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INFECTION AS A RISK FACTOR FOR GALLBLADDER CANCER- LITERATURE REVIEW.

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KEYWORDS :

INTRODUCTION-

Gallbladder cancer (GBC) is the 6th most common gastrointestinal malignancy and most common hepatobiliary malignancy representing 85-90% worldwide with an annual incident of 2/1,00,000 and marked geographical and ethnic variability[1] and known for late diagnosis and poor outcome. High rates of gallbladder carcinoma are seen in different parts of the world like South America (Chilli, Bolivia, and Ecuador)[2] as well as in some parts of India (specifically in north India- UP, Bihar, Delhi, West Bengal, Assam, and Madhya Pradesh, mostly in Gangetic belt), Pakistan, Japan, and Korea. In north India, gallbladder cancer is 10 times more common in comparison to south India. R Kanthan et al[3]. (2015) divides the risk factor for GBC into four broad groups: i)Patient demography, ii) GB abnormalities, iii)patient exposure to a specific chemical, genetic and molecular factors, iv) Infection. This study says that not only gallbladder stones but also infection by some microbial agents like salmonella and helicobacter also having contributory risk factors.

The studies demonstrating the association of bacterial infection in increase risk of Gallbladder cancer have been investigated by many reaserchers[4]. The role of cholelithiasis and the development of Gallbladder cancer has been established in various previous studies[5]. Annually 22 million cases of Salmonella typhi (S.typhi) and 5.4 million cases of Salmonella paratyphi (S.paratyphi) fever and death of approximately 2,00,000 worldwide. In the S.typhi endemic area of the world, approximately 1 to 4 % of infected individuals become chronic and asymptomatic carriers despite proper use of antibiotics (dose, days) of which 90% are also harbouring gallbladder stone and their combined association is a major predisposing factor for the development of gallbladder cancer[6]. Carcinogenesis is associated with genetics (mutation in multiple tumour suppressor gene and oncogene - p53 and k-RAS)[7] and lifestyle but S. Typhi infection and Gall stone represent the most important risk factors. Gall stone size also increases the risk > 3 cm size increase the risk by 10 fold[8].

The number of various infections is associated with cancers of different organs:

Cancer/ Malignancy	*Parasite/ virus*	*Evidences*
Burkitts lymphoma	EBV	Magrath I, Jain V, Bhatia K. Epstein-Barr virus and Burkitt's lymphoma. Semin Cancer Biol. 1992 Oct;3(5):285-95. PMID: 1335792.
Hepatocellular carcinmoma	HBV & HCV	El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012 May;142(6):1264-1273.e1. doi:10.1053/j.gastro.2011.12.061PMID: 22537432; PMCID: PMC3338949.
Kaposi's sarcoma	HHV-8	Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. Nat Rev Cancer. 2010 Oct;10(10):707-19. doi: 10.1038/nrc2888. PMID: 20865011; PMCID: PMC4721662.
Carcinoma Cervix, Head & neck, Anal, oral, pharyngeal ,penile cancer, vaginal & vulval cancer	HPV	Münger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, Grace M, Huh K. Mechanisms of human papillomavirus-induced oncogenesis. J Virol. 2004 Nov;78(21):11451-60. doi: 10. 1128/JVI.78.21.11451-11460.2004. PMID: 15479788; PMCID: PMC523272.
Adult T-cell Lymphoma	HTLV-1	Mahieux R, Gessain A. HTLV-1 and associated adult T-cell leukemia/ lymphoma. Rev Clin Exp Hematol. 2003 Dec;7(4):336-61. PMID: 15129647.
Skin cancer (Merkel cell carcinoma)	Merkel cell polyoma virus	Liu W, MacDonald M, You J. Merkel cell polyomavirus infection and Merkel cell carcinoma. Curr Opin Virol. 2016 Oct;20:20-27. doi: 10.1016/j.coviro.2016.07.011. Epub 2016 Aug 10. PMID: 27521569; PMCID: PMC5102790.
Stomach cancer & Maltomas	H.Pylori, EBV	Floch P, Mégraud F, Lehours P. Helicobacter pylori Strains and Gastric MALT Lymphoma. Toxins (Basel). 2017 Apr 8;9(4):132. doi: 10.3390/toxins9040132. PMID: 28397767; PMCID: PMC5408206.
Non-Hodgkin's lymphoma	EBV, HBV& HCV	Engels EA. Infectious agents as causes of non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev. 2007 Mar;16(3):401-4. doi: 10.1158/1055-9965.EPI-06-1056. Epub 2007 Mar 2. PMID: 17337646.
Urinary bladder cancer	Schistosoma Hematobium	Botelho MC, Alves H, Richter J. Halting *Schistosoma haematobium* - associated bladder cancer. Int J Cancer Manag. 2017;10(9):e9430. doi: 10.5812/ijcm.9430. Epub 2017 Sep 30. PMID: 29354800; PMCID: PMC5771257.
Cholangiocarcinoma	Opisthorchis viverrini & Clonorchis sinensis	Choi BI, Han JK, Hong ST, Lee KH. Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. Clin Microbiol Rev. 2004 Jul;17(3):540-52, table of contents. doi: 10.1128/CMR.17.3.540-552.2004.
Mesotheliom a, some brain tumor, bone tumor & lymphomas	Simian virus 40 (SV40)	Fraumeni JF, Ederer F, Miller RW. An evaluation of the carcinogenicity of simian virus 40 in man. *J Am Med Assoc* 1963; **185**: 713– 18

