RESEARCH

# TO CORRELATE THE EXPRESSION OF CD97, CD55 & CCK-AR WITH GRADE AND STAGE OF GALLBLADDER CANCER

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Corresponding Author:
**Dr. Sharique Ahmad,**
Email: diagnopath@gmail.com
ORCID: 0000-0002-9637-8838

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**Mohd. Anwar[1], Priya Singh[2], Sachin Kumar[3], Sharique Ahmad[4], Parul Gupta[5], Saba Naziya[6]**

[1]Senior Resident, Department of Transfusion Medicine, SGPGI, Lucknow, Uttar Pradesh, India
[2]Senior Resident, Dr RMLIMS, Uttar Pradesh, India.
[3]Senior Resident, Department of Pulmonary and critical care Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India.
[4]Professor, Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India.
[5]Professor, LNCT Medical College Bhopal Madhya Pradesh. India.
[6]Junior Resident, Department of Pathology, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh. India.

## Abstract

**Background**: India is a high incidence region for gallbladder carcinoma accounts for 9-11% burden of gall bladder carcinoma worldwide. In the recent years, in order to understand the pathogenesis and progression of gall bladder cancer in a better way, emphasis is being laid on assessing its link with various molecular and genetic markers. **Material and Method**: In this case control study we target that on the co-expression of CD97, CD55 and CCK-AR for any diagnostic and prognostic significance in the Gall bladder cancers with Grade and Staging. Expression of CD97, CD55 & CCK-AR with in all non-neoplastic and neoplastic lesions of gall bladder was evaluated and compared. Out of all cases expression of CD97, CD55 & CCK-AR was significantly higher in malignant cases as compared to benign cases. **Results:** Expression, intensity and score of CCK-AR were found in order mucinous adenocarcinoma, papillary adenocarcinoma and adenocarcinoma respectively. **Conclusion:** Out of 56 cases expression of CD97, CD55 & CCK-AR was significantly higher in malignant cases as compared to benign cases. Expression, intensity and score of CCK-AR were found in order mucinous adenocarcinoma, papillary adenocarcinoma and adenocarcinoma respectively.

## INTRODUCTION

Gallbladder(GB) cancer is very aggressive and untreatable neoplasm representing the commonest malignancy of biliary tract.[1] In North India population, it is the 3rd most common malignancy in female with an overall 5-year survival of <5%. No adjuvant chemotherapy is widely accepted due to the toxic effect of drug, resistance and less efficacy and therefore surgical approach is followed for cure. Both environmental and epidemiology factors play a critical role in cancer developing in gallbladder cancer, best illustrate by chronic inflammation and cholelithiasis.[2] In the recent years, in order to understand the pathogenesis and progression of gall bladder cancer in a better way, emphasis is being laid on assessing its link with various molecular and genetic markers. Recently Wu et al.[3] Expression of CD55 and CD97 in primary gallbladder cancer and their prognostic significance in a Chinese population. CD97 is expressed in series of epithelial cancer and also normal tissues. It is a member of epidermal growth factor seven span transmembrane (EGF TM7) families with adhesive properties.[4] CD97 exists in a variety of splice forms, each of which binds CD55 with different affinity. CD97 is expressed in many of human neoplasm like stomach, colon, thyroid and brain.

CD97 expression shows an invasive phenotype and also correlate with tumor grade, metastatic spread, lymph node invasion, and overall prognosis. CD97 have role in tumor invasion, signaling and novel therapeutic target.[5] Similarly, decay accelerating factor (DAF/CD55) is a major negative regulator of the complement cascade expressed by cells to prevent complement mediated destruction and was the first known ligand of CD97. CD55 binds to the small CD97 isoforms and this interaction is calcium dependent and mediated by short consensus repeat (SCR) domain of CD55 and EGF like domain of CD97.[6,7,8,9] Surrounding inflammation leads to upregulation of CD55 expression among epithelial

and endothelial cells. Co- localization of CD97 with CD55 appear to play an important role in malignant state.[10] Regulatory peptide hormones are Cytokeratin and gastrin having a wide range of physiological actions in gastrointestinal tract. Cytokeratin have shown growth stimulation in several neoplasms and their receptors have been found to be expressed in variety of human neoplastic tissues.[11,12] Further, CCK is a gall bladder modulator having motility by activating CCK-AR distributed on gall bladder smooth muscle cells and abnormal processing of the CCK-AR gene is associated with the gall bladder lesions.[13,14] A search of biological markers associated with advanced stage of tumor progression is necessary for early diagnosis and a discovery of a therapeutic target. Hence, in present study we target that on the co-expression of CD97, CD55 and CCK-AR for any diagnostic and prognostic significance in the GB cancers.

