Response to Reviewers and Editors

Dear Reviewers and Editors,

Many thanks for your valuable comments regarding our article entitled "**Pathological, Molecular, and Clinical Characteristics of Cholangiocarcinoma - A Comprehensive Review**". We appreciate your interest and valuable time spent in going through our article. We have now revised our manuscript taking in to account the comments, critiques and questions highlighted in your review. We believe that the revised manuscript now reads well and fulfils the requirements for publication in ***World Journal of Gastrointestinal Oncology.*** If you have any further queries or comments, please do not hesitate to contact us.

Kind Regards

Mukul vij

Reviewer comments

Reviewer(s)' Comments to Author:

Response to reviewers

Reviewer #1:

Although there are dozens of published reviews focusing on cholangiocarcinoma, such as recent work by Rodrigues PM, et al. (Pathogenesis of cholangiocarcinoma. Annual review of Pathology-Mechanisms of Disease. 2021;16:433-463. doi: 10.1146/annurev-pathol-030220-020455), the manuscript by Dr Mukul Vij is expected to give many new knowledge to readers. The review is well organized and written, it can be accepted after minor revision. Minor comments:

1. There are several errors in or inconsistencies in writing, for example, "A losses of SMAD4" on page 21, and "EPCAM" and "EpCAM" on page 10.

Authors' reply: The corrections as mentioned by the reviewer have been made in the manuscript.

2. The format of references needs to be re-edited.

Authors' reply: The corrections as mentioned by the reviewer have been made in the manuscript.

3. Some currently published articles regarding the pathology and tumorigenesis of cholangiocarcinoma are suggested to be cited, such as 1. Huang YH, et al. J Hepatol. 2021;74(4):838-849. doi: 10.1016/j.jhep.2020.10.037. and 2. Zhou YJ, et al. Hepatology. 2021;74(2):797-815. doi: 10.1002/hep.31780.

Authors' reply: Both the references have been added to the manuscript

Reviewer #2:

This review focuses on the current knowledge of pathological characteristics, molecular alterations of cholangiocarcinoma and its precursor lesions (including biliary intraepithelial neoplasia, intraductal papillary neoplasms of the bile duct, intraductal tubulopapillary neoplasms and mucinous cystic neoplasm). It gives a comprehensive review of all pathological types of cholangiocarcinoma. There are some minor concerns about this review article.

1. Molecular basis of different risk factors may be different. This could be described in detail.

Authors' reply: The section in the manuscript has been extensively rewritten to include a detailed description of risk factors (as shown below).

"RISK FACTORS

Most cases (70%) of cholangiocarcinoma are sporadic, occurring without any probable or known risk factors. Table 1 lists all known risk factors for cholangiocarcinoma. Parasitic infections like *Opisthorchis viverrini* and *Clonorchis sinensis* liver flukes induces chronic bile duct inflammation, and periductal scarring which increases risk of biliary tract malignancy.[12] In the Western world, primary sclerosing cholangitis (PSC) remains the most prevalent risk

factor.[4] PSC includes chronic inflammation, biliary epithelial proliferation, and production of endogenous bile mutagens leading to biliary tumorigenesis.[5] Malignant transformation in epithelial lining of biliary cysts occur as there is reflux of pancreatic enzymes, bile stasis and increased bile acid concentration.[5] Increased risk is also reported in Caroli disease and hepatolithiasis as there is bile stasis, chronic inflammation, bacterial infection, and recurrent cholangitis.[12] In cirrhotic patients there is increased risk of cholangiocarcinoma as there is increased cell proliferation, release of inflammatory cytokines and scarring.[5] In patients with HBV and HCV direct carcinogenic effect of viruses on hepatic progenitor cells as well as cirrhosis increases the risk of cholangiocarcinoma. Obesity increases the risk of cancer, by affecting the levels of leptin, adiponectin and proinflammatory cytokines.[5] Non-alcoholic fatty liver disease may promote cholangiocarcinoma development directly by induction of hepatic inflammation or, indirectly, via cirrhosis."

