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Infection as a Risk Factor for Gallbladder Cancer

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Gallbladder cancer is a common hepato-biliary malignancy with poor prognosis. The main associated risk factors identified so far include cholelithiasis (especially mixed gall stone), chronic infections of the gall bladder, obesity, reproductive factors, diet, hepato-biliary anamolies, and environmental exposure to specific chemicals. Genetic and molecular predisposing factors have also been described. This article reviews the association of chronic infection and gall bladder cancer. Most of the studies have shown a good association of mixed bacterial and Salmonella infections in the carcinogenesis of cancer gall bladder especially in the area of high endemicity of typhoid. Bacterial degradation of bile and chronic inflammation may also play some role in the carcinogenic process. Mutations in multiple tumor suppressor gene and oncogenes (P53 and K-ras) have also been found in a few studies. This review seeks to bring out many hidden infective etiological aspects of the pathogenesis of gall bladder cancer. Review of the entire published literature suggests a need for further studies for better understanding of the disease.
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KEY WORDS: **infection; enteric fever—Salmonella; Helicobacter; gallbladder cancer**

Gallbladder cancer is a common hepato-biliary malignancy with poor prognosis. The main associated risk factors identified so far include cholelithiasis (especially mixed gall stone), chronic infections of the gall bladder, obesity, reproductive factors, diet, hepato-biliary anamolies, and environmental exposure to specific chemicals. Genetic and molecular predisposing factors have also been described. This article reviews the association of chronic infection and gall bladder cancer.

Most of the studies have shown a good association of mixed bacterial and Salmonella infections in the carcinogenesis of cancer gall bladder especially in the area of high endemicity of typhoid. Bacterial degradation of bile and chronic inflammation may also play some role in the carcinogenic process. Mutations in multiple tumor suppressor gene and oncogenes (P53 and K-ras) have also been found in a few studies. This review seeks to bring out many hidden infective etiological aspects of the pathogenesis of gall bladder cancer. Review of the entire published literature suggests a need for further studies for better understanding of the disease.

The etiology of gall bladder cancer is not well known. The putative risk factors described are gall stones [1], diet, [2–4], and congenital hepato-biliary anomalies [5–7] besides the genetic and the molecular predisposition factors [8]. Several studies have shown high risk of gall bladder cancer associated with chronic infection and inflammation [9]. Several studies are discrete utilizing various biological evidences and study designs with no combinability of data. Hence, a meta-analysis has not been performed. Nevertheless, not a single study has refuted increased association of chronic infection with gall bladder carcinoma. The present review provides a brief description of studies correlating different chronic infections leading to gallbladder cancer.

Various infections are associated with cancers of different organs. For example *Helicobacter* species with gastric cancer [10], *bilharziasis* with urinary bladder cancer [11,12], *Papilloma* virus with uterine cervical cancer [13–15], *Hepatitis* '*B*' with hepato-cellular carcinoma [16–19], *liver fluke* with cholangiocarcinoma [20], etc. Chronic infection, inflammation, and irritation play a possible role in carcinomatous change, which may

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lead to gallbladder carcinoma. The evidence to this effect may come from experimental carcinogenesis models, ecological studies, cohorts, and case control studies. So far, there have been only two studies on animal models of gall bladder cancer in which diet has been implicated in its etio-pathogenesis [2,3]. However, no experimental carcinogenesis study has been conducted to show the pathogenesis of chronic infection.

There is substantial geographical variability of incidence of gall bladder cancer. Population-based data indicate that the incidence of gall bladder cancer was relatively high in northern Indian states [21,22] (Uttar Pradesh, Bihar, Orissa, West Bengal, and Assam). The rates of gall bladder cancer were higher among women than men in all populations. The incidence rates were higher in the women from Bolivia (15.5 per 100,000) and lower in Peru, Ecuador, Columbia, Brazil, Paraguay, and Uruguay (from 3.7 to 9.1 per 100,000). The incidence rates of carcinoma of gall bladder were low in North America (<2 per 100,000) with the exception of high rates among Indians in New Mexico (11.3 per 100,000) and among female immigrants from Latin America. In Europe, the highest incidence was found in the countries of Eastern Europe; Poland, the Czech Republic, and Slovakia. Except for some high-risk areas such as Nagasaki, Japan, the rates in other regions of Japan and the developed world were relatively low (<2 per 100,000) [23]. Ecologically, such geographical distribution of high incidence of gall bladder cancer has been reported from the areas where typhoid infection is more likely to occur such as Chile [24] and northern India [25,26]. In addition, gall bladder cancer occurs more frequently in the areas where proportion of mixed gall stone disease is higher as compared to pure cholesterol stones. The mixed varieties of gall stones are likely to originate from a nidus of infection [25].