**Aim**

To evaluate and compare the expression of CD97, CD55 & CCK-AR in non-neoplastic and neoplastic lesions of gall bladder cancer.

The expression of these markers was compared and correlated with respect to grade and stage of cancer.

## MATERIALS AND METHODS

A case control study with total sample size of 56 patients which included cholecystectomy specimens received in the pathology department Era's Lucknow Medical College and Hospital, Lucknow. It comprised 28 cases of gallbladder adenocarcinoma and 28 randomly selected cases of cholelithiasis associated chronic cholecystitis. Tissue sections were fixed in 10% buffered formalin and embedded in paraffin wax and retrieved from our archives, re-cut, stained with hematoxylin & eosin and reviewed by 3 pathologists using a light microscope. In gallbladder cancer cases, tumor differentiation was assessed. Tumors were divided into 3 groups regarding differentiation (well, moderate and poor). The immunohistochemistry was applied for CD97, CD55, CCK-AR on formalin fixed paraffin embedded tissues. The results were evaluated according to the intensity of staining pattern of the scoring system used for breast cancer, as there is no standard scoring system for gall bladder cancer. Intensity of staining was graded as, Grade 0- Negative, Grade 1- Weak, Grade 2-Moderate, Grade 3 -Strong. The percentage of cells showing staining was graded as: -None - 0, Grade 1 - <1%, Grade 2 -1%.

10%, Grade 3 -11%-33%, Grade 4 -34%- 66%, Grade 5 - >66%. Total staining score was calculated by adding the intensity score and the percentage score as Negative-0, Weak = + (2), Moderate=2+ (3-5), Strong=3+ (6-8).

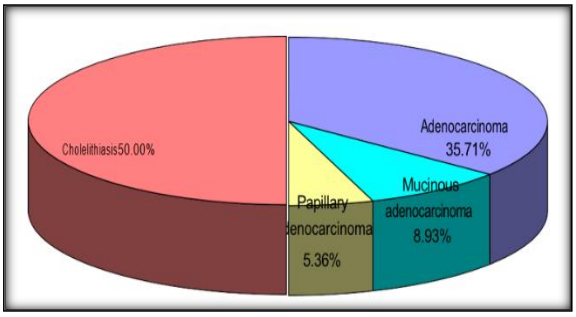
## RESULTS

Expression of CD97, CD55 & CCK-AR within 56 non-neoplastic and neoplastic lesions of gall bladder was evaluated and compared.

[Table 1] shows the histopathological diagnosis of study population.

**Table 1: Histopathological Diagnosis of Study Population**

| Diagnosis | No. of cases | Percentage |
|---|---|---|
| Cholelithiasis | 28 | 50.00 |
| Adenocarcinoma | 20 | 35.71 |
| Mucinous adenocarcinoma | 5 | 8.93 |
| Papillary adenocarcinoma | 3 | 5.36 |




Out of 56 cases of gall bladder lesions, histopathological diagnosis of 28 (50.0%) was Cholelithiasis, only 3 (5.36%) patients were diagnosed as Papillary adenocarcinoma, 5 (8.93%) as mucinous adenocarcinoma and rest 20 (35.71%) as adenocarcinoma. All the cases of cholelithiasis were classified as Benign lesions (50.0%) and rest of the cases were classified as Malignant lesions (50.0%). Age of patients in the present study ranged between 21 and 70 years, median age of patients was 42 years and mean age of patients was 42.45±14.56 years. In the present study age of 50.0% patients was up to 40 years and of rest 50.0% were aged above 40 years. All the patients with benign lesions were lower aged i.e. 21-30 years (64.28%) and 31-40 years (35.72%) while all the patients with malignant lesions were aged above 41 years and this was found to be statistically significant ($p<0.001$). Out of 56 cases of gall bladder lesions, 47 (83.93%) were females and rest 9 (16.07%) were males. Male to female ratio in the present study was 0.19. Though proportion of males was higher among benign (21.43%) as compared to malignant (10.71%) but this difference was not significant. All the patients of gall bladder lesions were subjected to USG investigation, Cholelithiasis was seen in majority of the patients (n=46; 82.14%) cases. All the benign cases had cholelithiasis whereas a total of 10 out of 28 (35.72%) cases with malignancy did not have cholelithiasis. Statistically, this difference was significant ($p<0.001$).


