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Assessment of Dietary Habit Associated with Gallbladder Cancer and Gallbladder Diseases: A Case-control Study

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ABSTRACT

Gallbladder Cancer (GBC) is an uncommon and aggressive neoplasm of gastrointestinal tract cancers with infrequent geographic variations and increasing incidence in northern India. The aim of this investigation was to identify the possible dietary or environmental risk factors associated with GBC and Gallbladder Diseases (GBD). The authors prepared a questionnaire filled by the patient mentioning the details of dietary habits, family history and other parameters, likely to be associated with or being risk factors for GBC and GSD. Details of ultrasonography, CT, FNAC and Histopathological Examination (HPE) of gallbladder cases (n=103) registered at CHRI hospital, Gwalior were also studied. Healthy, age and sex matched control subjects (n=50) were recruited. Data were analyzed by using SigmaPlotv3.5 and GraphPad. Among the protective factors, the consumption of iodized salt, fruits and dry fruits, while consumption of tobacco, non-filtered cooking oil, non-iodized salt and stale food appeared as significant risk factors in our study. Thus, increasing the proportion of the protective dietary items or significantly reducing those which may enhance the risk can effectively reduce the incidence of GBC and GBD in such endemic areas where they are more common.

Keywords: Gallbladder cancer; Gallbladder disease; Cholecystitis; Risk factor; Protective factor; Cholelithiasis

INTRODUCTION

Gallbladder cancer is a discrete type of biliary tract cancer, caused due to yet unclear reasons, which is rare, aggressive and with limited treatment option [1]. A marked geographical and demographic variation in its incidence is reported [2-4]. Its incidence is found to be high in Chile and Valdivia and Northern India [5-8]. The highest incidence rates were reported among females in Delhi, (21.5/100,000), followed by South Karachi, Pakistan (13.8/100,000) and Quito, Ecuador (12.9/100,000) [9]. GBC is still considered as an "orphan cancer", because of the least known environmental conditions associated with the disease progression [10]. Among the

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gallbladder diseases, GBC and Gallstone (GS) are considered as the foremost type [11]. The typical statement of four Fs': "Fat-fair-fertile-forty", for GS and the predominance of GBC arises a logical question to studying GBC in relation to dietary pattern [12]. Environmental factors and lifestyle are more prominent risk factors however multiple factors are linked to the occurrence of gallbladder cancer [13]. To distinguish the phenotypic effect of the disease, it is essential to identify the role of the surroundings. The environment plays an important role in causing the disease. The dietary habits of Indians vary among the states and regional areas. North central India has its own specific dietary pattern and eating habits, which are likely to be associated with gallbladder cancer. In view of this, we prepared a questionnaire related to the education, living conditions, socioeconomic status, dietary aspects, clinical parameters, etc. of the patients to critically analyze their association as risk factors for GBC and GBD, if any.

MATERIALS AND METHODS

Case-control

A total of 103 cases of GBC and GBD (females; n=72 and males; n=31) admitted at CHRI, Gwalior, were recruited in the last 2-3 years. The selection criteria of the patient were based on the record file in the patients' wardroom. After discussing with the duty-in-charge doctor, the patients were asked questions based on the questionnaire. Most of these patients were from various districts of North-central Madhya Pradesh and from the adjoining states (Rajasthan and Uttar Pradesh). Diagnosis of the patients was confirmed by ultrasonography, CT scan, FNAC and HPE of gallbladder specimens. Control subjects were healthy, age and gender-matched subjects (n=50), recruited from the same region and having no past clinical history of any cancer, stone, diabetes, jaundice, infection or other gastrointestinal diseases.

Questionnaire

A questionnaire was prepared to collect data on various food habits, clinical parameters, and other demographic characteristics. After obtaining well-informed consent from the patient, the questionnaire was filled enduring face to face interviews with the patients or their close relatives by the PhD scholars in the Hindi language for better explanation. The questionnaire comprised mainly three sections;

- Socio-economic-demographic.
- Dietary patterns and habits.
- Clinicopathological conditions.

Briefly, socioeconomic and demographic details were recorded for age, gender, district-wise incidence, caste, education, occupation status, marital status etc. Questions on dietary patterns and habits included information regarding, the type and condition of food, source and condition of drinking water, meal time (breakfast, lunch and dinner) habits of tobacco chewing, smoking and consuming alcohol. Clinico-pathological information gathered data on clinical symptoms, size and site of the tumour, family history of any illnesses, and haematological and biochemical details.

Data Analysis

Both univariate and multivariate logistic regression analysis were applied to calculate Odds Ratio (OR), and Confidence Interval (CI) at 95% Relative Risk (RR) to assess the risk and protective parameters. P value was calculated to test the significance of our data by using SigmaPlot and GraphPad software. For demographic and socioeconomic aspects, percentages were calculated. P value less than 5% was considered to be statistically significant.