Pathomicrobial development of Gallbladder cancer

Carcinogenic activity of salmonella typhi: A study from Bolivia and Mexico reported 12 fold increasing gallbladder cancer in subjects with a history of typhoid fever[9]. S.typhi releases *beta*-Glucouronidase and Nitroso compounds that are potential for carcinogenesis and also produce toxic molecules belong to the group of cytolethal distending toxins (CDTs) which induces DNA damage and activates irreversible cell cycle arrest and apoptosis[10]. CDTs encodes three different genes, cdt A, cdt B, cdt C, they encodes three different polypeptides (CdtA, CdtB, CdtC) together forms heterotrimeric toxine[11]. Cdt-B has functional homology with mammalian DNAase-I also causes a double stranded break in host cell DNA and activating the DNA damage response pathway(DDR)[12]. CdtA and CdtC acts as binding molecules to help in the binding of heterotrimeric holotoxin to the plasma

membrane of a target cell and facilitates CdtB penetration into the target cell and promotes double-strand break (DSB) and activation of Ataxia-telangiectasia mutation (ATM) dependent DNA damage response[13]. The activity of CdtB depends on its two-component which resembles with B component of pertussis like toxin named as pertussis like toxin A (*pltA)* pertussis like toxin B (*pltB)*[14]. The S. Typhi CdtB-pltA-pltB complex is being delivered into the target cell nucleus and induces DNA damage by one mechanism and by another mechanism after internalization into host cell it goes into vacuole after that via Golgi apparatus and Endoplasmic reticulum secreted into the extracellular medium and targets adjacent cells inducing DNA damage and cell cycle arrest[15].

Role of biofilm mediated Salmonella typhi carrier persistence in the development of gallbladder cancer:

S. Typhi chronic carrier state is associated with approx. 80% cases with the persistence of cholesterol gallstone[16]. The use of a high dose of prolonged antibiotic course is not capable of eradicating S. Typhi colonisation of gallbladder cancer in chronically infected patients. The chronic persistence of S. Typhi colonies can evade host immune response in carriers. There is two processes of biofilm formation over gall stone are Biofilm initiation and biofilm maturation[17]. The biofilm initiation process depends on two-component of bacterial appendages Flagella and fimbriae found on S. Typhi species and other Enteric pathogens for attachment and after the attachment phase starts the formation of biofilm and development of mature biofilm. The initiation and maturation phases are mediated by the presence of Extracellular polymeric substance (EPS) help in biofilm structure formation and cell-cell interaction[18]. Colonization of gall bladder with S. Typhi, bile, bile acid, cholesterol, phospholipids and bilirubin together forms strong antimicrobial properties and acts as the central pathogenic process in acute and chronic gall bladder infection, strongly suggest the presence of bio-film related microbial diseases[17]. So, biofilm formation is the key strategy adopted by s. typhi to allow microbial persistence, protection against antibiotics and host immune response. Chronic persistence posses the mutagenic effect of bacterial toxins on target cells and bile also induce pleiotropic effects regulating the expression of certain genes leads the cumulative damage and transformation[18].