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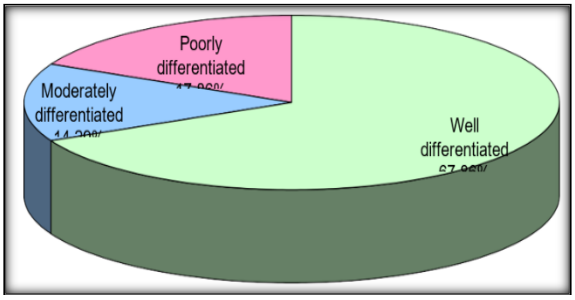
**Table 2: Histopathological Grade of Malignant Cases (n=28)**

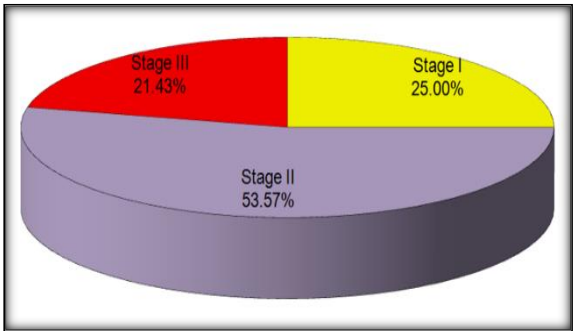
| Grade | No. of cases | Percentage |
|---|---|---|
| Well differentiated | 19 | 67.86 |
| Moderately differentiated | 4 | 14.29 |
| Poorly differentiated | 5 | 17.86 |




Out of 28 malignant cases, histopathological grade of 19 (67.86%) cases was Well differentiated, of 5 (17.86%) cases was Poorly differentiated and rest of the cases were diagnosed as Moderately differentiated (n=4; 14.29%).

**Table 3: Stage wise distribution of Malignant Cases (n=28)**

| Stage | No. of cases | Percentage |
|---|---|---|
| Stage I | 7 | 25.0 |
| Stage II | 15 | 53.6 |
| Stage III | 6 | 21.4 |




Majority of patients were Stage II (n=15; 53.6%), followed by Stage I (25.0%) while least common stage was Stage III (n=7; 24.1%). Difference in CD97 expression among malignant (82.86%) and benign lesions (82.14%) was not found to be statistically significant. Mean Expression of CD97 was found to be significantly higher among cases with malignant lesion (2.61±1.03) as compared to benign lesions (1.50±0.97). Mean Intensity of CD97 was found to be significantly higher among cases with malignant lesion (1.89±0.79) as compared to benign lesions (1.18±0.72). Mean Score of CD97 was found to be significantly higher among cases with malignant lesion (5.36±2.88) as compared to benign lesions (2.11±1.45). Based on the direction of assessment, CD97 total score was evaluated for prediction of malignancy of gall bladder lesion at a cut-off with a larger value indicating positive result. Area under curve findings were 0.851 (indicating a projected accuracy of 85.1%) for CD97 total score. On evaluating CD97 total score, a cut off value ≥3.50 was predicted to be 78.6% sensitive and 75.0% specific. Cut- off value of CD97 total score with high sensitivity was >1.50 which was 92.9% sensitive and 35.7% specific while at a cut off value with high specificity was ≥5.00 which was 46.4% sensitive but 100.0% specific. Difference in CD55 expression among malignant (82.86%) and benign lesions (82.14%) was not found to be statistically significant. Mean expression of CD55 was found to be significantly higher among cases with malignant lesion (2.57±1.07) as compared to benign lesions (1.50±0.96). Mean Intensity of CD55 was found to be significantly higher among cases with malignant lesion (1.93±0.77) as compared to benign lesions (1.18±0.72). Mean Score of CD55 was found to be significantly higher among cases with malignant lesion (5.36±2.88) as compared to benign lesions (2.11±1.45). Based on the direction of assessment, CD55 total score was evaluated for prediction of malignancy of gall bladder lesion at a cut-off with a larger value indicating positive result. Area under curve findings were 0.851 (indicating a projected accuracy of 85.1%) for CD55 total score. On evaluating CD55 total score, a cut off value ≥3.50 was predicted to be 78.6% sensitive and 75.0% specific. Cut- off value of CD55 total score with high sensitivity was >1.50 which was 92.9% sensitive and 35.7% specific while at a cut off value with high specificity was ≥5.50 which was 46.4% sensitive but 100.0% specific. CCK-AR expression among malignant cases (85.71%) was significantly higher as compared to Benign cases (39.29%). Mean expression of CCK- AR was found to be significantly higher among cases with malignant lesion (2.54±1.40) as compared to benign lesions (0.32±0.46). Mean Intensity of CCK-AR was found to be significantly higher among cases with malignant lesion (1.96±1.03) as compared to benign lesions (0.32±0.46). Mean Score of CCK-AR was found to be significantly higher among cases with malignant lesion (4.50±2.30) as compared to benign lesions (0.64±0.91). Based on the direction of assessment, CCK- AR total score was evaluated for prediction of malignancy of gall bladder lesion at a cut-off with a larger value indicating positive result. Area under curve findings were 0.895 (indicating a projected accuracy of 89.5%) for CCK-AR total score. On evaluating CCK-AR total score, a cut off value ≥2.50 was predicted to be 82.1% sensitive and 96.4% specific. Cut-off value of CCK-AR total score with high sensitivity was >1.50 which was 85.7% sensitive and 78.6% specific while at a cut off value with high specificity was ≥3.50 which was 75.0% sensitive but 100.0% specific.


