CCKAR expression in the neoplastic and non-neoplastic gall bladder lesion-A comparative study

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***Abstract*- Gall bladder carcinoma is considered as the fifth most common gastro-intestinal neoplasm and most common biliary tract malignancy in world. Spread of gall bladder carcinoma (GBC) to the liver parenchyma and the adjacent internal organs is due to lack of serosa in gall bladder wall and cholecystic veins draining into liver portal vein. Also, Incidence of Gall bladder cancer shows striking geographical predilections, with highest figures found in India and Chile and relatively low level seen in many western countries. Regulatory peptide receptors have now attracted interest of oncologists as a new promising approach for cancer pathology, imaging and therapy. CCKAR encodes a G-protein-coupled receptor that binds cholecystokinin (CCK) family of peptide hormones and also is a major physiological mediator of pancreatic growth and enzyme secretion, smooth muscle contraction of the gallbladder and stomach. Although cholecystokinin (CCK) is a potent modulator of gallbladder contractility and plays a potential role in pancreatic carcinogenesis through CCK type-A receptor (CCKAR), its role in gallbladder cancer (GBC) is still unknown and immunohistochemical detection of CCKAR in the gallbladder has been less evaluated. The aim of the study is to compare the expression of CCKAR in neoplastic and non-neoplastic lesions of gall bladder. This case-control study included 100 samples: 50 from GBC and 50 from Chronic cholecystitis. The expression of CCKAR was analysed by immunohistochemistry. There was a significant difference in CCKAR expression between chronic cholecystitis and GBC. So, it has future prognostic and therapeutic implications in the management of GBC**

***Keywords*- CCKAR, Carcinoma gall bladder**

INTRODUCTION

Gall bladder carcinoma is considered as the fifth most common gastrointestinal neoplasm and the most common biliary tract malignancy in the world. [1] As clinical symptoms of carcinoma can be non-specific, the diagnosis is usually late, which results in a poor prognosis. Spread of gall bladder carcinoma (GBC) to the liver parenchyma and the adjacent internal organs is due to a lack of serosa in gall bladder wall, cholecystic veins draining into liver portal vein, and lymphatics from GB draining into the liver. [2] Gallbladder carcinoma is highly lethal, as anatomic factors promote early local spread. The tumour spreads easily, and invades the liver and surrounding structures including the biliary tree contributing to its high mortality. The median survival is 6 months, indicating that the majority of patients present with advanced disease. Despite the widespread use of modern imaging techniques, early diagnosis is rare because there are no specific signs and symptoms, and many gallbladder carcinomas are not diagnosed preoperatively.

Gallbladder cancer shows a three folds higher incidence in females, [3] and also shows striking geographical predilections in its incidence. Highest figures are found in India and Chile but a relatively low level is seen in many of western countries. [4] There is a high incidence of in northern India, along the Gangetic belt, [5] for this tumour. Also, incidence of carcinoma gallbladder varies in different geographic regions and racial and ethnic groups. In India, it is the most common form of biliary malignancy and fifth most common gastrointestinal carcinoma in women. [6-8] and third most common carcinoma of the digestive tract in Eastern UP and Western Bihar.

Cholelithiasis, especially untreated chronic symptomatic gallstones, with inflammation is considered as one of the main risk factors of gallbladder cancer. Most of gallbladder carcinomas have regional disease or distant metastases at presentation with poor prognosis.

