival in GBC patients in curative surgery group (n=54). (A) Kaplan Meier curve showing TG2 negative patients had better survival compared to TG2 positive patients (log rank test, p=0.04). (B) Recurrence affecting survival in weakly (score: 3-6) and strongly TG2 (score: 6-9) positive patients.

Table 4. Correlation of TG2 expression with disease free survival (DFS) in curative surgery group demonstrating statistically significant difference in survival based on TG-2 expression (log rank test)

Marker	Marker status	Median survival	95% confidence interval	p value
TG2	Positive	21.3	17.20-25.6	0.04*
	Negative	33.33	26.77-39.8	
TG2	Strongly positive	20	10.9-29	0.84
	Weakly positive	21.7	17.1-26.3	

*Statistically significant difference in median survival between TG2 positive and negative group

evaluated in both curative and palliative subgroup. In Univariate analysis, none of these factors were significantly associated with TG2 expression except for its association with cholelithiasis. TG2 positive patients were associated with cholelithiasis in 60% (24/40) and 100% (22/22) of curative and palliative GBC group respectively. These findings were statistically significant (p=0.04 & 0.01).

On Univariate analysis, various factors were analyzed for predicting Overall Survival (OS). Out of the five clinic-pathological variables studied for correlation with OS, (AJCC stage, T stage, LN status, Histological grade, TG2 status), AJCC stage (p=0.017), LN status (p=0.019), Histological grade (p=0.015) and TG2 status (p=0.04) were found to illustrate significant correlation with OS in Univariate analysis. On multivariate analysis, only two of these four variables, lymph node status (p=0.03) and TG2 expression (p=0.037), were found to be significant predictor of recurrence and eventual survival (Table 5).

In Palliative group, median follow up was 6 months (4-24 months). Weakly TG2 positive had mean Disease Specific Survival (DSS) of 6.3 months (95% confidence interval 5.3-6.6) as compared to 3 months in strongly positive patients (95% confidence interval 3-3), this difference being statistically significant (log rank test, p=0.01). Survival in relation to weakly and strongly TG2 positive expression was plotted by Kaplan Meier curve and demonstrated that weakly TG2 positive patients had better survival as compared to strongly TG2 positive patients (Fig. 4).

DISCUSSION

Currently, there are no specific biomarkers or therapeutic molecular targets that can be used as predictive or prognostic indicators to either predict the risk or for use as therapeutic targets in metastatic GBC. Lack of effective chemotherapy agents for treatment of GBC in neoadjuvant/adjuvant setting further accentuates this clinical

Table 5. Univariate and multivariate analysis of clinico-pathological variables with OS in curative surgery group (n=54)

Clinical parameters	Univariate analysis (*p*-value)	Multivariate analysis (*p*-value)
AJCC stage	0.017	NS
T stage	0.019	NS
Lymph node (LN) positive status	0.015	0.03
Histological grade	0.019	NS
TG2 expression	0.04	0.037

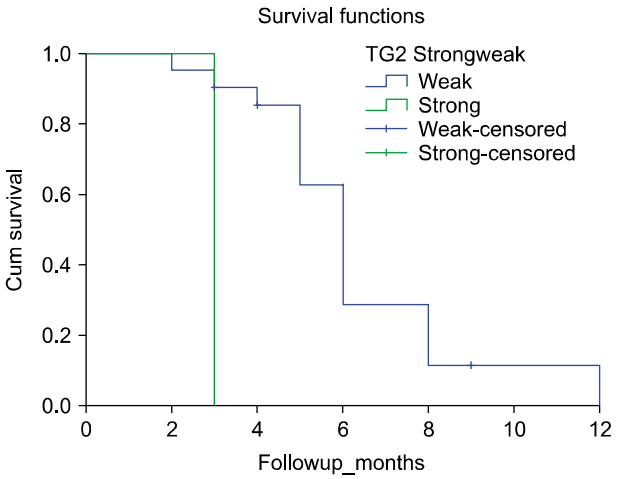

Fig. 4. Weak and strong TG2 positive expression effecting survival in palliative group (n=46).