EVIDENCE FROM COHORT STUDY

The most convincing evidence, however, came from a long-term cohort study by Cargill et al. [27] based on Aberdeen (Scotland) typhoid outbreak of 1964 (Table I). A total of 507 typhoid and para-typhoid cases were reported. Of the 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 tract, lung, and all other neoplasm, were analyzed as observed/expected cases (O/E). The carriers of typhoid and paratyphoid showed greater probability of gall bladder cancer (167; 95% CI 54.1–389), cancer of the pancreas (8.1; 95% CI 1.67–23.7), colo-rectum cancer (3; 95% CI O.62–8.77), lung cancer (2.5; 95% CI 0.82–5.89), and all other neoplasm (2.6; 95% CI 1.57–3.96). This study provides strong evidence of high risk for gall bladder cancer (167 times O/E) in chronic typhoid carriers as opposed to acute typhoid patients. It may be noted, however, that the risk is not only for gall bladder cancer but to some extent to other cancers also. The authors suggested that some of the non-carriers in their study were those who must have had a cholecystectomy and therefore the calculated excess risk is an underestimate. The authors did not comment on gall stone formation in this cohort. Gall stones associated with typhoid carrier status were more common in females in other studies which were mostly case control studies only [9,28]. Another multicentric case control study reported a 12-fold increase in risk of gallbladder cancer in subjects with history of typhoid fever (12.7; 95% CI 1.5–598), which unfortunately could not be correlated with serological assays [29].

EVIDENCE FROM CASE CONTROL STUDY

The possible role of bacterial degradation of primary bile acids in gall bladder carcinogensis was examined [30]. High performance liquid chromatography (HPLC) analysis for biliary bile acids, aerobic and anaerobic cultures were carried out in 10 patients, each with carcinoma gallbladder and cholelithiasis. Bacteria was seen in the bile of 4/10 (40%) patients with carcinoma gallbladder and 3/10 (30%) with cholelithiaisis. Among the carcinoma patients, culture showed two aerobic, one anaerobic, and one mixed infection. Patients with carcinoma of gallbladder significantly had high level of secondary bile acids (lithocholate and deoxycholate) compared to cholelithiasis. Among the cancer patients, those having positive bile culture had a significantly higher level of secondary bile acids compared to culture negative patients ($P < 0.001$). Carcinoma gallbladder patients with negative bile culture too, had significantly high secondary bile acids compared to culture positive cholelithiasis ($P < 0.0052$) and culture negative cholelithiasis patients ($P < 0.000056$). The study suggested that bacterial degradation of primary bile acids in the gallbladder may be responsible for gall bladder carcinogenesis [30].

A case-control study from northern India was performed on patients with biliary diseases and healthy controls to detect typhoid carrier state [31]. Typhoid carrier state was detected in patients with cholelithiasis, carcinoma of gall bladder, and control. This was indeed the first study of its kind in an area of high endemicity for both typhoid infection and carcinoma of gall bladder. An indirect hemagglutination assay measuring antibodies 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 controls (5%) ($\chi^2 = 6.325$, $P < 0.004$, OR = 7.19) and patients with cholelithiasis (10.7%) ($\chi^2 = 5.066$, $P < 0.01$, OR = 3.86). There was 8.47 times greater risk of developing carcinoma of gallbladder in culture positive typhoid carriers than in non-carriers. The study concluded that typhoid carrier state was a risk factor for gall bladder cancer development.