RESULTS

Case-control Study of Demographic and Life Styles

Among the total of 103 cases, GBC (n=84), Cholecystitis (CC, n=07), Gallstone (GS, n=09) and GBC with Gallstone (GBCS, n=3) and a total of 50 controls were included. The mean age of cases was 55 ± 1.5 (mean ± SD) years (**Table 1**). Gwalior and adjoining districts showed the highest number of cases among the North-central region. The demographic details of the subjects are shown in **Figures 1 and 2**. The gallbladder tumors at different sites were found at different rates. The Fundus region possessed the highest number of cases 56.63% (47/83), followed by the neck 20.48% (17/83), and lumen 9.64% (8/83). The average tumour size was 1.5 and 1.3 cm in females and males, respectively (**Table 2**). Most of the cancer patients were illiterate (50.49%; 52/103) and belonging to the lower middle socio-economic group (54.37%; 56/103). The parameters, smoking 11.637 (CI 1.51-89.688); p<0.0185, RR (Relative Risk) 1.531, tobacco chewing 6.633 (CI 1.887-23.342); p<0.0032, RR-1.604, non-filtered cooking oil 18.222 (CI 5.396-61.533); p<0.0001, RR-2.872, non-iodized salt 10.118 (CI 2.894-35.368); p<0.0003, RR-1.805, stale foods (food that is kept overnight at room temperature) 8.6 (CI 2.922–25.307); p<0.0001, RR-3.794, cold food (not frozen but kept at room temperature for a long time) 10.083 (CI 1.657-61.332); p<0.012, RR-2.397 and alcohol consumption 6.363 (CI 0.789-51.276); p<0.0822, RR-1.487 were found to be as risk factors for GBD (**Table 3**). While the parameters like the use of iodized salt 0.0988 (CI 0.028-0.345); p<0.0003, RR-0.554, fresh fruits 0.0026 (CI 0.0003–0.020); p<0.0001, RR-0.157, dry fruits 0.0378 (CI 0.037-0.132); p<0.0001, RR-0.404, consuming freshly cooked/ warm food 0.0992 (CI 0.0163-0.603); p<0.0121, RR-0.417 and filtered water 0.0349 (CI 0.01-0.12); p<0.0001, RR-0.1366 and also, having breakfast 0.479 (CI 0.183–1.253); p<0.133, RR-0.766, consumption of tea and coffee 0.193 (CI 0.023-1.598); p<0.127, RR-0.684, vegetables 0.221 (CI 0.026-1.856); p<0.164, RR-0.694, leafy vegetables 0.422 (CI 0.084–2.121); p<0.295, RR-0.767, and pickles 0.017 (CI 0.020-1.384); p<0.0978, RR-0.672 were observed as protective factors for GBD as compared to controls (**Tables 4 and 5, Figure 3**).

Table 1: Demographic and pathological details of the cases participated.

	Tumor sample	Gallstone	Tumor with stone	Tumor without stone
		Gender		
Male	32.09% (26/81)	16.12% (5/31)	8.33% (1/12)	36.23% (25/69)
Female	67.91% (55/81)	83.87% (26/31)	91.66% (11/12)	63.77% (44/69)
		Age		
≤ 45 years	28.75% (23/80)	13.79% (4/29)	16.67% (2/12)	26.92% (21/78)
>45 years	71.25% (57/80)	86.21% (25/29)	83.33% (10/12)	73.08% 57/78)
		Max tumor diameter		
≤ 3 cm	24.49% (12/49)			
>3 cm	75.51% (37/49)			
		Location of GB mass/gallstone		
Neck	20.48% (17/83)	8.33% (1/12)		
Body	3.62% (3/83)	8.33% (1/12)		
Fundus	56.63% (47/83)	50% (6/12)		
Fundus+Body	2.41% (2/83)	0% (0/12)		
Lumen	9.64% (8/83)	33.34% (4/12)		
Neck+Body	1.2% (1/83)	0% (0/12)		
Common hepatic duct	2.41% (2/83)	0% (0/12)		
GB wall	2.41% (2/83)	0% (0/12)		
Lumen+Fundus	1.2% (1/83)	0% (0/12)		

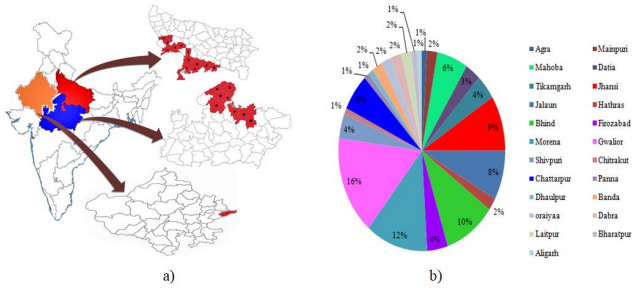

Figure 1: a) Geographic distribution of the participating subjects (map not to scale); b) District-wise distribution.