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**Table 4: Comparison of CD55, CD97 and CCK-AR Total Scores for different carcinoma types**

| | Adeno- carcinoma (n=20) | | Mucinous adeno-carcinoma (n=5) | | Papillary adeno-carcinoma (n=3) | | Kruskal Wallis Test | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | H | 'p' |
| CD97 | | | | | | | | |
| Expression | 2.65 | 1.18 | 2.40 | 0.55 | 2.67 | 0.58 | 0.815 | 0.665 |
| Intensity | 1.95 | 0.83 | 2.00 | 0.71 | 1.33 | 0.58 | 2.735 | 0.255 |
| Score | 5.85 | 3.22 | 4.60 | 1.34 | 3.33 | 0.58 | 4.232 | 0.121 |
| CD55 | | | | | | | | |
| Expression | 2.60 | 1.23 | 2.80 | 0.45 | 2.00 | 0.00 | 2.381 | 0.304 |
| Intensity | 2.00 | 0.79 | 1.60 | 0.89 | 2.00 | 0.00 | 2.030 | 0.362 |
| Score | 5.85 | 3.22 | 4.20 | 1.64 | 4.00 | 0.00 | 3.339 | 0.188 |
| CCK-AR | | | | | | | | |
| Expression | 2.15 | 1.42 | 3.80 | 0.45 | 3.00 | 1.00 | 7.245 | 0.027 |
| Intensity | 1.60 | 0.99 | 3.00 | 0.00 | 2.67 | 0.58 | 11.362 | 0.003 |
| Score | 3.75 | 2.27 | 6.80 | 0.45 | 5.67 | 1.15 | 10.873 | 0.004 |

Among different markers, CCK-AR was found to have a significant association with different carcinoma types, with mean values of expression scores, Intensity scores and total scores being in order mucinous adenocarcinoma followed by papillary adenocarcinoma and adenocarcinoma respectively.

**Table 5: Comparison of CD55, CD97 and CCK-AR Total scores for different histopathological grades**

| | Well differentiate d (n=19) | | Moderately differentiate d (n=4) | | Poorly differentiate d (n=5) | | Kruskal Wallis Test | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | H | 'p' |
| CD97 | | | | | | | | |
| Expression | 2.16 | 0.90 | 3.50 | 0.58 | 3.60 | 0.55 | 13.941 | 0.001 |
| Intensity | 1.68 | 0.82 | 2.00 | 0.00 | 2.60 | 0.55 | 6.292 | 0.043 |
| Score | 3.95 | 1.87 | 7.00 | 1.15 | 9.40 | 2.61 | 15.369 | <0.001 |
| CD55 | | | | | | | | |
| Expression | 2.11 | 0.94 | 3.50 | 0.58 | 3.60 | 0.55 | 13.750 | 0.001 |
| Intensity | 1.74 | 0.81 | 2.00 | 0.00 | 2.60 | 0.55 | 6.104 | 0.047 |
| Score | 3.95 | 1.87 | 7.00 | 1.15 | 9.40 | 2.61 | 15.369 | <0.001 |
| CCK-AR | | | | | | | | |
| Expressio n | 2.89 | 1.41 | 1.50 | 1.29 | 2.00 | 2.89 | 5.568 | 0.062 |
| Intensity | 2.00 | 1.11 | 1.50 | 1.00 | 2.20 | 2.00 | 1.357 | 0.507 |
| Score | 4.89 | 2.40 | 3.00 | 2.16 | 4.20 | 4.89 | 3.853 | 0.146 |

With increasing grade there was a significant increase in expression of CD97 and CD55 markers but not for CCK-AR.

**Table 6: Comparison of Sensitivity and Specificity of different markers for malignancy (Based on Positive Expression)**

| Marker | TP | FP | FN | TN | Sens | Spec | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| CD97 | 26 | 2 | 23 | 5 | 92.9 | 17.9 | 53.1 | 71.4 |
| CD55 | 26 | 2 | 23 | 5 | 92.9 | 17.9 | 53.1 | 71.4 |
| CCK-AR | 24 | 4 | 11 | 17 | 85.7 | 60.7 | 68.6 | 81.0 |

CD97 vs CD55: $\chi^2$=0.000; p=1.000(NS) CD97 vs CCK-AR: $\chi^2$=11.5; p=0.009(Sig) CCK-AR vs CD55: $\chi^2$=11.5; p=0.009(Sig)

CD97 and CD55 had higher sensitivity as compared to CCK-AR while CCK-AR had higher specificity as compared to CD-97 and CD-55 and the difference among these was significant statistically.

