Gallbladder Adenocarcinoma, Potential Target for Anti-Her-2 Therapy

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Abstract- Her-2 (ErbB-2) is an oncogene frequently overexpressed in breast and gastric adenocarcinomas, and anti-Her-2 targeted therapy can be given to such patients. Her-2 overexpression and role of anti-Her-2 targeted therapy in cases of gallbladder adenocarcinomas (GBC) are still debatable. Scoring protocols for Her-2 expression in breast and gastric carcinomas are standardized, however, not for carcinomas arising in other body organs like the gallbladder. This study is conducted to evaluate the expression of Her-2 in patients with GBC, which may benefit from targeted therapy. It is a cross-sectional study conducted on patients with GBC (n=63; 53 women and ten men). An automated immunohistochemical technique was used with an anti-ErbB2 antibody. Scoring was conducted according to the CAP (College of American Pathologists) criteria for breast cancer. Positive (3+) Her-2 staining was observed in 8/63 (12.7%). Nine cases (14.3%) showed equivocal staining (2+) pattern. All of the tumors showing Her-2 overexpression were moderately differentiated. This study indicates that a significant number of GBC cases show Her-2 overexpression. This subgroup may benefit from inhibitors of the Her-2 pathway. Standardization of scoring protocol for Her-2 expression in GBC is needed to better evaluate the predictive potential of Her-2 for the treatment of these tumors.

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Introduction

Gallbladder cancer (GBC) is the most common malignancy of the biliary tract, representing 80%-95% of biliary tract cancers worldwide. It ranks sixth among gastrointestinal cancers (1). The frequency of the gallbladder tumor is higher in Pakistan as compared to other countries. The high prevalence of gallbladder tumors in females is also notable, and it has been reported as the second commonest malignancy of gastrointestinal origin in Pakistani females (2).

GBC has an abysmal prognosis. This malignancy remains asymptomatic until aggressive disease has progressed to an advanced and non-curative stage (3,4). The overall mean survival rate for patients with gallbladder cancer is six months, with a 5-year survival rate of 5% (5).

There are a number of risk factors that may increase the chances of developing GBC that include gallstones, polyps, porcelain gallbladder, diabetes, old age, obesity, female gender, and family history. Gallstones appear to be one of the strongest risk factors for GBC (6,7,8). The risk increases with an increase in the size of the stone and duration of disease. Some drugs have also been implicated in biliary carcinogenesis, including methyldopa, oral contraceptives, and isoniazid. Others have found no convincing evidence for an association between oral contraceptive use and GBC (9).

Her-2 gene is a proto-oncogene located on the 17q12 chromosomal region. Overexpression of this receptor in breast cancer is associated with increased disease recurrence and worse prognosis (10). Overexpression is also noted in other cancers, such as gastric cancer and ovarian cancers (11,12,13,14).

In patients with advanced breast and gastric cancer, Her-2 receptor blockers (Trastuzumab) have already been incorporated into conventional chemotherapy with good results. In gallbladder cancer, there have been reports regarding the overexpression and amplification of Her-2 (15,16,17). Scoring protocols for Her-2 expression in breast and gastric carcinomas are standardized, however, not for carcinomas arising in other body organs like the gallbladder.

Few studies have been done worldwide about overexpression of the Her-2 gene in gallbladder carcinoma (14-17) but have never been performed in our country even though Pakistan has a high incidence of gallbladder cancer. This study was conducted in our
population to determine the over-expression of Her-2 in
gallbladder adenocarcinoma to determine the subset of
patients who can get benefit from anti-Her-2 therapy. A
study conducted in India by Ashai F showed Her-2 was
strongly positive in five cases (9.25%) of gallbladder
cancer, and 14 cases (25.92%) showed moderate
positivity (17).