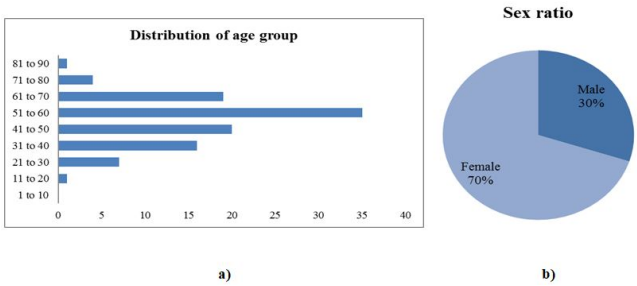

Figure 2: a) Distribution of age groups of cases; b) Percentage of gender (male and female).

Table 2: Details of tumors/mass in gallbladder.

Gender	No. of tumors	Mean ± SE (Size of tumor in cm)	S.D.	C.I. of mean	Range (cm)	Max to Min (cm)	Median (cm)	25% percentile	75% percentile
Female	32	1.5 ± 0.200	0.728	0.904	1.9	2.3 to 0.4	1.8	1	1.925
Male	17	1.3 ± 0.326	0.283	2.541	0.4	1.5 to 1.1	1.3	1.1	1.5

Table 3: Demographic details of cases and control subjects.

	Cases		Controls	
	N	%	N	%
Age class				
1 to 20	02/103	1.94	03/50	6.00
21 to 40	22/103	22.54	32/50	64.00
041 to 60	55/103	53.92	13/50	26.00
61 to 80	23/103	22.54	02/50	4.00
81 to 90	01/103	0.98	00/50	0.00
Education				
Illiterate	52/103	50.49	00/50	0.00
Primary	23/103	22.33	01/50	2.00
Secondary	21/103	20.39	02/50	4.00
Sr. secondary	02/103	1.94	09/50	38.00
College and above	05/103	4.85	38/50	76.00
Socio-economic condition				
Poor	34/103	33.01	00/50	0.00
Lower middle class	56/103	54.37	47/50	94.00
Upper middle class	13/103	12.62	03/50	6.00
Living location				
Pond	11/103	10.68	01/50	2.00
Cattle	32/103	31.06	00/50	0.00
Farming	12/103	11.65	00/50	0.00
Road	48/103	46.61	49/50	98.00
Living condition				
Pucca house (Brick house)	53/90	58.98	50/50	100.00
Kuccha house (Mud house)	37/90	41.11	00/50	0.00
Toilet				
Personal	40/84	47.62	50/50	100.00
Sarvajanik (Public)	03/84	3.57	00/50	0.00
Open	41/84	48.81	00/50	0.00

Table 4: Parameters found as protective factors between cases and controls.

Characteristics		Total	Numbers (%)	p value	OR	95% CI	RR
Breakfast	Cases	71	53 (74.64%)	0.133	0.479	0.183-1.253	0.766

	Control	50	43 (86%)				
Tea and coffee	Cases	84	76 (90.47%)	0.127	0.193	0.023-1.598	0.684
	Control	50	49 (98%)				
Seasonal vegetables	Cases	83	76 (91.5%)	0.164	0.221	0.026-1.856	0.694
	Control	50	49 (98%)				
Leafy vegetables	Cases	78	71 (91.02%)	0.295	0.422	0.084-2.121	0.767
	Control	50	48 (96%)				
Iodized salt	Cases	79	48 (60.75%)	0.0003	0.0988	0.028-0.345	0.554
	Control	50	47 (94%)				
Fruits	Cases	81	9 (11.11%)	0.0001	0.0026	0.0003-0.020	0.157
	Control	50	49 (98%)				
Dry fruits	Cases	78	29 (37.17%)	0.0001	0.0378	0.037-0.132	0.404
	Control	50	47 (94%)				
Pickles	Cases	84	75 (89.28%)	0.0978	0.017	0.020-1.384	0.672
	Control	50	49 (98%)				
Hot temperature foods	Cases	17	6 (35.29%)	0.0121	0.0992	0.0163-0.603	0.417
	Control	13	11 (84.61%)				
Filtered water	Cases	41	4 (9.75%)	0.0001	0.0349	0.01-0.12	0.136
	Control	45	34 (75.55%)				

Table 5: Parameters found as risk factors between cases and controls.