**Table 7: Comparison of CD55, CD97 and CCK-AR Total scores for different histopathological grades**

| | Stage I (n=7) | | Stage II (n=15) | | Stage III (n=7) | | Kruskal Wallis Test | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | H | 'p' |
| CD97 | | | | | | | | |
| Expression | 2.29 | 0.49 | 2.40 | 1.18 | 3.50 | 0.55 | 7.794 | 0.020 |
| Intensity | 1.71 | 0.76 | 1.73 | 0.80 | 2.50 | 0.55 | 5.413 | 0.067 |
| Score | 3.71 | 1.25 | 4.73 | 2.40 | 8.83 | 2.71 | 11.405 | 0.003 |
| CD55 | | | | | | | | |
| Expression | 2.29 | 0.49 | 2.27 | 1.16 | 3.67 | 0.52 | 10.027 | 0.007 |
| Intensity | 2.00 | 0.58 | 1.67 | 0.82 | 2.50 | 0.55 | 5.736 | 0.057 |
| Score | 4.43 | 1.13 | 4.27 | 2.34 | 9.17 | 2.40 | 11.897 | 0.003 |
| CCK-AR | | | | | | | | |
| Expression | 2.71 | 1.38 | 2.60 | 1.59 | 2.17 | 0.98 | 1.288 | 0.525 |
| Intensity | 2.00 | 1.00 | 1.87 | 1.19 | 2.17 | 0.75 | 0.097 | 0.953 |
| Score | 4.71 | 2.21 | 4.47 | 2.67 | 4.33 | 1.63 | 0.430 | 0.807 |

With increasing stage there was a significant increase in expression and score of CD97 and CD55 markers but not for CCK-AR.


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## DISCUSSION

One of the reasons for poor outcome related with gall bladder carcinoma is the fact that it is diagnosed at an advanced stage. The commonly performed histopathological evaluation is a cumbersome task and has a limited prognostic value. Keeping in view of the emerging role of biological markers in evaluation of gall bladder carcinoma, the present study was planned to study the expression of CD97, CD55 and CCK-AR with grade and stage of gall bladder cancer in order to mainly evaluate its role as a diagnostic and prognostic marker. For this purpose, a study was carried out in which a total of 56 cases (28 cholelithiasis and 28 gall bladder carcinoma) were enrolled. The sampling was done using a purposive sampling design as incidence of gall bladder carcinoma is quite low ranging from 0.5-1.5% cases undergoing cholecystectomy,[15] and hence it was difficult get the adequate number of gall bladder carcinoma cases in a cross-sectional or prospective evaluation. In present study, all the cases of gall bladder carcinoma were adenocarcinoma. These findings are in consonance with the epidemiological studies that show that adenocarcinoma is the major type of gall bladder cancer found in nearly 75-85% of total gall bladder cancer cases.[16] Among different subtypes of adenocarcinoma non-papillary adenocarcinoma is most common in present study to 20/28 (71.4%) of cases were non-papillary adenocarcinoma while 5/28 (17.9%) were mucinous adenocarcinoma and 3/28 (10.7%) were papillary adenocarcinoma. With respect to expression of different biological markers, expression of CD97 was seen in 82.14% of benign and 92.86% of malignant cases. Statistically, this difference was not significant. Compared to this, Wu et al.3 and Meng et al. (2017),[17] both absence of expression or a weak expression were treated at par as absence of CD97 expression. In present study, in order to increase the sensitivity of CD97, weak expression was also categorically accepted as expression, however, the overall discriminant classification of expression was done on the basis of combined quick score derived as product of expression and intensity and through receiver operator characteristic curve analysis derived a cut-off value >3.50 i.e. 3 to be 78.6% sensitive and 75% specific in differentiating malignant from benign gall bladder disease. The findings of present study are promising from the point of view that they were able to transform the non- discriminant role of CD97 into a discriminant one.[18] However, the method of CD97 expression used in present study is being employed for the first time and hence validation of same is essential.