The role of chronic bacterial infection in gallbladder cancer was evaluated in a study where the evidence of *Salmonella* infection was found in serum, bile, and surgically removed gallbladder tissue specimens [32]. The methods applied included detection of Vi antibodies from serum, microbial culture from bile, and tissue detection of *Salmonella* DNA by PCR. A total of 395 samples from all the three components of study were obtained (gallbladder cancer—107, gall stone—118, and normal subjects—170). Vi antibodies were found to be significantly higher ($P < 0.001$) in gall bladder cancer patients (68%) compared to gallstone patients (44%) and normal subjects (28%). Microbial culture was performed on samples obtained from patients of gall bladder cancer (bile—31, tissue—23), gall stone (bile—11, tissue—47), and normal subjects (bile—0, tissue—7). Growth of *Salmonella* was not obtained in cultures of the tissue and bile owing probably to the use of antibiotics. Hence there was no demonstrable direct evidence. Presence of *Salmonella* DNA was tested by PCR in gall bladder cancer patients (bile—6, tissue—11) that were positive in two cases. It may be noted, however, that the low detection rate of *Salmonella* DNA by PCR could be due to inhibitory presence of bile in the tissue. Overall, the study concluded that chronic *Salmonella* carrier state is strongly associated with gall bladder cancer as well as gall bladder stones [32].

The bacteriological features were analyzed in the bile of controls, gall stones and carcinoma gall bladder patients [33]. Bile cultures were obtained from a total of 372 patients (controls—36, symptomatic gall stones—165, acute cholecystitis—46, common bile duct stone—67, cancer gall bladder—58). No pathogenic bacteria were grown from the bile of controls but positive bile culture was grown from the bile of symptomatic patients of gall stones 52/165 (32%), acute cholecystitis 19/46 (41%), common bile duct stones 39/67 (58%), and cancer gall bladder 47/58 (81%). The most commonly associated bacteria were *Escherichia coli*, *Streptococcus faecalis*, *Klebsiella*, and *Enterobacter* but *Salmonella* was uncommon in cancer gall bladder 4/58 (8.5%). However, more sensitive tests like scanning electron microscopy and molecular genetic techniques for chronic infection, were not used. Patients over the age of 60 years tended to be more likely to have organisms in their bile than patients aged 60 or less, and the difference was significant for symptomatic gall stone disease ($P < 0.003$). Significant differences were also found between patients with symptomatic gall stone disease and those with carcinoma gall bladder in both age groups ($P < 0.002$ in each case). The advanced age associated with positive bacterial culture signifies chronicity of infection in the process of carcinogenesis [33].

In yet another significant prospective study [34] conducted from northern India, cultures were obtained from the core of gall stones after breaking a freshly removed stone on a culture plate. The stone was sterilized at surface in order to remove the bile and culture the bacteria inside the stone. Of the 100 patients studied (chronic cholecystitis—85, cancer gall bladder—14, periampullary carcinoma—1) who underwent cholecystectomy, the results demonstrated positive culture in 81% of cholelithiasis and 77% of cancer gall bladder patients of which 51.5% were enteric and 20% non-enteric bacteria. Only enteric organisms were cultured from 11 patients of cancer gall bladder. The mean age of patients with cholelithiasis was lower than that of patients with cancer gall bladder (43.48 ± 14.07 vs. 57.45 ± 12.51, $P = 0.005$). The mean age of patients harboring enteric bacteria in their calculi was higher than that of patients harboring non-enteric bacteria (47.52 ± 12.86 vs. 36.57 ± 12.63, $P = 0.0069$). The *Salmonella* organism, however, was not isolated from inside the stone contrary to popular belief that bacteria inside gall stones are dead (*gall stones as tombs*). The gall stones were shown to harbor live bacteria from inside (*gall stones as wombs*). The authors did not simultaneously culture the bile or the surface of the gall stone. Therefore, the study, though important for the pathogenesis of gall stones, fails to throw light on its impact on the etiopathogenesis of cancer of the gall bladder. Also, the authors have not done a sub-group analysis of the bacteria grown depending upon the type of stone whether mixed or cholesterol [34].