Characteristics		Total	Numbers (%)	p value	OR	95% CI	RR
Smoking	Cases	99	19 (19.19%)	0.0185	11.637	1.510-89.688	1.531
	Control	50	1 (2%)				
Tobacco	Cases	84	25 (29.76%)	0.0032	6.633	1.887-23.342	1.604
	Control	50	3 (6%)				
Alcohol	Cases	87	10 (11.49%)	0.0822	6.363	0.789-51.276	1.487
	Control	50	1 (2%)				
Non-filtered cooking oil	Cases	50	41 (82%)	0.0001	18.222	5.396-61.533	2.872
	Control	25	5 (20%)				
Non-iodized salt	Cases	79	31 (39.24%)	0.0003	10.118	2.894-35.368	1.805
	Control	50	3 (6%)				

Stale foods	Cases	48	43 (89.58%)	0.0001	8.6	2.922-25.307	3.794
	Control	50	25 (50%)				
Cold foods (frozen)	Cases	17	11 (64.7%)	0.012	10.083	1.657-61.332	2.397
	Control	13	2 (15.38%)				

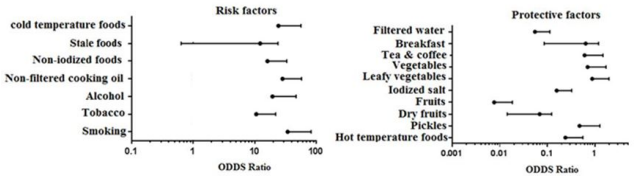

Figure 3: Odds ratios of different parameters as risk and protective factors between cases and controls subjects.

Liver Function Test Profile

The levels of total bilirubin, direct bilirubin, albumin, SGOT, SGPT and ALP were elevated in all pathological conditions of the gallbladder, *i.e.*, GBC, GS, CC, and GBCS, compared to the control subjects. Elevated levels of total bilirubin ($p<0.009$), direct bilirubin ($p<0.009$), albumin ($p<0.009$), SGOT (Serum Glutamic Oxaloactetic Transaminase) ($p<0.009$), SGPT (Serum Glutamic Pyruvic Transaminase) ($p<0.009$) and ALP (Alkaline Phosphatase) ($p<0.009$) were also observed in GBC. In the case of GS, significantly elevated values of total bilirubin ($p<0.012$), direct bilirubin ($p<0.012$), albumin ($p<0.012$), and ALP ($p<0.012$) were observed significantly elevated. In CC, significantly increased levels of albumin ($p<0.003$), SGOT ($p<0.003$) and SGPT ($p<0.003$) direct bilirubin, and direct sugar were observed. In the case of GBCS, random sugar ($p<0.004$) and ALP ($p<0.004$) levels were found elevated as compared to normal values. A remarkable increase in ALP, SGPT and SGOT levels were observed in GBC (**Figures 4 and 5**).

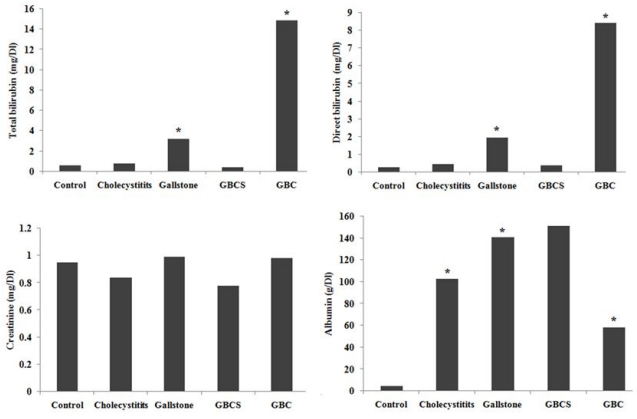

Figure 4: Histogram showing the comparative profile of direct bilirubin, total bilirubin, creatinine and albumin in GBC, GBSC, gallstone, cholecystitis and control subjects.

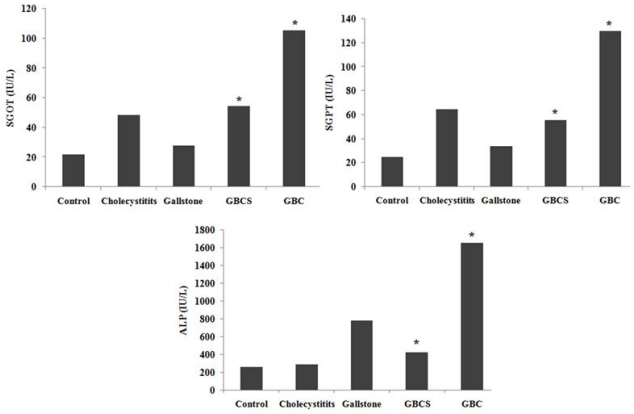

Figure 5: Histogram showing a comparative profile of SGOT, SGPT and ALP in GBC, GBSC, gallstone, cholecystitis and control subjects.