In present study, the role of CD55 marker was also evaluated in terms of categorical expression, expression scores, intensity and combined scores. As far as categorical expression was concerned, it failed to discriminate between benign and malignant gall bladder diseases, however, expression scores, intensity and combined scores of malignant cases were found to be significantly higher as compared to that in benign cases. In present study, CD55 expression was seen in 82.14% of benign and 92.86% of malignant cases. Compared to this Wu et al.[3] in their study evaluated only malignant cases and found the CD55 expression in 90/138 (65.2%) cases. However, the criteria for positive expression was relatively strict in their study, comparable to quick score 4 or above. Meng et al,[17] (2017) too in their study found CD55 expression in 70.4% of cholangiocarcinoma patients. A comparison of CD55 expression between benign and malignant cases was observed in endometrium tissue by Murray et al,[19] who assessed the expression of CD55 in terms of optical density in quantitative terms and found a significant difference between malignant and benign lesions. In present study too, using a semi quantitative approach we were able to differentiate between malignant and benign lesions. Subsequently, in order to derive a cut-off value for discrimination between malignant and benign lesions with the help of CD55 expression scores (quick scores) we performed a receiver operator characteristic curve analysis and derived an area under curve value of 0.851. The analysis provided a cut-off value of >3.50 (>3) which showed to have a projected sensitivity and specificity of 78.6% and 75% respectively. The Area under curve value derived for CD97 and CD55 showed a similar trend, thus showing that the two markers are generally complementary. CD55 is identified as a ligand to CD976 and binding of CD97 to its ligand might be responsible for development of cancer. Meng et al,[17] in their study showed coexpression of CD55 and CD97 in 47/71 (66.2%) of their malignant cases. In their study, independent expression of CD97 and CD55 was seen in 7/71 (9.9%) and 3/71 (4.2%) cases only.

In present study we did not perform any such analysis as we had performed a semi quantitative analysis instead of a qualitative assessment performed by Meng et al,[17] however the close area under curve values and projected sensitivity and specificity values of CD97 and CD55 the possibility of coexpression cannot be ruled out. CCK is a potent modulator of gall bladder motility by activating CCK-AR distributed on gall bladder smooth muscle cells and abnormal processing of the CCK-AR gene is associated with the gall bladder lesions.[20] In present study, CCK-AR expression was seen in 39.29% of cholelithiasis (benign) cases as compared to 85.71% of gall bladder cancer (malignant) cases. Similar to present study, Rai et al,[21] too compared CCK-AR expression between gallstone disease and gall bladder cancer patients and found CCK-AR expression in 44.1% of gall stone disease and 76.6% of gall bladder cancer patients, thereby showing a significant difference between two.[22,23,24] Xu et al,[25] in their study also showed that CCK polymorphism increases the gall bladder susceptibility. Kazmi et al,[22] in their study showed


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that the CCK-AR expression is affected by the age and aggressiveness of gall bladder disease. Unfortunately, there are limited studies on this biomarker using the evaluation method similar to ours. However, its potential in causation and progression of gall bladder cancer is accepted by a number of workers.[17,21,22,23] In one such study evaluating expression of CCK-AR between normal, gallstone and gall bladder cancer disease, the expression levels were found to be 62.5%, 86.6% and 52.6% respectively for normal, gallstone and gall bladder cancer respectively.[23] Thus showing higher expression in gallstone disease as compared to that in malignant disease, as observed in present study.

In present study, on performing ROC analysis, we obtained area under curve value of 0.895 and at projected cut-off for quick score >2.50(>2) the projected sensitivity and specificity of CCK-AR in distinguishing malignant from the benign cases was 82.1% and 96.4% respectively. This finding shows that CCK-AR probably has a higher utility in distinguishing benign from malignant cases. Most of the literature on CD97, CD55 and CCK-AR biomarkers has revolved around their prognostic value and not as a diagnostic marker and these assessments were mainly limited to assessment within the malignant group only. In present study too, we studied the association of these biomarkers for different carcinoma types, histological grades and clinical stages. When evaluating the usefulness of three biomarkers under study (CD97, CD55 and CCK-AR) for differentiation among different carcinoma types, we found that only CCK-AR expression, intensity and quick scores had a significant difference in mean values among different types of carcinoma. It was seen that mean values were maximum for mucinous adenocarcinoma and minimum for non-papillary adenocarcinoma. However, CD97 and CD55 did not show any such association. On evaluating the literature, we did not find any study exploring and reporting any such association, however, given the higher potential of mucinous adenocarcinoma to metastasize, the increased levels of CCK-AR in mucinous carcinoma cannot be ruled out. With respect to association with histological grades, in present study, both CD97 and CD55 showed a significant increase in expression, intensity and quick scores when evaluating them in well differentiated, moderately differentiated and poorly differentiated grades. A similar association between CD97 and CD55 expression with histological grade has also been reported by Meng et al,[17] who reported CD97 expression to be 28.6%, 77.3% and 90.0% respectively in histological grade 1, 2 and 3 respectively and CD55 expression to be 28.6%, 65.9% and 95.0% respectively for the corresponding histopathological grades. In another study Wu et al,[3] also showed a significant increasing expression of both CD97 and CD55 with the increasing histopathological grade. In present study, although we could not find a significant difference in mean expression, intensity and quick scores of CCK-AR, however, Rai et al,[21] in their study found that CCK-AR levels in poorly differentiated form were significantly lower as compared to that in moderately differentiated cases. However, on evaluating the association of expression of three biomarkers being studied with the clinical stage, the only significant association was seen for CD97 expression and quick scores only which showed a significantly increase with increasing clinical stage of cancer. On the other hand, Meng et al,[17] in their study did not evaluate the association of CD97 and CD55 with clinical stage, however, on evaluation this association with tumor size they did not find a significant association between tumor size and CD97 or CD55 expression. However, Wu et al,[3] in their study similar to our study also reported a significant association between increased expression of CD97 and CD55 with increasing stage of cancer. In present study, although we could not derive a significant association between clinical stage and CCK-AR expression, however, Rai et al,[21] in their study reported a significant difference in CCK-AR expression between TNM stage II and III but failed to find out significant difference between stage II and IV and III and IV.