STUDIES ON HELICOBACTER SPECIES

To see the association of *Helicobacter species*, the bile and gall bladder tissue of 46 patients with chronic cholecystitis who underwent cholecystectomy, was studied. [35] These specimens were cultured for *Helicobacter* species and subjected to PCR analysis taking *Helicobacter*-specific 16s ribosomal RNA primer. Recovery of *Helicobacter* species from frozen specimens was unsuccessful. However, using PCR analysis, 13/23 bile samples and 9/23 gall bladder tissues were found positive for *Helicobacter.* Eight of the *Helicobacter*-specific PCR amplicons were sequenced and subjected to phylogenetic analysis. Five sequences represented strains of *H. bilis*, two strains of *Floxisipira rappini*, and one of *H. pullorum*. This study also supported an association of bile

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TABLE I. Studies on Infections as a Cause of Gallbladder Cancer: Methodology, Results, and Level of Evidence

S No.	Authors	Methodology	Interpretation	Outcome level of evidence
TABLE IA.				
1	Caygill et al., 1994 [27]	Risk of cancer billiary tract, lung, pancreas, colorectal, and others were studied in typhoid carriers	↑↑Risk in GBC, colorectal, lung, and others	++++
2	Strom et al., 1995 [29]	Risk of GBC in subjects with history of typhoid fever	↑↑ Risk of GBC in subjects with history of typhoid fever	++
TABLE IB.				
3	Pandey et al., 1995 [30]	Analysis of secondary bile acids, aerobic and anaerobic cultures was carried in 10 patients each with GBC and GS	+ Bile culture GS 3/10(30%) GBC-↑↑ level of sec. bile acids GBC (+ve culture) versus GS(−ve culture) → ↑↑ level of sec. bile acids in GBC ($P < 0.001$) GBC (−ve culture) versus GS (+ve and −ve culture)→ ↑↑ level of sec. bile acids in GBC patients ($P < 0.0052$) and (0.000056)	Bacterial degradation of primary bile acids in the gall bladder may be responsible for gall bladder carcinogenesis ++
4	Shukla et al., 2000 [31]	Case control study. Typhoid carrier state in patients of GS, GBC, and normal subjects using indirect hemagglutination assay measuring antibodies against highly purified *S. typh*i Vi polysaccharide antigen	Vi polysaccharide→ significantly high in GBC (29.4%), Controls (5%) ($\chi^2 = 6.325$, OR = 3.86, $P < 0.004$) GS (10.7%) ($\chi^2 = 5.066$, OR = 3.86, $P < 0.001$)	8.47 times↑ risk of GBC in carriers ++
5	Singh et al., 2001 [32]	Vi antibodies, bile culture, and Salmonella DNA by PCR were detected in 395 patients (GBC—107, GS—118, Controls—170)	Vi antigen significantly high in GBC (68% $P <$ 0.001) versus GS (44%) and Controls (28%) Microbial culture −Salmonella in 2	−ve microbial culture, may be due to use of antibiotics. Low DNA detection rate probably due to inhibitory effect of bile. +
6	Csendes et al., 1994 [33]	A case control study, bile culture were obtained from 372 subjects (Controls—36, acute cholecystitis—46, GS—165, CBD stone—67, GBC—58)	No culture were seen in controls Positive culture in Ac cholecystitis 19/46 (41%) CBD stone-39/67(58%) GS 52/165 (32%) GBC 47/58 (81%) ($P < 0.001$)	Bacteria may have role in development of carcinoma gallbladder but S. *typhi* cultures were uncommon. ++
7	Hazrah et al., 2004 [34]	Prospective study in which bacterial cultures were obtained from the core of gall stones in 100 patients (GS and CBD stone—85, GBC—14, Periampullary cancer 1) who had cholecystectomy.	+ve cultures GS 81% GBC 77% Enteric 57% Non-enteric 20% No *Salmonella* was grown in GBC	No Salmonella was grown although this has long been considered a causative agent. +

TABLE IC.