Study on other microbial agents in the causality of gallbladder cancer

A study was done in Brazil by C.P. Silva and Lima et al. In 2003 showed the presence of Helicobacter DNA in the biliary epithelium of patients of biliary tissue and bile. In some studies, the presence of helicobacter or H.pylori DNA has been seen in bile and Gallbladder tissue of patients with Benign or malignant biliary diseases only when a more sensitive PCR 16S rRNA method was used for 46 Brazilian patients with and without cholelithiasis. Study shows that Helicobacter is associated with human cholelithiasis and cholecystitis but results are conflicting due to the small number of patients[19].

Pieces of evidence from case-control studies

Several studies (Kumar et al. 2006, Andia et al 2008, Nagaraja and Eslick et al. 2015, Kanthan et al. 2015) establishes the association of S. Typhi infection and cancer gall bladder development[20,21,22,2,23]. And the association of H. Pylori infection and GBC was established by some studies (Martel etal.2009, Pandey et al. 2010, Yakoob et al. 2011) by using the DNA PCR method[24]. A study was conducted in VMMC Hospital (New Delhi) in collaboration with BITS, Goa (India) patients were taken from north India (New Delhi), the histopathological study of 40 patients tissue sample was taken mostly suffered from cholelithiasis and cholecystitis. Histopathological sample sent for microbial assay showed the presence of E. Coli in three patients (7.5%) and the sample showing S. Typhi. The microbial tissue of seven patients was taken for culture none of them showed the presence of S.typhi DNA on PCR, four patients (10%) PCR positive for tissue, two patient PCR positive for bile sample and one patient was PCR positive for Gall stone disease. The isolation of S. Typhi in patients with chronic cholelithiasis did not support the hypothesis of bacterial persistence factor in gall bladder cancer development[25]. The fact that may be S. Typhi specific DNA present in the viable state but a non-cultivable state. So that to confirm the presence of chronic S. Typhi in the gall bladder was revealed by using molecular methods and by next-generation sequencing method for confirmation.

A case-control study from BHU (Varanasi, India) was performed on patients with the biliary disease and healthy control to detect the typhoid carrier state[26]. The study was conducted in an area of high endemicity of acute typhoid fever, asymptomatic chronic carrier and gall bladder cancer. An Indirect Haemagglutination assay measuring antibody against highly purified S. Typhi Vi polysaccharide antigen was used. Vi polysaccharide positivity was significantly higher in patients with gall bladder cancer (29.4%) compared to control (5%) (x2=6.325, p<0.0004, OR=7.19) and patient with cholelithiasis (10.7%)(x2=5.066, p<0.01,OR=3.86). There was an 8.47 times greater risk of developing cancer of gall bladder in culture-positive typhoid carriers than in non-carrier. The study concluded that typhoid carrier state was a risk factor for gall bladder cancer development.