problem. Transglutaminase 2 (TG2) is the most widely distributed and most abundantly expressed member of transglutaminase family of enzymes, which are known to catalyze the Ca^{2+}-dependent post-translational modification of proteins.[14] TG2 is a multifunctional protein and its enzymatic and non-enzymatic activities have been implicated in wide gamut of patho-physiological processes such as wound healing, cell growth and survival, extracellular matrix modification, apoptosis and autophagy: all of these attributes are known to play an important role in wound healing and inflammation. Thereby, TG2 may have a facilitator role in inflammation mediated cancer growth and progression.[15]

TG2 mediated signaling pathways enable the cancer cells to proliferate, resist cell death, invade, to reprogram glucose metabolism and to metastasize: all these characteristics are considered important hallmarks of aggressive cancer.[16,17] Role of TG2 expression in inflammation induced carcinogenesis is well established in various cancers including ovary, colorectal, breast, renal and lung.[8,10-13] Its role in GBC has not yet been studied. Our study, comprising 100 patients of GBC and 28 patients of cholelithiasis (control group) is the first study to evaluate expression of TG2 in a large cohort of GBC patients. We attempt to analyze TG2 expression and its correlation with various clinic-pathological features and to evaluate its role as a potential predictive/prognostic marker.

On Univariate analysis, only cholelithiasis was shown to have statistically significant correlation with TG2 expression in our series. It underlines the important role of TG2 in inflammation induced carcinogenesis which is known to be the main carcinogenic pathway in GBC. Higher TG2 expression correlated with higher recurrence rates and shorter DFS post curative resection. On similar lines, median survival was shorter in strong TG2 expression cohort as compared to weak TG2 positive patients in the palliative treatment group (3 versus 6.3 months, p=0.01).

Various studies have demonstrated TG2 as a potential negative prognosticator, and is often associated with advanced disease stage, early recurrence, and chemo-resistance. Erdem et al.[12] studied the role of TG2 expression in metastatic (n=33) and non-metastatic (n=33) renal cell carcinoma (RCC) and patients were stratified into two groups using median primary tumor staining score as the cutoff value: Group 1 (high risk, n=41) and Group 2 (low risk, n=22). The percentage of metastatic patients was significantly higher in Group 1 compared to Group 2 (68.3 vs. 18.2%, $p<0.001$). 5-year disease-free (34.9 vs. 92.9%, p=0.001) and cancer-specific (47.4 vs. 86.5%, p=0.04) survival rates were significantly lower in high-risk group thus demonstrated increased expression of TG2 in primary tumor associated with a decrease in disease-free and cancer-specific survival outcomes in RCC.[12] Similar results were observed in our study with patients with TG2 positive expression showed higher likelihood of developing recurrence as compared to those with TG-2 negative expression, in curative surgery group (p=0.03). It was also noted that TG2 positive patients had shorter survival as compared to TG2 negative patients (21.3 vs 33.3 months, p=0.04), in our series.

Similarly in colorectal cancer, Miyoshi et al.[10] analyzed TG2 expression in 91 paired cases of colorectal cancer

(CRC) and noncancerous regions. They showed poorer overall survival rate in high TG2 expression group than those in the low expression group (p=.001), indicating that increased TG2 expression was an independent poor prognostic factor.[10]

Chihong et al.[13] have demonstrated the role of TG2 in cell survival and cancer progression in 194 patients diagnosed with non-small cell lung cancer (NSCLC) and found that TG2 expression was significantly higher in lung cancer tissues as compared to paired marginal tissues or normal tissues. They also postulated that patients with low TG2 expression levels had longer disease free survival and overall survival as compared those with higher TG2 expression and also found that high TG2 expression correlated with NSCLC recurrence.[13] Findings in our study corroborated the above findings with high TG-2 expression demonstrated in GBC group (63%) as compared to control (21%) and this differential expression was statistically significant (p=0.001).