There were three major limitations in present study, first was evaluation of association of CD97, CD55 and CCK-AR biomarkers between benign and malignant group. In fact, diagnostic role of these markers is not much discussed rather their prognostic role or association with clinicopathological variables predictive of outcome has been evaluated so far. Hence, we did not have ample evidence to compare the results of present study with earlier studies with respect to discriminant role of CD97, CD55 and CCK-AR in distinguishing benign from malignant gall bladder carcinoma cases. Secondly, the sample size for gall bladder cancer group was too small, owing to which assessments with clinical stage, histopathological grade and carcinoma type, though performed, could not provide exactly comparable results as depicted in previous studies owing to fewer number of cases for each grade, stage and cancer type. Moreover, we have limited number of clinical variables available. The third major limitation was availability of limited data depictive of prognosis and clinicopathological profile of patients. In present study we did not have data related with lymph node involvement and other relevant parameters. In view of these limitations further studies should be conducted on a larger sample size with inclusion of more variables and survival as an outcome.

## CONCLUSION

Out of 56 cases expression of CD97, CD55 & CCK-AR was significantly higher in malignant cases as compared to benign cases. Expression, intensity and score of CCK-AR were found in order mucinous adenocarcinoma, papillary adenocarcinoma and adenocarcinoma respectively. This association was supported statistically. Sensitivity, specificity, PPV,


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NPV of CD97 and CD55 were found to be similar, while sensitivity of CD97 and CD55 was higher as compared to CCK-AR and specificity. PPV and NPV of CCK-AR was higher as compared to CD97 and CD55. With increasing grade and stage there was a significant increase in expression of CD97 and CD55 markers but not for CCK-AR.