Pieces of evidence from cohort studies

A prospective cohort study by Caygill et al. based on Scotland typhoid outbreak of 1964[27]. A total of 507 typhoid and paratyphoid cases were reported of the total traceable patients (386) 76% were cured and were non-carriers, (83) 16% were reported as chronic carriers. After excluding those dead (139), non-traceable (121) and still carrying the disease (6) the cancers of pancreas biliary track lung and all other neoplasm were analysed. This study provides strong evidence of the high risk of gallbladder cancer in chronic typhoid carriers as opposed to acute typhoid patients. The carrier of typhoid and paratyphoid showed greater risk of gall bladder cancer (167; 95% CI:54.1-389), cancer of Pancrease (8.1; 95% CI:1.67-23.7), Colorectal cancer (3; 95% CI:0.62-8.77), Lung cancer (2.5; 95% CI:0.82-5.89) and all other neoplasm (2.6; 955 CI:1.37-3.96). The study concluded a high risk of gall bladder cancer in chronic typhoid carriers with other site cancer a well, the author did not tell about gall stone formation and typhoid carrier association in this study. Another multicentric case-control study reported a 12 fold increase in the risk of gallbladder cancer in subjects with a history of typhoid fever (12; 95% CI:1.5-5980 which is not correlated by serological assay in bile sample and biliary tissue[28].

Evidence from the systematic review and Meta-analysis

A systematic review was done by Nagaraja and Eslick et al. 2014 include 17 studies for systematic review and meta-analysis[22]. The overall OR for chronic s. Typhi carrier state was 4.28(95% CI: 1.84-9.96), most of the studies were from southeast Asia specifically India, Pakistan and China. The demographic association are significant in south-east Asia (OR: 4.13, 95% CI: 2.87-5.94, P-value <0.01), chronic S. Typhi carrier state was associated with carcinoma of gall bladder based on detection method of S. Typhi antibody level (OR:3.52, 95% CI: 2.48-5.00, P Value<0.01) and detection of S. Typhi in culture (OR4.14, 95% CI:2.41-7.12, P value<0.01). The association was predominant in control without gall stone (OR:5.86, 95% CI:3.84-8.95, P-value,0.01) when compared with controls with gall stone (OR:2.71, 95% CI: 1.92-3.83, P value<0.01). The study concluded that chronic S. Typhi carrier is an important risk factor for patients with cancer gall bladder and high-risk association with gall stone disease.

CONCLUSION

Gall bladder cancer is uncommon in the developed world but the variable presentation in developing world with geographical, ethnic and etiological variability[29]. Gall bladder cancer is known for its usually late presentation and poor outcome. Chronic infection by certain organisms like Salmonella and H. Pylori and other Enterobaceriace group microbes with cholelithiasis is a major risk factor. Diagnosis usually at the time of cholecystectomy for gall stone disease with incidental pathological findings, although advances in radiological imaging with transabdominal and endoscopic imaging help in diagnosis. Surgery is the main modality for curative treatment but usually due to late presentation surgery is not possible in all cases, managed by Preoperative chemotherapy followed by assessment for surgery if feasible, some cases also need Adjuvant Chemotherapy[30]. So, the early diagnosis is imperative and need of assessment of high-risk patients in an area of high endemicity of acute typhoid cases for the search of chronic carriers and do early needful intervention and areas of high endemicity of gall stone and cholecystectomy and complete histopathological examination of all cases.

We also use some primary and secondary prevention strategies in high-risk areas. Primary prevention will be applied once the high-risk gene and environmental toxins like salmonella toxin with the chronic carrier are found. Secondary prevention is applied in areas of high endemicity and the patient is at risk[31]. Chronic salmonella infection is still considered as the primary predisposing factor in high endemic areas of typhoid fever independently or together with gall stone disease with biofilm formation over it. So we need additional studies to evaluate the strategies for the prevention of biofilm formation over gall stone surfaces.

Future Perspective of review literature

Previous studies demonstrate that PCR analysis was the most sensitive

method of detecting salmonella and other bacteria from microbial and bile samples but nowadays in the new era of diagnostics Next-generation sequencing(NGS) technology could be used to detect and identify in the natural tissue environment or in a human biological sample (blood etc) without a need for isolation and confirmation by culture[32]. This new technique also detects and identify the uncultivable microorganism in the biological sample. Also, need for a strengthening of vaccination strategy in the high endemic area of typhoid infection and to cut the development of chronic typhoid carrier state[33].

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