Study by Fisher et al.[18] demonstrated elevated TG2 levels in epidermal cancer stem cells (ECS cells) and important role TG2 plays in maintaining cancer stem cell survival, invasive, and metastatic behavior. They also showed that inhibitors induced TG2 knockdown or suppression of TG2 function can markedly reduce ECS cell survival. Thus TG2 inhibition can be an important therapeutic anti-cancer target by facilitating reduced survival of cancer stem cells in epidermal squamous cell carcinoma.[18]

Few studies have tried to study the correlation of some other biomarkers (EGFR, VEGFR, HER-2neu, p53) expression and its clinico-pathological correlation in GBC. Most of these studies are characterized by small number of GBC patients with incomplete clinical data and limited follow up details. Also most of these studies were performed on biliary tract cancers in general, with GBC comprising only a small proportion. Misra et al.[19] studied the correlation of p53 expression in operated GBC patients (n=20) and observed significant correlation between gall stone, T stage, grade of tumor and liver invasion with p53 over expression. In contrast, Ajiki et al.[20] found no significant correlation between p53 expression and prognosis or recurrence.

In a study of EGFR and HER-2neu expression in 124 advanced biliary tract cancer patients, GBC patients accounted for only 27% of the study cohort (34/124) and found no statistical association between grade, stage, overall survival and marker expression thus having no prognostic significance.[21] Tian et al.[22] found that positive VEGFR expression was present in 63.3% of GBC patients and revealed that positive VEGFR expression were more common in higher Nevin stage group as compared to lower Nevin stage group ($p<0.05$).

Despite various advancements in molecular biology, GBC treatment continues to be hampered by lack of effective chemotherapy options and targeted agents. Therefore, development of agents inhibiting TG2 may offer a novel therapeutic approach for treatment of GBC.

Strength of our study is the sample size of GBC patients undertaken for evaluation with all relevant clinic-pathological details and follow up. Our study has some limitations. First, the use of core biopsy samples in metastatic patients for immunohistochemistry, the small specimens may not represent the accurate TG2 status. Second, our sample size is small to show the association between drug resistance and the expression of TG2.

Present study is the first to demonstrate the clinic-pathological significance of TG2 expression in a large cohort of GBC patients (n=100). Positive TG2 expression was related to higher recurrence rates post curative surgery, shorter disease free and overall survival and ultimately portended poor prognosis. Further studies may be undertaken to better evaluate its role as novel prognostic/predictive marker in GBC and may be helpful in better prognostication and tailoring therapeutic approach for better management of GBC.

Novelty and impact

TG2 is a marker for inflammation induced carcinogenesis pathway. Strength of our study is large sample size (n=100) of GBC patients with all relevant clinic-pathological details and follow up. We studied TG2 expression and its correlation with various clinic-pathological factors. In our study, Positive TG2 expression correlated with higher recurrence rates post curative surgery, shorter disease free/overall survival and ultimately portended poor prognosis.