## REFERENCES


1. Piehler JM, Crichlow RW. Primary carcinoma of the gallbladder. Surg Gynecol Obstet. 1978;147(6):929-42.
2. Rakić M, Patrlj L, Kopljar M, Kliček R, Kolovrat M, Loncar B, et al. Gallbladder cancer. Hepatobiliary Surg Nutr. 2014;3(5):221-6. doi: 10.3978/j.issn.2304-3881.2014.09.03.
3. Wu LC, Chen LT, Tsai YJ, Lin CM, Lin CY, Tian YF, et al. Alpha-methylacyl coenzyme A racemase overexpression in gallbladder carcinoma confers an independent prognostic indicator. J Clin Pathol. 2012;65(4):309-14. doi: 10.1136/jclinpath-2011-200489.
4. Kwakkenbos MJ, Kop EN, Stacey M, Matmati M, Gordon S, Lin HH, et al. The EGF-TM7 family: a postgenomic view. Immunogenetics. 2004;55(10):655-66. doi: 10.1007/s00251-003-0625-2.
5. Safaee M, Clark AJ, Ivan ME, Oh MC, Bloch O, Sun MZ, et al. CD97 is a multifunctional leukocyte receptor with distinct roles in human cancers (Review). Int J Oncol. 2013;43(5):1343-50. doi: 10.3892/ijo.2013.2075.
6. Hamann J, Vogel B, van Schijndel GM, van Lier RA. The seven-span transmembrane receptor CD97 has a cellular ligand (CD55, DAF). J Exp Med. 1996;184(3):1185-9. doi: 10.1084/jem.184.3.1185.
7. Liu J, Miwa T, Hilliard B, Chen Y, Lambris JD, Wells AD, et al. The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. J Exp Med. 2005;201(4):567-77. doi: 10.1084/jem.20040863.
8. Niu M, Xu S, Yang J, Yao D, Li N, Yan J, Zhong G, Song G. Structural basis for CD97 recognition of the decay-accelerating factor CD55 suggests mechanosensitive activation of adhesion GPCRs. J Biol Chem. 2021;296:100776. doi: 10.1016/j.jbc.2021.100776.
9. Lublin DM, Atkinson JP. Decay-accelerating factor: biochemistry, molecular biology, and function. Annu Rev Immunol. 1989;7:35-58. doi: 10.1146/annurev.iy.07.040189.000343.
10. Li L, Spendlove I, Morgan J, Durrant LG. CD55 is over-expressed in the tumour environment. Br J Cancer. 2001;84(1):80-6. doi: 10.1054/bjoc.2000.1570.
11. Thomas RP, Hellmich MR, Townsend CM Jr, Evers BM. Role of gastrointestinal hormones in the proliferation of normal and neoplastic tissues. Endocr Rev. 2003;24(5):571-99. doi: 10.1210/er.2002-0028.
12. Baldwin GS, Shulkes A. CCK receptors and cancer. Curr Top Med Chem. 2007;7(12):1232-8. doi: 10.2174/156802607780960492.
13. Ivy AC, Oldberg A. A hormone mechanism for gallbladder contraction and evacuation. Am J Physiol 1928; 86: 599-613.
14. Miller LJ, Holicky EL, Ulrich CD, Wieben ED. Abnormal processing of the human cholecystokinin receptor gene in association with gallstones and obesity. Gastroenterology. 1995;109(4):1375-80. doi: 10.1016/0016-5085(95)90601-0.
15. Lai CH, Lau WY. Gallbladder cancer--a comprehensive review. Surgeon. 2008;6(2):101-10. doi: 10.1016/s1479-666x(08)80073-x.
16. Brandt-Rauf PW, Pincus M, Adelson S. Cancer of the gallbladder: a review of forty-three cases. Hum Pathol. 1982;13(1):48-53. doi: 10.1016/s0046-8177(82)80138-x.
17. Meng ZW, Liu MC, Hong HJ, Du Q, Chen YL. Expression and prognostic value of soluble CD97 and its ligand CD55 in intrahepatic cholangiocarcinoma. Tumour Biol. 2017;39(3):1010428317694319. doi: 10.1177/1010428317694319.
18. Nabhan F, Ringel MD. Thyroid nodules and cancer management guidelines: comparisons and controversies. Endocr Relat Cancer. 2017;24(2):R13-R26. doi: 10.1530/ERC-16-0432.
19. Murray KP, Mathure S, Kaul R, Khan S, Carson LF, Twiggs LB, et al. Expression of complement regulatory proteins-CD 35, CD 46, CD 55, and CD 59-in benign and malignant endometrial tissue. Gynecol Oncol. 2000;76(2):176-82. doi: 10.1006/gyno.1999.5614.
20. Miller LJ, Holicky EL, Ulrich CD, Wieben ED. Abnormal processing of the human cholecystokinin receptor gene in association with gallstones and obesity. Gastroenterology. 1995;109(4):1375-80. doi: 10.1016/0016-5085(95)90601-0.
21. Rai R, Tewari M, Kumar M, Singh TB, Shukla HS. Expression profile of cholecystokinin type-A receptor in gallbladder cancer and gallstone disease. Hepatobiliary Pancreat Dis Int. 2011;10(4):408-14. doi: 10.1016/s1499-3872(11)60069-6.
22. Kazmi HR, Chandra A, Baghel K, Singh A, Nigam J, Parmar D, et al. Differential expression of cholecystokinin A receptor in gallbladder cancer in the young and elderly suggests two subsets of the same disease? Biomed Res Int. 2014;2014:625695. doi: 10.1155/2014/625695.
23. Faridi MS, Jaiswal MS, Goel SK. Expression of CCK Receptors in Carcinoma Gallbladder and Cholelithiasis: A Pilot Study. J Clin Diagn Res. 2015;9(7):PC04-7. doi: 10.7860/JCDR/2015/12697.6152.
24. Rustagi T, Dasanu CA. Risk factors for gallbladder cancer and cholangiocarcinoma: similarities, differences and updates. J Gastrointest Cancer. 2012;43(2):137-47.
25. Xu HL, Hsing AW, Vogtmann E, Chu LW, Cheng JR, Gao J, et al. Variants in CCK and CCKAR genes to susceptibility to biliary tract cancers and stones: a population-based study in Shanghai, China. J Gastroenterol Hepatol. 2013;28(9):1476-81. doi: 10.1111/jgh.12278.




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