Hemogram Test Profile

The levels of platelets, monocytes, Haemoglobin (Hb), lymphocytes, eosinophils, lymphocytes and neutrophils were found to decrease in all pathological conditions of the gallbladder, including GBC, GS, CC, and GBCS. In GBC, significantly decreased levels of platelets ($p<0.001$), monocytes ($p<0.001$), Hb ($p<0.001$), and lymphocytes ($p<0.001$) were noted, whereas, basophils ($p<0.001$) were found to be significantly increased. In GS also, the levels of basophils ($p<0.0013$), platelets ($p<0.0013$), eosinophils ($p<0.0013$), monocytes ($p<0.0013$) and neutrophils ($p<0.0013$) were observed significantly low. In CC too, significantly decreased levels of platelets ($p<0.001$), eosinophils ($p<0.001$), monocytes ($p<0.001$), and lymphocytes ($p<0.001$) were observed. In cases of GBCS, the levels of eosinophils ($p<0.004$), and platelets ($p<0.004$) were observed at lower levels. TLC ($p<0.004$) was found to increase in all pathological cases, from CC to GBC. Moreover, the per cent of neutrophils was found gradually increase, from CC to GS to GBC (**Figures 6 and 7**).

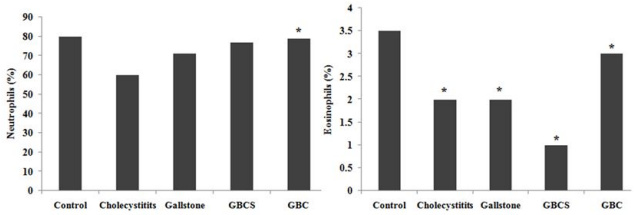

Figure 6: Histogram showing the comparative pro ile of neutrophils, eosinophils, monocytes and basophils in GBC, GBSC, gallstone, cholecystitis and control subjects.

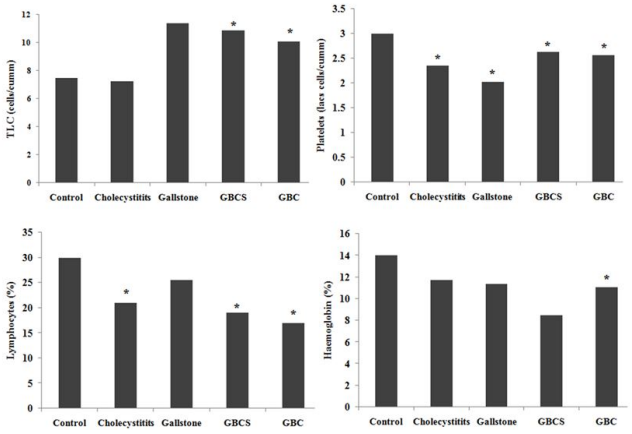

Figure 7: Histogram showing the comparative profile of TLC, platelets, lymphocytes and hemoglobin in GBC, GBSC, gallstone, cholecystitis and control subjects.

DISCUSSION

The present case-control study provides significant information on the possible protective and risk factors for GBC and GBD. Out of all cases, females (70%) were more in number compared to males (30%). Earlier studies have also reported the incidence of GBC to be high in females than in males [14]. We observed that middle-aged married women usually do not have proper food habits, especially meal timings. Inadequate and impure water intake could be a major cause of stone formation. Cholesterol stones are mainly found in most gallstone cases [15-20]. Adequate water intake increases the solubility of cholesterol with bile salt. If there is an imbalance between these bile salts and cholesterol, then the bile fluid turns into sludge. Impure water contains very fine stones and pebbles that may cause the solid stones [21]. In some of the studies, subjects (patients) were also found suffering from diabetes, typhoid fever, non-Hodgkin's lymphoma, acute pancreatitis, jaundice, and gastric problems. The predisposition of cancer is supposed to be a strong risk factor that may trigger the development of GBC. In our study, we noted the maximum number of masses to be present in the fundus region of the gallbladder. Most (>80%) the gallbladder cancers are adenocarcinomas that originate from fundus (60%), body (30%), or neck (10%).

Case-control Study of Life Styles

GBC is highly malignant, and there are both genetic as well as environmental factors that contribute to it. A gradual increase in the mortality rate of GBC is observed. We, therefore, need to execute advanced diagnostic and preventive strategies for reducing mortality rates. The foremost step towards this is the identification of the associated protective and risk factors.