ETHICS APPROVAL

This study was performed in accordance with the

Ethical Principles for Medical Research Involving Human Subjects outlined in the Helsinki Declaration in 1975 (revised in 2000). Approval was granted by the Ethics Committee of King George's Medical University vide letter no *3176*/*Ethics*/*R*. *Cell-15* dated 7/01/15.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Misra S, Chaturvedi A, Misra NC. Gallbladder cancer. Curr Treat Options Gastroenterol 2006;9:95-106.
2. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. Lancet Oncol 2003;4:167-176.
3. Silk YN, Douglass HO Jr, Nava HR, Driscoll DL, Tartarian G. Carcinoma of the gallbladder. The Roswell Park experience. Ann Surg 1989;210:751-757.
4. Perpetuo MD, Valdivieso M, Heilbrun LK, Nelson RS, Connor T, Bodey GP. Natural history study of gallbladder cancer: a review of 36 years experience at M.D. Anderson hospital and tumor institute. Cancer 1978;42:330-335.
5. Begg GE, Carrington L, Stokes PH, Matthews JM, Wouters MA, Husain A, et al. Mechanism of allosteric regulation of transglutaminase 2 by GTP. Proc Natl Acad Sci U S A 2006;103:19683-19688.
6. Kumar A, Xu J, Brady S, Gao H, Yu D, Reuben J, et al. Tissue transglutaminase promotes drug resistance and invasion by inducing mesenchymal transition in mammary epithelial cells. PLoS One 2010;5:e13390.
7. Szondy Z, Korponay-Szabó I, Király R, Sarang Z, Tsay GJ. Transglutaminase 2 in human diseases. Biomedicine (Taipei) 2017;7:15.
8. Shao M, Cao L, Shen C, Satpathy M, Chelladurai B, Bigsby RM, et al. Epithelial-to-mesenchymal transition and ovarian tumor progression induced by tissue transglutaminase. Cancer Res 2009;69:9192-9201.
9. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-867.
10. Miyoshi N, Ishii H, Mimori K, Tanaka F, Hitora T, Tei M, et al. TGM2 is a novel marker for prognosis and therapeutic target in colorectal cancer. Ann Surg Oncol 2010;17:967-972.
11. Agnihotri N, Kumar S, Mehta K. Tissue transglutaminase as a central mediator in inflammation-induced progression of breast cancer. Breast Cancer Res 2013;15:202.
12. Erdem S, Yegen G, Telci D, Yildiz I, Tefik T, Issever H, et al. The increased transglutaminase 2 expression levels during initial tumorigenesis predict increased risk of metastasis and decreased disease-free and cancer-specific survivals in renal cell carcinoma. World J Urol 2015;33:1553-1560.
13. Chihong Z, Yutian L, Danying W, Ruibin J, Huaying S, Linhui G, et al. Prognostic value of Transglutaminase 2 in non-small cell lung cancer patients. Oncotarget 2017;8:45577-45584.
14. Mehta K, Kumar A, Kim HI. Transglutaminase 2: a multi-tasking protein in the complex circuitry of inflammation and cancer. Biochem Pharmacol 2010;80:1921-1929.
15. Odii BO, Coussons P. Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family. ScientificWorldJournal 2014;2014:714561.
16. Agnihotri N, Mehta K. Transglutaminase-2: evolution from pedestrian protein to a promising therapeutic target. Amino Acids 2017;49:425-439.
17. Gundemir S, Colak G, Tucholski J, Johnson GV. Transglutaminase 2: a molecular Swiss army knife. Biochim Biophys Acta 2012;1823:406-419.
18. Fisher ML, Keillor JW, Xu W, Eckert RL, Kerr C. Transglutaminase is required for epidermal squamous cell carcinoma stem cell survival. Mol Cancer Res 2015;13:1083-1094.

19. Misra S, Chaturvedi A, Goel MM, Mehrotra R, Sharma ID, Srivastava AN, et al. Overexpression of p53 protein in gallbladder carcinoma in North India. Eur J Surg Oncol 2000;26:164-167.
20. Ajiki T, Onoyama H, Yamamoto M, Asaka K, Fujimori T, Maeda S, et al. p53 protein expression and prognosis in gallbladder carcinoma and premalignant lesions. Hepatogastroenterology 1996;43:521-526.
21. Harder J, Waiz O, Otto F, Geissler M, Olschewski M, Weinhold B, et al. EGFR and HER2 expression in advanced biliary tract cancer. World J Gastroenterol 2009;15:4511-4517.
22. Tian Y, Ding RY, Zhi YH, Guo RX, Wu SD. Analysis of p53 and vascular endothelial growth factor expression in human gallbladder carcinoma for the determination of tumor vascularity. World J Gastroenterol 2006;12:415-419.