2. In the section of "Sarcomatoid Cholangiocarcinoma", hpayperchromatic should be corrected as hyperchromatic.

Authors' reply: The mentioned typo has been corrected

3. In the section of "Sarcomatoid Cholangiocarcinoma", a "survival" is missing after "shorter overall".

Authors' reply: The mentioned typo has been corrected

4. The sentence "NCAM and EMA are often negative or weakly positive for tumour cell cytoplasm in HCC-like IHCC, but are are strongly positive for stem cell makers, including TROP2, EpCAM and Nestin" in section "IMMUNOHISTOCHEMICAL FEATURES" should be corrected for grammatic error.

Authors' reply: Thank you for highlighting the error, the mentioned typo has been corrected.

Reviewer 3

Reviewer Comments: I read with interest this comprehensive review that effectively summarises the current knowledge on clinico-pathological characteristics of

cholangiocarcinoma by Dr Vij and colleagues. I would like to anyway point out some concerns: - There are several typos and mistakes, and the English language needs a review

Authors' reply: The manuscript has been thoroughly reviewed and revised by a native English speaker for grammar, typos and syntax.

Reviewer Comments: In the text some parts are repetitive and do not help the logical flow of the manuscript (e.g., regarding liver flukes role as risk factor)

Authors' reply: The manuscript has been reviewed for repetitive texts and the same have been removed.

Reviewer Comments: In the "EPIDEMIOLOGY AND RISK FACTORS" section the authors state that CCA incidence is rising. This does not appear accurate, since it is true for intrahepatic CCA but not quite for the extrahepatic ones. Moreover, it would be worth mentioning that CCA subtypes appear to show distinct epidemiological trends.

Authors' reply: We thank the reviewer for the insightful comment. The section has been extensively re-written to incorporate epidemiological trends for extrahepatic CCA.

Reviewer Comments: The intraductal papillary mucinous neoplasm are described with different acronyms throughout the text (IPNB, which is preferable, and IPMN-B). This should be uniformed –

Authors' reply: The corrections have been made within the manuscript and only IPNB acronym has now been uniformly used.

Reviewer Comments: The Authors should add a section/paragraph on liquid biopsies and their role in the diagnosis/management of CCA (e.g. in the Role of Molecular Pathology in Diagnosis and Management section) –

Authors' reply: We thank the reviewer for the suggestion. On retrospect, we agree it a topic which needed addressing. We have now added the following paragraph to the manuscript.

"The term liquid biopsies comprise a diverse group of methodologies, centering around detection and analysis of tumour cells or tumour cell products obtained from blood or other body fluids.[109] Various types of liquid biopsies include circulating tumour cells (CTCs), cell free nucleic acids (DNA, mRNA, non-coding RNA such as micro-RNA or long non-coding RNA),

"tumour-educated platelets" (TEPs) or vesicles such as exosomes.[110] Clinical application of liquid biopsies includes early detection of cancer or tumour recurrence, individual risk assessment and treatment monitoring. Few studies have evaluated role of liquid biopsies in cholangiocarcinoma. Yang et al showed that CTCs were associated with more-aggressive tumour characteristics and independently associated with survival in patients with cholangiocarcinoma.[111] Wintachai et al investigated diagnostic and prognostic values of plasma cf DNA levels from 62 cholangiocarcinoma patients, 33 benign biliary disease patients and 30 normal controls and showed superior diagnostic efficacy in detecting cholangiocarcinoma as compared to CEA and CA19-9.[112] Most commonly identified genetic alterations were in ARID1A (30%), PBRM1 (30%), MTOR (30%), and FGFR3 (30%). The role of liquid biopsies in cholangiocarcinoma is still very limited and further research is required to appreciate its full potential.[109]

Reviewer Comments: In the Clinical management section would be worth expanding resection criteria and role of lymphadenectomy

Authors' reply: We have rewritten the whole section incorporating details of resection criteria and the role of lymphadenectomy.

"Surgical resection and Lymphadenectomy:

Treatment of choice is surgical resection for these tumours. Proximal and distal involvement of bile duct is critical in decision making in PHCC.[124] Arterial and portal vein local resection is indicated when R0 resection is possible. When the tumour is IHCC anatomical resection of involved segment of liver is sufficient but in cases of PHCC an extensive locoregional lymphadenectomy is indicated. Although data for extent of lymphadenectomy is not sufficient and conclusive. Perihilar standard lymphadenectomy extends to periaortic lymph node. Involvement of Para-aortic lymph nodes is bad prognostic indicator and lymphadenectomy should not be extended to the same.[125, 126] "