Materials and Methods

A descriptive cross-sectional study was performed
using a non-probability consecutive sampling technique
after approval from the institutional ethical committee.
The study was performed from January 2018 to February
2019 at the Chughtai Institute of Pathology, Lahore. Only
gallbladder adenocarcinoma cases were included
diagnosed on excisional or incisional biopsies. Blocks
from outside laboratories for review were also included.
All autolyzed/unfixed specimens were not included in the
study. There is no established protocol for reporting Her-
2 overexpression in gallbladder adenocarcinoma; therefore, reporting criteria for breast carcinoma were
used. Only cases showing complete, intense, circumferential membrane staining in >10% of invasive
tumor cells (3+) were considered positive (18). No
fluorescent in situ hybridization (FISH) was performed
for cases showing an equivocal (2+) staining pattern. The
clinical parameters, like age and gender, were recorded.
The histological slides were prepared by the classical
method for inclusion in paraffin, followed by
hematoxylin-eosin staining. The immunohistochemical
analysis was performed on serial sections using the
immune-enzymatic soluble complex method. The
antibody used was Her2 polyclonal antibodies from
DAKO, with positive control taken as breast carcinoma
3+ (positive) cases. Data were analyzed using SPSS
version 20. The mean and standard deviation was
calculated for quantitative variables, including the age
and size of the tumor. Qualitative variables, including
gender, tumor grade, tumor stage, perineural invasion,
and immunohistochemical staining (overexpression), were
presented in the form of frequencies and percentages.
Effect modifiers like age and gender were controlled
through stratification. The post-stratification chi-square
test was applied by taking P of 0.05 as significant.

Results

A total of 63 cases fulfilling the selection criteria were
enrolled in the study. The mean age of the patients was
55.44 +/- 12.21 years. The youngest patient in the study
was 22 years, while the oldest patient was 80 years old.
Most of the patients were greater than 45 years (77.8%),
while only 3.2% (n=2) were less than 30 years (Table 1).

| Age Group | Frequency (n) | Percentage |
|-----------|---------------|------------|
| <30 years | 2             | 3.2%       |
| 30-45 years | 12           | 19.0%      |
| 46-60 years | 26           | 41.3%      |
| >60 years | 23            | 36.5%      |

Gender distribution shows that 84.1% (n=53) were
females and 15.9% (n=10) were males (Table 2).

The mean tumor size of the patients was calculated as
4.46 +/- 2.72 cm. The tumor size was available for 59
cases. In 4 cases, tumor size was unavailable due to the
incisional nature of biopsy and lack of clinic-radiological
data. The smallest tumor included in the study was 0.9cm,
while the largest tumor was of 13.0cm. Tumor size
stratification showed that most of the tumors were larger
than 2.1 cm (n=46, 73.0%, Table 3).

| Tumor size | Frequency (n) | Percentage |
|------------|---------------|------------|
| 0-2.0cm    | 13            | 20.6%      |
| 2.1-5.0cm  | 26            | 41.3%      |
| >5.0cm     | 20            | 31.7%      |
| Cannot be determined | 4 | 6.4% |

Most of the tumors (n=44, 69.8%) were moderately
differentiated, while 22.3% (n=14) were poorly
differentiated and only 7.9% (n=5) were well
differentiated (Table 4).

| Histologic Grade | Frequency (n) | Percentage |
|------------------|---------------|------------|
| Well-differentiated | 5             | 7.9%       |
| Moderately
differentiated | 44            | 69.8%      |
| Poorly
differentiated | 14            | 22.3%      |

In our study, 14.3% (n=9) of the tumors were pT1,
44.4% (n=28) were pT2 and 36.5% (n=23) were pT3. It
was not possible to determine the stage of 3 cases due to
the non-availability of data (Table 5).

Perineural invasion was noted in 41.3% (n=26) of the

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Gallbladder adenocarcinoma cases. Most of the tumors involved fundus either as a single area or as a component of a rather diffuse process. 63% (n=34) of the cases were diffusely involving the gallbladder wall, with 50.8% (n=32) involving fundus as the component. Only fundus involvement was noted in 20.6% (n=13) of the cases (Table 6).