Cholecystokinin (CCK) and gastrin families of peptides act as hormones and neuropeptides on central and peripheral CCK receptors to mediate secretion and motility in GIT in the physiological response to a normal meal. CCK-A receptor, found predominantly in the GI system and selective areas of the CNS, have a high affinity for CCK and the non-peptide antagonist L-364,718. The physiological functions of gall bladder are done through different receptors. The main neurohormonal mechanisms regulating the motility of the gallbladder are the vagus and splanchnic nerves and the hormone CCK. The subtypes of receptors for CCK in the human pancreas and gallbladder are different. The human pancreas predominantly expresses CCK-B receptors, whereas CCK-A receptors are localized in the human gallbladder muscle. [9] It is now a known fact that the gallbladder has a high concentration of CCK-A receptor. [10] Although, the CCK-A receptor does not modulate the susceptibility of cancer gallbladder.[11] But their role

in gallbladder malignancy and other gallbladder lesions still remains undecided. Molecular studies in high-incidence areas, and in subsets of high-risk gallbladder disease patients, may help us to predict the possibility of gall stone disease developing into severity and increasing ailments of patients through social, economic, financial and emotional stigma. This may show for measures to be taken in developing new therapeutic strategies or screening for developing gall bladder pathologies at the earliest.
The aim of the study was to evaluate and compare the expression of the CCK-A receptor in non-neoplastic and neoplastic lesions of gall bladder.

MATERIALS AND METHODOLOGY

Patients

This case-control study included patients undergoing surgery for gall bladder carcinoma and gall stone disease from November 2021 to December 2022 in the Department of Pathology in Rajkiya (Government) Medical College, Jalaun. The sample size was calculated as 50 for each group. The study population comprised of patients undergoing a surgical procedure for gall bladder diseases either through open or laparoscopic procedures. Specimens were subjected to routine histopathological processing, and diagnosed cases of adenocarcinoma of the gall bladder were taken. Non-neoplastic cases which included chronic cholecystitis with or without cholelithiasis were taken as control. Patients with double malignancy, immunodeficiency diseases or any other associated chronic debilitating disorder which is likely to interfere with detection of marker were excluded.

Immunohistochemistry

3-4 µm sections from paraffin-embedded blocks were cut and placed on polylysine-coated slides and used for immunohistochemical staining. The primary antibody and a secondary kit used for the detection of CCK-A receptor (CCK-A receptor (H-60) antibody; sc- 33220) were from Santa Cruz, and the Super Sensitive Link-Lable IHC Detection System (QD000-5L) was from BiogGenex, San Ramon, CA, USA.
Briefly, all sections were dewaxed and rehydrated in xylene and graded alcohol, and placed under slow-running tap water for 15 minutes followed by citrate buffer (pH 6.0) retrieval by the microwave method. The sections were allowed to cool at room temperature and washed 3 times with Tris buffer (TBS, pH 7.6). Then they were incubated with peroxidase block for 20 minutes to check internal peroxidase activity. After washing with TBS, these sections were incubated with power block for 15 minutes to block nonspecific staining. The excess power block was removed and then the sections were immediately incubated with primary antibody at 1:200 dilution in TBS overnight at 4°C. After washing with TBS, the sections were incubated with multilink for 30 minutes and washed with TBS followed by secondary antibody incubation again for 30 minutes. After washing, colour was developed using diaminobenzidine (DAB) as the chromogen. Finally, slides were washed and counterstained with Harris hematoxylin. For positive control, a section known to stain positively on the gallbladder muscle layer component was included in each batch of staining, and for negative control primary antibody was replaced with TBS. Evaluation of IHC staining pattern. The results were evaluated qualitatively, with staining pattern as positive or negative.

RESULTS

Out of 100 subjects enrolled in the study, a total of 50 neoplastic were taken as cases and whereas the remaining 50 were non-neoplastic controls. Among neoplastic cases, all adenocarcinomas were taken and non-neoplastic chronic cholecystitis was taken. The age of patients ranged from 30 to 70 years. CCKAR expression, in neoplastic cases, by immunostaining showed 76% positive cases while in non-neoplastic only 42% cases showed positivity. On statistical analysis, there was a significant difference in CCKAR expression between both groups. P-value came out to be significant in comparison between neoplastic and non-neoplastic groups.

Table 1: CCKAR expression in the neoplastic and non-neoplastic groups.