The present study revealed many factors related to food habits to be protective for GBC, which suggested that seasonal fruits, dry fruits, filtered water, iodized salt and freshly cooked foods may be playing a significant protective role in GBC. An earlier study carried out in Japan reported that intake of fruits and vegetables reduces the risk of biliary tract cancer [22]. Our finding is in corroboration with another report also, where the regular intake of fruits and vegetables was suggested to prevent the risk of GBC [23]. The plants consist of phytochemicals like phenolic compounds (catechin, quercetin, gallic acid and cyanidin) and also flavonoids are present among the indigenous tropical fruits which play-potent a role as antioxidants. The phytochemicals provide anti-inflammatory and antimicrobial effects and protection against cancer [24]. Some factors, like tea and coffee, seasonal vegetables, leafy vegetables, having breakfast and pickles were also identified as protective factors for GBC; however, these findings did not appear statistically significant. Tea was previously reported, to be a protective factor for GBC and carries antioxidant, which provide protection against cancer [25,26]. While coffee drinkers showed a low risk of GBC in Japan. Most individuals do not take fruits on a daily basis, but only occasionally, and this may contribute to the development of the disease. In the Chilean population, it was found that a low intake of fresh fruits has an association with GBC [27]. In our study, we found that excluding fruits from the diet could be a significant factor contributing to the development of cancer/stone/cholecystitis. It is claimed that vegetables, especially of the cruciferous family, and fruits have a significant protective role. High consumption of vegetables is shown to be associated with alimentary tract cancer [28]. Our analysis also revealed that having regular and timely breakfast could be a protective factor in GBC. Breakfast fills up the gap between the meals, which decreases the contact time of bile with the mucosa of the gallbladder. It was observed that GBC patients often do not follow this specific habit. Most of them merged breakfast and lunch. Poor water intake is definitely an important risk factor towards developing gallstone diseases, followed by gallbladder cancer. In most cases, water is taken directly from the well or hand pump, which is not properly filtered. A detailed list of associated factors is given in **Table 1**. We also investigated many other presumed risk factors, like socioeconomic condition, living status, diet, tobacco, alcohol, smoking, etc., which may be related to environmental influences. Various clinical parameters or factors like Liver Function Tests (LFT) and hemograms were also studied. We observed 70% of patients to be female. Several other studies from India also support this, female, are more prone to GBC [29]. Our study also covered the living conditions of most GBC patients, which revealed that maximum patients were from low and middle socioeconomic groups, illiterate, unaware of health-related issues and located in odd places (such as livestock, industrial region, near roads or ponds, etc.). These are likely to act as other potential risk factors for GBC. Most of the patients used open toilets, which could lead to susceptibility to unhygienic conditions, including bacterial infections, such as *Helicobacter pylori* and *Salmonella typhii*, which are reported as potential risk factors for GBC. Rural areas have shown a high risk of Helicobacter pylori infection than urban [30-32]. Diet habits and lifestyles also play an important role in the risks of GBC [33]. Smokers being at a higher risk for GBC was also suggested by previous studies [34].Tobacco chewing has also been found to be a highly significant risk factor for GBC or associated with increased risk of it, since nicotine triggers

mutagenesis in the genome, which can lead to tumorigenesis. The smoke of tobacco contains several toxic chemicals that may have detrimental effects on the gallbladder [35,36]. Tobacco smoking increased the GBC risk and in the case of diabetes, even a small amount of smoking increased GBC risk in individuals [37]. Alcohol consumption was also found to be a risk factor for GBC, however previous studies were paradoxical with regard to the effect of alcohol on GBC [38]. Furthermore, certain additives in foods, such as non-filtered cooking oil, non-iodized salt, stale foods and the habit of consuming cold (frozen) foods were found to be significant risk factors for GBC as revealed by our study. Consumption of stale food has been associated as a risk factor for cancer [39]. An earlier study from Japan showed an increased risk of biliary tract cancer [40]. The non-filtered mustard oil consumed by GBC patients is likely to be adulterated with some carcinogens. In north India, mostly non-filtered mustard oil is consumed, which is suggested to be mixed with carcinogenic impurities. This may also be considered an etiological factor for GBC.

LFT in Gallbladder Diseases

Several GBC patients possessed increased levels of total bilirubin, direct bilirubin, albumin and SGOT. SGPT and ALP were significantly elevated. Albumin, SGOT and SGPT values were found to be exceeding the normal range in CC. Total bilirubin, direct bilirubin, albumin and ALP values were also found to be elevated in gallstones patients. On the contrary, the random sugar levels were below the normal range, but SGOT and SGPT were observed to be normal. SGOT and SGPT are the aminotransferase enzymes, which catalyze the chemical reactions involving the amino acids, where an amino group is transferred from the donor amino acid to the recipient molecule. During any tissue damage, these are released into the bloodstream. The dysfunction of the liver or gallbladder or the simultaneous effect of these organs trigger to release of biochemical enzymes. The patients with gallstones with GBC had elevated values of SGPT, SGOT, ALP and random sugar but total bilirubin, direct bilirubin, and albumin were found to be normal. Our present findings validate a previous study by Singh, et al. [41].