Table 5. Tumor Stage

| Tumor Stage | Frequency (n) | Percentage |
|-------------|---------------|------------|
| pT1         | 9             | 14.3%      |
| pT2         | 28            | 44.4%      |
| pT3         | 23            | 36.59%     |
| Cannot be determined | 3          | 4.8%       |

Table 6. Tumor site

| Tumor site                  | Frequency (n) | Percentage |
|-----------------------------|---------------|------------|
| Neck                        | 2             | 3.2%       |
| Body                        | 6             | 9.5%       |
| Fundus                      | 13            | 20.6%      |
| Diffuse disease involving fundus | 32        | 50.8%      |
| Diffuse disease not involving fundus | 8          | 12.7%      |
| Cannot be determined        | 2             | 3.2%       |

Frequency of Her-2 immunohistochemical stain positivity (3+) in patients of gallbladder adenocarcinoma was recorded in 12.7% (n=8) (Figure 1a). While 14.3% (n=9) of the cases showed equivocal (2+) staining pattern (Figure 1b), 42.9% (n=27) were negative as 1+ (Figure 1c) and 30.1% (n=19) of the cases were completely negative and scored 0 (Table 7).

Table 7. Her-2 scoring in GBC

| Score                  | Frequency (n) | Percentage |
|------------------------|---------------|------------|
| Negative (0)           | 19            | 30.1%      |
| Negative (1+)          | 27            | 42.9%      |
| Equivocal (2+)         | 9             | 14.3%      |
| Positive (3+)          | 8             | 12.7%      |

No significant association was noted between Her-2 expression and tumor stage, tumor size, or histologic grade (Table 8).

Table 8. Her-2 expression in histologic grade, tumor stage and tumor size

| Parameter              | Her-2 Score (n) |           |           |           |
|------------------------|-----------------|-----------|-----------|-----------|
|                        | Negative (0)    | Negative (1+) | Equivocal (2+) | Positive (3+) |
| Histologic Grade       |                 |           |           |           |
| Well Differentiated    | 3               | 2         | 0         | 0         |
| Moderately differentiated | 11         | 18        | 7         | 8         |
| Poorly Differentiated  | 5               | 7         | 2         | 0         |
|                        | P=0.372         |           |           |           |
| Tumor Stage            |                 |           |           |           |
| pT1                    | 2               | 3         | 1         | 3         |
| pT2                    | 11              | 11        | 4         | 2         |
| pT3                    | 4               | 12        | 4         | 3         |
|                        | P=0.432         |           |           |           |
| Tumor Size             |                 |           |           |           |
| 0.2-0.5cm              | 4               | 5         | 1         | 3         |
| 2.1-5.0cm              | 9               | 8         | 5         | 4         |
| >5.0cm                 | 3               | 14        | 2         | 1         |
|                        | P=0.103         |           |           |           |

Discussion

Her-2 belongs to the tyrosine kinase group having a well-established role in the pathogenesis of different tumors like breast and gastric cancers. Other tumors with overexpression of this gene include colorectal adenocarcinoma, lung adenocarcinoma, and pancreatic adenocarcinoma (10-14). The pathogenic role of Her-2 in
the development of GBC is not well established in humans. However, a study conducted by Kiguchi et al., demonstrated overexpression in biliary epithelium leading to the development of GBC (19).

The rationale of the study was that only a few studies had been performed worldwide about overexpression of Her-2 gene in gallbladder carcinoma but has never been performed in our country even though Pakistan has a high incidence of gallbladder cancer. The present study was conducted in our population to evaluate the overexpression of Her-2 in gallbladder adenocarcinoma to identify the subset of patients who can get benefit from anti-Her-2 therapy.

A study conducted in India by Ashai et al., showed that Her-2 was strongly positive (3+) in five cases (9.25%) of gallbladder cancer and 14 cases (25.92%) showed moderate positivity (2+), (17). The findings of their study are almost in agreement with our study.