8	Fox et al., 1998 [35]	Bile and gall bladder tissue Of 46 patient of chronic cholecystitis was cultured for helicobacter and subjected to PCR analysis.	+ve *Helicobacter* amplicon (39.1%)	Gallbladder	9/23	+
9	Matsukura et al., 2002 [36]	Bile samples from 45 Japanese and 40 Thais subjected to PCR analysis using *H bilis* specific primers	Bile 13/23(56.5%) Bile duct and GBC Japanese 13/15 (87%) → +ve for *H bilis* *Thai 11/14 (79%)→ + ve for H bils* *GS/Cholecystitis Japanese 8/16 (50%)* *Thai 10/226 (38%) tested +ve*			
10	Muarta et al., 2004 [37]	DNA extraction of *H bilis* by PCR from 34 subjects (GBC—11, Bile duct cancer—3, GS—16, and Pancreatic cancer—4) for *H bilis*	Japanese 8/16(50%)→ +ve *H bilis* GBC (3/11, 27.2%), Biliary duct cancer (1/3, 33.3%) Biliary tract cancer (4/14, 28.6%)			
11	Lu Y et al., 2004 [38]	The bacterial gene fragment was detected in 46 patients (tissue from resected GBC—28, bile—18) by PCR	+ ve bacterial DNA GBC tissue (36/46–78.3%)			++ ++

resistant *Helicobacter species* with gall bladder disease including cancer.

The association between *Helicobacter bilis* in bile and biliary tract malignancies in Japan and Thailand, was shown to have had high incidence of bile duct carcinoma and gall stones [36]. Bile samples from 45 Japanese and 40 Thai patients were subjected to PCR analysis using *H. bilis*-specific primers and six of the *H. bilis* amplicons were sequenced. Japanese 13/15 (87%) and Thai 11/14 (79%) patients with bile duct or gall bladder cancer were positive for *H. bilis* in their bile. Of the Japanese 8/16 (50%) and Thai 10/26 (38%) patients with gall stone and/or cholecystitis tested positive for *H. bilis*. The comparative analysis of Japanese patients having bile duct and gall bladder cancer with non biliary disease demonstrated significantly high positive rates ($P < 0.01$) of *H bilis*. The odds ratio for bile duct or gall bladder cancer with *H bilis* in comparison with gall stone and/or cholecystitis were 6.5 (95% CI 1.09–38.63) in Japanese and 5.86 (95% CI, 1.31–26.33) in the Thai patients. The study supports an association of *H bilis* infection of bile with biliary tract and gall bladder cancer.

Some investigators in Osaka, Japan, studied archival gall bladder specimens from 34 patients (gall bladder cancer 11, bile duct cancer 3, cholelithiasis 16, and pancreatic cancer 4). [37] DNA was extracted by PCR using 16S rRNA of *H. bilis* as primer. Amplification was observed in gallbladder cancer cases (3/11 27.2%) and biliary duct cancer (1/3–33.3%). A total 4 of 14 (28.6%) were positive for *H. bilis*. This was indeed suggestive of the possible role of *H. bilis* in biliary tract diseases, particularly biliary tract cancer.

In a study of 46 patients, the relationship between infection of different bacteria and gall bladder carcinogenesis, was studied [38]. The bacterial gene fragments were detected by PCR using common gene primer of bacteria 16s ribosomal RNA. In 28 patients, tissue samples were taken from resected gall bladder cancer on paraffin blocks and in remaining 18 patients, bile was taken during surgical procedure. The positive rate of bacterial DNA in gall bladder cancer tissue was (36/46–78.3%). Bacterial gene fragments of multiple kinds included *collibacillus*, *B fragilis*, *klebsiella*, *C perfringens*, and *Clostridium*. This study also indicates that gall bladder mucosa stimulated by anaerobic and aerobic bacteria might be a cause of the development of carcinoma.