Group	N	Mean	Standard Deviation
Neoplastic	50	4.42	2.408
Non neoplastic	50	1.78	1.250

p value=<0.001 (S)

DISCUSSION

Gallbladder carcinoma is considered the fifth most common neoplasm of the digestive tract and it has an overall incidence of 3 per 100000 people. Also, it is suspected preoperatively in only 30% of all patients. [11] It is the most common cancer of the biliary tract. [11] Gall bladder Carcinoma is found in 0.2%-3% of all cholecystectomies and 0.09%-2% of all laparoscopic cholecystectomies. [12,13]
Nearly 70% of cases are diagnosed using postoperative incidental findings by a pathologist and are termed incidental or occult gallbladder carcinomas. It is observed that only 15%-47% of the preoperatively known gallbladder carcinomas are suitable for resection. [14] The majority of symptomatic patients with malignant gallbladder disease have an incurable tumour. So, the outcome of gallbladder carcinoma is poor, and the overall 5-year survival rate is less than 5%.[15]
Gallbladder carcinoma is described in up to 3.4% of autopsies conducted on cholelithiasis patients over 60 years of age. [16] 2 peaks are usually observed in gallbladder tumour incidence. The first peak occurs at 50- 60 years of age and the second at 70-80 years of age, with a higher prevalence among women. [17-19] The incidence rates are extraordinarily high in Mapuche Indians in Chile, South America. This population exhibits the highest rate of gallbladder cancer which is 12.3/100000 for males and 27.3/100000 for females. [20] So, it is observed that countries with a higher rate of cholecystectomy have a lower rate of gallbladder carcinomas.
Different modes of lymphatic spread were described by Fahim et al. [21] based on the anatomical work in the foetuses of Clermont in 1909 and the 3 pathways described by Ito et al[22] in adult cadavers.

Shirai et al., [23] identified the regional lymphatic system of the gallbladder by intraoperative vital staining.
Also, a series of studies by Kano M et al.,[24] in 2002 had shown that the formation of cholesterol supersaturated bile in subjects with cholesterol gallstone disease is causatively related to decreased gallbladder contractility and mucin hypersecretion by the gallbladder. Supersaturated bile may modify the Composition of gallbladder membranes so that the transduction of smooth muscle regulatory signals is impaired, and also may enhance the inflammation-induced mucin secretion by gallbladder. This showed that CCKAR has a strong association with gall stone formation which may indirectly lead to carcinogenesis.
Also, a study conducted by Schulz S et al., [25] in 2005 showed that the presence of CCK1 receptors was rarely detected in human tumours except for carcinoids, insulinomas, pituitary adenomas, and meningiomas. So, it was observed that overexpression of the CCK receptor is definitely association with some form of carcinogenesis.
The findings of this study, have thus concluded that the positivity of CCKAR expression has a significant role in differentiating neoplastic and non-neoplastic lesions of gall bladder. Detection of CCKAR in normal gall bladder and in epithelial dysplasia should also be done to determine whether CCKAR expression progressively increases with the onset of changes in the gallbladder epithelium. So, we found that the coexpression of CCK, in situ detection of CCKAR, secondary signalling pathways linked to it and the mechanism of its up-regulation, should also be examined to explore the involvement of this receptor in the development and progression of GBC.

CONCLUSION

The incidence of gall bladder carcinoma remains much higher in Indian than in the Western world. Also, a more delayed presentation thus carrying a worse prognosis is observed. So, It is essential that it is recognized early and differentiated from other benign pathologies. Out of all available modalities, image-guided biopsies play the most important role in diagnosis and follow-up.
CCK is admittedly one of many regulatory peptides or hormones involved in GBC. The present study thus showed that the positivity of CCKAR expression has a significant role in differentiating non-neoplastic from neoplastic lesions of the gallbladder. CCKAR is significantly overexpressed in GBC and encourages the inclusion of more peptides and hormones in future studies. In addition, the over-expression of CCKARs in most cases of GBC may suggest the use of receptor antagonists for tumour localization, clinical assessment, and receptor-based delivery of therapeutic agents to treat GBC.

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