Hemogram in GBC and GBD

From the hematological observation, we found hemoglobin to be slightly lower in the case of GBC, whereas, GBCS showed a marked low percentage of Hb compared to the normal range of control subjects. All other cell types, such as neutrophils, basophils, eosinophils, lymphocytes, leukocytes, and platelets were in the normal range. This may also be due to the fact in our study that most of the subjects are females, and most of the females showed a low percentage of Hb.

CONCLUSION

Our analysis revealed that taking seasonal fruits, dry fruits and filtered water in adequate amounts, and using iodized salt might be developed strong protection against GBC and GBD, whereas, the use of stale food, non-filtered cooking oil, non-iodized salt, chewing tobacco and consuming alcohol are the major risk factors for GBC and GBD. Increasing awareness of people on these factors may provide aid in the management and prevention of gallbladder cancer. Further studies by increasing the sample size may reveal more conclusive results.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

1. Hu ZI, Lim KH (2022) Evolving paradigms in the systemic treatment of advanced gallbladder cancer: Updates in year 2022. Cancers. 14(5):1249.
2. Strom BL, Soloway RD, Rios-Dalenz JL, Rodriguez-Martinez HA, West SL, et al. (1995) Risk factors for gallbladder cancer. An international collaborative case-control study. Cancer. 76(10):1747-1756.
3. Pandey M, Shukla VK (2002) Diet and gallbladder cancer: A case-control study. Eur J Cancer Prev. 11(4):365-368.
4. Kai K, Aishima S, Miyazaki K (2014) Gallbladder cancer: Clinical and pathological approach. World J Clin Cases. 2(10):515-521.
5. Sharma JD, Kalit M, Nirmolia T, Saikia SP, Sharma A, et al. (2014) Cancer: Scenario and relationship of different geographical areas of the globe with special reference to North East-India. Asian Pac J Cancer Prev. 15(8): 3721-3729.
6. Barbhuiya MA, Singh TD, Gupta S, Shrivastav BR, Tiwari PK (2009) Incidence of gall bladder cancer in rural and semiurban population of north central India: A first insight. Internet J Epidemiol. 7(2):1820-2155.
7. Barbhuiya MA, Singh TD, Poojary SS, Gupta S, Kakkar M, et al. (2015) Gallbladder cancer incidence in Gwalior district of India: Five years trend based on the registry of a regional cancer center. Indian J Cancer. 52(3):430-437.
8. Hundal R, Shaffer EA (2014) Gallbladder cancer: Epidemiology and outcome. Clin Epidemiol. 6:99-109.
9. Randi G, Franceschi S, La Vecchia C (2006) Gallbladder cancer worldwide: Geographical distribution and risk factors. Int J Cancer. 118(7):1591-1602.