CONCLUSION

The classical geographical distribution supports the hypothesis that areas with high endemicity of typhoid carriers and mixed gall stones have also reported higher prevalence of carcinoma of gall bladder. However, there

may be areas of high prevalence of communicable diseases like salmonella and helicobacter infectivity where cancer prevalence rates have not been studied. The best evidence so far has come from a long-term cohort study reported from an otherwise low prevalence area. The case control studies utilizing bacterial cultures, HPLC, detection of carrier state by anti-bodies measurements, PCR, etc. have shown convincing evidence to prove the hypothesis. What remains to be shown are the time trend changes in the occurrence of cancer gall bladder? Control over communicable diseases should result in reducing the rates of incidence of gall bladder cancer. Bacterial degradation of bile and chronic inflammation, may be responsible in the pathogenesis of cancer gall bladder. The possible carcinogenic mechanism involved on account of chronic infection has not yet been clarified. It is important to note that salmonella species have β glucuronidase activity. The hepatic inactivation of exogenous carcinogenic xenobiotics by glucoronidation could be reversed after bacterial hydrolysis of β glucuronides. Like the paradigm shift in gastric cancer, now considered to be caused by *H pylori* infection, the recognition of biliary tract infections in the etio-pathogenesis of gall bladder cancer, may help in re-shaping the present knowledge. Nevertheless, the jury of medical experts in the world community will allow *cancere-en-bacteria* theory, only after a few more convincing studies in this area. The studies on genes and viruses in hepatobiliary neoplasia were described and multiple tumor suppressor genes and oncogenes including P53 and K-ras were shown to be altered in biliary tract cancers. Furthermore, molecular characterization of hepatobiliary cancers in case control models, in patients with chronic gall bladder infections, may lead to better understanding of the role of infection in the pathogenesis of gall bladder cancer. The bio-medical technologies of genomics and transcriptomics, which examine genetic complement and gene expression, respectively; the technique of proteomics, which involves the analysis of protein synthesis and metabonomics that provides the global determination of bio-chemical profiles in body fluids and tissues, are yet to be utilized seriously in the understanding of physio and morpho pathology of carcinoma gallbladder.

REFERENCES

1. Lowen fels AB, lindstrom CG, Conway MJ: Gall stones and risk of gall bladder cancer. JNCI 1985;75:77–80.
2. Kowalewski K, Todd EF: Carcinoma of the Gall bladder induced in hamsters by insertion of cholestrol pellets and feeding di methyl nitrosamine (35293). Proc Soc Ext Bio Med 1971;136: 482–486.
3. Enomoto M, Naoe S, Harada M: Carcinogenesis in extrahepatic bile duct and gall bladder; carcinogenic effect of N-H hydroxy-Z Acetamide fluorene in mice fed a 'gall stone inducing diet.' Jap J of Exp Med 1999;44:37–45.
4. Arundhati Rai, Mohapatra SC, Shukla HS: A review of association of dietary factor in gall bladder cancer. IJC 2004;41: 147–152.
5. Chijiwa K, Kimura H, Tanaka M: Malignant potential of the gall bladder in patients with anomalous pancreatico biliary junction: The difference in risk between patients with and without choledochal cyst. Int Surg 1995;80:61–65.
6. Chao TC, Jan YY, Chen MF: Primary carcinoma of the gall bladder associated with anomalous pancreatio biliary ductal junction. J Clin Gastoenterol 1995;21:306–309.
7. Kimura K, Ohto M, Saisho H: Association of gall bladder cancer and anomalous pancreatio biliary ductal union. Gastroenterology 1985;54:1258–1265.
8. Reevs ME, DeMatteo RP: Genes and viruses in hepatobiliary neoplasia. Semin Surg Oncol 2000;19:884–893.
9. Welton JC, Marr JS, Friedman SM: Association between hepatobiliary cancer and typhoid carrier stage. Lancet 1979;1: 791–794.
10. Parsonnet J, Friednam GD, et al.: Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
11. EL-Bolkainy MN, Mokhtar NM, Ghoneim MA, et al.: The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer 1981;129:2643–2648.
12. Brand KG: Schistosomiasis-Cancer; etiological consideration. A review, Acta Trop 1979;36:203–214.
13. Arende MJ, Buckley CH, Wells M: Aetiology, Pathogenesis and pathology of cervical neoplasia. J Clin Pathol 1998;51:96–103.
14. Chen M, Wang H, Woodworth CD, et al.: Detection of human herpes virus 6 and HPV 16 in cervical cancer. Am J Pathol 1994; 145:1509–1516.
15. Walboomers JM, Jacobs MV, Manos MM, et al.: HPV is a necessary cause of invasive cervical cancer world wide. J Pathol 1999;189:12–19.
16. Szmuness W: Hepatocellular carcinoma and hepatitis 'B' virus: Evidence for a causal association. Prog Med Virol 1978;24:40–69.
17. Lam KC, Yu Mc, Leung JW, et al.: Hepatitis 'B' Virus and cigarette smoking; risk factors for hepatocellular carcinoma in Hongkong. Cancer Res 1982;42:5246–5248.
18. Beasley RB: Hepatitis 'B' Virus as the etiologic agent in hepatocellular carcinoma. Epidemiological considerations. Hepatology 1982;2:21S–26S.
19. Prince AM: Hepatitis 'B' virus and hepatocellular carcinoma: molecular biology provides further evidence for an etiologic association. Hepatology 1981;1:73–75.
20. Watanpa P: Cholangio carcinoma in patient with opisthorachiasis. Br J Surg 1996;83:1062–1064.
21. Indian Council Of Medical Research (ICMR). Annual report of population based cancer registries of the national cancer registry programme 1996: New Delhi: ICMR publication; 18.
22. Shukla HS, Avasthi K, Naithani Y: A clinicopathological study of carcinoma gall bladder. Ind J Cancer 1981;18:198–200.
23. Lazcano-Ponce EC, Miquel JF, Nubia M, et al.: Epidemiology and molecular pathology of gall bladder cancer. Ca Cancer J Clin 2001;51:349–364.
24. Nervi F, Duarte I, Gomez G, et al.: Frequency of gall bladder cancer in Chile, a high risk area. Int J Cancer 1988;41:657–660.
25. Shukla VK, Khandelwal C, Roy SK, et al.: Primary carcinoma of the gall bladder: A review of a 16 years period at the University Hospital. J Surg Oncol 1985;28:32–35.
26. Prakash A, Sharma LK, Pandit PN: Primary carcinoma of gall bladder. Br J Surg 1975;62:32–36.
27. Caygill CPJ, Hill MJ, et al.: Cancer mortality in typhoid and paratyphoid carrier. Lancet 1994;343:83–84.
28. Mellemgard A, Gaarslev K: Risk of hepatobilliary cancer in carriers of Salmonella typhi. J Natl Cancer Inst 1998;80:288.