Iván Roa et al., determined the frequency of Her-2 overexpression and to identify a subgroup of patients with gallbladder cancer who would benefit from targeted therapy, they recorded that overexpression (3+ score) of Her-2 was observed in 12.8% of the cases. Among them, 0% were mucosal, 14.3% muscular, 12.8% subserosal, and 10.6% serosal. In the same study, it was suggested that overexpression was more frequent in the advanced cancers and in the better-differentiated tumors (13.8% and 17.4%, respectively). However, the difference was insignificant (15). In the present study, Her-2 overexpression is noted in 3.7% of pT1 tumors, 1.8% pT2 tumors, and 5.5% of pT3 tumors. No significant association of Her-2 overexpression and tumor stage was established. In our study, however, Her-2 overexpression was only seen in moderately differentiated tumors (12.7%, n=8). No significant association was found between tumor stage and Her-2 overexpression.

In order to introduce a new therapeutic option for any particular disease, it is of utmost importance to determine the frequency and role of such a molecular target in the pathogenesis of that disease. The reported prevalence of Her-2 overexpression has varied widely across different studies. It ranges from 9% to 70% in different studies (20,21,22,23). These erratic results can be explained in part due to the lack of any standardized criteria for reporting Her-2 in GBC while also use of altered criteria in different studies. It is also to be noted that in our study, a significant number of cases showed an equivocal (Score 2+) staining pattern, which requires fluorescent in-situ hybridization (FISH) to confirm Her-2 overexpression. Even in our study, the results would have been different if reporting criteria for gastric cancer were used instead. There would have been more clear cut positive (Score 3+) cases. This finding clarifies the wide variation in results noted in different studies.

As of now, there are no consensus criteria for reporting Her-2 overexpression in GBC. It is highly possible that the criteria used for reporting Her-2 in breast and gastric cancer are not applicable in GBC due to many biological and molecular differences (20). It is necessary to develop consensus criteria for reporting Her-2 overexpression in GBC to establish the validity of the above results.

Her-2 is a promising option for targeted therapy because the introduction of anti-Her therapy in gastric and breast cancer has revealed very good results and improved overall survival of patients (24,25). There are very few studies done worldwide to see the effect of anti-Her-2 therapy in GBC. A study conducted by Kiguchi et al., on transgenic mice suggested that targeting both the EGFR and erbB2 (Her-2) may be an effective strategy for the treatment of GBC (26). Another study performed by Pignochino et al., demonstrated that the Her-2 pathway is suitable therapeutic targets for biliary tract cancers (27). In yet another study, Milind Javle et al., are of the view that Her-2 blockade is a promising treatment strategy for gallbladder cancer patients with gene amplification and deserves further exploration in a multi-center study (28).

A phase II clinical trial was performed by the National Cancer Institute, the USA, from May 2007 to November 2011 to determine the efficacy of trastuzumab in GBC. This trial included only four patients and showed poor results as three patients showed progressive disease (29). However, in the above trial, trastuzumab was used as a single agent without any neoadjuvant therapy.

Our study has two major limitations. Firstly, FISH has been not performed on cases showing an equivocal (2+) staining pattern to determine the exact percentage of tumors showing Her-2 gene overexpression. Another major limitation of the study is the limited sample size. Considering the significance of GBC in Pakistan and the potential therapeutic impact of targeted therapy for patients with advanced GBC, a comprehensive assessment of Her-2 overexpression in our population is needed. More clinical trials are also required to determine the efficacy of anti-Her-2 therapy in GBC.

This study indicates that a significant number of GBC cases show Her-2 overexpression. This subgroup may benefit from inhibitors of the Her-2 pathway. Standardization of scoring protocol for Her-2 expression in GBC is needed to better evaluate the predictive potential of Her-2 in the treatment of these tumors.
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