10. Kumari N, Corless CL, Warrick A, Beadling C, Nelson D, et al. (2014) Mutation profiling in gallbladder cancer in Indian population. Indian J Pathol Microbiol. 57(1):9-12.
11. Stinton LM, Shaffer EA (2012) Epidemiology of gallbladder disease: Cholelithiasis and cancer. Gut Liver. 6(2):172-187.
12. Kumar JR, Tewari M, Rai A, Sinha R, Mohapatra SC, et al. (2006) An objective assessment of demography of gallbladder cancer. J Surg Oncol. 93(8):610-614.
13. Perez-Moreno P, Riquelme I, Garcia P, Brebi P, Roa JC (2022) Environmental and lifestyle risk factors in the carcinogenesis of gallbladder cancer. J Pers Med. 12(2): 234.
14. Alkhayyat M, Abou Saleh M, Qapaja T, Abureesh M, Almomani A, et al. (2021) Epidemiology of gallbladder cancer in the Unites States: A population-based study. Chin Clin Oncol. 10(3):25.
15. Wang HH, Portincasa P, Afdhal NH, Wang DQ (2010) *Lith* genes and genetic analysis of cholesterol gallstone formation. Gastroenterol Clin North Am. 39(2):185-207.
16. Jaruvongvanich V, Sanguankeo A, Upala S (2016) Significant association between gallstone disease and nonalcoholic fatty liver disease: A systematic review and meta-analysis. Dig Dis Sci. 61(8):2389-2396.
17. Shabanzadeh DM, Holmboe SA, Sorensen LT, Linneberg A, Andersson AM, et al. (2017) Are incident gallstones associated to gender dependent changes with age? A cohort study. Androl. 5(5):931-938.
18. Idowu BM, Onigbinde SO, Ebie IU, Adeyemi MT (2019) Gallbladder diseases in pregnancy: Sonographic findings in an indigenous African population. J Ultrason. 19(79): 269-275.
19. Grigoreva IN, Romanova TI (2020) Gallstone disease and microbiome. Microorganisms. 8(6):835.
20. Granel-Villach L, Gil-Fortuno M, Fortea-Sanchis C, Gamon-Giner RL, Martinez-Ramos D, et al. (2020) Factors that influence bile fluid microbiology in cholecystectomized patients. Rev Gastroenterol Mex (Engl Ed). 85(3):257-263.
21. van Erpecum KJ (2005) Biliary lipids, water and cholesterol gallstones. Biol Cell. 97(11):815-822.
22. Kato K, Akai S, Tominaga S, Kato I (1989) A case-control study of biliary tract cancer in Niigata Prefecture, Japan. Jpn J Cancer Res. 80(10):932-938.
23. Pandey M, Shukla M, Shukla V (2008) Diet and gallbladder cancer. Indian J Med Paediatr Oncol. 29(01): 6-7.
24. Khoo HE, Azlan A, Kong KW, Ismail A (2016) Phytochemicals and medicinal properties of indigenous tropical fruits with potential for commercial development. Evid Based Complement Alternat Med. 2016:7591951.
25. Tyagi BB, Manoharan N, Raina V (2008) Risk factors for gallbladder cancer: A population based case-control study in Delhi. Indian J Med Paediatr Oncol. 29(01): 16-26.
26. Zatonski WA, La Vecchia C, Przewozniak K, Maisonneuve P, Lowenfels AB, et al. (1992) Risk factors for gallbladder cancer: A polish case-control study. Int J Cancer. 51(5): 707-711.
27. Serra I, Yamamoto M, Calvo A, Cavada G, Baez S, et al. (2002) Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. Int J Cancer. 102(4):407-411.
28. Moerman CJ, de Mesquita HB, Smeets FW, Runia S (1995) Consumption of foods and micronutrients and the risk of cancer of the biliary tract. Prev Med. 24(6):591-602.
29. Shukla VK, Chauhan VS, Mishra RN, Basu S (2008) Lifestyle, reproductive factors and risk of gallbladder cancer. Singapore Med J. 49(11):912-915.
30. Mishra RR, Tewari M, Shukla HS (2011) *Helicobacter pylori* and pathogenesis of gallbladder cancer. J Gastroenterol Hepatol. 26(2):260-266.
31. Tewari M, Mishra RR, Shukla HS (2010) *Salmonella typhi* and gallbladder cancer: Report from an endemic region. Hepatobiliary Pancreat Dis Int. 9(5):524-530.
32. Ueda M, Kikuchi S, Kasugai T, Shunichi T, Miyake C (2003) *Helicobacter pylori* risk associated with childhood home environment. Cancer Sci. 94(10):914-918.
33. Zatonski WA, Lowenfels AB, Boyle P, Maisonneuve P, de Mesquita HB, et al. (1997) Epidemiologic aspects of gallbladder cancer: A case-control study of the search program of the international agency for research on cancer. J Natl Cancer Inst. 89(15):1132-1138.
34. McMichael AJ, Baghurst PA, Scragg RK (1992) A case-control study of smoking and gallbladder disease: Importance of examining time relations. Epidemiol. 3(6): 519-522.
35. Wenbin D, Zhuo C, Zhibing M, Chen Z, Ruifan Y, et al. (2013) The effect of smoking on the risk of gallbladder cancer: A meta-analysis of observational studies. Eur J Gastroenterol Hepatol. 25(3):373-379.
36. Aune D, Vatten LJ and Boffetta P (2016) Tobacco smoking and the risk of gallbladder disease. Eur J Epidemiol. 31(7):643-653.
37. Park JH, Hong JY, Han K (2022) The dose response association between smoking and the risk of gallbladder cancer differs by glycemic status: A nationwide cohort study. 1-24.
38. Khan ZR, Neugut AI, Ahsan H, Chabot JA (1999) Risk factors for biliary tract cancers. Am J Gastroenterol. 94(1):149-152.
39. Dorzhgotov B (1989) Risk factors in the manifestations of the 5 principal forms of cancer in the people's republic of Mongolia. Sante Publique (Bucur). 32(4):361-367.
40. Hai AA, Sinha DN, Shah SC (1994) Carcinoma gallbladder-possible aetiology. Proceedings of the XVI International Cancer Congress. MonduzziEditore. 20:69-72.
41. Singh TD, Barbhuiya MA, Poojary S, Shrivastav BR, Tiwari PK (2012) The liver function test enzymes and glucose level are positively correlated in gallbladder cancer: A cancer registry data analysis from north central India. Indian J Cancer. 49(1):125-136.