29. Strom B, Solowar R, Rios-Dalenz J, et al.: Risk factors for gall bladder cancer. An international collaborative cases control study. Cancer 1995;76:1747–1756.
30. Pandey M, Vishwakarma RA, Khatri AK, et al.: Bile bacteria and gall bladder carcinogenesis, J Surg Oncol 1995;58:282–283.
31. Shukla VK, Singh H, Pandey M, et al.: Carcinoma of the gall bladder is it a sequel of typhoid? Dig Dis Sci 2000;45:900–903.
32. Singh MK, Goel SK, Prasad KN, et al.: Gall bladder cancer: Is chronic infection responsible? Ind J Gastroenterology 2001;20: 63–64.
33. Csendes A, Becerra M, Burdiles P, et al.: Bacteriological studies of bile from the gall bladder in patients with carcinoma of the gall bladder, cholelithiasis, common bile duct stones and no gall stones disease. Eur J Surg 1994;160:363–367.
34. Hazrah P, Oahn KTH, Tewri M, et al.: The frequency of live bacteria in gallstones. HPB 2004;6:28–32.
35. Fox JG, Dewhirst FE, Shen Z: Hepatic Heliobacter species identified in bile and gall bladder tissue from Chileans with chronic choleystitis Gastroenterology 1998;114:255–263.
36. Matsukura N, Yokomuro S, Yamada S, et al.: Association between H. billis in bile and biliary tract malignancies: H. Billis in bile from Japanese and Thai patients with benign and malignant diseases in the billiary tract. Jpn J Cancer Res 2002;93:842–847.
37. Murata H, Tsujii M, Fu HY, et al.: Helicobactor billis infection in biliary tract cancer. Aliment Pharmacol Ther 2004;20:90–94.
38. Lu Y, Zhang BY, Shi JS, et al.: Expression of bacterial gene in gall bladder carcinoma tissue and bile. Hepatobiliary Pancrerat Dis Int 2004;3:133–135.